Biodefense Program Committee

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USAMRIID, Frederick, MD

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University of Texas, San Antonio, TX

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University of New Mexico School of Medicine, Albuquerque, NM

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Southwest Foundation for Biomedical Research, San Antonio, TX

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Reneé Woodland, Program and Abstracts Coordinator
Lisa Nalker, Manager, ASM Conferences and SCOPE Program
Bianca Blanks, CME Services Manager
Audrey Senn, Exhibits Assistant
Traci Williams, Logistics Coordinator
Latonya Nichols, Conferences Coordinator

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Dear Colleagues,

Welcome to Baltimore, Maryland, for the American Society for Microbiology’s 2009 Biodefense and Emerging Disease Research Meeting. The ASM Biodefense Research Planning Committee has assembled an expanded program including many of the top experts in the field presenting in several session formats. The 2009 program is expanded so that attendees have an opportunity to attend educational programming that is of most value and relevance to their individual professional needs.

Since October 2001, ASM has focused many of its efforts on policy issues, legislation, and gaps related to biodefense. Since the first meeting in March 2003, ASM’s biodefense research meetings have received an overwhelming positive response. For this year’s meeting we’ve 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, recognizing that many of the agents identified and studied also cause naturally occurring disease.

The meeting opens Sunday with four in-depth Focus Sessions followed by the Keynote Session on Sunday evening. Keynote speaker Dr. Stuart Levy, from the Tufts University School of Medicine in Boston, Massachusetts, will present “Studying Bacterial Transcription Factors Involved in Virulence and Antibiotic Resistance: Dual Use Research of Concern?” We cordially invite meeting attendees to the Opening Reception immediately following the conclusion of Dr. Levy’s talk.

Additional keynote speakers, including Dr. Bruce Levin, and Dr. Ralph Baric will be featured throughout the meeting as part of the daily Plenary Sessions. Concurrent symposia are scheduled for each afternoon and topics include: reversing pathogenesis, aerosol biology, arthropod-borne infections, toxin structure and function, Burkholderia, and imaging. Continuing at this year’s meeting are two discussion/Q&A sessions dealing with diagnostic testing and the select agent process. Please check the details in this Final Program to identify the presentations that most appeal to you.

Again, the program committee welcomes you and looks forward to meeting you as we continue work together to address the challenges that lie ahead.

Sincerely,

Theresa M. Koehler, Ph.D.
ASM Biodefense Research Meeting Co-Chair

Connie S. Schmaljohn, Ph.D.
ASM Biodefense Research Meeting Co-Chair
# Program-at-a-Glance

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<th>Sunday February 22</th>
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<tr>
<td><strong>Focus Sessions</strong></td>
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<tr>
<td><strong>Keynote Session</strong></td>
<td>6:30 pm – 8:00 pm</td>
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<tr>
<td><strong>Plenary Sessions</strong></td>
<td>8:30 am – 12:00 noon</td>
<td>8:30 am – 12:00 noon</td>
<td>8:30 am – 12:00 noon</td>
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<td><strong>Lunch</strong></td>
<td>12:00 noon – 1:00 pm</td>
<td>12:00 noon – 1:00 pm</td>
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<tr>
<td><strong>Exhibits</strong></td>
<td>8:00 pm – 10:00 pm</td>
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<tr>
<td><strong>Poster Sessions</strong></td>
<td>1:00 pm – 3:00 pm</td>
<td>1:00 pm – 3:00 pm</td>
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<tr>
<td><strong>Highlight Oral Abstract Presentations</strong></td>
<td>3:00 pm – 4:00 pm</td>
<td>3:00 pm – 4:00 pm</td>
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<tr>
<td><strong>Symposia</strong></td>
<td>4:15 pm – 6:15 pm</td>
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<tr>
<td><strong>Discussion/Q&amp;A Sessions</strong></td>
<td>6:30 pm – 7:30 pm</td>
<td>6:30 pm – 7:30 pm</td>
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<tr>
<td><strong>Receptions</strong></td>
<td>8:00 pm – 10:00 pm</td>
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</tbody>
</table>
Keynote Session

Sunday evening’s Keynote session will be followed by a reception to which all meeting attendees are invited. The session will begin at 6:30 pm in the Harborside Ballroom.

Dr. Stuart Levy, MD, will present his Keynote Address “Studying Bacterial Transcription Factors Involved in Virulence and Antibiotic Resistance: Dual Use Research of Concern?” in this Session.

