As the world’s largest independent research and development organization, Battelle is uniquely equipped to solve the challenges that our customers face today, and the ones waiting for them in the future. From Security to Life Sciences and from Energy to Environment, Battelle is investing in what matters most.

Experience the Breadth.
Vision. Impact.

Join 9,000 microbiologists from more than 80 countries as they come together in Denver, Colorado to share their diverse perspectives and expertise on the various disciplines of microbiology.

Scientific Program
- 12 Plenary Sessions on Interdisciplinary Topics of Broad Interest
- 36 Symposia on Specialized Topics of General Microbiology
- 26 Sessions on Diagnostic Microbiology and Epidemiology
- 23 Workshops on Basic Science, Clinical Microbiology, and Career Development
- Presentations and Networking Events for Students and Postdocs
- Poster Sessions

Register before April 1, 2013 to take advantage of discounted registration rates.

www.asm.org/asm2013
Dear Colleagues,

Welcome to the American Society for Microbiology's 11th Annual Biodefense and Emerging Diseases Research Meeting in Washington, D.C. The ASM Biodefense Research Program Committee has assembled a program that includes experts in multiple relevant fields presenting in several different session formats. The 2013 program is intended to provide each attendee with the opportunity to participate in educational programming that has the most value and relevance to their individual professional needs. For this year’s meeting we have brought together individuals who are carrying out research to defend against the growing threats of bioterrorism and emerging infectious diseases and decision makers shaping the future of the biodefense research agenda.

The meeting opens Monday morning with the Plenary Session “H5N1: One Year Later” and is followed by four in-depth Focus Sessions. W. Ian Lipkin, M.D., from Columbia University, will present this year’s Keynote session, “Teaching Biodefense to Hollywood.” Dr. Ian Lipkin, a modern day microbe hunter and an acknowledged world expert in emerging infections, was recruited as a senior technical advisor by Contagion director Stephen Soderburgh and screenwriter Scott Z. Burns. We cordially invite all meeting attendees to the Opening Reception immediately following the conclusion of Dr. Lipkin’s talk. The Opening Reception provides an opportunity to mingle with old friends and new acquaintences and get a preview of the new products and services on display.

Daily Plenary Sessions are followed by diverse concurrent symposia Tuesday and Wednesday afternoon. Continuing in 2013 is the early morning Global Threats, Collaborative Solutions session featuring the Honorable Dr. Richard Danzig, Chairman of the Board, Center for a New American Security and Former Secretary of the Navy. In addition, for the first time we have added a late-breaker early morning session that will focus on the novel coronavirus recently identified in the Middle East. Please check the details in this Program and Abstracts book to identify the presentations that most appeal to you.

From over 230 accepted abstract submissions, the Program Committee has organized two Poster Sessions and six Highlighted Oral Abstract Sessions. Poster presentations are scheduled from 3:15 p.m. – 4:45p.m. Tuesday and Wednesday in the Exhibit Hall. The Highlighted Oral Abstract Sessions are scheduled Tuesday and Wednesday from 5:00 p.m. – 6:15 p.m.

We would also like to thank this year’s exhibitors. The generous support provided by exhibitors is significant to the success of our meeting. Let’s demonstrate our appreciation for the long-standing patronage by visiting the Exhibit Hall on Monday, Tuesday and Wednesday. More than 30 exhibitors will display their products and services for deterring bioterrorism and infectious diseases.

Again, the Program Committee welcomes you and looks forward to meeting you as we continue to work together in addressing the challenges that lie ahead.

Sincerely,

Marshall Bloom, MD
ASM Biodefense Research Meeting Co-Chair

Jean L. Patterson, PhD
ASM Biodefense Research Meeting Co-Chair
11th ASM Biodefense and Emerging Diseases Research Meeting Program Committee

Marshall E. Bloom, MD, Co-Chair  
NIH/NIAID, Hamilton, MT

Jean L. Patterson, PhD, Co-Chair  
Texas Biomedical Research Institute, San Antonio, TX

Soren Alexandersen, DVM, PhD, DVSc, FRCPath, MRCVS  
NCFAD, Winnipeg, Canada

Catharine Bosio, PhD  
NIH/NIAID, Hamilton, MT

Elisabeth Carniel, MD, PhD  
Institut Pasteur, Paris, France

Miles Carroll, PhD  
Health Protection Agency Center for Emergency Preparedness & Response, Wiltshire, United Kingdom

Andrew Hayhurst, PhD  
Texas Biomedical Research Institute, San Antonio, TX

Colleen B. Jonsson, PhD  
Center for Predictive Medicine for Biodefense and Emerging Infectious Disease Clinical & Transitional, Louisville, KY

Carol D. Linden, PhD  
Office of the Biomedical Advanced Research & Development Authority, Washington, DC

C. Rick Lyons, MD  
Colorado State University, Fort Collins, CO

Petra Oyston, PhD  
B07A Microbiology, Wiltshire, United Kingdom

Theodore C. Pierson, PhD  
NIH/NIAID, Bethesda, MD

Anne Rimoin, PhD  
UCLA School of Public Health, Los Angeles, CA

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ASM Headquarters  
American Society for Microbiology  
Meetings and Exhibits Department  
1752 N Street, NW  
Washington, DC 20036-2904  
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Fax: (202) 942-9340  
E-mail: biodefense@asmusa.org  
Website: www.asmbiodefense.org
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<td>Keynote Session</td>
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<td>Global Threats, Cooperative Solutions</td>
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<td>Late-Breaker Session</td>
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<td>Lunch Break (on own)</td>
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<tr>
<td>Exhibits &amp; Poster Hall</td>
<td>7:30 a.m. – 4:00 p.m.</td>
<td>7:00 p.m. – 8:30 p.m. (Exhibits Only)</td>
<td>11:30 a.m. – 5:00 p.m.</td>
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Daily Schedule-at-a-Glance

MONDAY, February 25

**Plenary Session**
8:30 a.m. – 11:30 a.m. | Lincoln 2-4 | See Pages 17, 103
001 H5N1: One Year Later (A, G)

**Focus Sessions**
1:00 p.m. – 2:30 p.m. | Lincoln 6 | See Pages 17, 103
002 Using Social Networks for Tracking Health and Disease (K)

1:00 p.m. – 2:30 p.m. | Lincoln 5 | See Pages 18, 104
003 Environmental Infection Levels: What’s Out There and is it Relevant? (E)

3:45 p.m. – 5:15 p.m. | Lincoln 6 | See Pages 18, 104
004 Clinical Research Networks and the Global Response to Public Health Emergencies (G)

3:45 p.m. – 5:15 p.m. | Lincoln 5 | See Pages 19, 105
005 Potential Strategies for Curing BoNT Intoxication (B, G)

**Keynote Session**
5:30 p.m. – 7:00 p.m. | Lincoln 2-4 | See Page 5, 19
006 Teaching Biodefense to Hollywood (K)

TUESDAY, February 26

7:15 a.m. – 8:15 a.m. | Lincoln 2-4 | See Pages 5, 19
007 Global Threats, Collaborative Solutions

**Plenary Session**
8:30 a.m. – 11:30 a.m. | Lincoln 2-4 | See Pages 20, 106
008 Bats as Sources of Emerging Infectious Diseases (A, B, F)

**Symposia**
1:00 p.m. – 3:00 p.m. | Lincoln 5 | See Pages 20, 107
009 Filovirus Control and Prevention Strategies (A, E, G, H, I)

1:00 p.m. – 3:00 p.m. | Lincoln 4 | See Pages 21, 108
010 Biodefense-Related Intracellular Bacterial Pathogens: Are Suitable Vaccines Within Our Reach? (B, H, I)

1:00 p.m. – 3:00 p.m. | Lincoln 6 | See Pages 21, 109
011 Skin: Maginot Line or Early Warning System? (I)

WEDNESDAY, February 27

**Late-Breaking Symposium**
7:15 a.m. – 8:15 a.m. | Lincoln 2-4 | See Page 28
016 Emergence of a New Pathogenic Coronavirus: Are We Witnessing the Beginning of Another SARS-Like Pandemic, or Not?

**Plenary Session**
8:30 a.m. – 11:30 a.m. | Lincoln 2-4 | See Pages 28, 110
017 Developing Vaccines for Biodefense and Emerging Disease Pathogens (A, B, G)

**Symposia**
1:00 p.m. – 3:00 p.m. | Lincoln 5 | See Pages 29, 111
018 Emerging and Re-Emerging Bunyaviruses: Biggest RNA Virus Group, but Least Attention? (A)

1:00 p.m. – 3:00 p.m. | Lincoln 2-4 | See Pages 29, 112
019 Progress Towards a Dengue Vaccine: Killing Four Birds with One Stone (A, H)

1:00 p.m. – 3:00 p.m. | Lincoln 6 | See Pages 30, 113
020 Yersinia Pathogenesis: Latest Advances in Regulation and Understanding of Virulence (B)

**Poster Session**
3:15 p.m. – 4:45 p.m. | Hall B | See Pages 30, 65
021 Wednesday Posters

**Highlighted Oral Abstract Presentations**
5:00 p.m. – 6:15 p.m. | Lincoln 5 | See Pages 35, 97
022 Therapeutics and Immune Response

5:00 p.m. – 6:15 p.m. | Lincoln 2-4 | See Pages 35, 98
023 Vaccines

5:00 p.m. – 6:15 p.m. | Lincoln 6 | See Pages 35, 99
024 Diagnostics, Informatics and Genomics
Keynote Session

Monday, February 25
5:30 p.m. – 7:00 p.m.  |  Lincoln 2-4
Teaching Biodefense to Hollywood

W. Ian Lipkin, MD; Columbia University, New York, NY

The movie Contagion chronicled the identification, spread, investigation, and ultimate control of a globally emerging viral disease with unparalleled accuracy and penetrating authenticity. Columbia University professor, Dr. Ian Lipkin, a modern day microbe hunter and an acknowledged world expert in emerging infections, was recruited as a senior technical advisor by Contagion director Stephen Soderburgh and screenwriter Scott Z. Burns. Lipkin felt that “this was an effort to accurately represent the science and to make a movie that would entertain as well as educate.” Over the course of the project, Lipkin assisted with story line development, set design and staging, even modeling a novel paramyxovirus. He worked closely with the cast and staff, coaching them on how to deal with lethal disease and to navigate a modern infectious disease lab. In his keynote address, Ian Lipkin will describe the lessons he learned “Teaching Biodefense to Hollywood.”

Global Threats, Collaborative Solutions

Tuesday, February 26
7:15 a.m. – 8:15 a.m.  |  Lincoln 2-4

The Honorable Dr. Richard Danzig
Chairman of the Board, Center for a New American Security
Former Secretary of the Navy
Washington, DC

As a thought leader on the topic of Bioterrorism, Richard Danzig will explore the value of investment in bioterrorism defense, and the ways to make the most of these investments.

Late-Breaking Session

Wednesday, February 27
7:15 a.m. – 8:15 a.m.  |  Lincoln 2-4

016 Emergence of a New Pathogenic Coronavirus: Are We Witnessing the Beginning of Another SARS-Like Pandemic, or Not?

Coronaviruses are enveloped RNA viruses with a global distribution, they are found in humans, other mammals and birds. While most infections caused by human coronaviruses are relatively mild, the zoonotic outbreak of severe acute respiratory syndrome (SARS) caused by SARS-CoV in 2002–2003 caused the deaths over 900 people. In September 2012, less than a decade after the SARS pandemic, the World Health Organization (WHO) reported two cases of severe community-acquired pneumonia which bore significant similarity with SARS. Subsequent laboratory tests revealed the emergence of a novel human coronavirus. This virus has continued to cause human disease and extra cases have been confirmed in the Middle East. While the number of cases remains low at this stage, research preparedness against another SARS-like pandemic is an important precautionary strategy.

Moderators:
Miles Carroll; Hlth. Protection Agency, Salisbury, United Kingdom
Jean L. Patterson; Texas Biomedical Res. Inst., San Antonio, TX

Presentations:
164a An Ethical Frontier: Global Public Health
Gwen Stephens; Saudi Arabia Ministry of Hlth., Riyadh, Saudi Arabia

164b Novel Human Coronavirus Causes Pneumonia in a Macaque Model Resembling Human Disease
Vincent Munster; Rocky Mountain Lab., Hamilton, MT

164c Emerging Infections: UK Response to the Novel Coronavirus 2012
Alison Bermingham; Microbiol. Reference Services Hlth. Protection Agency, London, United Kingdom
The ASM Biodefense Student Travel Postdoctoral Grant Program was established to support the travel of students to ASM meetings. Recipients are selected by the Biodefense Research Program Committee on the basis of the scientific quality of the submitted abstracts.

**Congratulations to this year’s recipients:**

Caitlin Briggs  
Arbovax, Inc., Raleigh, NC  
*Session 023; Presentation 292*

Clair Marie Filone  
Boston University, Boston, MA  
*Session 013; Presentation 154*

Andrew Kocsis  
University of Missouri, Columbia, MO  
*Session 014; Presentation 158*

Rui Liu  
Institute for Immunology and Informatics,  
University of Rhode Island, Providence, RI  
*Session 023; Presentation 295*

Stanley Okoli  
Genøk-Centre for Biosafety; Faculty of Health Sciences,  
University of Tromsø, Tromsø, Norway  
*Session 023; Presentation 294*

Jennifer Spence  
Tulane University School of Medicine, New Orleans, LA  
*Session 022; Presentation 287*

**Visit the Membership Booth to find out how to Advance with ASM!**

*Join or renew during BioDefense and receive a free gift! Stop by ASM’s membership booth for information about ASM’s membership categories and how you can advance your science, career, network and society.*

Can’t wait to find out more? Visit us online at asm.org/advance.
Exhibit Hall Information

Scientific exhibits are located in Exhibit Halls B-South and C, co-located with poster sessions.

The Exhibit Hall will be open:

- Monday, February 25 | 7:30 a.m. – 4:00 p.m.
- Monday, February 25 | 7:00 p.m. – 8:30 p.m. (Opening Reception)
- Tuesday, February 26 | 11:30 a.m. – 5:00 p.m.
- Wednesday, February 27 | 11:30 a.m. – 5:00 p.m.

Additionally, coffee will be provided in the Exhibit Hall on Monday, February 25, from 7:30 a.m. – 9:00 a.m. Attendees can walk the Exhibit Hall and get the most current information on scientific products and services relating to Biodefense.

Opening Reception in the Exhibit Hall
Monday, February 25 | 7:00 p.m. – 8:30 p.m.

Network with colleagues, investigate the amazing variety of exhibits, and visit with industry representatives at your leisure, all while enjoying light hors d’oeuvres and drinks.

Name badges required.

The American Society for Microbiology expresses special thanks to all exhibitors and supporters of the 11th ASM Biodefense and Emerging Diseases Research Meeting. We regret if any supporters have been omitted from this Exhibitor List due to printing deadlines, but that does not lessen our appreciation for their support. The generous support provided by exhibitors is significant to the success of our meeting, so let’s demonstrate our appreciation for the long-standing patronage by visiting as many exhibit booths as possible during the designated breaks.

Exhibitors: (Listed in alphabetical order)

A2LA – American Association for Laboratory Accreditation ........................................ Booth #25
Frederick, MD | www.A2LA.org
A2LA is an internationally recognized accreditation body, whose primary mission is to provide comprehensive accreditation and training services for laboratories, inspection bodies, proficiency testing providers, reference materials producers and product certification bodies. Assesments are conducted using international standards and field specific technical requirements developed in cooperation with government and industry.

Advanced Instruments, Inc. ................................. Booth #23
Norwood, MA | www.AICompanies.com
Advanced Instruments, Inc., a leader in the automated microbiology field, presents the Advanced Instruments Autoplate® Spiral Plating System. The Autoplate SPS features a touchscreen interface. Also showing: the QCount® colony counter with ColorCount™ color recognition technology and the Advanced Instruments Anoxomat™ anaerobic system – fast, dependable environments in a jar.

Battelle .............................................................. Booth #39
Columbus, OH | www.battelle.org
Battelle provides GLP compliant research to pharmaceutical and medical device companies in addition to NIAID, BARDA, DOD and other agencies. Our capabilities include animal model development, safety and efficacy testing in BSL3 laboratories; pathology; and assay development and validation. Battelle has experience with multiple pathogens and supports numerous product development efforts.

BEI Resources ................................................. Booth #29
Manassas, VA | www.beiresources.org
BEI Resources was established by NIAID to provide reagents, tools and information for studying priority pathogens, emerging infectious agents and other microbiological materials relevant to the research community. BEI Resources acquires, authenticates and produces reagents that scientists need to carry out basic research and develop diagnostic tests, vaccines, and therapies.

BioFire Diagnostics, Inc. ................................. Booth #28
Salt Lake City, UT | www.biofiredx.com
BioFire Diagnostics produces the most sensitive and reliable BioSurveillance products that span operations from the lab to the field and clinical diagnostics to environmental surveillance. The hand-carry RAZOR EX allows frontline military troops to detect bioterrorst in the field and the FilmArray identifies biological pathogens in clinical or environmental samples.

BioStat Solutions, Inc. ................................. Booth #26
Mount Airy, MD | www.biostatsolutions.com
BioStat Solutions, Inc. (BSSI) is a statistical consulting corporation in service to the pharmaceutical, biotechnology and biodefense industries, with an emphasis on development and implementation of analytical strategies for biomarker and biodefense countermeasure development.

In the biodefense space, BSSI develops analytical strategies to address the requirements of the Animal Rule.

...continued on following page
CH Technologies (USA) Inc. ......................... Booth #36
Westwood, NJ | www.inhalation.org
CH Technologies offers turnkey systems for nose-only and whole body use. Including generation systems, measurement devices, restraints, and cigarette smoke equipment. Along with Biaera Technologies, BGI and Palas of Germany, we offer a collaboration of industrial hygiene, environmental, bio-defense, pharmaceutical and microbiological applications. Our technology is applied within the industry worldwide.

Data Sciences International .......................... Booth #34
St. Paul, MN | www.datasci.com
Data Sciences International is a pioneering biomedical research company focused on preclinical systems physiology and pharmacology. Our telemetry, instrumentation, software and service offerings help scientists working in the biodefense, pharmaceutical and basic research communities advance science. We offer solutions that are tailored specifically to meet the unique research needs of our customers.

DynPort Vaccine Company, LLC A CSC Company .......... Booth #4
Frederick, MD | www.csc.com/duv
DynPort Vaccine Company, LLC A CSC Company manages product development programs for government agencies, and provides consulting, technical and program management services to companies in the biotechnology and pharmaceutical industries. DynPort’s portfolio includes vaccines and therapeutics to protect against emerging infectious diseases including biological warfare threat agents and seasonal and pandemic influenza.

emka TECHNOLOGIES INC ......................... Booth #32
Falls Church, VA | www.emkatech.com
emka TECHNOLOGIES specializes in non-invasive Telemetry systems as well as Software for data acquisition, analysis, and study management. Our products and services provide medical countermeasures researchers the tools to achieve their demanding and critical goals, such as fever onset/recovery with diurnal correction, non-invasive edema detection, SMS/email notifications, and more!

Federal Select Agent Program ....................... Booth #24
Atlanta, GA | www.selectagents.gov
The Federal Select Agent Program regulates the possession, use, and transfer of biological agents and toxins that could pose a severe threat to public health and safety. The Program promotes laboratory safety and security by enforcing select agent regulations, providing guidance to the regulated community and inspecting select agent facilities.

GeneReach Biotechnology Corporation .................. Booth #2
Lexington, MA | www.genereach-us.com
GeneReach has launched a novel insulated isothermal PCR detection system, POCKIT Nucleic Acid Analyzer and its compatible reagents, which is the first commercialized on-site detection device based on single heat source and capillary based nucleic acid amplification method. Convection PCR can shorten reaction time from hours to about 30 minutes.

Georgetown University Biomedical Graduate Education .................. Booth #8
Washington, DC | www.biomedgrad.georgetown.edu
Georgetown University Biomedical Graduate Education offers an outstanding educational experience within one of the world’s leading academic and research institutions. Our location in the nation’s capital provides unparalleled access to leading scientific centers, institutes and government agencies such as, National Institutes of Health, the Walter Reed Army Institute of Research, the Naval Medical Research Institute, the Food and Drug Administration, and the Department of Homeland Security.

IIT Research Institute ................................. Booth #40
Chicago, IL | www.iitri.org
IITRI has a proud history of supporting government-commercial-academic sponsors in animal model development and assessment of medical countermeasures. Research is underway to test immunogenicity, efficacy, and safety of vaccines, biologics and small molecule therapeutics for protection against various biological agents. Select Agent Registered ~ Biological Surety Program ~ Bioaerosol ~ BSL2-3

List Biological Laboratories, Inc. ..................... Booth #3
Campbell, CA | www.listlabs.com
List Biological Laboratories, Inc produces bacterial toxins for research: anthrax, botulinum, pertussis, cholera, difficile, diphtheria, tetanus, staphylococcal and shiga toxins. Products include lipopolysaccharides, botulinum chains, toxoids, antibodies and conjugates. Toxins may be used to investigate disease models and vaccines. SNAPtide® fluorescent substrates used in inhibition and detection studies. Contract manufacturing.

Lovelace Respiratory Research Institute ................ Booth #27
Albuquerque, NM | www.liri.org
The Lovelace Respiratory Research Institute is a private, nonprofit biomedical research organization that specializes in aerosol and bioaerosol science, inhalation exposure technology, small and large animal infectious disease models, toxicology, pharmacokinetics, biocontainment studies and clinical trials. In vitro or in vivo research can be performed under GLP.

MO BIO Laboratories, Inc. ............................. Booth #41
Carlsbad, CA | www.mobio.com
MO BIO Laboratories, Inc. is a global leader in solutions for nucleic acid purification, offering innovative tools for research in molecular biology. MO BIO’s line of soil and microbial isolation kits are now the method of choice among environmental and microbiology researchers studying microbial DNA and RNA.

NanoLogix Incorporated .............................. Booth #38
Hubbard, OH | www.nanologix.com
NanoLogix is a biotechnology company specializing in live cell, rapid diagnostics. Our kits are simple and reliable rapid test solutions that provide the ability to detect active threat bacteria and other microorganisms 4x-12x faster than traditional Petri culture technology, and more cost effectively than other methods.

National Biosafety & Biocontainment Training Program (NBBTP) .................... Booth #1
Bethesda, MD | www.nbbtp.org
The National Biosafety and Biocontainment Training Program (NBBTP), a partnership between the Division of Occupational Health and Safety (DOHS) and the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health includes 2-year post-doctorate and post-baccalaureate biosafety fellowships, NBBTP certificates, and professional development courses. www.nbbtp.org

National Counterproliferation Center (NCPC) .................. Booth #21
Washington, DC | www.phe.gov/S3
The National Counterproliferation Center continues to focus on our inter-agency outreach program aimed at engaging and partnering with the broad life sciences community on the critical topics of biosecurity and biosafety. Our goal is to maintain a two-way conversation with life scientists that raises awareness, shares information and offers best-practice guidance on these topics.

New England Biolabs ................................. Booth #35
Ipswich, MA | www.neb.com
New England Biolabs, Inc. leads the industry in the discovery and production of enzymes for molecular biology. NEB’s global reputation for industry-leading product quality and technical support makes NEB the first choice for applications such as library preparations for next gen sequencing and DNA amplification.

NIH Office of Biotechnology Activities .................... Booth #30
Bethesda, MD | http://oba.od.nih.gov/oba/index.html
NIH Office of Biotechnology Activities staff will be available to answer questions about dual use research in the Life Sciences. Informative materials and handouts related to dual use research will be available.

OpGen, Inc. provides whole genome analysis systems and services for sequence assembly, strain typing and comparative genomics applications. Genomics research and public health labs use OpGen’s Whole Genome Mapping technology to produce high-resolution, ordered, restriction maps from single DNA molecules that provide a comprehensive view of genomic architecture.

Reagent Proteins  San Diego, CA  www.reagentproteins.com

Reagent Proteins is the source for proteins. From custom protein production to our over 5,000 proteins, we are the leading supplier of reagent, pre-clinical and cGMP grade proteins. We ensure the cost-effective and rapid delivery of high quality recombinant proteins to enable your research and development efforts.

RURO Inc.  Frederick, MD  www.RURO.com

RURO Inc. provides LIMS, ELN and Sample Management software systems and develops RFID solutions for research, biotechnological, pharmaceutical, healthcare, and government laboratories worldwide. The RURO Smart RFID program was designed to bring the accuracy, safety, security and productivity benefits of the technology to the life sciences.

Southern Research Institute  Birmingham, AL  www.Southernresearch.org

Southern Research is a preclinical contract research organization with over 50 year’s experience. Services include efficacy testing with special expertise in therapeutics and vaccines in the areas of bioterrorism, emerging pathogens and public health, with a complete range of safety testing including: bioanalytical, PCR, immunology, and pathology services.

Tetracore, Inc.  Rockville, MD  www.tetracore.com

Tetracore is a biotechnology organization that develops assays, and devices for the rapid detection of BioThreat agents. We now offer a portable realtime PCR instrument, the T-COR 4. Combined with our BioThreat Alert Strip Reader, Tetracore offers a powerful mobile detection platform using both realtime PCR and antibody based technology.

The University of Chicago – Howard T. Ricketts Laboratory  Lemont, IL  www.htrl.uchicago.edu

The Howard T. Ricketts Regional Biocontainment Laboratory, operated by the University of Chicago and located at Argonne National Laboratory, was designed by the NIAID to conduct biodefense-related and emerging infectious disease research. The HTRL conducts BSL-3, ABSL-3, and aerobiology experiments for RG3 microorganisms, utilizing the on-site Animal Research Immunology Core.

UTMB-National Biocontainment Training Center  Galveston, TX  www.utmb.edu/nbtc

The National Biocontainment Training Center (NBTC) prepares infectious disease scientists to work safely in high and maximum – containment environments. The NBTC offers fellowship tracks for scientists and biocontainment engineers and The University of Texas Medical Branch Laboratory Biosafety Training Program provides specialized instruction in BSL2 to 4 environments. www.utmb.edu/nbtc

ViroCyt, LLC  Denver, CO  www.virocyt.com

ViroCyt’s mission is to provide the tools that deliver meaningful improvements to critical biotechnology processes such as vaccine manufacturing, protein expression, antiviral development and other settings where viruses play a significant role. The Virus Counter 2100 is a compact, easy to use instrument that enables rapid quantification of virus particles.
## Tuesday Poster Key

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<thead>
<tr>
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<tr>
<td>A</td>
<td>045 (A) - 061 (A)</td>
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<td>B</td>
<td>062 (B) - 077 (B)</td>
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<td>D</td>
<td>079 (D) - 095 (D)</td>
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<td>E</td>
<td>096 (E) - 103 (E)</td>
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<td>K</td>
<td>146 (K) - 149 (K)</td>
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</tbody>
</table>

### Tuesday Posters Floor Plan

- **Floor 1**: Room 13-12
- **Floor 2**: Room 3-4-5
- **Floor 3**: Room 6-7-8-9
- **Floor 4**: Room 10-11-12
- **Floor 5**: Room 13-14-15
### Wednesday Poster Key

<table>
<thead>
<tr>
<th>Category</th>
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<tbody>
<tr>
<td>A</td>
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<tr>
<td>K</td>
<td>282 (K) – 286 (K)</td>
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</tbody>
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Washington Marriott Wardman Park Hotel
General Information

American with Disabilities Act
Persons requiring special services at the hotel are asked to stop by the registration desk located in the atrium of the Washington Marriott Wardman Park Hotel.

ATM
There is an ATM located on the Lobby Level of the hotel across from the gift shop.

Badge Policies
For security reasons, all attendees are required to wear a badge to attend sessions or enter the Exhibit Hall. Please note that badges are the property of ASM and must be relinquished at any time at the request of ASM staff or security.

Attendee Badges
• Access into all scientific sessions
• Access to the Opening Reception
• Access to the Exhibit Hall and Poster Sessions

Guest Badges
• Access to the Exhibit Hall and Poster Sessions
• May attend the Opening Session and Reception
• Attendees wearing guest badges will not be permitted into scientific session rooms

Exhibitor Badges
• Entrance into the Exhibit Hall during installation and dismantling hours
• Entrance into the Exhibit Hall 30 minutes prior to the opening of exhibits
• Access into all scientific sessions

Press Badges
• Access into all scientific sessions
• Access to the Exhibit Hall and Poster Sessions
• Members of the press must register for the meeting and pay the appropriate registration fee to receive continuing education credit

Business Center
A full-service business center is located in the Exhibition Level of the Washington Marriott Wardman Park Hotel.

Cameras and Recording
Digital recorders, cameras (including camera phones), and video cameras (including video phones) are prohibited in the Poster Hall and session rooms.

Certificate of Attendance
Certificate of Attendance will be available at the registration desk starting on February 26.

Child Policy
Children are not permitted in session rooms. Also, children under the age of 18 are not permitted on the Exhibition Hall floor during setup or dismantling hours. During scheduled show hours, exceptions are made when parents or guardians complete and sign a minor release form. This form can be found at the registration desk located in atrium of the Washington Marriott Wardman Park Hotel. Childcare may be arranged through the concierge of the Washington Marriott Wardman Park Hotel.

Contact Information
To contact ASM staff of site at the conference, please visit staff at the registration desk located in the Atrium of the Washington Marriott Wardman Park Hotel.

Lost and Found
Unattended personal belongings will be removed and taken to hotel security.

Meeting Announcements
A bulletin board will be available in the Atrium registration area for those attendees wishing to post one-page flyers announcing upcoming meetings.

Messages
All mail and communications to meeting registrants should be directed to the individual at the hotel where he or she is staying. The phone number for the Washington Marriott Wardman Park Hotel is (202) 328-2000.
Registration

Registration will be located in the Atrium from Sunday, February 24 – Wednesday, February 27.

Registration Hours:
Sunday, February 24 | 4:00 p.m. – 7:00 p.m.
Monday, February 25 | 6:30 a.m. – 7:00 p.m.
Tuesday, February 26 | 6:30 a.m. – 3:30 p.m.
Wednesday, February 27 | 6:30 a.m. – 3:30 p.m.

Smoking Policy

Smoking is prohibited in all areas of the Washington Marriott Wardman Park Hotel.

Speaker Ready Room

Lincoln 1, Exhibition Level
Hours
Monday, February 25 | 7:00 a.m. – 5:30 p.m.
Tuesday, February 26 | 6:15 a.m. – 5:00 p.m.
Wednesday, February 27 | 6:15 a.m. – 5:00 p.m.

Oral presenters are requested to bring their PowerPoint presentations to the Speaker Ready Room at least one hour prior to their session to be uploaded. A technician will be available to help ensure your presentation is properly uploaded.

Transportation and Parking

Self Parking is available at a reduced rate of $26.00 per day for local and overnight attendees.

Valet parking is also available at a fee of $39.00 per day. The Washington Marriott Wardman Park Hotel is located less than 0.1 miles from the Woodley Park – Zoo/Adams Morgan Metro station on the red line.

Scientific Program Information

Symposia

Symposia consist of 4-5 faculty, invited by the Biodefense Program Committee, who present lectures on a specific topic followed by a moderated Q&A period.

Focus Sessions

Focus sessions are in-depth educational activities that highlight a specific topic area. These sessions precede the Keynote session and are an opportunity for attendees to explore new and emerging issues with a group of faculty experts.

Plenary Sessions

Daily Plenary Sessions will be held Monday, Tuesday, and Wednesday mornings from 8:30 a.m. to 11:30 a.m. These sessions provide all attendees an opportunity to collaborate on presentations of science, policy, and public health issues. The speakers were invited by the Biodefense Program Committee based on their professional expertise and achievement. Plenary Sessions may have a moderated Q&A period.

Poster Sessions

Accepted abstracts are scheduled for poster presentation based on the December 2012 review of all abstract submissions by the Biodefense Research Program Committee. Approximately 87% of accepted abstracts are presented as posters. Posters presentations will take place in the Hall B Tuesday, February 26, and Wednesday, February 27. Each presenter will be available at his or her poster to answer questions from 3:15 p.m. – 4:45 p.m. either Tuesday or Wednesday as scheduled in this Program and Abstracts book.

Highlighted Oral Abstract Sessions

These sessions highlight accepted abstract research submitted during the 2012 abstract submission for the ASM Biodefense and Emerging Diseases Research Meeting. Approximately 13% of accepted abstracts are presented in these sessions. Authors are invited by topic to give a 10 to 15 minute presentation of their research followed by a moderated Q&A period.

For specific presentation times and additional details, please see the session information listed by date in this Program and Abstracts book.
Monday Sessions

For the most up-to-date program information, visit the Online Program Planner at www.asmbiodefense.org

**Plenary Session**

**001 H5N1: One Year Later (A, G)**

Monday, February 25, 2013   |   8:30 AM - 11:30 AM
Lincoln 2-4

At the time of the 2012 ASM Biodefense Meeting, the NSABB recommended about whether to publish papers on the studies investigating the determinants of H5N1 influenza transmissibility from birds to mammals dominated scientific and lay media and conversations worldwide. The NSABB reviewed its recommendation and revised its recommendation to oppose publication of the initial papers, but to support publication in full of 2 revised manuscripts. A year later, discussion on the publication of the papers, the impact on global influenza surveillance, a moratorium on performing H5N1 studies, the need for evaluating Dual Use Research of Concern (DURC), and the resulting impact on scientific inquiry continue to be dominant issues. This session will offer updates and perspectives on selected aspects of the H5N1 debate.

**Moderators:**

Marshall E. Bloom; DIR/NIAID/NIH, Hamilton, MT.
Jean L. Patterson; Texas Biomedical Res. Inst., San Antonio, TX.

**Presentations:**

8:30AM

001 An Overview of the H5N1 Controversy
Arturo Casadevall; Albert Einstein Coll. of Med., Yeshiva Univ., Bronx, NY.

8:55AM

002 The Importance of Understanding Influenza Host Switching, and Dual Use Implications of Such Studies
Jeffery K. Taubenberger; NIH/NIAID, Bethesda, MD.

9:20AM

003 A Journal’s Perspective on the H5N1 Controversy
Barbara Jasny; American Association for the Advancement of Sci., Washington, DC.

9:45AM

004 The NSABB Perspective: Lessons Learned
Michael Imperiale; Univ. of Michigan, Ann Arbor, MI.

10:10AM

Coffee Break

10:40AM

005 The Ecology of Avian Influenza: Understanding the Emergence of Highly Pathogenic Avian Influenza Viruses
Vincent J. Munster; Rocky Mountain Lab., Hamilton, MT.

11:05AM

006 Implications of H5N1 Studies on Influenza Surveillance
Nancy J. Cox; CDC, Atlanta, GA.

**Focus Session**

**002 Using Social Networks for Tracking Health and Disease (K)**

Monday, February 25, 2013   |   1:00 PM - 2:30 PM
Lincoln 6

This program will provide an update to attendees on using elements of the social media to monitor health and disease during epidemics and states of crisis. The program will discuss some of the latest development in applying social networks to health and infectious disease tracking. The different elements of social network will be demonstrated for their utility in managing health related events.

**Moderators:**

Brian Lee; CDC, Atlanta, GA.
Rick Lyons; Colorado State Univ., Collins, CO.

**Presentations:**

1:00PM

008 Using Social Media “Big Data” for Disease Surveillance Purposes
Brian Norris; Social Hlth. Insights LLC, Westfield, IN.

1:30PM

009 Leveraging Crowd Sourced Mapping Big Data and Social Media for Biodefense
Jen Ziemke; John Carroll Univ., University Heights, OH.

2:00PM

010 A Landscape Analysis of the Use of Social Media for Biosurveillance
Jody Ranck; PwC Advisory Services, Tysons Corner, VA.
Focus Session

003 Environmental Infection Levels: What’s Out There and is it Relevant? (E)
Monday, February 25, 2013 | 1:00 PM - 2:30 PM
Lincoln 5

This session will focus on the infectious pathogens that reside in the environment, often emerging to cause sporadic episodes or more serious outbreaks of infectious disease. The questions of how they are maintained, at what levels and when these become a problem will be discussed. This will provide insight into the given examples and how much risk they pose to the human population, and the key aspects which are more widely applicable to other infectious diseases. The session will be of interest to researchers who work on the pathogens themselves, clinicians dealing with cases and also those focused on epidemiology and interventions.

Moderators:
Richard Vipond; Hlth. Protection Agency, Salisbury, United Kingdom.
Connie S. Schmaljohn; USAMRIID, Frederick, MD.

Presentations:

1:00PM
010a Hantaviruses: Out There, Relevant and Emerging
Connie S. Schmaljohn; USAMRIID, Frederick, MD.

1:10PM
011 Ecology of Hantaviruses in North America
Brian R. Amman; CDC, Atlanta, GA.

1:35PM
012 Will the Real U.K. Hanta Please Stand Up?
Lisa Jameson; Hlth. Protection Agency, Porton, United Kingdom.

2:00PM
013 Coxiella burnetii in the Netherlands
Bart J. van Rotterdam; Natl. Inst. for Publ. Hlth. and the Environment, Bilthoven, Netherlands.

Focus Session

004 Clinical Research Networks and the Global Response to Public Health Emergencies (G)
Monday, February 25, 2013 | 3:45 PM - 5:15 PM
Lincoln 6

A key element of optimal response to public health emergencies is the delivery of effective, timely medical care to the affected population. Unfortunately, despite advances in understanding of the biology, transmission dynamics, and diagnostics of many emerging infectious diseases, we still lack a basic understanding of the pathophysiology of many disease syndromes and thus cannot develop effective countermeasures. The obstacles to such understanding include the lack of clinical research infrastructure in many settings, the difficulties of conducting research under emergency settings, and institutional barriers to cooperation and communication. This program will set out the current state of preparedness and response to EID outbreaks from the international perspective, update the audience on the rapidly-evolving state of international collaboration in clinical research in EID, highlight the possibilities of novel trial design in developing rapid interventions during outbreaks, demonstrate the link between improved patient management and the development of countermeasures. The program will also illustrate the role of clinical research as an element in the U.S. government’s international biopreparedness strategy.

Moderators:
Lisa Hensley; FDA OCS, OCET, Silver Spring, MD.
Matthew Lim; Dept. of Hlth. and Human Services, U.S. Navy, Washington, DC.

Presentations:

3:45PM
014 WHO IMAI Triage, Differential Diagnosis, and Management Severe Illness: Opportunity Begins Here
Nahoko Shindo; WHO, Geneva, Switzerland.

4:00PM
015 Update on International Clinical Research Networks in Public Health Emergencies
Gail Carson; ISARIC, Oxford, United Kingdom.

4:15PM
016 InFACT: Global Collaboration in Acute Care Research
John Marshall; St. Michael’s Hosp., Toronto, Canada.

4:30PM
017 Naval Medical Research Center, Frederick: Targeting Enhanced Sepsis Outcomes
James V. Lawler; Naval Med. Res. Ctr., Frederick, MD.

4:45PM
018 Clinical Research and Global Health Security: U.S. Government Perspective
Matthew Hepburn; Natl. Security Staff, Office of Med. Preparedness, Washington, DC.
Focus Session

005 Potential Strategies for Curing BoNT Intoxication (B, G)
Monday, February 25, 2013  |  3:45 PM - 5:15 PM
Lincoln 5

The paralytic effects of BoNT intoxication can be severe enough to require long term life-support until the toxins are slowly dissipated and neurotransmitter release returns to normality. While monoclonal antibodies have shown great promise in clearing circulating BoNT, targeting which has been already taken up by the neurons and/or bypassing the impacts of neurotransmitter cleavage will be required to reverse the effects of intoxication. This session will introduce some of the novel routes that are under study to address this very challenging problem.

Moderators:
Andrew Hayhurst; Texas Biomedical Res. Inst., San Antonio, TX.
Brenda A. Wilson; Univ. of Illinois, Urbana, IL.

Presentations:
3:45PM

019 Approaches and Challenges in the Development of Therapeutics for Botulinum A Intoxication
Kim D. Janda; The Scripps Res. Inst., La Jolla, CA.

4:05PM

020 Engineering BoNT-Based Inhibitor Delivery Platforms against Botulism
Brenda A. Wilson; Univ. of Illinois, Urbana, IL.

4:25PM

021 A General Strategy for Rapidly Developing Simple Antitoxin Products and the Potential for Their Genetic Delivery
Charles B. Shoemaker; Tufts Cummings Sch. of Vet. Med., North Grafton, MA.

4:45PM

022 Harnessing the Neuronal Ubiquitin Proteasome System for BoNT Therapeutics
George A. Oyler; Tufts Cummings Sch. of Vet., Baltimore, MD.

Keynote Session

006 Teaching Biodefense to Hollywood (K)
Monday, February 25, 2013  |  5:30 PM – 7:00 PM
Lincoln 2-4

The movie Contagion chronicled the identification, spread, investigation, and ultimate control of a globally emerging viral disease with unparalleled accuracy and penetrating authenticity. Columbia University professor, Dr. Ian Lipkin, a modern day microbe hunter and an acknowledged world expert in emerging infections, was recruited as a senior technical advisor by Contagion director Stephen Soderburgh and screenwriter Scott Z. Burns. Lipkin felt that "this was an effort to accurately represent the science and to make a movie that would entertain as well as educate.” Over the course of the project, Lipkin assisted with story line development, set design and staging, even modeling a novel paramyxovirus. He worked closely with the cast and staff, coaching them on how to deal with lethal disease and to navigate a modern infectious disease lab. In his keynote address, Ian Lipkin will describe the lessons he learned “Teaching Biodefense to Hollywood.”

Moderators:
Marshall E. Bloom; DIR/NIAID/NIH, Hamilton, MT.
Jean L. Patterson; Texas Biomedical Res. Inst., San Antonio, TX.

Presentation:
5:30PM

023 Teaching Biodefense to Hollywood
W. Ian Lipkin; Columbia Univ., New York, NY.

Global Threats, Collaborative Solutions

007 Should We Invest in Defenses against Bioterrorism? How Should We Invest in Defenses against Bioterrorism?
Tuesday, February 26, 2013  |  7:15 am - 8:15 am
Lincoln 2-4

As a thoughtful leader on the topic of Bioterrorism, Richard Danzig will explore the value of investment in bioterrorism defense, and the ways to make the most of these investments.

Presentation:
Richard J. Danzig; Chairman of the Board, Ctr. for a New American Security, Former Secretary of the Navy, Washington, DC.
Plenary Session

008 Bats as Sources of Emerging Infectious Diseases (A, B, F)
Tuesday, February 26, 2013    |    8:30 AM - 11:30 AM
Lincoln 2-4

Bats have received much attention as potential reservoir hosts for several human pathogens including rabies, SARS-CoV, Marburgvirus and Bartonella spp. Surveys of the potential viromes associated with bats indicate that bats may come into contact with a remarkable diversity of viruses. Although a worryingly bountiful source of emerging human health threats bats may also point the way to new directions to counter these agents since they often show no overt signs of severe disease and may have evolved mechanisms to minimize pathogenesis and/or resolve infection rapidly.

Moderators:
Anthony Griffiths; Texas Biomedical Res. Inst., San Antonio, TX.
Andrew Hayhurst; Texas Biomedical Res. Inst., San Antonio, TX.

Presentations:
8:30AM
025 Bats and Emerging Lyssaviruses: Surveillance, Prevention and Control
Amy T. Gilbert; CDC, Atlanta, GA.
9:00AM
026 Analyzing, Predicting, and Preventing the Emergence of Infectious Diseases from Bats
Peter Daszak; EcoHlth. Alliance, New York, NY.
9:30AM
027 Sources of Mammalian Viruses: Bats and Beyond
Christian Drosten; Univ. of Bonn Med. Ctr., Bonn, Germany.
10:00AM
Coffee Break
10:30AM
028 Bartonella Species in Bats and Bat Ectoparasites
Michael Y. Kosoy; CDC, Fort Collins, CO.
11:00AM
029 Experimental Methods for Bat-Borne Infectious Diseases
Tony Schountz; Univ. of Northern Colorado, Greeley, CO.

Symposium

009 Filovirus Control and Prevention Strategies (A, E, G, H, I)
Tuesday, February 26, 2013    |    1:00PM – 3:00 PM
Lincoln 5

Recent advances in the understanding of filovirus biology have resulted in the identification of potential new targets and strategies for filovirus infection control. Presentations will focus on mechanisms of filovirus immune modulation and evasion, virus entry and trafficking, and virus maintenance in the natural reservoir. This session will present a mix of applied and basic science with an emphasis on promising antiviral therapies, vaccine development and outbreak prevention.

Moderators:
Jonathan S. Towner; CDC, Atlanta, GA.
Elke Muehlberger; Boston Univ. Sch. of Med., Boston, MA.

Presentations:
1:00PM
030 Filoviral Immune Evasion Mechanisms: Implications for Pathogenesis and Treatment
Christopher F. Basler; Mount Sinai Sch. of Med., New York, NY.
1:20PM
031 Interplay of Ebola Virus with the Host Defense Machinery
Judith Olejnik; Natl. Emerging Infectious Diseases Lab., Boston Univ. Sch. of Med., Boston, MA.
1:40PM
032 A Syrian Hamster Model Recapulating Ebola Hemorrhagic Fever
Hideki Ebina; Rocky Mtn. Lab, NIAID, NIH, Hamilton, MT.
2:00PM
033 Ebola Virus Ins and Outs: Designing Therapies Around the Trafficking Requirements of a Pathogen
Robert A. Davey; Texas Biomedical Res. Inst., San Antonio, TX.
2:20PM
034 Preventing Marburg Virus Outbreaks: Understanding Virus Spillover from the Natural Reservoir
Brian R. Amman; CDC, Atlanta, GA.
2:40PM
035 Progress in the Development of Vaccines against Ebola and Marburg Viruses
Tom W. Geisbert; Univ. of Texas Med. Branch, Galveston, TX.
**Symposium**

**010 Biodefense-Related Intracellular Bacterial Pathogens: Are Suitable Vaccines within Our Reach? (B, H, I)**

Tuesday, February 26, 2013 | 1:00 PM – 3:00 PM
Lincoln 2-4

Developing medical countermeasures, such as vaccines, that can protect the public from infectious biodefense-related bacterial threats, has been a research priority in research years. Quick progress in basic science has generated a greater understanding of these bacterial diseases as well as new concepts about their prevention, based on studies of the disease-causing pathogen and the human immune response to them. This session will present recent progress in the identification and the advancement of successful candidates into advanced vaccine testing and product development, which some of these products entering the evaluation stage for safety and efficacy in animals and humans.

**Moderators:**
Alfredo G. Torres; Univ. of Texas Med. Branch, Galveston, TX.
Catharine Bosio; RML/NIAID/NIH, Hamilton, MT.

**Presentations:**
1:00PM
**036 Nanoparticle Vaccines for Melioidosis**
Richard Titball; Univ. of Exeter, Exeter, United Kingdom.
1:20PM
**037 Protective Efficacy and Safety of the Brucella Vaccine**
Thomas A. Ficht; Texas A&M Univ., College Station, TX.
1:40PM
**038 Molecular Basis of Immunity to Rickettsial Infection**
Juan J. Martinez; Univ. of Chicago, Chicago, IL.
2:00PM
**039 Components of Protective Immunity in Coxiella burnetii Infection**
James E. Samuel; Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX.
2:20PM
**040 TBD**

**Symposium**

**011 Skin: Maginot Line or Early Warning System? (I)**

Tuesday, February 26, 2013 | 1:00 PM – 3:00 PM
Lincoln 6

The multifunctional nature of mast cells (MCs) has been revealed through their involvement in both innate and adaptive immune responses. Recent insight into the various functions of MCs has shown that these innate immune effectors possess the dual ability to kill microbes and to modify classical adaptive immune responses. Members of the cathelicidin family of antimicrobial peptides (AMPs) are expressed by MCs and epithelial cells at sites of injury. The granular localization of the cathelicidin peptides, their extracellular release, and their capacity to modify inflammatory responses suggests that cathelicidin plays an important role in the capacity of MCs to combat skin infection. Preliminary data inside this proposal show that MCs are sentinels in the skin for defending against bacterial and viral infections. Mast cell-deficient (KitW-sash-/-) mice are more susceptible to skin infection than the wild-type mice, while KitW-sash-/- mice reconstituted with skin MCs show a normal response. Using MCs derived from mice deficient in cathelicidin, we showed that antimicrobial peptides (AMPs) are critical anti-pathogenic granule components. Signaling through toll-like receptor (TLR)-2 increased the level of antimicrobial peptide MCs, enhancing their capacity to fight skin infections.

**Moderators:**
Anna Dinardo; Univ. of California, La Jolla, CA.
Colleen B. Jonsson; Univ. of Louisville, Louisville, KY.

**Presentations:**
1:00PM
**041 Mast Cell Role in Skin Infections**
Anna Dinardo; Univ. of California, La Jolla, CA.
1:20PM
**042 Mechanisms that Promote Neutrophil Recruitment against Staphylococcus aureus Skin Infections**
Lloyd S. Miller; Johns Hopkins Univ., Baltimore, MD.
1:40PM
**043 The Skin Microbiome and Immunity: Who Shapes Whom?**
Julia Segre; NIH, Bethesda, MD.
2:00PM
**044 Bioinformatic Approaches to Investigating Mycobacterial Infection to Gain Insights into Mechanisms of Immunity**
Delphine Lee; John Wayne Cancer Inst., Santa Monica, CA.

**Poster Session**

**012 Tuesday Posters**

Tuesday, February 26, 2013 | 3:15 PM - 4:45 PM
Hall B

**Presentations:**
**045 (A) Characterisation of Rotavirus Causing Acute Diarrhea in Children in Kathmandu, Nepal: Dominance of G12**
046 (A) Withdrawn
047 (A) Withdrawn
048 (A) Withdrawn

049 (A) Assessment of Human Health Risks from Adenoviruses, Hepatitis A Virus, Rotaviruses and Enteroviruses in the Buffalo River and Three Source Water Dams in the Eastern Cape Province, South Africa
V. N. Chigor1,2, A. I. Okoh3; 1Univ. of Nigeria, Nsukka, Nigeria, 2Univ. of Fort Hale, Alice, South Africa.

050 (A) Classical Swine Fever Virus and Pasteurella multocida Interaction Can Be Augmented by Magnetic Fields
B. Andriy, IV; IECVM, Kharkiv, Ukraine.

051 (A) Rift Valley Fever Virus Nose-Only Aerosol Characterization
R. C. Layton, R. Tuttle; MRIGlobal, Kansas City, MO.

052 (A) Fatal Encephalitis in African Green Monkeys and Common Marmosets after Aerosol Infection with Rift Valley Fever Virus

053 (A) Epizootic Monitoring of Swine Epizootic Fever
S. Nychyk, M. Sytyuk; IVM, Kyiv, Ukraine.

054 (A) Phylogenetic Analysis of Virus Isolates of PRRS
N. Gavrasieva; SSCIBMS, Kyiv, Ukraine.

055 (A) Oxidative Stress-Induced Responses in RVFV-Infected Liver Cells
A. Narayanan, M. Amaya, M. Chung, K. Kehn-Hall, S. Senina, C. Carpenter, C. Bailey, R. Hakami, F. Kashanchi; George Mason Univ., Manassas, VA.

056 (A) West Nile Virus and Chronic Kidney Disease: A Case-Control Evaluation
M. S. Nolan1, C. Yan1, A. S. Podoll1, K. Finkel1, K. O. Murray1; 1Baylor Coll. of Med., Houston, TX, 2Univ. of Texas Hlth. Sci. Ctr., Houston, TX.

057 (A) Spatial and Temporal Patterns of West Nile Incidence, 2003-2010

058 (A) Discovery and Analysis of Herpes B Virus-Encoded MicroRNAs
M. Amen1,2, A. Griffiths1,2; 1Texas BioMed. Res. Inst., San Antonio, TX, 2Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX.

059 (A) Human and Viral Genetic Determinants of Lassa Fever
S. Gire1,2; 1Viral Hemorrhagic Fever Consortium, Iruka Specialist Teaching Hosp., Kenema Government Hosp., Robert Garry Lab, Christian Happi Lab, P. C. Sabeti1,2, Pardis Sabeti Lab; 1Harvard Univ., Cambridge, MA, 2Broad Inst., Cambridge, MA.

060 (A) Avian Influenza Disease in Georgia Republic
N. Vepkhvadze, M. Donduashvili, M. Kokhreidze, T. Onashvili; Lab. for the Ministry of Agriculture, Tbilisi, Georgia.

061 (A) The Critical Role of the IKK-Complex in VEEV Infection
A. Narayanan, M. Amaya, K. Voss, S. Senina, C. Carpenter, K. Kehn-Hall, C. Bailey, F. Kashanchi; George Mason Univ., Manassas, VA.

062 (B) Dissemination of Inhaled Yersinia pestis in the African Green Monkey Pneumonic Plague Model
W. MEGA1, T. Brasel1, P. Kuehl1; 1LRI, Albuquerque, NM, 2UTMB, Galveston, TX.

063 (B) Novel RNAIII-Inhibiting Peptide Derivatives Increase Survival in MRSA Sepsis by Inhibiting Nlrp3 Inflammasome Activation
Z. Hou, F. Da, X. Xue, X. Luo; Fourth Military Med. Univ., Xi'an, China.

064 (B) Importance of Brucella Global Serology in the Study to Check Recent Upsurge of Psychiatric Diseases
S. Das; Peerless Hosp. & B. K. Roy Res. Ctr., Kolkata, India.

065 (B) Development of a Yersinia pestis A1122 Knock-Out Library Through Conjugative Transposon Mutagenesis
D. R. Pawlowski1,2, A. Howlett1; 1CUBRC, Buffalo, NY, 2State Univ. of New York, Buffalo, NY.

066 (B) A System Causing Maintenance of Bacillus anthracis Plasmid pX01
A. P. Pomerantsev, C. Rappole, Z. Chang, S. H. Leppa; NIH/NIAID, Bethesda, MD.

067 (B) Proteomic Analysis of Atypical F. tularensis Strains Isolated from Natural Foci in Georgia
R. Solomonia1, M. Nozadze1, M. Meparishvili1, E. Mikautadze1, E. Zhgenti2, G. Chanturia2, M. Kekeklidze2, S. Francesconi1; 1Inst. of Chemical Biol., Ila State Univ., Tbilisi, Georgia, 2Natl. Ctr. for Disease Control, Tbilisi, Georgia, 3Naval Med. Res. Ctr., Frederick, MD.

068 (B) Improvement of Epidemiological Data on Leishmaniasis in Natural and Recently Recognized VL Focuses in Tbilisi
G. Babuadze1, M. Iosava1, M. Kekelidze1, P. Imnadze1, M. Ejov2, J. Alvar2; 1Natl. Ctr. for Disease Control, Tbilisi, Georgia, 2WHO, Copenhagen, Denmark.

069 (B) Combined SNP and MLVA Subtyping of Bacillus anthracis Strains from Georgia
E. Khalada1, G. Chanturia1, E. Zhgenti2, N. Abazashvili1, M. Zakalashvili1, M. Kekelidze1, P. Imnadze1, S. Tsanava1, L. Malania1, N. Tsertsvadze1, D. Birdsel1, T. Pearson1, D. Wagner1, M. Nikolich2, P. Keim2; 1Natl. Ctr. for Disease Control, Tbilisi, Georgia, 2Ctr. for Microbial Genetics and Genomics, Northern Arizona Univ., Flagstaff, AZ, 3Ctr. for Microbial Genetics and Genomics, Northern Arizona Univ., Flagstaff, CO.

070 (B) Molecular Typing of Human and Animal Brucella Isolates from Georgia
K. Sidamonidze1, E. Zhgenti1, M. Zakalashvili1, L. Malania1, M. Rasmishvili1, M. Grdzelidze1, S. Tsanava1, T. Akhvlediani1, N. Trapaidze1, I. Kokaia1, T. Onashvili1, E. Mamisashvili1, X. Huang2, R. Rivard2, P. Elzer3, M. Nikolich3; 1Natl. Ctr. for Disease Control, Tbilisi, Georgia, 2CWRIR/USAMRIID Clin. Res. Unit/ TMC, Tbilisi, Georgia, 3Med. Parasitology and Genomics, U.S. Army Med. Res. Inst. of Infectious Diseases, Frederick, MD.

071 (B) Emerging Fluoroquinolone Resistance among Typhoidal Salmonella Serovars in Nepal
D. R. Bhatta; Tribhuvan Univ., Kathmandu, Nepal.
072 (B) Mapping Outbreaks and Modeling Niches: Genetic Diversity, SNP Discovery and Ecological Modeling of *Bacillus anthracis* in Azerbaijan


073 (B) Identification of *Burkholderia pseudomallei* Near-Neighbors in the Northern Territory of Australia


074 (B) *Paramaecium* Consumes and DIGests *Francisella novicida* with No Apparent Mortality

W. E. Bell,1 C. V. Sayer; Virginia Military Inst., Lexington, VA.

075 (B) In Vitro Susceptibility Activity of Tigecycline against a Panel of Highly Pathogenic Bacteria

G. Ezepeleta,1 O. Telleria, R. Cisterna; Basurto Univ. Hosp., Bilbao, Spain.

076 (B) Structural Studies of the Type III Secretion System Transcriptional Activator ExsA, a Homolog of LcrF in *Yersinia pestis*

R. C. Bernhards,1 Y. Xiao, F. D. Schubot; Virginia Tech, Blacksburg, VA.

077 (B) Insights Into Anthrax Toxemia in Vivo: Relationship between Lethal Factor and Lethal Toxin Complex

A. E. Boyer,1 M. Gallegos-Candela1, R. C. Lins2, A. R. Woolfltt3, C. P. Quinn4;1 CDC, Atlanta, GA, 2 Battelle Analytical Services, Atlanta, GA.

078 (C) Comparison of Cryptococcal Antigenemia between Antiretroviral Naïve and Antiretroviral Experienced HIV Infected Patients at Two Hospitals in Ethiopia


079 (D) Collection of the Standard Set for Brucellosis Positive and Negative Sera Diagnostics with Intent to Control Quality of Immune Enzymatic Test-Systems

Y. Rodina1, SSCIIBMS, Kyiv, Ukraine.

080 (D) Detection of Coxiella burnetii in Dairy Camels in Saudi Arabia

M. F. Hussein, O. B. Mohammed, R. S. Aljumaah, M. A. Alshaikh; King Saud Univ., Riyadh, Saudi Arabia.

081 (D) Clinical and Diagnostic Features of Cutaneous Anthrax in Georgia


082 (D) Development of an IS-Based One-Tube Multiplex Conventional PCR Method of Simultaneous Detection, Identification and Typing of *Yersinia pestis*

A. A. Filippov, M. P. Nikolich; Walter Reed Army Inst. of Res., Silver Spring, MD.

083 (D) Lethal Factor Assay Development and Validation

S. E. Carpenter1, E. Bollin,2 L. S. Casey1, E. F. O’Connor1, S. F. Bungo2, P. R. Coniliffe1, A. Shadiak1;1 Elusys Therapeutics, Pinebrook, NJ, 2Avanza Lab., Inc., Gaithersburg, MD.

084 (D) Development of Sample Ready Multiplex Real-Time PCR Assay for Detection of Malaria: Application in Diagnosis, Clinical Trials and Epidemiological Surveillance

E. Kamau1, S. Alemayehu1, K. Feghalri,1 C. Ockenhouse2;1 USAMRU-K, Kisumu, Kenya, 2WRAIR, Silver Spring, MD.

085 (D) Serodiagnosis of Human Melioidosis Using Rapid Latex Agglutination Assays

M. N. Burtnick1, C. Vikraiphat1, N. Chantratita1, P. J. Brett4;1 Univ. of South Alabama, Mobile, AL, 2Mahidol Univ., Bangkok, Thailand.

086 (D) Use of InDevR ampliPHOX Colorimetric Detection Technology and Custom Low-Density Microarrays for the Identification and Differentiation of Crimean-Congo Hemorrhagic Fever Virus

L. J. Hartman1,2, P. D. Craw1, T. D. Minogue1, D. A. Kuleshi1;1 USAMRID, Ft. Detrick, MD, 2Clinical RM, Hinckley, OH.

087 (D) The Use of NanoTrap Particles to Enhance Viral Diagnostics

N. Shaafagati1, A. Narayanan1, A. Baer1, K. Fite,1 C. Bailey1, F. Kashanchi1, B. Lepene1, K. Kehn-Hall1;1 George Mason Univ., Manassas, VA, 2Ceres NanoSci., Manassas, VA.

088 (D) Development of Multiplex 3D Tests for Warfare Agents Detection

T. Berthelot1, Chemistry of Surfaces and Interfaces Lab., J. Dano1, Immunoanalysis Studies and Res. Lab., J. Creou1, H. Volland1,2, Immunoalysis- Studies and Res. Lab.;1 Commissariat à l’Énergie Atomique, GIF sur Yvette, France, 2Immunoanalysis Studies and Res. Lab. and Chemistry of Surfaces and Interfaces Lab., GIF sur Yvette, France.

089 (D) Rapid, Sensitive and Specific Detection of *Bacillus anthracis* on the POCKIT: A Field Deployable Polymerase Chain Reaction Detection System

J. D. TRUIJILLO,1 U. Donnett1, A. Herrick1, L-Y. Tsai1, J. L. Ma2, S.-H. Weng3, K. Victry1, C. Bruckner-Lea1, I. Morozov4, T. Wang5, R. Ozanich1, P. Nara1;1 Ctr. for Advanced Host Defenses, Immunobiotics and Translational Comparative Med., Iowa State Univ., Ames, IA, 2GeneReach, Boston, MA, 3Pacific Northwest Natl. Lab., Richland, WA, 4DHSS Ctr. of Excellence for Emerging and Zoonotic Animal Diseases, Kansas State Univ., Manhattan, KS, 5Pacific Northwest Natl. Lab., Richland, IA.

090 (D) Enhanced Stabilization of a Stable Single Domain Antibody for SEB Toxin by Random Mutagenesis and Stringent Selection


091 (D) Isolation of Single Domain Antibodies Targeting Vaccinia Virus


092 (D) Highly Sensitive Loop-Mediated Isothermal Amplification for the Detection of *Coxiella burnetii*

093 (D) Impedance Measurements Could Accelerate Phage-Based Identification of Bacillus anthracis and Other Bacteria

094 (D) High Sensitivity Assay to Quantify Anthrax Toxins and Screen Anti-Toxin Antibodies
N. Kamal, 1, 2 M. Eppeison, 1, 2 P. Maniatis, 1, 2 C. Quinn; 1 Atlanta Res. and Ed. Fndn., Decatur, GA, 2 CDC, Atlanta, GA.

095 (D) Optimization of a Lateral Flow Immunoassay (LFI) for the Rapid Diagnosis of Melioidosis
D. AuCoin, 1 M. Dillon, 1 S. Raychaudhuri, 1 J. Chen, 1 D. Limmuthurosakul, 1 B. J. Currie, 1 A. R. Hoffmaster, 1 B. D. Duval, 1 D. E. Reed, 1 N. Chantratita, 1 S. J. Peacock, 1 R. Bowen, 1 P. J. Brett, 1 M. N. Burtnick, 1 T. R. Kozel; 1 Ft. Detrick, MD, 2 BioSense Technologies, Inc., Woburn, MA.

096 (E) Detection of BioThreat Agents in Food Matrices Using a Multiplex PCR and the Bio-Plex Suspension Array System
K. G. Jarvis, 1 J. Jean-Gilles Beaubrun, 1 L. Ewing, 1 K. Dudley, 1 D. E. Hanes; 1 FDA/Ctr. for Food Safety and Applied Nutrition, Laurel, MD.

097 (E) Laboratory Preparedness: A Multi-Laboratory Validation of Non-Typhoidal Salmonella in Drinking and Surface Water
K. Shen, 1 L. Mapp, 1 S. R. Shah; 1 U.S. EPA, Washington, DC.

098 (E) Microbial Biofilms as an Environmental Sampling Tool: Retention and Detection of DNA from Bacillus anthracis in a Sink Drain
E. M. Winder, 1 G. T. Bonheyo; 1 Pacific Northwest Natl. Lab., Sequim, WA.

099 (E) Comparison of Nutrient Media for Primary Isolation of Francisella tularensis from Contaminated Samples
A. Skrypnyk, 1 N. Vydakyov, 1 M. Nikolich, 1 J. Farlow; 1 Black & Veatch Special Projects Corp., Kyiv, Ukraine, 2 Central Sanitary Epidemiologic Station, Kyiv, Ukraine, 3 Walter Reed Army Inst. of Res., Silver Spring, AE, 4 Arizona State Univ., Tempe, AZ.

100 (E) Hemorrhagic Fever with the Renal Syndrome (HFRS) in the West Kazakhstan Oblast (WKO)
F. G. Bidadasho, 1 F. G. Bidadasho, 1 F. K. Grazhdanov, 1 M. V. Pak, 1 V. Surov, 1 L. Belonojkina, 1 A. V. Andriushenko, 1 B. G. Kdysikh, 1 A. K. F. G. Bidashko, 1 F. K. Grazhdanov, 1 M. V. Pak, 1 V. V. Surov, 1 L. Kuspanov; 1 Uralsk Anti-Plague Station, Uralsk, Kazakhstan.

101 (E) Classical Swine Fever Virus and Pasteurella multocida Interaction

102 (E) Application of Multiplex 3-D Rapid Tests for Detection of Foodborne Pathogens
C. Feraudet-Tarisse, 1 J. Dano, 1 N. MOREL, C. Goulard-Huet, 1 J. Laporte, 1 P. Lamourette, 1 T. Berthelot, 1 C. Créminon, 1 H. Volland; 1 CEA, Gif sur Yvette, France.