Symposia

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

Poster Sessions

All accepted abstracts are scheduled for poster presentation based on the December 2008 review of all abstract submissions by the Biodefense Research Program Committee. Posters are on display in the Grand Ballroom from 12:00 Noon–3:30 pm Monday, February 23, and Tuesday, February 24. Please refer to the map on page 7. Presenting authors have been instructed to leave their posters up for both days. However, each presenter will be available at his or her poster to answer questions from 1:00–3:00 pm either Monday or Tuesday (as scheduled in this program).

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. These sessions offer all attendees a chance to come together for presentations of science, policy, and public health issues. The speakers were invited by the Program Committee based on their professional expertise and achievement. Plenary Sessions also feature a moderated Q&A period.

Highlighted Oral Abstract Sessions

These sessions highlight research submitted for the ASM Biodefense and Emerging Diseases Research Meeting. Authors are invited by topic to give a 10 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 Final Program.

Discussion/Q&A Sessions

A one hour session dedicated to providing the attendee with information about selected government programs to include how to apply for grants and other financial support.

Exhibit Hall

The Exhibit Hall is located in the Grand Ballroom. Nearly 50 companies will be staffing their booths during exhibition hours: 8:00–10:00 pm, Sunday, February 22, and 12:00 noon–3:30 pm on Monday-Tuesday, February 23-24.

ASM Press

ASM Press, the book publishing division of the American Society for Microbiology, will be exhibiting its broad selection of texts and references in molecular biology and the microbiological sciences. Stop by to see these and other great offerings from ASM Press. And remember there’s a 10% discount on all purchases made at the meeting.
General Information

Registration Hours
Sunday, February 22 ..................... 11:00 am – 7:00 pm
Monday, February 23 .................... 7:30 am – 5:00 pm
Tuesday, February 24 ................... 7:30 am – 5:00 pm
Wednesday, February 25 — see an ASM staff member for assistance

Registration will be located in the Grand Ballroom Foyer from Sunday, February 22 – Tuesday, February 24, of the Baltimore Marriott Waterfront Hotel.

Telephone Numbers
To contact ASM staff on site at the conference, please call the Baltimore Marriott Waterfront Hotel, at 410-385-3000, and ask to be transferred to the ASM Registration Desk located at the Grand Ballroom Foyer.

Guests
Unregistered guests are not permitted in session rooms or in poster sessions. However, guests may attend Sunday’s Opening Reception. (Meeting attendees can gain entry to the reception with their meeting badges.)

Special Needs
Please contact ASM staff at the Registration Desk to request special needs accommodations.

Child Policy
Please Note: Children are not permitted in session rooms. Childcare may be arranged through the Concierge of the Baltimore Marriott Waterfront Hotel.

Baltimore Marriott Waterfront Business Center
Hours of Operation:
Sunday – Saturday, 7:00 am– 4:00 pm

Poster Storage
Posters may be stored when not displayed at the Registration Desk. Posters must be clearly marked with the presenter’s name when placed in storage. Please note that registration closes at 5 pm on Tuesday, February 24. All posters must be collected by that time or they will be discarded.

Speaker Ready Room
A speaker ready room will be available during registration hours in the Essex Room. Appropriate equipment will be provided. Limited Internet access and laptop internet cables are also available.

Cameras and Recording
Cassette recorders, cameras (including camera phones), and video cameras (including video phones) are prohibited in the Poster Hall and session rooms. Anyone found photographing, videotaping or recording in the prohibited areas will be asked to surrender their badge immediately and leave the conference. No refund will be provided. This rule is strictly enforced.

Certificate of Attendance
Certificates of Attendance are available at the Registration Desk during registration hours beginning Tuesday, February 24. Attendees may also print a Participation Statement of Credit directly from your computer, by accessing the ASM CE Portal (ce.asm.org). You will need your Badge ID from your registration badge to obtain your statement in the CE Portal. The Participation Statement of Credit documents each session you attended at the 7th ASM Biodefense and Emerging Diseases Research Meeting.

Food and Beverage
There are many dining options close to the hotel, some within walking distance, some a short cab ride away. A list of restaurants is available through the hotel Concierge. Please note that lunch will be provided Monday, February 23, and Tuesday, February 24, beginning at 12:00 Noon in the Exhibit Hall (Grand Ballroom).
**Lost and Found**

Unattended personal belongings will be removed and taken to the ASM Headquarters Office then turned into the hotel security office after the conference.

**Meeting Announcements**

A handout table displaying previously approved future meeting and other brochures is located in the registration area. A bulletin board will be available 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 Baltimore Marriott Waterfront Hotel is 410-385-3000.

**Press Room**

The Press Room is located in the Essex Room, on the Harbor View Ballroom level.

**Smoking Policy**

Smoking is prohibited in all areas of the Baltimore Marriott Waterfront Hotel.

**Meeting Venue**

All scientific presentations and poster sessions will take place at Baltimore Marriott Waterfront Hotel. Scientific presentations will take place in the Harborview and Exhibits/Posters in the Grand Ballroom.

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**Student Travel Grants**

The following students were awarded Student Travel Grants to attend the meeting. Please take a moment to congratulate them.

- **Nicholas A. Eisele**  
  Univ. of Missouri, Columbia, MO.

- **Brendan M. Giles**  
  University of Pittsburg, Pittsburg, PA

- **Robert C. McRae**  
  Wright State University, Dayton, OH

- **Meghan A. Wynosky-Dolfi**  
  Drexel University College of Medicine, Philadelphia, PA
2009 Biodefense Exhibits

Exhibitors are here to see you: the Biodefense Meeting attendee. Visit their booths between poster session presentations. Get the latest information on new products while you have a drink with colleagues during receptions, or simply pick up literature as you stroll through the hall. Exhibits, posters, lunch and the opening receptions will be held in the Grand Ballroom.

The American Society for Microbiology welcomes the following exhibitors:

Abbott Molecular ............... #30
www.abbott.com

Alliance Biosciences ............. #6
www.alliancebiosciences.com

Arbor Vita Corporation ........... #23
www.arborvita.com

ASM Press ..................... #38
http://estore.asm.org

BARDA ........................................ #25
www.hhs.gov/aspr/barda

BEI Resources ..................... #8
www.beiresources.org

BIAERA Technologies, LLC ......... #44
www.biaera.com

bioMerieux ..................... #46
www.biomerieux-usa.com

BioSafety Solutions LLC ........... #42
www.bsisolutions.com

Biosearch Technologies ............ #4
www.biosearchtech.com

Bio-Synthesis, Inc. ................. #7
www.biosyn.com

BioTrove ................................... #26
www.biotrove.com

BNBI, LLC .................. #3
www.bnbi.org

CH Technologies (USA) ............ #36
www.inhalation.org

Critical Reagents Program ......... #47
www.peocbd.osd.mil/

D.I.S.S., LLC ................... #33
www.dissdata.com

DSI .................................. #29
www.datasci.com

DTRA RD-CB ...................... #27
www.dtra.mil/rd/cbt

DynPort Vaccine Company LLC ...... #41
www.csc.com/dvc

Germfree Labs, Inc. ............... #16
www.germfree.com

Idaho Technology, Inc. .......... #20
www.idahotech.com

IIT Research Institute ............. #5
www.iitr.org

JPT Peptide Technologies GmbH ....... #37
www.jpt.com

KinemAtik ........................ #35
www.kinematik.com

List Biological Laboratories, Inc. ... #9
www.listlabs.com

Lovelace Respiratory Research Institute ...... #40
www.lrrl.org

Meso Scale Discovery, a division of Meso Scale Diagnostics .... #45
www.mesoscale.com

Microbial-Vac Systems .......... #10
www.m-vac.com

Microfluidic Systems Inc. ....... #17
www.microfluidicsystems.com

MIDI, Inc. ........................ #39
www.midi-inc.com

Midwest Research Institute ......... #34
www.mrriresearch.org

National Biosafety & Biocontainment Training ............ #11
www.nbbtp.org

National Institute of Allergy & Infectious Diseases ........... #14
www.niaid.nih.gov

New Brunswick Scientific ........ #18
www.nbsci.com

NIH Office of Biotechnology Activities ..................................... #24
www.oba.od.nih.gov

OpGen ................................ #22
www.opengn.com

Paragon Bioservices .............. #48
www.paragonbioservices.com

Puritan Medical Products Company .................................. #19
www.puritanmedproducts.com

QIAGEN, Inc. ...................... #1
www.qiagen.com

R & F Laboratories ............... #21
www.rf-labs.com

Roche Applied Science .......... #49
www.roche-applied-science.com

Roche-Roche Applied Sciences .... #50

SA Scientific ...................... #32
www.sascientific.com

Seattle Biomedical Institute & Center for Structural Genomics of Infectious Diseases .......... #15
www.ssgcd.org

Sequenom, Inc. ................... #12
www.sequenom.com

Tetracore, Inc. ................... #13
www.tetracore.com

Transonic Systems, Inc. .......... #31
www.transonic.com

University of Pittsburgh Regional Biocontainment Laboratory .......... #43
www.cvri.pitt.edu
Continuing Medical Education Credit

The following ASM Biodefense and Emerging Diseases Research Meeting session types offer continuing medical education credit as noted in this Final Program by the CME symbol.