103 (E) A Cost-Effective Biosurveillance Approach to Identify Novel and Emerging Vector-Borne Pathogens by Deep Sequencing
S. A. Langevin, 1 J. D. Curtis, 1 S. S. Wheeler, 1 O. D. Solberg, 1 Z. W. Bent, 1 V. A. Vandenoot, 1 W. K. Reisen, 1 T. W. Lane; 1 Sandia Natl. Lab., Livermore, CA, 2 Univ. of California, Davis, CA.

104 (F) Rare Variant Detection in Viral and Bacterial Populations
V. Fofanov, 1 M. Shin, 1 D. Kim, 1 H. Koshinsky; 1 Eureka Genomics, Sugarland, TX, 2 Eureka Genomics, Hercules, CA, 3 Eureka Genomics, Hercules, TX.

105 (F) Diversity among Viruses Isolated from Mosquitoes in the Peruvian Amazon Basin
L. L. Lofts, 1 M. J. Turell, 1 R. Kugelman, 1 T. L. Ladner, 1 G. I. Koroleva, 1 M. S. Lee, 1 C. A. Whitehouse, 1 G. F. Palacios; 1 USAMRIID, Frederick, MD.

106 (F) In Silico Identification and Analysis of miRNAs in Bacterial Infected Human Peripheral Blood Mononuclear Cells

107 (F) Enriching Pathogen Transcripts from Infected Samples: A Capture Based Approach to Improved Host-Pathogen RNA-Seq
Z. Bent, 1 M. Tran-Gyamfi, 1 S. Langevin, 1 D. Brazenel, 1 R. Hamblin, 1 S. Brandt, 1 T. Lane, 1 V. VanderNoot; 1 Sandia Natl. Labs, Livermore, CA, 2 Univ. of California, Davis, CA.

108 (G) Antiviral Susceptibility of Highly Pathogenic Influenza A (H5N1) Viruses Isolated Worldwide In 2002-2012
E. A. Govorkova, 1 A. Burnham, 1 T. Baranovich, 1 P. Seiler, 1 J. Armstrong, 1 R. J. Webby, 1 R. G. Webster; 1 St. Jude Child. Res. Hosp., Memphis, TN.

109 (G) LED209-PAMAM G3.0 Dendrimer Conjugate as a Novel Antibacterial Agent with Low Toxicity and Ability to Target QseC Receptor
X-Y. Xue, 1 Z. Hou, 1 J-R. Meng, 1 Z. Li, 1 F. Da, 1 X-X. Luo; 1 The Fourth Military Med. Univ., Xi’an, Shaanxi Province, China.

110 (G) Host-Directed Antimicrobial Therapeutics for Coxiella burnetii
D. Czyz, 1 H. A. Shuman, 1 L. Potluri, 1 D. Arvans, 1 P. Robins, 1 J. E. Gabay; 1 Howard T. Ricketts Lab., Lemont, IL, 2 Univ. of Chicago, Chicago, IL.

111 (G) Antibiotic Susceptibility of Human Brucella Isolates in Georgia

112 (G) Antibacterial Activity of Biapenem/RPX7009 against Biodefense Pathogens
O. Lomovskaya, 1 L. Lynda Miller, 1 S. Stephanie Halasohoris, 1 J. R. Hershfield; 1 Rempex Pharmaceuticals, Inc., San Diego, CA, 2 U.S. Army Med. Inst. of Infectious Diseases, Ft. Detrick, MD.

113 (G) Autolysin LytM as an Anti-S. aureus Agent
D. C. Osipovitch, 1 K. E. Griswold; Dartmouth Coll., Hanover, NH.

114 (G) Eravacycline (TP-434) is Potent against Category A and B Pathogens
J. Hershfield, 1 A. Howlett, 1 H. P. Schweizer, 1 M. Goodly, 1 N. L. Podnecky, 1 L. Miller, 1 S. Halasohoris, 1 J. Sutcliffe; 1 USAMRIID, Ft. Detrick, MD, 2 CUBRC, Inc., Buffalo, NY, 3 Colorado State Univ., Fort Collins, CO, 4 Tetraphase Pharmaceuticals, Inc., Watertown, MA.
115 (G) Identification of Broad-Spectrum Therapeutics against Filoviruses
L. M. Evans DeWald1, S. Stronsky1, C. Lear1, L. Pierce1, J. M. Grenier2, A. Stossel3, L. M. Johansen4, P. J. Glass5, G. O. Olgin6; 1U.S. Army Med. Res. Inst. of Infectious Diseases, Frederick, MD, 2Zalicus Inc., Cambridge, MA.

116 (G) A Broad-Spectrum Host-Based Antiviral Drug Platform for Emerging Viral Diseases
U. Ramstedt1, S. Perry1, M. Buck1, R. Penmasta2, H. Batra1, D. Alonzi3, E. Plummer1, T. Butters1, S. Lada1, K. King1, E. Stavale1, S. Enterlein1, K. Warfield1, B. Klose1, S. Shresta1; 1Unither Virology, Silver Spring, MD, 2LaJolla, CA, 3Oxford Glycobiology Inst., Oxford, United Kingdom.

117 (G) Safety and Pharmacokinetics of a Novel Co-Mixture of Three Monoclonal Antibodies against Botulism in Healthy Subjects

118 (G) Monoclonal Antibodies against Dengue Virus Type 4 and Their Epitopes in the Association with Viral Neutralization and Enhancement
C-T. Tang1, M-Y. Liao1, W-F. Shen1, P-C. Cheng1, H-C. Wu1; 1Inst. of Cellular and Organismic Biol., Academia Sinica, Taipei, Taiwan.

119 (G) Antisense Inhibition of Growth in Methicillin-Resistant Staphylococcus aureus by Locked Nucleic Acid Conjugated With Cell-Penetrating Peptide Inhibit as a Novel FtsZ Inhibitor
M. Jingru1, F. Holtsberg1, A. Guajardo1, C. Slee1, J. Warfield1, A. Lee1; 1Institute for Vaccine Innovation (G), Emory University, Atlanta, GA, 2IITRI, Chicago, IL.

120 (G) Efficacy of Human Monoclonal Antibodies as an Immunotherapeutic in a Guinea Pig Model of Lassa Fever

121 (G) GC-072 is Effective against the Biothreat Agent Burkholderia pseudomallei and Other Select Agents
S. Goldman1, G. Salerno1, P. Roussel1, R. Bowen1, N. L. Podnecky1, H. P. Schweizer7, J. Heim1; 1Evolve SA, Reinach, Switzerland, 2Colorado State Univ., Fort Collins, CO, 3Colorado State Univ., Fort Collins, CO.

122 (G) Efficacy of Intramuscularly (IM) Administered Anti-Toxin in Preventing Inhalational Anthrax-Related Mortality in the Cynomolgus Model of Bacillus anthracis Infection
N. Serbina1, A. Shadiack1, E. O'Connor1, D. Sanford2, L. Casey1; 1Elusys Therapeutics, Inc., Pine Brook, NJ, 2Battelle BioMed. Res. Ctr., Columbus, OH.

123 (G) FDA-Approved, Host Targeted Drugs with Broad Spectrum Antiviral Activity

124 (G) Recombinant Monoclonal Antibody Based Antitoxins for Treatment of Type A, B, and E Botulism
M. Tomic1, J. Freeberg1, A. Wajid1, A. H. Horwitz2, A. B. Ahene3, K. Der4, A. C. Dadson, Jr.5, J. D. Marks6; 1XOMA (US) LLC, Berkeley, CA, 2Univ. of California, San Francisco, CA.

125 (H) On the Influence of Immunomodulation on the Early Phase of Antigen-Specific Response of Rabbits Immunized a Living Plague Vaccine EV
B. V. Karalnik1,2, T. S. Pomona, G. A. Denisova, P. N. Deryabin, T. L. Tugambaev, B. B. Atshabar; 1Scientific Ctr. of Hygiene and Epidemiology, Almaty, Kazakhstan, 2Kazakh Scientific Ctr. of Quarantine and Zoonotic Diseases, Almaty, Kazakhstan.

126 (H) The Burkholderia mallei tonB Mutant Exhibits an Attenuated Phenotype and Provides Protective Immunity against Acute Inhalational Murine Glanders
T. M. Mott1, K. Johnston1, E. Sbrana1, A. Torres1; 1Univ. of Texas Med. Branch, Galveston, TX, 2Naval Hlth. Res. Ctr., San Diego, CA.

127 (H) Animal Models Supporting Bridge ELISA Titors as a Correlate of Protection for a Recombinant Plague Vaccine (r1FV)
P. Fellows1, J. Price1, W. Lin1, H. Lockman2, R. Kri1, S. Marshall3, S. Martin1; 1DVC, Frederick, MD, 2IITRI, Chicago, IL, 3Battelle, Columbus, OH, 4BSSI, Mt. Airy, MD.

128 (H) Phase 1 Safety and Immunogenicity Study of 4 Formulations of Anthrax Vaccine Adsorbed Plus CPG 7909 (AV7909) in Healthy Adult Volunteers

129 (H) Novel Approaches to Inactivate Chikungunya Virus: Potential Strategies for Disease Elimination
M. Gayen1, A. Sharma1, P. Gupta2, E. K. Gaidamakova1, M. J. Daly2, R. K. Madeshwari1; 1Uniformed services Univ. of Hlth. Sci., Bethesda, MD.

130 (H) A Francisella Lipid A Mutant Confers Sterilizing Immunity as Well as Therapeutic Antibodies Capable of Protecting Mice after Lethal Challenge
D. A. Powell1, M. R. Pelletier1, A. J. Scott1, R. K. Ernst2; 1Univ. of Maryland, Baltimore, Baltimore, MD.

131 (H) Stability Study of an Attenuated Smallpox Vaccine LC16m8
H. Yokote1, S. Maruno1, K. Ohkuma1, Y. Ogata1; Kaketsukuen, Kumamoto, Japan.

132 (H) Characterization of Humoral and Cell-Mediated Immune Responses to Formalin-Inactivated and Gamma-Irradiated EEEV Vaccine Candidates
E. M. Morazzani1, L. I. Prugar1, R. R. Bakken1, J. W. Cohen1, L. T. Eccleston1, P. J. Glass1; USAMRIID, Ft. Detrick, MD.

133 (H) Evaluation of a Burkholderia pseudomallei Outer Membrane Vesicle Vaccine in Non-Human Primates
**136 (H)** Vaccination with Cross-Conserved H1N1 Influenza CD4+ T-Cell Epitopes Reduces Viral Load in HLA Transgenic Mice


**137 (I)** Withdrawn

**138 (I)** Commensal Bacteria Lipoteichoic Acid Increases Mast Cell Antimicrobial Activity against Vaccinia Viruses

Z. Wang, D. T. MacLeod, A. Di Nardo; Univ. of California, La Jolla, CA.

**139 (I)** Characterization of Memory T-Cell Responses in *Vibrio cholerae* O1 Infected Patients


**140 (I)** Withdrawn

**141 (J)** Evaluating Chemical and Heat Mediated Inactivation of Viruses Using Dengue Virus Types 1, 2 and Influenza A as Models

M. E. Mayda, Z. Erwin, M. Boley, S. Rashid, K. Langenbach; BEI Resources/ATCC, Manassas, VA.

**142 (J)** Testing, Registration and Activation of Biocontainment Laboratories at the NBACC

J. P. Fitch, J. Burans, M. Hevey, P. Weaver; Natl. Biodefense Analysis and Countermeasures Ctr., Natl. Interagency Biodefense Campus, MD.

**143 (J)** Current Laboratory Bio-Safety and Bio-Security Problems in Armenia

N. Bakunts1, A. Vanyan1, A. Melkonyan2, L. Avetisyan3, S. Melikjanian4; 1State Hygenic and Anti-Epidemic Inspectorate, Yerevan, Armenia, 2Ministry of Health, Yerevan, Armenia, 3Ctr. for Disease Control and Prevention, Yerevan, Armenia.

**144 (J)** Recalcitrant Biological Agents and Survival in Landfill Leachates Over Several Years: Experiments and Modeling

W. J. Davis-Hoover; U.S. Environmental Protection Agency, Cincinnati, OH.

**145 (K) Withdrawn**

**146 (K)** The Animal Model Qualification Process: The Process in Depth

H. Lee-Lewis1, J. Beren1, J. Davis1,2, J. Flaherty1, R. Roberts1; 1Food and Drug Admin., Silver Spring, MD, 2Purdue Univ., West Lafayette, IN.

**147 (K)** The Role of the US Army Medical Command in the National Laboratory Response Network: A Phased Approach to National Biodefense

J. G. Keary1, D. Nauschuetz2; 1Irwin Army Community Hosp., Fort Riley, KS, 2U.S. Army Med. Command, Fort Sam Houston, TX.

**148 (K)** Quality System Integration in the BSL-4 Laboratory During Regulated Nonhuman Primate Filovirus Challenge Studies

T. Brasel, M. Eltzen, D. Beasley, D. Trent; Univ. of Texas Med. Branch, Galveston, TX.

**149 (K)** Guideline, Recommendation, or Regulation?: The Administrative Procedure Act and Lab Inspections

H. N. Carson, C. Davenport, V. Sutton, S. Trinh, A. Franklin; Texas Tech Univ. Sch. of Law, Lubbock, TX.

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**Highlighted Oral Abstract Presentations**

**013 Viral Agents**

**013 Viral Agents**

**Tuesday, February 26, 2013 | 5:00 PM - 6:15 PM**

**Lincoln 5**

**Moderator:**

Andrew Hayhurst; Texas Biomedical Res. Inst., San Antonio, TX.

**Presentations:**

**5:00PM**

**150 Investigating the Dynamics of Filovirus Evolution in Cell Culture**

A. M. Nour, J. Wolenski, Y. Li, Y. Modis; Yale Univ., New Haven, CT.

**5:15PM**

**151 Viral Membrane Fusion and Nucleocapsid Delivery into the Cytoplasm are Two DISTINCT Events in Flaviviruses**

A. M. Nour, J. Wolenski, Y. Li, Y. Modis; Yale Univ., New Haven, CT.

**5:30PM**

**152 Unusually Broad Ephrin Receptor Tropism of Cedar Virus, a Newly Discovered Henipavirus**


**5:45PM**

**153 A Novel Adenovirus Species Associated with an Outbreak of Fatal Pneumonia in a Baboon Colony**


**6:00PM**

**154 Identifying New Host Factors: Stress Regulated Control of Poxvirus Replication**

C. Filone1, K. Dower1, G. Cowley1, D. Root1, N. Hacohen1, L. Hensley1, J. Connor1; 1Boston Univ., Boston, MA, 2Pfizer, Boston, MA, 3Broad Inst., Cambridge, MA, 4FDA, Silver Spring, MD.
Highlighted Oral Abstract Presentations

014 Bacterial Agents
Tuesday, February 26, 2013 | 5:00 PM - 6:15 PM
Lincoln 2-4
Moderator: Catharine Bosio; RML/NIAID/NIH, Hamilton, MT.
Presentations:
155 Withdrawn
156 Functional Discovery of Regulatory Small RNAs in Infectious Disease
N. Li, C. J. Stubben, B. Hu, P. S. Chain, E. Hong-Geller; Los Alamos Natl. Lab., Los Alamos, NM.
5:00PM
157 Characterisation of the Natural History of Inhalational Melioidosis in Cynomolgus and Rhesus Macaques
J. Vipond, G. Hatch, J. Kane, S. Bate, S. Funnell, A. Roberts, H. Shuttleworth; Hlth. Protection Agency, Salisbury, United Kingdom.
5:15PM
158 Mutation of Core Oligosaccharide Attenuates Virulence of Yersinia pestis Through TLR4
5:30PM
159 Withdrawn

Highlighted Oral Abstract Presentations

015 Environmental Detection, Decontamination, Biosafety, Containment and Other
Tuesday, February 26, 2013 | 5:00 PM - 6:15 PM
Lincoln 6
Moderator: Carol D. Linden; Office of the Biomedical Advanced Res. and Dev. Authority, Washington, DC.
Presentations:
160 Summary of October 2012 Changes to the Select Agent Regulations
R. S. Weyant, D. Gangadharan, L. Bane; CDC, Atlanta, GA.
5:00PM
161 Detection of Microorganisms in Water Using Online Sensors
S. P. Sherchan, C. P. Gerba, I. L. Pepper; Univ. of Arizona, Tucson, AZ.
5:15PM
162 D-Cycloserine or Similar Compounds May Be Uniquely Suited for Bacillus anthracis Spore Decontamination Strategies
J. D. Heffron, C. Marchand, L. Miller, S. Halasohoris, J. Bozue, S. Welkos, C. Cole; USAMRIID, Ft. Detrick, MD.
5:30PM
163 Inactivation of Bacillus Spores Using Acidified Nitrite
5:45PM
164 Removal of Ricin and Pathogenic Bacteria Surrogates from Water, Material Surfaces and Reusable Medical Devices
E. Gonzalez, A. Lucas, V. Hitchins; FDA, White Oak, MD.
Wednesday Sessions

For the most up-to-date program information, visit the Online Program Planner at www.asmbiodefense.org

Late-Breaking Symposium

016 Emergence of a New Pathogenic Coronavirus: Are We Witnessing the Beginning of Another SARS-Like Pandemic, or Not? (A)

Wednesday, February 27, 2013 | 7:15 AM - 8:15 AM
Lincoln 2-4

Coronaviruses are enveloped RNA viruses with a global distribution, they are found in humans, other mammals and birds. While most infections caused by human coronaviruses are relatively mild, the zoonotic outbreak of severe acute respiratory syndrome (SARS) caused by SARS-CoV in 2002–2003 caused the deaths over 900 people. In September 2012, less than a decade after the SARS pandemic, the World Health Organization (WHO) reported two cases of severe community-acquired pneumonia which bore significant similarity with SARS. Subsequent laboratory tests revealed the emergence of a novel human coronavirus. This virus has continued to cause human disease and extra cases have been confirmed in the Middle East. While the number of cases remains low at this stage, research preparedness against another SARS-like pandemic is an important precautionary strategy.

Moderators:
Miles Carroll; Hlth. Protection Agency, Salisbury, United Kingdom.
Jean L. Patterson; Texas Biomedical Res. Inst., San Antonio, TX.

Presentations:
7:15am
164a An Ethical Frontier: Global Public Health
Gwen Stephens; Saudi Arabia Ministry of Hlth., Riyadh, Saudi Arabia.
7:35am
164b Novel Human Coronavirus Causes Pneumonia in a Macaque Model Resembling Human Disease
Vincent Munster; Rocky Mountain Lab., Hamilton, MT.
7:55am
164c Emerging Infections: UK Response to the Novel Coronavirus 2012

Plenary Session

017 Developing Vaccines for Biodefense and Emerging Disease Pathogens (A, B, G)

Wednesday, February 27, 2013 | 8:30 AM - 11:30 AM
Lincoln 2-4

Over the last decade, a significant investment has been made into the development of safe and effective vaccines to Biodefense and emerging disease pathogens. This plenary session will overview the critical steps in vaccine research and development, discuss the optimal immune response required and highlight the unique challenges of developing vaccines to diseases with a relatively low incidence. The session will focus on a critical assessment of progress made in the development of new licensed vaccines including; smallpox, plague, anthrax, filoviruses and other emerging diseases.

Moderators:
Miles Carroll; Hlth. Protection Agency, Salisbury, United Kingdom.
Soren Alexandersen; Natl. Ctr. for Animal Disease, Winnipeg, Canada.

8:30AM
164d Vaccine Design: Dissection of Immune Responses to Vaccine Ags
Phil Felgner; Univ. of California, Irvine, CA.

8:55AM
165 Tackling the Henipavirus Transboundary Threats by Active and Passive Immunization Approaches
Christopher C. Broder; Uniformed Services Univ. of Hlth. Sci., Bethesda, MD.

9:20AM
166 Filovirus Vaccine R&D
Heinz Feldmann; NIAID Rocky Mountain Lab, Hamilton, MT.

9:45AM
167 Smallpox Vaccine Development: Why, How and Where are We Now
Miles Carroll; Hlth. Protection Agency, Salisbury, United Kingdom.

10:10AM
Coffee Break

10:40AM
168 Plague Vaccine R&D
Diane Williamson; Defense Sci. and Technology Lab., Salisbury, United Kingdom.

11:05AM
169 Anthrax Vaccine Development: Addressing the Needs of the Strategic National Stockpile
Gerald R. Kovacs; Biomedical Adv., Res. and Dev. Authority, Dept. of Hlth. and Human Services, Washington, DC.

11th ASM Biodefense and Emerging Diseases Research Meeting
Viruses belonging to the family Bunyaviridae (the largest family of RNA viruses) cause a number of important human and animal diseases. Many of these viruses are listed as NIAID category A, B, or C pathogens for priority research on virology, pathogenesis, ecology, and the development of countermeasures. In addition, members of the Bunyaviridae are found worldwide, and their host range is very broad, including arthropods, mammals, and birds. Since bunyaviruses possess a genome consisting of three negative-strand RNA segments, the evolution of bunyaviruses is complex and driven by accumulation of mutations, genome reassortment, and virus/host interactions, making it difficult to understand the mechanisms of biogenesis and emergence/re-emergence of highly pathogenic viruses. However, almost all of these viruses and their associated diseases have been neglected, due in part to a lack of information for most members of this family. Recently, three novel emerging bunyaviruses, Severe Fever with Thrombocytopenia virus in China, Schmallenberg virus in EU countries, and Heartland virus in the USA, have been causing disease in humans and livestock. In order to fill the gaps in our knowledge of this important and emerging group of viruses, this session will stimulate discussion on the current state of bunyavirus research, its future directions, and the development of countermeasures.

**Moderators:**
Robert B. Tesh; The Univ. of Texas Med. Branch, Galveston, TX.
Hideki Ebihara; Rocky Mountain Lab., NIAID, NIH, Hamilton, MT.

**Presentations:**

1:00PM

**170 Overview of the Virus Family Bunyaviridae (The Bunyaviruses)**

1:30PM

**171 Current Update on Molecular Biology of Bunyavirus and Emergence of Schmallenberg Virus in EU Countries**
Richard M. Elliott; Univ. of St. Andrews, St. Andrews, United Kingdom.

2:00PM

**172 Discovery of a New Phlebovirus Associated with Severe Febrile Illness in Missouri**
William L. Nicholson; CDC, Atlanta, GA.

2:30PM

**173 Molecular Determinants of Tick-Borne Phlebovirus Virulence**
Hideki Ebihara; Rocky Mountain Lab., NIAID, NIH, Hamilton, MT.

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**Symposium**

**019 Progress towards a Dengue Vaccine: Killing Four Birds with One Stone (A, H)**
Wednesday, February 27, 2013 | 1:00 PM - 3:00 PM
Lincoln 2-4

Dengue virus (DENV) is a mosquito-borne Flavivirus that has a global impact on public health. Each year, more than 50 million individuals are infected by DENV, with clinical manifestations ranging from a self-limiting acute, febrile illness to a potentially fatal syndrome characterized by plasma leakage and shock. Eliciting a neutralizing antibody response is a major goal of ongoing DENV vaccine development efforts. However, these efforts are complicated by a requirement to simultaneously elicit protection against four different DENV viruses that while antigenically related, share only some of the determinants thought to contribute to protection. With field trials of candidate vaccines underway, identifying unique features of protective and non-protective responses will provide relevant correlates of protection. In addition, understanding the dynamics of the neutralizing antibody repertoire will provide insight into the pathogenesis of secondary DENV infection with respect to the complex role of antibody in exacerbating disease.

**Moderator:**
Stephen Whitehead; NIAID, NIH, Bethesda, MD.

1:00PM

**174 Dengue Vaccines: Are We Getting Closer?**
Thomas P. Monath; Kleiner Perkins Caufield & Byers, Harvard, MA.

1:30PM

**175 A Single Dose of the NIH Dengue Vaccine is Safe, Immunogenic, and Capable of Neutralizing Vaccine Challenge**
Stephen Whitehead; NIAID, NIH, Bethesda, MD.

2:00PM

**176 Structure, Dynamics and Composition of Dengue Viruses**
Richard Kuhn; Purdue Univ., West Lafayette, IN.

2:30PM

**177 Genetic and Structural Basis for Recognition of Dengue Viruses by Human Monoclonal Antibodies**
James E. Crowe Jr.; Vanderbilt Univ., Nashville, TN.
**Symposium**

**020 Yersinia Pathogenesis: Latest Advances in Regulation and Understanding of Virulence (B)**

Wednesday, February 27, 2013  |  1:00 PM – 3:00 PM  
Lincoln 6

This session will emphasize the tie from basic research into the biology of a microbial pathogen to the development of medical countermeasures against it. Yersinia pestis is particularly problematic in that it has a complex lifestyle involving multiple hosts and various niches and there is still a limited understanding of its pathogenesis. Therefore, this session will focus on the recent findings in Yersinia molecular pathogenesis and how this microbe interacts with its various hosts which will aid our understanding of the pathogen and in the development of novel countermeasures.

**Moderator:**

Elisabeth Carniel; Inst. Pasteur, Paris, France.

**Presentations:**

1:00PM

178 Adaptation to Life in the Flea: Understanding the Microbe-Flea Interaction  
Viveka Vadyvaloo; Washington State Univ., Pullman, WA.

1:20PM

179 Early Events in Bubonic Plague  
Virginia L. Miller; Univ. of North Carolina, Chapel Hill, NC.

1:40PM

180 Molecular Mechanisms of Disease During Pneumonic Plague  
Wyndham W. Latham; Northwestern Univ., Chicago, IL.

2:00PM

181 Regulation of *Yersinia* Virulence by Small and Sensory RNAs  
Petra Dersch; Helmholtz Ctr. for Infection Res., Braunschweig, Germany.

2:20PM

182 The Role of Divalent Cation Transport Systems in Plague Pathogenesis  
Jacqueline D. Fetherston; Univ. of Kentucky, Lexington, KY.

**Poster Session**

**021 Wednesday Posters**

Wednesday, February 27, 2013  |  3:15 PM - 4:45 PM  
Hall B

**Presentations:**

183 (A) Subcellular Localization of Hantavirus RNA in Viral Nucleocapsid Filaments  
M. Lindquist; C. S. Schmaljohn, USAMRIID, Frederick, MD.

184 (A) Hepatitis B Virus among Potential Blood Donors in Ibadan, Nigeria  

185 (A) Impact of IL-12B and TGF-β1 Polymorphism and Levels in HBV-HCC Disease Progression in India  
J. Kaur, R. Saxena, Y. Chawla, I. Verma; PGIMER, India, Chandigarh, India.

186 (A) Anti-HIV-1 Activities of Kaempferol and Kaempferol-7-O-Glucoside Isolated from the Medicinal Plant Securigera Securidaca, In Vitro  
M. Behbahani; Univ. of Isfahan, Isfahan, Iran, Islamic Republic of.

187 (A) Genome Analysis of Cowpox Viruses Reveals a Clade Closely Related to Variola Virus  

188 (A) Multi-Faceted Proteomic Profiling of Host Proteins Associated with Rift Valley Fever Virus (RVFV) Virions and Identification of Specific Heat Shock Proteins (HSPs) as Essential Host Factors  

189 (A) Modulation of Viral and Host Proteins During Infection Using Nuclear Import and Export Inhibitors  
C. Pinkham1, L. Lundberg1, A. Narayanani1, A. Baer2, K. Wagstaff3, D. Jans3, K. Kehn-Hall3; INCBID, Manassas, VA, 4Monash Univ., Clayton, Australia.

190 (A) Monoclonal Antibodies Derived from Lassa Fever Survivors  
J. E. Robinson1, L. Branco1, K. Hastie1, R. Yenni2, S. Igneti3, D. Elliott1, R. F. Garry1, J. Rouelle1, C. Kannadka1, A. Reyna1, M. Gleaton1, M. Zandonatti1, A. Lee1, E. Sapphire1, R. Garry1; 1Viral Hemorrhagic Fever Consortium; Tulane Univ., New Orleans, LA, 2The Scripps Res. Inst., La Jolla, CA, 3UT Southwestern Med. Ctr. Med., Dallas, TX.

191 (A) Control and Diagnostics of African Swine Fever (ASF) in Ukraine  
A. Golovko1; 1, O. Nevolko2, L. Marushchak2; 1, NAAS, Kyiv, Ukraine, 2SS-RILDVSE, Kyiv, Ukraine.

192 (A) Viral Hemorrhagic Fever (VHF) Cases in Georgia  
T. Kuchuloria1, P. Imnadze2, T. Tsertsvadze1, M. A. Faddei3, D. Clark1, C. Bautista1, M. J. Hepburn3, R. G. Rivard5, G. Pimentel3, B. House3, S. Makino4, A. Garry1; 1Tulane Univ., New Orleans, LA, 2UT Southwestern Med. Ctr., Dallas, TX.

193 (A) Production of a Mycoplasma Free Stock of Taï Forest Ebolavirus  

194 (A) Pathogenesis of Hendra Virus in a Mouse Model  
B. Rockx1, J. Smith, V. Borisevich, T. Yun, O. Escaffre, T. Juelich, G. Campbell1, A. Freiberg; Univ. of Texas Med. Branch, Galveston, TX.

195 (A) Evaluation and Characterization of the AG129 Mouse Model of Subcutaneous Challenge with Dengue Strain D2Y98P  
A. T. Russo, S. Dechnik, K. Dearen, D. N. Mitzel, R. Baker; LRRI, Albuquerque, NM.
196 (A) Epidemiology of Lassa Fever in Post-Conflict Sierra Leone: Seasonal Demographics
J. G. Shaffer1, D. S. Grant1, A. Goba2, M. L. Boisen1, L. M. Branco1, D. Ottomassathi1, D. Levy1, R. F. Garry1, J. S. Schieffelin1; 1Tulane Univ., New Orleans, LA, 2Kenema Government Hosp., Kenema, Sierra Leone, 3Corgenix Med. Corp., Broomfield, CO, 4Autoimmune Technologies, LLC, New Orleans, LA.

197 (A) Household Case Investigation of HFRS in Georgia, 2010

198 (A) SOCS3 is Required for Rift Valley Fever Virus Replication
B. M. Fried1, J. Langford1, G. G. Olinger1, D. R. Smith1; 1USAMRIID, Ft. Detrick, MD, 2Colorado State Univ., Fort Collins, CO.

199 (A) Prevalence and Drug Resistance Patterns of Salmonella Concord among Children in Selected Orphanages and Visiting Health Institutions in Addis Ababa, Ethiopia
B. H. Woldeyohanes; AAU, Addis Ababa, Ethiopia.

200 (B) Pathogenicity Modeling of the C. trachomatis T3SS Effector Protein CT694 in Fission Yeast Identifies the IKAP Complex as a Novel Host Target
H. D. Bullock, K. A. Fields; Univ. of Miami Miller Sch. of Med., Miami, FL.

201 (B) Examination of Burkholderia Biofilms from Clinical Isolates
S. Ruiz, A. Nalca; USAMRIID, Ft. Detrick, MD.

202 (B) Genomic Diversity of Brucella Over Space and Time
N. A. Hasan1, 2, S. Y. Choi3, 2, E. Castro-Nallar1, R. Robison1, K. A. Chandall1, J. Chun1, T. A. Cebula1, 2, R. R. Colwell1, 2; 1Univ. of Maryland, College Park, MD, 2CosmosID Inc., College Park, MD, 3George Washington Univ., Washington, DC, 4Brigham Young Univ., Provo, UT, 5Seoul Natl. Univ., Seoul, Korea, Republic of.

203 (B) Structure and Molecular Interactions of a Monoclonal Antibody Specific for the Polypeptide Capsular Antigen of Bacillus anthracis
M. A. Hubbard, W. H. Welch, T. R. Koze1, Univ. of Nevada, Reno, Reno, NV.

204 (B) Investigation the Role of Biofilm Formation in Tigecycline Resistance of Acinetobacter baumannii
J. Qu, X. Lü, X. Wang; West China Hosp., Sichuan Univ., Chengdu, China.

205 (B) Brucella Infection of Livestock in Georgia
M. Kokheidze, M. Doudaushvili, E. Mamisashvili, M. Zakareishvili, N. Vepkhvadze, K. Goginashvili; Lab. for the Ministry of Agriculture, Tbilisi, Georgia.

206 (B) Detection of Rickettsia, Ehrlichia and Borrelia Species in Ticks from Different Regions of Georgia Using Real-Time PCR Assay
R. Sukhishvili1, E. Zhgenti1, E. Khaladze1, N. Tsretsvadze1, M. Alkhazashvili1, S. Francesconi1, E. Wallace1, A. Richards1, S. Pilsarck1; 1Natl. Ctr. for Disease Control, Tbilisi, Georgia, 2Naval Med. Res. Ctr., Frederick, MD, 3USAMRIID, Ft. Detrick, MD.

207 (B) Anthrax Surveillance Data of 2012 in Georgia
N. Abazashvili1, M. Broladze1, L. Malania1, M. Zakshashvili1, E. Zhgenti1, G. Chanturia1, E. Khaladze1, N. Tsretsvadze1, S. Francesconi1; 1Natl. Ctr. for Disease Control, Tbilisi, Georgia, 2Naval Med. Res. Ctr., Frederick, MD.

208 (B) Analysis of Virulence and Virulence Factors in Acinetobacter baumannii A118
M. Tolmasky1, M. Ramirez2, W. Penwell1, N. Nikolaidis3, C. Adams1, B. Arivett1, R. Munson, Jr.1, R. Bonomo1, L. Actis1; 1California State Univ., Fullerton, Fullerton, CA, 2IMPaM, UBA, Buenos Aires, Argentina, 3Miami Univ., Oxford, OH, 4Ohio State Univ., Columbus, OH, 5Case Western Reserve Univ., Cleveland, OH.

209 (B) Structural Characterization of the Yersinia pestis Type III Secretion Protein YscD and Evaluation of the Potential Public Health Threat Posed by Y. pestis

210 (B) Genetic Analysis of Bacillus anthracis S-Layer Assembly
O. Schneewind, J.M Lunderberg, S-M. Nguyen-Mau, Y. Wang, S-Y. Oh, D. M. Missiakas; Univ. of Chicago, Chicago, IL.

211 (B) Chitinases: Negative Regulators of Francisella Novicida Biofilm
M-C. Chung, M. L. van Hoek; George Mason Univ., Manassas, VA.

212 (B) Francisella tularensis and Pseudomonas aeruginosa Interspecies Communication
S. N. Dean, A. Nwabueze, M. L. van Hoek; George Mason Univ., Manassas, VA.

213 (B) Influence of Serial Passaging on Carbohydrate Profiles of Yersinia pestis
H. W. Kreuzer1, B. H. Clowers1, J. R. Hutchison1, C. Ehrhardt1, O. P. Leiser1, J. Foster1; 1Pacific Northwest Natl. Lab, Richland, WA, 2Virginia Commonwealth Univ., Richmond, VA, 3Northern Arizona Univ., Flagstaff, AZ.

214 (B) Determining the Fate of YpeB During Germination of Bacillus anthracis Spores and its Effect on SleB Activation for Cortex Degradation
C. B. Bernhards, D. L. Popham; Virginia Tech., Blacksburg, VA.

215 (B) E5564 Enhances Prophylactic Antibiotic Efficacy in a Murine Model of Inhalational Anthrax
C. L. Marchand, III1, L. L. Miller1, S. A. Halasohoris1, J. R. Hershfield1, M. P. Everson1, F. P. Duncanson1; USAMRIID, Ft. Detrick, MD, 2Eisai Inc., Woodcliff Lake, NJ.

216 (C) Histopathological Analysis of Chronic Necrotizing Pulmonary Aspergillosis

217 (D) Efficacy of Ribavirin Therapy in Lassa Fever
J. Schieffelin1, D. Grant1, J. Shaffer1, M. Boisen1, L. Branco1, A. Goba1, B. Fonnie2, D. Ottomassathi1, D. Levy1, D. Bausch1, R. Garry1; 1Tulane Univ., New Orleans, LA, 2Kenema Government Hosp., Kenema, Sierra Leone, 3Corgenix Inc., Broomfield, CO, 4Autoimmune Technologies, LLC, New Orleans, LA.

218 (D) Analysis of Laboratory Preparations of Bacillus Spores Using Direct Analysis in Real-Time Mass Spectrometry (DART-MS): Implications for Rapid Taxonomic Identification and Forensic Attribution of Bacterial Threat Agents
E. J. Dolak, K. Asal, J. Haithcock, C. Stanciu, C. J. Ehrhardt; Virginia Commonwealth Univ., Richmond, VA.
219 (D) Production and Characterization of Monoclonal Murine Antibodies against Type III Secretion System of Yersinia pestis
S. Simon, Sr., J. Laporte, Jr., C. E. Demeure, Sr., H. Volland, Sr., P. Lamourette, Jr., K. Devilliers, Jr., M. Plaisance, Sr., C. Créminon, Sr., E. Carniel, Sr.; 1CEA, Gif sur Yvette Cedex, France, 2Inst. Pasteur, Paris, France.

220 (D) Cell Surface Hydrophobicity of Bacillus Spores: Novel Forensic Signatures for the Holistic Characterization of Bacterial Threat Agents
J. M. Haltcock, C. E. Stanciu, C. J. Ehrhardt; Virginia Commonwealth Univ., Richmond, VA.

221 (D) Quantification of Anthrax Edema Factor Activity: Comparison of Cyclic AMP Detection by ELISA and LC-MS/MS
R. C. Lins, A. E. Boyer, C. P. Quin, Z. Kuklenyik, J. Barr; 2Battelle Analytical Services, Atlanta, GA, 3CDC, Atlanta, GA.


223 (D) Rpob Gene Can Be Used as a Marker for Rapid Detection of Bacillus cereus Group

224 (D) Two Step RT-PCR for Rapid Diagnosis of Francisella spp. Infection
O. Telleria, G. Ezepeleta, R. Cisterna; Basurto Univ. Hosp., Bilbao, Spain.

225 (D) URPI: Ultra-Rapid Pathogen Identification and Discovery in Clinical Metagenomic Samples

226 (D) DNA-Chromatography for Rapid Screening BSL3 Pathogens
T. Ezaki, S. Yoshida, M. Hayashi, T. Mizuno, N. Tatori, I. Kanazawa, J. Zhang, and K. Okusu; Gifu Univ. Graduate Sch. of Med., Gifu, Japan.

227 (D) Sentinel Clinical Laboratories Assess Preparedness Competency with a Biannual Exercise on Biothreat Agent Detection
C. L. Chadwick; Assoc. of Publ. Hlth. Lab., Silver Spring, MD.

228 (D) Rapid Blood Flow Immunoassay for Early Diagnosis of Anthrax

229 (D) Detection of Q Fever Specific Antibodies with Recombinant Antigens Com1 and AdaA

230 (D) Rapid Anthrax Assay: Proof of Concept for Point of Care Diagnostics
N. Kamali, M. Epperson, P. Maniatis, C. Quinn; 1Atlanta Res. and Ed. Fndn., Decatur, GA, 2CDC, Atlanta, GA.

231 (D) A Novel Approach to Unknown Pathogen Detection in Clinical Samples

232 (D) ReLASV Diagnostic Assays Improve of Detection and Surveillance of Lassa Fever in West Africa

233 (E) Immune Cell-Assisted Culturing of Bacterial Pathogens Collected from Biocrimes
S. Sorrell, N. Moritz, J. Bortzner, R. Winegar PhD, K. Brown PhD; MRI-Global, Palm Bay, FL.

234 (E) The First Bat Survey for Emerging Zoonotic Pathogens in the Republic of Georgia

235 (E) Withdrawn

236 (E) Using Preparations of Cosmetic Botulinum Toxin (A and B) for Evaluation of Commercial Quick Tests
H-C. Slotved, A. Lindqvist, J. T. Tanussi, N. H. Heegaard, N. Steenhard; Ctr. for Biosecurity and Biopreparedness (CBB), Statens Serum Univ., Copenhagen, Denmark.

237 (E) Streptavidin-Nucleic Acid Network as Signal Enhancer in a Portable Detection Platform Using Electrochemical Biochips
C. Pühlmann, T. Elßner; Bruker Daltonik GmbH, Leipzig, Germany.

238 (E) Contamination of Ticks with Tick-Borne Encephalitis (TBE) Virus and Crimean-Congo Hemorrhagic Fever (CCHF) Virus in the Southern and Southeastern Regions of Kazakhstan

239 (E) Development of Process of Especially Dangerous Pathogens Detection in Environment in Armenia, 2011
S. Melikjanyan, A. Vanyan, A. Melkonyan, L. Avelitsyan; 1Ctr. for Disease Control and Prevention, Yerevan, Armenia, 2State Hygienic and Envi-Epidemic Inspectorate, Yerevan, Armenia, 3Ministry of Hlth., Yerevan, Armenia.

240 (E) Utilisation of Ultrafiltration for Sampling of Water at an Anthrax Outbreak Site
241 (F) Genetic activity within Francisella tularensis A.I and A.II Clades
M. A. Larson1, E. B. Zentzi2, S. H. Hinrichs1; 1Univ. of Nebraska Med. Ctr., Omaha, NE, 2OpGen Inc., Gaithersburg, MD.

242 (F) Virus Pathogen Resource (ViPR): A Public Bioinformatics Database and Analysis Resource for Human Virus Pathogen Research
Y. Zhang1, B. E. Picketti1, D. S. Greer1, B. D. Aevermann1, L. Stewart1, L. Zhou1, Z. Gu1, S. Kumar1, S. Zaremba1, G. Sun1, C. N. Larson1, W. Jenii, E. B. Klem1, R. H. Scheuermann1, J. Craige Venter Inst., San Diego, CA, 2Northrop Grumman Hlth. Solutions, Rockville, MD, 3Vecna Technologies, Greenbelt, MD.

243 (F) Genotyping and Trace-Back-Analysis of Bacillus anthracis Isolates Related to Injectional Anthrax
G. Grassi1, S. Klee1, D. M. Wagner1, T. Pearson1, R. Grunow1, M. Hanczarak1, P. Keim1; 1Bundeswehr Inst. of Microbiol., Munich, Germany, 2Robert Koch-Instit., Ctr. for Biol. Security, Berlin, Germany, 3Ctr. for Microbial Genetics and Genomics, Northern Arizona Univ., Flagstaff, AZ.

244 (F) A High Resolution Framework for the Phylogenomic Analysis of Escherichia coli 0157:H7
M. Eppinger1, T. H. Hazen1, M. Rodriguez2, S. Agrawal1, K. Galens3, S. S. Koenig1, S. Daugherty2, L. Sadzewicz2, L. Tallon2, D. R. Aasko2, C. Fraser2, J. Ravel1; 1UTSA, San Antonio, TX, 2Univ. of Maryland, Baltimore, MD.

245 (F) A System for Rapid Pathogen Detection and Outbreak Integration Based Solely on NGS Reads

246 (G) A Phase 1C Clinical Trial of DAS181-F03: A Sialidase for Prophylaxis and Treatment of Influenza and Parainfluenza Infections
R. McKenzie1, E. J. Fuchs1, C. W. Hendrix1, C. Radebaugh1, R. Jura1, R. B. Moss1, J. M. Griffiss1, J. M. Zenilman2; 1Johns Hopkins Univ., Baltimore, MD, 2NexBio Inc., San Diego, CA, 3Clinical RM, Inc., Hinckley, OH.

247 (G) Withdrawn

248 (G) Detection of Endogenous Anti-PA Antibodies in Anthrax-Infected Rabbits after Treatment with ETI-204
A. Shadiack1, E. O’Connor1, S. Carpenter1, H. Tang1, D. Yaskanin2, C. Rivera2, L. Casey1; 1Ellusys Therapeutics, Pine Brook, NJ, 2Tandem Labs, West Trenton, NJ.

249 (G) Assessment of the Post-Exposure Prophylactic Efficacy of Orally Administered Levofoxacin in a Bacillus anthracis Aerosol Infection Model

250 (G) Interpretation of Nitric Oxide Precursors and Antibiotic against Typhoid
S. S. Haque, II; Indira Gandhi Inst. of Med. Sci., Patna, India.

251 (G) Evaluation of Some Medicinal Plants Effect on Growth, Biofilm Formation and Pyocyanin Production of Pseudomonas aeruginosa
E. Sepahi, Sx.; Ferdowsi Univ. of Mashhad, Mashhad, Iran, Islamic Republic of.

252 (G) PANACEA Broad-Spectrum Antiviral Therapeutics
T. H. Rider; Massachusetts Inst. of Technology, Cambridge, MA.

253 (G) In Vitro Activity of Omadacycline (OMC) against Biothreat Bacteria
M. Draper1, L. Miller1, S. Halasohoris2, O. Kim1, J. R. Hershfield1; 1Paratek Pharmaceuticals, Boston, MA, 2US. Army Med. Inst. of Infectious Diseases, Ft. Detrick, MD.

254 (G) In Vitro Characterization of a Potent and Novel Broad-Spectrum Antiviral (BSAV) Agent Targeting RNA Polymerase
B. R. Taubenheim1, S. Banta1, D. Barnard1, C. Parker1, R. Upshaw1, Y. Lou1, J. Williams1, D. Kellogg2, P. Kotian1, W. Sheridan1, Y. S. Babu1; 1BioCryst Pharmaceuticals, Inc., Durham, NC, 2Inst. of Antiviral Res. Utah State Univ., Logan, UT.

255 (G) Discovery of a New Class of Small Molecules with Anti-Gram-Positive, (S. aureus, MRSA, P. acnes), and Tumor Suppression Activity
B. Memarzadeh1, S. Noonan1, H. N. Duong1, H. Lopez2; 1Common Pharma, San Carlos, CA, 2MuriGenics, Inc., Vallejo, CA, 3MuriGenics, Vallejo, CA.

256 (G) Protein Phosphatase 1 Inhibitors Effect on Rift Valley Fever Virus
A. Baer; George Mason Univ., Manassas, VA.

257 (G) Studies on the Therapeutic Efficacy of Thoridazine against Active and Latent Tuberculosis

258 (G) Therapeutics, Prophylactic and Diagnostic Applications of Phages in Biodefense

259 (G) Plant-Based Expression and In Vivo Evaluation of Three Neutralizing, Chimeric Monoclonal Antibodies against Ricin Toxin

260 (G) Assessment of Therapeutic Efficacy: Anthrax Immune Globulin Antitoxin Interferes With Protective Antigen but Not Lethal Factor Dependent Analyses
M. G. Candela1, A. E. Boyer1, K. Isbell1, A. R. Woolfit1, R. C. Lins1, J. Heitz1, C. P. Quinn1, J. R. Barr1; 1CDC, Atlanta, GA, 2Batelle Analytical Services, Atlanta, GA.

261 (G) Plant-Manufactured Monoclonal Antibodies for Pre- and Post-Exposure Prophylaxis of Ebola Zaire Infection
L. Zeilm1, J. Pettitt1, N. Bohorova1, O. Bohorov1, B. Bratcher1, C. Goodman1, L. Hensley1, E. Hiatt1, S. Hume1, A. Johnson1, D. Kim1, C. Lear1, J. Morton1, M. H. Pauly1, C. Scully1, J. Velasco1, K. J. Whaley1, C. Working1, G. Olinger1; 1Mapp Biopharmaceutical, Inc., San Diego, CA, 2USAMRIID, Frederick, MD, 3Kentucky BioProcessing, Owensboro, KY.

262 (G) Nano-Aerosol Therapeutic Intervention against Francisella novicida Infection
A. O. Nwabueze1, V. N. Morozov2, M. L. van Hoek1; 1George Mason Univ., Manassas, VA, 2Inst. of Theoretical and Experimental Biophysics, Pushchino, Russian Federation.
263 (H) Novel Nanogel as Adjuvant for Vaccine Development

264 (H) Improved Potency Assay for Quantification of Immuno-reactive Hemagglutinin in Trivalent Influenza Vaccines Using Isotope Dilution Mass Spectrometry
C. L. Pierce; W. Wang, T. L. Williams, J. R. Barr; CDC, Atlanta, GA, Battelle Mem. Inst., CDC, Atlanta, GA.

265 (H) Testing of Inactivated Vaccine against Highly Pathogenic Avian Influenza in Ukraine

266 (H) Live-Attenuated Tetra-valent Dengue Virus Host Range Vaccine Elicits Immune Response in African Green Monkeys

267 (H) Assay Verification and Optimization of the IgG Anti-Pertussis Toxoid ELISA for Use in Outbreak Investigations

268 (H) Correlates of Protection in Lethal Monkeypox Virus Challenge Models of Cynomolgus Macaques

269 (H) Mucosal Immunization of Bacillus anthracis Surface Protein EA1 Protects Mice from Intranasal Challenge of B. anthracis Spores

270 (H) yscPext, A Mutation That Extends Type III Needle Complexes, Cannot Endow Yersinia pestis with Escape from Plague Protective Immunity
K. Given Lichtenberg, L. Quenee, O. Schneewind; Univ. of Chicago, HTRL Argonne Natl. Lab., Argonne, IL.

271 (H) Characterization of Immune Correlates of Protection for a Burkholderia pseudomallei Outer Membrane Vesicle Vaccine Co-Administered with CpG Adjuvant

272 (H) Yersinia pestis Live Attenuated Vaccine Strains with Enhanced Safety and Efficacy
L. E. Quenee, K. Given-Lichtenberg, N. A. Ciletti, D. Elli, R. Perry, O. Schneewind; The Univ. of Chicago, Chicago, IL, The Univ. of Kentucky, Lexington, KY.

273 (H) A Multiagent DNA Vaccine Delivered by Electroporation Elicits Protective Immunity against Eastern and Western Equine Encephalitis Viruses in Nonhuman Primates

274 (H) Intranasal Vaccination with Integrate-Defective Lentiviral Vectors Expressing Influenza Nucleoprotein Induce Protective Immunity against Influenza Virus

275 (I) Francisella novicida Alters Cellular and Exosomal mRNA Expression in Murine Macrophages In Vitro

276 (I) The Effectiveness of Rabies Immunoglobulin against Street Rabies Virus Isolates Belonging to Different Genetic Clusters

277 (I) Development of a Flow Cytometric Assay to Assess Filovirus Vaccine Efficacy in Non-Human Primates

278 (I) Control Efficiency Sterilization of Biological Indicators as Elements in Ensuring Biosafety
N. Pinchuk; SSCIBMS, Kyiv, Ukraine.

279 (J) Plant Manufactured Monoclonal Antibodies as Immunoprotectants for Category B Toxins

280 (J) Functionalized, Carbon Nanotube Catalyst for the Degradation of Nerve Agents
M. M. Bailey, J. M. Heddeleston, J. Davis, J. L. Staymates, A. R. Hight Walker; NIST, Gaithersburg, MD.

281 (J) Medical Hazardous Waste Disposal in Kazakhstan

282 (K) Toxins and Contaminated Drugs: A Report of Selected Biological Events in Germany 2012
M. H. Richter, M.-H. Lee, C. Herzog; Robert Koch-Instit., Berlin, Germany.

283 (K) Extreme Biological Events in the Military: Effect and Response: Lessons Learned from the Israeli Biological Preparedness Project — The “Orange Flame”

284 (K) Clinician Training in Select Agent Outbreak Response is Potentially Sustainable in Resource-Limited East African Countries
M. Chambers, J. Ahluwalia, C. Williams; USAMRIID, Ft. Detrick, MD.

285 (K) A Comprehensive Method of Risk Analysis for Civil Protection: The German Infectious Disease Example
C. Uhlenhaut; Robert Koch-Instit., Berlin, Germany.
286 (K) Physiologically Relevant In Vitro Models to Study Infectious Diseases
T. M. Straub, J. R. Hutchison, H. Kreuzer; Pacific Northwest Natl. Lab., Richland, WA.

Highlighted Oral Abstract Presentations

022 Therapeutics and Immune Response
Wednesday, February 27, 2013 | 5:00 PM - 6:15 PM
Lincoln 5
Moderator:
Andrew Hayhurst; Texas Biomedical Res. Inst., San Antonio, TX.

Presentations:

5:00PM
287 Premature Activation of the Arenavirus Fusion Protein by a Glycoprotein-Derived Antiviral Peptide
J. S. Spence, R. F. Garry; Tulane Univ. Sch. of Med., New Orleans, LA.

5:15PM
288 Identification of Potent Anti-VEEV Compounds Through a Cell-Based HTS and Hit Optimization Study
D. Chung1, N. A. Tower2, J. B. Sotsky1, E. Sahin1, E. White1, J. W. Noah2, S. N. McKel- lipp1, M. Sosa1, C. B. Jonsson1, Y-K. Chu1, C. Schroeder1, J. E. Golden1, C. Schmaljohn4; 1Univ. of Louisville, Louisville, KY, 2Southern Res. Inst., Birmingham, AL, 3Univ. of Kansas, Lawrence, KS, 4USAMRIID, Ft. Detrick, MD.

5:30PM
289 Oral Aminoglycosides with Cell-Targeted Delivery for the Treatment of F. tularensis

5:45PM
290 Post-Exposure Therapeutic Efficacy of COX-2 Inhibition against Pneumonic Melioidosis

6:00PM
291 Two-Photon Imaging of Pulmonary Anthrax Infection Reveals Novel Immunological Synapses
J-N. Tournier1, P. Deman1, J. Douady2, D. Fiole1; 1Inst. de Recherche Biomédicale des Armées, Grenoble, France, 2Lab. Interdisciplinaire de Physique, UMR 5588 CNRS/Univ. Joseph Fourier, Grenoble, France.

Highlighted Oral Abstract Presentations

023 Vaccines
Wednesday, February 27, 2013 | 5:00 PM - 6:15 PM
Lincoln 2-4
Moderator:
Miles Carroll; Hlhs. Protection Agency, Salisbury, United Kingdom.

Presentations:

5:00PM
292 Chikungunya Virus Host-Range E2-Transmembrane Deletion Mutants Induce Protective Immunity against Challenge in C57BL/6J Mice
C. M. Briggs1, A. Piper1, M. Ribeiro2, K. M. Smith1, E. Huitt1, K. Nanda1, C. J. Spears1, M. Ques1, J. Cullen1, M. E. Thomas1, D. T. Brown2, R. Hernandez1; *Abxovax, Inc., Raleigh, NC, 1North Carolina State Univ., Raleigh, NC.

5:15PM
293 Development of Second Generation Inactivated Alphavirus Vaccine
P. Gupta1, A. Sharma1, S. Honnold1, M. Gayen1, E. K. Gaidamakova1, Y. Raviv2, M. Viard2, K. B. Spurgers2, R. M. Blumenthal1, P. J. Glass1, M. J. Daly1, R. K. Maheshwari1; 1USUHS, Bethesda, MD, 2Birla Inst. of Technology and Sci., Pilani, India, 3USAMRIID, Ft. Detrick, MD, 4NCI, NIH, Frederick, MD.

5:30PM
294 Host Cell Response to a Recombinant Modified Vaccinia Virus Ankara-Based Vaccine
A. Okoli1,2, M. Okeke1,2, U. Moens1, Ø. Nilsen1, M. Tryland1, J. Angsar-Bruun1, C. Tummler1, T. Behni2, T. Trazvick1; 1Genøk-Ctr. for Biosafety, Tromsø, Norway, 2Univ. of Tromsø, Norway.

5:45PM
295 Immunogenicity Assessment of In Silico-Selected T-Cell Epitopes for a Burkholderia Biodefense Vaccine
R. Liu1, J. Desrosiers1, B. A. Martin1, L. Moise1,2, A. D. Groot1,2; 1Inst. for Immunology and Informatics, Univ. of Rhode Island, Providence, RI, 2EpiVax, Inc., Providence, RI.

6:00PM
296 Host Factors Involved in Burkholderia Phagocytosis and Intracellular Survival
S. Micheva-Viteva1, K. L. Nowak-Lovato, Y. Shou, K. Ganguly, E. Hong-Geller; Los Alamos Natl. Lab., Los Alamos, NM.

Highlighted Oral Abstract Presentations

024 Diagnostics, Informatics and Genomics
Wednesday, February 27, 2013 | 5:00 PM - 6:15 PM
Lincoln 6
Moderator:
Soren Alexandersen; Natl. Ctr. for Animal Disease, Winnipeg, Canada.

Presentations:

5:00PM
297 Optimization of High-Throughput Sequencing Technology and its Application in Clinical Virology Diagnosis
N. Zhi1, B. Xu1, G. Hu2, K. Zhao2, N. S. Young1; 1Hematology Branch, NHLBI, NIH, Bethesda, MD, 2Systems Biol. Ctr., NHLBI, NIH, Bethesda, MD.

5:15PM
298 Rapid Pathogen Detection and Identification Using a Microbial Detection Array
5:30 PM

**299 Mobilome Analysis of *Yersinia pestis* Using Comparative Genomics**

S. Y. Choi¹,², N. A. Hasan¹,², J. Chun¹, A. Huq¹, T. A. Cebula¹,², R. R. Colwell¹,²,³; ¹Maryland Pathogen Res. Inst., Univ. of Maryland, College Park, MD, ²CosmosID Inc., College Park, MD, ³Sch. of Biol. Sci. and Inst. of Microbiol., Seoul Natl. Univ., Seoul, Korea, Republic of, ⁴Johns Hopkins Univ., Baltimore, MD, ⁵Inst. for Advanced Computer Studies, Univ. of Maryland, College Park, MD, ⁶Johns Hopkins Bloomberg Sch. of Publ. Hlth., Baltimore, MD.

5:45 PM

**300 Haplotype Analysis Advances Bacterial Rare Variant Detection in Next-Generation Sequencing**

R. E. Colman¹, J. Schupp¹, D. Smith¹, J. Gillece¹, A. Rawat¹, J. Usher¹, D. M. Engelthaler¹, J. Foster¹, P. Keim¹,²; ¹Translational Genomics Res. Inst., Flagstaff, AZ, ²Ctr. for Microbial Genetics & Genomics, Northern Arizona Univ., Flagstaff, AZ.

6:00 PM

**301 Viral Metagenomic Sequencing of Non-Malarial Febrile Illness in West Africa**

M. Stremlau¹, K. G. Andersen¹, S. Gire¹, C. Matranga¹, R. Taryial¹, O. Ikpanwomsa¹, P. Ehlaghe¹, W. Omoniwa¹, C. Happi¹, R. Garry¹, P. Sabeti¹; ¹The Broad Inst., Cambridge, MA, ²Harvard Univ., Cambridge, MA, ³Irrua Specialist Teaching Hosp., Irrua, Nigeria, ⁴Univ. of Ibadan, Nigeria, ⁵Univ. of New Orleans, LA.
Diarrheal diseases are a major problem in developing countries. Though precise data on childhood mortality associated with diarrheal diseases in Nepal is not available, it has been estimated that approximately 25% of child deaths are associated with diarrheal disease, particularly acute diarrhea. The purpose of this study was to assess the incidence of rotavirus causing acute diarrhea in children under 5 years of age. A total of 525 children with acute diarrhea in a children’s hospital of Kathmandu, Nepal were enrolled between April 2011 to September 2011. The incidence of acute diarrhea due to rotavirus was 25.9% (136/525) by ELISA. The percentage of rotavirus infected males were higher (64.5%) than females (35.5%). The frequency of rotavirus cases were higher in the children below 2 years of age among which the highest case 80.2% were found in age between 6-24 months (P<0.01). The genotypic characterization by reverse-transcriptase polymerase chain reaction(RT-PCR) revealed that the serotype G12 represented 55.9% in this study associated with P-types either P[6], P[8] or P[8]. Further a total of eight G/P combination were identified, with G12P[6] 46.4% being the most common strain type of rotavirus in Nepal. The aim of this study was to find out the major genotypes of rotavirus causing acute diarrhea in children.

046 (A) Withdrawn

047 (A) Withdrawn

048 (A) Withdrawn

049 (A) Assessment of Human Health Risks from Adenoviruses, Hepatitis A Virus, Rotaviruses and Enteroviruses in the Buffalo River and Three Source Water Dams in the Eastern Cape Province, South Africa

V. N. Chigor,1,2 A. I. Okoh;1 Univ. of Nigeria,Nsukka,Nigeria,1 Univ. of Fort Hare, Alice, South Africa

Buffalo River is an important water resource in the Eastern Cape Province of South Africa. We assessed the potential risks of infection constituted by exposure to human enteric viruses in the Buffalo River and three source water dams along its course, using mean values and static quantitative microbial risk assessment (QMRA). Hepatitis A virus (HAV), adenoviruses (Adv), rotaviruses (RoV) and enteroviruses (EnV) were detected using real-time qPCR/qRT-PCR in 43.1%, 34.7%, 13.9% and 9.7% respectively of a total of 72 water samples tested. Two or more viruses were detected in 22.2 % of the samples. The daily risks of infection determined by the exponential model (for Adv and EnV) and the beta-Poisson model, for HAV and RoV, varied with sites and exposure scenario and ranged from 7.31x10^-1 (for Adv), 4.23x10^-2-6.54x10^-1 (RoV), 2.32x10^-1-1.73x10^-1 (HAV) and 1.32x10^-5-5.70x10^-7 (EnV). The yearly risks of infection in individuals exposed to the river/dam water via drinking, recreational, domestic or irrigational activities were unacceptably high, exceeding the acceptable risk of 0.01% (10^-4 infection/person/year) recommended by the US EPA for drinking water. The risks of illness and of death from infection ranged from 6.58x10^-3-5.0x10^-1 and 6.58x10^-7-5.0x10^-3 respectively. Therefore, we conclude that the Buffalo River and its source water dams are a public health hazard. The QMRA presented here is the first of its kind in the Eastern Cape Province and provides the building block for a quantitatively oriented local guideline for water quality management in the Province.

050 (A) Classical Swine Fever Virus and Pasteurella Multocida Interaction Can Be Augmented by Magnetic Fields

B. Andiry, IV, IECVM, Kharkiv, Ukraine.

Background: Classical swine fever (CSF) often manifests as a mixed-infection. Current research has suggested that magnetic field(s) may be a factor, which affects bacterial and viral pathogenicity. To better understand any potential influences of magnetic fields on pathogenesis, we have employed the use of a “Grabbina device” (GD), which creates asymmetric rolling magnetic fields generation for biological use. Objective: To study the potential change in physical interactions between CSFV and Pasteurella multocida following exposure to right (RM-) and left (LM-) rolling magnetic fields. Methods: We used GD to generate asymmetric magnetic fields, and subsequently exposed virus-bacteria mixes (V=5,0cm³; [Un-supported Character - Symbol Font & #61553;]=0,2 mT) which consisted from 4,5 IgFU of CSFV ("IECV/UM-03" strain, CSFV) and the 5x10⁴ BC of Pasteurella multocida "7" strain on buffered saline (BS). Mixes were exposed by RM- (n=3) and LM- (n=3) rolling fields during 25 min. Control mixes (CM-samples, n=3) were exposed on switch-off device. After exposures all mixes settlements were rinsed and RM-samples divided on 3 part - 1st parts controlled immediately altogether with LM- and CM-samples. 2nd & 3rd of RM-samples parts controlled after exposition under 40⁰C in 3 and 5 days. The virus quantity in all settlements was titrated in PK-15 cell with standard procedure of indirect PLA colored-calculation. Results: CSFV was revealed only in bacterial settlements of all RM-samples. The virus titers in these samples were 2,52±0,9 IgFU after RM-application (n=3, P<0,01). On 3rd and 5th day after RM-bacterial settlements exposition under 40⁰C CSFV titers were 1,1±0,8 IgFU (n=3). A free CSFV was inactivated at 2nd day of 40⁰C exposition completely (n=3, P<0,01). Conclusion: RM-rolling field ([Un-supported Character - Symbol Font & #61553;]=0,2 mT) promote the CSFV adsorption on Pasteurella cells. Adsorbed virus rise of resistance to 40⁰C significantly. This data explain the events which may be to come during natural CSF outbreaks.

051 (A) Rift Valley Fever Virus Nose-Only Aerosol Characterization

R. C. Layton, R. Tuttle, MRIGlobal, Kansas City, MO.

The objective of the study was to characterize the aerosol delivery characteristics of a nose-only exposure system for the delivery of Rift Valley Fever Virus (RVFV) in animal models. This system will be used to expose Wistar-Furth rats to determine the phenotypic effects of multi-passage, laboratory culture, on RVFV. RVFV (genus Phlebovirus, family Bunyaviridae) is a spherical, enveloped, RNA virus which is 90 to 110 nm in diameter with a single-stranded tripartite RNA genome. RVFV is a "Category A" agent due to its lethality, stability in the environment, ease of preparation, and importantly, transmissibility through inhalation of aerosolized virus. RVFV ZHS01 produced on Vero cells for system characterization was aerosolized using a Collison 3-jet nebulizer. Aerosol collection was performed using three low flow and low pressure midget impingers in series. The plaque assay using low passage Vero cells was used to determine the viral concentrations of the nebulizer solutions both before and after aerosolization, and from each of the three impingers. Aerosol characterization demonstrated increased aerosol delivery efficiency from 0.7 % to 2.0% with increased serum concentrations. An average of 38%, 57% and 5% of total virus was collected from the first, second, and third tandem impingers (respectively). These data show that RVFV can be effectively and reproducibly aerosolized at high concentrations within a controlled environment using this system. Other systems use whole body exposure, large displacement volume, guidance formulas, and a single impinger with high sampling pressures and velocity. These factors may substantially underestimate the exposure concentration, and thus the actual challenge dose. Therefore, the use of a nose-only system and tandem impingers with the addition of real-time plethysmography control of exposure provides a more accurate estimate of the challenge dose.
052 (A) Fatal Encephalitis in African Green Monkeys and Common Marmosets after Aerosol Infection with Rift Valley Fever Virus


Background: Rift Valley Fever virus (RVFV) outbreaks occur in eastern Africa and the Middle East, with epidemics of severe disease in livestock and human populations. In humans, RVFV causes a self-limiting febrile illness, but a small percentage of people develop severe complications including hepatitis, retinitis, hemorrhagic fever, and encephalitis. Although predominantly a mosquito-borne disease, RVFV is infectious by other routes including inhalation. Because RVFV is infectious by the respiratory route, it is considered a potential bioterror agent. Animal models are needed to evaluate potential medical countermeasures. Methods: African green monkeys (AGM) and common marmosets were exposed to small particle aerosols containing RVFV. Radiotelemetry was used to monitor and record changes in body temperature. An Auto-Regressive Integrated Moving Average (ARIMA) model was used to quantify fever responses to infection. Tissue samples were taken at necropsy for pathological and virological analyses. Results: Two marmosets exposed to aerosolized RVFV developed a biphasic febrile response followed by clinical signs of neurological disease (drooling, unsteady gait, seizures) and were euthanized. Virological and pathological findings confirmed viral encephalitis. Four AGM exposed to aerosolized RVFV developed a fever after exposure. Three of the four developed neurological signs indicative of viral encephalitis and were euthanized. Virological and pathological findings confirmed viral encephalitis. Gross pathological findings in the AGM included vasculitis in the brain and hemorrhaging in the lungs. Conclusions: Both AGM and marmosets appear to be good models of the encephalitis caused by RVFV. The additional clinical findings in AGM (vasculitis, hemorrhaging in the lungs) suggest that AGM might also be a good model of other aspects of RVFV disease.