NOTE: Sessions marked with no-CE offered symbol did not receive credit for the 7th ASM Biodefense and Emerging Diseases Research Meeting.

<table>
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<tr>
<th>Session Type</th>
<th>Maximum Credit</th>
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<tr>
<td>Focus Sessions</td>
<td>2.5</td>
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<tr>
<td>Plenary Sessions</td>
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<tr>
<td>Keynote Session</td>
<td>1.5</td>
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<td>Symposia Sessions</td>
<td>2.0</td>
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<tr>
<td>Discussion Roundtable Sessions</td>
<td>1.0</td>
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Continuing Medical Education

The American Society for Microbiology is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. The American Society for Microbiology takes responsibility for the content, quality, and scientific integrity of this CME activity.

The ASM designates this educational activity for a maximum of 20.5 AMA PRA Category 1 Credits™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Diplomates of the American Board of Medical Microbiology, Diplomates of the American Board of Medical Laboratory Immunology, and Registrants of the National Registry of Microbiologists may earn AMA PRA Category 1 Credits™ toward re-certification. Each professional should claim credit commensurate with their level of participation in the activity.

Other CE Licenses

A Participation Statement of Credit can be obtained by completing the activity evaluation. This is a detailed statement that documents your participation for each session you attended at this activity.

How to Obtain Your CME Certificate of Credit

ASM has centralized all continuing education activities that are eligible for continuing education credit in the ASM CE Portal. The ASM CE Portal allows individuals to:

- Print their own CE or participation certificates for activities they take part in,
- Evaluate the activities they attend,
- Browse the library of ASM CE activities available,
- Review and print a transcript of all ASM CE activities attended, and
- Participate in online learning activities.

To claim your credit and print your Statement of Credit for participating in the 7th ASM Biodefense and Emerging Diseases Research Meeting, please follow these steps:

- First, visit http://estore.asm.org/cme/createaccount.asp?FormID=101 and create an account. You must use ASM’s eStore to register your name and password prior to accessing the CE Portal. If you already have an eStore account, you may go directly to the ASM CE Portal via this link: http://ce.asm.org/
- Access the ASM CE Portal (link above) and use your login and password you created at the ASM eStore.
- Select the 7th ASM Biodefense and Emerging Diseases Research Meeting from your “My Activities” page after which you will be directed to enter your Badge ID for the activity. The Badge ID should be obtained from your printed badge or from your emailed confirmation letter.
- From the “Claim Credits” tab, you will be able to select each session attended, indicate how many credits you are requesting, and complete the evaluation for each session.
- Follow the instructions to print your certificate.

When completing your online evaluation, please do not press Print until you have entered your information for each session attended at 7th ASM Biodefense and Emerging Diseases Research Meeting.

…continued on page 10
Once you print, your Statement of Credit is considered your final transcript and sessions cannot be added or changed.

- Once you have claimed your credit for this activity it will be added to your transcript which can be viewed at any time by logging into the ASM CE Portal and selecting the “Transcript” button at the top right of any page in the portal.

Should you have questions or need additional information about the continuing education component of this program, please email cmeservices@asmusa.org. This e-mail address is protected from spam bots; you need JavaScript enabled to view it or you may contact Bianca Blanks by phone at 202.942.9223.

Attendees are responsible for recording their attendance at ASM Biodefense and Emerging Diseases Research Meeting sessions and obtaining a CE Statement of Credit.

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**Educational Disclaimer**

The primary purpose of the ASM Biodefense and Emerging Diseases Research Meeting is education. Information presented, as well as publications, technologies, products, and/or services discussed, are intended to inform you about the knowledge, techniques, and experiences of professionals who are willing to share such information with colleagues. A diversity of professional opinions exists in the fields discussed at the ASM Biodefense and Emerging Diseases Research Meeting and the views of the faculty are offered solely for educational purposes. Faculty’s views neither represent those of ASM nor constitute endorsement by the association. The ASM disclaims any and all liability for damages to any individual attending the ASM Biodefense and Emerging Diseases Research Meeting and for all claims which may result from the use of this information, publications, technologies, products, and/or services at the ASM Biodefense and Emerging Diseases Research Meeting.

If you have concerns (e.g., commercially-biased presentations, promotional materials distributed by presenters and/or participants, etc.), please visit the ASM Biodefense Research Meeting Headquarters Office and ask to speak to an ASM staff person so that ASM can respond immediately to your concerns.

**Disclosure of Relevant Financial Relationships and Unlabeled/Unapproved Uses of Drugs or Devices**

As a provider accredited by the Accreditation Council for Continuing Medical Education (ACCMCE), the ASM requires that all faculty members participating in continuing education activities sponsored by ASM disclose any relevant financial relationships related to the content of their presentation(s), as well as any off-label uses of a drug. The ASM then takes steps to resolve any identified conflict of interest. A listing of faculty disclosures is available in this Final Program on page 11. This policy is intended to make you aware of faculty disclosures so you may form your own judgments about material discussed during the 7th ASM Biodefense and Emerging Diseases Research Meeting.

**Statement of Need**

Organizations around the world are involved in research pertaining to biothreat agents, vaccines, detection and diagnostic procedures, animal and plant pathogens, biosecurity facilities, global surveillance, the training of individuals in BL3 and BL4 labs, and other vital topics. As technology changes and research evolves, professional involved in biodefense are being challenged to move ever faster to respond to the growing threat of bioterrorism.

**Target Audience**

It is intended for scientists, physicians, public health researchers and policy makers who need to be informed about the latest scientific developments.

**Learning Objectives**

The ASM Biodefense and Emerging Diseases Research Meeting offers an educational forum which allows the attendee to:

- Discuss new data from the research of microbiological sciences related to biodefense and bioterrorism.
- Discuss with fellow physicians, scientists and researchers the latest information on clinical diagnoses, preventative modalities and therapeutics related to biothreat agents.
- Describe the most recent trends in the management and planning of biodefense programs.
Disclosures

1. Disclosure Statement

As a provider accredited by the Accreditation Council for Continuing Medical Education (ACCME), the ASM requires that all faculty members participating in continuing education activities sponsored by ASM disclose any relevant financial relationships related to the content of their presentation(s), as well as any off-label uses of a drug. This policy is intended to make you aware of faculty disclosures so you may form your own judgments about material discussed during the 7th ASM Biodefense and Emerging Diseases Research Meeting.

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The following are faculty members of the ASM Biodefense and Emerging Diseases Research Meeting who have declared that they have no financial relationships relevant to the content of their presentation to disclose:

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<th>Session #</th>
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<td>Baric, Ralph</td>
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<td>Barnewall, Roy</td>
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<td>Bloom, Marshall E.</td>
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<td>Buchmeier, Michael J.</td>
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<td>Gibson, Daniel</td>
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<td>Golding, Hana</td>
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<td>Goldman, William</td>
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<td>Grinshpun, Sergey A.</td>
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<td>Haas, Charles N.</td>
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<td>Hensley, Lisa</td>
<td>01, 03, 07, 11</td>
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<td>Hepburn, Matthew J.</td>
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<td>Hotchkiss, Richard S.</td>
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<td>Jahrling, Peter B.</td>
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<td>Janda, Kim D.</td>
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<td>Johnson, Eric A.</td>
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<td>Koehler, Theresa</td>
<td>05, 06</td>
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2. Disclosure Statement

As an organization accredited by the Accreditation Council for Continuing Medical Education (ACCM), the ASM has specific policies regarding the disclosure and resolution of a speaker’s conflict(s) of interest for all educational activities. All speakers must disclose financial relationships with commercial interests that are relevant to their presentation and these disclosures are provided to the activity participants. Should a speaker disclose a relevant financial relationship, ASM takes steps to resolve that conflict through a variety of resolution mechanisms and ensure that all presentations are evidence based and free of commercial bias.

The following speakers disclosed financial relationships:

These speakers have disclosed that they or their spouse have a relevant financial relationship that is relevant to their presentations. These disclosures have been reviewed by the ASM Biodefense and Emerging Diseases Research Meeting Program Committee and ASM staff. All speakers have signed Speaker Attestation Forms indicating that they will provide balanced, evidence-based presentations and that they will not slant their presentations commercially. If you have a concern regarding the content of a speaker’s presentation or if you feel that a speaker has not provided an evidence-based presentation, please visit the ASM Biodefense and Emerging Diseases Research Meeting Headquarters Office and ask to speak to an ASM staff person.