053 (A) Epizootic Monitoring of Swine Epizootic Fever

S. Nychyk, M. Sutyuk; IVM, Kyiv, Ukraine.

In swine infectious pathology the most dangerous disease in epizootic aspect is African Swine Fever. ASF was first registered on the African continent by Hutchens and Stockman in 1903, and in 1921 was officially studied and described by English explorer R.E.Montgomery. In 1957, ASF had spread to the European continent to Portugal; in 1960 - Spain; 1964 - France; 1967 - Madeira, Italy, 1975 - Andorra, 1978 - 1984 - Sar dinia, 1978 - Malta, 1985 - Belgium 1986 - The Netherlands and in North America (Cuba 1971, 1980, Haiti 1978-1981) and South America (Brazil 1973-1978). African Republic 1979-1980). Currently ASF disadvantaged are 18 countries in the African continent, some areas in Caucasus region, Russia, Sardinia and Ukraine. Goal: To analyze retrospectively ASF epizootological manifestation in disadvantaged countries within 10-year period (2000-2010), with the definition of morbidity, mortality and lethality of pigs from ASF. Depict graphically the ASF manifestations dynamics in Africa, Asia and regions of the Russian Federation. Describe the specific ASF manifestation in Ukraine. Materials and Methodology: In our researches we used and analyzed information of Organization International d’Epizootologie (OIE) concerning numbers of ASF outbreaks, infected animals, dead, killed and slaughtered animals in foci within a period of 1997-2012. We used intensive methods of epizootic study - definition of morbidity, mortality, lethality. Research Results: ASF historical information was analyzed, characterized and submitted. It was determined that ASF morbidity ranged from 6.6 to 22.4%, mortality - 6.1 to 19.8%, lethality - 88.4 to 93.5%. We presented graphical data concerning ASF retrospective manifestations in the territory of different nozoea areas with manifestation tendencies. The processed results have been reflected in diagrams containing elements of retrospective comparison of epizootic process in different ASF nozoea areas.Conclusion: Historical and contemporary analysis of ASF epizootiology since 1903 indicates unpredictability of nozoea expansion of the disease to new regions with non-specific climatic and biocenotical conditions and too remote from endemic areas of “parental” African continent.

054 (A) Phylogenetic Analysis of Virus Isolates of PRRS

N. Gavrasieva, SSSCBMS, Kyiv, Ukraine.

Background: Reproductive and respiratory syndrome swine (PRRS) - one of the very common contagious diseases in the world, is characterized by abortion in sows, stillborn piglets, premature farrowing, respiratory disorders, and appearance of bluish adornment on ears and other organs. Objective: The purpose of the work was to determine the genetic relationship between field samples of PRRS virus isolated from pathological material collected at farms in the Kyiv region and the genotypes of European and American origins. Method: Phylogenetic analysis and dendrogram plotting characterizing the degree of homology of the studied genome areas was performed using the “Mega” software ver. 3.1. With PCR and nucleotide sequencing we determined the primary structure of ORF7 nucleocapside gene in samples that are specific for the PRRS virus. Results: Study of multiple alignment of partial sequences of PRRS viruses of European genotype showed the presence of three major groups of sequences corresponding to subtype 1.3. Dendogram analyses (Neighbors Joining) suggested probable (over 60%) difference of nucleotide sequences of the PRRS virus of European genotype versus isolate North. PRRS isolates, collected in the Kiev region, belonged to subtype 2 of European genotype and had a divergence from 2% to 12% within the group. The most phylogenetically close to them (92-94% homology) were isolates from Moldova and the Republic of Tatarstan, to a less extent (88-90% homology) - isolates from Kemerovo and the Nizhny Novgorod region, which correspond to topographical subtypes 2 of European genotype and are identical RRSS pathogens circulating in Central and Eastern Europe and Asia. Data suggests that PRRS viral isolates collected in Ukraine have a genetic relationship with the European genotype and subtype with nucleotide composition similar to Russian isolates. Conclusion: The result of phylogenetic analysis established that the epizootic RRSS virus isolates that circulate in the economy of Ukraine have expressed genetic homology (92-94%) with the reference strain Lelystadt that is a prototype of European genotype of RRSS pathogen and PRRS virus isolates with Russian origin.

055 (A) Oxidative Stress-Induced Responses in RVFV-Infected Liver Cells

A. Narayanan, M. Amaya, M. Chung, K. Kehn-Hall, S. Senina, C. Carpenter, C. Bailey, R. Hakami, F. Kashanchi; George Mason Univ., Manassas, VA.

Rift Valley fever virus (RVFV) is an arbovirus belonging to the genus Phlebovirus, family Bunyaviridae. RVFV infection often results in pronounced damage to the liver. Previously, our studies had revealed that RVFV infection induces the up-regulation of pro-inflammatory cytokines and pro-apoptotic gene expression at early time points post infection. The activation of these transcription factors leads to alterations in host-phospho-signal signaling responses including NFkB, MAPK and DNA damage responses that correlate with the host response to cellular stress. In our more recent studies, we have observed that RVFV infection of liver cells leads to a strong increase in reactive oxygen species (ROS) in infected cells by measuring free superoxide in infected cells. Increased ROS levels correlated with activation of NFkB (p65) responses at early time points and p53 responses at late time points after infection. The activation of these transcription factors in conjunction with infection was also reflected as macromolecular rearrangements observed using size fractionation of protein lysates from infected cells. As a consequence of the activation of these transcription factors, we documented an increase in cytokine expression and pro-apoptotic gene expression at early and late time points post infection respectively. When we treated infected cells with antioxidants, we observed a reversal of the cytokine and apoptotic gene expression profiles in addition to inhibition of viral multiplication in both in vitro and in vivo systems. These observations therefore identify ROS and oxidative stress as critical contributors to liver pathology during RVFV infection.
West Nile virus (WNV) evidence of renal persistence in animals has been well described, but the long-term pathology of persistent WNV infection in human kidneys is poorly understood. We previously reported an association between WNV infection and chronic kidney disease. Our previous observational study of only WNV participants, found a prevalence of CKD in 40%. Traditional risk factors, such as diabetes, age, or hypertension, were not found to be statistically associated with having CKD. In our current study, we aimed to further distinguish CKD clinical characteristics in those with and without history of WNV infection. WNV negative controls (n=83) were matched to WNV positive cases (n=40) in a 2:1 ratio by age, race, gender, and CKD estimated glomerular filtration rate (eGFR) significantly different. We performed retrospective chart abstractions on WNV negative controls and used previously collected data on WNV positives. We compared the two groups for longitudinal progression of CKD, presence of CKD risk factors, and clinical characteristics of CKD. Odds ratios were calculated to test associations of these variables between the case and control groups. We found prevalence of diabetes and hypertension were significantly lower in the WNV positive case group, and incidence of proteinuria and hematuria were significantly higher compared to the WNV negative controls. eGFR decline in WNV positive cases proceeded at a rate of -10.77 ml/min/1.73 m²/year versus -5.04 ml/min/1.73 m²/year for WNV negative controls. eGFR decline was significantly quicker in the WNV positive group than in the WNV negative group (p<0.03). In light of our findings, we cannot rule out the influence of WNV with declining renal functionality. Physicians should monitor the kidney health of patients with a history of WNV infection.

**057 (A) Spatial and Temporal Patterns of West Nile Incidence, 2003-2010**


Background: West Nile entered New York City in 1999. Despite massive containment efforts, it reached Colorado by 2002. A minority of infections result in severe long-term neurological illness or death. Since no effective drug treatment exists for severe disease, vector elimination is essential. Understanding disease distribution patterns may aid in direction of such preventative programs. This study examines incidence in western U. S. in 2003-2010 in order to determine spatial or temporal disease patterns.

Methods: West Nile incidence data were obtained from the CDC West Nile Virus site for 2003-2010 and plotted by year at county, state, or regional levels. County-level G-statistics were used to calculate and map disease hot spots. Population data was obtained from the U. S. Census Bureau.

Data: Location, size, and number of hot spots differed yearly and may or may not encompass major population centers. Hot spots were found around Los Angeles, San Francisco, Tucson, Phoenix, Denver, El Paso, and Dallas. Hot spots in less populated areas included northern Kansas; southern/ western Nebraska; SE/central Wyoming; western Colorado; NW Utah; southern/SW Idaho, SE/eastern/western Oregon, NE Nevada, North Dakota, western Minnesota, and eastern Washington. One hot spot was detected in 2003, 3 in 2004, 2 in 2005, 3 in 2006, 6 in 2007, 3 in 2008, 7 in 2009, and 4 in 2010. When divided into 4 regions, the SW had 9701 cases from 2002-2010 (peak incidence in 2003, 2006); NE - 6701 cases (peak in 2003); SE - 4488 cases (peak in 2003); and NW - 2584 cases (peaks in 2003, 2006). Peaks did not always occur in the same year, even in contiguous states. Summary: Spatial and temporal analysis of West Nile incidence indicated great yearly variation. Hot spots frequently occurred outside of population centers in regions with greatly differing climatic and geographical features. Conclusions: Lack of clear-cut spatial and temporal patterns of West Nile incidence complicates efforts to predict future outbreaks, however further analysis may reveal differing patterns within set climatic or geographical parameters.
**060 (A) Avian Influenza Disease in Georgia Republic**

N. Vepkhvadze, M. Donduashvili, M. Kokhareidze, T. Onashvili; Lab. for the Ministry of Agriculture, Tbilisi, Georgia.

Avian Influenza (AI) is a worldwide problem of domestic and wild birds. It is contagious and characterized as an often-fatal infectious disease affecting all species of domestic and wild birds, which infects serious economical damage on the Agriculture including Poultry. Most Avian Influenza (AI) viruses are of low pathogenicity and lead to sub-clinical infections in aquatic birds. However, these low pathogenic avian influenza (LPAI) viruses can evolve into highly pathogenic avian influenza (HPAI) viruses, (such as H5N1, N1H1 viruses causing high mortality in chickens, and other Galliformes); therefore it is very important to conduct monitoring in wild birds. Avian Influenza (AI) viruses are classified as Especially Dangerous Pathogens. Diagnostic assays for influenza are based on real-time reverse transcriptase polymerase chain reaction (rRT-PCR). Avian Influenza virus matrix RNA strains can be tested with these methods. Matrix testing is used for sample screening in case of positive samples require additional testing for H5 and H7 strains. In 2011, the Laboratory of the Ministry of Agriculture (LMA) screened 1,000 wild bird samples for AI virus. The AI virus RNA was extracted from AI suspected samples (tissues, tracheal and cloacal swabs) by using RNA extraction Kits. Samples were tested using rRT-PCR using three unique primer/probe combinations (matrix gene, H5 and H7 genes). Most of the samples were negative for AI viruses and only 13 were positive for AI. Further testing of the Avian Influenza matrix RNA positive samples confirmed that these viruses belong to the group of Avian Influenza virus low pathogenic strains. Low pathogenic AI strains are circulating throughout Georgia. Continued surveillance of AI in Georgia is warranted and is part of the Threat Agent Detection and Response Network. Testing results show that low pathogenic AI in wild birds circulates in Georgia. Accordingly, permanent monitoring should be carried out in wild birds as well as in domestic ones. The Laboratory surveillance of the above mentioned disease will allow the country to carry out complex measures for controlling AI.

**061 (A) The Critical Role of the IKK-Complex in VEEV Infection**

A. Narayanan, M. Amaya, K. Voss, S. Senina, C. Carpenter, K. Kehn-Hall, C. Bailey, F. Kashanchi; George Mason Univ., Manassas, VA.

Venezuelan equine encephalitis virus (VEEV) belongs to the genus Alphavirus, family Togaviridae. VEEV infection can cause a fatal neurological disease in humans with no significant therapeutic intervention strategies currently available. Our studies to determine the interactions between VEEV and its human host have revealed that the host IKK (I Kappa KInase) complex may play a critical role in VEEV multiplication. Our analysis commenced with the identification of VEEV infection resulted in phosphorylation of IkBa and p65 proteins at early time points which was not observed during infection by a UV-inactivated virus. We also observed nuclear translocation of p65 which is a prelude to activation of host transcription. The upstream event that contributes to phosphorylation and nuclear translocation of p65 is the activation of the IKK complex. Our current studies reveal that inhibition of the IKK complex results in a decrease in viral multiplication in infected cells as measured by plaque assays. Time of addition experiments indicated that inhibition of the IKK complex at 6 hours post initial exposure continues to be effective at inhibiting viral multiplication. Correlating with inhibition of viral replication, we observed decreased capsid expression and caspase cleavage in infected cells thus suggestive of decreased apoptosis. Animal experiments also reveal that mice treated with IKK inhibitors exhibit increased survival in the face of VEEV infection. Thus, our studies so far have revealed that the host IKK complex may be critically involved in VEEV multiplication. Ongoing studies are aimed at determining using proteomic analyses how the IKK complex is altered during VEEV infection.
063 (B) Novel RNAIII-Inhibiting Peptide Derivatives Increase Survival in MRSA Sepsis by Inhibiting Nlrp3 Inflammasome Activation
Z. Hou, F. Da, X. Xue, X. Luo; Fourth Military Med. Univ., Xi’an, China.

Previous researches have shown that RNAIII-inhibiting peptide (RIP) could effectively inhibit the quorum sensing (QS) system in staphylococci. However, it remains ill-understood why RIP can markedly decrease the bacterial load in vivo while not influencing the number of bacteria in vitro. Here, both inflammasome formation and the release of mature IL-1β were assessed after treatment with RIP derivatives in a murine sepsis model. Secretions of hemolysin, survival rates, bacterial titers and histological changes were determined. We found the secretion of hemolysin and the mRNA expression of RNAIII, Nlrp3 and ASC were markedly suppressed by RIPs in vivo. Moreover, caspase-1 and IL-1β protein level were markedly reduced. More importantly, RIP1183 produced the greatest improvement in mice survival and inhibition of pathological damage. In addition, no antibacterial activities were displayed by any of the RIPs in vitro. These findings firstly demonstrate that RIPs could increase the survival rate of MRSA-infected mice by reducing the secretion of bacterial virulence factors and inhibiting Nlrp3 inflammasome activation, and indicate that RIPs may be a useful anti-infective agent, especially against multidrug-resistant Gram-positive bacteria.
064 (B) Importance of Brucella Global Sero-Epidemiology Study to Check Recent Upsurge of Psychiatric Diseases


Brucellosis is a common disease throughout the world particularly in developing countries. One important manifestation of Brucellosis is psychiatric and there are at least one important documentation showing ~80% patients admitted in a psychiatric hospital cured after Brucellosis treatment. At present there are about 90 million patients suffering from psychiatric diseases and in 2025 this number will be more than cardiac diseases. In most of the countries doctors are not aware about this peculiar manifestation of Brucellosis and thousands of persons who are really suffering from Brucellosis are diagnosed and treated as conventional psychiatric diseases and many such patients die without getting proper treatment.

In this study we have analyzed retrospectively Brucellosis cases which were detected between 2010 to 2012 in a tertiary care hospital in Eastern India where the burden of this disease is largely unknown. Records of 257 patients diagnosed as brucellosis over a period of above-mentioned 3 years are analyzed and this revealed that 126 (49.02%) patients were associated with psychiatric problems and along with other manifestations and specific medicines were also used to treat such psychiatric symptoms.

One such patient after getting psychiatric treatment for several months was admitted in ITU and became normal after getting only two days Brucellosis treatment. The analysis also showed that the number of patients with brucellosis increased over the years.

Thus in conclusion we may say that Brucella global sero-epidemiology study is an essential step to find out real burden of Brucellosis and preventive measures in selected areas will definitely check the recent upsurge of psychiatric diseases.

065 (B) Development of a Yersinia pestis A1122 Knock-Out Library through Conjugative Transposon Mutagenesis

D. R. Pawlowski1,2, A. Howlett1; 1CUBRC, Buffalo, NY, 2State Univ. of New York at Buffalo, Buffalo, NY.

Yersinia pestis, the etiological agent of the plague, is a Tier 1 Select Agent with globally distributed endemic foci. In the last decade, research has revealed a large amount of information regarding the plague bacterium's genomics, evolution and pathology however there is still much to learn.

Two areas where our understanding lags are with regards to the plague bacterium's ecology and growth requirements. We've recently published a report describing an antagonistic interaction between Y. pestis and the ubiquitous, freshwater bacterium, Hylemonella gracilis [1]. In order to fully delineate and understand the mechanism behind this interaction we've constructed a library consisting of 8,448 unique, knock-out mutants of the avirulent Y. pestis A1122 strain.

This library was constructed through conjugation with a F+ E. coli strain harboring the pFD1 suicide vector and provides approximately 2x coverage over the 4,333 ORF genome of the A1122 strain. We will utilize this library to identify genes in Y. pestis that are responsible for mediating H. gracilis' antagonistic activity. Importantly, this library may become an important tool to confirminformatics-based hypotheses and for other high throughput studies including growth and nutrient utilization studies.

066 (B) A System Causing Maintenance of Bacillus anthracis Plasmid pX01

A. P. Pomerantsev, C. Rappole, Z. Chang, S. H. Leppa; NIH/NIAID, Bethesda, MD.

A Bacillus anthracis pX01 minireplicon plasmid (pMR) containing ORFs GBAA_pX01_0020 to GBAA_pX01_0023 is not stably maintained in B. anthracis, whereas the full-size parent pX01 plasmid is extremely stable under the same growth conditions. Two genetic tools developed for DNA manipulation in B. anthracis (Cre/loxP and Rip/FRT systems) were used to identify regions of pX01 that are required for plasmid stability during B. anthracis vegetative growth. Genetic analyses of these regions allowed identification of three non-contiguous genes that constitute a plasmid maintenance system (MS): amsP, minDP, and sojP. Analysis of conserved domains in the corresponding proteins indicated that AmsP (activator of maintenance system from pX01) is a DNA-binding protein having a strong helix-turn-helix (H-T-H) motif. Two conserved domains were found in the MinDP protein (MinD from pX01): the N-terminal part of the protein is similar to the B. anthracis septum-site-determining protein MinD, while the C-terminal part contains a single strand DNA-binding domain similar to a baculovirus single strand DNA-binding protein. The SojP protein (Soj from pX01) contains putative Walker A/B motifs and belongs to the Soj family: ATPases involved in chromosome and plasmids partitioning. Assembly of the three genes of the MS with pMR produced a stably maintained recombinant plasmid pMS12 (15 Kbp). This plasmid, containing a loxP-site, could be used as a vector for the creation of a multivalent vaccine, providing protection from bacterial and viral infectious diseases.
069 (B) Combined SNP and MLVA Subtyping of *Bacillus anthracis* Strains from Georgia


*Bacillus anthracis* causes an acute fatal disease and is a Class A select agent. Anthrax is distributed worldwide and endemic in Georgia, a small Eurasian country of geopolitical significance, where human and animal cases are reported annually and morbidity is on the rise. Very little was known about the genetic diversity of *B. anthracis* in Georgia, though the genus is known to have a low level of genetic variability. To address this we used two tools, Single Nucleotide Polymorphisms (SNP) and a 15-marker multiple-locus variable-number tandem repeat analysis (MLVA 15), to subtype Georgian *B. anthracis* isolates and place them into a global phylogeographic context. We discovered two populations of *B. anthracis* that belong to different genetic groups defined by canonical SNPs. Novel SNPs were discovered and converted into assays, which were then used to screen Georgian isolates to provide further genetic differentiation. We identified a new sub-lineage that contains eight new genetic subgroups, which are spread across east and west parts of country. In this study, we present this novel molecular typing system incorporating more rapidly evolving variable-number tandem repeat (VNTR) loci. Georgian isolates were screened with the MLVA 15 system to determine the level of genetic diversity within each identified SNP subclade, at NAU and at NCDC using ABI and Beckman Coulter systems respectively. VNTR patterns showed little genetic diversity among Georgian isolates, with a few highly variable markers and all others constant across the all isolates. One of the phylogenetic basal SNP subclades contained a majority of the MLVA genotypes seen. The most important application of this research is to develop a system for Georgia to track sources of infection in outbreaks or in case of bioterrorism.

070 (B) Molecular Typing of Human and Animal Brucella Isolates from Georgia


Brucellosis, an ancient worldwide zoonosis and significant economic and public health problem in Georgia, is caused by Brucella, a genus with Category B agents because of their high infectivity in mammals. Biotyping Brucella by microbiological methods alone has limitations, so molecular typing was implemented in this study to confirm species. Isolates from human blood and ruminant milk or blood were identified by a bacteriological algorithm and confirmed by real-time PCR (Brucella T1, Idaho Tech). 86 strains (48 human, 38 animal isolates) obtained 2009-2011 were confirmed as Brucella by real-time PCR. AMOS PCR supported biochemical test results for 53 Brucella melitensis and four Brucella abortus strains, but not for 29 suspected *B. abortus* human and animal isolates. SNP typing of all 86 isolates supported the AMOS PCR results but also confirmed the species of the 29 strains not amplified by AMOS PCR. Combined AMOS PCR and SNP typing in this study provided the first genetic confirmation that both *B. abortus* and *B. melitensis* are actively circulating in humans and animals in Georgia. Since these SNP assays successfully determined species of Georgian Brucella isolates, this approach should be incorporated for this purpose in future surveillance.

071 (B) Emerging Fluoroquinolone Resistance among Typhoidal Salmonella Serovars in Nepal


We have been monitoring the changing antibiotic resistance pattern among blood isolates and waterborne isolates of *Salmonella enterica* serovar Typhi and S. Paratyphi A. During November 2009 to March 2012 we isolated *Salmonella enterica* serovar Typhi and S. Paratyphi A, from blood samples of clinically diagnosed enteric fever patients with an aim to evaluate antibiotic susceptibility pattern of the isolates. Isolates were identified by standard microbiological procedures including serotyping. Antibiotic susceptibility testing was performed by disc diffusion method and minimum inhibitory concentration (MIC) to ciprofloxacin, ofloxacin and nalidixic acid was determined by agar dilution method following CLSI guidelines. A total of 400 isolates of *Salmonella* were obtained from 223 male & 177 female patients. In all isolates 256 were MDR. *Salmonella* Typhi were more prevalent than salmonella Paratyphi A. They were prevalent in all the seasons even in winter months. Most of the *Salmonella* isolates were Nalidixic acid resistant and Ciprofloxacin resistant. Since these antibiotics are first choice to treat Typhoid and other enteric infections in Nepal. The increasing multidrug resistance among *Salmonella* isolates specially resistance to fluoroquinolone, and cephalosporin in Nepal has created a serious public health concern.

072 (B) Mapping Outbreaks and Modeling Niches: Genetic Diversity, SNP Discovery and Ecological Modeling of *Bacillus anthracis* in Azerbaijan


Anthrax is an acute zoonosis of domestic and wild herbivores, with secondary human cases, often from spillover associated with slaughtering animals. The disease is caused by the spore-forming bacterium *Bacillus anthracis*, which has potential for persistence and reoccurrence in some soils, as well as long-distance transmission events. Though Azerbaijan has a long record of human and animal outbreaks, little data are available on the geography of anthrax or genetic relationships between local strains and global diversity. Villages reporting human and veterinary anthrax were each aggregated by decade to map hotspots of disease reporting from 1940-2000 using GIS. An exploratory kernel density estimation was used to map concentrations of disease. Thresholds of 75%, 90% and 95% were used to map areas of greatest anthrax concentration in each group by decade. A collection of strains was genotyped using the MLVA-25 and SNR-4 systems. All genotyped isolates were mapped in the GIS and used to construct an ecological niche model of the Azerbaijani *B. anthracis* sub-lineage. Azerbaijan had sustained hotspots in the south, central and northwest across the time period. These areas partially correspond to rayon-level zones of anthrax risk defined by the State Veterinary Service. Genotyping efforts assigned all available isolates into a single lineage, with MLVA-25 and SNR-4 identifying 3 genotypes. The niche model predictions broadly correspond to epidemiological hotspots. MLVA types were most closely related to neighboring Iran. Sequencing of the MLVA loci identified Azerbaijan specific SNPs, which may be useful for rapid identification of regional strains. These results identify surveillance priorities and add to our understanding the genetic relationships between Azerbaijan and other parts of the world.
**073 (B) Identification of Burkholderia pseudomallei Near-Neighbors in the Northern Territory of Australia**

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Identification and characterization of near-neighbor species is critical to development of robust molecular diagnostic tools for bioterror agents. One such agent, *Burkholderia pseudomallei*, a soil bacterium and the causative agent of melioidosis, is lacking in this area because of its genomic diversity and widespread geographic distribution. The *Burkholderia* genus contains over 60 species and occupies a large range of environments including soil, plants, rhizospheres, water, humans and animals. Recently, two non-pathogenic bacterial species, *B. ubonensis* and *B. thailandensis*, were found in Australia, an area of the world where it had never been documented before. This discovery demonstrates the need to identify the true global distribution of *Burkholderia* species, especially the members that are closely related to *B. pseudomallei*. In our current study we analyzed recently characterized species of *Burkholderia* to determine the effectiveness of a previously published Burkholderia-specific recA sequencing assay. We modified the primers to include new species like *B. oklahomensis*. We used this characterization technique to identify over 150 unknown soil isolates to the species level. Samples were isolated from the Darwin area in the northern Territory of Australia where melioidosis is endemic. *Burkholderia* recA PCR negative samples were further characterized using 16s rDNA sequencing. Phylogenetic analysis demonstrated that over 70% of the bacterial isolates were identified as *B. ubonensis* indicating that this species is prevalent in the soil where *B. pseudomallei* is endemic. Bayesian analysis reveals many novel branches within the *B. cepacia* complex as well as one novel *B. oklahomensis*-like species. This is the first account of a *B. oklahomensis*-like species in Australia. This study concludes that the recA sequencing technique is an effective tool to classify soil organisms in a melioidosis endemic area and will identify novel *Burkholderia* species.

**074 (B) Paramecium Consumes and Digests Francisella novicida with No Apparent Mortality**

W. E. Bell, C. V. Sayer; Virginia Military Inst., Lexington, VA.

*Francisella tularensis* is a virulent pathogenic bacterium that is the causative agent of the disease tularemia. Although it has been widely studied, little is known about its ecology. One hypothesis is that *Francisella* may have an association with fresh water habitats. The nature of the specific micro-habitat these bacteria may occupy is unknown. It has been reported that Protists, such as *Acanthamoeba* and *Tetrahymena*, are able to harbor pathogenic bacteria such as *Legionella*, *Salmonella* and *Francisella*. The purpose of this study was to test the ability of another ubiquitous fresh water Protist, *Paramecium tetraurelia* to be a natural reservoir for *Francisella*. We used a Green Fluorescent Protein transformed sub-species of *F. tularensis, Francisella novicida* (kind gift from the Klose lab) to track movement and viability of bacteria. We observed *Paramecium* readily ingest *novicida* and incorporate the bacteria into membrane-bound food vacuoles. Furthermore, we noted co-localization of lysosomes with vacuoles containing *Francisella* and an associated decrease in pH within these vacuoles. Attempts to recover *Francisella* from *Paramecium* post-ingestion were unsuccessful. This suggests that *Francisella* are fully digested, or at least killed in the *Paramecium* endocytic pathway. These observations indicate that *Paramecium* may not be a viable natural reservoir for *Francisella novicida*.

**075 (B) In Vitro Susceptibility Activity of Tigecycline against a Panel of Highly Pathogenic Bacteria**


**Introduction:** Tigecycline proved good activity against a wide range of bacteria including those which exhibit resistance to multiple antibiotics. **Objective:** Determine the in vitro activity of tigecycline against an established panel of 35 strains within the framework of an EU project (QUANDHIP) to improve testing procedures in highly pathogenic bacteria. **Material and Methods:** A panel comprising 5 *B. anthracis*, 5 *B. mallei*, 5 *B. pseudomallei*, 5 *Brucella* spp., 10 *Francisella* spp. and 5 *Y. pestis* strains was set up to measure the in vitro activity of 16 antibiotics using the microbroth microdilution testing plates. The 96 well plate was customized under the specifications set up at the project. Each plate contained a 2 - fold serial dilution of amoxicillin - clavulanic acid, cefazidime, chloramphenicol, ciprofloxacin, doxycycline, gentami-
077 (B) Insights into Anthrax Toxemia In Vivo: Relationship Between Lethal Factor and Lethal Toxin Complex
A. E. Boyer1, M. Gallegos-Candel1, R. C. Lins, A. R. Woolfitt1, C. P. Quinn, J. R. Barr1; 1CDC, Atlanta, GA, 1Battelle Analytical Services, Atlanta, GA.

Lethal factor (LF) toxemia is an important diagnostic target for systemic anthrax. LF has trisphic kinetics during inhalation anthrax (IA) in rheus macaques and is present in most animals by 18 hours post exposure. Protective antigen (PA) toxemia develops later. Lethal toxin (LTx) comprises PA which binds and transports LF into target cells. To evaluate the relationship between LTx and LF toxemia during systemic anthrax the LF and LTx were differentially purified and quantified in sequential serum samples from a nonhuman model of IA. The toxemia profiles were compared to clinical samples from human inhalation (IA) and injection (InjA) anthrax treated with anthrax immune globulin (AIG). Total LF was purified with anti-LF monoclonal antibodies (mAb) and quantified by synthetic peptide substrate cleavage using MALDI-TOF mass spectrometry. LTx (PA-LF complex) was quantified by differential purification of LTx with an anti-PA mAb followed by quantification of PA-associated LF activity. Samples analyzed included serum, plasma and pleural fluid in 3 IA case-patients and 13 InjA cases. All but one were treated with AIG in nonhuman primates during early infection the %LTx was less than 22% of the total LF; during the moribund-terminal stages this increased to 22-100%. Elevated LTx (>20% of the total LF) may identify late stage IA. In humans the %LTx in serum prior to any treatment was <10% for both IA and InjA. In pleural fluid, the %LTx, when available, was >10 times higher than in serum. AIG treatment decreased total toxin load but was associated with transient increases in the %LTx. In fatal IA, the %LTx in pleural fluid was 9.4% prior to AIG, increased to 18% at 22 h post-AIG, then declined but remained elevated at 9% at 48 h post-AIG treatment. In contrast, the %LTx in pleural fluid in a survivor declined from 11% pre-AIG to 2.6% at 48 h post-AIG. The kinetics of both LF and LTx were trisphic. Correlations of total LF and LTx revealed differences in slopes that may relate to stage of disease progression and responses to treatment.

078 (C) Comparison of Cryptococcal Antigenemia between Antiretroviral Naïve and Antiretroviral Experienced HIV Infected Patients at Two Hospitals in Ethiopia


Background: Cryptococcal meningitis is a major cause of HIV/AIDS-related death in Africa. It is a neglected killer in Sub-Saharan African countries. The purpose of the present study was to compare the prevalence of cryptococcal antigenemia among HIV infected antiretroviral naïve and experienced patients and compare with their CD4 cell counts to identify the target population for cryptococcal antigen early screening. Methods: Questionnaires were administered to 254 consenting HIV positive patients. The polysaccharide antigen was detected in the serum of HIV infected individuals by latex agglutination test and the positive results were further confirmed by culture, urease and India ink staining. Results: Out of 254, 127(50.0%) were ART naive and 121(47.6%) were ART experienced and 6(2.4%) were defaulters. The range of CD4 count was 2-884 cells/μL. Most of patients (79.5%) were between 16-45 years old; 139 (54.7%) patients were women; 160(63.0%) were patients from rural, and (61.4%) of them were married. The overall prevalence of cryptococcal antigenemia was 26(10.2%) and 18(14.3%) among ART naive, 5(4.3%) among ART experienced and 3(30.0%) among defaulters. The cryptococcal antigenemia positivity rates were similar in sex and marital status but, it was high (12.5%) in patients from urban. It was similar in different ages of age but, low (6.7%) in children. It is also varies with different ranges of CD4+ counts; ≤100, 101-200, 350 cells/μL, 13(22.0%), 10(14.7%), 23(18.1%), 3(5.8%) and 0(0.0%) respectively. Out of 26 CRAG+ 9 showed agglutination at titers ≥256≥1024. Out of 26 samples only 4(15.4%) were positive for yeast of cryptococcus. Conclusion: In the present study, we report a high CRAG+ prevalence (18.11%) in ART naïve and defaulters, particularly in those with CD4+counts ≤ 200 cells/μL. This group is the target populations for CRAG screening at entering ART programmes.

079 (D) Collection of the Standard Set for Brucellosis Positive and Negative Sera Diagnostics with Intent to Control Quality of Immune Enzymatic Test-Systems

Y. Rodina; SSCIBMS, Kyiv, Ukraine.

Background: Development and introduction of the standard set for brucellosis positive and negative animal serum diagnostics into general practice, which represents assessment system of immune enzymatic research. Purpose: Characteristics of immune enzymatic test-systems and sets by such indexes as specificity, sensitivity and reproducibility applied for brucellosis diagnostics in Ukraine by means of the standard set. Methods: To ground approaches for collecting the standard set for brucellosis positive and negative sera diagnostics tests were performed by indirect ELISA for detection of brucellosis antibodies in serum. Results: Standard diagnostic set or “panels” enables to assess basic technical parameters of immune enzymatic test-systems. To that effect the standard set should be complemented with “positive” and “negative” samples on the basis of selected sera of infected, sick or healthy animals by defined criteria. Collection of the standard set is based upon the following principles: 1. Blood sampling and serum preparation. 2. Determination of certain concentration of antibodies by maximum dilution of serum by means of the following reactions of ELISA, Bengal-Rose Probe, HIR (heamagglutination inhibition reaction) and complement binding. 3. Standard storage regime (-20°C) of prepackaged 0,5 ml of serum. 4. Number of serum samples must be no less than 20 units. 5. Volume of serum samples should be sufficient for no less than four two-phase reactions (with 0,5 ml of each sample). 6. Equal correlation of positive and negative serum samples (10 negative and 10 positive sera) in the set. 7. Required addition of positive serum with no less than 1/3 of serum to the stage of early seroconversion (3 low-titer and 7 high-titer sera). 8. Required addition to negative serum no less than 1/4 false-negative serum (2 false-negative and 8 negative sera). Conclusion: Introduction of the standard set for brucellosis positive and negative sera diagnostics into the domestic practical veterinary medicine has undoubtedly become an important milestone for improvement of laboratory research quality within the country.

080 (D) Detection of Coxiella burnetii in Dairy Camels in Saudi Arabia
M. F. Hussein, O. B. Mohammed, R. S. Aljumaah, M. A. Alshaikh; King Saud Univ., Riyadh, Saudi Arabia.

Background: Coxiella burnetii, which causes Q-fever, is holoendemic among inhabitants of Saudi Arabia. However, its prevalence and significance in indigenous livestock is largely unknown. Investigating the role of the Arabian camel (Camelus dromedarius) as a potential reservoir of C. burnetii in that country is long overdue, not only as camels are a major source of food and folk medicine for a large sector of the population but also because of the widespread tradition of consuming raw camel milk. This study describes serological prevalence of anti-C. burnetii antibodies and detection of C. burnetii DNA in blood and milk samples from dairy camels in Saudi Arabia. Methods: Sera from 100 apparently healthy adult female camels raised for milk production in Saudi Arabia were screened for antibodies against C. burnetii. The sera were tested by immunofluorescence (IF) using IF-conjugated goat anti-camel IgG, and by enzyme-linked immunosorbent assay (ELISA) using goat anti-camel IgG peroxidase conjugate. DNA extraction was carried out on milk and whole blood samples of 38 serologically positive animals using the Qiagen DNeasy blood and tissue extraction kit. Nested polymerase chain reaction (PCR) was performed using two sets of primers which amplify com1 gene encoding 27-kDa outer membrane protein. Results: IF and ELISA screening revealed anti-C. burnetii antibodies in 52.1% and 50.5% of the samples tested, respectively. All ELISA-positive samples were also positive by IF. The ODs in ELISA ranged from 0.40 - 240, while IF titers ranged from 1:64 to 1: 2048. Nested PCR results revealed that C. burnetii DNA was detected in 11 (28.9%) milk samples and 9 (23.7%) blood samples. Conclusions: These results constitute the first record of C. burnetii shedding in camel's milk in Saudi Arabia. The widespread habit of consuming raw camel milk for nutritional and medicinal purposes could be an important contributing factor to the hyperendemicity of C. burnetii among Saudis and other inhabitants of the Arabian Peninsula.
**081 (D) Clinical and Diagnostic Features of Cutaneous Anthrax in Georgia**


Cutaneous anthrax caused by *B. anthracis* is endemic in Georgia. Patients admitted to the two regional reference hospitals were assessed on enrollment and at seven follow-up time points. Blood and skin lesion swabs were collected on enrollment and up to 16 days later to conduct molecular and PCR testing. To date, 69 patients have been enrolled. Typical skin lesions, regional lymphadenopathy, fever, and headache were the most frequent clinical findings. A majority of patients received antibiotic therapy prior to enrollment; only one culture positive skin lesion swab was observed at day 2-3, however 57% of collected skin lesion swabs on day 8-11 and one on day 12-16 were PCR positive. Blood cultures and PCR performed on whole blood were all negative. All patients recovered without significant complications with the exception of one case with scarring/restricted hand motion. PCR of skin lesion swabs demonstrated its utility as a diagnostic test particularly when the volunteer had already received antimicrobial therapy. Recovery of the patients with systemic symptoms without further complications shows the effectiveness of the prescribed treatment. Negative bacteriology and PCR of blood supports existing knowledge that it takes time for *B. anthracis* to invade blood in cutaneous anthrax cases. Early antibiotic therapy probably contributed to this finding. Studying anthrax prospectively provides benefit for both public health and biodefense community; optimization of anthrax molecular diagnostic platforms targeting skin lesions will improve detection and response to this pathogen contributing to biodefense preparedness.

**082 (D) Development of an IS-Based One-Tube Multiplex Conventional PCR Method of Simultaneous Detection, Identification and Typing of *Yersinia pestis***

A. A. Filippov1, M. P. Nikolich2; Walter Reed Army Inst. of Res., Silver Spring, MD.

There are many publications on the detection and strain characterization of *Yersinia pestis* by using a number of high-technology methods. However, globally there is a need for simpler, more economical techniques that would allow simultaneous detection, identification and typing of *Y. pestis* strains, particularly for field studies in plague foci in developing countries. We have previously used molecular probes targeting mobile genetic elements IS285 and IS100 to detect restriction fragment length polymorphisms for genotyping *Y. pestis* strains, which showed high discriminative power. The purpose of this work was to develop a simple one-tube method of coupled detection, identification and typing of *Y. pestis* using multiplex PCR targeting chromosomal and plasmid insertions of IS285 and IS100. One primer in each pair targeted the IS element and the second primer was complementary to the insertion flank. The primers were designed to avoid self- and cross-complementarity and to provide a set of DNA amplicons of distinct sizes from 76 to 1,853 bp. Reactions were run using AccuPower® Gold Multiplex PCR PreMix (Bioneer, Alameda, CA) containing all components except primers and template DNA. A 13-plex assay was developed that allowed differentiation of *Y. pestis* from its closest relative, *Yersinia pseudotuberculosis*, discrimination of epidemic strains from endemic variants, determination of main biotypes (Antiqua, Orientalis and Medievalis), detection of atypical pesticidal strains from the Caucasus, and recognition of the three canonical plasmids of *Y. pestis*. Work is in progress to further enhance multiplexing to 20 targets and to develop a premix including all components except template DNA.

**083 (D) Lethal Factor Assay Development and Validation**

S. E. Carpenter1, E. Bollin2, L. S. Casey3, E. F. O’Connor1, S. F. Bungo4, P. R. Conniffe1, A. Shadiak1; 1Elusys Therapeutics, PineBrook, NJ, 2Avanza Lab., Inc., Galthersburg, MD.

**Background:** *Bacillus anthracis* (Ba) excretes proteins that can be fatal. Lethal Factor (LF), a protease enzyme, is one of three excreted proteins which include LF, Edema Factor (EF), and Protective Antigen (PA). LF combines with PA to form Lethal Toxin (LeTx) which is required for cell entry. Once inside the cell, LeTx causes cell death by cleaving the N terminal of most dual specificity mitogen activated protein kinase kinases. Here, we report the development and validation of immunooassays which measure BaLF, rabbit and cytoxin monomer with two new MesOx Scale Discovery (MSD) platform.

**Assay Design:** LF is captured with biotinylated anti-LF bound to a streptavidin coated plate. Ruthenylated anti-LF is added to complex immobilized LF. MSD read buffer is added and immobilized complexes detected by electrochemiluminescence. Data are regressed in Watson LIMITS and the concentration of Lethal Factor is extrapolated from a standard curve.

**Results:** The method was validated in both species with an analytical range of 25-4,000 ng/mL. Cynomolgus inter-assay precision (CV) ranged from 3.3% to 11.3%, with mean bias ranging from -14% to 3.3%. Rabbit CV ranged from 3.7 % to 10.9%, with mean bias ranging from -7.8% to 3.7. Interference from PA, EF, and ETI-204 (a MAβ to PA) at 1500 ng/mL LF was tested. The % bias values are presented below.

**Conclusion:** This method is suitable for routine use to quantitate LF in both rabbit and cynomolgus serum. Initial application will be to determine the utility of LF as a biomarker of disease progression along with PA after treatment with ETI-204, a monoclonal antibody with anti-PA activity. Supported by Federal funds from ASPR/BARDA, Contract No. HH-S0100201000026C.

**Interference % Bias**

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**084 (D) Development of Sample Ready Multiplex Real-Time PCR Assay for Detection of Malaria: Application in Diagnosis, Clinical Trials and Epidemiological Surveillance**

E. Kamau1, S. Alemayehu2, K. Feghali3, C. Ockenhouse4; 1USAMRU-K, Kisumu, Kenya, 2WRAIR, Silver Spring, MD.

We have developed ultra-sensitive real-time PCR assays for detection of Plasmodium at genus and species level (P. falciparum, P. vivax, P. ovale and P. malariae). The assays target the 18S rRNA genes and we have shown that amplification of total nucleic acid (DNA and RNA) significantly increases the analytical sensitivity of the assays by more than a log-fold over detecting DNA only. The assays can be multiplexed without any interference or reduction in individual assay sensitivity. Data shows the assays highly accurate, reproducible, robust, sensitive and specific. The genus-specific assay has Limit of Detection (LOD) of 2 parasites/ml using 3D7 cultured parasite and less than 1 parasite/ml in clinical samples. For plasmid DNA and in vitro transcript RNA, the assay has LOD of 1 molecules/μL. To further improve the performance and application of the assays, we converted the wet assays into a lyophilized, sample ready format. Two assays were converted: 1) a multiplex of genus-specific, P. falciparum, P. vivax and indigenous control human RNase P and 2) a genus-specific only assay. The Sample-ReadyTM format contains all the components required for real-time PCR reaction in a tube, only 5μL water and 1μL sample are added into the tube. We tested and showed that the sample ready format performs the same as the wet assay. We are in the process of validating the multiplex assay in a CLIA certified lab for use in support of a clinical trial and as a first step towards FDA approval process. We are also working with our collaborator, BioGX to develop a miniaturized real-time PCR instrument that is inexpensive (<$5,000), portable and battery operated. This miniaturized real-time PCR will be configured as a portable laboratory unit for laboratory based platform such as AB17500 or SmartCycler. The genus-specific, sample ready assay will be optimized for this instrument.
**085 (D) Serodiagnosis of Human Melioidosis Using Rapid Latex Agglutination Assays**

M. N. Burtnick1, C. Wikaiphat1, N. Chantaritla2, P. J. Brett1; 1Univ. of South Alabama, Mobile, AL, 2Mahidol Univ., Bangkok, Thailand.

**Background:** Burkholderia pseudomallei, the etiologic agent of melioidosis, is a CDC Tier 1 select agent that causes severe disease in humans and animals. Diagnosis and treatment of infections caused by this organism can be challenging and, in the absence of chemotherapeutic intervention, acute disease is frequently fatal. One of the long term objectives of our research, therefore, is to identify and characterize immunogens expressed by *B. pseudomallei* for the intended purpose of developing rapid serodiagnostic tests. Recently, studies in our laboratories have demonstrated that the O-polysaccharides (OPS) and capsular polysaccharides (CPS) expressed by this pathogen are well suited for this task. Because of this, we have begun to evaluate the use of OPS- and CPS-based latex agglutination assays for serodiagnosing human melioidosis.

**Methods:** OPS and CPS antigens were isolated from derivatives of the select agent excluded strain, *B. pseudomallei* Bp82, using a modified hot aqueous-phenol extraction procedure. Following purification by size-exclusion chromatography, the carbohydrate antigens were chemically activated with sodium periodate and covalently linked to aliphatic amine-derivated latex beads via reductive amination to produce the agglutination assays. Recently, studies in our laboratories have demonstrated that the O-polysaccharides (OPS) and capsular polysaccharides (CPS) expressed by this pathogen are well suited for this task. Because of this, we have begun to evaluate the use of OPS- and CPS-based latex agglutination assays for serodiagnosing human melioidosis.

**Results:** The specificity of the agglutination assays was confirmed by mixing the OPS- and CPS-coated beads with *B. pseudomallei* OPS- or CPS-specific rabbit antiserum. Testing of melioidosis patient and healthy donor serum samples with the polysaccharide-coated beads indicated that the sensitivity and specificity of the assays was comparable to indirect hemagglutination assays.

**Conclusions:** Preliminary studies suggest that our rapid latex agglutination assays may be useful as cost, effective, point-of-care tests for serodiagnosing melioidosis in endemic regions. Additionally, they may also represent useful countermeasures for combatting disease in the event that *B. pseudomallei* and/or *B. mallei* are intentionally released as agents of biological warfare and terrorism.

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**086 (D) Use of InDevR ampliPHOX Colorimetric Detection Technology and Custom Low-Density Microarrays for the Identification and Differentiation of Crimean-Congo Hemorrhagic Fever Virus**

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease that is exceedingly virulent. The strains are genotypically highly variable and sequencing is the classical method for differentiation. A new approach for strain identification and discrimination was recently published by Wölfel et al. (J. Clin. Microbiol. 2009; 47:1025-1030) using low-density macroarray (LDM) technology from PharmGenomics (Germany). This method involved the use of a LDM containing 20 CCHF-specific oligonucleotide probes spotted onto a filter membrane. A reverse transcription-PCR reaction using primers upstream/downstream of the probe sequences was run to produce biotin-labeled cDNA amplicons which were then hybridized to the LDM. The result was a unique hybridization pattern for each CCHF strain. We have adapted the Wölfel CCHF virus primer and probe sequences for use on a substantially different microarray platform from InDevR (Boulder, CO) which employs the ampliPHOX® colorimetric detection system. The ampliPHOX® system includes probes spotted on a glass slide, a reader, ampliVIEW™ software and reagents. ampliVIEW™ is unique in that it applies proprietary algorithms to the hybridization spots thereby allowing a quantitative positive/negative “calling” for each spot. In addition, a biotin-labeled dATP and a λ exonuclease are used to increase assay sensitivity. Our results indicate that the ampliPHOX® system is uniquely capable of identifying and differentiating multiple strains of CCHF virus based on the Wölfel primers/probes. The InDevR microarray provided a rapid and specific analytical report for each of the 18 CCHF virus strains tested, three of which were a direct comparison to the Wölfel results. This direct comparison indicates that while both systems are capable of differentiating multiple CCHF virus strains, the hybridization patterns of each system are considerably different.

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**087 (D) The Use of NanoTrap Particles to Enhance Viral Diagnostics**

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The recent appearance and worldwide spread of various viruses has highlighted the need to reexamine widely used diagnostic tests. Various findings have demonstrated that while currently available diagnostic tests detect virus from specimens containing high viral copies, sensitivity levels can be low and decrease significantly as virus levels decrease. Therefore, a negative result does not rule out infection. This lack of sensitivity must be addressed. NanoTrap particles are a novel technology that can address critical analytical challenges. NanoTrap particles contain internal affinity baits and perform three functions in one step, in solution, in complex biologic fluid: molecular size sieving, target analyte affinity sequestration, and complete protection of captured analytes from degradation. Targeted classes of protein analytes sequestered by the particles can be concentrated in small volumes to amplify (up to 100 fold or greater) the sensitivity of mass spectrometry, western blotting, and immunoassays. Up until now, NanoTrap particles have been used only in the capture of proteins. Our data demonstrate that NanoTrap particles can bind and concentrate whole virus, nucleic acids, and proteins. To date, our lab has demonstrated the whole virus capture of Rift Valley Fever Virus, Venezuelan Equine Encephalitis, Human Immunodeficiency Virus, Influenza A, Influenza B, Adenovirus, and Coronavirus using NanoTrap particles. Our results reveal that the virus can be inactivated while still maintaining its integrity, rendering it safe for testing at BSL2 laboratories. While there are other commercially available beads that also capture virus, we have shown that NanoTrap particles are the only beads that can protect the viral RNA from enzymatic degradation. Lastly, we have demonstrated that NanoTrap particles can capture and enrich protein analytes in serum.

Our future goal is to develop novel diagnostic methods with bait and shell chemistries tailored to each virus that can detect viruses at low concentrations previously undetectable by current methods.

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**088 (D) Development of Multiplex 3D Tests for Warfare Agents Detection**

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**Background:** The development of powerful detection systems for biological warfare agents, as early as their dispersion, is crucial to be efficient against those. These systems will have to be easy to use for non-specialists. Among the various methods of detection developed, immunochromatography presents several advantages: fast, ease of use, adapted to measurements on the field and low cost. Unfortunately the detection of several agents on one strip implies the realization of several test lines which could induce misinterpretation. Moreover non-specific interactions could occur between the nitrocellulose and some agents (anthrax spore for instance). **Methods:** In our new device, we built 3D fluidic pathway instead of a 1D pathway for classical immunochromatographic tests. This 3D fluidic pathway is obtained by overlapping several layers with different designs. Based on marquetry work, each layer is obtained by combining polymer stencil and hydrophilic matrix such as cellulose. The sample migrates through the layers by capillarity following the hydrophilic pathway. These 3D devices will allow us to detect simultaneously several agents in the same sample without ambiguity. **Results:** To obtain a functional device, test spots are realized by localized drops of antibodies modified beads in the hydrophilic matrix. Depending of the final application, the number of these spots could be easily adapted (at least 16). Currently, the tracer antibodies (fonctionnalized with gold colloid) are mixed directly with the sample within the tank. The first results highlight that the 3D fluidic works perfectly and that the device is able to detect several toxins or bacteria in the same time and within the same sample. Work is currently in progress to(i) integrate the tracer antibodies in a tank layer, to (ii) use other labeling systems and to (iii) improve the detections limits of our device.
089 (D) Rapid, Sensitive and Specific Detection of *Bacillus anthracis* on the POCKIT: A Field Deployable Polymerase Chain Reaction Detection System

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*Background:* PCR based diagnostic assays have been developed for detecting *B. anthracis*, *Coxiella burnetii*, *Listeria* species, *Brucella* species, and *P. aeruginosa. PCR technology has enabled the development of rapid, sensitive and specific tests which require specialized equipment and extensive training. Their use has been limited due to the lack of portable and rugged diagnostic tools. *B. anthracis* and *Coxiella burnetii* are listed as select agents by the U.S. Department of Health and Human Services and are potential biowarfare agents and are listed as critical agents for field result confirmation. POCKIT can serve as a rapid, affordable tool for the specific detection of *B. anthracis* and *C. burnetii* for first responders, public health officials and veterinarians.

**Methods:** Libraries of *sdAbs* were constructed from the mRNA of the peripheral blood lymphocytes from llamas immunized with the recombinant L1 and recombinant L2. The isolated *sdAbs* were used to detect vaccinia virus in immunoassays and to determine the limits of detection (LOD) for *sdAbs* using either DNA plasmid containing the *IS* element or DNA plasmid containing the *IS* element. The POCKIT yielded equivalent sensitivity for *B. anthracis* and *C. burnetii*. Three targets were amplified using either DNA plasmid containing the *IS* element or DNA plasmid containing the *IS* element, and the limits of detection were determined. The POCKIT can serve as a rapid, affordable tool for the specific detection of *B. anthracis* and *C. burnetii* for first responders, public health officials and veterinarians.

**Conclusions:** Immunization with either a recombinant L1 or vaccinated L1 enabled construction of *sdAbs* libraries from which virus binding elements could be isolated. These *sdAbs* have the potential to provide rugged reagents for the detection of vaccinia.

**090 (D) Enhanced Stabilization of a Stable Single Domain Antibody for SEB Toxin by Random Mutagenesis and Stringent Selection**


*Staphylococcal* enterotoxin B (SEB), an enterotoxin produced by *Staphylococcus aureus*, is listed as a select agent by the U.S. Department of Health and Human Services and is a common cause of food poisoning. As a result, there is a demand for sensitive, accurate assays which are also rugged and portable to facilitate on-site testing. To that end, highly stable single domain antibodies (sdAbs) have been developed capable of selectively binding SEB. We were interested to see if we could start with an antibody that was already highly stable and enhance that stability even further. If successful, we might eliminate the need for specialized storage conditions for the proteins altogether. The gene for a disulfide-free *sdAb* (A3-ds) was amplified by random mutagenesis PCR to produce a library of mutants then cloned into the phage-display vector (pECAN21). Phage from this library was panned against SEB at stringent conditions using a combination of increased concentration of guanidine hydrochloride, a protein denaturant, and elevated temperature. Selected phages were collected and the genes for captured mutants sequenced and cloned into an expression vector for protein expression, purification, and characterization. Native A3-ds antibody has a melting temperature of 62.2 °C as determined by circular dichroism (CD) spectroscopy. Several mutants have been produced with similar melting temperatures to A3-ds. One mutant, A3-ds-H2, has been produced with a melting temperature of 69.5 °C, an increase of 7.3 °C relative to A3-ds. By creating a random mutagenesis library of a disulfide-free *sdAb* we successfully identified a mutant, A3-ds-H2, with a melting temperature improved by 7.3 °C. Ongoing isolation and characterization is being performed to identify more mutants and determine their melting temperatures and binding affinity to SEB. To the isolated mutants, site-directed mutagenesis will be performed to restore the disulfide bonds present in the original antibody, further increasing thermostability.

**091 (D) Isolation of Single Domain Antibodies Targeting Vaccinia Virus**

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*Background:* Single domain antibodies (sdAbs), recombinantly expressed variable domains from the heavy-chain only antibodies of camelds, are emerging as excellent alternatives to conventional antibodies for use in field deployable sensors and assays in more austere environments. sdAbs routinely exhibit high affinities and their reduced complexity frequently translates to an inherent ability to refold to an active detection molecule following denaturation. Though vaccinia virus is considered non-pathogenic, other members of the poxviridae family are classified as potential bioweapons. L1 is a transmembrane protein found on the surface of the mature vaccinia virion and is highly conserved among orthopoxviruses. Llamas were immunized with either inactivated virus or recombinant L1 to allow for the enrichment of sdAbs capable of recognizing vaccinia. *Methods:* Libraries of sdAbs were constructed from the mRNA of the peripheral blood lymphocytes from llamas immunized with either inactivated virus or recombinant L1. The L1 was immobilized to magnetic beads to facilitate enrichment of binders through biopanning. The sdAbs were also biopanned on inactivated vaccinia virus. Target binders were identified through a monoclonal phage ELISA then subsequently characterized for binding ability via direct binding and sandwich assays on a bead-based multiplexing platform. Thermal stability was assessed using circular dichroism. *Results:* Biopanning the libraries yielded multiple sequence families based on amino acid composition of the CDRs; 9 from llamas immunized with recombinant L1 and 10 from those immunized with inactivated virus. Representative clones showed specific and high affinity binding to both recombinant L1 and inactivated virus. The isolated sdAbs were able to detect vaccinia virus in immunomas when incorporated into sandwich assays. *Conclusions:* Immunization with either a recombinant L1 or inactivated vaccinia enabled construction of sdAb libraries from which virus binding elements could be isolated. These sdAbs have the potential to provide rugged reagents for the detection of vaccinia.

**092 (D) Highly Sensitive Loop-Mediated Isothermal Amplification for the Detection of *Coxiella burnetii***


*Coxiella burnetii*, the bacterium causing Q fever, is an obligate intracellular biosafety level 3 agent. Detection and quantification of these bacteria with conventional culturing methods is time consuming and dangerous. PCR based diagnostic assays have been developed for detecting *C. burnetii* DNA in cell cultures and clinical samples. Because PCR method requires specialized equipment and extensive end user training, it is not suitable for routine work especially in the resource-constrained area. We have developed a loop-mediated isothermal amplification (LAMP) assay to detect the presence of *C. burnetii* in plasma. It is an auto-cycling strand displacement DNA synthesis method that can be performed at a single temperature around 60°-65°C. Set of primers were designed using the transposase gene of the *IS* element *IS1110*. The amplification reactions were incubated at 60°C for 60 min. We were able to detect less than 20 copies of bacterial DNA (or equivalent of one organism) in the reaction using either DNA plasmid containing the *IS* element or DNA plasmid spiked plasma. The sensitivity of the LAMP assay was also verified by qPCR. Our results suggested this assay has the potential to be used as a rapid, robust, and easy-to-perform assay in the endemic regions.
093 (D) Impedance Measurements Could Accelerate Phage-Based Identification of Bacillus anthracis and Other Bacteria


The high degree of specificity that phage and phage-derived lysins exhibit for their host has made them valuable tools for identifying bacterial species and subspecies. This is particularly true in the field of biodetection where phage-based assays have a long history of being used to identify Bacillus anthracis and Yersinia pestis isolates. However, these assays generally require that suspect colonies be sub-cloned onto a fresh agar plate to generate a dense lawn against which the plaques can be observed. After a potential exposure to bacterial threat agents, this additional time step can consume valuable time. BioSense Technologies has previously shown that the electrical impedance across bacterial micro-cultures differs for chemically- or physically-stressed and unstressed cells. In this study, this distinctive shift in impedance can be detected as early as 1 hour after exposing as few as 10^7 CFU bacteria to the stressor. We predicted that impedance could be similarly used to detect phage-related stress in susceptible bacterial micro-cultures and thereby reduce the time and biomass required to perform these diagnostic assays. After exposing small quantities of B. anthracis and other bacteria to phage or phage-derived lysins, we tracked the cultures for several hours using microscopy, impedance, and plated serial dilutions. Our results show that the bacteria exhibit distinctive shifts in impedance when exposed to their cognate phage. These data suggest that impedance measurements can be used to improve phage-based identification assays, including those used in the field of biodetection.

094 (D) High Sensitivity Assay to Quantify Anthrax Toxins and Screen Anti-Toxin Antibodies

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Background: Critical to the success of an emergency response is the ability to confirm anthrax at the earliest possible disease stage and in the shortest possible time. Current diagnostics include culture isolation of B. anthracis and direct PCR on clinical samples, both of which can be impeded by antimicrobial therapy. Early detection markers for anthrax include the toxin components EF, LF, and PA. This study describes the development of an enhanced electrochemiluminescence (ECL) assay that can both quantify low levels of anthrax toxins and be used to screen and select pivotal reagents for point of care (POC) diagnostic development.

Methods: Two anti-PA monoclonal antibodies (mAbs) and 9 anti-LF mAbs were generated to develop an ECL assay to detect PA, LF, or lethal toxin (Ltx). These mAbs were screened to determine multiple optimal non-competitive antibody pairs. Capture mAbs were optimized to 0.1-2 μg/mL, antigen was titrated from 0.5 to 50 ng/mL, labeled detection antibodies were used at 1-2 μg/mL. Results: Two anti-PA mAbs and 4 anti-LF mAbs were selected. The limit of detection (LOD) for each of PA, LF, and Ltx was < 1 ng/mL in a 25 μL sample. Orientation of the mAbs as either capture or detector species affected signal output. Antibody orientations which produced the highest signal in the ECL assay also had the highest analytic sensitivity in a point of care assay. Conclusions: The ECL assay described detects anthrax antigens below 1.0 ng/mL in only 25 μL of sample. This quantitative test is well within the target range (10-50 ng/mL) for inhalation anthrax. These data show that clinically relevant concentrations of anthrax toxin proteins can be measured in monomeric or complex form. This assay is also an effective tool to screen antibodies to determine optimal antibody pairs and orientations for use in a rapid POC assay.

095 (D) Optimization of a Lateral Flow Immunoassay (LFI) for the Rapid Diagnosis of Melioidosis

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Burkholderia pseudomallei is a soil-dwelling bacterium that is the causative agent of melioidosis. Detection of B. pseudomallei by microbiology laboratories is difficult due to a lack of validated diagnostic reagents. Isolation of B. pseudomallei from cultures of patient samples remains the “gold standard”. Culturing for B. pseudomallei is labor intensive, multiple patient samples must be collected. Isolation of B. pseudomallei from any one of these cultures is diagnostic for melioidosis; unfortunately this takes 3-7 days. Therefore the goal of our research is to develop a rapid point-of-care immunoassay for the diagnosis of melioidosis. We employed a novel approach termed in vivo Microbial Antigen Discovery (InMAD) to identify the capsular polysaccharide (CPS) and numerous protein antigens that could be targeted for diagnosis. Our initial efforts have focused on the CPS. Following production of a CPS-specific monoclonal antibody (mAb), we have established the presence of CPS in melioidosis patient serum and urine samples. The same mAb was used to produce a prototype lateral flow immunoassay (LFI) that is capable of detecting CPS in a variety of patient samples. The LFI is currently undergoing pre-clinical testing in Thailand and Australia; the focus here is to optimize and validate testing procedures on melioidosis patient samples. Finally, our laboratory has generated a large panel CPS specific mAbs that have increased the performance of the prototype LFI.

096 (E) Detection of Biothreat Agents in Food Matrices Using a Multiplex PCR and the Bio-Plex Suspension Array System

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After the 2011 anthrax attacks in letter mail in the United States it became apparent that there was a need for the rapid and reliable detection and identification of biothreat agents. In 1984, a deliberate contamination of salad bars at ten local restaurants in Oregon with Salmonella increased our awareness that microbial pathogens can be used as biothreat agents and administered through our food supply. The CDC categorized biothreat agents according to the risk levels posed by each agent to the public. Some of the organisms in the category A list are Anthrax (Bacillus anthracis), Botulism (Clostridium botulinum) toxin, Plague (Yersinia pestis), Tularemia (Francisella tularensis). These pathogens can cause gastrointestinal disease, can be disseminated through food, and have the potential for major public health impacts. A multiplex PCR assay using the BioPlex (Luminex) suspension array® system developed by Biosearch Technologies and Lawrence Livermore National Laboratory was used to detect B. anthracis (Ba), Y. pestis (Yp), and F. tularensis (Ft) in foods. The assay includes PCR primers for nine gene targets specific to these pathogens including three for Ba, four targets for Yp, and two Ft targets. The assay also includes a fluorescence control, a hybridization control, and an instrument control. This flow cytometry based detector uses fluorescent beads coupled to unique nucleic acid probes in a multiplex PCR based assay and can be used to identify up to 100 different targets. In this study, the BioPlex assay was successfully used to detect Ba, Yp, and Ft in green bean and banana baby foods, infant formula, and eggbeaters. The assay was also successful in detecting Yp in ground beef. Future studies to include additional targets and to test detection capabilities in additional food matrices.

097 (E) Laboratory Preparedness: A Multi-Laboratory Validation of Non-Typhoidal Salmonella in Drinking and Surface Water

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The EPA Water Laboratory Alliance (WLA) is a nationwide laboratory network that provides the capability to analyze water samples during a contamination event. In 2011 the WLA completed a multi-laboratory validation of non-typhoidal Salmonella. The multi-laboratory validation study involved ten commercial, utility, and public health laboratories from eight states. The Study included the analysis of PBS, drinking water, and surface water samples spiked with S. typhimurium BioBall® spikes. The results of this study were used to assess method performance (i.e., recovery and precision) across multiple laboratories and matrices, compare effect of holding time (0 - 8 hours after sample spiking, compared to 0 - 12 hours after sample spiking), assess reproducibility of results from analyses of “blind” samples collected from each of two sites per matrix (drinking water and surface water), and develop QC acceptance criteria. This Method is needed to address gaps that exist within standardized methods of isolation and analysis. Ultimately, the validation of this protocol enables laboratory capabilities, improving the ability of the Water Sector to respond to water contamination.
098 (E) Microbial Biofilms as an Environmental Sampling Tool: Retention and Detection of DNA from Bacillus anthracis in a Sink Drain
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Bioremediation is ubiquitous in nature and comprised mainly of an array of cells and extracellular polymeric substances released from microorganisms, which have an innate ability to capture and retain passing material. Sink drains were selected as a model system to test whether established biofilms could be used as a means to record and detect signatures of biological agent production. A controlled parallel drain system was assembled to assess signature capture, retention, and degradation. This design allows the total influx and efflux of material to be recorded, while also allowing multiple tests to be performed in parallel on biofilms initiated using a single mixed microbial drain community. To determine the persistence and detectability of DNA in a sink drain biofilm, Bacillus anthracis strain Ames35 was cultured (1.5 L at 6.5 x 10^7 CFU/mL), sterilized by use of an autoclave, and subsequently disposed of by passage through the artificial sink drain apparatus containing a previously established biofilm. The sink drain apparatus was sampled before and after addition of the sterilized cell culture to confirm the influx of the B. anthracis DNA. The drain system was flushed, daily, with 0.5-18 L of wastewater to simulate regular biofilm. Primers targeting shorter sequences, <500 bp, showed greater amplification efficiency and positive detection over those targeting sequences >500 bp. PCR amplification and detection of target sequences indicated persistence of the DNA signatures 1-2 weeks post-disposal. These studies demonstrate an ability and some limitations in using naturally occurring biofilms as in situ collection matrices for biological signatures.

099 (E) Comparison of Nutrient Media for Primary Isolation of Francisella tularensis from Contaminated Samples
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Background: Tularemia has natural reservoirs in rodents and ticks and these were collected to define the presence of F. tularensis within UP-2 project “Incorporating GIS, Remote Sensing, and Laboratory Diagnostics into Human and Veterinary Disease Surveillance for Tularemia and Anthrax in Ukraine.” Due to contamination of collected tissues from rodents and ticks with additional microorganisms the aim of the study was to define selective nutrient medium. Materials and Methods: 3 nutrient media were compared: 1) FT-agar, no antibiotics; 2) ADET-agar with polymyxin M, ampicillin, amphotergiculin, and tetracycline; 3) CHAB-PACCV. The results suggest seasonal epizootic activity associates with the four species Clethrionomys glareolus, Microtus arvalis, Apodemus silvaticus, and the domestic mouse. Specifically, Clethrionomys glareolus have been found to be more active in the cold months, whereas the three other rodent species are more active in the warmer seasons. The Clethrionomys glareolus activities correspond with the seasonal distribution of human cases, which would suggest that the bank vole is one of the main sources for infection of people in WKO.

100 (E) Hemorrhagic Fever with the Renal Syndrome (HFRS) in the West Kazakhstan Oblast (WKO)
Hantaviruses, a genus of the Bunyaviridae Family, are known to cause hemorrhagic fever with renal syndrome in humans exposed to rodent excreta. Since 2000, Hantavirus has been documented in the West Kazakhstan Oblast (WKO) with little understanding of the ecology in which this virus flourishes. The objective of this work was to study and understand the ecology of Hantavirus associated with HFRS that are known to circulate in the WKO by screening small field mammals. For several years, GPS coordinates of field rodents collected were recorded; then the rodents were collected, identified, and tested for Hantaviruses, Puumala and Dobrava, via ELISA and immunofluorescent assays. Hospital data of rates of human HFRS were also collated. A total of eleven different species of field mammals were found for a total sum of 55,000 rodents collected. The results suggest seasonal epizootic activity associates with the four species Clethrionomys glareolus, Microtus arvalis, Apodemus silvaticus, and the domestic mouse. Specifically, Clethrionomys glareolus have been found to be more active in the cold months, whereas the three other rodent species are more active in the warmer seasons. The Clethrionomys glareolus activities correspond with the seasonal distribution of human cases, which would suggest that the bank vole is one of the main sources for infection of people in WKO.

101 (E) Classical Swine Fever Virus and Pasteurella multocida Interaction
Background: Classical swine fever (CSF) often manifests as a mixed-infection. Current research has suggested that magnetic field(s) may be a factor, which affects bacterial and viral pathogenicity. To better understand any potential influences of magnetic fields on pathogenesis, we have employed the use of a “Grabbin device” (GD), which creates asymmetric rolling magnetic fields generation for biological use. Objective: To study the potential change in physical interactions between CSFV and Pasteurella multocida following exposure to right (RM-) and left (LM-) rolling magnetic fields. Methods: We used GD to generate asymmetric magnetic fields, and subsequently exposed virus-bacteria mixes (V=5,0cm; B=0,2 mT) which consisted from 4,5 lgFU/m^2 of CSF virus (“IECV-03” strain, CSFV) and the 5x10^10 BCF[unit] of Pasteurella multocida “77” strain on buffered saline (BS). Mixes were exposed by RM- (n=3) or LM- (n=3) rolling fields during 25 min. Control mixes (CM-samples, n=3) were exposed on switch-off device. After exposures all mixes were rinsed and RM-samples divided on 3 part - 1st parts controlled immediately altogether with LM- and CM-samples. 2nd and 3rd of RM-samples were controlled after exposition under 40oC in 3 and 5 days. The virus quantity in all settlements was titrated in PK-15 cell with standard procedure of indirect PLA colored-calculation. Results: CSFV was revealed only in bacterial settlements of all RM-samples. The virus titers in these samples were 2,5+0,9 lgFU/m^2 after RM-application (n=3, P<0,01). On 3rd and 5th day after RM-bacterial settlements exposition under 40°C CSFV titers were 1,1+0,8 lgFU/m^2 (n=3). A free CSFV was inactivated at 2nd day of 40°C exposition completely (n=3, P<0,01). Conclusion: RM-rolling field (B=0,2 mT) promote the CSFV adsorption on Pasteurella cells. Adsorbed virus rise of resistance to 40°C significantly. This data explain the events which may be to come during natural CSF outbreaks.
**102 (E) Application of Multiplex 3-D Rapid Tests for Detection of Foodborne Pathogens**


**Background:** Natural contamination of foods or water by pathogenic bacteria, viruses, parasites or toxins frequently occurs throughout the world and affects hundreds of millions of people every year all around the world. Therefore, the ability to detect the responsible pathogenic agents is essential for ensuring food safety and public health. Moreover, the food supply could be a target for terrorism and an attack could occur at any point in the food chain. These contaminations have to be detected quickly because even if the resulting diseases only seldom put people life in danger, the social and/or economic impact is generally quite important.

**Methods:** Monoclonal antibodies directed against numerous foodborne pathogens (enterohemorrhagic *Escherichia coli* O157:H7, *Shigella sonnei*, *Shigella flexneri*, and *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis* and Shiga toxins) were produced and characterized in the laboratory. All these mAbs were evaluated for the specific detection of the corresponding food poisoning agents by multiplex 3-D rapid tests (see abstract by Berthelot et al. for the description of this technique).

**Results and Conclusion:** Our study confirms the feasibility of multiplex 3-D tests to detect simultaneously and rapidly toxins and bacteria with the same device. Different matrices were also evaluated and further improvements are under investigation.

**103 (E) A Cost-Effective Biosurveillance Approach to Identify Novel and Emerging Vector-Borne Pathogens By Deep Sequencing**

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Monitoring infections in vectors such as mosquitoes, sandflies, and ticks to identify human pathogens may serve as an early warning detection system to direct local government preventive measures (vector-control, public outreach). One major hurdle in detection is the ability to screen large numbers of vectors for human pathogens without the use of genotype-specific molecular techniques. Current vector surveillance programs test for only a subset of pathogens known to circulate in a given geographic area and do not have the diagnostic capacity to screen for novel or emerging public health threats except for using inefficient and specific cell culture systems. Deep sequencing provides an unbiased platform capable of identifying known and unknown pathogens circulating within a vector population, but utilizing this technology is time-consuming and costly for vector-borne disease surveillance programs. We developed novel deep sequencing protocols to rapidly generate Illumina® compatible cDNA libraries and remove abundant host genetic background to enrich for microbial sequences circulating in vectors at the population level.

These methodologies were implemented to characterize the microbiome and virome of Culex mosquitoes during the 2012 West Nile virus (WNV) outbreak in California. We identified novel and well-documented proteobacteria, eukaryotic parasites, and viruses present in spatially defined Culex populations and performed phylogenetic analyses on assembled mosquito-borne viruses (including WNV) found in pooled Culex samples thereby comparing viral genotypes from different geographic locations. This data provides insight into the complexity of microbial species circulating in medically important vectors and their potential impact on transmission of vector-borne human pathogens.

**104 (F) Rare Variant Detection in Viral and Bacterial Populations**

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Identification of variants within relatively homogeneous biological samples, such as single-species bacterial or viral populations, is important in many fields, including forensics, infectious disease research, biodefense, and biologics quality control. Advances in High Throughput Sequencing (HTS) allows sensitive detection of SNPs at previously unattainable frequencies - potentially down to ultra-rare variants present in < 0.1% frequency of the test population. It is generally recognized that errors can and will be introduced at every step of the sequence data generation process, from DNA fragmentation and through to base assignment by the HTS machine. In addition to sequencing errors, reference sequence-based effects and mapping algorithm errors have two significant impacts on rare variant (SNP) detection accuracy: (1) some SNPs are more difficult to detect (loss of sensitivity), (2) some SNPs are more likely to be incorrectly detected (loss of specificity). We have developed an exhaustive approach to identify and quantify all one-mismatch-away effects of reference genome and mapping algorithm associated errors. This approach was tested on in silico simulated viral and bacterial populations and on plasmid mixtures, with known SNP containing plasmids present in as low as 0.1% frequency. Multiple HTS platforms (GAIIx, SOLiD, and HiSeq) and library preparation protocols were evaluated on the basis of accuracy of rare variant detection. The results suggest that the reference genome features, for a given n-mer length, have a significant impact on both the sensitivity (through silencing effects) and specificity (through ambiguous called variant) of variant detection. When proper correction procedures are applied, these errors can be significantly mitigated, making rare variant detected feasible - potentially even at ultra-rare variant frequencies.

**105 (F) Diversity among Viruses Isolated from Mosquitoes in the Peruvian Amazon Basin**


Members of the genus *Orthobunyavirus* (family *Bunyaviridae*) are segmented, negative-sense, single-stranded RNA viruses that are responsible for mild to severe disease in humans. As part of a long-term field study of arbovirus ecology in the Amazon Basin of Peru, more than 160 viral isolates were serologically identified in mosquitoes captured at three collection sites within a ten kilometer radius of Iquitos, Peru. *De novo* next-generation sequence of approximately half of these mosquito isolates identified several different virus species among the genus *Orthobunyavirus*. Phylogenetic genomic analysis has genotyped these viruses into orthobunyavirus antigenic serogroup complexes Capim, Group C, Oropouche, as well as novel viruses within the Wyeomyia group. Intra-species or reassortant and recombination analysis within each of these complexes will also be discussed. This comprehensive *de novo* orthobunyavirus full genome sequence analysis has 1) developed a core capability pipeline for high-throughput pathogen discovery, 2) revised the phylogeny of the human pathogenic virus genus *Orthobunyavirus*, 3) provided details of the diversity of emerging viruses isolated from several mosquito species that exist within close proximity to humans, and 4) provided confirmed virus genomic identification of orthobunyaviruses to design rational diagnostic assays and therapeutics.
106 (F) In Silico Identification and Analysis of miRNAs in Bacterial Infected Human Peripheral Blood Mononuclear Cells


MicroRNAs (miRNAs) are ~22 nt long non-coding RNA sequences implicated in a wide range of physiological as well as pathological processes, including inflammatory responses, apoptosis, growth and cancer, neurodegenerative and cardiovascular diseases. There has been increasing evidence that miRNAs play an important role in the immune response against infectious agents. In this study, human PBMCs were infected with four pathogens: E. coli DH5α, B. pseudomallei K92643, F. tularensis ShuS4 and B. anthracis Ames (spores). Total RNA samples were collected at infection intervals, 30, 60 and 120 minutes; libraries were prepared using Illumina TruSeq Small RNA protocol and sequenced on the MiSeq with a 2 x 51 read length configuration. The FASTQ files were analyzed using Flicker 3.0 that uses several packages included in CASAVA 1.8.2. The adapter sequences were trimmed using trimmer; post-trimmed sequencing reads meeting 15 bp minimum length requirement were aligned using Eland against contaminants such as mitochondrial DNA, 5S ribosomal RNA, adapter, poly A, poly C, phage phiX174 sequences, snoRNA, lincRNA, and snRNA obtained from Illumina iGenome and Ensembl. The remaining reads which did not match to contaminants were then aligned to human genome (NCBI build 37.2) and the corresponding bacterial genomic DNA databases. Concurrently, the same reads were screened against mirBase (http://mirbase.org/) for human-specific mature miRNAs as well as precursor miRNAs. Hits normalized abundance values of miRNAs among different infection times were analyzed to identify differential expression. On average, we obtained ~100 Mb per sequencing run and quality score 92% bases higher than Q30. Of the average 1.7 million reads collected, ~60% of them passed minimum read length for downstream analyses. We have utilized Flicker small RNA workflow to identify and analyze miRNAs in bacterial infected human PBMCs and our findings on the up and down regulation of human miRNAs in relation to increasing infection time are discussed.

107 (F) Enriching Pathogen Transcripts from Infected Samples: A Capture Based Approach to Improved Host-Pathogen RNA-Seq

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To fully understand the interactions of a pathogen with its host, it is necessary to analyze the RNA transcripts of both the host and the pathogen throughout the course of an infection. While this can be accomplished relatively easily on the host side, the analysis of pathogen transcripts is complicated by the overwhelming amount of host RNA isolated from an infected sample. Even with the read depth provided by second-generation-sequencing (SGS), it is extremely difficult to get enough pathogen sequences for an effective analysis of the genes involved in pathogenesis. In this study we describe a novel capture-based technique that significantly enriches for pathogen transcripts from infected samples by up to 100 fold. This versatile method can, in principle, enrich for any pathogen of interest in any infected sample. To validate the technique, we performed time course tissue culture infections using Rift Valley Fever Virus (RVFV) and Francisella tularensis. RNA-Seq analysis was performed on samples from each infection time point and the results of the captured samples were compared to untreated controls. The capture of pathogen sequences, in all cases, led to over an order of magnitude enrichment of pathogen reads, greatly increasing the number of genes hit, the coverage of those genes, and the depth at which each transcript was sequenced. This increased information led to the identification of several, previously uncharacterized, genes involved in Francisella pathogenesis.

108 (G) Antiviral Susceptibility of Highly Pathogenic Influenza A (H5N1) Viruses Isolated Worldwide In 2002-2012


Background: Highly pathogenic avian H5N1 influenza viruses remain a potential pandemic threat and have caused significant morbidity and mortality in avian species. These viruses are emerging as human pathogens with >60% mortality. Continuous evolution of the highly pathogenic H5N1 influenza viruses results in the emergence of multiple clades, some of which have a distinct geographic distribution. The use of antiviral drugs would play an important role during the early stages of a pandemic, but drug-resistant variants would most likely emerge, thereby limiting our options for control. The aim of this study was to determine the susceptibility of different HA clades of H5N1 viruses that were isolated in 2002-2012 to the FDA-approved neuraminidase inhibitors (NAIs) oseltamivir and zanamivir, the M2 inhibitor amantadine, and the investigational drug T-705. Methods: Susceptibility of H5N1 viruses to NAIs was assayed in phenotypic fluorescence-based assay, and to T-705 in plaque inhibition assay in MDCK cells. Molecular markers for NAI and amantadine resistance were determined by sequence analysis. Results: Both human and avian H5N1 viruses were susceptible to NAIs (mean IC50, 0.65-1.53 nM) with little variability over time, and the susceptibility of clade 2 viruses was about 5.5-fold less than that of clade 1 viruses. H5N1 clades were susceptible to T-705 with similar mean IC50 values over time. The frequency of amantadine-resistant variants was higher among human viruses (62.2%) than avian viruses (31.6%). Phylogenetic analysis revealed that equal proportions of human amantadine-resistant variants belonged to HA clades 1 and 2 (46.2% and 53.8%, respectively), but avian variants were most frequently isolated from clade 1 (~70%). Conclusion: Highly pathogenic H5N1 influenza viruses are susceptible to NAIs and T-705, and some are still susceptible to adamantanes. Continued monitoring of H5N1 viruses is needed to evaluate the antiviral susceptibility of clades with pandemic potential and to maintain therapeutic approaches for control of the disease.
109 (G) LED209-PAMAM G3.0 Dendrimer Conjugate as a Novel Antibacterial Agent with Low Toxicity and Ability to Target QseC Receptor

It has been confirmed that amino-terminated generation 3.0 poly(amideamine) dendrimer (PAMAM G3.0) had broad-spectrum antibacterial activity against Gram-positive/negative and even multi-resistant strains, while the high cytotoxicity hindered its further application. LED209 is a newly discovered, potent, non-toxic inhibitor of QseC receptor and endow it with new antibacterial features. We therefore synthesized LED209-COOH (Fig 1A) and conjugated it to PAMAM G3.0 (LED209-PAMAM G3.0, Fig 1B), and further examined the cytotoxicity and antibacterial activity of LED209-PAMAM G3.0. H-NMR and elemental analysis revealed about 6.25% coverage of LED209 on the surface of PAMAM G3.0. MIC test showed that LED209-PAMAM G3.0 had strong antibacterial activity against almost all strains tested, and the MIC values were 1- or 2-fold higher than that of unmodified PAMAM G3.0. MTT assays showed that the toxicity of LED209-PAMAM G3.0 to GES-1, SW480 and MC3T3 cells was much lower than that of unmodified PAMAM G3.0. After incubated with 10 μM of LED209-PAMAM G3.0, the effect of norepinephrine (NE) induced activation of QseC receptor in Enterohemorrhage Escherichia coli (EHEC) was significantly antagonized, and the expression of key virulence genes, including stx2A, flhD, flIC and ler, was inhibited (Fig 1C). Therefore, LED209-PAMAM G3.0 might be a novel kind of antibacterial agent with low cytotoxicity and potency to inhibit the expression of bacterial key virulence genes by targeting QseC receptor.

110 (G) Host-Directed Antimicrobial Therapeutics for Coxiella burnetii
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Rationale and Scope: Although antibiotic therapy is often successful, it is clear that alternatives to conventional pathogen-directed antibiotic therapy must be developed in the face of increased antibiotic resistance. Our overall goal is to identify host functions required for successful infection by a variety of intracellular bacterial pathogens. We hypothesize that novel host-directed antimicrobial therapies, which circumvent the problem of developing drug resistance, can be developed by targeting specific host pathways used by individual or groups of pathogens. One approach to identify these therapeutics is to screen libraries of small molecules whose targets and mechanisms of action are well known for compounds that make human cells resistant to infection in vitro.

Experimental Procedures: We screened two libraries of small molecules; ICBN known bioactive compounds (n=480) and BIOMOL FDA-approved drugs (n=640) for the ability to prevent intracellular growth of Coxiella burnetii (Cxb) in PMA-differentiated THP-1 human mononuclear cells; distinct antibiotic activity for Cxb growing axenically and for THP-1 cytotoxicity. We utilized mCherry expressing Cxb to assess growth over the course of several days. Secondary assays on a subset of compounds confirmed the screening results and dose dependency assays were used to quantitate the effects on Cxb intracellular growth and cytotoxicity. Data: We found a variety of compounds, including several FDA-approved drugs, were effective at preventing Cxb intracellular growth with no effect on axenic growth and with minimal cytotoxicity on the THP-1 cells. The three largest classes of compounds appear to affect intracellular trafficking of cholesterol, lysosomal pH regulation or tyrosine kinase signaling pathways.

Principal Conclusions: We conclude that host cells can be manipulated in a predictable manner that renders the cells less permissive for infection by Coxiella burnetii. Additional studies are underway to extend this work to additional intracellular pathogens.

111 (G) Antibiotic Susceptibility of Human Brucella Isolates in Georgia

Brucellosis is a debilitating febrile illness with substantial morbidity. Treatment of brucellosis in Georgia consists of three, 10-day antibiotic courses with 10 days intervening. Antibiotics of choice are doxycycline, chloramphenicol, rifampin, tetracycline or streptomycin. Antibiotic susceptibility data on Georgian Brucella isolates is very limited. To address this, the susceptibility of human B. melitensis and B. abortus isolates obtained at the National Center for Disease Control and Public Health of Georgia in 2009-2011 was investigated by testing 42 clinical isolates against penicilin (6 μg), kanamicin (30 μg), rifampin (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), streptomycin (10 μg), tetracycline (30 μg), erythromycin (15 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), doxycycline (30 μg), chloramphenicol (30 μg), cefazolin (10 μg) and ofloxacin (5 μg) using BBL Sensi-Disc Antimicrobial Susceptibility Tests. All but one B. abortus had similar susceptibility patterns and were sensitive to the antibiotics tested with a few exceptions: All strains tested were resistant to cefazolin (11/11); 95% (60/62) to trimethoprim/sulfon; 60% (25/42) were resistant, 31% (13/42) intermediate and 9% (4/42) sensitive to erythromycin. Two strains were sensitive and 19 resistant to penicilin, while 24% (10/42) had intermediate sensitivity and 10% (4/42) resistance to rifampin, a commonly used treatment. The data suggest that Georgian Brucella isolates are mostly susceptible to antibiotics used for treatment. Further routine testing will identify emerging resistance and inform treatment regimens.

BIODEFENSE PROGRAM & ABSTRACTS
**112 (G) Antibacterial Activity of Biapenem/RPX7009 against Biodefense Pathogens**  
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**Background:** Antibiotic resistance in pathogenic bacteria, particularly the bioterror pathogens, is a widespread concern that requires continual updating of existing treatment options. RPX7009 is a new beta-lactamase inhibitor with potent inhibitory activity against Class A carbapenemases. It is being developed as a fixed combination with carbapenem biapenem, a broad-spectrum antimicrobial. This combination therapy was evaluated in vitro at USAMRIID against geographically diverse sets of *Burkholderia pseudomallei* (BP), the causative agent of melioidosis, and *Yersinia pestis* (YP), the causative agent of plague. **Methods:** Minimum inhibitory concentration (MIC) values were determined for each compound by the microdilution method in 96-well plates using CLSI reference methods. The antibiotic concentration tested for biapenem was 0.031 - 64 μg/mL, based on a final well volume of 100 μL after inoculation. The β-lactamase inhibitor RPX7009 was tested at fixed concentrations of 4 or 8 μg/mL. Plates were incubated at 35ºC in ambient air. **Results:** Similar to the other carbapenems, biapenem alone demonstrated solid in vitro activity against the YP isolates (MIC90 = 0.5 μg/mL) and was slightly enhanced by the addition of RPX7009 (MIC90 = 0.25 μg/mL). The biapenem/RPX7009 combination was more active against BP isolates (MIC90 = 1 μg/mL for biapenem alone and 0.25 μg/mL for the combination). In particular, two β-lactam-resistant strains of BP were markedly more susceptible to the combination (Strain #275: MIC = 8 μg/mL for biapenem and 2 μg/mL for the combination). **Conclusion:** The combination of biapenem and RPX7009 beta-lactamase inhibitor shows great potential for treating infections caused by *Y. pestis* and *B. pseudomallei*, as well as the broader potential for treating other natural or man-made outbreaks of β-lactam-resistant bacterial infections.

**113 (G) Autolysin LytM as an Anti-*S. aureus* Agent**  
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Autolysins are bacterial enzymes that catabolize peptidoglycan during autolysis, cell division, and peptidoglycan recycling, and deletion of autolysin genes can lower cell fitness. We propose that autolysins can be leveraged to create novel, bacteriolytic therapies with higher specificity and reduced resistance compared to conventional antibiotics. We recently have evaluated the active form of *S. aureus* autolysin LytM, a zinc-containing glycosylglycine endopeptidase whose catalytic activity is similar to the well-characterized lytic enzyme lysostaphin. Both enzymes degrade *S. aureus* cell wall peptidoglycan by cleaving its pentaglycine crosslinks. We cloned the *lytM* gene from *S. aureus* SA113, appending an N-terminal His6 coding sequence. In an effort to enhance the enzyme’s antibacterial activity, we also created a second construct in which LytM was fused to the C-terminal cell wall binding domain of lysostaphin. Both proteins were expressed in *E. coli* and purified in a two-step chromatographic procedure. Activity towards *S. aureus* strain SA113 was assessed by a fluorogenic kinetic assay and determination of minimal inhibitory concentrations (MICs). LytM showed poor kinetics compared to a lysostaphin positive control, and its MIC value could not be determined (>110 μg/mL). However, LytM cleared turbid *S. aureus* suspensions during long incubations, demonstrating low but non-negligible lytic activity. In contrast, the fusion protein exhibited reasonable kinetics, with a specific activity of 34±5 units/s/μg compared to 272±8 units/s/μg for lysostaphin. Importantly, the MIC for the fusion was equivalent to that of lysostaphin (1.3 μg/mL). These data suggest that LytM requires a cell wall binding domain to manifest useful levels of lytic activity when applied exogenously. We have developed a novel fusion of the *S. aureus* autolysin LytM and the cell wall binding domain of lysostaphin. Compared to lysostaphin itself, the fusion exhibited moderately reduced kinetics but equivalent growth inhibition. Future studies will quantify rates of resistance development, anti-biofilm activity, and in vivo efficacy.

**114 (G) Eravacycline (TP-434) is Potent against Category A and B Pathogens**  

**Background:** Eravacycline (ERA) is a novel broad-spectrum tetracycline being developed for the treatment of serious gram-negative and gram-positive aerobic and anaerobic bacterial infections. ERA is impervious to known tetracycline-specific-resistant mechanisms for efflux, ribosomal protection, and inactivation. **Methods:** ERA and comparators were tested against 167 strains of *Bacillus anthracis*, *Yersinia pestis*, Francisella tularensis, *Burkholderia mallei*, and *Burkholderia pseudomallei* using standard CLSI microtiter-based methods to determine MIC values. Quality control of experimental conditions was established using *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213. **Results:** MIC90 results for ERA and comparators against bioterror pathogens are shown in Table 1. To determine the impact of well characterized resistance-nodulation-cell-division (RND) efflux pumps on susceptibility, ERA and comparators were tested in isogenic strains of *B. pseudomallei* 1026b expressing 0-3 up-regulated or disabled RND pumps. Similar to doxycycline (DOX), ERA was effluxed by BpeEF-OpcR and to a lesser extent by the AmrAB-OprC and BpeAB-OprB efflux pumps. However, the degree of efflux of ERA by a strain overexpressing BpeEF-OprC was significantly lower than that observed with DOX and the observed MIC (2 μg/mL) may well be within the therapeutic range.

### Table 1: Susceptibility (MIC90 in μg/mL) of bioterror pathogens to ERA and comparators

<table>
<thead>
<tr>
<th>Compound</th>
<th>B. anthracis (35)</th>
<th>B. mallei (39)</th>
<th>B. pseudomallei (35)</th>
<th>F. tularensis (32)</th>
<th>S. aureus (34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERA</td>
<td>0.016</td>
<td>0.25</td>
<td>2</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>TET</td>
<td>0.021</td>
<td>0.125</td>
<td>8</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>PEN</td>
<td>0.125</td>
<td>nd</td>
<td>nd</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>CIP</td>
<td>0.06</td>
<td>2</td>
<td>nd</td>
<td>0.125</td>
<td>0.031</td>
</tr>
<tr>
<td>GEN</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>CAZ</td>
<td>nd</td>
<td>4</td>
<td>4</td>
<td>nd</td>
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<tr>
<td>BLM</td>
<td>nd</td>
<td>nd</td>
<td>2</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

*DOX: doxycycline; TET: tetracycline; PEN: penicillin G; CIP: ciprofloxacin; GEN: gentamicin; CAZ: ceftazidime; BLM: imipenem*
115 (G) Identification of Broad-Spectrum Therapeutics against Filoviruses
L. M. Evans DeWald1, S. Stronsky1, C. Lear1, L. Pierce1, J. M. Grenier1, A. Stosse1, L. M. Johansen1, P. J. Glass1, G. G. Olinger2; 1U.S. Army Med. Res. Inst. of Infectious Diseases, Frederick, MD, 2Zalicus Inc., Cambridge, MA.

Ebolavirus and Marburgvirus, genera of the family Filoviridae, are classified as category A agents due to their high morbidity and mortality rates and their potential for misuse in biological warfare or as terrorism threats. Currently, there are no approved therapeutics or vaccines for filovirus infections in humans. Even if therapeutics were available, the fast onset and similarities in symptoms make it difficult to identify the species responsible within a therapeutic window. Therefore, identifying broad-spectrum drugs is advantageous because it decreases the necessity to identify the filovirus species before commencing treatment and may provide a therapeutic defense against known, emerging, and possible engineered bioterrorism threats. FDA-approved compounds are enticing because of their potential to rapidly move into clinical testing and their availability for rapid distribution in the case of an outbreak or a biological attack. Previously, we screened a set of ~2,600 approved drugs and biologically active molecules during which we identified a set of 43 FDA-approved compounds with potent antiviral activity. Using antibodies against the species-specific glycoprotein (GP), we developed a screening system to evaluate this set of drugs for their potential broad-spectrum activity against different filovirus species and strains, including Ebola, Sudan, Marburg, and Ravn. These cell-based ELISA screening systems are advantageous over traditional plaque assays and yield reduction assays due to their higher-throughput nature. They also allow for a majority of the assay, other than the infection period, to be performed in a biosafety level-2 setting thereby minimizing risk to the researcher. Using this approach, we identified 40 FDA-approved compounds that have broad-spectrum antiviral efficacy against filoviruses. We are currently investigating the mechanism of anti-filovirus activity of these compounds as well as working to identify any possible synergies when used in combinations.

116 (G) A Broad-Spectrum Host-Based Antiviral Drug Platform for Emerging Viral Diseases
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Unither Virology is developing a host-based platform of iminosugar compounds which shows promise for developing broad spectrum antiviral drugs with potential to treat viruses from multiple virus families. These iminosugars inhibit processing of viral glycoproteins and impair viral assembly, secretion and infectivity. In vitro, compounds from the platform have shown activity against virus families including arena, buny, filo, flavi, orthomyxoviruses, and show protection against each of these families. Therapeutic protection has been demonstrated against dengue and influenza virus. Candidate compounds from the platform are safe and are orally bioavailable. Thus, drugs based on this platform could provide important defense against known and emerging virus threats.

117 (G) Safety and Pharmacokinetics of a Novel Co-Mixture of Three Monoclonal Antibodies against Botulism in Healthy Subjects
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Background: Botulism is a Category A bioterrorism agent. Current therapies use antitoxins from scarce pooled human plasma or equine serum, which have multiple adverse effects. New approaches are needed. XOMA 3AB consists of 3 IgG monoclonal antibodies (mAbs), each with a distinct humanized variable region against botulinum neurotoxin serotype A. This Phase I study evaluated safety and pharmacokinetics (PK) of escalating doses of XOMA 3AB administered intravenously (IV) to healthy adults. Methods: Double-blind, placebo-controlled, dose-escalation study of 3 cohorts of 8 subjects, each with 6 active and 2 placebo recipients. The initial cohort dose was 0.033 mg/kg, and subsequent groups received 0.165 mg/kg and 0.33 mg/kg of XOMA 3AB, administered IV in 100 mL of saline over one hour. PK measurements were collected through day 90 for the first cohort and through day 120 for later groups. PK parameters were estimated using non-compartmental methods. Safety evaluations included physical exams, clinical chemistries and EKGs at Days 1, 2, 3, 7, 14, 28, 42, 56, 90, and 120 (for later groups). Results: There were no infusions discontinuations or hypersensitivity reactions. Two or more subjects experienced headache, hyperglycemia, transient hypereosinophilia and anemia. All adverse events (AEs) were mild to moderate except for an episode of exercise-induced rhabdomyolysis deemed unrelated to XOMA 3AB. There were no dose-dependent trends in AEs. Concentration-time plots indicated a rise in MAb that did not coincide with end of the infusion, but lagged by another 1 to 2 hours. After the rise to peak concentration, MAb concentration declined in a biexponential decay pattern, for all 3 analytes. Cmax and AUC generally increased as the dose increased with each cohort, for all 3 MAbs. None of the MABs were found to be immunogenic. Conclusions: Administration of XOMA 3AB was well tolerated by healthy subjects at all doses.

118 (G) Monoclonal Antibodies against Dengue Virus Type 4 and Their Epitopes in the Association with Viral Neutralization and Enhancement

The four serotypes of dengue virus (DENV1-4) infect 50 to 100 million humans annually worldwide and pose a threat to global health. DENV infection causes a spectrum of clinical syndromes, including dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). In severe cases, mortality rate among infected patients is high, particularly in patients with secondary infection with a heterologous serotype. To date, there is no approved vaccine that helps to alleviate the symptoms. In order to investigate the roles of anti-DENV4 antibodies, we developed a panel of 17 new monoclonal antibodies (mAbs) and characterized their specificity by Immunofluorescent assay (IFA) and Western blotting. Furthermore, we evaluated the neutralizing activity through plaque reduction neutralization test and suckling mice protection assay. One of the serotype-specific mAbs displays strong neutralizing activities in vitro and in vivo. On the other hand, the enhancement of heterologous DENV infection was examined by in vitro ADE assay. Two of the cross-reactive and poorly neutralizing mAbs highly enhance DENV infection. Finally, the epitopes of neutralizing and enhancing mAbs were identified by structure prediction by computer, and epitope mapping by VLP mutants. In conclusion, these results provide information for epitopes in immune responses against DENV4 and development of safety vaccine in the future.
119 (G) Antisense Inhibition of Growth in Methicillin-Resistant *Staphylococcus aureus* by Locked Nucleic Acid Conjugated with Cell-Penetrating Peptide Inhibit as a Novel FtsZ Inhibitor

**M. Jingru**, D. Fei, S. Guojun, M. Yue, J. Min, L. Xiaoxing; Sch. of Pharmacy, Fourth Military Med. Univ., Xi An, China.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is One Of The Most Frequent Causes Of Life Threatening Infections In Hospital- And Community-acquired patients. MRSA infections are becoming increasingly difficult to treat owing to acquired antibiotic resistance. The emergence and spread of multi-drug resistance MRSA limits therapeutic options and requires new therapeutic strategies and develops novel MRSA-active antibiotics. Filamentous temperature-sensitive protein Z (FtsZ) is a highly conserved bacterial tubulin homologue, which is essential for controlling bacterial cell division process in different species of *S. aureus*. In this study, the peptide (KFF) K-conjugated locked nucleic acid (PLNAs) were synthesized to target ftsZ mRNA, which encodes FtsZ protein. We identified PLNA787, which is complementary in sequence to nucleotide 787-808 in the coding region of ftsZ mRNA, being the most active among PLNAs by further optimization. PLNA787 exhibited promising antisense bactericidal effect against four pathogenic *S. aureus* strains with different drug-resistance profiles, including clinical vancomycin-intermediate resistance *S. aureus* MU50 in concentration-dependent manner, whereas a PLNA with a scrambled base sequence had no effect on bacterial growth. Bactericidal effects of PLNA787 were associated with specific and potent suppression of ftsZ mRNA and FtsZ protein in a concentration-dependent manner. PLNA787 (12.5μM) completely inhibited bacterial growth cured lethal Mu50 infection in epithelial cell cultures, meanwhile didn't show appreciable cytoxicity against human cells. The SEM analyses of Mu50 shown in PLNA787 treated group the size of Mu50strain enlarged in a tented manner. PLNA787 (12.5μM) completely inhibited bacterial growth fixed for residual bacterial count. **Results:** GC-072 showed MIC values of 0.125 mg/L against Bps while ciprofloxacin gave an MIC of 4 mg/L. Against five Bps clinical isolates GC-072 showed MIC values of between 0.125-6.5 mg/L while Dmoxycycline gave a MIC of 1 mg/L. In the case of select agents (*B. abortus, B. melilii, T. tularenis* and *Y. pestis*), GC-072 showed a MIC90 of 0.125 mg/L while ciprofloxacin MIC90 was 4 mg/L. In an acute murine melioidosis model, survival rates at 21 days after infection were; 12.5% (1/8) for excipient only, 87.5% (7/8) for mice treated with Cef and 75% for animals treated with GC-072. Clinical scores were comparable in the treated groups. Analysis of spleen residual Bps loads showed data below the limit of detection for all but one mouse in each of the GC-072 and Ceftazidime treated groups. **Conclusions:** GC-072 is efficacious against *B. pseudomallei* in vitro MIC studies. In an in vivo lethal/acute murine melioidosis protection study, GC-072 was delivered orally and showed clinical survival rates and survival times comparable to Cef, which was given at higher doses and intraperitoneally. This novel molecule may become a valuable drug against *B. pseudomallei* and other biothreat agents.

120 (G) Efficacy of Human Monoclonal Antibodies as an Immunotherapeutic in a Guinea Pig Model of Lassa Fever

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Lassa virus (LASV), an arenavirus, is the causative agent of Lassa fever (LF) and a Biosafety Level 4 and NIAID Biodefense category A agent. LF is a major public health concern causing widespread loss of life and social disruption across West Africa. There is no approved LF vaccine, and ribavirin, the only available therapeutic, has modest efficacy and severe side-effects. Monoclonal antibodies have been derived from Lassa fever survivors with sustained IgG titers against recombinant LASV antigens treated at Kenema Government Hospital in Sierra Leone. Production of two LASV neutralizing huMabs to over 100 mg scale in a transient mammalian cell system allowed for a study of passive transfer studies in a newly developed uniformly lethal guinea pig LF model. To pilot whether hMabs may serve as an immunotherapeutic outbred guinea pigs were injected with a single dose of approximately 30 mg/kg and 15 mg/kg of 19.7E and 10.4B, respectively, on the same day as LASV challenge. 100% of GP (4/4) in the control group injected with antibody diluent had succumbed by day 16 post-infection, whereas none of the guinea pigs (4/4) in the hMab treated group have displayed any signs or symptoms of LF. A LF immunotherapeutic could provide critical layers of protection for the survivors with sustained IgG titers against recombinant LASV antigens treated at Kenema Government Hospital in Sierra Leone. Production of two LASV neutralizing huMabs to over 100 mg/kg. The antibody diluent has succumbed by day 16 post-infection, whereas no sign of the guinea pigs (4/4) in the hMab treated group have displayed any signs or symptoms of LF. A LF immunotherapeutic could provide critical layers of protection for the LF warfighter, first responders, and conducting research in development of LF countermeasures. It could also be used to treat those suspected of being exposed both in the event of an intentional release and in public health settings.

121 (G) GC-072 is Effective against the Biothreat Agent *Burkholderia pseudomallei* and Other Select Agents

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**Background:** GC-072 is a novel bacterial topoisomerase inhibitor with excellent activity in vitro and in vivo activity against the *B. pseudomallei* (Bps) and other biothreat agents. GC-072 had excellent therapeutic efficacy, delivered orally, in an acute murine melioidosis model. **Methods:** GC-072 was tested in vitro against Bps and other biothreat bacteria. MIC determinations were performed according to CLSI guidelines. In an animal protection study, groups of 8 BALB/c mice were challenged intranasally with 5,500 CFU of *B. pseudomallei* strain 1026b. Treatment was initiated 8 hours later with either excipient only, GC-072 orally at 30 mg/kg, or ceftazidime (Ceft) intraperitoneally (i.p.) at 50 mg/kg, TID for seven days. Survival, clinical score and weights were recorded through day 21 when surviving animals were sacrificed for residual bacterial count. **Results:** GC-072 showed MIC values of 0.125 mg/L against Bps while ciprofloxacin gave an MIC of 4 mg/L. Against five Bps clinical isolates GC-072 showed MIC values of between 0.125-6.5 mg/L while Doxycycline gave a MIC of 1 mg/L. In the case of select agents (*B. anthracis, B. abortus, B. malilii, T. Tularenis* and *Y. pestis*), GC-072 showed a MIC90 of 0.125 mg/L while ciprofloxacin MIC90 was 4 mg/L. In an acute murine melioidosis model, survival rates at 21 days after infection were; 12.5% (1/8) for excipient only, 87.5% (7/8) for mice treated with Cef and 75% for animals treated with GC-072. Clinical scores were comparable in the treated groups. Analysis of spleen residual Bps loads showed data below the limit of detection for all but one mouse in each of the GC-072 and Ceftazidime treated groups. **Conclusions:** GC-072 is efficacious against *B. pseudomallei* in in vitro MIC studies. In an in vivo lethal/acute murine melioidosis protection study, GC-072 was delivered orally and showed clinical survival rates and survival times comparable to Cef, which was given at higher doses and intraperitoneally. This novel molecule may become a valuable drug against *B. pseudomallei* and other biothreat agents.

122 (G) Efficacy of Intramuscularly (IM) Administered Antitoxin in Preventing Inhalational Anthrax-Related Mortality in the Cynomolgus Model of *Bacillus anthracis* Infection

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**Background:** Inhalational anthrax is a serious bioterrorist threat. It is anticipated that large numbers of people may be impacted in an event of bioterrorism emergency, and there is an unmet need for an antitoxin that can be easily administered to people in the post-exposure scenario. ETI-204 is a humanized de-immunized antibody against the protective antigen (PA) component of anthrax toxins. ETI-204 is being developed for intravenous treatment of patients with inhalational anthrax. Study was designed to determine protective efficacy of ETI-204 when administered IM to cynomolgus monkeys at increasing times post anthrax spore exposure. **Study Design:** Cynomolgus monkeys were aerosol challenged with a targeted 200 LD50 of *B. anthracis* (Ames strain) spores. A single dose of 16 mg/kg ETI-204 was administered IM to monkeys at 24, 36, or 48 hours following exposure. Survival was the primary study endpoint; secondary endpoints included analyses of bacteraemia and toxemia. Animals confirmed bacteraemic prior to treatment were considered symptomatic. **Results:** IM ETI-204 administration 24, 36 and 48 hrs following challenge protected 92.8%, 42.8%, and 28.5% of animals, respectively. The survival rate of the control group was 10%. Irrespective of the time of administration, 100% of ETI-204 treated animals that were non-bacteraemic and ~30% of those that were bacteraemic survived. In animals that were not bacteraemic at the time of treatment, ETI-204 completely prevented bacteraemia development. **Conclusions:** IM ETI-204 was highly efficacious in protecting cynomolgus monkeys against inhalational anthrax when administered IM up to 48 hrs post spore challenge. In addition, ETI-204 administered IM may be efficacious in treatment of bacteraemic animals. Our results support the feasibility of further assessment of IM ETI-204 efficacy under both non-symptomatic and symptomatic disease scenarios. Supported by Federal funds from ASPR/BARDA under Contract No. HHSO10020100026C.
123 (G) FDA-Approved, Host Targeted Drugs with Broad Spectrum Antiviral Activity

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Background: Viral pathogens can cause widespread disruption of world economies and public health systems. Antiviral drugs are an important means to mitigate the impact of biodefense threats and emerging infections. Our objective is to develop a broad spectrum antiviral therapy that can be used to treat multiple etiologic agents. We hypothesize that: 1) within the pharmacopoeia of approved drugs there are compounds that inhibit cellular pathways important for virus replication, and 2) multiple viruses share a reliance on common cellular pathways for replication, and thus inhibition of these pathways will result in broad spectrum antiviral activity. Method: To identify host targeted drugs with antiviral activity, a library of FDA-approved drugs was screened against influenza. Drugs identified in the initial screen were categorized by mechanism of action and prioritized by both in vitro activity and clinical toxicity. Selected drugs with influenza antiviral activity were also evaluated against a panel of unrelated viruses, including category A, B, and C viruses. Results: Over seventy compounds were initially identified as having antiviral activity. Putative cellular pathways inhibited by the drugs included nucleoside synthesis, cell membrane biosynthesis, tubulin polymerization, protein folding, and receptor signaling. Forty of the drugs were retested to confirm activity and ten drugs demonstrated sub- to low micromolar activity and good selective index against influenza. When tested against other viruses, most drugs were active against more than one virus, with drugs targeting nucleoside metabolism having the broadest spectrum of activity. For instance, one nucleoside synthesis inhibitor had strong activity (EC50 from 0.002 to 9 mcM) against thirteen different viruses, including pox, arena, and flaviviruses. Conclusion: Inhibition of cellular pathways by FDA-approved drugs result in potent and broad spectrum antiviral activity.

124 (G) Recombinant Monoclonal Antibody Based Antitoxins for Treatment of Type A, B, and C Botulism

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Background: XOMA is developing anti-BoNT monoclonal antibody (mAb) cocktails to treat poisoning by BoNT/A, B and E mechanisms and iron utilization with and without iron supplementation. Using a platform process for antibody engineering, cell culture, purification, and ELISA characterization and formulation has been accomplished. XOMA 3AB, the lead monoclonal product with broad anti-BoNT/A properties, has been evaluated in humans for safety in a Phase I clinical trial. GMP anti-Botulinum antibodies have been filled and placed on stability. Methods: Candidate antibodies were characterized with respect to epitope, affinity, and in vitro and in vivo toxin neutralization. Cell membrane biosynthesis, tubulin polymerization, protein folding, and receptor signaling. Forty of the drugs were retested to confirm activity and ten drugs demonstrated sub- to low micromolar activity and good selective index against influenza. When tested against other viruses, most drugs were active against more than one virus, with drugs targeting nucleoside metabolism having the broadest spectrum of activity. For instance, one nucleoside synthesis inhibitor had strong activity (EC50 from 0.002 to 9 mcM) against thirteen different viruses, including pox, arena, and flaviviruses. Conclusion: Inhibition of cellular pathways by FDA-approved drugs result in potent and broad spectrum antiviral activity.

125 (H) On the Influence of Immunomodulation on the Early Phase of Antigen-Specific Response of Rabbits Immunized a Living Plague Vaccine EV

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12 rabbits immunized EV vaccine at 3 x 10^9 bacterial cells in 0.5 ml of physiologically solution. Simultaneously with immunization of rabbits NoNe 1 - 4 Polyoxidonium administered intravenously at a dose of 0.3 mg / kg body weight, rabbits NoNe 5 - 8 was injected Betaleukin (IL-1) in / at a dose of 0.5 mg, rabbits NoNe 9 - 12 were administered physiologic solution in the same volume (the control group). In the individual suspensions of lymphocytes isolated by density gradient of 1.077 blood collected in dynamics - before and after 2, 4, 7, 14, 21, 28, 35 and 42 days after immunization, determined the relative content of lymphocytes with receptors (LIR) antigen F1 Y. pestis in response to lymphocytes of adhesion immunoreagents optimal and suboptimal sensitivity, we obtained by conjugation with rivanol F1 c bovine erythrocytes, fixed acetaldehyde. In parallel performed such analyzes of the reagent erythrocyte, not laden F1. As indicators of influence on the dynamics of immunomodulation in LIR was used the first and last days of detection LIR, achievements to date and the amount of the maximum of their content, the total (for the entire period of detection) content LIR identified by immuno reagents optimal and suboptimal sensitivity. For each of these indicators is determined by the rank of the group. Overall immunomodulation accelerated appearance and disappearance LIR, maximum achievement of their content, reducing the amount of the maximum and total LIR. Earlier in several experimental models and in the clinic has been shown that these changes reflect the activation of the early phase of antigen-specific responses and determine the intensity of the subsequent effector phase of the immune response. This study also found the advanced appearance of anti-F1 antibodies in rabbits receiving immunomodulators. Thus, the use of IL-1 and Polyoxidonium may enable accelerate the development antigen-specific response to the EV vaccine in the early and effector phases.

126 (H) The Burkholderia mallei tonB Mutant Exhibits an Attenuated Phenotype and Provides Protective Immunity against Acute Inhalational Murine Glanders

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Background: Burkholderia mallei, the causative agent of glanders, has been classified by the CDC as a category B bioagent due to its highly infectious nature and the lack of an available vaccine. Iron is only present in growth limiting concentrations within the host; therefore, to establish infection, most Gram negative bacteria employ TonB-dependent active transport for iron uptake. Thus, we hypothesize that deleting TonB will generate an attenuated B. mallei mutant that can elicit protective immunity. Methods: The B. mallei tonB mutant was created using a suicide plasmid that allowed the insertion of an unmarked deletion via allelic exchange. The tonB mutant was then assessed for changes in growth kinetics and iron utilization with and without iron supplementation. Using an optimized acute respiratory challenge model of B. mallei infection in BALB/c mice, the tonB mutant was evaluated for degree of attenuation and potential use as a vaccine. Using a B. mallei luminescent reporter strain, the dynamics of wild-type infection were monitored using in vivo imaging technology while bacterial burden was assessed by CFU counts and histopathology of organs. Results: In media not supplemented with iron, the tonB mutant displayed reduced ability to grow and utilize various iron sources compared to wild-type. Although, the tonB mutant displayed wild-type fitness when grown in iron supplemented media. The attenuated phenotype was also seen in vivo resulting in decreased killing when grown in the absence of iron supplementation. BALB/c mice vaccinated with the tonB mutant were 100% protected against acute inhalational glanders and free of detectable reporter strain. Conclusion: 1. The tonB mutation results in the reduced fitness of B. mallei by impairing its ability to acquire iron. 2. The degree of fitness/virulence of the tonB mutant is correlated with the amount of iron present during growth. 3. The tonB mutant may be a promising live attenuated vaccine strain.
127 (H) Animal Models Supporting Bridge ELISA Titors as a Correlate of Protection for a Recombinant Plague Vaccine (rF1V)  
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Objective: The primary objective of these studies was to establish the relationship between antibody titers and survival in rF1V-vaccinated cynomolgus macaques (CM) and Swiss Webster mice following aerosol challenge with Yersinia pestis CO92. A secondary objective was to evaluate amelioration of disease in vaccinated and challenged CMs.

Methods: CMs were vaccinated on Days 0, 56, and 121 with dosages of rF1V ranging from 0.016 μg to 20 μg per CM. Serum was collected for evaluation of antibody titers on Day 149 prior to aerosol exposure to a lethal dosage of Y. pestis CO92. Mice were vaccinated on Days 0, 14, and 28 with dosages of the rF1V vaccine ranging from 0.0032 μg to 16 μg per mouse. Serum was collected for evaluation of antibody titers on Day 43. Mice were challenged on Day 44 by aerosol exposure to a lethal dosage of Y. pestis CO92. Control animals were infected with saline using the same vaccination schedule and challenged with CO92 as described for the vaccinated CMs and mice. Clinical signs of disease and survival were observed in mice and CMs for 14 and 21 days post-challenge, respectively. Pathology evaluations were performed in CMs at the end of the study.

Results: A dose response curve was observed in both CMs and mice. Logistic regression modeling showed a statistically significant relationship between increased antibody titers and probability of survival (p<0.0001) in both models. The highest dosage of rF1V administered to CMs and mice provided 87% survival, respectively. Significant amelioration of disease in a dose-dependent manner with a delay in onset of fever and fewer lung lesions observed in CMs receiving higher dosages of rF1V.

Conclusion: The rF1V vaccine provides protection in a dosage-related manner to CMs and mice following aerosol exposure to a lethal dosage of Y. pestis. Data generated in these studies supports the use of titers measured by Bridge ELISA as a correlate of protection. The results also guide the selection of vaccine dosages for pivotal efficacy studies supporting vaccine licensure using the Animal Rule.

128 (H) Phase 1 Safety and Immunogenicity Study of Four Formulations of Anthrax Vaccine Adsorbed Plus CPG 7909 (AV7909) in Healthy Adult Volunteers  

In a previous Phase 1 study of 3 doses (Days 0, 14, and 28) of an admixture of BioThrax® (Anthrax Vaccine Adsorbed) and 1 mg of the vaccine adjuvant CPG 7909, toxin neutralizing antibody response was higher than with BioThrax alone. In a second Phase 1 study, the safety and immunogenicity of 4 formulations of AV7909 (AVA plus CPG 7909), BioThrax, or saline placebo were evaluated using 2 IM doses (Days 0 and 14). A total of 105 healthy adults 18 to 50 years of age were randomized to 1 of 6 study groups: BioThrax (0.5 mL), AV7909 Formulation 1 (0.5 mL AVA + 0.5 mg CPG 7909), AV7909 Formulation 2 (0.5 mL AVA + 0.25 mg CPG 7909), AV7909 Formulation 3 (0.25 mL AVA + 0.5 mg CPG 7909), AV7909 Formulation 4 (0.25 mL AVA + 0.25 mg CPG 7909), or saline placebo. Subjects completed the trial. In the AV7909 groups, 73.7% to 100% of subjects experienced adverse events (AEs) related to vaccination compared with 83.3% for BioThrax and 26.7% for saline placebo. Most of these events were injection site reactions. The percentages in the AV7909 groups were not associated with the amount of AVA or CPG 7909 dose. Most AEs were mild or moderate in severity. The subject incidences of local and systemic reactions recorded in diaries for 7 days after each injection appeared highest with Formulation 1. After 2 doses, peak geometric mean toxin neutralizing antibody values (TNF NF50) in the AV7909 groups (achieved on Day 28) were 13- to 30-fold higher than in the BioThrax group (achieved on Day 35). Formulation 2 elicited the highest peak titer, followed by Formulation 1, although differences among the AV7909 groups were not statistically significant. AV7909 Formulation 2 was selected for further development in Phase 2 based on its immunogenicity and reactogenicity profiles.

129 (H) Novel Approaches to Inactivate Chikungunya Virus: Potential Strategies for Vaccine Development  
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Chikungunya virus (CHIKV), an arbovirus, has recently caused several epidemics in the Indian Ocean Islands with high morbidity and fatality. Though the typical symptoms of CHIKV infection are fever and rash, several patients develop severe chronic symptoms of long lasting joint pain and polyarthralgias. The US Army had developed a live-attenuated vaccine candidate, CHIKV-181/25 (TSI-GSD-218), but it failed during phase II clinical trial due to high rate of transient arthralgia in vaccinees. Thus, circulation of highly virulent CHIKV strains, ease of their transmission and absence of any effective vaccine or specific antiviral therapy project a major global health concern and highlight the urgent need for developing a highly potent and immunogenic vaccine against CHIKV. We have evaluated two different novel strategies for CHIKV inactivation. First, UV-based inactivation using a photocative compound, 1,5 iodonaphthyl azide (IANA), which covalently binds to the viral proteins upon irradiation, inactivating the virus without altering the overall structure of its epitopes. In another approach, CHIKV was gamma irradiated in presence of radioprotective Mn-DP-PI complexes which protect the viral epitopes from the oxidative damage caused by high doses of irradiation without interfering with complete genome degradation. Both inactivation strategies resulted in completely inactivated virus particles. Mn-DP-PI complexes significantly protected the viral epitopes from any oxidative damage at supraletal doses of irradiation which completely destroyed the viral genome. Thus, our studies provide new approaches of potential inactivated viral vac- cine candidate development. We plan to evaluate the protective efficacy of these inactivated preparations against challenge with virulent CHIKV in vivo. This work was supported by funding from the Defense Threat Reduction Agency. Opinions presented here are of the authors and should not be construed as that of USUHS and BITs.

130 (H) A Francisella Lipid a Mutant Confers Sterilizing Immunity as well as Therapeutic Antibodies Capable of Protecting Mice after Lethal Challenge  
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Francisella tularensis tularensis (Ft) is an intracellular gram-negative bacterium and the causative agent of the severe human disease tularemia with potential for use as a bioweapon. Francisella lipid A, the biologically active component of lipopolysaccharide (LPS) has extremely low endo- toxic activity. A Francisella tularensis novicida (Fn) lipid A biosynthesis mutant was generated that lacked the 4-phosphatase enzyme (LpxF). Analysis of lipid A isolated from this mutant strain, compared to WT Fn showed retention of the phosphate moiety at the 4’ position and the N-linked fatty acid at the 3’ position on the diglucosamine backbone. This mutant was previously shown in our laboratory to be avirulent and confer protective immunity to a lethal WT Fn challenge. The role of B cells in this protection was examined using μMT-/- mice, which lack functional B cells. All vaccinated μMT-/- mice succumbed to the lethal challenge. This complete lack of protection shows that B cells are absolutely required for this protective response. While development of an effective vaccine to Francisella remains a priority, we decided to address the therapeutic potential of serum obtained from immunization with the LpxF-null mutant in the possibility of a Francisella outbreak. Mice were infected with Fn and then were treated with serum either from naive or immune mice. Mice receiving immune serum were completely rescued out to 36 hours post-infection, whereas mice receiving naive serum started succumbing to in fection at 60 hours post-infection, indicating the serum has therapeutic potential even late in an infection. The identity of the proteins recognized by this protective serum was further investigated. Our work identified known Francisella immunogens as well as proteins previously unknown to be antigenic. Serum from LVS LpxF-null vaccinated-mice showed a similar protective capacity when given as a therapeutic. Current work is being expanded to generate monoclonal antibodies to these identified proteins and assay their ability to be used in the event of a Francisella outbreak.
131 (H) Stability Study of an Attenuated Smallpox Vaccine
LC16m8
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Introduction: LC16m8 is an attenuated smallpox vaccine and was licensed in 1975 in Japan. However, due to the cessation of routine vaccination against smallpox in 1976, the LC16m8 vaccine has never been placed on the market at that time. Recently, in response to the increasing threat of bioterrorism using smallpox virus, the Japanese Government has started stockpiling the LC16m8 vaccine for an emergent use as a countermeasure against bioterrorism. From this point of view, it is desirable that the LC16m8 vaccine has a high stability and maintains it as long as possible for a long storage. Methods: In accordance with ICH guidelines, we’ve been performing long-term stability studies on three lots of LC16m8 vaccine in two storage conditions. One is at -20ºC and another is at between 2 and 8ºC. In addition, we examined the stability of reconstituted vaccine stored at ambient temperature for 30 days after reconstitution. The long-term stability studies will be continued to be up to 120 months. Results: Regarding the long-term stability studies, each of vaccines stored at -20ºC or at between 2 and 8ºC passed all the specification testing up to 72 months of storage. No significant change was observed in the potency of the vaccines examined. As for the stability of the reconstituted vaccine, the vaccine maintained a high level of potency during storage at ambient temperature for 30 days. Conclusion: The results of the stability studies indicated that LC16m8 vaccine has a highly stability and is suitable as a stockpiled vaccine for an out break event. Based on the results, storage at -20ºC for 4 years was additionally authorized in Japan.

132 (H) Characterization of Humoral and Cell-Mediated Immune Responses to Formalin-Inactivated and Gamma-Irradiated EEEV Vaccine Candidates

Recent studies have demonstrated 80-100% protection from aerosolized virulent eastern equine encephalitis virus (EEEV) FL93-939 strain challenge in mice administered two doses of a formalin- or gamma-irradiation-inactivated EEEV vaccine candidate. It has long been accepted that neutralizing antibody responses correlate with protection against alphavirus disease. This appears to be the correlate of protection against subcutaneous infection with alphaviruses; however, current studies show that this may not be the case for protective efficacy against aerosolized alphaviruses. To more clearly define the immune response(s) involved in the protective efficacy of next-generation inactivated EEEV vaccine candidates, the cellular and humoral immune responses in mice administrated with one or two doses of a formalin- or gamma-irradiation-inactivated EEEV vaccine candidate were characterized. Splenic T-cell populations and cytokine profiles were compared in vaccinated versus unvaccinated mice after in vitro re-stimulation of T cells. Serum-neutralizing antibody responses and virus-specific Ig responses were examined by plaque reduction neutralization assay and ELISA, respectively. Evaluation of the immune responses in mice vaccinated against EEEV will aid in assessing and improving the efficacy of current and future alphavirus vaccines.

133 (H) Evaluation of a Burkholderia pseudomallei Outer Membrane Vesicle Vaccine in Non-Human Primates

Burkholderia pseudomallei (Bps), the causative agent of melioidosis, is inherently resistant to multiple antibiotics and is considered a potential biological warfare agent by the U.S. DHHS. An effective vaccine is urgently needed to combat infection and to safeguard against biological attack with Bps. In previous work, we showed that immunization with outer membrane vesicles (OMVs) provides significant protection against lethal aerosol and systemic challenge in mice. Here we evaluated the safety and immunogenicity of escalating doses of OMV vaccine in rhesus macaques. We hypothesize that immunization of primates with Bps OMVs will generate protective antibody and cell-mediated immune responses without any associated toxicity or reactogenicity. Two groups of macaques were immunized with either Bps OMVs admixed with 600 μg CpG or 400 μg CpG alone. Immunizations were performed on days 0, 28, and 56. OMV immunizations were given using a dose escalation with the first dose containing 25 μg OMV, the second dose 50 μg, and the third dose 100 μg. Safety of the OMV vaccine was evaluated by complete blood chemistry and clinical observation. OMV-specific IgM, IgG, and IgA were measured in plasma of OMV-immunized and control animals by ELISA. T cell responses were measured using PBMCs isolated from blood. PBMCs were restimulated with inactivated whole bacteria or OMVs and cell proliferation was measured by CFSE stain. No toxicity was observed in any animal that received up to 50 μg OMV. Toxicity results for the third dose are pending. Vaccination after a single dose of 25 μg OMV elicited Bps-specific serum IgG superior to that induced by experimental infection. Analyses of antibody responses to the second and third doses as well as T cell responses to OMV immunization are underway. In conclusion, this work demonstrates the safety and immunogenicity of a Bps OMV vaccine in non-human primates at doses equivalent or higher to that previously used in humans (Naess et al, 1998). Future studies will evaluate the protective efficacy of the OMV vaccine against Bps in this large animal model.

134 (H) Preclinical Development of a Dual-Purpose Biodefense Vaccine for Yersinia pestis and Orthopoxviruses
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Background: The adoption of MVA as a second-generation smallpox vaccine makes recombinant MVA vaccines attractive candidates for biodefense. We have developed a recombinant MVA vaccine expressing antigen from Yersinia pestis that protects against plague and monkey poxvirus. Methods: Efficacy of an MVA recombinant vaccine (MVA/C13L/V307) expressing a truncated version of the virulence (V 307) antigen from Y. pestis (CO92) fused to a putative secretory signal from vaccinia virus (C13L) was tested in a mouse and a monkey challenge models. Immunized mice and monkeys were challenged intranasally with lethal doses of Y. pestis (CO92) or monkey poxvirus (Zaire). Passive transfer of immune serum as well as longevity of the vaccine’s efficacy was also assessed against challenge with Y. pestis in mice. Results: A single or two doses of the vaccine conferred complete protection against challenge (100LD50) with monkey poxvirus in mice or monkeys respectively. A single dose of the vaccine conferred partial (50%) protection in monkeys but failed to protect immunized mice against 100LD50 of Y. pestis. A prime and booster immunizations conferred complete protection against lethal challenge with 100LD50 of Y. pestis in both mice and monkeys. The vaccine elicited strong antibody titers (60895 ± 163 GMT) to Y. pestis (CO92) following prime and booster immunizations in mice. Antibody titer of 179 ± 22 GMT was elicited to monkey poxvirus (Zaire) following a single dose of the vaccine in mice. Passive transfer of antibodies with a titer of 6400 GMT conferred 75% protection in immunized mice following challenge with Y. pestis. Eight months following prime and booster immunizations, the vaccine conferred 87.5% protection against lethal challenge with Y. pestis. Serological analysis shows monkeys are infected from samples of monkeys is ongoing. Conclusion: MVA/C13L/V307 is a promising candidate vaccine currently undergoing toxicological testing and further preclinical and clinical development.

135 (H) Membrane Vesicle Vaccine in Non-Human Primates
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Background: Originally developed for vaccine candidates for Bacillus anthracis and Yersinia pestis, OMV vaccines have been shown to be safe and immunogenic in a variety of animal models. Methods: We hypothesize that immunization of primates with Bps OMVs will generate protective antibody and cell-mediated immune responses without any associated toxicity or reactogenicity. Two groups of macaques were immunized with either Bps OMVs admixed with 600 μg CpG or 400 μg CpG alone. Immunizations were performed on days 0, 28, and 56. OMV immunizations were given using a dose escalation with the first dose containing 25 μg OMV, the second dose 50 μg, and the third dose 100 μg. Safety of the OMV vaccine was evaluated by complete blood chemistry and clinical observation. OMV-specific IgM, IgG, and IgA were measured in plasma of OMV-immunized and control animals by ELISA. T cell responses were measured using PBMCs isolated from blood. PBMCs were restimulated with inactivated whole bacteria or OMVs and cell proliferation was measured by CFSE stain. No toxicity was observed in any animal that received up to 50 μg OMV. Toxicity results for the third dose are pending. Vaccination after a single dose of 25 μg OMV elicited Bps-specific serum IgG superior to that induced by experimental infection. Analyses of antibody responses to the second and third doses as well as T cell responses to OMV immunization are underway. In conclusion, this work demonstrates the safety and immunogenicity of a Bps OMV vaccine in non-human primates at doses equivalent or higher to that previously used in humans (Naess et al, 1998). Future studies will evaluate the protective efficacy of the OMV vaccine against Bps in this large animal model.
Development of Characterization and Serology ELISA Assays to Support VLP-Based Vaccine Studies in Nonhuman Primates


The filoviruses, ebola- and marburgvirus cause highly lethal hemorrhagic fever in humans and nonhuman primates. There are no licensed vaccines or treatments for filovirus hemorrhagic fever and a safe, effective vaccine is the best way to protect from these lethal diseases. Virus-like particles (VLP) are safe and effective vaccines being developed for protection against a wide variety of public health threats including HIV, pandemic and seasonal influenza, rotavirus, norovirus, and human papillomavirus. We have previously demonstrated that noncovalent and blended combinations of EBOV (Zaire or Sudan) and MARV VLP can protect nonhuman primates from multiple strains of EBOV (Zaire, Sudan and Ivory Coast) or MARV (Musoke, CI67, and Ravn isolates) using 'Double' VLP vaccines expressing glycoprotein (GP), nucleoprotein (NP), and viral matrix protein (VP40). To demonstrate whether the NP component of the vaccine was critical for protection, IGT generated baculovirus vectors expressing the ZEBOV glycoprotein (GP) and the viral protein (VP40) to produce 'Double' VLP vaccines. A pilot dose-response study was performed in nonhuman primates to assess immunogenicity and efficacy of 'Double' ZEBOV VLPs compared to the activities of the 'Triple' VLPs in a lethal challenge model. To support the advancement of the multivalent VLP 'Double' ZEBOV VLPs compared to the activities of the 'Triple' VLPs in a lethal challenge model. To support the advancement of the multivalent VLP vaccines, critical reagents and assays have been developed that include standard and novel ELISAs for the quantitation of each matrix protein (VP40) and glycoprotein (GP). Epitope-specific ELISAs were developed as potential stability-indicating assays and serological ELISAs were developed to measure humoral immune response to key filovirus proteins. Results from the developed assays will be discussed in relation to the outcome of the 'Double' versus 'Triple' VLP efficacy study.