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*Single presentation (not on Speaker’s bureau)
3. The following speakers disclosed off-label use(s):

**Speaker .......................................................... Session # .... Disclosure**

Nuzum, E .......................................................... 2 .... Presentation may include discussion of Pharmathene Recombinant Protective Antigen (rPA)vaccine. This is an IND vaccine intended for prevention and post exposure prophylaxis of anthrax.

Schweitzer, H .................................................... 21 .... I will discuss efficacy of a monobactam, BAL30072, that is still investigational.

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### CME Relationship Code Table

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Sunday, February 22, 2009

Focus Session

01. Threat Reduction/Counter-Proliferation: Part I - Policy

Location: Harborview B
Session Time: 12:00 noon - 2:30 PM

In recent years, the number of biocontainment laboratories in the United States and around the world has increased to support the research needs of investigators working with emerging infectious diseases and agents of biothreat. Development and implementation of policy regulation regarding biosafety, as well as biosurety and biosecurity of domestic and international programs will be discussed.

Upon completion of this Focus Session, participants should be able to:
- Identify key components of effective biosecurity and biodefense policy and regulation;
- Explain the role of cooperative threat reduction programs in the present political and social environment.

Moderators:
L. HENSLEY, USAMRIID, Ft. Detrick, MD.
S. M. JONES, Special Pathogens Program, Natl. Microbiology Lab., Winnipeg, MB, CANADA.

Presentations
12:00 Noon 001. International Collaborations on Emerging and Dangerous Pathogens
B. GOBLE, Chief Executive Officer, TDV Global, Inc., Ottawa, ON, CANADA

12:30PM 002. U.S. Department of State Efforts to Enhance Biological Security Globally
J. RAO, U.S. Dept. of State, Washington, DC.

1:00PM 003. Science-Based Support to Attribution and Awareness in Biodefense
J. P. FITCH, Natl. Biodefense Analysis and Countermeasures Ctr., Fort Detrick, MD.

1:30PM 004. The Biological Threat Reduction Program of the U.S. Department of Defense
A. WEBER, Office of the Secretary of Defense, Department of Defense, Washington, DC.

02. Correlates of Protection

Location: Harborview C
Session Time: 12:00 Noon - 2:30 PM

A major goal in vaccine research is to find immunological response parameters that predict vaccine efficacy. Such correlates of protection facilitate licensure of vaccines and can help guide immunization practices. Correlates of protection may also expedite licensure of vaccines for which human efficacy trials are not feasible or not ethical, as is the case for a number of vaccines against bioterror/biodefense agents. In those cases, correlates of protection could strengthen the extrapolation of animal protection data to humans. In this session, established correlates of protection and the processes by which they were determined will be reviewed. In addition, mechanisms for derivation of new correlates of protection will be discussed.

Upon completion of this Focus Session, participants should be able to:
- Explain the public health importance of correlates of protection;
- Identify vaccines for which correlates of protection exist; and
- Describe how correlates of protection are derived.

Moderators:
D. L. BURNS, FDA/CBER, Bethesda, MD.
S. A. PLOTKIN, Sanofi Pasteur, Doylestown, PA.

Presentations
12:00 Noon 005. An Overview of Correlates of Protection
S. A. PLOTKIN, Sanofi Pasteur, Doylestown, PA.

12:30PM 006. Correlates of Protection against Seasonal and Pandemic Influenza
H. GOLDING, Laboratory of Retroviruses, FDA/CBER, Bethesda, MD.

1:00PM 007. Correlates of Protection against Anthrax and Extrapolation to Humans
E. NUZUM, Office of Biodefense Research Affairs, NIH/NIAID/DMID, Bethesda, MD.

1:30PM 008. Mechanisms of Protection against Botulinum Toxin
J. D. MARKS, Anesthesia and Perioperative Care, Univ. of California, San Francisco, CA.