Vaccination with Cross-Conserved H1N1 Influenza CD4+ T-Cell Epitopes Reduces Viral Load in HLA Transgenic Mice


The outbreak of novel H1N1 influenza in 2009 was associated with a w-shaped age-related susceptibility curve that was also observed in the 1918 pandemic. Because of this unique epidemiology, we hypothesized that immune response to cross-conserved T-cell epitopes might have contributed to diminished reports of influenza-like illnesses and confirmed novel H1N1 infection among older adults, in the absence of cross-reactive humoral immunity. We used immunoinformatic tools to identify cross-conserved CD4 and CD8 T-cell epitopes in the sequences of the three hemagglutinin (HA) and neuraminidase (NA) proteins contained in 2008-2009 conventional influenza vaccine (CIV) to their counterparts in A/California/04/2009 (H1N1). &gt;50% conservation of CD4 and CD8 T-cell epitopes between pandemic H1-HA and CIV HA was observed, with conservation lower among NA epitopes. Pandemic H1-HA contains sixteen promiscuous helper T-cell epitopes, of which nine (56%) were 100% conserved in 2008-2009 CIV; 81% were either identical or had one conservative substitution. Cross-conserved CD4 T-cell epitopes were validated for their affinity for multiple Class II HLA alleles in binding assays and their antigenicity in cytokine secretion studies using peripheral blood leukocytes obtained from influenza vaccinees. When formulated for vaccination in DNA and peptide formats, the epitopes elicited de novo T cell responses in HLA DR3 transgenic mice and significantly lowered viral loads four days post-infection with 106 PFU A/California/07/2009 (H1N1). Results of these studies suggest that conserved influenza sequences, important to viral fitness, may also be immunologically significant contributors to protection against yet to emerge strains of influenza. The conserved epitope approach promises to answer the need for prompt preparedness and delivery of a safe and efficacious vaccine without requiring a new vaccine for every emergent influenza strain.

137 (I) Withdrawn

138 (I) Commensal Bacteria Lipoteichoic Acid Increases Mast Cell Antimicrobial Activity against Vaccinia Viruses

Z. Wang, D. T. MacLeod, A. Di Nardo; Univ. of California, La Jolla, CA.

MaSt cells (MSCs) are considered sentinels in the skin and mucosa. In this study, we demonstrate that MSCs react to vaccinia virus (V) and degranulate using a membrane-activated pathway that leads to antimicrobial peptide discharge and virus inactivation. And cathelicidin expression is induced in MSCs by the activation of TLR2 from bacterial products (lipoteichoic acid, a TLR2 ligand) produced by commensal bacteria at the epithelial surface. Our research shows that signaling through TLR2 increases the production and expression of cathelicidin in MSCs, thereby enhancing their capacity to fight vaccinia virus. MSCs deficient in cathelicidin were less efficient in killing vaccinia virus after lipoteichoic acid stimulation than wild-type MSCs. Moreover, the activation of TLR2 increases the MSC recruitment at the skin barrier interface. Taken together, our findings reveal that the expression and control of antimicrobial peptides and TLR signaling on MSCs are key in fighting viral infection. Our findings also provide new insights into the pathogenesis of skin infections and suggest potential roles for MSCs and TLR2 ligands in antiviral therapy.

Characterization of Memory T-Cell Responses in Vibrio cholerae O1 Infected Patients


Cholera caused by Vibrio cholerae O1 is most prevalent in young children as well as adults, if untreated leads to fluid loss and death. Efforts are being carried out to understand the mechanisms of adaptive immune responses to V. cholerae O1 natural infection. This study has attempted to characterize memory T cell and its subsets in V. cholerae O1 infections and adult patients. T cell dependent antigens, MP and G33D mCt have been used to proliferate lymphocytes by flow cytometric assay specific cell mediated immune response in activated cells. G33D mCt shows better T cell proliferative response than MP at day 7 compared to day 2 after cholera infection. Adults have long lasting response than child patients. Follicular helper T cells were significantly elevated at day 7 which may provide help to B cell for long lasting immunity to V. cholerae following infection. In the context of mucosal immune response, αβ T integrin and CCR9 were studied with a particular focus on effector T-cell migration to the intestinal mucosa. The effector β7+ T cell responses significantly increased at day 7 and day 30 compared to day 2 in response to G33D mCt and MP, the former had better response than the latter. In this study, the β7+ cells in both child and adult patients have been well characterized. However, it has found that the effector gut-homing CCR9+ T cell response is under represented in children. G33D mCt has a potent immunogenic that would help better understanding the mechanisms of protective immunity in terms of various T-cell subsets and eventually would help to design a more effective vaccine as well as provide long-lasting immunity as natural V. cholerae O1 infection.
**141 (J) Evaluating Chemical and Heat Mediated Inactivation of Viruses using Dengue Virus Types 1, 2 and Influenza A as Models**

**M. E. Mayda, Z. Erwin, M. Boley, S. Rashid, K. Langenbach; BEI Resources/ATCC, Manassas, VA.**

**Background:** Inactivated viral preparations are generated using various physical and chemical agents that alter the chemistry of the virus inactivating it. These treatments allow for manipulation of biological threat agents under reduced biosafety levels, are associated with a decrease in biosecurity requirements and involve less stringent federal regulations for material possession and transportation. However, the inactivation treatment itself may alter the surface components of the virus or can denature the virus completely, thereby making them inappropriate samples for investigations or research. Further, the cytotoxic nature of the chemical agents may pose a challenge for innocuity testing. **Objective:** Various inactivation compounds (chemicals and heat) were evaluated for their ability to render organisms non-replicative yet able to retain the virus’s genetic and antigenic integrity such that they were still suitable for molecular biology and immunological applications. **Study and Design Methods:** Heat, paraformaldehyde, solvent/detergent (S/D), RNA-LD (RNA stabilizer, Biomatrica), DNAplus (DNA stabilizer, Biomatrica) and Theralin (tissue fixative, Theranostics) were tested individually for their ability to inactivate Dengue virus type 1, Dengue type 2 and Influenza A viruses. Samples were tested for inactivation by various approaches including real time monitoring of their ability to induce cytopathic effect (CPE) using the xCELLigence™ impedance based system. PCR tests and immunoassays were performed to verify the integrity of the viral genomic and protein components. **Results and Conclusions:** Viral inactivation was achieved using several treatments (i.e. treatment for > 5 minutes at 65°C). However, as time and temperature or chemical concentration increased, the genomic material and protein become degraded. Our data provide the basis for us to expand our studies on the viral inactivation treatments using an extended panel of viruses, and support utilization of real-time measurement of cellular parameters to assess virus replication.

**142 (J) Testing, Registration and Activation of Biocontainment Laboratories at the NBACC**

**J. P. Fitch, J. Burans, M. Hevey, P. Weaver; Natl. Biodefense Analysis and Countermeasures Ctr., Natl. Interagency Biodefense Campus, MD.**

The National Biodefense Analysis and Countermeasures Center (NBACC) just completed a three year testing, registration and activation process of its laboratory facility. While bioforensic and biological threat characterization mission requirements influenced aspects of the approach taken at NBACC, many of the successes, challenges, and lessons learned are potentially of use to other biocontainment laboratories initiating or continuing operations. An assessment of process, schedule and outcome is presented for activation of the 162,000 square foot facility. Processes including facility endurance testing, establishment of infrastructure capabilities (e.g. Personnel Reliability Program), and registration (e.g. CDC, USDA) are presented as well as overall coordination of the activities. As we were activating our facility, we also had on-going operations in leased laboratories and staff deployed to other biocontainment laboratories. These activities were conducted at BSL-2, 3 and 4 and allowed us to a) meet our mission requirements in bioforensics and biological threat characterization, b) establish draft SOPs that could later be customized to our facility, and c) establish NBACC’s scientific and technical team. The NBACC laboratory facility became available to the scientific and operations teams in January of 2010. An NBACC-specific endurance testing process was implemented for the entire facility. Biosafety Level 2 (BSL-2) laboratories completed endurance testing, met other internal activation criteria, and scientific experiments began in May 2010. Endurance testing identified issues requiring remediation for the BSL-3 and BSL-4 laboratories prior to registration. NBACC received CDC/USDA Select Agent Registration on September 21, 2011 for BSL-3 and 4 operations in the BSL-4 space and later for BSL-3 laboratories. As a result of the successful registration, NBACC has been utilizing laboratory spaces to perform bioforensics and threat characterization work for DHS and other Federal agencies.

**143 (J) Current Laboratory Bio-Safety and Bio-Security Problems in Armenia**

**N. Bakunts1, A. Vanyan1, A. Melkonyan2, L. Avetisyan1, S. Melikjanyan1; 1State Hygienic and Anti-Epidemic Inspectorate, Yerevan, Armenia, 2Ministry of Hlth., Yerevan, Armenia, 3CDC, Yerevan, Armenia.**

Laboratory capacities, including laboratory bio-safety and bio-security, that determine capabilities requested by International Health Regulations. We performed laboratory system evaluation in partnership with the IQLS as part of a DTRA’s CBEP program/ CDC partnership and in collaboration with the WHO to detect gaps and make recommendations. WHO laboratory assessment tool, adapted for Armenia, was used. For each indicator a score in the range of 65-85% was indicative of a functional system. Sites were chosen by the ministry of health, WHO and CDC. The 25 laboratories were evaluated and indicators were scored in accordance to the responses. National bio-safety manuals and procedures, bio-safety practices, waste disposal practices are in use, although not always reflect the international requirements. Bio-safety measures were found and disease specific regulations and guidelines also exist. Existing 19 bio-safety cabinets (BSC) were not tested and certified, definitions and conditions of bio-safety levels (BSLs) not established. Bio-safety officer is not included in the list of medical professions and doesn’t have Terms of Reference (ToRs). Summary score of the bio-safety for laboratories was calculated 8%, although for different labs it varied from 57% to 8%. Bio-safety and bio-security requirements and measures are in place, but are not sufficient and not in accordance with the international requirements. The requirements to test and certify BSC and to have a bio-safety officer are absent, BSLs levels not defined. None of the laboratories scored the range; the highest score was 8% (65%-57%) below minimum. We recommend developing and implementing the systematic approach to the bio-safety and bio-security, including the requirements for testing and certification of BSC, BSLs definitions and conditions, including Bio-safety officer in the list of medical professions and developing the ToR.

**144 (J) Recalcitrant Biological Agents and Survival in Landfill Leachates over Several Years: Experiments and Modeling**

**W. J. Davis-Hoover; U.S. EPA, Cincinnati, OH.**

The permanence of the final disposal of inactivated or active biological agents of terrorism bound in contaminated debris generated from response activities following a biological contamination incident must be examined by looking at the fate of various agents in the most likely medium of escape. In this study, fate is being determined by looking at the activation status of the agent in landfill leachate over a period of time. These trace contaminants may either remain behind in the waste generated from cleanup operations, or due to lack of available laboratory capacity, may be present at undetermined levels. Mediums of escape from a landfill, according to likelihood, would be leachate, leachate discharge to wastewater treatment plant, air, groundwater, and soil. The objective of this study is to determine the duration of survival of these agents in municipal solid waste leachate. Although it would be hoped that biological agents of concern would not survive in landfill leachates, the persistence of some of these agents must be considered before deciding to place them into landfills. Evaluation of the permanence of biological warfare agents in municipal solid waste landfills is being undertaken by the United States EPA, Office of Research and Development, National Homeland Security Research Center in collaboration with the Department of Defense Edgewood Chemical Biological Center. The majority of the experiments in this study on the fate of biological agents in a single, municipal solid waste landfill leachate have been completed, and although some additional measurements are planned, with the results obtained thus far some projections are able to be made. The results show that existing computer models do not predict the actual survivability of agents when tested. Vegetative agents do not survive while spores are surviving over multiple years. Survivability of bacterial agents over years of exposure, long term mathematical predictions from the data, and the potential modes of destruction will be discussed.

**145 (K) Withdrawn**
**146 (K) The Animal Model Qualification Process: The Process in Depth**

**H. Lee-Lewis**, J. Beren, J. Davis, J. Flaherty, R. Roberts; 1FDA, Silver Spring, MD, 2Purdue Univ., West Lafayette, IN.

The Animal Model Qualification Program (AMQP) was established as part of a multi-faceted response by the United States Food and Drug Administration (FDA) to address the “recent slowdown, instead of the expected acceleration, in innovative medical therapies”, as published in FDA’s Critical Path Initiative (2004). This report, as well as FDA’s Medical Countermeasures (MCM) initiative, identified a significant challenge in MCM development as the lack of product-independent animal models in which to evaluate product efficacy when human trials are unethical or infeasible. The AMQP serves as a voluntary program where submitters engage in an iterative process with FDA throughout the development, comprehensive characterization, and refinement of a model for the ultimate goal of qualification. MCM development would be greatly supported by the availability of these reproducible defined models, whether proprietary or public, due to increased economies of time, animals, and resources in drug development under the Animal Rule. The mission and processes of the AMQP are described in the draft guidance, Qualification Process for Drug Development Tools, and a public web page. Engagement with potential submitters has allowed us to identify specific areas of the program and instructions that required clarification. Several submitters have begun the qualification process and, from these interactions, we have been able to modify the process to increase efficiency and be more user-friendly. We have developed additional public resources such as instructions for the structure of a Letter of Intent and the Initial Briefing Document; the web page has also been updated with more current information and a Frequently Asked Questions section. It is our hope that as interest in the AMQP grows and more questions are brought forth, we can continue to improve and develop our program. We encourage contacting the AMQP (AnimalModelQualification@fda.hhs.gov) for additional information.

**147 (K) The Role of the U.S. Army Medical Command in the National Laboratory Response Network: A Phased Approach to National Biodefense**


The US Army Medical Command (USAMEDCOM) is an integral part of our National biodefense plan through its role in the Laboratory Response Network (LRN). With its first line of defense being the Army Community Hospitals and Army Health Clinics as “sentinel laboratories”, USAMEDCOM is prepared to assume a major role in the LRN, culminating in the highest level of laboratory response, the US Army Medical Research Institute for Infectious Disease (USAMRIID). This presentation will examine the roles of each echelon in the USAMEDCOM LRN response and how each of these coordinate with relevant Federal and State health agencies. Participants will expand their knowledge of the potential role of USAMEDCOM in a local, regional or national event. We will include the response by select units and Soldiers from the Army National Guard and Army Reserve as members of the “Army Team”. Specific examples of these coordination steps will be presented in order to clarify the role that USAMEDCOM plays in the comprehensive National LRN. This will include the role that USAMEDCOM plays in the Defense Support of Civil Authorities and the collaboration with the US Navy and US Air Force Medical Departments. Special attention will be paid to the function of USAMRIID in researching novel methods of rapid identification of biologic agents which might be of interest to the biodefense community.

**148 (K) Quality System Integration in the BSL-4 Laboratory During Regulated Nonhuman Primate Filovirus Challenge Studies**

**T. Brasel, M. Eitzen, D. Beaasley, D. Trent; Univ. of Texas Med. Branch, Galveston, TX.**

The 2012 Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) Strategy defines four main goals, one of which is to “establish and communicate clear regulatory pathways to facilitate Medical Counter Measure (MCM) development and use.” One of PHEMCE’s objectives is to identify scientific and regulatory issues that challenge MCM development including conduct of studies under BSL3 and BSL4 containment. Numerous studies with BSL3 threats (e.g., anthrax, plague, and botulinum toxin) have been successfully conducted, however significant gaps exist in the conduct of regulated studies with BSL4 agents including Ebola virus (EBOV). Due to the highly specialized nature of BSL4 facilities and lack of trained personnel, conduct of regulatory science and GLP studies in these unique environments has been hindered. Implementation and oversight of quality systems, including standard operating procedures, data and record management, test and control article handling, laboratory setup, documentation practices, personnel training files, and protocol development, presents a unique challenge. The University of Texas Medical Branch at Galveston (UTMB) has established an institutional Office of Regulated Nonclinical Studies (ORNcS) to overcome these challenges and provide infrastructure supporting BSL4 regulated studies. The ORNcS partners with scientific teams and BSL4 facility operations personnel to provide regulatory strategy and risk mitigation to facilitate licensure, documentation, and data integrity. These quality improvements represent essential steps to facilitate transition from traditional basic discovery research, which remains a focus of the UTMB BSL4 program, to successful execution of pivotal studies in support of MCM licensure. Here, we present the approaches taken by UTMB through the ORNcS to integrate quality systems and mitigate regulatory risk in the BSL4 during a nonhuman primate EBOV challenge study.

**149 (K) Guideline, Recommendation, or Regulation?: The Administrative Procedure Act and Lab Inspections**

**H. N. Carson**, C. Davenport, V. Sutton, S. Trinh, A. Franklin; Texas Tech Univ. Sch. of Law, Lubbock, TX.

By circumventing the notice and comment requirements of the Administrative Procedure Act (APA), federal agencies often abuse their regulatory abilities, causing confusion for microbiological and biomedical researchers during laboratory inspections and unfair punishment when violations occur. Background: The Administrative Procedure Act, 5 U.S.C § 500 (2012) (APA), was enacted on June 11, 1946, in order to allow executive departments and agencies to enact regulations while still protecting the separation of powers doctrine under the U.S. Constitution. The APA requires that all regulations, with a few exceptions, be subject to notice and comment by those that the regulation would apply. Many agencies, however, including the Centers for Disease Control and Prevention, also publish recommendations and guidance, which are not subject to notice and comment. When such recommendations and guidance are used as enforcement tools during inspection, it can create confusion and when used to exact penalties raises constitutional due process concerns. Methods: A thorough review of the APA text and key case law was conducted. The appropriate process for promulgating regulations, as well as the exceptions, was identified and mapped. This process was compared with recent practices of agencies. Results: Using material not subject to notice and comment violates the APA. Case law, however, reveals that it is unlikely a court will overturn this practice because the courts have historically demonstrated a wide amount of deference to the agencies, and these actions would not likely rise to an arbitrary and capricious level that is required to overturn the practice. Conclusion: While using guidance not subject to notice and comment is contrary to the APA, many federal agencies regulate microbiological and biomedical activity in this manner, causing compliance issues for researchers. It is unlikely this practice will change without the benefit of a court case it an issue that could also be addressed legislatively in order to clarify requirements of researchers.
021 Wednesday Posters
Poster Session
Wednesday, February 27, 2013 | 3:15 PM - 4:45 PM | Hall B

183 (A) Subcellular Localization of Hantavirus RNA in Viral Nucleocapsid Filaments
M. Lindquist, C. S. Schmaljohn; USAMRIID, Frederick, MD.

Background: Hantaan virus (HTNV) and Andes virus (ANDV) are pathogenic hantaviruses responsible for causing two types of severe disease in humans, hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome, respectively. The multifunctional hantavirus nucleocapsid (N) protein is involved in viral replication, assembly, and modulation of the host immune response. Previous immunofluorescence studies have shown that N is localized in viral inclusion bodies or in poorly characterized intracellular filamentous structures. We hypothesized that the distinct N cytoplasmic structures are regions of unique functions in the viral life cycle.

Methods: HeLa cells were transfected with ANDV-N or HTNV-N for 24 h using Fugene HD. For infections, A549 cells were infected with ANDV or HTNV for 5 days. EU was detected using Click-IT RNA staining (Invitrogen). ANDV and HTNV RNA probes were generated by Biosearch Technologies.

Results: To ascertain the specific function of nucleocapsid filaments (NFs), we first attempted to identify host proteins that co-localize with NFs. We transfected cells with ANDV-N or HTNV-N and co-stained for NFs and a panel of host proteins. We detected co-localization between NFs and P-body components, similar to results previously described with Sin Nombre virus. We next determined if NFs are sites of viral transcription or replication by staining for the presence of RNA. We detected sites of RNA localization in infected cells by incorporating 5-ethynyl-uridine (EU) into nascent RNA. These results clearly demonstrated that NFs contain RNA, however, EU staining did not discern between host and viral RNA. To specifically identify viral RNA, we generated fluorescently-tagged RNA probes complementary to genomic segments of ANDV or HTNV. We hybridized RNA probes in cells that had been infected and then immunostained for viral protein. These results showed prolifer amounts of viral RNA specifically localized to NFs for both ANDV and HTNV.

Conclusions: These experiments clearly demonstrated that ANDV and HTNV RNA specifically localizes to NFs. Given the abundance of genomic RNA found within NFs, they are likely the sites of viral replication.

184 (A) Hepatitis B Virus among Potential Blood Donors in Ibadan, Nigeria

Hepatitis B Virus (HBV) is one of the agents of transmissible transfusion infection (TTI) and causes threat to blood safety for recipients. Blood transfusion is one of the pathways in which HBV is being transmitted. This study aimed to determine the seroprevalence of HBV among potential blood donors at Blood Bank of University College Hospital (UCH) Ibadan, Nigeria. Over a period of 6 month (February - July, 2010) 507 consenting blood donors at Blood Bank of University College Hospital (UCH) Ibadan, Nigeria. were tested for anti-HIV1 activity of Kaempferol and Kaempferol-7-O-glucoside by use of the real-time polymerase chain reaction (PCR) assay and HIV1 p24 Antigen kit. Results: According to the results of real time PCR, these anti-HIV1 compounds by 20 μg/ml exerted good inhibitory effect and time of addition studies showed that this inhibitory effect is higher in pre HIV1 infection compared to post-infection. Antiviral activity of Kaempferol-7-O-glucoside was more than Kaempferol. Conclusion: These findings demonstrate these compounds that isolated from S.securidaca may prevent HIV from entering cells by inhibiting HIV1 viral replication in host cells.

185 (A) Impact of IL-12B and TGF-β1 Polymorphism and Levels in HBV-HCC Disease Progression in India
J. Kaur, R. Saxena, Y. K. Chawla, I. Verma; Pgimer, Chandigarh, India.

Objectives: Hepatocellular carcinoma (HCC), the dominant primary liver cancer, is the third most common cause of cancer-related death in the world. The hepatitis B virus (HBV) infection induced chronic inflammation, is considered to be the major etiological factor for the disease chronicity. Previous study was undertaken to analyze the association of IL-12B(+1188) and TGF-β1(-509) polymorphisms and their expression with HBV/HCC risk in Indian population.

Methods: Five groups of subjects were enrolled viz. control (n=153), HBV-carriers (n=67), chronic-active HBV (n=61), HBV-cirrhosis (n=61) and HBV-related HCC (n=59). PCR-RFLP, ELISA and RT-PCR, were done for assessing polymorphism, protein and the mRNA levels, respectively, of the two cytokines. Genotype distributions were compared using chi square analysis and the odds ratios (ORs) and 95% CI were calculated to express the relative risk.

Results: In TGF-β1(-509>C), both the CT and TT genotypes acted as potential risk factors for HCC development, with control as the referent group. However, the TT genotype was found to be significantly in negative association with cirrhosis and the subsequent HCC risk, among carriers. While, in case of IL-12B(+1188A>C 3’UTR), the CC genotype shared a significant positive association with hepatitis, among controls. TGF-β1 levels were observed to be significantly high in all the categories, as compared to controls, with maximum levels in the HCC group. On the contrary, a consistent decrease in IL-12B levels was evident in the diseased categories, as the disease progressed from carrier to the HCC state.

Conclusions: Polymorphic forms of IL-12B and TGF-β1 and their levels might act as key determinants in HBV-HCC development in Indian population and thus, should be further evaluated as candidate genes to determine individual susceptibility for the same. Authors acknowledge the financial grant received from ICMR, New Delhi.
Zoonotic infections by several orthopox viruses like Monkeypox virus or Vaccinia virus represent a threat to human today. In Europe the number of diagnosed infections with Cowpox virus (CPXV) is increasing in animals as well as in humans. Despite its name Cowpox, no infections of cattle have been documented for decades. Instead, infections are being transmitted from cats or exotic zoo animals to humans, and the viruses involved are obviously a very heterogeneous population. Both animals and humans reveal generally self-limited local exanthema on the site of contact, although lethal systemic disease resembling smallpox has been documented in immunosuppressed patients. As observed in several infections of animals, the pathogenicity depends on the individual virus strain and host species interaction. This raises the question of whether a CPXV variant might emerge from an animal reservoir, that is highly pathogenic to humans. A reliable risk assessment could be performed based on genomic information of viruses with known pathogenicity. However, to date only limited information on the complex and sparsely conserved CPXV genome is available. Therefore, the genomes of independent CPXV isolates from ten human cases, four rats, two cats, two jaguarundis, one beaver, one elephant, one marah and one mongoose (in total 22 isolates) were sequenced using massive parallel sequencing. Extensive phylogenetic analysis showed that the strains cluster into several distinct classes some of which are closer related to other orthopox viruses than to each other. Particularly one CPXV clade is closer related to VARV than other known OPV except for Taterapox virus. These results support recent data from other groups who postulate that CPXV is not a monophyletic group but rather should be divided into multiple lineages and, above all, it includes virus strains closely related to Variola virus.

#### 188 (A) Multi-Faceted Proteomic Profiling of Host Proteins Associated with Rift Valley Fever Virus (RVFV) Virions and Identification of Specific Heat Shock Proteins (HSPs) as Essential Host Factors


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The Gel LC/MS/MS approach together with alternative techniques that preserve protein complexes were used to profile the host protein complement of highly pure RVFV virions, followed by targeted functional studies. Over 300 host proteins, including multiple macromolecular complexes were identified. Bioinformatic analysis showed that HSPs were among over-represented protein families. siRNA gene silencing, and treatment with small molecule HSP inhibitors specific for Hsp90 (17-AAG) and Hsp70 (KNK437), identified several HSPs as essential host factors. Time-of-addition studies with 17-AAG and KNK437 demonstrated that the HSP effects occur early, manifesting within hours after infection. Consistent with these results, real-time studies by FACS monitoring of cells treated with HSP inhibitors and infected with GFP-expressing virus showed that 17-AAG causes a significant decrease in viral load in early infection (4 and 8 hours post-infection). These findings suggest HSP effects on viral replication/transcription. Specific effects of HSP inhibitors on RVFV protein levels were also demonstrated. Interestingly, while 17-AAG significantly decreased both viral NSs and N-protein levels, KNK-437 caused a significant decrease only in NSs levels, indicating that the HSPs act through distinct functional mechanisms. As 17-AAG and several other Hsp90 inhibitors are already in clinical trials for cancer treatment, there is the exciting potential of repurposing them to treat RVF. Furthermore, Hsp90 has been shown to play a role during infections by other RNA viruses. Therefore, the strategy of targeting Hsp90 also presents broad spectrum therapeutic options for other RNA viruses.
191 (A) Control and Diagnostics of African Swine Fever (ASF) in Ukraine
A. Golovko1, O. Nevolko2, L. Marushchak3; 1NAAS, Kyiv, Ukraine, 2SS-RLIDVSE, Kyiv, Ukraine.

Background: There are 8 million pigs in Ukraine, including 3.5 million pigs in agricultural enterprises and 4.5 million pigs in private households. Threat of transmission of African swine fever (ASF) into Ukraine from endemic regions of the Caucasus and Russia, where there are periodic outbreaks of ASF among domestic pigs and the population of wild boars. The introduction of ASF into Ukraine’s pig populations would be economically catastrophic. Objective: Epizootological monitoring of Ukrainian pig populations is being carried out to detect ASF. This will give possibility to identify the virus circulation in a particularly hazardous areas and in case of animals death. In 2011, the State Emergency Antiepizootic Commission identified the several areas of concern where wild boar migrate from across Ukrainian borders. Laboratory tests for ASF were conducted on all hunted wild boar. Methods and Results: SSRLIDVSE is a center of ASF diagnostics in Ukraine. During 2010-2011, DNDILDVSE tested 1,154 serum from wild boars by ELISA (623 samples in 2010, 531 samples in 2011) and 1,560 samples of pathological material by molecular genetic methods (PCR in real time) (647 samples in 2010 and 913 samples in 2011). During 2010-2011 regional lab tested 1,236 biological material by FTA. In July 2012, outbreak of ASF was registered for the first time in Ukraine. Pathological material was tested at DNDILDVSE by FTA and RT-PCR with positive diagnostic for ASF. Diagnosis was confirmed in the Center for Animal Health Research, National Institute for Agricultural and Food Research and Technology (CISA-INIA, Madrid). Discovered ASF virus was 100% homologous to Caucasus isolates responsible of the outbreaks occurred in East Europe since its introduction in Georgia in 2007, and it gave possibility to attribute the strain of the virus, that caused the disease, to genotype II. Conclusion: Early diagnosis and effective measures gave possibility to localize the disease and prevent its spreading in Ukraine. Complete measures of epizootic surveillance and early diagnostics give possibility to control ASF in Ukraine effectively.

192 (A) Viral Hemorrhagic Fever (VHF) Cases in Georgia

Background: In recent years more frequent and larger outbreaks of filovirus hemorrhagic fevers in central Africa have increased international public health concerns. The acquisition of well characterized virus stock is essential for research in this area. As part of our program to produce well characterized stocks of selected species of Ebola virus it was noted that the Taï Forest virus (TAFV) stocks were contaminated with mycoplasma. Here we describe how we have attempted to remove the contamination and produce a characterized stock of the virus. Methods: Viruses Virus was propagated in Vero E6 cells. Sequencing Genomic analysis was performed by Sanger sequencing. Results: Several commercially available mycoplasma eradication kits were used in this study to allow the production of a stock in which mycoplasma could not be detected by a PCR assay. The ‘cleaned’ isolate was sequenced and only 12 point mutations were found when compared to the original isolate. Conclusions: We have successfully grown a stock of TAFV which is mycoplasma free by the methods used in this study. Despite the multiple passages in vero E6 cells required to remove the mycoplasma contamination, this stock only differed from the original isolate by 12 point mutations. We believe that this is the first documented mycoplasma free stock of TAFV and can now be used in future studies.

193 (A) Production of a Mycoplasma Free Stock of Taï Forest Ebolavirus

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A patient showed improvement and another was lost to follow-up. It is important to make the Georgian medical community aware of the characteristics of VHF infections in Georgia. Further studies of CCHF and Hantavirus infection in Georgia. From 2008-2011, 537 patients meeting the criteria for CCHF infection were enrolled into a prospective study to investigate the incidence of nine pathogens including CCHF and hantavirus as causes of F0U using ELISA. Positive results were confirmed by IgM/IgG IFA and IgM/IgG immunoblotting for Hantavirus. Three of fourteen patients with hemorrhagic fever syndrome on enrollment retrospectively tested positive for CCHF IgM antibodies. All three patients initially diagnosed as F0U cases were from the southwest districts of Adigeni and Akhalsikhe bordered by Turkey. Bleeding, petechial rash, hepatomegaly and splenomegaly were the major symptoms observed together with abnormal CBC findings. Two patients showed improvement on follow-up at 2-6 weeks without antiviral therapy. The third patient was lost to follow-up. Two patients from Tbiliki diagnosed as F0U cases with acute renal failure were confirmed as acute Hantavirus infections. Both had febrile illness with progressive deterioration of renal function without hemorrhagic manifestation. Renal pathology results of one case revealed acute tubular necrosis with mild grade arteriolosclerosis. One patient showed improvement and another was lost to follow-up. It is important to make the Georgian medical community aware of the characteristics of VHF infections in Georgia. Further studies of CCHF and Hantavirus strains in Georgia, as well as sentinel lab surveillance are needed to improve diagnosis and treatment in Georgia.

194 (A) Pathogenesis of Hendra Virus in a Mouse Model
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Hendra virus (HeV) is an emerging zoonotic virus and member of the genus Henipavirus (family Paramyxoviridae) that causes severe respiratory illness and encephalitis in humans. HeV was identified as the causative agent of an acute respiratory disease in horses in 1994 and to date there have been 7 human infections with HeV, of which 4 were fatal (57%). Clinical signs include severe respiratory distress, encephalitic manifestations (mild confusion, ataxia) and seizures. Little is known about the mechanisms that govern the development of respiratory and neurological disease. Using a novel mouse model of lethal HeV infection, we determine virus tropism and host responses following infection. Infection of animals via the intraperitoneal route resulted in the development of severe neurological signs within 7 days. Infectious virus could be detected in the brain by day 6 post infection. Low levels of virus replication could also be detected in lung and spleen on day 3 post infection. Histopathologic changes in the brain included perivascular cuffs, acute necrosis of neurons and meningitis. The development of neurological signs coincided with disruption of the blood-brain barrier and peak expression levels of tumour necrosis alpha (TNF-α) and interleukin 1β (IL-1β) by day 5 and 7 post infection, respectively. In lungs, a transient peak in cytokine levels was observed on day 5 post-infection and included IL-1β, IL-6, Interferon γ, MCP-1 and TNF-α. These studies reveal novel information on the development and progression of HeV clinical disease, and identify specific cytokines and chemokines that might serve as important targets for treatment.
195 (A) Evaluation and Characterization of the AG129 Mouse Model of Subcutaneous Challenge with Dengue Strain D2Y98P

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The range of Dengue virus and the incidence of Dengue infections have increased over recent decades. Dengue now affects most tropical and sub-tropical regions of the world. Estimates place up to 2.5 billion people at risk of Dengue infection worldwide with 50-100 million infections per year including up to 500,000 cases of dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS), resulting in up to 25,000 deaths. The NIH currently considers Dengue to be a serious public health threat to the United States. Two studies, LD and natural history, were run on Dengue strain D2Y98P in interferon (Type I and II) receptor knockout mice (AG129). Mice were challenged subcutaneously with 1.5x10^5 to 1.1x10^7 (LD) or 3.8x10^6 (Natural History) FFU of virus. Observations were collected for up to 21 days, or mice were euthanatized on Study Day 6 for collection of samples for hematology, cytokine analysis, and blood and tissue viral burden analysis. Survival in the LD study was <50% at all doses tested. The highest mortality rate of 90% was observed in the 5.2x10^6 FFU challenge group and the lowest (70%) was in the 1.1x10^7 challenge dose group. All challenged mice showed signs of illness. All mice in the natural history succumbed to infection. Mice showed weight loss and a notable drop in subcutaneous temperature two to three days prior to death. Total white blood cell count was elevated in challenged mice and this was reflected in the neutrophil, lymphocyte and large unstained cell levels. Reduced platelet counts were observed in all challenged mice. A Luminex analysis of cytokine levels in serum showed notable elevation of FGF-basic, IFN-α, IL-1β, IL-2, IL-4, IL-5, IL-6, and KC in challenged mice. Challenged mice showed a decrease in serum IL-10 levels related to unchallenged controls. Results are consistent with published characteristics of the AG129 mouse model of Dengue D2Y98P infection. We extend the range of cytokines evaluated in this model, and define multiple model endpoints useful for evaluation of medical countermeasures to Dengue infection.

196 (A) Epidemiology of Lassa Fever in Post-Conflict Sierra Leone: Seasonal Demographics

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Lassa fever (LF) is an acute and often fatal illness that is endemic in the West African countries of Sierra Leone, Liberia, Guinea, and Nigeria. Lassa virus (LASV), an arenavirus, is transmitted to humans by Mastomys natalensis, a ubiquitous rodent in sub-Saharan Africa. Kenema Government Hospital (KGH) in eastern Sierra Leone maintains a 25-bed Lassa ward where up to 600 suspected LF cases are seen yearly. At the KGH, our team has developed a new class of state-of-the-art LF diagnostics, including antigen (Ag)-capture enzyme-linked immunosorbant assays (ELISAs) and lateral flow immunoassays (LFIs) to detect LASV viremia, and immunoglobulin M (IgM)- and immunoglobulin G (IgG)-capture ELISAs to detect convalescent or prior LASV infections. We analyzed the diagnostic test results for suspected Lassa patients presenting to the KGH from 2008 to 2011. We found that acute, viremic LF cases (Ag+/IgM+) most frequently presented to the KGH during the dry season (November to May) with peak frequencies occurring during the month of May. We also observed a significant spike in the proportion of suspected cases with evidence of prior LASV infection (Ag+/IgM+) over the months of June and July, which corresponds to the beginning of the rainy season. These results suggest that patients with current or prior LASV infection, even without severe hemorrhagic manifestations, may be predisposed to other febrile illnesses, particularly at the beginning of the rainy season.

197 (A) Household Case Investigation of HFRS in Georgia, 2010

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A lethal case on suspected Hemorrhagic Fever with Renal Syndrome (HFRS) was reported from Ozurgeti region, Western Georgia, subtropical climate, by the regional Public Health Center, on November 13th, 2010. The National Center for Disease Control and Public Health considered it as an unusual and unexpected event and conducted an epidemiological investigation of the possible outbreak. For this case, based on the CDC case definition, HFRS case definition were developed. Information about the case and patient were obtained using a standard questionnaire to find epidemiological link to exposure or source of the infection. A specific anti-hantaviral IgM and IgG enzyme-linked immunosorbent assay (ELISA) was used for lab confirmation. The patient was referred to the hospital with one month of history of fever, abdominal pain and cough and symptomatic self-treatment. There was an initial diagnosis of bilateral pneumonia; the patient died on the same day of admission. The household included 9 members (3 males, 6 females) with an age distribution from 6 to 70. All were exposed to rodents. Out of the 9 members, 3 required hospitalization. Most frequent symptoms were malaise - 6; arthralgia - 5; headache -4; myalgia - 3; high fever - 2; abdominal pain- 2; cough- 1; sweating -1; polyuria - 1. According to ELISA test results, three of the household members were positive; two cases were negative; two samples had equivocal test results. This was classified as a cluster outbreak with three confirmed HFRS and three probable cases. Over the last several years, Hantavirus infections have increased in Georgia. The investigation findings suggest that Hantavirus is endemic in the region where the outbreak occurred and it is important to further investigations and implement preventive activities.

198 (A) SOCS3 is Required for Rift Valley Fever Virus Replication

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Rift Valley fever virus (RVFV) is an important natural and intentional biological threat that is classified as a Category A agent by the CDC due to its ability to cause high mortality rates, ease of dissemination, and its potential to cause major public panic and social disruption. There are no approved vaccines or therapeutics. The RVFV BALB/c mouse model mimics the acute-onset hepatitis and delayed-onset encephalitis that are important features of severe human disease. Little is known about the host immune response to RVFV infection. Therefore, we used the murine model to examine mRNA expression of 85 signature immune response genes after infection with RVFV. Mice were exposed either to an aerosolized or peripheral RVFV challenge. Peripheral blood mononuclear cells and splenocytes were isolated and total RNA was extracted for gene expression analysis using the nCounter analysis system (NanoString Technologies). Briefly, RNA was hybridized with NanoString customized Reporter CodeSet and Capture ProbeSet according to the manufacturer’s instructions. SOCS3 was one of the genes shown to be significantly upregulated during RVFV infection. Downregulation of SOCS3 with siRNA significantly inhibited RVFV in vitro. Additionally, zoledronic acid, a bone cancer drug that was also shown to inhibit SOCS3, inhibited RVFV in vitro in a dose-dependent manner. Further in vivo testing is needed and planned; however, this demonstrates the potential for zoledronic acid (and other SOCS3 inhibitors) as an effective antiviral against RVFV.
199 (B) Prevalence and Drug Resistance Patterns of Salmonella Concord among Children in Selected Orphanages and Visiting Health Institutions in Addis Ababa, Ethiopia

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Background: A few studies conducted in Ethiopia and abroad including U.S.A and European countries among adopted children reported the emergence of multidrug resistance S. Concord. Objective: To determine the prevalence and drug resistance of S. Concord among children in Addis Ababa and identify possible source(s) of infection.

Materials and Method: This cross-sectional study was conducted from March to July 2011. A total of 572 fecal specimen and 209 food sample were collected and examined. All the samples were then processed at EHNRI using standard methods and for all salmonella isolated Serogrouping and drug susceptibility tests using standard disk diffusion method was done. Complete serotyping was done at National Institute of Health, Thailand. Data entry and analysis was carried out using SPSS version 12.0. A difference was considered statistically significant with a P value of less than 0.05.

Result: The prevalence of S. Concord in children in this study was found to be 6.9% (29/420). No S. Concord was isolated from adult subject studies and the food sample examined. In general the isolated S. Concord showed a significantly high level of resistance to clinically important drugs including third generation cephalosporin. The significant risk factor identified was cessation of breast feeding before six months of age. The probability of infection by S. Concord among children who cease breast feeding after the age of six month was 0.23 times less likely than those cease before six month (Adjusted Odds Ratio, 0.23 (P-value is 0.03 at 95% C.I of 0.061-0.868)).

Conclusion and Recommendation: The high prevalence of MDR S. Concord isolates in this study is a major public health concern since this will have a major adverse impact on control of child mortality in Ethiopia and also Globally as well. Thus we recommend further larger scale studies and urgent priority in Ethiopia to initiate active salmonella surveillance system. In addition developing effective preventive measures in particular in orphanages should be given priority since promoting breast feeding would not be possible in orphanages.

200 (B) Pathogenicity Modeling of the C. trachomatis T3SS Effector Protein CT694 in Fission Yeast Identifies the IKAP Complex as a Novel Host Target

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Chlamydia trachomatis encodes an ancient, highly-conserved type three-secretion system (T3SS) closely related to other bacterial T3SS, including those encoded by Yersinia pestis and Y. pseudotuberculosis. These T3SS and their respective secreted substrates are key virulence determinants in these pathogens. The C. trachomatis T3SS effector protein CT694 is expressed during late cycle development and translocated into host cells early during the infection cycle. Upon introduction into the host cell, CT694 localizes to the host cell plasma membrane, where it exerts an as-yet unidentified effect that results in toxicity after over-expression. This membrane localization-dependent toxicity closely mimics localization-dependent toxicity in other T3SS effectors such as Pseudomonas aeruginosa ExoS and Yersinia spp. YopE. CT694 toxicity occurs in both human cells as well as a fission yeast model of pathogenesis, indicating that the target pathway similarly conserved in both organisms. We took advantage of this conservation and employed a synthetic genetic analysis screen in Schizosaccharomyces pombe to identify host pathways which CT694 may target during infection. S. pombe expressing thiamine-suppressible GFP-Ct694 was crossed to a yeast deletion library covering 98% of the yeast genome and analyzed for suppressor mutants. We selected mutants with a log growth ratio of 1.5 or less when comparing strains on suppressing versus inducing media for further analysis. Through this screen, we identified 5 of the 6 members of the IKAP complex, a highly conserved complex which has been demonstrated to be involved in cytoskeleton regulation, transcriptional elongation, MAPK signaling, and other cellular processes. These results suggest that CT694 may differentially regulate a variety of cellular processes through modulation of the IKAP complex in host cells. Multiple drug resistance S. Concord carries mutations in each of these host components. Given the apparent pleiotropism of the IKAP complex, it is possible that other intracellular pathogens likewise perturb IKAP signaling.

201 (B) Examination of Burkholderia Biofilms from Clinical Isolates

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Burkholderia pseudomallei, the causative agent of melioidosis, is a gram-negative bacterium that is predominately found in the water and wet soil of Southeast Asia and Northern Australia. Symptoms of melioidosis typically include fever, pneumonia, and potentially osteomyelitis. Glanders, which is caused by Burkholderia mallei is endemic to Asia, Africa, the Middle East, and South America. Typically a disease of horses, humans whom contract the disease develop lesions in their lungs and upper respiratory tract that can eventually lead to septiciemia. Interest in both of these bacteria has increased over the years given their potential to be used in bioterrorism due to the inherent ease of aerosol transmission, resistance to common antibiotics, and ability to establish both an acute and chronic infection. Currently, there is no effective vaccine and antibiotic therapy is limited. Antibiotic resistance is a hallmark feature of both B. pseudomallei and B. mallei and in vitro studies have shown that biofilm formation is a contributing factor. To date, in vivo studies on the influence of biofilm formation in the disease course and subsequent immune response has yet to be performed. Utilizing a crystal violet biofilm quantification assay, it was determined that human clinical strains possessed differing capabilities to form biofilms with B. mallei FMH23344 having the least biofilm formation than any other tested strain. A correlation between disease severity and biofilm formation has yet to be determined. Biofilms can be visualized in non-human primate lungs infected with B. pseudomallei upon staining with fluorescent markers. The presence of a biofilm during the natural course of disease implies that it is an important bacterial state to consider in assessing novel treatment options. Given that the immune response is influenced by the bacterial state, proteomic analysis is currently underway to identify novel drug targets.

202 (B) Genomic Diversity of Brucella Over Space and Time

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Brucella is one of the world’s most pronounced zoonotic pathogens, yet it represents a poorly understood collection of microbes. We thus applied whole genome sequencing to 20 Brucella strains to provide insight into this genus. Comparative genomics was used to predict, locate and characterize mobile genetic elements (MGEs) and to reconstruct the core Brucella genome. 47 MGEs, ranging from 1.8 to 68 kb, were identified among 45 Brucella genomes using a GI-definition of ≥ 5 contiguous ORFs. While others were paraphyletic. When rooted with uncharacterized Brucella isolates, phylogenetically distant from other Brucella spp., showed fully resolved bifurcating patterns with varying levels of diversity and placed B. abortus, B. ovis and B. microti into monophyletic clades, though others were paraphyletic. When rooted with uncharacterized Brucella isolates, B. microti was basal in this lineage, with B. suis as highly divergent species. When decorated with MGEs and associated metadata, clade differences appeared to be correlated with potential hosts jumps among Brucella spp. and their respective MGE content. Four uncharacterized Brucella isolates, phylogenetically distant from other Brucella spp., contained a battery of GIs (G1-30 to G7), not found in other Brucella isolates. And, although B. abortus and B. melitensis clades are closely related, they could readily be differentiated by the presence or absence of a 29 kb MGE. Despite genome-wide spans indicates relatively low genetic diversity and recombination rates in Brucella, whole genome alignments provide clear evidence of genomic rearrangements in different regions of Brucella genomes, especially at the species boundaries. Our study thus provides a comprehensive analysis of Brucella phylogeny, mobiome dynamics, genetic diversity, and recombination; further characterization of the MGEs will provide a resource to further improve typing tools for forensic attribution.
203 (B) Structure and Molecular Interactions of a Monoclonal Antibody Specific for the Polypeptide Capsular Antigen of Bacillus anthracis
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Bacillus anthracis is the etiologic agent of anthrax and a Tier 1 biothreat. The bacterium produces an anti-phagocytic capsule entirely composed of y-linked D-glutamic acid (yDPGA). Several monoclonal antibodies (mAbs) that react with yDPGA have been isolated. These mAbs provide both immunoprotection and a means of disease diagnosis. For example, targeting yDPGA with mAb-based assays, we demonstrated that yDPGA is present in the serum of mice and rhesus monkeys at 24 hours post infection. Importantly, yDPGA is detectable before the emergence of bacteria into the bloodstream, which may allow for faster diagnosis. yDPGA is an unusual antigen because it contains a uniform negative charge, repeating subunits, and is remarkably flexible. Generally, molecular recognition of such antigens is problematic. Given the many useful applications of yDPGA-specific mAbs, we wished to determine how these interactions occur. For this reason, a molecular model of the yDPGA-reactive mAb F26G3 was constructed. A Fab fragment of mAb F26G3 was created using homology modeling. Docking experiments between mAb F26G3 and a 25-residue yDPGA antigen resulted in spontaneous binding. Following docking experiments 12 mAb residues were identified within 4Å contact of the antigen; 1-A, 1-H, 2-K, 2-R, 5-Y and 1-W. Given the acidic nature of the antigen, the comparatively high content of basic residues in the binding site is not surprising; however, the interactions are not merely due to non-directional ionic forces. Molecular modeling showed the formation of specific salt bridges between the basic mAb residues and the carboxyl moieties along the antigen backbone. Although yDPGA is highly disordered in solution, the formation of these specific salt bridges restricted its mobility to where distinct bound antigen conformers, with a lifetime of thirty picoseconds, could be observed. In contrast, molecular dynamic simulation of non-bound yDPGA did not result in the identification of conformers with thirty picosecond lifetimes. Together, these results provide a basis for understanding the interactions between mAb and the unique yDPGA antigen.

204 (B) Investigation of the Role of Biofilm Formation in Tigecycline Resistance of Acinetobacter baumannii
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Objective: To investigate the role of biofilm formation in tigecycline resistance of Acinetobacter baumannii. Methods: Nonrepetitive 81 Acinetobacter baumannii isolates were collected from 14 hospitals in western China from 2010 to 2012. Susceptibility of Acinetobacter spp. to tigecycline was determined by the broth microdilution method. Detection of biofilms was done by the spectrometric microfiltration plate method and crystal violet staining method. Results: Of the 81 isolates, the resistance rate to tigecycline was 9.88% (8/81) according to the British society for antimicrobial chemotherapy (BSAC) non-specific MIC breakpoints and 40.7% (33/81) were in vitro positive for biofilm production. Among 8 tigecycline resistant strains, 37.5% (3/8) were positive for biofilm production. There was no significant difference in the biofilm-forming ability between the tigecycline-susceptible and the tigecycline-resistant strains (P=0.579). Conclusion: Acinetobacter baumannii have biofilm formation ability to a certain degree. However, the resistance mechanism of Acinetobacter baumannii to tigecycline seems not relevant with biofilm formation. Further studies are required in order to demonstrate the resistance mechanism of Acinetobacter baumannii to tigecycline.

205 (B) Brucella Infection of Livestock in Georgia
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Brucellosis is one of the most common livestock zoonoses in Georgia, resulting in significant economic losses. Multiple species of Brucella, the causative bacterial genus, contribute to chronic and acute health complications in animals and humans. In this study, from 2008 - 2011, the Laboratory of the Ministry of Agriculture (LMA) collected animal specimens seasonally (Spring and Fall) in three regions designated: Kakheti, Kvemo Kartli and Imereti. Samples included blood, serum, and milk from cattle, sheep, and goats. Samples were tested using serology and molecular diagnostics (Rose-Bengal, classic bacteriology, RT-PCR, and AMOS PCR). GIS was also used to map positive cases and suspect isolates to construct maps based on strain location and epidemiological data from the livestock. In total, the field sampling efforts yielded 5,684 blood/serum samples and 2,164 milk samples from cattle, 3,966 blood/serum samples, and 638 milk samples from sheep; and 1,183 blood/serum samples and 401 milk samples from goats. Testing included serology and molecular diagnostics (Rose-Bengal, Bacteriological, RT-PCR, and AMOS PCR). In total, 735 serological tests across 16 rayons were positive on Rose-Bengal. An additional 32 bacterial isolates were recovered and identified as Brucella melitensis n=11 or Brucella abortus n=21 obtained from AMOS PCR. A majority of positive cases came from Kvemo Kartli (46%), 30% were from Kakheti, and 15% were from Imereti. The project gave Georgia capability for better detection and surveillance of Brucella, as well as planning control efforts for brucellosis in the above mentioned three regions of Georgia.

206 (B) Detection of Rickettsia, Ehrlichia and Borrelia Species in Ticks from Different Regions of Georgia Using Real-Time PCR Assay
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Rickettsiae are important arthropod-borne pathogens which may infect humans causing various types of clinical diseases. Some species of Rickettsia is classified by CDC as a category B bioterrorism agents. Human rickettsioses represent important public health problems with potentially significant morbidity and mortality. The clinical features of rickettsial diseases can often overlap with other tick-borne diseases, particularly those of ehrlichioses and borrelioses. Besides, these organisms can share the same transmitting vector and therefore co-infection in a single vector is possible, further complicating the diagnosis and treatment of these diseases. There are no reliable epidemiological data to estimate the prevalence of pathogenic species of Rickettsia, Ehrlichia and Borrelia species in Georgia. The objectives of the study was to estimate the prevalence of pathogenic species of Rickettsia, Ehrlichia and Borrelia among ticks in Georgia. Pooled tick sample collected from different regions of Georgia were used in this study. Real-time PCR assay have been validated and applied to detect nucleic acids of Rickettsia, Ehrlichia and Borrelia spp. 200 tick samples were screened from different regions of Georgia on genus-specific Rickettsia, Ehrlichia and Borrelia spp. More than 500 tick samples were processed. The results from this study will expand laboratory and epidemiological capacity for the early identification of especially dangerous pathogens related to tick-borne infections and timely response to public health threats posed by Rickettsial pathogens in our country.
207 (B) Anthrax Surveillance Data of 2012 in Georgia

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Anthrax is an infectious disease and a category A agent. The control of anthrax infections is one of the main goals of Public Health and Veterinary Service in Georgia. Anthrax is endemic in Georgia, affecting almost the entire country. Many agriculturally acquired human cases are registered annually. During last several years, the geography of anthrax infections have changed—cases among the human population and cattle were registered in areas previously not considered as affected sites. Expansion of those areas has become an important problem for the country. We are currently observing an increase in the incidence rates of anthrax cases. In 2012, 134 human cases were registered; the majority were sporadic cases, although there appeared to be 15 small outbreaks. All cases were of the cutaneous forms, and all the patients recovered with the exception one whose lethal outcome was due to a delayed start of treatment. In a vast majority of cases (86%), the source of infection was from cattle (slaughtering, processing meat), and of those most (115) of patients were male. Anthrax cases peaked in June (60/134). 133 cases had undergone clinical, laboratory (bacteriology, Real-Time PCR) and epidemiological investigations. In many cases material was sampled after antibiotic treatment had been initiated, affecting the outcome of bacteriological investigation. Approximately 75% of cases were laboratory confirmed: 40% (53/133) were culture positive, 78% (104/133) were PCR positive. Additionally, more than 2000 environmental samples were cultured for B. anthracis prevalence, 34 isolates of B.anthracis were obtained from these samples. The data indicate that the incidence of anthrax in humans in 2012 has increased by 170% compared to previous year in Georgia. DTRA program activities have facilitated the strengthening of the Georgian disease surveillance system, resulted in the improvement in rapid detection, timely reporting/notification, and response of anthrax cases in Georgia.

208 (B) Analysis of Virulence and Virulence Factors in Acinetobacter baumannii A118

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Acinetobacter baumannii (Ab) A118 is a clinical isolate susceptible to most antibiotics used in genetic studies, which makes it an ideal model for genetic studies. In this work we identify potential virulence factors and evaluate its virulence. Genomic comparisons with Ab ATCC 17978 and ATCC 19606 were carried out using MapSolver and BLAST. Acinetobactin production and iron uptake from ferric-acinetobactin were assessed using specific bioassays. The receptor BauA was identified by immunoblot. Virulence was determined using Galleria mellonella killing assays. Biofilm formation was estimated combining the material attached to the walls of tubes and the pellets on the liquid surface. Cell motility was assessed using semi-solid plates. Analysis of high-resolution restriction maps and nucleotide sequence showed the Acinetobacter iron Uptake System (AUIS) genetic makeup in Ab A118. Bioassays and immuno blot analyses confirmed that this strain secretes acinetobactin, produces the outer membrane receptor protein BauA, and takes up iron from the ferric-acinetobactin complex. Ab A118 includes all genes involved in adhesion and forms biofilms as the other 2 strains. The motility of strain A118 was similar to that of strain 19606 but lower than that of strain 19798. As strain 19606, Ab A118 lacks a fimbral protein gene, which could explain the reduced motility. Killing assays showed that strain A118 is less virulent than strains 19798 and 19606. In conclusion, Ab A118 possesses a functional AUIS and forms biofilm but showed reduced motility with respect to one of the model Ab strains, probably due to a fimbral protein missing. However, this does not seem to be an impediment for virulence since Ab ATCC 19606 shows the same characteristic and is highly virulent. Ab A118 is less virulent than strains ATCC 19798 and ATCC 19606. Experiments are being carried out to determine the molecular basis of its reduced virulence.

209 (B) Structural Characterization of the Yersinia pestis Type III Secretion Protein YscD and Evaluation of Potential Public Health Threat Posed by Y. pestis


This project consists of two parts. First, seeking to improve our understanding of the molecular mechanism of type III secretion in Y. pestis, we used surface entropy reduction mutagenesis to create mutant forms of the periplasmic domain of the type III secretion protein YscD that may be more amenable to crystallization. The mutant proteins were expressed in Escherichia coli, purified and screened for crystals. Thus far, no crystals of the mutant proteins have been obtained. The second part of the project is an evaluation of the public health threat posed by Y. pestis in the southwestern United States and Madagascar, along with a discussion of the potential ramifications of the release of a weaponized plague in a highly populated area.

210 (B) Genetic Analysis of Bacillus anthracis S-Layer Assembly

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The envelope of Bacillus anthracis encompasses a peptidoglycan layer with attached secondary cell wall polysaccharide (SCWP) and poly-D-xylocarboxylic acid (PDGA) capsule. In addition, B. anthracis assemble a proteaceous S-layer with two S-layer proteins (BslA and BslA) and twenty-two S-layer associated proteins (BSLs). S-layer assembly in the envelope of B. anthracis requires S-layer homology domains (SLH) within S-layer proteins and BSLs, which associate directly with the SCWP. Mutations in csaB, encoding the ketal-pyruvyl transferase for the SCWP, abolish not only S-layer assembly but also abrogate bacterial control of cell separation, leading to chains of incompletely separated vegetative forms with dramatically increased length. Chain length control requires at least one S-layer protein and several BSLs, which assume discrete positions in the envelope of B. anthracis. Further, B. anthracis has evolved genes that aid in the specific secretion and assembly of S-layer proteins. Other factors involved in chain length control are defined by genes that contribute to the synthesis and modification of the SCWP. We discuss the hypothesis that S-layer assembly pathways enable B. anthracis escape from host immune responses by controlling the length of vegetative chains without interference of capsule synthesis and cell division.

211 (B) Chitinases: Negative Regulators of Francisella novicida Biofilm

M-C. Chung, M. L. Van Hoek; George Mason Univ., Manassas, VA.

Biofilms, multicellular communities of bacteria, can be both environmental survival and transmission mechanisms of bacteria. We are studying Francisella tularensis which causes the zoonotic disease tularemia. Chitinases (ChiA, ChiB) of F. tularensis ssp. novicida (F. novicida), a less virulent strain which is widely used as a model organism for the more virulent F. tularensis, have been suggested to regulate biofilm formation on chitin surfaces such as crab shell. However, the underlying mechanisms of how chitinases regulate biofilm production in diverse environmental conditions are not fully determined. We hypothesized that F. novicida chitinases modulate bacterial surface properties resulting in the alteration of bacterial attachment and biofilm formation. We analyzed biofilm formation under diverse environmental conditions using chitinase mutants compared to the wild-type strain. Substratum surface charges, density of biofilm formation, and mutant bacteria. Attachment and biophysical analysis of bacterial surfaces confirmed that the chi mutant bacteria had a net-negative charge. Congo red binding assays suggested that chitinase could be at least required for modification of surface ζ-glucans levels that alter its charge. Chitinase activity is required for its anti-biofilm property and for dispersion of pre-formed biofilms. Exogenous chitinase increased drug susceptibility of F. novicida biofilms to gentamicin, suggesting that during biofilm formation chitinase altered bacterial properties for drug susceptibility. In addition, chitinase modulated bacterial adhesion and invasion to A549 cells as well as intracellular bacterial replication, possibly by altering the expression of capsule-like complex or other exopolysaccharide chitinase substrates. Our results support a key role of the chitinase(s) in biofilm formation by modulating bacterial surface properties. Our findings position chitinases as excellent anti-biofilm candidates in Francisella species. This study was supported by HDTRA1-12-C-0039 Translational Peptide Research for Personnel Protection.
212 (B) Francisella tularensis and Pseudomonas aeruginosa Interspecies Communication
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The goal of this study is to determine the interspecies communication of Francisella tularensis in the context of the natural polymicrobial environment. These results will enhance our knowledge of the persistence of this organism in its natural habitat. In this study we focused on Francisella and its interaction with Pseudomonas aerugiosa, a well-studied opportunistic pathogen with high prevalence in soil. *P. aeruginosa* produces HQNO (2-heptyl-4-hydroxy quinoline-N-oxide) as well as monounsaturated fatty acids and other signals that cause significant effects on the metabolism, growth, and biofilm formation of *F. tularensis* novicida. We hypothesize that small soluble factors produced by *P. aeruginosa* may have specific effects on *F. tularensis* lifestyle through dysregulation of Francisella gene expression. In our studies, we have shown the effect of 100 μg/mL HQNO and *P. aeruginosa* conditioned media on static *F. tularensis* novicida biofilm (Figure 1), and that these small soluble signals significantly decrease biofilm mass (p < 0.01) as quantified by COMSTAT (without affecting bacterial growth). The metabolism and growth of Francisella was also studied in response to *P. aeruginosa* signaling molecules. We have shown that *P. aeruginosa* produces signals that significantly alter *F. tularensis* novicida metabolism, growth, and biofilm. In future work, we will assess the effect of HQNO and other small signaling molecules on Francisella virulence and gene expression. These studies may lead to a greater knowledge of *F. tularensis* persistence in the natural polymicrobial environment. This work was supported by HDTRA1-12-1-030 Francisella interspecies interaction with Pseudomonas, a soil microbe.

*Figure 1*

![Image](image-url)

213 (B) Influence of Serial Passaging on Carbohydrate Profiles of Yersinia pestis

We are studying how long-term laboratory culturing changes the genome and expressed characteristics of *Yersinia pestis*. Two independent, wild isolates of *Y. pestis* were isolated from frozen fleas harvested from infected prairie dog burrows, one in Arizona and one in Texas, USA. Twelve independent lineages from each strain were established in the BSL3 facility at Northern Arizona University and grown to stationary phase and passed 60 times in brain-heart infusion broth at 26 degrees C. Cells were collected by centrifugation, and the cell pellet and supernatant were processed separately. Following inactivation by ethanol treatment, monosaccharides were liberated by acid hydrolysis and converted to alditol acetate derivatives to volatilize them. Carbohydrate profiles were generated by gas chromatographic-mass spectrometric analysis. Six different monosaccharides were quantified in each sample in addition to one polyamine, putrescine. While not technically a carbohydrate, putrescine was monosaccharides were liberated by acid hydrolysis and converted to alditol acetate derivatives to volatilize them. Carbohydrate profiles were generated by gas chromatographic-mass spectrometric analysis. Six different monosaccharides were quantified in each sample in addition to one polyamine, putrescine. While not technically a carbohydrate, putrescine was quantitatively analyzed of select carbohydrates and related compounds as potential markers to distinguish laboratory-grown organisms from naturally occurring isolates.

![Image](image-url)

214 (B) Determining the Fate of YpeB During Germination of Bacillus anthracis Spores and its Effect on SleB Activation for Cortex Degradation
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*Bacillus anthracis*, the causative agent of the disease anthrax, is classified as a Category A bioterrorism agent, in part, due to the ease in which it can be aerosolized and the resistance characteristics that have made spore eradication from contaminated sites exceedingly difficult. Spore resistance is dependent on spore dehydration, which is maintained by the cortex peptidoglycan. During spore germination, the cortex is degraded by germination-specific lytic enzymes (GSLEs) resulting in spore rehydration and loss of resistance properties. The objective of this study is to better understand the relationship between SleB, a GSLE, and YpeB, a protein expressed with SleB from an operon in *B. anthracis* and a potential regulator of SleB activity. We aim to determine the fate of YpeB during spore germination and ascertain its effect on SleB activation. In this study, western blots revealed that these proteins are dependent upon one another for their stable incorporation within dormant spores. The co-dependent relationship between SleB and YpeB supports the hypothesis that SleB and YpeB physically interact, yet the exact role of YpeB has yet to be elucidated. One possibility is that YpeB holds SleB inactive until germination is triggered. Western blots were also used to demonstrate that during germination, YpeB is cleaved to form two products both containing the C-terminus and PepSY domains - domains thought to inhibit enzymatic activity. This finding opens the possibility that YpeB cleavage during germination is important for SleB activation. The position of one cleavage site was determined, and mutated, and deletion strains were constructed to remove stretches of residues where the processing events are likely to occur. Ongoing studies aim to determine what effect a non-cleavable YpeB has on SleB activation and if YpeB cleavage is the mechanism by which SleB becomes active during germination. With a more complete understanding of GSLEs and the proteins that regulate them, it may be possible to activate germination of *B. anthracis* spores, thereby facilitating decontamination efforts.

215 (B) E5564 Enhances Prophylactic Antibiotic Efficacy in a Murine Model of Inhalational Anthrax

**Background:** Preparedness is required to protect military and civilians against bioterrorism attacks due to pathogens/toxins that are rarely seen in the U.S. *Bacillus anthracis* (BA), the causative agent of anthrax, is a high-priority (Cat. A) agent that poses a severe health risk and can result in high mortality rates. BA evades the host innate immune system by killing macrophages via apoptosis. Infection with BA induces Toll-Like Receptor 4 (TLR4)-dependent macrophage apoptosis. Treatment with TLR4 antagonist E5564 (eritoran) should minimize apoptosis, facilitate BA clearance, and increase survival. **Methods:** Three E5564 doses were tested with suboptimal doses of ciprofloxacin (Cipro) to assess potential for an added benefit in prophylactic treatment of mice prior to BA exposure (mice given a mean of 30.08 LD50s of BA [Ames] via whole body aerosol on Day 0). Six cohorts of 10 mice each were treated for 14 days using these dosing regimens: Vehicle (-) control, Standard dose antibiotic (+) control = Cipro 30 mg/kg (mpk) every 12 h (q12h); Cipro 7.5 q12h (Test Rx) + E5564 (doses [3/10/30 mpk] in 3 cohorts; single IV injections given prior to BA exposure) on Day 0. Cipro was dosed every 12 hours over the 14-day treatment period. Survival was monitored daily for 14 days.

**Results:** Survival was monitored daily for 14 days.

**Conclusion:** E5564 given prophylactically prior to BA exposure provides added benefit (up to 100% survival) to treatment with a suboptimal course of Cipro alone.
216 (C) Histopathological Analysis of Chronic Necrotizing Pulmonary Aspergillosis


Summary: Chronic necrotizing pulmonary aspergillosis (CNPA) is defined as the condition between invasive pulmonary aspergillosis and aspergiloma. However, the pathophysiology of CNPA still remains obscure. We histologically analyzed surgically removed lungs with CNPA, and found the relation of bronchiolar erosion and Splendore-Hoeppli phenomenon, which is the amorphous eosinophilic material around the fungus material. Material: Nine cases were male including a lung cancer patient. None had both hematopoietic malignancy and chemotherapy. No severe immune compromised host was confirmed. However, mild immune system downregulation was seen caused by diabetes mellitus or steroid using. We observed all of the histopathological slides using light microscope.

Results: In all cases, there had been caveat formation, a part of which was covered with ciliated epithelium. Splendore-Hoeppli phenomenon was found in three cases, and pigmented conidiospore, associated was covered with ciliated epithelium. Splendore-Hoeppli phenomenon was then used on whole spore suspensions and mass spectra collected in the range of 50-1000 m/z Results showed that mass spectral profiles of whole spore suspensions consisted of complex lipid assemblages that included branched-chain odd, branched-chain even, and straight-chained fatty acids. The relative ratios of fatty acid structures were indicative of each species. For example the ratio of 15:0 iso (m/z 243) to 17:0 iso (m/z 257) was higher in B. cereus and B. anthracis spores compared to B. thuringiensis (~2.5 and 1 respectively). The ratio of 15:0 iso (m/z 243) to 16:1 straight chain (m/z 241) differentiated B. anthracis spores from B. cereus. Lastly, meglumine ion (m/z 196) was detected on all spores purified with a Renografin gradient. Both fatty acids and meglumine were detected in bacterial concentrations as low as 1x10^4 cells/mL. Taken together, these results suggest that DART-MS can be a powerful tool for taxonomic characterization of unidentified spores as well as detecting compounds that are specific for a spore’s production process.

217 (D) Efficacy of Ribavirin Therapy in Lassa Fever

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Lassa virus (LASV), a member of the Arenaviridae family, is the etiologic agent of Lassa fever (LF), an often fatal viral illness endemic in West Africa. Case-fatality rates for LF can reach 50% and may approach 90% for pregnant women. There is currently no licensed vaccine or immunotherapy available. Ribavirin is considered the standard treatment of LF in endemic areas. Previous studies of ribavirin demonstrated efficacy in reducing mortality among LF patients. The Kenema Government Hospital (KGH) in eastern Sierra Leone is situated in a hyperendemic area for LF. All suspected cases at the KGH are tested using antigen (Ag)-capture ELISAs to detect LASV viremia, and IgM ELISAs to recent LASV infections. Treatment decisions based on clinical presentation with limited guidance from LF test results. We reviewed 161 patient medical charts for suspected LF cases presenting to the KGH from 2009 to 2012 to ascertain their survival outcomes and treatment status. Due to intermittent shortages, ribavirin treatment was not available to all patients determined by a physician to be in need of treatment. Our results showed a modest, significant reduction in case-fatality rates for patients receiving ribavirin in the acute, viremic stages of LF (Ag+/IgM-). Those with prior LASV exposure (Ag-/IgM+) or non-Lassa febrile illnesses (Ag-/IgM-) did not show any survivorship benefit from ribavirin. These results underscore the need for more thorough studies of ribavirin therapy to establish guidelines on the use of ribavirin for treating LF patients.

218 (D) Analysis of Laboratory Preparations of Bacillus Spores Using Direct Analysis in Real-Time Mass Spectrometry (DART-MS): Implications for Rapid Taxonomic Identification and Forensic Attribution of Bacterial Threat Agents

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Direct Analysis in Real Time-Mass Spectrometry (DART-MS) is a promising technique for rapid characterization of unidentified bacterial agents. Because there is little to no sample preparation and ionization occurs under ambient conditions, DART-MS may be used in a variety of biodefense and forensic applications. The goal of this study was to test whether DART-MS could be used to analyze Bacillus spore preparations by identifying the species present and any compounds that are uniquely associated with the culturing method. Three different species of Bacillus spores (anthracis, cereus, and thuringiensis) were cultured and then purified by centrifugation through a Renografin (Meglumine Diatrizoate) gradient. DART-MS was then used on whole spore suspensions and mass spectra collected in the range of 50-1000 m/z Results showed that mass spectral profiles of whole spore suspensions consisted of complex lipid assemblages that included branched-chain odd, branched-chain even, and straight-chained fatty acids. The relative ratios of fatty acid structures were indicative of each species. For example the ratio of 15:0 iso (m/z 243) to 17:0 iso (m/z 257) was higher in B. cereus and B. anthracis spores compared to B. thuringiensis (~2.5 and 1.3 respectively). The ratio of 15:0 iso (m/z 243) to 16:1 straight chain (m/z 241) differentiated B. anthracis spores from B. cereus. Lastly, meglumine ion (m/z 196) was detected on all spores purified with a Renografin gradient. Both fatty acids and meglumine were detected in bacterial concentrations as low as 1x10^4 cells/mL. Taken together, these results suggest that DART-MS can be a powerful tool for taxonomic characterization of unidentified spores as well as detecting compounds that are specific for a spore’s production process.
220 (D) Cell Surface Hydrophobicity of Bacillus Spores: Novel Forensic Signatures for the Holistic Characterization of Bacterial Threat Agents
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The goal of this study was to test whether the cell surface hydrophobicity could be used to identify different species/strains of biothreat agents and characterize forensically relevant aspects of the organisms' cultivating environment. Spores from four different species of Bacillus (B. cereus, B. thuringiensis, B. anthracis, B. subtilis) were cultured using three different medium recipes (G Medium, Casein Acid Digest, and 1x Schaefer's Medium). The cell surface hydrophobicity of all samples was then measured using Microbial Adherence to Hydrocarbon (MATh) and Hydrophobic Interaction Chromatography (HIC) techniques. Results from the MATh assay showed that most Bacillus species showed a distinct range of hydrophobic retention values to hexadecane during an organic extraction. B. cereus spores ranged from 30 to 38%, B. anthracis from 35 to 40%, B. thuringiensis from 42 to 51%, and B. subtilis from 0% to 6%. Conversely, HIC tests revealed that growth in different medium formulations changed the surface chemistry of the spore such that each culture displayed different adsorbance capacities to sepharose beads. Growth in G Medium (formulation without enzymatically digested protein source) yielded spores with significantly higher HIC hydrophobicity compared to spores grown in media supplemented with peptone or tryptone (80-88% and 25-40%, respectively). Taken together, this indicates that MATh and HIC are powerful techniques for determining both the taxonomic identity of an unknown bioterror agent and phenotypic signatures that can assist in the forensic attribution of the organism.

221 (D) Quantification of Anthrax Edema Factor Activity: Comparison of Cyclic Amp Detection by ELISA and LC-MS/MS
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Bacillus anthracis toxins consist of lethal factor and edema factor (EF) that combine with protective antigen to form lethal toxin and edema toxin, respectively. EF is a highly active calmodulin-dependent adenyl cyclase that converts ATP to cyclic AMP (cAMP) causing edema and immune dysfunction during anthrax. We developed a fast (<5 hr), high sensitivity (0.019 pg/ml in plasma) quantitative EF method. Analytic sensitivity is derived from monoclonal antibody (mAb) purification/concentration of EF, EF reactivity with ATP producing cAMP, and cAMP quantification by LC-MS/MS. There is a need for converting highly sensitive diagnostic tests, such as those developed for MS, to a platform that is widely available. Here we report a comparison between detection of EF-generated cAMP by LC-MS/MS and ELISA EF spiked in plasma from 2.5-0.000019 ng/ml was analyzed both with and without prior magnetic dual EF-mAb purification. EF was then reacted with ATP/calcium/calmodulin generating cAMP. The reaction mixtures were split for analysis by LC-ESI-MS/MS and a commercially available cAMP ELISA kit. Quantification of EF activity in samples from infected rhesus macaques were also compared. The cAMP ELISA kit used was a competitive immunomassay that quantifies cAMP with reported detection range of 200-0.78 pmol/mL cAMP within 2-6 hrs. We determined that VLP capture was more efficient with gentle rotator incubation than with high speed orbital shaking. VLPs were detected by their respective capture antibodies at 4:1 to 9:1 signal to noise ratios in the high nanogram range. Cross-reactivity was not detected in the fully multiplexed assay. Ongoing research is aimed at optimizing the speed and sensitivity of the current assay, developing and engineering antibodies against more filovirus variants and developing a broad diversity of VLPs for testing.

222 (D) A Multiplexed Approach for Rapid Filovirus Detection

Filoviruses present a re-emerging threat to military personnel, healthcare workers and the public in Sub-Saharan Africa and Southeast Asia. Rapid, simple, sensitive and specific diagnostic tests are preferred in resource-limited settings. Despite the advantages, one of the shortcomings of immunodiagnostic tests is their narrow tolerance for antigenic variation, thereby limiting their ability for detection of pathogens with broad antigenic diversity. Recombinant engineered antibodies may serve as powerful components of configurable diagnostic systems. These antibodies are rapidly produced and ideal as interchangeable components of immunodiagnostic assays. We used an engineered antibody fragment, KZ52, and commercial full-length antibodies coupled to Luminex microspheres (MagPlex®) to build a multiplex assay to detect filoviruses. Virus-like particles (VLPs) expressing glycoproteins from Zaire ebolavirus, Sudan ebolavirus, and marburgvirus were used as surrogate assay targets. VLPs were diluted to various concentrations, and exposed to microspheres in 96-well plates using either individual assays or in multiplex configurations. VLPs were captured using incubations at room temperature from 2-6 hrs. We determined that VLP capture was more efficient with gentle rotator incubation than with high speed orbital shaking. VLPs were detected by their respective capture antibodies at 4:1 to 9:1 signal to noise ratios in the high nanogram range. Cross-reactivity was not detected in the fully multiplexed assay. Ongoing research is aimed at optimizing the speed and sensitivity of the current assay, developing and engineering antibodies against more filovirus variants and developing a broad diversity of VLPs for testing.
224 (D) Two Step RT-PCR for Rapid Diagnosis of Francisella spp. Infection
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Introduction: Tularaemia is a zoonosis caused by Francisella tularensis. Diagnosis using conventional techniques lacks on sensitivity and is slow, so a rapid method for diagnosis is important to guide successful antimicrobial treatment and prevent dissemination. The genus Francisella only has two species: F. philomiragia and F. tularensis spp. but the last one is virulent for humans. Objective: To develop a real-time PCR (RT-PCR) screening assay targeting the 16S rRNA and pdpD genes for accurate identification of different Francisella spp. in complex biological matrices to reduce the hands-on time and increase the sensitivity. Materials and Methods: Within the framework of an EU project (QUANDHIP), 15 clinically relevant Francisella spp. samples, from different matrices and 5 controls were tested to develop a two step real-time protocol using SYBR Green in a LC 480. The first step consisted in the amplification and sequencing of a 16S rRNA region of a 15 ng of DNA extracted from samples mentioned above following the recommendations provided by CLSI and. After that, we optimized the RT-PCR reaction using specific primers targeting the pdpD gene for identification using melting temperature (Tm) and melting profile of all samples previously identified as Francisella spp. To guarantee the maximum difference between the obtained melting profiles from different Francisella spp, we added different quantities of DMSO. Results: The amplification and sequencing of 16S RNA genes allowed the identification between F. philomiragia from the F. tularensis subspecies. The assay targeting pdpD was negative for F. tularensis subsp. holocattica and positive for the other subspecies. The best obtained melting profile was with the PCR master mix which contained 5% of DMSO, so as to achieve the discrimination F. tularensis, tularensis among F. tularensis subsp. spp. since, to be a Tm, 77 ± 0.11 and 76 ± 0.08 for F. tularensis and F. tularensis subsp. spp, respectively. Conclusion: This combined study shows that RT-PCR amplification in 16S RNA and pdpD region have been successfully applied using complex matrices diagnosing both F. tularensis subsp and F. philomiragia.

225 (D) URPI: Ultra-Rapid Pathogen Identification and Discovery in Clinical Metagenomic Samples
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Metagenomics by next-generation sequencing (NGS) is a powerful tool for pathogen detection and discovery. However, practical deployment of the technology has been hampered by the bioinformatics challenge of rapidly discriminating pathogen from host background sequences. We have developed URPI (“Ultra-Rapid Pathogen Identification”), a computational pipeline integrating two analysis modes: (1) Sequedex, a tool for pathogen detection from unprocessed NGS reads by signature peptide matching (8 min for 100 million reads on a 64-core computational server), and (2) SNAP, a hash-based alignment tool for computational subtraction of host background sequences followed by in-depth identification of pathogen sequences for de novo genome assembly (1 hr for preprocessing and <5 min for SNAP). By ROC curve analysis, SNAP exhibited comparable sensitivity and specificity to BLASTn, BOWTIE, and BWA using synthetic datasets of random human, bacterial, and viral sequences. Processing times for SNAP and Sequedex were scalable and considerably faster than existing algorithms (image). For clinical samples, SNAP and Sequedex were equivalent to BLASTn and BLASTx, respectively, in detection of influenza, sapovirus, parechovirus, and hantavirus. URPI also correctly identified a recombinant HIV strain at a concentration <100 copies/ml, divergent outbreak viruses (Bas-Congo virus, <334 aa identity to nearest neighbor; titi monkey adenovirus, <50% aa identity), and a pathogenic Lyme bacterium (Borrelia burgdorferi). Given its speed and accuracy, URPI is suitable for numerous NGS applications ranging from routine diagnosis in the clinical microbiology laboratory to global surveillance of unknown outbreaks.

226 (D) DNA-Chromatography for Rapid Screening BSL3 Pathogens

DNA chromatographic detection system was established for multiple BSL3 Bacterial pathogens. Ready to use cocktail primers set dried in a single low profile PCR tube was prepared for quick detection of respiratory pathogens. After collecting gargling water, the specimen was just boiled and the 5ul of the boiled solution was directly used for Quick PCR with ready to use dried cocktail primers. After 30 min, DNA probe printed paper strip was inserted in the PCR tube to identify the PCR amplicon. Within 5 min, the amplicon was captured to specific probe printed on the DNA strip and visualized with colored Latex beads. Using this crude gargo- lling specimen, 100 to 1000/ml of pathogens was detected in our spiked experiments. Patients exposed BSL3 bacterial pathogens usually visit clinics with mild upper respiratory symptoms at early stage. City hospitals are not ready to diagnosis these BSL3 pathogens because laboratories need special media and diagnostic reagents to confirm these BSL3 pathogens. To notify the infection of BSL3 pathogens among daily outpatients, our dried ready to use cocktail primers and DNA strips might be useful tools for screening of BSL3 pathogens. Enzyme for PCR was selected from commercially available kits, popular 2x fold PCR premix reagent, which can be stocked at 4C for more than 1 year and also, can be kept at room temperature for 2 weeks. Cocktail primers to amplify agents of common outpatient pneumonia such as Legionella, Mycoplasma, Chlamydia, Orientialia, and Rickettsia, were also mounted in the primer sets to differentiate these common outpatient pneumonia from BSL3 pathogens such as Bacillus anthracis, Yersinia pestis, Brucella spp, Francisella tularensis, and Burkholderia mallei-pneumobacter.
**227 (D) Sentinel Clinical Laboratories Assess Preparedness Competency with a Biannual Exercise on Biothreat Agent Detection**

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The College of American Pathologists (CAP), the Centers for Disease Control and Prevention (CDC), and the Association of Public Health Laboratories (APHL) developed the biannual Laboratory Preparedness Exercise (LPX) to prepare sentinel laboratories for the detection of pathogens of epidemiologic importance, including bioterrorism (BT) agents. Laboratories receive three specimens of potential BT agents to detect, rule-out, or refer to a Laboratory Response Network (LRN) Reference Laboratory for further characterization. The laboratories are to utilize LRN protocols and American Society for Microbiology (ASM) Sentinel Laboratory Guidelines throughout the exercise. In April 2012, approximately 1300 sentinel laboratories participated in LPX-A to receive specimens for *Yersinia pestis*, Bacillus megaterium with Staphylococcus epidemidis, and *Bacillus anthracis* with viridians Streptococcus. • For *Y. pestis*, 94% reported it as a potential or confirmed BT agent while 6% reported it as a non-BT culture. • For *B. megaterium*, 86.5% reported it as a non-BT culture; however, Gram stain results suggest laboratories were unable to detect both organisms in the mixed culture as only 60% reported cocci. • For *B. anthracis*, 97.7% recognized and evaluated the Gram-positive bacillus, but 15.5% reported it as a non-BT culture. Also, 8% of participants and 9% of participants did not perform catalase and motility tests, respectively, both of which are key reactions for the identification of *B. anthracis*. The results of the LPX-A exercise indicate that sentinel laboratories performed acceptably thus they are capable of evaluating culture for potential BT agents. The results do suggest that the laboratories should continually review LRN protocols and ASM guidelines for testing algorithms and key tests and reactions, especially when evaluating mixed cultures. Additionally, these results indicate the importance of timely communication with an LRN Reference Laboratory in the event that the sentinel laboratory is unable to rule-out a potential BT agent (e.g., for the *Y. pestis* and *B. anthracis* cultures from this exercise).

**228 (D) Rapid Lateral Flow Immunoassay for Early Diagnosis of Anthrax**


Anthrax is the most serious bioterror threat facing the civilian and military populations in the United States. *Bacillus anthracis* infection presents a narrow treatment window and current diagnostic methods are limited by the time required to obtain results. Our laboratory previously created a monoclonal antibody (mAb), F26G3, that specifically binds to the capsular polypeptide, poly-gamma-D-glutamic acid (PGA). This mAb was used to construct a lateral flow immunoassay (LFA) for the detection of disseminated *B. anthracis* antigen. The goal of this study was to measure circulating PGA levels during a rabbit model of infection to establish the required sensitivity for diagnosis at or before the time when corresponding blood cultures retrospectively turn positive and to verify that the anthrax LFA meets the required sensitivity. F26G3 was used in an ELISA format to quantify and monitor PGA levels in rabbit plasma and urine collected in a time-point series post inhalation exposure to virulent *B. anthracis* spores. These findings were then compared to parallel blood culture results. The LFA limit of detection was determined by visual evaluation of strips tested with serum samples containing serially diluted purified PGA. Results using the ELISA format detected PGA in plasma from infected rabbits at the same time blood cultures turned positive and frequently identified the infection sooner, when cultures were still negative. While PGA was readily detectable in plasma, urine proved to be a less reliable source for identifying the antigen. Limit of detection analysis found that the anthrax LFA readily meets the required sensitivity to detect PGA at or before the time when cultures become positive. With blood cultures taking up to 24 hours to yield results, the rapid point-of-care format of the LFA presents a clear opportunity for a more timely diagnosis; shortening treatment delay time and consequently improving chances of a positive patient outcome.

**229 (D) Detection of Q Fever Specific Antibodies with Recombinant Antigens Com1 and AdaA**


*Coxiella burnetii*, the etiological agent of Q fever, is an obligate intracellular pathogen with worldwide distribution and a broad host range that includes livestock and humans. Symptoms of acute Q-fever are highly nonspecific, diagnosis can be very difficult. The accepted method of diagnosis is indirect immunofluorescence assay (IFA) using the whole cell antigen. Due to the hazard and difficulty of culturing and purifying *C. burnetii* in a biosafety level (BSL)-3 laboratory, the antigens are not available in most clinical laboratories. Earlier studies focused on the identification of immunogenic antigens of *C. burnetii* have discovered several protein immunogens. In this study, we prepared the recombinant antigens of the 27-kDa outer membrane protein (Com1) and the 28-kDa acute disease antigen A (AdaA) which have been shown to be recognized by Q fever patient sera. The performance of recombinant antigens was evaluated in ELISA by IFA confirmed serum samples. Due to the low titers of IgG and IgM in Q fever patients, the standard ELISA signals were further amplified by using biotinylated anti-human IgG or IgM plus streptavidin-HRP polymer. There was a good correlation between the O.D. readings of the amplified ELISA and the titers of IFA on the confirmed Q fever positive sera. None of the sera from patients with other febrile diseases reacted with the Com1 and AdaA. These results suggested that modified ELISA using recombinant Com1 and AdaA may prove to be a potential method for the detection of Q fever specific antibodies especially in resource-constrained areas.

**230 (D) Rapid Anthrax Assay: Proof of Concept for Point of Care Diagnostics**

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**Background:** *Bacillus anthracis* is a Category A priority biological threat agent. Following a release of *B. anthracis* spores, there is an unmet public health need for a point of care (POC) device to rapidly diagnose anthrax. Current detection methods, such as PCR and culture, are time consuming, require laboratory settings, and may be sensitive to antibiotic therapy. Early culture-independent detection candidates for anthrax diagnosis include EF, LF, and PA antigens in serum. This study establishes proof of concept that a POC assay can be used diagnose anthrax. **Methods:** Monoclonal antibodies (mAbs) generated against PA (AVR1046, AVR1162) and LF (AVR1675) were used to develop a gold based lateral flow immunoassay to detect PA or LTx (lethal toxin). Each mAb was conjugated to 40 nm colloidal gold spheres for visualization. During development, selection of the nitrocellulose membrane, quantities of mAb on the test line, and selection of conjugate pads were each optimized. PA and LTx antigen detection was developed in a phosphate buffered saline matrix and optimized in a serum matrix. **Results:** Visual limits of detection (LOD) were 2.5 ng/mL for PA and 5 ng/mL for LTx using AVR1162 as the test line and AVR1046 as the detector in buffer, LOD were 5 ng/mL for PA and 10 ng/mL for LTx in the reverse orientation. Detection of LTx using AVR1675 as detector gave an LOD of 50 ng/mL with AVR1046 as the test line. In serum, the LOD for PA was 5 ng/mL. Results in both matrices are obtained in less than 15 minutes. **Conclusions:** The proof of concept device described can detect anthrax antigens as low as 2.5 ng/mL, an LOD within the target range (10-50 ng/mL) for inhalation anthrax. This rapid, low resource POC shows that mAbs generated against anthrax antigens may be used to detect such antigens as monomers (i.e. PA) or in complex with other antigens (i.e. LTx as a complex of PA and LF), providing a multi-target test that has enhanced analytic sensitivity with clinical relevance. Antibodies generated against EF and LF will be exploited to expand this assay.
A Novel Approach to Unknown Pathogen Detection in Clinical Samples


High-throughput sequencing has opened up new avenues in science and medicine. Among these possibilities is diagnostics, particularly early infection diagnostics. There is a great deal of interest among the medical and biodefense communities in early, rapid, and accurate pathogen detection. This is particularly difficult in the case of unknown and poorly characterized pathogens such as emerging infectious disease and bioterrorism. Current technologies in pathogen diagnostics such as PCR and ELISA require prior knowledge of what pathogens are expected to be in the sample. A major challenge in development of this technology has been significant background problems. Low signal-to-noise ratios in identifying trace pathogens in blood or other patient samples frequently result in insufficient pathogen sequence detection to conclusively identify the actual pathogen in a sample. Sandia National Laboratories has made significant progress toward this challenge in recent projects, reaching 10-fold human background reduction. However, this needs further improvement to realize success using this technology. We are developing a novel approach to this problem that would allow separation of virus-infected cells in a blood sample from those that are not infected based on distinct fluorescent biomarker profiles correlated with viral infection. Hyperspectral imaging allows classification of cells based on spectral and morphological/spatial differences. Hyperspectral imaging will be combined with microfluidic cell sorting in order to rapidly isolate infected and uninfected cells within a single sample. The cells identified as potentially infected can then be further analyzed by single cell deep sequencing to identify potential pathogens not present in apparently uninfected cells. This technology has the potential to revolutionize not only early clinical and field diagnostics but also improve understanding of how viral pathogens cause disease and facilitate improved countermeasures against emerging and engineered pathogens.

Improved Detection and Surveillance of Lassa Fever in West Africa using ReLASV LF Diagnostic Assays


Lassa fever (LF) is an acute viral illness caused by Lassa Virus (LASV) which is endemic to West Africa and causes high morbidity and mortality. Currently there are no commercial LF diagnostic tests available in West Africa. The Viral Hemorrhagic Fever Consortium has developed the ReLASV™Ag RDT, a rapid antigen based immunodiagnostic test for LF detection and surveillance. Preliminary clinical performance data indicates ReLASV™Ag RDT is capable of identifying LF patients in the acute viremia stage of infection. ReLASV™Ag, IgM, IgG ELISAs have been incorporated into the clinical effectiveness testing of the RDT to aid in confirmation of acute viremia, monitor therapy effectiveness, and track LF patients humoral immune response. The ReLASV™Ag RDT is capable of detecting LASV nucleocapsid (NP) in 5 - 15 mins with a detection range of 0.18 to 300ng/test. Overall mortality rate of suspected casess is 10% with 55% mortality when RT-PCR positive and 54% for RDT positives. However, increased mortality rates in the RT-PCR positive/ RDT positive (77%) indicates RDT capacity to identify the most acute LF cases and aid in patient management. The ReLASV™ assays have demonstrated sensitivity to Sierra Leone and Nigerian strains of LF and are not cross-reactive with New World Arenaviruses, and other febrile illnesses such as Chikungunya, Dengue fever, Malaria and West Nile Virus. Interfering substances testing has also demonstrated no affect on signal development by Ribavirin, Quinine, Artemether, Proguanil, and other drugs in common use. We evaluated LF and non-LF patients for symptoms, blood chemistry and non-LF infectious disease (FUOs). Our survey revealed that LF diagnosis based on symptoms or blood chemistry was not adequate and is confounded by FUOs which can also present with similar symptoms. We believe the use of these new LF diagnostic tests can fill the current vacuum of LF point of care testing for healthcare facilities in West Africa.

Immune Cell-Assisted Culturing of Bacterial Pathogens Collected from Biocrimes

S. Sorrell, N. Moritz, J. Bortzner, R. Vinegar PhD, K. Brown PhD; MRI-Global, Palm Bay, FL.

Successful recovery of bacterial pathogens from biocrime scenes is an important aspect of a criminal investigation. Bacterial pathogens, expanded in culture, provide a potentially valuable source of information relevant to source attribution. However, bacteria collected from biocrimes are sometimes fastidious in nature and may even be stressed due to aerosolization procedures or environmental exposure. These fastidious and/or stressed bacteria are highly recalcitrant to culturing from environmental matrices using classical culturing approaches. Immune cell-assisted culturing is a novel culturing approach that exploits host immune cells for stimulation of bacterial growth. During immune cell-assisted culturing, bacteria are co-cultured with macrophages under prescribed conditions prior to recovery on standard laboratory media. We tested this process with Bacillus anthracis Sterne and Francisella tularensis LVS. In addition, a small extraction study was performed to determine the robustness of this method on LIVS. The authors gratefully acknowledge the Department of Homeland Security for their support of this research.

The First Bat Survey for Emerging Zoonotic Pathogens in the Republic of Georgia


Bats are recognized as reservoirs of emerging zoonotic pathogens, such as lyssaviruses, paramyxoviruses, coronaviruses, filoviruses. NCDC in collaboration with CDC, US initiated new program funded by DTRA to strengthen bat surveillance, and implement laboratory diagnostic methods along with binding laboratory capacity. Collaborators from CDC visited Georgia and conducted first round of trainings in field sampling of bats and laboratory methods. Although the significance of bats as reservoirs of zoonotic diseases in the Caucasus region is unknown, we do know that West Caucasian bat virus circulates in Miniopeters schreibersii bats in the area. The same bat species was recently implicated as reservoir of a novel filovirus in Spain. Furthermore, other bat species that maintain circulation of European bat lyssaviruses, paramyxoviruses and coronaviruses in different parts of Europe are present in Caucasus. Our goal was to initiate surveillance of bats for these and other pathogens of public health and veterinary significance. Surveillance was initiated in 5 regions of Georgia during June, 2012. 236 bats from 8 species were sampled randomly from different roosts, manually or using nets using CDC protocols. The numbers per roost and species where reviewed by expert zoologists, to avoid harmful consequences for bat populations. Seven bats were found sick or dead. Bats were sedated, measured, identified to species, weighed and subjected to euthanasia via cardiac exsanguination. Further, oral and fecal swabs, and tissues were harvested. Samples were sent to the CDC (Atlanta), for screening for multiple pathogens. Georgian staff were trained, local sampling protocol developed for further surveillance. By inferences from other parts of Europe and the world, bats from the Caucasus are expected to maintain circulation of important zoonotic pathogens. This surveillance project is the first step in appreciation of the significance for veterinary and public health in the area in conjunction with monitoring.

Program & Abstracts

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BIODEFENSE PROGRAM & ABSTRACTS
235 (E) Withdrawn

236 (E) Using Preparations of Cosmetic Botulinum Toxin (A and B) For Evaluation of Commercial Quick Tests
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Background: Botulinum neurotoxin (BoNT) secreted from Clostridium botulinum is a well-known toxin of concern as a biological weapon. There are seven different serotypes of BoNTs, named A to G. Human botulism is caused mainly by BoNT/A /B /E and occasionally /F, with BoNT/A being the most poisonous followed by BoNT/B. There are different commercially available quick tests for the identification of BoNT, and selecting the optimal test can therefore be difficult. In this study, we present data using cosmetic preparations of BoNT A and B in the evaluation of quick tests.

The advantage of using cosmetic toxins is that they are well defined and easily available. Methods: For reference we used an in-house ELISA procedure with a starting dilution of 1 μg/ml of in-house toxins of BoNT A and 0.1 μg/ml B (two-fold dilution curves). Four commercially available cosmetic toxins, Botox (BoNT A, 10 ng/ml), Myobloc®/Neurobloc® (BoNT B, 27.5 ng/ml), Dysport® (BoNT A, 8.7 ng/ml), and Xeomin (BoNT A, 1.2 ng/ml), were tested in the assay. Based on these data commercially available quick tests will be evaluated. Results: The in-house ELISA was able to detect and measure the cosmetic toxins, Botox, Dysport and Myobloc®/Neurobloc®. The toxin Xeomin was not detectable, probably because of a too low concentration. The toxin Myobloc®/Neurobloc® showed a very high reaction with the ELISA for B as expected; however, a relatively high cross-reaction was also observed for the A test in which the ELISA measured 5.5 ng/ml. Commercial kits were tested with the cosmetic toxins as a test for their limit detection and specificity (data will be presented at the conference).

Conclusions: This study shows that cosmetic BoNT products have enough toxin contents to be used as a convenient means of testing commercially available quick tests. The advantage is that the test material is readily available, well-defined and under strict pharmacological specifications. The study will furthermore present data from the testing of several commercial kits.

237 (E) Streptavidin-Nucleic Acid Network as Signal Enhancer in a Portable Detection Platform Using Electrochemical Biochips
C. Pöhlmann, T. Elbner; Bruker Daltonik GmbH, Leipzig, Germany.

Rapid and accurate monitoring of biological toxins such as botulinum neurotoxins (BoNTs), staphylococcal enterotoxins (SES) or ricin in environmental or food samples is an important task in biosecurity and defense. Electrochemical biosensors offer the possibility for parallel analysis of several agents along with a high sensitivity. However, due to the high toxicity of biological toxins ultrasensitive detection methods are necessary to enable detection of these toxins in a relevant concentration range. One possibility to improve sensitivity and reliability of biochips is the introduction of a signal enhancement step such as application of dendrimer technology or nanosystems. Recently, we presented an electrochemical detection platform applying biochips with immobilized antibodies against BoNT/A, BoNT/B, BoNT/F, ricin and SEB. The detection platform allows simultaneous identification of these toxins within less than 25 minutes in an automated ELISA process. Detection limits for BoNTs, ricin and SEB are in the low ng/ml-range. Detection of these toxins is possible in food and environmental sample matrices. Here, we introduce the application of multivalent β-galactosidase-streptavidin nucleic acid network for signal amplification in electrochemical sandwich immunoassays. The multivalent β-galactosidase-streptavidin-nucleic acid network causes amplification of the signal in terms of enzymatic hydrolysis of p-aminophenyl-β-D-galactopyranoside followed by an increased redox cycling rate of electroactive p-aminophenol. Consequently detection limit of 1 ng/ml model antigen goat IgG, representing tenfold improvement in comparison to monovalent β-galactosidase-streptavidin reporter conjugate, can be achieved. Finally, we demonstrate the application of streptavidin-nucleic acid network for the detection of BoNTs, SEB and ricin showing an influence on the sensitivity. These results demonstrate the potential of biochips in combination with streptavidin-nucleic acid network as signal enhancer for extremely sensitive on-site detection of biotreat agents.
240 (E) Utilisation of Ultrafiltration for Sampling of Water at an Anthrax Outbreak Site

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In 2011 there was an anthrax outbreak among cattle grazing a conservation area in Örebro county in Sweden. To investigate presence of anthrax spores in a water body bordering the pasture, 40 L samples of water were concentrated in hollow fiber ultra filters. Sampling was done at three different sites and the filters were retrieved for analysis at BSL 3 laboratory. Upon preparation of the concentrated water samples presence of anthrax at one of the sampling sites could be confirmed by real time PCR.

241 (F) Genetic Variation within Francisella tularensis A.I and A.II Clades

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Francisella tularensis is one of the most pathogenic bacteria known and is accordingly classified by the Centers for Disease Control and Prevention (CDC) as a Category A select agent. This intracellular pathogen is grouped into several subspecies. One of the subspecies, specifically F. tularensis subs. tularensis (also known as type A), is further subdivided into subtypes A.I and A.II, with the former clade having the highest infectivity among all the members of this pathogenic species. Although the various F. tularensis subspecies share considerable genomic content, they exhibit different degrees of virulence. To evaluate the genomic diversity within this monomorphic pathogen, we sequenced and assembled the complete chromosome of wild-type isolates from both the A.I and A.II clades for comparison to other sequenced F. tularensis strains. Due to the difficulty in closing gaps between contigs because of the numerous insertion sequence (IS) elements and repetitive DNA segments within the F. tularensis chromosome, Whole Genome Mapping was utilized to complete genome assembly. These results illustrated that several major chromosomal translocation occurred in both the A.I and A.II subtypes. The data also showed that while disparate in geographic origin and year of isolation, polymorphisms were limited in the fully sequenced A.I strains (SCHU S4, FSC198, and NEO6159B), indicating considerable sequence conservation and chromosomal synteny. In contrast, comparisons between a newly sequenced wild-type A.II isolate (WY-00W4114) and the only other sequenced A.II strain (WY96-341B8) revealed substantial genomic variation. Unlike the A.II isolates, the A.II strains have numerous indels and nucleotide substitutions, as well as a higher gene content with more IS elements than the A.I-I isolates. Overall, these results indicate a considerable difference in genome variation and plasticity between the A.I and A.II subtypes. The A.II clade appears to be evolving at a faster rate than the A.I clade with possible changes in fitness and virulence. Studies are currently underway to investigate the significance of these findings.

242 (F) Virus Pathogen Resource (ViPR): A Public Bioinformatics Database and Analysis Resource for Human Virus Pathogen Research

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The Virus Pathogen Resource (ViPR, www.viprbrc.org) is a freely available, NIAD-funded one-stop database and analysis resource to view, analyze, visualize, save and share data for a broad range of human virus pathogens and other related viruses, including Arenaviridae, Bunyaviridae, Caliciviridae, Coronaviridae, Flaviviridae, Filoviridae, Herpesviridae, Paramyxoviridae, Picornaviridae, PoXviridae, Reoviridae, and Rhinoviridae. ViPR provides access to genome sequences, enriched gene and protein annotations, Gene Ontology classifications, proteins domains and motifs, protein ortholog groups, experimentally-determined and computationally-predicted immune epitopes, Sequence Feature Variant Types, 3D protein structures, clinical metadata, data involving host factors involved in virus replication, and other data types through an intuitive web-based search interface. The information within ViPR, or custom user data, can be analyzed and visualized through web-based tools including: BLAST, multiple sequence alignment, phylogenetic tree inference, Metadata-driven Comparative Analysis, sequence format conversion, PCR primer design, sequence variation determination, Sequence Feature Variant Type analysis, 3D protein structure visualization, clinical annotation, and, in GBrowse. A personal Workbench space is also provided for ViPR users to save or share sequence, searches and analysis results for future use. Our current suite of tools can be used to facilitate the research and development of diagnostics, prophylactics, vaccines and therapeutics against these human viral pathogens.

243 (F) Genotyping and Trace-Back-Analysis of Bacillus anthracis Isolates Related to Injection Anthrax


Anthrax is a lethal zoonotic disease caused by Bacillus anthracis leading to several distinct manifestations, known as cutaneous, inhalation or intestinal anthrax, respectively. During recent years, a novel form of the disease has emerged, named injection or subcutaneous anthrax. This anthrax is supposedly caused by endospore-contaminated heroin injected by drug consumers. Thus far, about one hundred cases of injection and inhalation in heroin consumers have been observed in several European countries resulting in 22 deaths. This unusual manifestation of the disease is of great concern because of its high casualty-rate and because the origin and source of the pathogen remains obscure. To trace-back the origin of the bacteria, we conducted a comprehensive molecular-genetic bioforensic investigation of several B. anthracis strains related to injection anthrax. Comparative analysis of several independent genomic markers between recent drug-related and endemic strains of B. anthracis collected from all over the world revealed a possible connection of the endospore-contamination to international drug-trafficking.

244 (F) A High Resolution Framework for the Phylogenomic Analysis of Escherichia coli O157:H7

M. Eppinger1, T. H. Hazen2, M. Rodriguez2, S. Agrawal2, K. GaIiens3, S. S. Koenig1, S. Daugherty1, L. Sadowszicz2, L. Tallon1, D. A. Rasko2, K. Fraser2, J. Ravel1, 1UTSA, San Antonio, TX, 2Univ. of Maryland, Baltimore, MD.

Background: The rapid emergence of E. coli O157:H7 from an unknown strain in 1982 to the dominant enterohemorrhagic serotype in the United States and the cause of widespread outbreaks of human food-borne illness, heightened the critical need to understand its pathogenic potential, genomic plasticity and different evolutionary and ecological niches. Insights into the E. coli O157:H7 pathogenome is hindered by its genetically homogenous population genetics, which is often beyond the resolution threshold of investigative methodologies deployed by public health laboratories. Methods: This study follows outbreaks dynamics and plasticity in a global panel of more than 350 E. coli O157:H7 distributed in time and space recovered from the zoonic reservoir, contaminated produce and ill patients. To investigate the phenotypic variations on the level of individual polymorphisms, we have developed a high-throughput bioinformatics SNP discovery and validation pipeline taking into account coverage and quality of underlying sequence reads. Results: In-depth genome sequencing combined with high-resolution phylogenomic approaches allowed the dynamics of pathogenome evolution to be studied at a previously unprecedented level of phylogenetic accuracy and resolution. Many of the identified SNPs and structural biomarkers seem to be intimately associated with niche-adaptation, pathogenesis and outbreak assignment. Conclusions: The established refined phylogenomic framework and rich mutational database has major implications for improving public health. These resources provide a resource molecular epidemiology, clinical and forensic communities and provide critical insights into evolutionary and ecological processes and aid current molecular assays used in public health laboratories in order to better prepare for future outbreaks and improved risk assessment. This project is funded with federal funds from the National Institute of Allergy and Infectious Diseases under contract HHSN27220090007C.
245 (F) A System for Rapid Pathogen Detection and Outbreak Integration Based Solely on NGS Reads


The National Center for Biotechnology Information (NCBI) has long provided archive services to store pathogenic data at the molecular level. Over 7000 assemblies of bacterial genomes have been submitted to date and it is now possible for certain species to be detected directly from next generation sequencing (NGS) data. NCBI has designed a system by which reads from a set of samples of a putative outbreak are analyzed for likely clonality. First, libraries of raw reads are used to find a suitable reference genome from existing NCBI collections that can serve as an analysis substrate. This is done by comparing kmer spectra of the raw read sets with those of candidate reference genomes. The query libraries are then assembled against the substrate and combined with de novo assemblies of the same libraries. A set of single nucleotide polymorphisms (SNPs) is computed from those assemblies in a clonal cluster. These genotypes are analyzed for phylogenetic signal and a putative evolutionary distance tree is constructed. Additional genomes from the existing NCBI collections may be added in order to link the query genomes to previous outbreak clusters using both molecular information and sample metadata. Initially this system will be provided to users on request. Later, an automated submission service will be integrated into the existing NCBI high throughput submission portal. The system’s utility has been demonstrated on samples of Salmonella enterica involved in a food borne outbreak using three different NGS technologies, as well as on samples of hospital acquired infections of Klebsiella pneumoniae and Staphylococcus aureus.

246 (G) A Phase 1C Clinical Trial of DAS181-F03: A Sialidase for Prophyllaxis and Treatment of Influenza and Parainfluenza Infections

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Background: DAS181 is a fusion protein consisting of a recombinant sialidase derived from Actinomyces viscosus, fused to an anchoring domain which binds to respiratory epithelial cells. Delivered as an inhaled lyophilized powder, it cleaves the sialic acid receptors for influenza A and B and parainfluenza. This host-targeting mechanism reduces the potential for drug resistance. We examined a 10μ particle size (F03), formulated to deposit mainly in the upper airways to minimize systemic absorption. The larger particle size of the F03 formulation was associated to deposit mainly in the upper airways to minimize systemic absorption. The differences between the treatment groups are presented in a table. 

Methods: In this double-blind, randomized trial, 2 cohorts of 9 healthy adults received DAS181-F03 or placebo at a ratio of 2:1. Cohort 1 received one dose of 20 mg. Cohort 2 received 10 daily doses of 20 mg. Serum samples were obtained for PK analysis. Serum antibody was measured on Days 0, 30, and 90. Safety evaluations included CXRs, EKGs, and PFTs.

Results: Of the 18 subjects, 10 were African-American, 11 were male, and the mean age was 34 yr. A single dose was well tolerated by all subjects. In Cohort 2, 2 subjects developed symptoms after the 8th dose and stopped dosing early: one had wheezing and another had fever and a pulmonary infiltrate. Both had mild decreases in FEV1, which normalized within 1-5 days. In Cohort 1 plasma DAS181 concentrations were measurable in 4 of 9 subjects, and all levels were ≤ 0.33 ng/ml. In Cohort 2, DAS181 concentrations were measurable in 6 subjects and reached peak levels of 0.8-4.1 ng/ml before the final dose in all subjects. All levels fell below the assay sensitivity by 7 days after the last dose. 3 subjects in Cohort 1 and 4 of 9 in Cohort 2 developed serum IgG and IgM antibodies to DAS181 by Day 30. Conclusions: DAS181-F03 was well tolerated until after the 8th dose, when 2 subjects developed respiratory symptoms. Inhaled DAS181-F03 had low and variable systemic absorption. The larger particle size of the F03 formulation was associated with lower peak plasma levels than previous formulations of DAS181 with smaller particle sizes.

247 (G) Withdrawn

248 (G) Detection of Endogenous Anti-PA Antibodies in Anthrax-Infected Rabbits after Treatment with ETI-204

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Inhalational anthrax is associated with rapid disease progression and death. Anthrax toxins are comprised of protective antigen (PA) with lethal or edema factors, forming lethal or edema toxins, respectively. These toxins have immunosuppressive activity on the innate and adaptive immune system. ETI-204 is a humanized, monoclonal antibody against PA that neutralizes the toxins. We hypothesized that ETI-204 treatment of rabbits with inhalational anthrax would promote survival and allow development of an immune response against anthrax. NZW rabbits were exposed to B. anthracis spores and treated with a single IV dose of 0, 1, 4, 8, or 16 mg/kg ETI-204 at the first sign of anthrax symptoms (increased body temperature or circulating PA). The primary endpoint was mortality at Day 28. Serum samples were collected pre-dose and from 15 minutes after dosing through Day 28. Levels of ETI-204 and endogenous anti-PA IgG were assessed by two ELISA methods. ETI-204 was measured by capture with recombinant PA and selective detection by ruthenylated goat-anti-human kappa antibodies. In method two, the detection component was ruthenylated protein A/G which quantified any anti-PA IgG in the sample, regardless of species. Control animals succumbed to anthrax by Day 5 while ETI-204-treated animals showed a dose dependent increase in survival. ETI-204 had a T₅₀ in serum of 15 minutes post-dose (the first time point), a Vₙₐₐₙ of ~30 mL/kg and a tₜₘₜₜ of ~1-2 days. Rabbit anti-PA IgG levels in the serum were detectable approximately a week after exposure to B. anthracis in survivors and reached a plateau of 20-30 μg/mL by 2 weeks, irrespective of ETI-204 dose. The profile of the combined levels of both anti-PA antibodies showed Cₘₜₜ levels >10 μg/mL in survivors throughout the study. These results suggest that treatment with ETI-204, alone, is partially protective during the acute phase of inhalation anthrax and allows the development of adaptive immunity which may convey long term protection against anthrax. Supported by Federal funds from ASPR/BARDA under Contract No. HHS0100201000026C.

249 (G) Assessment of the Post-Exposure Prophylactic Efficacy of Orally Administered Levofloxacin in a Bacillus anthracis Aerosol Infection Model


Anthrax is a potentially fatal disease in man, and previous events have highlighted the possibility for the agent to be used as a biological weapon through deliberate release. Whilst outbreaks of anthrax occur in several countries throughout the world, there are only a few areas in which natural outbreaks of inhalational anthrax occur so it is not possible to test the prophylactic in clinical trials. To overcome these problems, the FDA published the ‘Animal Rule’ in 2002. This rule is designed to permit licensure of drugs and biologics that are intended to reduce or prevent serious or life-threatening conditions caused by exposure to biological, chemical, radiological, or nuclear substances. The work presented details the assessment of orally administered levofloxacin in a B.anthracis aerosol infection model. Methods: The challenge item, B.anthracis Ames, was produced at HPA and stocks at a concentration of approximately 8.0±09 CFU/mi were aerosolised and delivered to cynomolgus macaques using the AeromP-Henderson (n=4). The test item, Levofloxacin, was supplied as 500 mg tablets. Tablets were diluted to enable oral administration of 15mg/kg at 0h and 4mg/kg at 12h. Treatment was continued for 30 days. Daily monitoring and health scores were taken throughout the study and blood was sampled for bacterial load, anti-PA titres and haematology analysis. Upon necropsy, tissues were processed to paraffin blocks, sectioned, stained with haematoxylin and eosin and examined microscopically. Results: Both animals that received carrier only succumbed at 3 days post-challenge but all treated animals survived. Very high levels of bacteria were recorded in the blood and tissues of untreated animals but no clinical observations or health scores indicative of disease were detected. Levofloxacin treated animals had no bacteria detected in the blood at any of the bleed points from challenge through to antibiotic cessation. The differences between the treatment groups are presented in terms of bacterial load, weight loss and survival time.
250 (G) Interpretation of Nitric Oxide Precursors and Antibiotic against Typhoid
S. S. Haque, II; Indira Gandhi Inst. of Med. Sci., Patna, India.

Background: Typhoid caused by Salmonella typhi remains a major health concern worldwide. The emergence of multidrug-resistant (MDR) strains of Salmonella typhi increased virulence leading to increased morbidity and mortality has further complicated its management. Human typhoid is similar to the infection caused by Salmonella typhimurium in mice. Most of the antibiotic are resistant and vaccines have less-than-desired efficacy and certain unacceptable side effects, making it pertinent to search for new suitable formulation. Nitric oxide (NO) is a gaseous free radical molecules; produced in biological systems. During enzymatic conversion of L-arginine to L-citrulline by NO synthase (NOS) nitric oxide is produced. Ciprofloxacin one such fluoroquinolones have been shown to achieve high intracellular concentrations and least resistant antibiotic used against typhoid. Exogenous administration of L-arginine results in increased NO production, indicating that endogenous substrate is insufficient for maximal NO production. By considering these facts, it was thought to see the effect of oral administration of NO donor i.e. L-arginine along with the low doses of antibiotic (ciprofloxacin).

Material and Methods: NO estimation was done by the fluorometric method Misko et al, (1993) with slight modification. Results and Discussion: Hepatic nitrite level in mice infected with 0.6xLD50 of S. typhimurium was 8.33%, higher than control animals (treated with saline) at day 8, and in group B. ciprofloxacin showed better therapeutic induction of L-arginine and ciprofloxacin shows better therapeutic induction respectively as compared to only ciprofloxacin. This increase of nitrite level (metabolites of nitric oxide) is may be due to enhanced cytokine expression.

251 (G) Evaluation of Some Medicinal Plants Effect on Growth, Biofilm Formation and Pyocyanin Production of Pseudomonas aeruginosa
E. Sepahi, Sr.; Ferdowsi Univ. of Mashhad, Mashhad, Iran, Islamic Republic of.

Pseudomonas aeruginosa is an opportunistic pathogen which can cause different diseases in human, animal and plants. To cause disease this pathogen uses different virulence factors such as biofilm formation, pyocyanin and elastase production that their production is under control of Quorum sensing (QS). Extracts of six plants, cardamom, stinging assay, thyme, eucalyptus, cumin, and garlic, were examined in this study for their effects on growth and virulence factor production of P. aeruginosa. Thyme, eucalyptus and cumin caused a significant inhibition of growth on LB-agar plates and on liquid cultures. Additionally each plant presented a distinct effect on biofilm formation. Among them, eucalyptus and cumin could induce biofilm formation although in their presence no pyocyanin were detected in cultures. My results indicated that in lower dosage of eucalyptus and cumin, although we observed a good effect on pyocyanin production and biofilm formation but these results are contradictory with QS regulated behaviors.

252 (G) PANACEA Broad-Spectrum Antiviral Therapeutics
T. H. Rider; Massachusetts Inst. of Tech., Cambridge, MA.

Although there is great concern over emerging viruses and viruses on the category A-C priority pathogen lists, there are relatively few prophylactics or therapeutics for these viruses, and most which do exist are highly pathogen-specific or have undesirable side effects or other disadvantages. We have developed a radially new and very broad-spectrum antiviral therapeutic/prophylactic that has the potential to revolutionize the treatment of viral infections, including those due to emerging, category A-C, and common clinical pathogens. Our Double-stranded RNA (dsRNA) Activator (BCX4430) is a novel compound which selectively induces apoptosis in cells containing viral dsRNA, rapidly killing infected cells without harming uninfected cells. We have demonstrated that BCX4430 is nontoxic in all 11 cell types tested thus far, and effective against at least 15 different viruses, including DNA and RNA viruses, enveloped and nonenveloped viruses, viruses that replicate in the nucleus and in the cytoplasm, and viruses that use a variety of receptors. Among the viruses against which BCX4430 has proven effective in vitro are dengue, yellow fever, West Nile influenza, Amapari arenavirus, and Guama bunyavirus. We hope to optimize our DRACO designs and demonstrate them against additional viruses and in additional animal models. This work should greatly advance DRACOs toward ultimate utility as safe, broad-spectrum therapeutics/prophylactics for priority and emerging viral pathogens, filling a large gap in existing therapeutics.

253 (G) In Vitro Activity of Omacycline (OMC) against Biothreat Bacteria
M. Draper; L. Miller; S. Halaszhorzi; O. Kim; J. R. Hershfield; 1Paratek Pharmaceuticals, Boston, MA, 2U.S. Army Med. Inst. of Infectious Diseases, Fort Detrick, MD.

Introduction: Omacycline is a novel aminomethylcyclycline of the tetracycline family, designed to overcome antibiotic resistance, and is currently in advanced clinical development for community-acquired acute bacteraial skin and skin structure infections (ABSSSI) and community acquired bacterial pneumonia (CABP). OMC’s development is based on its demonstrated potent activity against key pathogens in these indications, including MRSA, MDRSP, and atypical pathogens, and its lack of cross resistance to older generation tetracyclines and other antibiotic classes. OMC is being developed as both an intravenous and oral formulation and would therefore be well suited for use in the treatment or post-exposure prophylaxis of infections of concern in both the biodefense and the public health settings. Methods: MICs were determined by the microbroth dilution method according to CLSI guidelines. Medium was supplemented as necessary. Test plates were incubated for 18-24 or 42 to 48 h, depending on the organism. Quality control for the testing was done using QC organisms and OMC QC MIC ranges established by CLSI. Thirty isolates of each pathogen, representing broad geographic diversity, were tested.

Results: OMC was active against Y. pestis (MIC90 = 1 μg/ml), B. anthracis (MIC90 = 0.06 μg/ml), F. tularensis (MIC90 = 2 μg/ml), and B. mallei (MIC90 = 0.25 μg/ml), but not against B. pseudomallei (MIC90 = 64 μg/ml). OMC was less active than comparator ciprofloxacin against Y. pestis (MIC90 = 0.03 μg/ml) and F. tularensis (MIC90 = 0.015 μg/ml), but slightly more active against B. anthracis (MIC90 = 0.12 μg/ml). OMC was more active than comparator azithromycin against B. mallei (MIC90 = 1 μg/ml). OMC activity was comparable to historical data for both tetracycline and doxycycline.

Conclusions: Based on the in vitro activity of OMC and its well-characterized IV and oral pharmacokinetics, safety and tolerability, further assessment of its utility in combating these biothreat organisms in vivo is underway.

254 (G) In Vitro Characterization of a Potent and Novel Broad-Spectrum Antiviral (BSAV) Agent Targeting RNA Polymerase B. R. Taubenheim; S. Bantia; D. Barnard; C. Parker; R. Upshaw; Y. Lou; J. Williams; D. Kellogg; P. Kotian; W. Sheridan; Y. S. Babu; 1BioCrystal Pharmaceuticals, Inc., Durham, NC, 2Inst. of Antiviral Res. Utah State Univ., Logan, UT.

Introduction: A BSAV agent can replace multiple pathogen-specific drugs for emergency use in response to both natural outbreaks and bioterrorist threats. Targeting viral RNA polymerase, a common and essential component of RNA viruses, is a proven approach. Objective: To demonstrate the broad-spectrum antiviral activity of BCX4430, a novel adenosine analog and putative RNA polymerase inhibitor. Methods: Antiviral activity was determined using cell-based cytotoxicity and virus-yield reduction assays or by high-content image analysis. BCX4430 triphosphate (BCX4430TP) measurements were performed by LC/MS/MS. 3H-BCX4430 was used to determine incorporation into mammalian RNA and DNA. UV spectroscopy was used to monitor adenosine deaminase activity. PK and tolerability studies were conducted in rats, mice, guinea pigs and monkeys. Liver BCX4430TP measurements were done in rats. Results: BCX4430 inhibited the growth of a number of viruses in cell culture studies. BCX4430 is readily anabolized to BCX4430TP in cell lines and primary hepatocytes. BCX4430TP is not incorporated into mammalian RNA or genomic DNA and is not deaminated by ADA. BCX4430 (at 30 mg/kg I.M. dose) is converted efficiently to BCX4430TP (70- 100 μg/g) in rat liver. Conclusion: BCX4430 is a promising BSAV agent demonstrating antiviral activity against high priority pathogens.
255 (G) Discovery of a New Class of Small Molecules with Anti-Gram-Positive, (S. aureus, MRSA, P. acnes), and Tumor Suppression Activity
B. Memarzadeh1, S. Noonan2, H. N. Duong1, H. Lopez1, Common Pharma, San Carlos, CA, 1MuriGenics, Inc., Vallejo, CA, 1MuriGenics, Vallejo, CA.

Background: The test compound (LTA-001) belongs to a new class of small molecule pharmacologically active compounds. Anti-bacterial (in vitro) and anti-tumor (in vivo) activities of LTA were evaluated.

Methods: Initially, the microbiological efficacy was evaluated against S. aureus and P. aeruginosa in a short-term exposure (15 minutes), then as part of several longer-term efficacy and MIC studies for LTA-001 which included evaluation of activity against S. aureus, MRSA (BAA-1720), P. aeruginosa and E. coli. A disc diffusion assay was also performed and evaluated the activity. The MIC for P. acnes was estimated following the CLSI procedures. Anti-tumor efficacy was evaluated against implanted HT-29 tumor cell line in NCr nude mice. Tumor volume measurements were recorded. Results: Short-term exposure study evaluated log-reductions at 5, 15, 30 and 60 minutes. S. aureus (6 x 10^6) had 4, 5.2, 5.7, 6.3 logs reductions. The MIC assay confirmed the initial data showing activity against S. aureus (ATCC 6538), MRSA (BAA-1720) for LTA-001 concentrations of ~0.1%. Disc diffusion assay also confirmed the previous data. There were no zones of inhibition against E.coli and P. aeruginosa. For S. aureus, the zones of inhibition were similar and independent of concentrations with highest concentrations having zones of 12 mm limited by the lipophilic nature of the LTA. During the testing against P. acnes, the low solubility of the LTA-001 in the media was observed but the activity against P. acnes was confirmed. Anti-tumor activity of LTA-001 was evaluated in vivo (Murine HT29). HT-29 tumor cells were implanted in NCr nude mice (3 treatments and 3 controls, 2 sites/mouse) and treated twice/day by IP injections. The average tumor size after one week was 50% less in the treatment group. A wash-out period following by the same treatment for one week confirmed this activity. Conclusions: LTA-001 shows efficacy against MRSA, S. aureus and P. acnes. Anti-tumor activity shows that this class of compounds is pharmaceutically active and further studies are warranted.

256 (G) Protein Phosphatase 1 Inhibitors Effect on Rift Valley Fever Virus
A. Baer; George Mason Univ., Manassas, VA.

Rift Valley fever virus (RVFV) is a highly pathogenic arthropod-borne virus infecting a wide range of vertebrate hosts. Many viruses such as HIV-1, Marburg, Ebola, and RVFV take advantage of phosphorylation signaling pathways in order to create an environment that is beneficial for viral replication. Previous studies have demonstrated that PP1 is able to regulate various MAP and Src Kinases such as p38, JNK and ERK, all of which become heavily phosphorylated during RVFV infection. While the significance of several cellular kinases in the phosphorylation state of viral proteins in the Bunyaviridae family have been partially established, little is known about the impact of cellular phosphatases on the viral replication cycle. Here we propose a model in which dynamic phosphorylation is an important mechanism in regulating RVFV’s replication cycle. The current study focuses on the role of PP1 inhibitor regulation in RVFV infection and viral replication. Past studies inhibiting PP1 with okadaic acid (OA) as a therapeutic model have met with limited success due to the promotion of tumor growth and genetic instability. Here we propose the use of a novel PP1 inhibitor 1E7-03, which has demonstrated negligible side effects and cytotoxicity, along with increased therapeutic efficacy in comparison to traditional PP1 inhibitors. Time of addition studies and internal viral RNA levels indicate that 1E7-03 inhibition likely plays a role during the first few hrs post entry, potentially acting on viral transcription. Importantly we have observed a significant decrease in viral titers in both ZH501 and MP12. Through the targeting of PP1 subsets we hope to elucidate and selectivity target potentially critical viral/cellular signaling pathways.

257 (G) Studies on the Therapeutic Efficacy of Thioridazine against Active and Latent Tuberculosis

Background: Tuberculosis remains important cause of mortality and morbidity throughout the world. Its therapy is complicated by the longer treatment schedule and ever increasing number of MDR /XDR and latent TB cases. New therapeutics are required to overcome these problems. In the present study we have evaluated the potential of thioridazine, a potent phenothiazine compound against active and latent tuberculosis.

Methods: Thioridazine was evaluated for its in vitro and in vivo inhibitory activity against active and latent M. tuberculosis. The chemotherapeutic efficacy of thioridazine along with antibacterial drugs was studied in murine model of active & latent TB. Results: In vitro studies showed that thioridazine had potent inhibitory effect on M. tuberculosis and it acted synergistically with rifampicin and isoniazid. In vivo studies confirmed the accumulation of thioridazine in the alveolar macrophages as well. Thioridazine when used in combination with rifampicin and isoniazid in a short-course chemotherapy model of experimental TB, showed more potent activity than individual drugs. Similar results were found during 6-week extended chemotherapy, wherein thioridazine when used as an adjunct drug in the standard regimen showed more clearance of the bacilli. Thioridazine also showed promising activity against in vitro and in vivo model of latent TB. Most importantly, addition of thioridazine to the latent TB regimen further shortened the therapy period. Conclusion: Our results confirm that thioridazine can augment the potency of conventional TB drugs and shorten the duration of therapy. It has the potential to be used as an adjunct drug to improve the chemotherapy of active as well as latent TB.

Figure 1: Chemotherapeutic efficacy of Thioridazine against murine models of TB
a) Active TB, b) Latent TB
UTC= Untreated Controls, R= Rifampicin, Z= Pyrazinamide, E= Ethambutol, T= Thioridazine, ND= Not Detected
Results are mean ± SD of 3-6 mice, **p<0.01, ***p<0.001
The increased prevalence of antibiotic resistant is a concern for many bacterial pathogens. Currently, the World Health Organization has declared antimicrobial resistance to be one of the greatest threats to human health. Global surveillance indicates that infectious microorganisms are emerging at an alarming rate. There is also a significant concern about the potential of generating highly virulent microorganisms using genetic engineering and synthetic biology techniques. Outbreaks caused by these types of natural or man-made variants can spread rapidly and must be stopped before diseases become epidemic. Quarantine, rapid diagnosis, proper use of therapeutics and rapid vaccination against the targeted pathogen is the only way to prevent epidemics during massive disease outbreaks. These facts encouraged us to employ modern biotechnological techniques for further enhancing the therapeutic prophylactic and diagnostic efficacy of phases. Phages are the most abundant biologically active organic entities on the surface of the earth. Isolation of new phages is rapid, facile and inexpensive and there is an abundant supply of available entities on the surface of the earth. Isolation of new phages is rapid, facile and inexpensive and there is an abundant supply of bio-entities that can be utilized as broad spectrum vaccines. Our presentation includes use of phages as expression and delivery systems for influenza specific major antigens to produce combinatorial Flu-vaccine.

We are currently defining the minimal amount of PB10 required to protect mice against a 10 LD50 toxin challenge. Here, we describe the production or binding (RTB) subunits that were each capable of passively protecting previously studies, we identified three murine monoclonal antibodies The overarching goal of this study is to develop an immune-based countermeasure for ricin toxin, a Category B bioterror agent. In a series of previous studies, we identified three murine monoclonal antibodies (mAbs), PB10, Syt7 and Syt8, directed against ricin’s enzymatic (RTA) or binding (RTB) subunits that were each capable of passively protecting mice against a 10 LD50 toxin challenge. Here, we describe the production and characterization of these monoclonal antibodies. The murine heavy and light chain variable regions were fused to human IgG frameworks and the resulting chimeric mAbs were expressed and purified using a Nicotiana-based system. The chimeric mAbs retained subunit specificity and relative affinities for ricin, as determined by ELISA. Moreover, the chimeric mAbs were each capable of neutralizing ricin in vitro at least as well as their respective parental murine mAb counterparts (Table 1). Passive administration of the mAbs (20 μg) to mice by intraperitoneal injection protected the animals against a 10 LD50 ricin challenge. Preliminary studies suggest that chimeric PB10 is the most effective toxin-neutralizing mAb among those that have been characterized to date. We are currently defining the minimal amount of PB10 required to protect mice against ricin challenge, as well as evaluating the potential of mAbs, individually or as a cocktail, to “rescue” the animals from toxin-induced death.
**262 (G) Nano-Aerosol Therapeutic Intervention against Francisella novicida Infection**

**A. O. Nwabueze, V. N. Morozov, M. L. van Hoek; George Mason Univ., Manassas, VA, Inst. of Theoretical and Experimental Biophysics, Pushchino, Russian Federation.**

Francisella tularensis is a pathogen endemic to the United States and Russia, and as a result of its virulence, is listed as a Category A Select Agent. Pulmonary infection via aerosol exposure of Francisella tularensis results in an especially severe disease form. Although conventional antibiotic treatments are available for tularemia, the antibiotic of choice (e.g. Ciprofloxacin) may not be safe for or well tolerated by subsets of the population, such as children or the elderly, at the prescribed dose. The objective of this research is to develop a novel device to generate nano-aerosols of therapies, taking advantage of all of the benefits of inhalational therapeutics (reduced systemic exposure, highly local concentration of drug delivery) plus the benefits of nano-aerosol (deep penetration, enhanced deposition and tremendous reduction in required dose), to treat tularemia infection of the lung. This is a novel approach to treatment of tularemia. Recently, we have developed a technology that enables us to generate nanometer-sized aerosols of antibiotics, which could theoretically penetrate into the alveolar spaces of the lung. Using a Scanning Mobility Particle Sizer, we have characterized the generated nano-aerosols which are predominantly in the 50nm to 100nm range. We have further shown that we can generate similar sized nano-aerosols from solutions of various antibiotics with known effectiveness against tularemia, including levofloxacin, streptomycin, kanamycin, and tetracycline. Our next studies will examine the effectiveness of nano-aerosol delivered antibiotics to treat mice infected with Francisella (i.n. and aerosol). Nano-aerosol drug delivery has the potential to reduce the dose required for treating pulmonary infections via its targeted deposition of antibiotics onto previously inaccessible bronchial regions. This joint US-Russian study was supported by HDTRA1-11-1-0054 Novel Nano-aerosol Device for Therapeutic Intervention against Francisella tularensis aerosol Infection.