2:00PM 009. Mechanisms of Immunity against Tuberculosis
D. F. HOFT, Internal Medicine & Molecular Microbiology, St. Louis Univ. Hlth. Sci. Ctr., St. Louis, MO.
03. Threat Reduction/Counter Proliferation: Part II - Science

Location: Harborview B
Session Time: 3:00 PM - 5:30 PM

In recent years, the number of biocontainment laboratories in the United States and in many areas of the world has increased to support the research needs of investigators working with emerging infectious diseases and agents of biothreat. However, due to political and economic changes, there has not been a global surge in supporting biodefense related work. In some parts of the world, financial support for work with select agents or biothreat agents has been lost. This is of particular concern from biodefense and public health aspects. Specifically, the state and condition of former laboratories and stocks of materials is questionable or unknown. The potential is now present for gaps in surveillance due to a lack of infrastructure and financial support. This session will highlight some of the current “on the ground efforts.”

Upon completion of this Focus Session, participants should be able to:

• Describe an existing program or effort. Explain the potential barriers to success; and
• Highlight one example of success. In broad terms describe the overall benefits of these efforts.

Moderators:
L. HENSLEY, USAMRIID, Ft. Detrick, MD.
J. L. PATTERSON, Virology and Immunology, Southwest Fdn. for Biomedical Res., San Antonio, TX.

Presentations:
3:00PM 010. Making the World Safer: Implementing a Biological Threat Reduction Program

3:30PM 011. Establishment and Operational Capacity of the Zonal Diagnostics Laboratory at Kutaisi, Republic of Georgia for the U.S. Department of Defense/Defense Threat Reduction Agency (DTRA) / Threat Assessment Detection Response (TADR) Program
B. K. PURCELL, Bacteriology Division, U.S. Army Medical Research Institute of Infectious Disease, Fort Detrick, MD.

4:00PM 012. Clinical Research with the Cooperative Threat Reduction Program: Opportunities and Challenges
M. J. HEPBURN, Biomedical Sciences, Defence Science and Tech. Lab. (DSTL), Salisbury, UNITED KINGDOM.

4:30PM 013. Public Health Science Support to Counter Proliferation and Counter-Terrorism Activities
S. M. JONES, Special Pathogens Program, National Microbiology Laboratory, Winnipeg, MB, CANADA.

5:00PM 014. Post Exposure Management of VHF Infection
M. CALLAHAN, DARPA/DSO, Arlington, VA.

04. Microbial Risk Assessment

Location: Harborview C
Session Time: 3:00 PM - 5:30 PM

Although much progress has been made from increased research activity in the biothreat arena in recent years, there are still significant uncertainties regarding our understanding of the risks from biological threat agents. Microbial risk assessment (MRA) of biological threat agents is an emerging field of applied microbiology spanning many diverse disciplines including, but not limited to, Mathematics, Public Health, Engineering, and Toxicology. The large and broad focus of this developing field has caused many of the traditional research Microbiologists to remain uninformed about MRA. However, several Federal Agencies, including the US Environmental Protection Agency and the Centers for Disease Control and Prevention, are mandated to conduct MRA research of biological threat agents and emerging infectious diseases. This critical MRA effort for these Agencies has been complicated by numerous, significant data gaps and continually requires the extensive expertise of the Microbiology research community.

Upon completion of this Focus Session, participants should be able to:

• Describe the current state-of-the-science and new avenues to address the priority data gaps in MRA;
• Identify the existing risk assessment paradigm steps and current techniques to characterize biological threat agent risk;
• Explain some of the challenges associated with hazard identification of biological threat agents with regards to sample to sample analysis and validation of laboratory methods;
• Discuss the use of animal models to determine doses in humans; and
• State the potential issues regarding the utility of biological surrogates to study exposures.

Moderators:
S. A. MORSE, Bioterrorism Preparedness and Response Program, CDC, Atlanta, GA.

Presentations:
3:00PM 016. Hazard Identification: From Environmental Sampling to the Laboratory
E. VAN GIESON, Special Applications Branch, Johns Hopkins Univ. Applied Physics Lab., Laurel, MD.

3:30PM 017. Hazard Identification: Validation of Laboratory Methods
S. A. MORSE, Bioterrorism Preparedness and Response Program, CDC, Atlanta, GA.

4:00PM 018. Dose-Response: Use of Animal Models to Determine Doses in Humans

4:30PM 019. Exposure Assessment: Comparing the Physical Properties of Anthrax Spore Surrogates
K. T. MADHUSUDHAN, Clean Earth Technologies, LLC, Winston-Salem, NC.

5:00PM 020. Monte Carlo Analysis Work on Microbial Risk Assessment
05. Keynote/Opening Session

Location: Harborview
Session Time: 6:30 PM - 8:00 PM

Stuart B. Levy, M.D., Professor of Molecular Biology & Microbiology and of Medicine is the Director of the Center for Adaptation Genetics and Drug Resistance at Tufts University School of Medicine and Staff Physician at the New England Medical Center.