**263 (H) Novel Nanogel as Adjuvant for Vaccine Development**

**V. Vlizlo, M. Kozak, O. Zaichenko, A. Oliynyk, Y. Kit, R. Stoika; Inst. of Animal Biol., Lviv, Ukraine.**

**Background:** Vaccination is the best cost-effective biomedical approach in the face of the threat of the emerging diseases. The latest trend towards novel and safer vaccines utilizes well-characterized antigens, like purified proteins, peptides, or carbohydrates. These so-called subunit vaccines are expected to potentiate the immune response to the coadministered antigen. The main purpose of our research - to determine the effectiveness of the vaccine against HPAI in backyard farms. Methods: Development of novel non-toxic type of adjuvants ensuring an efficient immune response with the prospect for their use in the vaccine. Results: The main purpose of our research - to determine the effectiveness of the vaccine against HPAI in backyard farms. Methods: To determine the antigenic properties of vaccine under lab condition it was worked out the inactivated vaccine against the HPAI “AviFluVac”, the objectives for trivalent influenza vaccine (TIV) require HA quantification based on the single radial immunodiffusion (SRID) assay, which is laborious, time-consuming and difficult to obtain reproducible results. We have developed a new influenza vaccine potency assay, immunocapture-isotope dilution mass spectrometry (IC-IDMS), to quantify immunoreactive hemagglutinin (HA), the primary antigen and only regulated component of influenza vaccines. This methodology has the potential to immediately influence influenza vaccine testing, which would have a global impact. IC-IDMS utilizes polyclonal antisera to strain-specific HA, which is provided by regulatory agencies as reagent standards for the SRID assay. These antibodies are coated on magnetic beads and used to capture the conformationally correct form of HA in purified influenza bulk material as well as final vaccine presentations. The captured proteins are enzymatically digested, and evolutionarily conserved tryptic peptides are quantified for each HA subtype. IC-IDMS provides accurate and specific quantification of immunoreactive H1, H3, and B HA in commercial TIV samples. IC-IDMS is valuable for evaluating binding specificity and efficiency of the antibodies presently used in regulatory quantification methods and for monitoring the stability of the final vaccine product. It has also been expanded to the use of monoclonal antibodies, which will alleviate the current requirement of annual production of strain-specific antisera. The IC-IDMS methodology has several advantages over the current SRID method, including higher throughput due to method automation as well as improved precision, accuracy, selectivity, and sensitivity.

**266 (H) Improved Potency Assay for Quantification of Immunoreactive Hemagglutinin in Trivalent Influenza Vaccines Using Isotope Dilution Mass Spectrometry**

**C. L. Pierce, W. Wang, T. L. Williams, J. R. Barr; CDC, Atlanta, GA, Batlle Mem. Inst., CDC, Atlanta, GA.**

Vaccination against influenza virus is the primary strategy to reduce the morbidity and mortality associated with seasonal influenza. There is an important public health need to develop methods to improve the quality of vaccines and to dramatically decrease the time required to get a protective vaccine to the public. Currently, regulatory potency requirements for trivalent influenza vaccine (TIV) require HA quantification based on the single radial immunodiffusion (SRID) assay, which is laborious, time-consuming and difficult to obtain reproducible results. We have developed a new influenza vaccine potency assay, immunocapture-isotope dilution mass spectrometry (IC-IDMS), to quantify immunoreactive hemagglutinin (HA), the primary antigen and only regulated component of influenza vaccines. This methodology has the potential to immediately influence influenza vaccine testing, which would have a global impact. IC-IDMS utilizes polyclonal antisera to strain-specific HA, which is provided by regulatory agencies as reagent standards for the SRID assay. These antibodies are coated on magnetic beads and used to capture the conformationally correct form of HA in purified influenza bulk material as well as final vaccine presentations. The captured proteins are enzymatically digested, and evolutionarily conserved tryptic peptides are quantified for each HA subtype. IC-IDMS provides accurate and specific quantification of immunoreactive H1, H3, and B HA in commercial TIV samples. IC-IDMS is valuable for evaluating binding specificity and efficiency of the antibodies presently used in regulatory quantification methods and for monitoring the stability of the final vaccine product. It has also been expanded to the use of monoclonal antibodies, which will alleviate the current requirement of annual production of strain-specific antisera. The IC-IDMS methodology has several advantages over the current SRID method, including higher throughput due to method automation as well as improved precision, accuracy, selectivity, and sensitivity.
266 (H) Live-Attenuated Tetravalent Dengue Virus Host Range Vaccine Elicits Immune Response in African Green Monkeys

K. M. Smith1, C. M. Briggs1, C. J. Spears1, M. Quiles2, A. Piper1, M. Ribeiro1, E. Huitt1, M. E. Thomas1, D. T. Brown1, R. Hernandez2; 1Arbovax Inc., Raleigh, NC, 2North Carolina State Univ., Raleigh, NC.

Dengue virus (DV) is becoming increasingly common in tropical and subtropical regions throughout the world and recent outbreaks in the continental USA highlight the widespread threat to public health. DV is transmitted by mosquitoes and as distribution of these insects has expanded, so have cases of dengue fever. DV is a member of the Flavivirus family and has four distinct serotypes (DV1, 2, 3, and 4). No cross protection is afforded to a heterologous serotype following infection by any one of the individual serotypes. In addition, the presence of antibodies to one serotype of DV can facilitate the occurrence of the more severe dengue hemorrhagic fever upon infection with a second serotype. For this reason, the development of a safe, tetravalent vaccine to produce a balanced immune response to all four serotypes is absolutely critical. Arbovax employs a novel dual-function platform technology to develop safe and effective live-attenuated vaccines coupled with a low cost system of manufacture to target DV. Host range (HR) mutants of each DV serotype were created by truncating the transmembrane domain of the E protein and selecting for strains of DV that replicated well in insect but not mammalian cells. Four experimental groups of African green monkeys were vaccinated with a tetravalent DV HR vaccine in four separate injections, one into each limb. Each group was challenged with one serotype of wild type DV. No vaccine related adverse events occurred. The vaccine strains were confirmed to be attenuated in vivo by infectious center assays. Vaccinated animals displayed a robust humoral immune response. The DV HR mutants are promising candidate vaccines that warrant further analysis. This method for the creation of safe, live-attenuated viral vaccines that generate effective immunity may also be applied to other insect-borne viral diseases for which no current therapies exist.

267 (H) Assay Verification and Optimization of the IgG Anti-Pertussis Toxin ELISA for Use in Outbreak Investigations

D. Oyuga Newby1,2, L. Trivedi1, Y. Washington1, G. Thakore2, T. Guy2, M. Paz Carlos1,2,3,4, John Hopkins Univ. Zanvyl Krieger Sch. of Arts and Sci., Baltimore, MD, 2Maryland Dept. of Hlth. and Mental Hygiene Lab. Admin., Div. of Virology and Immunology, Baltimore, MD, 3Johns Hopkins Univ. Bloomberg Sch. of Publ. Hlth., Baltimore, MD.

Background: Bordetella pertussis is a gram-negative coccobacillus that invades the respiratory tract causing pertussis (whooping cough). Laboratory confirmation of pertussis cases with clinical symptoms (onset of disease of 0-4 weeks) includes culture and PCR. IgG antibodies pertussis toxin (PT) are subsequently measured by ELISA. The CDC developed the IgG Anti-PT ELISA for use in strains of DV that replicated well in insect but not mammalian cells. Four experimental groups of African green monkeys were vaccinated with a tetravalent DV HR vaccine in four separate injections, one into each limb. Each group was challenged with one serotype of wild type DV. No vaccine related adverse events occurred. The vaccine strains were confirmed to be attenuated in vivo by infectious center assays. Vaccinated animals displayed a robust humoral immune response. The DV HR mutants are promising candidate vaccines that warrant further analysis. This method for the creation of safe, live-attenuated viral vaccines that generate effective immunity may also be applied to other insect-borne viral diseases for which no current therapies exist.

268 (H) Correlates of Protection in Lethal Monkeypox Virus Challenge Models of Cynomolgus Macaques


New generation smallpox vaccines like MVA-BN (Modified vaccinia virus Ankara, IMVAMUNE®) are investigated that have a highly improved safety profile compared to traditional smallpox vaccines. Since smallpox has been eradicated, clinical studies can only evaluate the immunogenicity and safety of new vaccines, while efficacy has to be demonstrated in animal models and then linked to immune parameters measurable in humans. There are comprehensive data demonstrating that both the innate and overlapping components of the adaptive immune response play an important role in the protective immunity against poxviruses. However, it will be important to identify suitable correlates of protection, which by definition do not explain the whole protective mechanism, but do allow the prediction of a fully protective response afforded by a smallpox vaccine. Thus, our studies in non-human primates (NHP) aimed at investigating whether antibody responses correlate with protection from lethal monkeypox virus (MPXV) challenge. In two separate studies, NHP were vaccinated on day 0 and 28 with suboptimal doses (1x102 to 1x107 TCID50) of MVA-BN to induce a wide range of neutralizing and total antibody levels measured by PRNT and ELISA. Five weeks after the second vaccination the animals were challenged with MPXV via the intravenous route or by aerosol exposure. Antibody responses were vaccine dose dependent. Antibody responses also correlated clinical signs, body weight loss, viral load and survival post challenge. Using logistic regression analysis as a robust, accepted statistical model to establish human immune correlates, we demonstrated that both PRNT and ELISA titers were significantly correlated (p<0.0005) with survival from MPXV challenge via the intravenous and aerosol route. Our data support the critical role of antibodies, including neutralizing antibodies, in the protection from poxvirus infections and offer two assays suitable to predict efficacy of smallpox vaccines in man.

269 (H) Mucosal Immunization of Bacillus anthracis Surface Protein EA1 Protects Mice from Intranasal Challenge of B. anthracis Spores


Bacillus anthracis spores germinate to vegetative forms in host cells, and produced fatal toxins. In this study, we examined protective effect of extractable antigen 1 (EA1), a major 5-layer component of B. anthracis, against anthrax. Mice were intranasally immunized with recombinant EA1 and/or protective antigen (PA) followed by a lethal challenge of B. anthracis spores. Mucosal immunization with EA1 resulted in a significant level of anti-EA1 antibodies in feces, saliva and serum. It also improved survival rates. The numbers of bacteria in organs of EA1-immunized mice were significantly decreased compared to those in the control and PA-alone immunized mice. The protective effect of EA1 alone was greater than in PA alone. Immunity to EA1 might contribute to protection at the early phase of infection, i.e., before massive multiplication and toxin production by vegetative cells. These results suggest that EA1 is a novel candidate for anthrax vaccine and provides a more effective protection when used in combination with PA.
**ABSTRACTS** — *Wednesday Poster Session*

**270 (H) yscP$_{ext}$, A Mutation That Extends Type III Needle Complexes, Cannot Endow *Yersinia pestis* with Escape from Plague Protective Immunity**

K. Given Ligtenberg, L. Quenee, O. Schneewind; Univ. of Chicago, HTL Argonne Natl. Lab., Argonne, IL.

The pathogenesis of *Yersinia pestis* relies upon its virulence plasmid encoded type III secretion machine. The LcrV capped type III needle complex acts as a conduit through which effector proteins are transported into host immune cells, thereby enabling escape from opsonophagocytic killing and other innate immune responses. YscP serves as a molecular ruler that determines the length of the needle complex. *Yersinia enterocolitica* yscP$_{ext}$ harbors a tandem duplication that is absent from *Y. pestis* yscP and triggers the production of type III needles with elongated shafts. In contrast to *Y. pestis*, the causative agent of plague, *Y. enterocolitica* does not elaborate an F1 capsule. F1 is a pilin-derived surface organelle that elicits robust antibody responses in infected hosts, which are the molecular substrate of plague protective immune responses. Previous work established that F1-specific antibodies interfere with *Y. pestis* type III translocation of effectors into host cells. To test whether YscP$_{ext}$ needle length variants provide for escape from plague protective immune responses, the duplication in the *Y. enterocolitica* yscP$_{ext}$ allele was introduced into *Y. pestis* yscP. The resulting strain, *Y. pestis* yscP$_{ext}$, was fully virulent and capable of effector translocation. When interrogated for its ability to escape plague protective immunity, *Y. pestis* yscP$_{ext}$ effector translocation in vitro was not perturbed by F1-specific antibodies, unlike the effector translocation of wild-type *Y. pestis*. Nevertheless, F1-specific antibodies provided for opsonophagocytic killing of both wild-type and yscP$_{ext}$. *Y. pestis* in blood, and mice vaccinated with purified recombinant F1 were protected from bubonic plague infection by *Y. pestis* yscP$_{ext}$. We conclude that F1-specific antibodies provide plague protective immunity in a manner that is independent on the needle length of *Y. pestis* type III machines.

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**271 (H) Characterization of Immune Correlates of Protection for a *Burkholderia pseudomallei* Outer Membrane Vesicle Vaccine Co-Administered with CpG Adjuvant**


*B. pseudomallei* (Bps) is the causative agent of melioidosis, a tropical disease characterized by high lethality and septicemic or pulmonary clinical manifestations. Due to its innate resistance to most antibiotics, the lack of a vaccine approved for human use, and the threat of its use in biological warfare, DHHS recently listed Bps as a Tier 1 select agent. We previously demonstrated that parenteral immunization with naturally-derived Bps outer membrane vesicles (OMVs) provided significant protection against lethal systemic challenge in the murine model. Protection was significantly enhanced by the co-administration of the Th1-promoting adjuvant, CpG oligodeoxynucleotide (ODN 2395). In this work, we repeated the immunization study in order to elucidate differences in immune responses between OMV- and OMV/CpG-immunized groups of mice. OMVs were purified from Bps strain 1026b using density gradient ultracentrifugation and vaccine preparations were confirmed to be free of bacterial contamination. Groups of BALB/c mice were immunized subcutaneously (s.c.) on days 0, 21, and 42 with 5 μg of Bps OMVs +/- 10 μg CpG ODN 2395, 10 μg CpG only or left unimmunized (naive). Serial bleeds were performed prior to each immunization and four weeks after the final dose (day 70) to measure OMV-specific antibody responses. On day 70, splenocytes were also harvested to measure memory T cell responses by antigen-restimulation assay. Both OMV- and OMV/CpG-immunized groups generated a significant increase in OMV-, LPS-, and CpG-specific serum IgG and IFN-γ producing CD4+ and CD8+ T cells. The increased protection in the OMV/CpG immunized animals could not be attributed to IFN-γ production alone, but may be related to enhanced Th1 responses which we are currently exploring further by T cell array. In conclusion, co-administration of OMVs with a Th1-promoting adjuvant improves OMV vaccine protection and is characterized by protective antibody and cell-mediated immune responses against Bps.

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**272 (H) *Yersinia pestis* Live Attenuated Vaccine Strains with Enhanced Safety and Efficacy**

L. E. Quenee, K. Given-Ligtenberg, N. A. Ciletti, D. Elli, R. Perry, O. Schneewind; Tulane Univ. of Chicago, Chicago, IL, “The Univ. of Kentucky, Lexington, KY.

*Yersinia pestis* causes plague, a zoonotic disease with high mortality rates that can be transmitted by flebitis or aerosol. A US Food and Drug Administration (FDA)-licensed plague vaccine is currently not available. Non-pigmented (*pgm*) *Y. pestis* strains, due to their attenuated virulence, have long been used as live vaccines against plague. However, the safety of this vaccine strategy has been questioned. More recently, a case of septicemic plague precipitated by a non-pigmented isolate of *Y. pestis* suggested that vaccine strains can regain virulence attributes in hosts with mutations causing increased iron load. In order to generate live-attenuated vaccine strains with improved safety profile, we examined the effect of deleting additional iron or metal-ion acquisition mechanisms from non-pigmented plague strains. *Y. pestis* C902 *pgm* strains lacking the HasA hemophore or the yfe operon encoded transporter system were tested for their ability to cause plague disease in wild-type animals as well as in mice presenting with hemochromatosis. Strains unable to cause lethal disease were subsequently interrogated for their ability to elicit F1-based protective immunity. Further molecular engineering is ongoing to promote the establishment of robust LcrV-based immunity mediated by immunization with live-attenuated strains.

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**273 (H) A Multiagent DNA Vaccine Delivered by Electroporation Elicits Protective Immunity against Eastern and Western Equine Encephalitis Viruses in Nonhuman Primates**


Next-generation vaccines that can safely and effectively protect against human infections caused by the biological threat agents Venezuelan, eastern, and western equine encephalitis virus (VEEV, EEEV, and WEEV) are needed. Previously, we demonstrated that DNA constructs expressing codon-optimized envelope glycoprotein genes of VEEV, EEEV, or WEEV delivered by intramuscular (IM) electroporation (EP) individually or as a multiagent combination elicited high levels of virus-neutralizing antibodies in multiple animal species and provided protective immunity against lethal aerosol homologous viral challenge in mice. Currently, we have completed studies to further assess the immunogenicity and protective efficacy of the individual and combined DNA vaccines delivered by IM EP in nonhuman primate (NHP) models of aerosol viral challenge. After EEVP exposure, all NHPs that received empty vector DNA developed severe neurological signs indicative of acute encephalitis and were euthanized on days 5-7 postchallenge. Although neurological signs of disease were observed in some NHPs vaccinated with the individual EEEV plasmid, these were of lesser severity than those observed in the negative control animals and all survived. Interestingly, no significant clinical signs of disease were observed in NHPs that received the combined DNA vaccines. Similarly, NHPs that received the combined DNA vaccines did not display significant clinical signs of disease after WEEV exposure. We are currently investigating the apparent synergistic protective effect achieved with the combined DNA vaccines in further detail. Taken together, the results of our current studies further demonstrate that IM EP delivery of a multiagent formulation of VEEV, EEEV, and WEEV DNA vaccines represents a potent means of protecting against aerosolized encephalitic alphavirus infections and support its continued development into a mature vaccine candidate suitable for future clinical testing.
**274 (H) Intranasal Vaccination with Integrase-Defective Lentiviral Vectors Expressing Influenza Nucleoprotein Induce Protective Immunity against Influenza Virus**


Influenza pandemics are unpredictable and can spread quickly in non-immune populations, causing devastating morbidity and mortality. Current vaccines based on humoral immunity to viral hemagglutinin require an antigenic match to circulating strains, and must be reformulated annually.

An alternative approach is to develop vaccines that promote cellular immunity and target conserved internal viral components, such as influenza nucleoprotein (NP), which may allow for cross-protection among strains. Lentiviral vectors (LV) induce cellular and humoral immune responses, but risk is associated with integration in the host genome. Integrase-defective LV (ID-LV) have a mutation in the integrase gene that prevents genomic integration. Instead, they are stably retained in the nucleus as episomal circular DNA that expresses functional proteins. We hypothesize that ID-LV will express sustained levels of NP (ID-LV-NP) capable of inducing robust immune responses, and can be exploited for vaccine development. ID-LV-NP were made by transfecting three plasmids into 293T cells: a packaging vector expressing all viral proteins but Env, rendering vectors replication-deficient, and with a non-functional integrase gene, an Env vector expressing the G protein of VSV and a transfer vector expressing NP and packaging elements. NP expression in vitro from ID-LV-NP was confirmed by flow cytometry and Western blot. Administration of ID-LV-NP induced persistent NP-specific CTL and antibody responses in mice, as measured by ELISPOT and ELISA. Mice receiving two intranasal doses of ID-LV-NP were fully protected against influenza A virus challenge. Partial protection was seen when only one dose of ID-LV-NP was administered. This suggests that ID-LV-NP can induce an immune response that protects against influenza virus infection, possibly due to induction of mucosal immunity.

**275 (I) Francisella novicida Alters Cellular and Exosomal miRNA Expression in Murine Macrophages In Vitro**


Macrophages in the lung of infected host organists are the first line of innate defense for many inhalational diseases. Phagocytosis of Francisella tularensis by these cells is the first step in the infectious process that leads to pneumonic tularemia. Intracellular communication between infected macrophages and cells of the innate immune system may be mediated via exosomes, small (50-90 nm) vesicles released from a variety of mammalian cell types. These microvesicles have been shown to play a role in cell-cell communication and priming of the immune system. The contents of these extracellular vesicles include microRNAs (miRNA), and their transfer to distant cells represents a robust mode of systemic signal transmission. We hypothesized that the cellular response to intracellular pathogens such as F. tularensis may be to modulate the amount or types of miRNAs that are packaged into exosomes when compared to naive, uninfected cells. We purified and characterized exosomes from naive and infected J774.A.1 murine macrophages. We analyzed the miRNA content of these exosomes by RNA sequencing and found differential miRNA expression patterns were indeed present. We demonstrate here that a well known miRNA, miR-155, is strongly up-regulated in infected cells and more abundantly packaged in the exosomes released from infected cells than from uninfected cells. Ongoing experiments are investigating the ability of the delivered miRNAs to alter the susceptibility of naive bystander cells to infection, and potentially modulate the host response.

**276 (I) The Effectiveness of Rabies Immunoglobulin against Street Rabies Viruses Isolates Belonging to Different Genetic Clusters**


To develop and implement an effective programme of rabies eradication in Ukraine it is necessary to research the molecular genetic characteristics of the causative agent of this disease in the country. The objective of our work was to establish antigenic complementarity of reference immunoglobulin which is produced using fix strain of rabies virus, derived from Paster Virus to street rabies virus circulating in Ukraine. For this purpose we used “The 2nd International Standard for Rabies immunoglobulin human” with a specific activity of 30.0 IU/sm). To assess its ability to neutralize street isolates that circulating in Ukraine and belonging to two genetic clusters identified in previous studies, four isolates of rabies virus (two from each genetic cluster) with high infectious activity (5.02-5.22 lg LD50/sm) were selected. Using selected rabies virus isolates from a pig, two foxes and badgers it was carried out neutralization test on white mice against Rabies immunoglobulin. Specific activity of Rabies immunoglobulin against street rabies virus isolates were expressed in - lg ED50. The results showed that the effective dose of Rabies immunoglobulin at infection of mice with street rabies virus belonging to cluster II was lower comparatively to infection with isolates belonging to cluster I (2.57±0.06 and 2.93±0.12 respectively). Analysis of the results showed that the Rabies immunoglobulin at the neutralization test “in vivo” using street rabies virus isolates in infecting dose (31.6-50.1 LD50) was at 1.51-3.47 times more effective for neutralizing isolates belonging to genetic cluster I. The results showed significantly less protection of Rabies immunoglobulin against street rabies virus isolates belonging to genetic cluster II compared with isolates belonging to cluster I. The obtained results indicate the need for highly efficient rabies immunoglobulins and vaccines and in future new regional vaccine strains of rabies virus.

**277 (I) Development of a Flow Cytometric Assay to Assess Filovirus Vaccine Efficacy in Non-Human Primates**


New filovirus vaccine candidates have shown efficacy in the non-human primate (NHP) model. Identifying correlate(s) of protection to support licensure of vaccine candidates under the FDA's animal rule is critical. Evaluating humoral immune correlates resulted in variable success. Therefore, development of assays to assess filovirus-specific NHP T cell responses were undertaken to facilitate development of vaccines. We developed an in vitro peptide stimulation culture with peripheral blood mononuclear cells (PBMC) from Cynomolgus macaques, vaccinated with either a vaccine containing Marburg Musoke glycoprotein (gp), or a multivalent vaccine containing gp from Ebola Species Zaire, Sudan/Gulu, Ivory Coast and Marburg Angola. PBMC were stimulated for 20-24 h with vaccine-relevant peptide pools, subjected to intracellular cytokine staining (ICS) and analyzed on a six-color flow cytometer. Cell surface markers CD3, CD4 and CD8 identified T cell subsets, producing cytokines IL-2, TNF and IFN-γ. PBMC cryopreservation techniques and freezing media were assessed. PBMC from one animal who received a vaccine containing Marburg Musoke gp consistently showed a higher magnitude cytokine response to C-terminal Marburg consensus peptide pool, compared to the N-terminal Marburg peptide pool. PBMC from animals vaccinated with a multivalent vaccine also showed virus-specific T cell cytokine response to relevant Ebola peptide pools. Comparing freshly isolated PBMC to cryopreserved PBMC in the flow cytometric assay revealed a minor difference in cytokine response. A cryopreservation media containing 85% fetal bovine serum (FBS) and 5% glucose resulted in slightly higher cytokine response compared to a cryo-media with no glucose and approximately 45% FBS. We developed an in vitro stimulation culture followed by flow cytometric ICS to assess T cell responses in NHPs after vaccination with filoviral vaccine candidates. Filovirus-specific cytokine responses from T cells were detected after stimulation with vaccine-relevant peptide pools.

**ABSTRACTS**

**BIODEFENSE PROGRAM & ABSTRACTS**
278 (I) Control Efficiency Sterilization of Biological Indicators as Elements in Ensuring Biosafety
N. Pinchuk; SSCIBMS, Kyiv, Ukraine.

**Background:** Spores of pathogenic bacteria can cause severe disease in humans and animals. Since pathogenic aerobic bacteria Bacillus anthracis, a pathogenic anaerobic bacteria Clostridium tetani and gas gangrene group of agents capable of forming spores that can cause serious complications and for years to pollute the environment. Toxigenic saprophytes, such as Cl.botulinum, pose a great danger to the food industry because of botulinistichnoho toxin. Today in Ukraine there are no current effective domestic biological controls for different sterilization in accordance with international requirements for quality. **Objective:** Improving the reliability of monitoring the effectiveness of sterilization process by identifying microorganisms to develop and use as biological indicators during the sterilization processes.

**Materials and Methods:** The selection of strains that could be used as a domestic biological controls various modes of sterilization by us were tested for thermal stability of Bac. anthracis M-71, Cl. perfringens, Bac. cereus 96, Bac.subtilis, Bac.licheniformis G VKM B-1711D, Bac. steatorhaphilus CMEs B-718, Bac.steatorhaphilus var. 2.

**Results:** Observed that dried spores of Bac. anthracis M-71, Bac. cereus 96, Bac.subtilis in (1-5)×10⁶ by the action of water fluid pair at (100±2)°C die in 7-8 min.; Cl. perfringens — 6-9 min.; Bac. steatorhaphilus VKM B-718 survive with 5 min. of autoclaving at (120±1)° C and die within 15 min.; Bac. steatorhaphilus var. 2 subjected to steam sterilization and isolated in the laboratory, killed only 60 min. autoclaving at (120±1)°C, Bac.licheniformis G VKM B-1711D under dry hot air (160±3)° C dies after 30 min.

**Conclusions:** Based on the findings, compiled the most resistant strains of bacteria (B. steatorhaphilus VKM B-718, B. steatorhaphilus var. 2 and B. licheniformis G VKM B-1711D), which can be used as biological indicators for local control of various modes of sterilization process — (120±1)° C (132±1)° C and (160±3) °C.

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279 (J) Plant Manufactured Monoclonal Antibodies as Immunoprotectants for Category B Toxins
L. Zeitlin1, M. J. Aman2, N. Bohorova1, O. Bohorov1, B. Bratcher3, J. Garcia1, C. Goodman1, E. Hiatt3, A. Johnson1, D. Kim1, J. Morton1, M. H. Paul1, C. J. Roy1, E. Stavale1, K. Swope3, F. Uzal4, K. Warfield1, K. J. Whaley1, 1Mapp Biopharmaceutical, Inc., San Diego, CA, 2Integrated BioTherapeutics, Gaithersburg, MD, 3Kentucky BioProcessing, Owensboro, KY, 4UC Davis, San Bernadino, CA, 5Tulane Natl. Primate Res. Ctr., Convington, LA.

Staphylococcal enterotoxin B (SEB) and Clostridium perfringens epsilon toxin (ETX), are potent, readily accessible, and easily disseminated toxins which can cause significant morbidity and mortality. Currently there are no options for preventing or treating exposure to these agents. To address this unmet need, monoclonal antibodies (mAbs) against these two toxins have been identified and expressed in a rapid, inexpensive and scalable Nicotiana benthamiana manufacturing system. c19F1 (anti-SEB) and c4D7 (anti-ETX) are mouse-human chimeric mAbs (igG1) that are highly potent neutralizers of their respective antigens (Table 1). Mortality after systemic challenge with these toxins was extremely rapid in mouse models (1 day for SEB, < 1 hour for ETX). The mAbs have been tested in these models of intoxication and found to be highly efficacious, with doses of less than 1 mg/kg providing 100% protection prophylactically against both toxins. The mAbs also conferred post-exposure protection against both toxins within a small window (0.5 - 1 hour post-exposure). Complete protection was also observed when the anti-SEB mAb was delivered after aerosol exposure in mice. These aerosol challenge experiments are now being expanded to non-human primates, where the time course of intoxication more closely reflects human exposure. The results to date suggest that continued development of these mAbs as immunoprotectants for human use is warranted.

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<td>Prophylactic 50% protective dose in mice - systemic challenge</td>
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<td>Prophylactic 100% protective dose in mice - systemic challenge</td>
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<td>Post-exposure window in mice (100% survival) - systemic challenge</td>
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<td>Time to death in untreated mice</td>
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<td>Post-exposure window in mice - aerosol challenge (100% survival)</td>
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**280 (J) Functionalized, Carbon Nanotube Catalyst for the Degradation of Nerve Agents**  
M. M. Bailey, J. M. Heddleston, J. Davis, J. L. Staymates, A. R. Hight Walker; NIST, Gaithersburg, MD.

Recent events have emphasized the need to develop functional materials that will safely neutralize chemical warfare (CW) agents in situ to protect military personnel from dermal exposure. Single-wall carbon nanotubes (SWCNTs) have unique mechanical properties, making them a desirable structural material for a variety of applications, including threads and fabric-like materials with high tensile strength. Chemical modification of SWCNT threads could lead to multifunctional materials that impart chemical functionality to a mechanically strong substrate, for example high strength threads containing catalytic sites that destroy CW agents immediately upon exposure. This study aims to develop a self-decontaminating, SWCNT material that can ultimately be incorporated into a fabric or protective material to minimize exposure to organophosphate nerve agents and to prevent accidental exposure during decontamination procedures. Carboxylated SWCNTs were functionalized with a polymer containing Cu-chelating bipyridine groups and their catalytic activity against an organophosphate simulant was measured. The material was characterized using Raman spectroscopy, and x-ray fluorescence confirmed the presence of Cu in the catalyst. Assuming zeroth-order reaction kinetics, the hydrolysis rate of the organophosphate simulant in the presence of catalyst was 63 times faster than the uncatalyzed hydrolysis rate. This study demonstrates the efficacy of a novel, proof-of-concept design for a Cu-containing catalyst chemically bonded to a SWCNT support that shows catalytic activity statistically better than an uncatalyzed hydrolysis reaction.

**281 (J) Medical Hazardous Waste Disposal in Kazakhstan**  
A. M. Alikimbayev, Sr.,1 M. A. Abdras,2 S. U. Zhandosov,2 G. A. Tuleushova,1 L. S. Kassymkanova2; 1Scientific Practical Ctr. for Sanitary Epidemiological Expertise and Monitoring, Almaty, Kazakhstan, 2Global Green USA, Washington, DC.

**Background:** Contaminated with pathogens, chemical and radioactive substances, medical waste represents a serious risk to a country’s epidemiological and environmental safety. The growing volume of medical waste in Kazakhstan and poor regulation hamper the ability of the biomedical community to cope with a major outbreak. **Methods:** Review of current medical waste management practices in Kazakhstan and laboratory research. **Results:** Kazakhstan has 14,434 health care institutions. About 50 tons of medical waste (22% are the most epidemiologically dangerous) are produced daily across the country, which contain animal corpses, infected anatomical material, and sharps. As of 2011, there are 70 incinerators and 40 muffle ovens in Kazakhstan. The capacity of these facilities does not match the entire volume of produced medical waste. While dumping infectious waste at landfills is prohibited, there are no regulations or standards for waste incineration. Kazakhstan lacks dedicated landfills for chemical and toxic waste such as acids, mercury and formaldehyde. Since the cost of medical waste processing is very high, medical institutions tend to minimize the amount of waste destined for processing. This leads to poor sorting and disposal of potentially infectious waste as household waste. **Conclusions:** Analysis of existing management practices and regulations in Kazakhstan shows a lack of uniform regulations for handling and monitoring the volume of medical waste. Irrational medical waste management poses a threat of pollution to Kazakhstan’s water, air, soils and sediments. There is a need to improve the infectious medical waste management regulations in Kazakhstan in order to protect local, national, and international public health, enhance biosafety, and better prepare the biomedical infrastructure for emergency situations. The project research was made possible by support provided by the ISTC under project K-1925 and by Green Cross Switzerland.

**282 (K) Toxins and Contaminated Drugs: A Report of Selected Biological Events in Germany 2012**  
M. H. Richter, M-H. Lee, C. Herzog; Robert Koch-Instit., Berlin, Germany.

As part of a national program to timely and efficiently respond to severe and extraordinary biological events a special operational unit has been established at the Center of Biological Security at the Robert Koch-Institute (RKI) located in Berlin, Germany. The unit has been established to offer support and assistance to regional or state authorities in case of highly unusual disease outbreaks, bio-accidents, or alleged bioterrorist events. The unit is also involved in preventive measures to exclude deliberate release of highly pathogenic agents in the run up to large public events or state visits. Its unique modular concept allows timely and most appropriate adjustments to manage selected biological events. Joint interdependencies with local and state authorities achieve greater efficiency especially with limited financial resources. In a federal system with decentralized responsibilities (e.g. Germany) highly specialized units are indispensable for management of low-probability, high-impact events. Several special biological events in 2012 from which a selection is described more detailed here have put the unit’s concept to the test. (1) In January 2012 the unit was called for support of local public health authorities, police- and firefighting-units in coping with an unusual case of intoxication involving a highly toxic biological agent (ricin). The case at the time had raised questions about whether bioterrorist activities had been performed. Further, immediate danger for the public and surroundings could not be ruled out. (2) On June 6th 2012 the Robert Koch-Institutes Bio-Incident Response-Unit secured traces of heroin from a drug user. The drug was suspected to be contaminated with Bacillus anthracis as the user recently became ill with a new form of anthrax after injecting the drug a couple days earlier. Both small and large scale operations highlight the need of a highly specialized unit to support local authorities with challenges on communication, risk assessment, sampling, decontamination medical countermeasures and policy decision making.
283 (K) Extreme Biological Events in the Military: Effect and Response: Lessons Learned from the Israeli Biodefense Preparedness Project - The "Orange Flame"


"Orange Flame" is an annual national drill aimed to prepare medical and relevant non-medical organizations in Israel to manage extreme biological events. The Israel Defense Forces (IDF) and its Medical Corps have participated in the "Orange Flame" drills since 2007. In the 2011 drill, the IDF has elaborated its involvement to include operational units outside the medical corps. This has granted valuable insights regarding the consequences of a bio-terror or natural occurring biological outbreak upon operative function and the medical, logistic and administrative efforts required in order to contain the outbreak. It was demonstrated that an extreme biological event has a major operative impact on maintaining military combat capabilities. Implementation of comprehensive measures is to be expected, including bases quarantine, postponing operative missions, transferring on-call missions, limiting gatherings and cancelling large forum discussions. Logistic consequences include the need for manpower and equipment reinforcement and food and water supply in case of suspected resource contamination. Major operational and logistics dilemmas were raised that could not be otherwise predicted "under the neon light". Lessons learned from the drill were implanted in order to revisit our pre-event national and military doctrine for extreme biological events. Military capacity building drills simulating major bio-events must integrate medical and operational elements in order to have a better sense of events impact and current containment ability. All organizations involved in the containment of such an event must keep an up to date contingency plan to be implemented in time of need. To our knowledge, this is the first paper discussing the effects and operative consequences of an extreme outbreak in the military.

284 (K) Clinician Training in Select Agent Outbreak Response is Potentially Sustainable in Resource-Limited East African Countries

M. Chambers, J. Aihuwalla, C. Williams; USAMRIID, Fort Detrick, MD.

Background: The United States Army Medical Research Institute of Infectious Diseases (USAMRIID) works closely with the Defense Threat Reduction Agency - Cooperative Biological Engagement Program (DTRA-CBEP) and the Ugandan NGO, Makerere University Walter Reed Project, to develop clinician training on early recognition, management and reporting of outbreaks of select agents (e.g. Ebola, Anthrax) that is host-nation sustainable in the East African region. Hypothesis: Kenyans and Ugandan leaders are (1) effectively engaged in designing and implementing training that improves clinician outbreak response (2) have the skills and human resources to conduct and sustain the training (3) committed to devoting time to training without remuneration. Methods: Key leaders from Kenyan and Ugandan government ministries, universities and non-governmental organizations met with USAMRIID during a workshop hosted over 3 days in Kampala, Uganda to: Provide constructive criticism of training material presented by USAMRIID. Delineate format and content of the curriculum identify and refine specific, measurable, attainable, relevant and timely goals. Develop criteria for selecting trainees. Define the target audience / end-user of the training. Define collaborator roles (consultation, accreditation, implementation, etc.). Establish a timeline for implementing the training. Begin logistical planning for the first "training of trainers" iteration. Participants filled out Target Asset Maps covering 9 areas of necessary training related activity to inventory their roles and contributions to the training. Results: All of the key objectives were addressed and consensus for a plan and timeline was achieved. Various participants had prior experience and willing to perform in all 9 areas of necessary training related activity; they were committed to working a range of 2-10 hours a week without remuneration. Conclusion: Kenyans and Ugandans consider the training necessary enough to develop their own plan and commit skills and time without remuneration. The training should proceed and funding be procured.

285 (K) A Comprehensive Method of Risk Analysis for Civil Protection: The German Infectious Disease Example

C. Uhlenhaut; Robert Koch-Instit., Berlin, Germany.

Comprehensive national risk management has to take all potential hazards regardless of the origin - natural, technical and anthropogenic - into account. For all hazards, the probability and the potential resulting damages have to be assessed. This assessment is then a corner stone for evaluating the status quo of preparedness and possible risk mitigation strategies. The Federal Office for of Civil Protection and Disaster Assistance (BBK) developed a method that allows comparing different hazards. Key aspects of this method are likelihood and impact. It can be applied on the regional, state and national level. So far, more than 20 hazards were identified that will be analyzed in the coming years. These separate analyses will be compiled in a matrix and thus allow a comprehensive, open-view and unbiased comparison of hazards. At national level the lead for each analysis will be given to the risk owner who develops a reasonable worst case scenario. This institution is supported by all relevant federal institutions in order to allow thorough understanding of the potential damage in five areas: education, environment, economy, supply, and immaterial. Damage parameters were identified for each area, e.g. for "man" the four damage parameters are fatalities, injured, persons requiring assistance (such as shelter, food) and missing persons. In 2012 the first two hazards were evaluated, severe flooding and a pandemic caused by a novel virus related to SARS CoV. We looked at the potential impact on Germany as a whole, including the potential number of lives lost and impact on critical infrastructures. The effects of the pandemic were modeled on a general level, yet it became obvious that given unfavorable conditions disasters cannot be fully prevented even with the best level of preparedness. Especially in the context of infectious diseases preventing an outbreak from becoming a pandemic by using surveillance and public health measures is our best option for risk management.

286 (K) Physiologically Relevant In Vitro Models to Study Infectious Diseases

T. M. Straub, J. R. Hutchison, H. Kreuzer; Pacific Northwest Natl. Lab., Richland, WA.

In vitro models for pathogenic microorganisms have advanced our understanding of infectious diseases. Especially for gut pathogens, there are several key factors that limit our understanding of the host - pathogen relationship including, but not limited to, 1) inability of clinical isolates to efficiently infect host cells (e.g. noroviruses and rotaviruses), and 2) limits to our understanding of mechanisms of pathogenesis in the context of the commensal microflora of the gut. The objectives of our research are to: make a stepwise approach towards construction of a physiologically relevant intestinal model system that takes into account the cellular architecture of the organ system, the anaerobic nature of the intestinal lumen, relevant commensal microflora, and peristaltic flow through the system. The first critical steps of the model are construction of the tissue system, and demonstrating that commensal microorganisms can co-exist with the host system without damage to the architecture of the host cell system. C2BBe1 cells were grown on collagen-coated microspheres in a rotating wall vessel bioreactor under conditions of physiological fluid shear to mimic the 3-D architecture of the host. Facultative bacteria were then introduced to the organ system: non-pathogenic (commensals) Escherichia coli, Lactobacillus reuteri, or pathogenic Escherichia coli O157:H7. Fluorescent antibody staining against villin showed the presence of brush borders in control and co-culture samples with the commensal bacteria. Conversely, membrane damage was observed when cells were cultured with E. coli O157:H7. Scanning electron microscopy revealed appropriate cellular structure and no apparent toxicity to the host for the controls and co-cultures with the commensal bacteria. As expected, cells exposed to E. coli O157:H7 displayed significant cellular damage. Successful co-cultivation of commensal bacteria with host cells will allow us to increase the complexity of the model that takes into account the true nature of the intestine and allows for robust studies regarding the role of the microflora in protecting against pathogens.
150 Investigating the Dynamics of Filovirus Evolution in Cell Culture


Filoviruses are highly lethal RNA viruses that cause hemorrhagic fever with fatality rates of up to 90%. No approved vaccines or therapies exist for filovirus infections. Evidence suggests that fruit bats may be the natural reservoir for filoviruses but they are capable of replicating in multiple species (e.g. humans, non-human primates, and pigs). Typically, RNA viruses have high spontaneous mutation rates due to error prone RNA-dependent RNA polymerases. A consequence of high spontaneous mutation and replication rates is populations composed of heterogeneous swarms of related variant sequences, often referred to as quasispecies. These swarms have important biological consequences as they allow viruses to evolve rapidly in response to selection pressures, which is critically relevant to viral-emergence, virulence, drug-resistance, and vaccine-development. Our preliminary data suggested that filoviruses have spontaneous mutation rates similar to other RNA viruses. The literature also supports the idea that filoviruses are capable of rapid evolution. However, the dynamics of this rapid evolution are poorly understood and little is known about the quasispecies present in filovirus populations. The following data and future experiments aim to investigate these unknowns. Currently, we are using deep sequencing to assess the genetic changes associated with filovirus passage in cell culture. These studies were performed using cultured cells derived from African green monkeys and fruit bats. The viruses used included Zaire ebolavirus, Sudan ebolavirus, and Marburgvirus. We are detecting interesting adaptive changes in the consensus sequence and in the quasispecies population associated with passaging the virus. Some of these changes appear to be cell line and virus dependent. It is likely that the observed genomic changes can also be correlated to phenotype and function. Our data suggest that filoviruses exhibit high genome plasticity and are able to rapidly evolve to different environments. This could have major implications for future filovirus research on emergence, virulence, drug-resistance, and vaccine-development.
153 A Novel Adenovirus Species Associated with an Outbreak of Fatal Pneumonia in a Baboon Colony


Adenoviruses (AdVs) are DNA viruses that infect and cause disease in a number of vertebrate hosts, including humans and nonhuman primates. Here we identify a novel AdV species, provisionally named “species H”, associated with an outbreak of rapidly fatal pneumonia in captive baboons at a primate research facility in Texas, with a case fatality rate of 50% (Image). By whole-genome sequencing, one of 4 AdVs (BaAdV-1) isolated from sick and healthy baboons during the outbreak was a member of a previously described monkey adenovirus species (SAdV-B), while the remaining 3 AdVs [BaAdV-2, BaAdV-3, and BaAdV-4 (genetically identical to BaAdV-2)] were members of species H. Experimental infection of infant baboons with BaAdV-2/-4 (species H), but not with BaAdV-1 (species SAdV-B), produced an acute respiratory illness in one animal. BaAdV-3, closely related to BaAdV-2/-4, was the only AdV among the 4 isolated from a clinically ill baboon, and thus thought to be the most likely causative agent of the pneumonia outbreak. Although BaAdV-3 shared >90% sequence identity overall with BaAdV-2/-4, the significant divergence in the short fiber protein (~58% amino acid identity) and bootscan analysis sequence identity overall with BaAdV-2/-4, the significant divergence in the short fiber protein (~58% amino acid identity) and bootscan analysis raised the possibility that BaAdV-3 is a rare species H recombinant. In support of this hypothesis, specific neutralizing antibodies to BaAdV-1 and BaAdV-2/-4, but not BaAdV-3, were detected in healthy captive baboons and human staff researchers. These results implicate a novel species H AdV in a fatal pneumonia outbreak at a primate research facility, and further establish the potential for cross-species transmission of AdVs between humans and nonhuman primates.

154 Identifying New Host Factors: Stress Regulated Control of Poxvirus Replication

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The genus Orthopoxviridae contains a diverse group of human pathogens including Cowpox, Monkeypox, Variola and Vaccinia. There are currently no FDA-licensed drugs to treat individuals infected with these viruses, which is a concern given the perceived threat of poxvirus weaponization and the steady increase in human infection with Monkeypox, which is endemic to Africa. Therefore, the identification of antiviral compounds and cellular factors important for poxvirus replication is of significant interest. To identify critical host factors required by poxviruses for the productive infection of cells, we developed an RNAi protocol in a pooled-cell format. Using a fluorescent reporter-based infection assay, we completed a FACS-based screen to investigate an shRNA library with over 90,000 hairpins for genes that are necessary poxvirus infection. Several interesting host targets were identified in the screen, and a subset were validated in secondary and tertiary assays. A particularly interesting gene identified through this approach is Heat Shock Factor 1 (HSF1). Knockdown of HSF1, the master regulator of heat-shock transcription, significantly inhibits both Vaccinia and Monkeypox infection; virus replication is also inhibited in HSF1 -/- cells. Furthermore, poxvirus replication can be inhibited by small molecules that block HSF1 function. These data strongly suggest that an induced heat-shock response is important for poxvirus replication. Development of inhibitors that block host factors involved in the heat shock response is a potential therapeutic opportunity.
157 Characterisation of the Natural History of Inhalational Melioidosis in Cynomolgus and Rhesus Macaques
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Burkholderia pseudomallei, the causative agents of melioidosis, is recognised as a pathogen of public health consequence and also as a potential biological warfare agent, especially in the aerosolised form. Due to the lack of proven pre- or post-exposure prophylaxis after inhalational exposure for use in humans, it is essential to have a robust animal model of this disease. In accordance with the FDA’s “Animal Rule”, the generation of data packages that could support the approval or licensure of medical countermeasures against diseases caused by pathogens of high consequence, in this instance specifically B. pseudomallei, are of significant importance. Here we present work performed to evaluate the aerosolisation of B. pseudomallei in rhesus and cynomolgus macaques using a well characterised strain. Methods: The challenge item, B. pseudomallei NCTC 13392, was aerosolised and delivered to cynomolgus and rhesus macaques using the AerolMP-Henderson (n=6). Animals were singly housed enabling continuous telemetry, monitoring core parameters including temperature, heart rate, respiration rate, blood pressure and activity. - Daily monitoring and health scores were taken throughout the study and blood was sampled for bacteriology, immunology and haematology analysis every three days. - Upon necropsy, tissues were processed to paraffin blocks, sectioned at 3-5µm thickness, and stained with haematoxylin and eosin and examined microscopically. Results: Animals received between 200 and 450 CFU of B. pseudomallei delivered via the aerosol route and started to develop clinical signs approximately three days post-challenge. The data presented will summarise the outcome of infection in both species and compare their potential value as a model to study the pathogenesis of B. pseudomallei and evaluate antibiotic efficacy.

158 Mutation of Core Oligosaccharide Attenuates Virulence of Yersinia pestis Through TLR4
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Yersinia pestis, the causative agent of plague, has caused more deaths and socioeconomic impact throughout history than any other infectious agent. The bacterium is endemic worldwide, including parts of the US, and, due to its documented ability to acquire DNA from other bacteria, continually threatens to reemerge as a deadly multi-drug resistant pandemic. Y. pestis is maintained in rodent reservoirs, preserving its eradication and providing a source of bacteria for malignant uses. These factors, combined with its extreme virulence, resulted in its classification as a category A select agent, placing it as a top priority in the US government’s biodefense research agenda for the development of novel treatments and vaccines. While screening a Y. pestis transposon library, one of the mutants, EC51, was shown to be hypocytopotoxic and was selected for further characterization as part of a search for novel virulence factors. Growth curves for EC51 demonstrated that growth at 37°C is comparable to wild type, while growth at 26°C is reduced. Both pneumonic and septicemic infections in mice demonstrated delayed onset of morbidity and increased overall survival. Histology demonstrated vastly altered host response and disease course. Sequencing of the transposon insertion site indicates disruption of the waaD-F-C operon, suggesting abnormal LPS is responsible. Y. pestis’s surface LPS, which is hexacylated or tetraacylated based on its growing temperature, is a critical factor in its ability to remain undetected by the immune system in the early stages of infection. TLR4 is the signaling pathway which most readily responds to LPS stimulation but is not triggered until late in infection by Y. pestis. We hypothesize that this pathway is triggered during infection by EC51. Using TLR4 deficient mice, a significantly increased virulence of EC51 compared to controls was shown. Therefore disruption of the waaD-F-C operon has altered LPS presentation to the host immune system, resulting in activation of an effective innate immune response.
Decontamination and subsequent remediation of a site contaminated by the accidental or intentional release of Bacillus anthracis spores is a problematic task. As observed in the aftermath of the anthrax attacks of 2001, decontamination is difficult, costly, and potentially damaging to the environment. The identification of novel strategies that is efficient at neutralizing the threat of the spore release, but is less hazardous to the environment remains a high priority. D-cycloserine (DCS) is an inhibitor of the alanine racemase enzyme responsible for converting L-alanine to D-alanine. This process is important for impeding germination of dormant spores, but also for subsequent cell wall synthesis. Due to this inhibition of cell wall synthesis, DCS has been historically used as an antibiotic for the treatment of tuberculosis. We characterized the impact of DCS on both spores and vegetative cells of fully virulent 

*Bacillus anthracis*. Both fluororesent-based kinetic assays and traditional plating assays showed that spores germinated to a significantly greater extent when the standard alanine-insoline germinant mixture was supplemented with DCS. A minimum inhibitory concentration analysis demonstrated that 10 mM DCS is bactericidal towards newly germinated spores and bacteriostatic towards vegetative cells. The anti-microbial activity was consistent against many strains from environmental and clinical sources. DCS (10 mM) was also able to protect murine macrophages in vitro from spore-associated killing with no associated toxicity. Our results confirmed that DCS does augment germination induced by L-alanine but also that DCS is quite efficient at killing the newly germinated spores, a characteristic possibly making DCS (and similar compounds) uniquely suited for decontamination strategies. While not necessarily a relevant antibiotic for the treatment of clinical anthrax, DCS, if applied with other germinants, could potentially aid in the decontamination efforts by augmenting the induction of spore germination and then rendering the germinated spores non-viable.

Acidified nitrite was evaluated as a disinfectant for Bacillus spores. If a drinking water system or a building were contaminated with *Bacillus anthracis* spores, waste water resulting from flushing or washing, respectively, would need treatment. Disinfectants like chlorine or chloride dioxide can inactivate spores, but large quantities of these chemicals may be needed and particulate matter can interfere with disinfection. Acidified nitrite, unlike chlorinated compounds, does not produce harmful disinfection by-products. Its’ use requires pH adjustment and application of a nitrite salt. *B. anthracis* Sterne (BaS) and *B. atrophaeus* subsp. globigii (Bg) were used as surrogates for virulent *B. anthracis*. Spore suspensions were added to reaction vessels containing pH 2 or pH 3 Butterfield’s buffer at 4°C or at ambient temperature in order to achieve a mean initial titer of 6.3 log10 spores per ml. After briefly stirring the suspensions, sodium nitrite was added to result in a concentration of 0.01 or 0.1M acidified nitrite. Buffers without nitrite served as controls for each species, pH, and temperature combination. Samples were removed from the test suspensions and neutralized in nutrient broth at timed intervals. Spore concentrations were determined using TSA spread plates incubated for 24 hours at 37°C. As expected, BaS spores were more sensitive to inactivation than Bg spores. Inactivation was more rapid at the lower pH, the higher temperature and the higher concentration of sodium nitrite. Inactivation to the < 5 CFU/ml minimum detection limit (MDL) was reached in less than 30 minutes in the tests of BaS in pH 2, 0.1M sodium nitrite at room temperature. Under the same conditions, inactivation of Bg to the MDL took approximately 5 hours. Under the least favorable inactivation conditions of pH 3, 0.01M sodium nitrite at 4°C, the mean log10 reduction was approximately 5.1 after 72 hours for BaS and approximately 0.2 after 72 hours for Bg. Although further testing is needed, acidified nitrite has potential to be an effective disinfectant against spores in water as long as certain conditions can be met.
289 Oral Aminoglycosides with Cell-Targeted Delivery for the Treatment of *F. tularensis*

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Infectious diseases caused by intracellular microorganisms are an increasingly serious problem and represent biosecurity threats, e.g. *F. tularensis* infection. A challenge to the treatment of such infections is difficulty in achieving efficient delivery of antimicrobial agents across the membrane of infected cells. Many existing, effective antimicrobials can currently only be delivered by injection, and demonstrate toxic side effects and inefficient intracellular delivery. The proposed solution is to reformulate existing drugs using cochleates. Cochleates mediate oral bioavailability, reduce toxicity, and significantly enhance intracellular drug delivery. Cochleates are stable, anhydrous, multilayered, crystalline precipitates composed of soy phosphatidylserine and calcium. Gentamicin and amikacin have been formulated into cochleates and tested in vitro against intracellular *tularensis* infections. The intracellular efficacy of aminoglycoside cochleate formulations against either *F. tularensis* LVS and Type A was tested using THP-1 macrophages, matured with PMA for 4 hrs at 5x10⁵ cells/well. 24 hours later, infection with *F. tularensis* LVS (1 x 106 bacteria) was carried out for 1 hr at 37°C. Monolayers were treated, for 3 days, lysed, plated and counted. “Encocleation” increases the effectiveness of aminoglycosides against intracellular microorganisms: Amikacin cochleates were 3x more effective against *F. tularensis* LVS. Gentamicin cochleates were 2x more effective against *F. tularensis* LVS and 4x more effective against *F. tularensis* Type A. Aminoglycoside cochleates achieved enhanced activity against *F. tularensis* Type A and LVS in macrophages. In other studies amikacin cochleates delivered orally to mice were efficacious against systemic M. avium infections, and AmB cochleates demonstrated oral bioavailability in humans. Thus, oral administration of aminoglycoside cochleates has potential for treating *F. tularensis* and other intracellular pathogens such as *Y. pestis.*

290 Post-Exposure Therapeutic Efficacy of COX-2 Inhibition against Pneumonic Melioidosis

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*Burkholderia pseudomallei* is a gram-negative, facultative intracellular bacillus and the etiologic agent of melioidosis, a severe disease in Southeast Asia and Northern Australia. Like other multidrug-resistant pathogens, the inherent antibiotic resistance of *B. pseudomallei* impedes treatment and highlights the need for alternative therapeutic strategies that can circumvent antimicrobial resistance mechanisms. In this work, we characterized macrophage innate immune responses to *B. pseudomallei* using a Toll-like receptor pathway array in order to identify potential host targets for immunotherapy. We observed that macrophage COX-2 mRNA is rapidly expressed (>16,000 fold) after treatment with live, but not heat-inactivated, bacteria. Subsequent production of prostaglandin E2 (PGE₂) by infected macrophages in a time- and dose-dependent manner was confirmed by ELISA. PGE₂ significantly enhanced *B. pseudomallei* intracellular survival within macrophages and treatment of macrophages with a COX-2 inhibitor suppressed *B. pseudomallei* growth. PGE₂-mediated immunosuppression of macrophage bactericidal effector functions was associated with increased arginase 2 expression and decreased nitric oxide (NO) production. Treatment of macrophages with an arginase inhibitor also significantly decreased bacterial intracellular survival and increased NO production. The regulatory role for PGE₂ in the pathogenesis of *B. pseudomallei* was confirmed in the mouse model of pneumonia melioidosis. Lung PGE₂ production significantly correlated with disease progression and bacterial virulence in vivo. Systemic administration of a commercially-available COX-2 inhibitor suppressed the growth of *B. pseudomallei* in the lung and afforded significant protection against rapidly lethal pneumonic melioidosis when administered post-exposure to *B. pseudomallei*-infected mice. COX-2 inhibition may represent a novel immunomodulatory strategy to control infection with *B. pseudomallei* and other intracellular pathogens.

288 Oral Aminoglycosides with Cell-Targeted Delivery for the Treatment of *F. tularensis*
291 Two-Photon Imaging of Pulmonary Anthrax Infection Reveals Novel Immunological Synapses
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The dynamic imaging is critical to revealing the spatial interactions of cells within the tissue environment. In the lung in vivo imaging is particularly difficult because of tissue motion arising from breathing and cardiac contraction. As a result, infected lung tissue has never been imaged in vivo thus far, and little is known concerning the kinetics of the mucosal immune system at the cellular level. We have developed an optimized post-processing strategy to overcome tissue motion, based upon two-photon and second harmonic generation (SHG) microscopy. Most of our strategy is based on post-processing of collagen signal in SHG by cross-correlation with a reference image. We used CX3CR1/gfp mice that express green fluorescent protein (GFP) in CD11b dendritic cell (DCs) and inflammatory monocytes, and Fck1+/gfp that expresses GFP in the endothelial capillaries in the lung. Using Fck1+/gfp we have shown in our model that we could image the lung over a period of time of 1 hour without inducing any leak. This would indicate that our strategy was not invasive and did not alter lung physiology. Next, we infected CX3CR1+/gfp mice by intra-tracheal route with Alexa 633 stained spores of Bacillus anthracis, the agent of anthrax. We observed striking connections between DCs and spores engulfed by alveolar cells. To determine what cells were connected to DCs between we instilled intra-nasally rhodamine-dextran in CX3CR1+/gfp mice, and infected them either by intra-tracheal or intra-nasal route by spores of Bacillus anthracis stained with Alexa-633. We demonstrated that alveolar macrophages phagocytosed spores in the first line and then made synapses with DCs. Synapses between alveolar macrophages and DCs were present at the homeostasis, but increased dramatically after infection. These synapses may participate in a better coordinate immune response. Our results not only demonstrate the phagocytizing task of lung DCs but also infer a cooperative role of alveo- lar macrophages and DCs.

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292 Chikungunya Virus Host-Range E2-Transmembrane Deletion Mutants Induce Protective Immunity against Challenge in C57BL/6j Mice
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Chikungunya virus (CHIKV) is an arthropod-borne disease transmitted to humans through the bite of an infected adenine mosquito and is a re-emerging human pathogen. Infection in humans is characterized by fever, headache, nausea and vomiting, rash, muscle pain, and arthralgia. In some instances, arthralgia produced by CHIKV infection can persist for months or years after clearance of the virus. Every year, millions of individuals are infected with CHIKV in more than 40 countries. There is currently no approved therapeutic or vaccine for CHIKV. A vaccine against CHIKV, which has been made by attenuating WT virus via truncation of the transmembrane domain (TMD) of E2 and selecting for host range (HR) mutants. Mice were inoculated to determine the ability of the mutant strains to elicit neutralizing antibody and protective immunity upon virus challenge. One mutant, ChikV TM17-2 fulfilled the criteria for a good vaccine candidate. It displayed no reactogenicity at the site of injections, no tissue disease in the foot/ankle and quadriceps, and no evidence of persistence in foot/ankle tissues 29 days after infection. Upon challenge with a highly pathogenic strain of ChikV, this mutant produced sterilizing immunity in all tissues tested. This study has identified a ChikV host range (HR) mutant that grows to high levels in insect cells but was restricted in the ability to assemble virus in mammalian cells in vitro. This study demonstrates that these HR strains are attenuated in the mammalian host and warrant further development as live-attenuated vaccine strains.

293 Development of Second Generation Inactivated Alphavirus Vaccine
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Alphaviruses are highly infectious envelope viruses and have been identified as emerging infectious pathogens and potential bioterror agents. Currently, there is no FDA approved alphavirus vaccine. Therefore, development of safe and efficacious alphaviral vaccine is urgently required. Traditional methods of virus inactivation have inherent drawbacks like poor immunogenicity due to loss or damage of epitopes and in some cases incomplete inactivation. Thus, here we describe two novel approaches of viral inactivation which result in better immunogenicity and complete inactivation of viruses. A photoactive compound, 1,5,6iodonaphthal azide (INA), was used to inactivate alphaviruses. Upon UV-irradiation, INA partitions into the hydrophobic domain of the biomembrane and covalently binds to the membrane proteins without affecting their ectodomains. In another approach, Venezuelan equine encephalitis virus (VEEV) was inactivated by using supra lethal doses of gamma irradiation (up to 50,000 kGy) in the presence of Mn-DP-Pi complexes derived from highly radioreistant bacteria, Deinococcus radiodurans. Both the inactivation strategies resulted in completely inactivated and safe virus particles. INA treatment resulted in inactivation of the infectious viral genome whereas gamma irradiation resulted in denaturation of the viral genome. INA-inactivated VEEV was found highly immunogenic and protected mice against lethal virus challenge. Our findings also show that INA has a dual mechanism of inactivating the viruses i.e. by targeting both the viral proteins and the genome. Our findings present novel approaches towards developing highly immunogenic and safe inactivated viral vaccine(s). This work was supported by funding from Defense Threat Reduction Agency. Opinions expressed here are of the authors and should not be construed as that of USUHS, USAMRRIID, NCI and BITS.
295 Immunogenicity Assessment of In Silico-Selected T-Cell Epitopes for a Burkholderia Biodefense Vaccine
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Background: Burkholderia pseudomallei (BPM) and Burkholderia mallei (BM), the causative agents of melioidosis and glanders respectively, have been designated as Category B pathogens due to their potential use in bioterrorism. Burkholderia cepacia (BC) is a life-threatening opportunistic pathogen affecting Cystic Fibrosis patients. Currently, there are no licensed vaccines available for any of these pathogens. Previously, we used IVAX vaccine design toolkit and in vitro HLA binding assays to select 70 promiscuous Class II T helper epitopes that are highly conserved in BPM, BM and/or BC. Our goal is to generate a single Burkholderia vaccine that will be effective against all three pathogens. Here we report new data on the immunogenicity of the vaccine candidate from studies in HLA transgenic mice. Methods: Epitopes were concatenated to form 3 multi-epitope gene constructs (PB1, PB2 and PB3). The VaxCAD algorithm was used to place epitopes in an order that minimizes potential immunogenicity at epitope junctions. Genes were synthesized and subcloned into a mammalian expression vector to produce a DNA vaccine and into an E. coli expression vector to produce a concanameric protein. A heterologous DNA-prime, peptide- or protein-boost strategy was used to immunize HLA DR3 transgenic mice. Vaccine immunogenicity was determined by measuring IFN-γ production to individual peptides by ELISpot assay using splenocytes from immunized mice. Results and Conclusion: Following DNA/peptide immunization, significant IFN-γ induction specific to multiple epitopes was found in PB3- but not PB1- and PB2-immunized mice compared to non-immunized mice. Only PB3 concanameric protein was successfully expressed. DNA/PB3 protein immunization studies are ongoing. These results indicate that immunoinformatics-derived epitopes are immunogenic but further construct design will be required before protein concatamers and multi-epitope DNA vaccines can be used in Burkholderia biodefense.

296 Host Factors Involved in Burkholderia Phagocytosis and Intracellular Survival

Given their resistance to many commonly-used antibiotics and relatively high mortality rate, pathogenic Burkholderia are considered to be potential biological warfare agents. Burkholderia spp are facultative intracellular pathogens and rely on host factors for efficient phagocytosis, survival, and spread. Identification of host factors essential for Burkholderia spp virulence will provide novel targets for therapeutic countermeasures against the pathogen. We have performed a functional genomic screen using a small interference RNA (siRNA) library against 718 human kinase genes. The screen was based on high throughput flow cytometry analysis of THP-1 cells infected with B. thailandensis DW503 expressing GFP. Our initial RNAi screen resulted in 49 hit genes. We have validated 16 genes representative of six distinct functional groups: adhesion and intracellular trafficking, mitosis, Ca2+/calmodulin signaling, receptor tyrosine kinase/protein kinase C signaling, phosphatidyl inositol signaling, and glucose metabolism. Using imaging flow cytometry and differential fluorescent labeling of intracellular and membrane-associated bacteria, we have identified 7 human genes critical for the internalization and proliferation of B. thailandensis (BTH) within THP-1 cells. Gene silencing of CALM1, CAMKKB, EphB2, STK38L, STK35, PTK7, and PKRCH, produced ≥60% reduction in intracellular BTH CDC27211, a rare clinical isolate identified as the causative agent of pneumonia and septicemia in humans. Additionally, invasion of BTH CDC27211 was significantly reduced in THP-1 cells treated with specific inhibitors of protein kinase AKT (H-89), Abl (PD180970), CKII (TBB), KDR (ABT-B69), and PKC (PKC20-28). We have further investigated the role of MARKCS, a PKC substrate and a scaffold protein involved in actin cross-linking, as a potential therapeutic target for host-directed inhibition of Burkholderia phagocytosis. We expect these studies to advance discovery of novel inhibitors of Burkholderia infection.

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297 Optimization of High-Throughput Sequencing Technology and its Application in Clinical Virology Diagnosis
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High-throughput sequencing technology is having a growing impact on biological research and clinical diagnosis by providing rapid and high resolution access to genome-scale information. We proposed an experimental and analytic procedure for virus identification in a variety of human specimens based on high-throughput sequencing. The method is initialized by filter sterilization, then digestion of samples with DNase and RNase to eliminate host nucleotide contamination, and subsequently remaining nucleic acids were extracted with carrier RNA. cDNA was synthesized from the extracted viral nucleic acids with random hexamers, which were designed for specific blocking of the reverse transcription of the carrier RNA. After double-stranded cDNA synthesis and fragmentation, multiplex adapters were ligated to each sample. The high capacity of Next Generation Sequencing enables us to perform multiplex sequencing of over 70 individual samples from a single sequencing run. By using serial dilutions of known DNA and RNA viruses, “spiked” into normal serum specimens, as few as ~100 viral particles could be detected by the assay. The short DNA sequences are filtered to eliminate potential DNA contamination from human, mouse, and known bacteria. With a focus on discovering novel virus, the short sequences are further filtered against virus with genome sequences available from public database and then were subjected to de novo assembly. The pipeline searched the resultant contigs against non-redundant (NR) protein database by blastx and defined the candidates as the contigs that show the highest similarity from kingdom of viruses. We applied the method to 92 serum specimens of patients with acute seronegative hepatitis to screen for potential viral infection and identified multiple known and new DNA and RNA viruses. Our work demonstrated that high-throughput sequencing is effective to identify highly divergent viruses in patient samples for clinical diagnosis, as well as discover novel viruses with potential disease associations.
298 Rapid Pathogen Detection and Identification Using a Microbial Detection Array

Background: Rapid and accurate detection of viral and bacterial pathogens for biodefense and public health require technologies that are broad-spectrum and cost-effective. We have developed a comprehensive microbial detection technology, the Lawrence Livermore Microbial Detection Array (LLMDA), to detect all sequenced viruses, bacteria, fungi, protozoa and archaea. LLMDA is significantly faster and more cost-effective than whole genome sequencing. Methods: A total of 357,700 unique probes were designed on the LLMDA to detect the target sequences representing more than 5,900 microbial species. We have applied this microarray to identify viral and bacterial pathogens from a variety of human and animal clinical samples. The LLMDA has also been used in identification of a contaminating pig virus in a childhood vaccine. We determined the sensitivity of the LLMDA and also compared the performance of LLMDA to next generation sequencing. Results: In a recent international unknown pathogen identification study, the LLMDA detected a Rift Valley fever virus from 1000 pfu/ml of human blood. In another study, the LLMDA was able to detect BK polyomavirus with as little as 5 DNA copies ≥1000 copies/ml from urine samples in combination with phi29 amplification. Additionally, viral agents such as HPV, human herpesviruses, enteroviruses, adenoviruses, etc. were detected from a variety of human sample types including nasal swabs, urine, stool, serum, and cerebrospinal fluid. When different concentrations of five pathogens were spiked into a complex blood or soil background, the LLMDA gave equivalent results to a full plate of 454 sequencing. Conclusions: The LLMDA is a cost-effective and rapid genomic technology that has been used for detection and genotyping of viral and bacterial pathogens for biodefense, public health, and food and drug safety. The LLMDA will enable informed responses to novel biological threats and provide a complement to high-throughput sequencing. Pathogen detection arrays such as the LLMDA have the potential to gain wider use both in research settings and in the regulatory and diagnostic environment.

299 Mobilome Analysis of Yersinia pestis Using Comparative Genomics
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Yersinia pestis, a Gram-negative bacterium, is the causative agent of plague, responsible for three human pandemics in history. Y. pestis strains were subdivided historically into classical biosvars (bv.) Orientalis, Medievalis and Antiqua. Currently, two groups of atypical Y. pestis, Pestoi des and Microtus are classified as new biosvars. All 24 available Y. pestis genomes were included in this study focused on phylogenetic analysis and genomic islands (GIs) identification. Y. pestis exhibits low genetic diversity and evolutionary rate as evidenced by very shallow branch lengths in its core genome phylogeny. All eight bv. Orientalis strains formed a monophyletic clade, while bv. Antiqua strains distributed into three subgroups. Four bv. Medievalis and bv. Antiqua Nepal516 comprised a single clade. Fourteen GIs were identified in Y. pestis using a GI-definition of five or more contiguous ORFs., While known high pathogenicity island, HPI, and regions associated with pathogenicity were identified by this study, most MGEs were novel. All bv. Medievalis strains and bv. Antiqua Nepal516 were GI-01 (14kb) negative, and GI-07 (8kb) was present in bv. Orientalis strains. Most GIs harbored IS elements, and FV-I isolated in USA had elements of the type IV secretory pathway, though its precise genomic location could not be determined. The study provides evidence that, despite having extremely low genetic diversity, a number of genome rearrangements have shaped Y. pestis. This study thus provides the starting point for phylogenetic analysis and study of mobilome dynamics in Y. pestis, for we expect that as more strains are analyzed, the number of examples of GIs and genome rearrangements in Y. pestis will only continue to increase.

300 Haplotype Analysis Advances Bacterial Rare Variant Detection in Next-Generation Sequencing
R. E. Colman1, J. Schupp1, D. Smith1, J. Gillece1, A. Rawat1, J. Usher1, D. M. Engelthaler1, J. Foster1, P. Keim1,2; 1Translational Genomics Res. Inst., Flagstaff, AZ, 2Cfr. for Microbial Genetics & Genomics, Northern Arizona Univ., Flagstaff, AZ.

Rare variant detection on complex biological mixtures is radically changing since next-generation sequencing (NGS) emerged. The majority of research has focused on detection of emergence of antibiotic or antiviral resistance, specifically with HIV. However, this type of work stands to transform the methods of forensic investigations, such as in the response to bioterrorist attacks for use of identifying particular preparations of agents. Unfortunately, rare variant detection from NGS data is not trivial, primarily due to the error rates in sequencing. This error associated with the sequencing platform sets the limit of discerning a rare variant from error. However, the use of SNP states of multiple known positions together will lead to lower probability of a haplotype occurring due to error. Using the Illumina platform, we sequenced several different known mixtures of synthetic plasmids to high coverage to explore the use of read count based haplotype analysis for identifying rare variants. We calculated the error rate of a given di-nucleotide haplotype based on average error (0.12%) per position across the plasmid. For a 0.1% mixture the calculated false haplotype rate of a known di-nucleotide haplotype would be 1.6x10^-7. The expected frequency of the true rare variant would be 1.0x10^-3 which is higher than the frequency due to error. In this study, we were able to detect a 0.1% mixture at a frequency of 1.5x10^-3 with no false positive haplotypes. In conclusion the use of haplotype analysis and NGS enables the detection of very low level mixtures down to at least 0.1% with appropriate coverage.

301 Viral Metagenomic Sequencing of Non-Malarial Febrile Illness in West Africa

Many infectious pathogens including emerging and weaponizable microbes produce non-specific symptoms, such as fever, making them difficult to diagnose clinically. This challenge is especially problematic in tropical developing countries where molecular diagnostic tools remain limited. Rapid social and demographic change in many of these countries is increasing the likelihood that infectious microbes will spread beyond their borders. Our laboratory seeks to elucidate the spectrum of viruses associated with non-malarial febrile illness in West Africa. To achieve this goal we developed molecular and computational methods to sequence the viral metagenome from healthy individuals and febrile patients at two hospitals in Sierra Leone and Nigeria. Through long-standing partnerships with these hospitals, we sequenced the bloodstream viral metagenome from 121 afebrile controls and 193 cases of non-malarial febrile illness. We found significantly more viral reads in samples from febrile patients. We will present data from this study and an update on our efforts to implement a viral metagenomics in West Africa to rapidly identify emerging human pathogens.
Invited Speakers

Plenary Session

001 H5N1: One Year Later (A, G)
Monday, February 25, 2013 | 8:30 AM - 11:30 AM
Lincoln 2-4

002 The Importance of Understanding Influenza Host Switching, and Dual Use Implications of Such Studies
Jeffery K. Taubenberger; NIH/NIAID, Bethesda, MD.

Influenza pandemics have apparently occurred since at least the Middle Ages. When pandemics appear, 50% or more of an affected population can be infected in a single year, and the number of deaths caused by influenza can dramatically exceed what is normally expected. Since 1500, there appears to have been at least 14 influenza pandemics. In the past 120 years there were undoubted pandemics in 1889, 1918, 1957, 1968, and 2009. It is currently not possible to predict the next pandemic, including when it will appear, where it will originate, or how severe it will be. Study of previous pandemics may help guide future pandemic planning and lead to a better understanding of the complex ecobiology underlying the formation of pandemic strains of influenza A viruses.

003 A Journal’s Perspective on the H5N1 Controversy
Barbara Jasny; American Association for the Advancement of Sci., Washington, DC.

The publication last year of two papers on enhancing transmissibility of H5N1 represented, for the journals involved, the culmination of a perfect storm formed by seemingly incompatible researcher priorities, public concerns, and political forces. What was clear was that the current systems for evaluating and communicating research with biosecurity concerns need improvement. Since the publications, the moratorium on certain kinds of H5N1 research has been extended, there have been some efforts at public education, and there have been steps towards increased regulation. This talk will evaluate the lessons learned from the experience and discuss future challenges.

004 The NSABB Perspective: Lessons Learned
Michael Imperiale; Univ. of Michigan, Ann Arbor, MI.

In the fall of 2011, two laboratories submitted manuscripts describing experiments demonstrating that HPAI H5N1 influenza virus could transmit directly from mammal to mammal. Prior to publication, the U.S. Government asked the National Science Advisory Board for Biosecurity (NSABB) to determine whether the results met the definition of dual use research of concern and, if so, to make recommendations about publication. This presentation will summarize the deliberative process undertaken by the NSABB, its conclusions, and the lessons learned.

005 The Ecology of Avian Influenza: Understanding the Emergence of Highly Pathogenic Avian Influenza Viruses
Vincent J. Munster; Rocky Mountain Lab., Hamilton, MT.

Highly Pathogenic Avian Influenza (HPAI) viruses can emerge when low pathogenic viruses of the H5 or H7 subtype are introduced from wild birds into poultry, through a change in the hemagglutinin cleavage site. HPAI viruses have a devastating impact on chickens and turkeys, with mortality rates of ~ 100%. Outbreaks of HPAI virus in poultry have occurred on a regular basis during the last decades and with the exception of HPAI H5N1 virus, these have all been contained. Compared to all other HPAI virus outbreaks, the current epizootic of HPAI H5N1 virus is highly unusual in many respects, such as the spread of HPAI H5N1 virus throughout Asia and into Europe and Africa, the large number of countries affected, the loss of hundreds of millions of poultry, the cross-species transmission to humans and other mammals, the continuously changing genotypes and the spill-back of the virus into wild birds.

Focus Session

002 Using Social Networks for Tracking Health and Disease (K)
Monday, February 25, 2013 | 1:00 PM - 2:30 PM
Lincoln 6

009 Leveraging Crowd Sourced Mapping Big Data and Social Media for Biodefense
Jen Ziemke; John Carroll Univ., University Heights, OH.

This talk will describe current and future projects in biodefense and epidemiology that leverage crowdsourcing, crisis mapping, and social media for enhanced early warning and response.

010 A Landscape Analysis of the Use of Social Media for Biosurveillance
Jody Ranck; PwC Advisory Services, Tysons Corner, VA.

Over the past several years we have witnessed a dramatic growth in the number of users of social media platforms as well as a proliferation of tools that leverage the power of social networks. This presentation will provide an overview and analysis of the platforms used for public health purposes. With the increasing use of mobiles for social networking combined with the sensor capabilities that are available we have new approaches for biosurveillance that are more bottom-up and participatory in nature. Early warnings for cholera outbreaks in Haiti have been detected in Twitter streams and geo-tagged data. The advent of big data tools that have made it possible to analyze large volumes of data from social media, mobile and traditional structured and unstructured data sources also offers expanded analytical capabilities. The National Retail Data Monitor that tracks sales of over-the-counter medications combined with data from social media, for example, offer the promise of real-time, early warning system capabilities. From the University of Rochester’s efforts to develop algorithms for data mining Twitter to understand social networks and food borne disease outbreaks to crowdsourcing platforms such as Ushahidi, GoogleFlu Trends or HealthMap, we are now at a moment to reflect on recent experiences with these platforms to address emerging challenges to established practices in biosurveillance in public health as well as consider strategies for optimizing their impact and offering new paradigms for public health and biosurveillance practices. This presentation will also consider a number of citizen science applications as well as smart networks for epidemiologists and public health professionals that can be leveraged with social media and data analytics tools to improve biosurveillance techniques.
Focus Session

**003 Environmental Infection Levels: What’s Out There and is it Relevant? (E)**

Monday, February 25, 2013 | 1:00 PM - 2:30 PM

Lincoln 5

**010a Hantaviruses: Out There, Relevant and Emerging**
Connie S. Schmaljohn; USAMRIID, Frederick, MD.

The first report of Hantaan virus, the etiologic agent of Korean hemorrhagic fever, appeared in 1978. Within the next 10 years, molecular, serological, epidemiological and ecological studies led to the establishment of the Hantaan virus genus in the family Bunyaviridae. Hantaan virus and three related viruses were shown to cause clinically similar diseases in Asia and Europe, which were collectively termed hemorrhagic fever with renal syndrome. Two interesting, serologically and genetically related, but apparently nonpathogenic viruses were also identified; one in native voles in Maryland, and the other in shrews in India. Although both were regarded mostly as anomalies at the time, they were actually clues to the ubiquity and variety of the hantaviruses and their hosts that we now recognize. The major research impetus leading to the recognition of the global presence of these viruses was the 1993 emergence of a novel hantavirus in the US, which caused an outbreak of highly lethal acute respiratory distress termed hantavirus pulmonary syndrome. Since then, numerous hantaviruses have been detected in a myriad of rodents, insectivores and bats all over the world. The discoveries that hantaviruses have experienced genetic reassortment, host spillover and host switching many times over their long evolutionary history have further complicated our understanding of these viruses, and have challenged the long held dogma of one hantavirus virus-one host. The taxonomic rules for placement of new hantaviruses is also evolving, and it is now clear that there are not well defined or easily separable groups of distinct hantaviruses, but rather a continuum of genetic variants that can only be classified using rather arbitrary criteria. For hantaviruses, “what is out there” is intrinsic to how they have been able to so successfully inhabit the natural world. This fascinating story is only being unraveled now and appears to involve circumventing and down regulating host immune responses in their hosts, with relatively infrequent, accidental and usually dead end encounters with humans, the sole natural victims of hantaviral diseases. The obvious potential for further emergence of these viruses suggests that it would be wise to continue to seek better insight into what and where they are.

**011 Ecology of Hantaviruses in North America**
Brian R. Amman; CDC, Atlanta, GA.

Since the discovery of Sin Nombre virus (SNV) in 1993 at least 17 hantavirus genotypes have been described in North America whose reservoir hosts belong the small mammalian orders Rodentia (subfamilies Muri-nae, Arvicolinae, and Sigmodontinae) and Soricomorpha (subfamilies Soricinae and Talpinae). Intensive studies during the last 19 years have contributed greatly to our knowledge of the factors influencing hantavirus transmission in host populations. From an ecological standpoint, components of hantavirus transmission involve environmental (weather and landscape characteristics), anthropogenic (habitat disturbance and biodiversity loss), and behavioral factors. Current ecological research is also challenging the paradigm that infection with SNV in the natural reservoir host does not impact the health and fitness of that host.

**012 Will the Real U.K. Hanta Please Stand Up?**
Lisa Jameson; Hlth. Protection Agency, Porton, United Kingdom.

Hantaviruses are emerging rodent-borne viruses. Human infection most often occurs when breathing in aerosols of excreta from infected rodents. In general, two clinical syndromes are recognised: haemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. In the United Kingdom there is growing evidence for human and animal exposure to hantaviruses demonstrated by the detection of specific antibodies and classic HFRS type disease. However no direct evidence for any hantavirus has ever been reported in the UK and some skepticism naturally exists. This presentation will detail the virus isolation and genetic characterisation of a UK hantavirus (Seoul virus) from wild caught Rattus norvegicus. These findings have implications for public health as Seoul virus is capable of causing moderate-severe HFRS type disease, and indeed, we were able to link this virus to human disease in a patient diagnosed with acute kidney injury. Our findings firmly prove the existence of a hantavirus in the UK and confirms the presence of a pathogenic Seoul virus in Europe.

Focus Session

**004 Clinical Research Networks and the Global Response to Public Health Emergencies (G)**

Monday, February 25, 2013 | 3:45 PM - 5:15 PM

Lincoln 6

**014 WHO IMAI Triage, Differential Diagnosis, and Management Severe Illness: Opportunity Begins Here**
Nahoko Shindo; WHO, Geneva, Switzerland.

The WHO Integrated Management of Adolescent and Adult (IMAI) District Clinician Manual for limited-resource settings addresses the care of acutely ill adolescents and adults, including people living with HIV and targets senior health workers. A 5-day training course based on the manual aims to enhance capacity of the district hospital team in the timely identification and emergency treatment of severely ill patients, and ongoing management of patients with severe respiratory distress from pneumonia or septic shock. A team-based approach is taught, and includes case-based coursework for clinicians, nurses, administrators and auxiliary staff covering infection prevention and control, diagnostic specimen collection, clinical reasoning and management, and their roles in disease surveillance-specifically, recognizing and reporting dangerous pathogens to enable more rapid and better responses to disease outbreaks.

**015 Update on International Clinical Research Networks in Public Health Emergencies**
Gail Carson; ISARIC, Oxford, United Kingdom.

The need for a clinical research infra-structure which can respond to a public health emergency to provide data to improve patient care and policy decisions is well overdue. ISARIC was formed post pandemic to try to ensure that the clinical research community can and will respond. An update will be provided on how ISARIC is trying to meet that challenge.
016 InFACT: Global Collaboration in Acute Care Research
John Marshall; St. Michael’s Hosp., Toronto, Canada.
Multicenter clinical research is challenging. But the challenges increase exponentially if attempts are made to conduct rigorous clinical trials during an evolving pandemic: the threat is still being defined, treatment options are unclear, and research resources are constrained. The recent H1N1 pandemic, and the SARS epidemic before it underline the virtual impossibility of launching research during an outbreak, and stimulated investigators to consider different models. The International Forum for Acute Care Trialists (InFACT) is a recently formed umbrella group representing some 20 investigator-led critical care research groups from around the world. InFACT coordinated 5 large regional observational studies during the H1N1 pandemic, and launched 3 randomized controlled trials assessing the utility of statins and steroids in the ICU patient with influenza. Building on the lessons of the 2009 pandemic, we are now working with the International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC) to identify and build the needed infrastructure to enable research during future major outbreaks. InFACT has launched an initiative to map global capacity for acute care, with the goal of creating a broad network for sentinel surveillance and for the conduct of large scale epidemiologic research. We are establishing an outcome measures working group to develop and validate biologic and clinical endpoints for acute care research. A core component of a pandemic preparedness strategy is the development of a proposal for an inter-panademic adaptive clinical trial of inexpensive and readily available interventions that might improve outcomes for patients with severe acute respiratory infection. Adaptive clinical trial designs are gaining acceptance in other disciplines such as oncology. In essence, the strategy establishes an analytic plan that permits ongoing assessment of the trial results, modifying aspects such as randomization to maximize the chances that patients will be enrolled in the arm generating superior outcomes. Multiple arms can be tested simultaneously, and recruitment to a given arm concludes once an a priori measure of efficacy or futility has been obtained. By establishing the rules for adapting the design in advance of the trial launch, the design is protected from the bias that might result from knowledge of the ongoing trial results. The model permits both flexibility and efficiency, and is ideally suited for a large international study that would employ a common design and analytic plan, but be run autonomously by participating research networks. As information is derived from the ongoing trial, it informs the management of patients in the control arm, with the result that the trial is a hybrid of a clinical trial and a quality improvement program. The emergence of large international networks such as InFACT and ISARIC is a product of the failure of previous pandemic research efforts. While the model is gaining traction with decision-makers and funders, the immediate challenges are substantial, and include funding, sustainability, and decision-making models that respect the autonomy of highly successful member groups. Nonetheless, it is apparent that success during a pandemic can only be accomplished by leveraging capacity that has been created during the inter-pandemic period.

017 Naval Medical Research Center, Frederick: Targeting Enhanced Sepsis Outcomes
James V. Lawler; Naval Med. Res. Ctr., Frederick, MD.
Severe sepsis is the common end-point for many diseases that might constitute public health emergencies. Improving our ability to recognize and effectively manage sepsis, particularly in the setting of scarce or overwhelmed resources, could significantly improve patient outcomes in a deliberate or naturally emerging event. More effective agent-agnostic treatment strategies would be especially useful in instances where rapid diagnosis is unavailable, for diseases without available drug options (e.g. most viral hemorrhagic fevers) or with potential drug-resistance, and for novel agents. Naval Medical Research Center, Frederick (NMRC-F) has been funded by the Defense Threat Reduction Agency, Joint Science and Technology Office to start a consortium to improve severe sepsis outcomes by establishing an international network of clinical research centers intended to develop improved clinical management algorithms and identify common pathways of pathogenesis that could be exploited for host-based diagnostic and therapeutic approaches. In this presentation, we will discuss NMRC-F’s plans for a severe sepsis research consortium.

Focus Session
005 Potential Strategies for Curing BoNT Intoxication (B, G)
Monday, February 25, 2013 | 3:45 PM - 5:15 PM
Lincoln 5

019 Approaches and Challenges in the Development of Therapeutics for Botulinum A Intoxication
Kim D. Janda; The Scripps Res. Inst., La Jolla, CA.
Botulinum neurotoxins (BoNT) are the etiological agents responsible for human botulism, a disease characterized by peripheral neuromuscular blockade and a characteristic flaccid paralysis. Of particular concern is the lack of clinical interventions that can reverse cellular intoxication. Targeting the BoNT light chain (BoNT/LC) is a logical strategy since it could provide an effective post-exposure remedy. Yet, virtually all efforts at finding inhibitors of the BoNT/LC have been met with limited achievement. We will discuss problematic issues seen with different forms of the LC in relationship to previously characterized inhibitors of the BoNT/A protease. A second therapeutic initiative is to uncover molecules that can provide transient relief from BoNT/A intoxication. Aminopyridines have shown clinical efficacy; yet, aminopyridines have a relatively short half-life and blood brain barrier (BBB) penetration producing neurotoxic side effects. A series of aminopyridines will be detailed that both delay the onset of paralysis for BoNT/A and lack BBB permeation.

020 Engineering BoNT-Based Inhibitor Delivery Platforms against Botulism
Brenda A. Wilson; Univ. of Illinois, Urbana, IL.
There is currently a keen need to develop post-exposure antitoxin therapeutics capable of reversing the paralytic effects of Clostridium botulinum neurotoxins (BoNTs). The short window of opportunity during which immunoglobulin therapy is effective and the long half-life of BoNT serotype A (BoNT/A) in cells poses a major challenge in developing post-exposure therapeutics complementary to existing antitoxin strategies. We have developed a platform for post-exposure antitoxin therapeutics in which the toxin heavy chain (BoNT/A-HC residues 544-1295) acts as a vehicle for delivery of potential inhibitory cargo, and we have demonstrated the delivery of prototype cargo GFP in cultured NG108-15 neuronal cells, in vivo in mouse nerve-muscle preparations, and in vivo in mouse EDL muscle nerve endings. Here, we have expanded and optimized this toxin-based delivery platform by modifying the pH-sensitivity of the toxin translocation domain (TD) and linkers to capitalize on toxin use of the pH-sensitive translocation machinery for gaining access to the cytosol such that translocation of the cargo occurs promptly after cell entry, thereby avoiding lysosomal degradation. We have made considerable progress at finding inhibitors of the BoNT/LC have been met with limited achievement. Targeting the BoNT light chain (BoNT/LC) is a logical strategy since it could provide an effective post-exposure remedy. Yet, virtually all efforts at finding inhibitors of the BoNT/LC have been met with limited achievement. We will discuss problematic issues seen with different forms of the LC in relationship to previously characterized inhibitors of the BoNT/A protease. A second therapeutic initiative is to uncover molecules that can provide transient relief from BoNT/A intoxication. Aminopyridines have shown clinical efficacy; yet, aminopyridines have a relatively short half-life and blood brain barrier (BBB) penetration producing neurotoxic side effects. A series of aminopyridines will be detailed that both delay the onset of paralysis for BoNT/A and lack BBB permeation.

Lincoln 5
**021 A General Strategy for Rapidly Developing Simple Antitoxin Products and the Potential for Their Genetic Delivery**

Charles B. Shoemaker; Tufts Cummings Sch. of Vet. Med., North Grafton, MA.

Toxins cause a wide variety of illnesses and pose serious biodefense threats. We have developed and successfully tested a novel antitoxin platform that performs the toxin neutralization and clearance functions of conventional polyclonal antitoxin serum by administering two simple recombinant proteins. One component is a ‘toxin neutralizing agent’ that binds the toxin at two unique sites and potently neutralizes its ability to intoxicate cells. Each toxin neutralizing agent is a heterodimer of two anti-toxin camelid heavy-chain-only antibody (Ab) V₃(VHH) binding domains. The second component of the antitoxin is the ‘clearing Ab’, a monoclonal Ab that binds to two copies of an epitopic tag present on each toxin neutralizing agent. Co-administration of the toxin neutralizing agent and the clearing Ab results in decoration of the toxin with up to four Abs which promotes its rapid clearance from serum. The same anti-tag clearing Ab results in decoration of the toxin with up to four Abs which promotes its rapid clearance from serum. The same anti-tag clearing Ab can be used in all therapeutics developed using this platform. Our antitoxin strategy has been successfully applied to two serotypes of *Botulinum* neurotoxin (BoNT), *Shiga toxins* Stx1 and Stx2, C. difficile toxins TcdA and TcdB, ricin and anthrax. For some animal models, employing the toxin neutralizing agent has proven fully protective even in the absence of clearing Ab. It has been possible to link VHHs targeting several toxins into a single functional protein chain, thus permitting protection from multiple toxins in mice by administering a single toxin neutralizing agent and, where necessary, the clearing Ab. Genetic delivery of a toxin neutralizing agent using a single dose of an adenovirus delivery vehicle fully protected mice from lethal BoNT/A challenge for more than two months. Use of this antitoxin platform should permit rapid and economic development of new antitoxin therapeutics to all toxin threat agents, leading to products having improved safety and shelf-life properties compared to conventional antiserum-based products. Genetic delivery options should permit protection from multiple toxin risks for long periods with a single treatment.

**022 Harnessing the Neuronal Ubiquitin Proteasome System for BoNT Therapeutics**

George A. Oyler; Tufts Cummings Sch. of Vet., Baltimore, MD.

Approaches that harness host cell processes to shorten the persistence of BoNT/A are being developed as potential antidotes for botulism. Differences in the intracellular stability of *Botulinum neurotoxin* (BoNT) serotypes, BoNT/A and BoNT/E light chains (LC), is in part due to differential susceptibility of these LCs to proteasome-mediated degradation. We have developed so-called ‘designer E3 ubiquitin ligase’ therapeutics directed against BoNT/A and BoNT/B LC and are developing LC mutants to deliver these proteins to cells. Additionally we have identified interactions between BoNT/A and the cellular proteins p47 and VCP135 which may promote deubiquitination and increase the persistence of BoNT/A LC. The interaction between BoNT/A LC and deubiquitinating enzymes may represent new targets for small molecule inhibition. Designer E3 ubiquitin ligases based on SNAP25 or camelid heavy-chain-only Ab VH (VHH) targeting domains have been developed by fusion of these proteins to a number of E3 ligase forming domains including RING, HECT, and the TrCP F-box. These constructs have been shown to promote BoNT LC degradation in cells. Methods for delivery of these therapeutic agents to intoxicated neurons are being developed; including the use of toxin-based proteins, liposomes, viral like particles, and viral vectors. Yeast two hybrid and other protein-protein interaction approaches are being used to evaluate the potential of perturbing deubiquitinating enzyme (DUB) interactions with BoNT/A LC as a therapeutic strategy. The candidate DUBs were further assessed for their roles in BoNT/A LC stability by half-life measurements after siRNA knockdowns. Designer E3 ubiquitin ligases directed toward BoNT/A and /B LC were demonstrated to accelerate neuronal cell recovery from BoNT intoxication. Development of delivery systems for such protein bases treatments is showing rapid progress. siRNA knock down of p47 and the DUB VCP135 shortens the half-life of BoNT/A LC. Delivery of designer E3 ligases directed toward BoNT/A and /B LC are promising therapies for BoNT intoxication. Inhibition or knockdown of BoNT/A associated DUBs represent a new host cell directed therapeutic strategy.

**Plenary Session**

**008 Bats as Sources of Emerging Infectious Diseases (A, B, F)**

Tuesday, February 26, 2013 | 8:30 AM - 11:30 AM

Lincoln 2-4

**026 Analyzing, Predicting, and Preventing the Emergence of Infectious Diseases from Bats**

Peter Daszak; EcoHlth. Alliance, New York, NY.

A series of clinically significant pathogens have emerged from bats over the past few decades, often from unexpected regions. To understand future risk of disease emergence from bats, we created a database of all reported bat parasites, corrected for biases inherent in sampling and surveillance, and analyzed it against expected drivers of pathogen spillover from bats. Our ‘hotspot’ map shows that disease emergence from bats is strongly correlated with human activity on the planet, including livestock production and bushmeat hunting. This hotspot map has critical value if we are to spend global resources to prevent the emergence of the, likely thousands of, potential zoonoses that bats harbor because it suggests high priority regions for pathogen discovery and preventive measures. In this talk I highlight some of the pathogen discovery that we are conducting through the USAID EPT PREDICT program, and report on some of the interesting, potentially zoonotic viruses that we have discovered. Finally, it is important to remember that bats are under threat globally from anthropogenic activity, and that they provide valuable ecosystem services via pollination and pest control. Conservation of bat populations that reduce human activity and contact with them, would benefit not only the services they provide, but also global health.

**027 Sources of Mammalian Viruses: Bats and Beyond**

Christian Drosten; Univ. of Bonn Med. Ctr., Bonn, Germany.

Studies in bats and other small mammals have recently yielded a wide range of new animal viruses. There is hope (and big promises) that virus discovery may give us a head start against the next pandemic emerging. Clearly it makes a lot of sense to make a census of all those viruses sleeping in animal reservoirs. These data can provide fundamental insights into the evolution of mammalian viruses, explaining how humans might have acquired some of their major infectious agents and providing new scenarios for the investigation of disease mechanisms. However, only in very selected cases findings of novel reservoir-borne viruses can have direct consequences for public health. The majority of discovered viruses have resided in their hosts for thousands or millions of years without affecting human health. Barriers that decide whether a virus can or cannot infect and spread in humans include complex processes such as viral entry, virus-host co-operation on cellular level, as well as the many components of epithelial and systemic host defense versus viral evasion. To achieve a real triage among reservoir-borne viruses in terms of their epidemic/epizootic risks, we need to invest much more work in comparative mechanistic virology. In this talk I will provide examples from our own work showing the use of virus discovery for the fundamental investigations of virus evolution, as well as challenges in mechanistic risk assessment of selected reservoir-borne viruses.

**028 Bartonella Species in Bats and Bat Ectoparasites**

Michael Y. Kosoy; CDC, Fort Collins, CO.

Recent investigations have confirmed the presence of hemotropic bacteria of genus *Bartonella* in bats from Africa, Europe, Southeast Asia, Central and South America. These studies reported that *Bartonella* infections are highly prevalent in bats and the *Bartonella* strains exhibit high genetic diversity. Analysis of *Bartonella* strains recovered from Kenyan and Thai bats exhibited specificity to their bat hosts whereas those obtained from Guatemalan and Peruvian bats showed very little specificity to their bat hosts. Co-infection with multiple *Bartonella* strains in a single bat species and even in an individual bat was reported. *New Bartonella* genotypes were detected in a global sampling of many species of blood-feeding bat flies suggesting an important role of bat flies in harboring bartonellae. Identification of *Bartonella* species in the vampire bats is epidemiologically important because of the possibility of direct transmission of *Bartonella* infection to animals and humans through biting.
A hallmark of Zaire Ebola virus (EBOV) infection is a dysregulated immune response. It has been suggested that early events in the host response to EBOV infection may be crucial to the outcome of the disease. Macrophages and dendritic cells not only orchestrate innate and adaptive immune responses but are also early target cells of EBOV. We have performed a comparative analysis of early cytokine and chemokine profiles in human primary cells infected with highly pathogenic or nonpathogenic Ebola viruses and found significant differences. EBOV has been shown to interfere with multiple host defense mechanisms, including the interferon response and activation of PKR. We will present data on the interaction of EBOV with additional cellular antiviral signaling pathways including stress response and cell death signaling.

The severity of filoviral, Ebola virus (EBOV) and Marburg virus (MARV), disease can be accounted for in part by the ability of these viruses to antagonize the host interferon (IFN) response, a major innate antiviral defense. Filoviral VP35 proteins inhibit IFN alpha/beta production by blocking signals transduced by RIG-I-like receptors which sense RNAs produced during viral infection, triggering IFN alpha/beta production. Re-combinant EBOVs with defective VP35 IFN-antagonist functions exhibit decreased replication in cell culture and do not cause disease in animal models. EBOV VP24 proteins block cellular responses to IFNs by impairing the nuclear accumulation of the critical IFN-activated transcription factor STAT1. In contrast, MARVs use their VP40 protein to target the same pathways, but at an upstream step where they block function of the tyrosine kinase Jak1. These latter immune evasion functions are also likely to contribute to virulence. Therapeutic approaches that either inhibit or subvert these immune evasion functions are likely to mitigate the severity of filoviral disease.

Remarkable progress has been made over the preceding decade in developing candidate vaccines against the filoviruses, Marburg virus and Ebola virus, in nonhuman primate (NHP) models. There are at least six different vaccine systems that have shown promise in completely protecting NHPs against filovirus infection. Among these prospective vaccines, two candidates, one based on a replication-defective adenovirus and the other on recombinant vesicular stomatitis virus (rVSV), have provided complete protection to NHPs when administered as a single injection. Recent sequencing studies of historical Zaire ebolavirus (ZEOBV) seed stocks has raised concerns since many of these studies used viruses predominantly containing a mutated BU residue at the GP editing site. Preliminary evaluation of several vaccines showed that only rVSV-based vaccines were able to provide complete protection against a ZEOBV stock with around 90% containing the wild type BU residue. This recent finding has important implications on filovirus vaccine development.
**036 Nanoparticle Vaccines for Melioidosis**

Richard Titball; Univ. of Exeter, Exeter, United Kingdom.

Both *Burkholderia pseudomallei* and *Burkholderia mallei* are potential bio threat agents causing serious and often fatal diseases in humans. The treatment of disease caused by *B. pseudomallei* and *B. mallei* is often difficult because the bacteria are inherently resistant to many antibiotics. Consequently, there has been interest in developing a vaccine which would protect against this disease. Such a vaccine would also have potential for the prevention of disease in melioidosis-endemic areas of the world. Over the past decade a range of polysaccharide and protein antigens have been identified which are able to induce partially protective immunity against experimental infections in mice. Our aim was to develop a gold nanoparticle platform which would allow the presentation of multiple antigens, including glyco-conjugates, to the immune system. Our results, with a range of modified by linking the antigens to gold nanoparticles. Further modulation of the immune response could be achieved by additionally tethering Toll ligands or agonists onto the nanoparticles. Our studies in mice indicate that vaccines devised using this technology will allow us to devise more effective glanders and melioidosis vaccines.

**037 Protective Efficacy and Safety of the Brucella Vaccine**

Thomas A. Ficht; Texas A&M Univ., College Station, TX.

Development of live, attenuated vaccines has indicated that enhanced immune protection is directly related to the endurance of the vaccine strain. However, in order to avoid symptoms of disease we have explored the use of encapsulation to enhance endurance of otherwise highly attenuated and rapidly cleared organisms. Microencapsulation and nanoparticle platform which would allow the presentation of multiple antigens, including glyco-conjugates, to the immune system. Our results, with a range of antigens, indicate that the immune response can be enhanced and modified by linking the antigens to gold nanoparticles. Further modulation of the immune response could be achieved by additionally tethering Toll ligands or agonists onto the nanoparticles. Our studies in mice indicate that vaccines devised using this technology will allow us to devise more effective glanders and melioidosis vaccines.

**038 Molecular Basis of Immunity to Rickettsial Infection**

Juan J. Martinez; Univ. of Chicago, Chicago, IL.

Spotted fever group (SFG) rickettsiae including *R. rickettsii* (RMSF) and *R. conorii* (Mediterranean spotted fever) are pathogenic organisms transmitted to humans through tick salivary contents during the blood meal. RMSF is one of the most severe spotted fever group rickettsioses in the western hemisphere, causing severe morbidity and up to 20% mortality in the absence of timely and appropriate antibiotic treatment. MSF exhibits an expansive geographic distribution, now including central Europe and central and southern Africa, and increased disease severity commensurate to RMSF, with mortality rates reported as high as 32% in Portugal in 1997. Using *R. conorii* and C3H/HeN mice as a model for SFG rickettsial pathogenesis, we demonstrated that the conserved autotransporter adhesin protein, OmpB is a protective antigen for this class of pathogens. These studies revealed that *R. conorii* can in part survive killing in blood and plasma in the absence of the appropriate class of neutralizing antibodies. Survival in blood and plasma was correlated with the absence of complement deposition suggesting that rickettsiae have an active mechanism(s) to inhibit this arm of the innate immune system. We have identified two proteins, the OmpB β-peptide (OmpB βp) and RC1281/Adr1 in *R. conorii* Malish 7 that are sufficient to mediate resistance to bactericidal effects of human serum in vitro. Homologues to OmpB, a factor H binding protein (Hfbp), and RC1281/Adr1, a vitronectin binding protein, are present in all pathogenic rickettsiae suggesting that complement evasion may be a conserved virulence strategy that will be important in the progression of various rickettsial diseases. Interestingly, purified recombinant subunit-based vaccines consisting of Hfbps are currently in clinical trials to elicit protective immunity against pathogenic Neisseria suggesting that Hfbps and other serum resistance factors in rickettsiae may also be viable vaccine targets.
**Symposium**

**011 Skin: Maginot Line or Early Warning System?**

**I**

Tuesday, February 26, 2013  |  1:00 PM - 3:00 PM

**Lincoln 6**

**041 Mast Cell Role in Skin Infections**

Anna Dinardo; Univ. of California, La Jolla, CA.

The multifunctional nature of mast cells (MCs) has been revealed through their involvement in both innate and adaptive immune responses. Recent insight into the various functions of MCs has shown that these innate immune effectors possess the dual ability to kill microbes and to modify classical adaptive immune responses. Members of the cathelicidin family of antimicrobial peptides (AMPs) are expressed by MCs and epithelial cells at sites of injury. The granular localization of the cathelicidin peptides, their extracellular release, and their capacity to modify inflammatory responses suggests that cathelicidin plays an important role in the capacity of MCs to combat skin infection. Our data show that MCs are sentinels in the skin for defending against bacterial and viral infections. Mast cell-deficient (Kit^W-sash-/-) mice are more susceptible to skin infection than the wild-type mice, while Kit^W-sash-/- mice reconstituted with skin MCs show a normal response. Using MCs derived from mice deficient in cathelicidin, we showed that antimicrobial peptides (AMPs) are critical anti-pathogenic granule components. Using human derived mast cells, we confirmed that Cathelicidin is also essential in human mast cells for fighting viral infections. Signaling through toll-like receptor (TLR)-2 increased the level of antimicrobial peptide MCs, enhancing their capacity to fight skin infections.

**043 Skin Microbiome and Immunity: Who Shapes Whom?**

Juliæ Segre; NIH, Bethesda, MD.

Culture-based methods have been the primary techniques used to study microbes inhabiting humans; however, many species are not successfully grown in culture. We performed high throughput genomic sequencing surveys to investigate the topographical and temporal complexity of skin microbial communities from 20 skin sites in healthy adults. Significant differences were observed in the species predominating in particular microenvironments: sebaceous, moist, and dry. Interpersonal variation and temporal changes in the microbiome were site-specific. While microbiota activate and educate the immune system, little is known of the extent to which the immune system shapes the microbiome and thus sets the stage for disease. Primary immunodeficient patients are vulnerable to recurrent bacterial infections, commonly attributed to a loss of immune regulation. We describe the microbial characteristics of the skin of primary immunodeficiency patients who share a common phenotype of skin eczema yet have different syndromes arising from monogenic mutations leading to loss of distinct lymphocytic populations. In parallel, with animal models we explore the role of the skin commensal bacteria to induce protective and regulatory responses that maintain host-microbial mutualism. Skin microbiota plays a non-redundant role in controlling the level of activation of skin resident lymphocytes at steady state and during pathogen challenge independent of the gut flora and via distinct mechanisms. Together, these results begin to deepen our understanding of the intricate relationship of the skin as niches fostering distinct microbial communities and immune cells.

**044 Bioinformatic Approaches to Investigating Mycobacterial Infection to Gain Insights into Mechanisms of Immunity**

Delphine Lee; John Wayne Cancer Inst., Santa Monica, CA.

To gain insights into skin immune cells in the context of infection, we will describe the approaches of three studies in which 1) bioinformatic pathways analysis investigated the mechanism of neutrophil recruitment in human infectious disease in the skin lesions of leprosy, 2) differential gene expression profiles compared the clinically progressive lepromatous form of leprosy with self-limited tuberculoid to demonstrate an association of galectin-3 with unfavorable host response in leprosy and a potential mechanism for impaired host defense in humans and 3) Rank-Rank Hypergeometric Overlap (RRHO) analysis identified genes induced by BCG that may be relevant for improved cutaneous host defense.
**Plenary Session**

**017 Developing Vaccines for Biodefense and Emerging Disease Pathogens (A, B, G)**

Wednesday, February 27, 2013 | 8:30 AM - 11:30 AM  
Lincoln 2-4

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**164d Vaccine Design: Dissection of Immune Responses to Vaccine AGS**

Phil Felgner; Univ. of California, Irvine, CA.

We developed an approach to construct and probe protein microarrays on a genome-wide scale. We applied the approach to more than 30 medically important infectious agents, made 42,500 plasmids, printed the encoded proteins on 25,000 microarrays and probed them with 15,000 serum specimens to determine disease-associated antibody profiles in people infected with each agent. The individual proteins printed on these arrays capture antibodies present in serum from infected individuals and the amount of captured antibody can be quantified using fluorescent secondary antibody. In this way a comprehensive profile of antibodies that result after infection or exposure can be determined that is characteristic of the type of infection, the disease stage. The goals of this research are to develop a more detailed understanding of how the immune system responds to infection, and to identify serodiagnostic antigens that predict acute infectious disease and past exposure. A prototype multiplex array containing serodiagnostic antigens from 18 different infectious agents will be described that is intended to be applied for determining the causes of undifferentiated febrile diseases. For vaccine applications individuals with immune responses against an infectious agent can be clinically classified, some of whom are protected against disease and others who are not. Comparing differential antibody reactivity between these protected and unprotected individual identifies a short list of vaccine antigens associated with protection. Several examples of this vaccine antigen discovery approach will be described.

*P. falciparum* protein microarray containing 4,528 proteins

**Naturally Exposed Resident of Mali West Africa**

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**165 Tackling the Henipavirus Transboundary Threats by Active and Passive Immunization Approaches**

Christopher C. Broder; Uniformed Services Univ. of Hlth. Sci., Bethesda, MD.

Hendra virus and Nipah virus are highly pathogenic bat-borne zoonotic agents that can cause a severe and often fatal neurologic and/or respiratory disease in a wide variety of mammals including humans. Together they comprise the genus Henipavirus, within the Paramyxoviridae family, and are classified as BSL4 agents. There are currently no approved antiviral therapeutics or vaccines available for use in people, and both remain a significant public health concern. A variety of initial Hendra and Nipah virus studies focused on virus entry, which uncovered many details of the attachment and membrane fusion events of the infection process including the identification of the B-class ephrin proteins employed by the viruses as entry receptors. Recent structural studies on the henipavirus attachment G glycoproteins have produced important details on both receptor binding and antibody based neutralization mechanisms. From these studies, along with the development of several now well-accepted animal models of henipavirus pathogenesis, two Hendra and Nipah countermeasures have emerged showing significant promise. One is a passive immunization approach with a cross-reactive neutralizing human monoclonal antibody (m102.4) targeting the viral G glycoprotein receptor binding site and the other is a soluble form of the Hendra G glycoprotein (HeV-sG) as a subunit vaccine. The human m102.4 antibody is now in clinical development for human use and to date it has been used in three people who experienced a significant risk of Hendra virus infection in Australia, through the implementation of a compassionate use protocol. The HeV-sG immunogen was recently launched as a vaccine against Hendra virus for horses in Australia; called Equivac® HeV (Pfizer Animal Health). The m102.4 antibody and the HeV-sG subunit vaccine have proven remarkably effective in treating or completely preventing Hendra and Nipah infection, respectively, in multiple animal species and together they now offer viable approaches to addressing Hendra and Nipah virus infection that are applicable to either people and/or livestock.

**166 Filovirus Vaccine R&D**

Heinz Feldmann; NIAID Rocky Mountain Lab, Hamilton, MT.

Viral hemorrhagic fever is caused by multiple pathogens including filoviruses, arenaviruses, bunyaviruses, flaviviruses and paramyxoviruses. Several of these viruses are important public health pathogens and agents of biothreat potential. Therefore, countermeasure development is a priority for many nations even so many of the viruses are fairly restricted in their endemicity zones. Vaccine development for these pathogens has utilized multiple strategies including DNA and subunit vaccination as well as replication-deficient and replication-competent viral vectors. Three of the most prominent platforms used in this field are replication-deficient adenovirus vectors, attenuated replication-competent vesicular stomatitis virus vectors and virus-like particles. Promising vaccine candidates based on these platforms will be discussed for filoviruses and selected other viral hemorrhagic fevers.

**168 Plaque Vaccine R&D**

Diane Williamson; Defense Sci. and Technology Lab., Salisbury, United Kingdom.

Plague, caused by the bacterium *Yersinia pestis*, is an ancient disease which is still present in the modern world. Currently, there is no licensed vaccine and significant effort is ongoing to develop a next generation of vaccines for clinical use. This presentation will review how an understanding of the natural history of *Yersinia pestis* and the basis of its virulence, has facilitated progress towards next generation vaccines. It will review the immunonalysis conducted on a range of animal models and the progress towards the derivation of immune correlates of protection which in turn facilitates the identification of surrogate markers of efficacy, essential for the successful conduct of clinical trials.
169 Anthrax Vaccine Development: Addressing the Needs of the Strategic National Stockpile
Gerald R. Kovacs; Biomedical Adv., Res. and Dev. Authority, Dept. of Hlth. and Human Services, Washington, DC.

The mission of the Biomedical Advanced Research and Development Authority (BARDA) is to develop and procure medical countermeasures that address the public health and medical consequences of chemical, biological, radiological, and nuclear (CBRN) accidents, incidents and attacks, pandemic influenza, and emerging infectious diseases. CBRN medical countermeasures are being developed for the highest priority threats as identified by the Department of Homeland Security. Anthrax is regarded as one of the most serious of all bioterror threats because of its stability in the environment, ease of dispersion, and high mortality rate. BARDA’s portfolio of anthrax medical countermeasures includes broad-spectrum antibiotics, antitoxin therapeutics, and vaccines. Our anthrax vaccine development and procurement strategy is based on HHS’ requirement for up to 25 million regimens of vaccine for use in a post-exposure prophylaxis campaign. A summary of HHS’ anthrax vaccine projects, the pathway to licensure under the FDA Animal Rule, dose- and antigen-sparing strategies, and requirements for next generation vaccines will be presented.

Symposium

018 Emerging and Re-Emerging Bunyaviruses: Biggest RNA Virus Group, but Least Attention? (A)
Wednesday, February 27, 2013 | 1:00 PM - 3:00 PM
Lincoln 5

170 Overview of the Virus Family Bunyaviridae (The Bunyaviruses)

All the well more than 200 viruses in the family Bunyaviridae (the bunyaviruses) have tripartite RNA genomes. These viruses have been placed in one of five genera: Orthobunyavirus, Nairovirus, Phlebovirus, Hantavirus, and Tospovirus, or have not yet been assigned to a genus. The orthobunyaviruses, nairoviruses, and phleboviruses are transmitted by various hematophagous arthropods and are able to alterate replicate in vertebrates and arthropods, whereas the tospoviruses are transmitted by plant-feeding arthropods; hantaviruses are transmitted vertebrate-to-vertebrate. Many of these viruses are significant human or livestock pathogens or are pathogens of plants and are widely distributed geographically. Some of the vertebrate bunyaviruses cause uncomplicated fevers or fevers with rash; others may cause encephalitis, congenital defects, abortion storms, blindness, hemorrhagic fevers with renal involvement, or pulmonary diseases. That Schmallenberg virus and other viruses actually are reassortants of RNAs of multiple viruses is a startling and disturbing development and will be discussed.

171 Current Update on Molecular Biology of Bunyavirus and Emergence of Schmallenberg Virus in EU Countries
Richard M. Elliott; Univ. of St. Andrews, St. Andrews, United Kingdom.

In 2011 farms in Germany and the Netherlands reported a disease in cattle causing transient fever, diarrhoea, loss of condition and reduction in milk yield. Animals recovered within a few days. Towards the end of 2011 reports of abortion, stillbirths and animals with congenital abnormalities appeared from cattle, sheep and goats. Researchers at the Friedrich-Loeffler Institute in Germany determined the pathogen, by metagenomic analysis, to be an orthobunyavirus of the Simbu serogroup, and named it Schmallenberg virus (SBV) after the town where the first isolation was made (Hoffmann et al., 2012. Emerg. Infect. Dis. 18, 469). Orthobunyaviruses (family Bunyaviridae) are characterised by a tri-segmented negative-sense RNA genome. The Simbu serogroup contains 25 viruses, of which Akabane, Aino, Sathuperi, Peaton, Shamonda and Shuni viruses are known to be teratogenic in ruminants, in Africa, Asia and Australia. The basic virology of orthobunyaviruses and the relationships between these viruses will be discussed. Simbu serogroup viruses are transmitted by midges and mosquitoes, and analysis of midges trapped in the Netherlands, Belgium and Denmark has shown evidence of Schmallenberg infection. In late summer 2011 it is likely that infected mides were blown across the North Sea/English Channel and transmitted the virus to cattle and sheep in the southeast of England and westwards to Cornwall. The virus has successfully over-wintered in both mainland Europe and the UK, where circulating virus has been detected in August 2012. Further spread of the virus is therefore expected. A reverse genetic system for Schmallenberg virus has been established which allows manipulation of the viral genome to introduce specific mutations. Exploitation of reverse genetics to create recombinant attenuated viruses with vaccine potential will be discussed.

172 Discovery of a New Phlebovirus Associated with Severe Febrile Illness in Missouri
William L. Nicholson; CDC, Atlanta, GA.

The discovery of a novel phlebovirus (Bunyaviridae) in the United States is described. Two men from northwestern Missouri presented in early June 2009 to a medical facility with fever, fatigue, diarrhea, thrombocytopenia, and leukopenia and a history of tick bite (s) within 5 to 7 days prior to onset of illness. Initially, ehrlichiosis was suspected as the cause, but was ruled out through serology, PCR, and lack of response to doxycycline. Cultures were initiated onto DH82 canine cells, the cell line used for growth of Ehrlichia chaffeensis. Although cytopathic effect was noted in inoculated cultures, evidence of bacterial morulae was not seen. Instead, the cultures yielded viruses tentatively identified by electron microscopy as members of the family Bunyaviridae. These were transferred onto Vero cells for further investigation. Next generation sequencing (NGS) and phylogenetic analysis showed these viruses to be similar to each other, but not identical in sequence. This virus was determined to be a unique virus of the genus Phlebovirus. This is the first report of the identification of a phlebovirus pathogenic to humans in the Americas. Ongoing investigations on the ecology of the virus will also be discussed.

173 Molecular Determinants of Tick-Borne Phlebovirus Virulence
Hideki Ebihara; Rocky Mountain Lab., NIAID, NIH, Hamilton, MT.

Recently, two novel human pathogenic phleboviruses, termed Severe Fever with Thrombocytopenia Syndrome virus (SFTSV) and Heartland virus (HRTV), have emerged in China and the United States, respectively. These emerging phleboviruses cause a severe febrile illness with thrombocytopenia in humans, and their emergence highlights the need for a greater understanding of the virology, ecology, and pathogenesis of these viruses. These novel viruses constitute a new species related to the tick-borne phleboviruses (TBPVs) in the Uukuniemi virus group within the genus Phlebovirus. Recently, we identified a taxonomically unassigned tick-borne bunyavirus, Bhanja virus that has a broad geographical distribution, causes sporadic cases of febrile illness and encephalitis in humans, and is genetically and serologically related to the novel human pathogenic SFTSV and HRTV. In this presentation, the molecular determinants of pathogenicity of TBPVs and their evolution will be discussed. This work was funded by the Division of Intramural Research, NIAID, NIH.
**Symposium**  
019 Progress towards a Dengue Vaccine: Killing Four Birds with One Stone (A, H)  
Wednesday, February 27, 2013 | 1:00 PM - 3:00 PM  
Lincoln 2-4

**174 Dengue Vaccines: Are We Getting Closer?**  
**Thomas P. Monath;** Kleiner Perkins Caufield & Byers, Harvard, MA.

Prevention of dengue by vaccination is an immensely important goal, as the incidence and geographic range of the disease continues to increase. The most advanced efforts in vaccine development are all live, attenuated vaccines, which have been in development for 15-20 years. Non-replicating vaccines, including recombinant subunits, inactivated whole virion, and plasmid DNA are also in development, but are at an earlier stage, and it remains to be seen if sufficiently durable immunity can be generated. The state of dengue vaccine development may be framed in terms of a number of immunological and virological challenges, including the lack of an immune correlate, which will be discussed in this presentation. The near-term hope is that a large-scale Phase IIb trial of the live dengue/yellow fever chimeric vaccine, currently underway in multiple countries, will show it to be protective against all serotypes (despite the absence of protection to dengue type 2 in a Phase IIb study). It is possible that an immune correlate of protection (probably based on a new in vitro assay) will ultimately be defined, in turn facilitating equivalence testing of other candidates in development.

**175 A Single Dose of the NIH Dengue Vaccine is Safe, Immunogenic, and Capable of Neutralizing Vaccine Challenge**  
**Stephen Whitehead;** NIAID, NIH, Bethesda, MD.

The NIH live attenuated dengue vaccine developed by the Laboratory of Infectious Disease has been evaluated in several Phase I trials in the U.S. and is poised to enter Phase II trials in Brazil and Thailand. In developing the tetravalent vaccine, we first evaluated the safety, replication kinetics, and immunogenicity of 8 different monovalent dengue vaccine candidates in 15 separate trials to identify those candidates suitable for inclusion in a tetravalent vaccine formulation. We subsequently evaluated 5 different tetravalent admixtures to determine which combination would generate the most suitable safety and immunogenicity profile. Although all 5 admixtures were well tolerated and elicited similar antibody responses in flavivirus-naïve adult subjects, tetravalent admixture TV003 performed the best overall. TV003 elicited a trivalent or better neutralizing antibody response in 90% of vaccinated subjects following a single subcutaneous dose and low level vaccine viremia to all four serotypes was detectable within the cohort. In addition, we evaluated the ability of this single dose of TV003 to protect against challenge with a second dose of vaccine given 6 months later. Following the challenge dose, vaccine viremia was not observed and primary neutralizing antibody titers were not boosted. Based on these promising results, we decided to further evaluate the safety and immunogenicity of TV003 in an additional 56 flavivirus-naïve adult subjects. As in previous studies, each of the components of TV003 was given at a dose of 1,000 PFU. Six months after receipt of the first dose of vaccine, subjects were challenged with a second dose of vaccine (or placebo). Following the single-dose immunization, the vaccine induced a tetravalent neutralizing antibody response in 68% of vaccinees and a trivalent or better response in 92% of vaccinees. Evaluation of TV003 in flavivirus-seropositive adults demonstrated a tetravalent response in 85% of vaccinees after a single dose. Complete safety and immunogenicity data for these studies will be presented. The safety, absence of viremia, and immunologic response of vaccinees to the challenge dose of vaccine will also be discussed.

**176 Structure, Dynamics and Composition of Dengue Viruses**  
**Richard Kuhn;** Purdue Univ., West Lafayette, IN.

The structure of the dengue virus has been determined using a combination of X-ray crystallography and NMR for component proteins and fitting of these high-resolution structures into cryo-Electron Microscopy reconstructions. Previous studies have described the maturation of immature dengue particles into fully or partially mature virions. Studies examining the binding of neutralizing antibodies have also suggested that particles have dynamic properties that result in movement of the envelope proteins away from the lipid bilayer surface. We now show that this process is temperature dependent and the solvent accessible surface of the E glycoprotein is significantly different depending upon temperature. Furthermore, mass spectrometry analysis of purified virions has revealed that the conversion of the prM protein to M is inefficient and other viral proteins may also be present in some virions. A discussion of the biological relevance of these findings and their implications for vaccine development will be presented.

**177 Genetic and Structural Basis for Recognition of Dengue Viruses by Human Monoclonal Antibodies**  
**James E. Crowe, Jr.;** Vanderbilt Univ., Nashville, TN.

A better understanding of the humoral immune response to natural dengue virus infection in humans is thus sorely needed. By coupling human hybridoma technology with a flow cytometric neutralization screening assay, we identified a large number of potently neutralizing antibodies without creating a bias toward particular epitopes. Using this approach, we isolated several major phenotypic groups of human mAbs that exhibited unique neutralization and functional properties. Interestingly, the mAbs with the most potent serotype-specific neutralizing activity identified thus far exhibit viron-only binding - targeting an epitope present only on the intact viral particle. Characterization of the binding sites and activity of these naturally-occurring human antibodies is underway, with recent mapping studies suggesting binding to a discontinuous epitope made up of two E protein molecules, spanning two neighboring homodimers. Fusion loop specific antibodies also appear to be important contributors to neutralization, but usually in a cross-reactive manner. In addition to defining critical human antibody epitopes, a better understanding of the genetic aspects involved in the antibody-heavy chain maturation of these antibodies is critical for vaccine development. To this end, we have begun to extensively dissect the genetic determinants of the human dengue-specific antibody response using high throughput antibody repertoire sequence analysis. By obtaining antibody sequence data from 50-100 different dengue specific hybridomas (for which functional information is known) and aligning these sequences with nextgen sequencing data from flow cytometrically sorted, dengue-specific B cells obtained by leukopheresis of the same subject, we are assembling the largest human B cell repertoire of functional antibodies ever. The long-term goal is to use such molecular information in the rational design of dengue vaccines that enhance the induction of protective neutralizing antibodies and reduce the risk of development of severe disease.
020 Yersinia Pathogenesis: Latest Advances in Regulation and Understanding of Virulence (B)
Wednesday, February 27, 2013 | 1:00 PM - 3:00 PM
Lincoln 6

178 Adaptation to Life in the Flea: Understanding the Microbe-Flea Interaction
Viveka Vadyvaloo; Washington State Univ., Pullman, WA.

Adaptation to the physiological environment of the flea host is essential for efficient biofilm blockage formation and transmission of Y. pestis by flea-bite. Sensory transcriptional regulator PhoP is highly induced during flea infection and a PhoP mutant is deficient in the ability to form biofilm blockage. Following sensing of specific environmental stimuli PhoP will coordinate gene expression that favors adaptation to that specific environmental condition. To elucidate PhoP dependent genes that support successful biofilm mediated flea gut blockage we compared whole genome transcriptional profiles between a PhoP mutant and the wildtype strain during flea infection. Here we show that absence of PhoP regulation leads to a transcriptional program that suggests stalling of essential cellular processes and induction of physiological stress coping mechanisms like the toxin-antitoxin molecules and heat shock gene expression. Our data suggests that Y. pestis is able survive in a unique alternate low metabolic state in the flea gut.

179 Early Events in Bubonic Plague
Virginia L. Miller; Univ. of North Carolina, Chapel Hill, NC.

Yersinia pestis is the causative agent of disease in a variety of mammals, and humans can become infected when human and animal ecologies intersect. This has led to several pandemics of plague in human history, and infection with Y. pestis is currently considered by the WHO as a re-emerging infectious disease because of the increased incidence in a wide number of countries. Bubonic plague is the most prevalent form of the disease and it develops after inoculation of Y. pestis into the skin. The bacteria traffic from the site of inoculation to a draining lymph node during early stages of infection, before they disseminate systemically. The successive events that occur during early stages of infection are under studied and largely speculative. In this talk we will present studies aimed at delineating early events post inoculation - i.e. trafficking of Y. pestis from the site of inoculation to the draining lymph node. To this end, we inoculated mice intradermally with a mix of oligonucleotide-tagged Y. pestis strains and established that only a fraction of the inoculated strains (10-40%) trafficked to the lymph node. This suggests the existence of a bottleneck early during infection. In contrast, 60-100% of the original inoculum appeared to survive at the site of inoculation and to be confined to this site throughout infection. These studies also demonstrated that Y. pestis at systemic sites (e.g. spleen) originate from the lymph node and not from the inoculation site.

180 Molecular Mechanisms of Disease During Pneumonic Plague
Wyndham W. Latham; Northwestern Univ., Chicago, IL.

Yersinia pestis is the etiological agent of plague and is considered to be a public health threat due to its high infectivity and rapid disease progression. Pneumonic plague is the most deadly form of disease caused by Y. pestis and can be transmitted by respiratory droplets containing bacteria or via the purposeful release of the plague bacilli as aerosols into the environment. The host response to primary pneumonic plague is characterized by an early quiescent phase that rapidly transitions to a highly inflammatory phase midway through the disease. The plasminogen activator Pla, a bacterial outer membrane protease, is essential for the full virulence of Y. pestis during pneumonic plague and is required for the transition to the pro-inflammatory phase of disease. We will discuss recent advances in understanding the mechanisms by which Pla enables the rapid outgrowth of Y. pestis in the lungs through the cleavage of specific host protein substrates and how Pla stimulates the development of pneumonia by altering the balance of cellular apoptosis, pyroptosis, and inflammation in the lungs.

181 Regulation of Yersinia Virulence by Small and Sensory RNAs
Petra Dersch; Helmholtz Ctr. for Infection Res., Braunschweig, Germany.

Pathogenic Yersinia species produce different sets of pathogenicity factors that guarantee efficient colonization, dissemination and persistence during the different stages of the infection. An important set of virulence-associated traits is activated by the global virulence regulator RovA which has been shown to be controlled by the carbon starvation regulatory system involving the ncRNAs CsrB and CsrC in Yersinia pseudotuberculosis. Recently, we found that the regulator YmoA of the Hha family of nucleoid-associated proteins controls expression of the counterregulated Csr-type RNAs through alterations of the CsrC RNA stability. YmoA-mediated stabilization of CsrC depends on CsrA and H-NS, but not on the RNA chaperone Hfq and involves a stabilizing stem-loop structure within the 5'-region of CsrC. In addition, YmoA controls expression of the global virulence regulator LcrF(VirF) in an opposite manner. LcrF activates the virulence factors encoded on the Yersinia virulence plasmid (pYV), including the type III secretion system and the antiphagocytic Yop effector genes. Microarray analysis further demonstrated that YmoA co-regulates the expression of these virulence factors with many bacterial stress responses and certain metabolic functions. Following oral infections in a mouse model, we demonstrate that a ymoA mutant is strongly reduced in its ability to disseminate to the Peyer’s patches, mesenteric lymph nodes, liver and spleen and exhibits a reduced mortality. We propose a model in which YmoA is a central part of a complex regulatory network and controls switching from a RovA-dependent early colonization phase towards a virulence plasmid (pYV)-dependent infection phase important for host defense and persistence.

182 The Role of Divalent Cation Transport Systems in Pliague Pathogenesis
Jacqueline D. Fetherston; Univ. of Kentucky, Lexington, KY.

Divalent cation transporters for iron, manganese, and zinc are required for virulence in a number of bacterial pathogens. We have examined the role of a variety of divalent cation transporters in the pathogenesis of Yersinia pestis, the causative agent of plague. Mutants in the ferrous iron transporter, Fee, are fully virulent by both a subcutaneous and intranasal route of infection. When combined with a mutation in the Yfe system, which transports both iron and manganese, the double mutant has an approximately 100 fold loss in virulence by a subcutaneous route. Similar results are obtained with the manganese transporter, MntH. Single mutants in yfe and mntH are still fully virulent. Finally, although in vitro zinc transport is entirely dependent upon the presence of the Znu ABC transporter, znu mutants are still fully virulent.
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