Dr. Levy is world renowned for his work on the tetracycline class of antibiotics. His pioneering research in this area led to the first description of genetic heterogeneity among drug resistance determinants and he was among the first to recognize the dangers associated with indiscriminate use of antibiotics. Today Dr. Levy serves as President of the Alliance for the Prudent Use of Antibiotics, which was founded as a non-profit global organization in 1981 to contain antibiotic resistance and to improve antibiotic effectiveness. He is also a former president of ASM, a member of the American Academy of Microbiology, and was awarded the Hoechst Hoechst-Roussel Award for esteemed research in antimicrobial chemotherapy by ASM.

Dr. Levy's recent work has centered on the phenomenon of multidrug resistance in bacterial and mammalian cells. His group made the breakthrough discovery of a regulatory locus mar for intrinsic multiple antibiotic resistance/susceptibility among the Enterobacteriaceae.

In 2004 Dr. Levy was appointed to the National Science Advisory Board for Biosecurity. This board was established by HHS to provide advice and recommend specific strategies for efficient and effective oversight of federally conducted or supported dual-use biological research taking into consideration both national security concerns and the needs of the research community. In 2006, Dr. Levy published a paper describing a gene in Yersinia pestis, the causative agent of plague, which was similar to the Escherichia coli gene known to cause multiple antibiotic resistance and contribute to virulence. He further demonstrated that the Y. pestis gene functions in the same manner and suggested that this type of gene is likely to be an important and common mechanism by which bacteria acquire resistance and virulence. These findings led Dr. Levy to question the implications for dual use of antibiotic resistance genes in relation to for dual use research of concern.

Upon completion of this Plenary Session, participants should be able to:

- Describe what is meant by “dual use” research;
- Describe how multidrug resistant bacteria emerge; and
- Explain the use of small molecule inhibitors of proteins controlling virulence and antibiotic resistance as a strategy for combating infectious disease.

Moderators:

T. M. KOEHLER, Microbiology and Molecular Genetics, Univ. of Texas Hlth. Sci. Ctr., Houston, TX
C. S. SCHMALJOHN, Molecular Virology, USAMRIID, Frederick, MD.

Presentation:

6:45PM 021. Keynote Address: Studying Bacterial Transcription Factors Involved in Virulence and Antibiotic Resistance: Dual Use Research of Concern?
S. B. LEVY, Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA

Monday, February 23, 2009

Plenary Session

06. Antimicrobial Resistance

Location: Harborview
Session Time: 8:30 AM - 12:00 Noon

Antimicrobial resistance, whether naturally acquired or intentionally created, jeopardizes preparedness and defense against agents of bioterrorism and emerging diseases. This session will address the challenges and potential solutions to this emerging public health threat. Topics to be discussed will include (1) molecular methods for detection of antimicrobial resistance, (2) the benefits and risks associated with widespread use of antimicrobials during a pandemic threat, (3) predictive models for emergence of resistance, and (4) the significance of the environmental reservoir of antibiotic resistance determinants in development of multiple antibiotic resistance in pathogens.

Upon completion of this Plenary Session, participants should be able to:

- State the public health significance of the emergence of antimicrobial resistance in bacteria and viruses;
- Distinguish the potential benefits and risks of extensive use of antimicrobials during a large outbreak;
- Recognize methods for detection of molecular signatures associated with antimicrobial resistance; and
- Explain the relationship between environmental exposure to naturally-produced antimicrobials and the development of resistance.

Moderators:

T. M. KOEHLER, Microbiology and Molecular Genetics, Univ. of Texas Hlth. Sci. Ctr., Houston, TX
C. S. SCHMALJOHN, Molecular Virology, USAMRIID, Frederick, MD.

Presentations:

8:30AM 022. Featured Speaker: Waiting for the Next Flu to Drop
B. R. LEVY, Dept. of Biology, Emory Univ., Atlanta, GA

9:30AM Coffee Break

10:00AM 024. Bacterial Utilization of Antibiotics
G. DANTAS, Genetics, Harvard Med. Sch., Boston, MA

10:30AM 025. MRSA
F. C. TENOVER, Cepheid, Sunnyvale, CA

11:00AM 026. Signatures for Genetically Engineered Organisms
T. R. SLEZAK, Global Security Program, Lawrence Livermore Natl. Lab., Livermore, CA

11:30AM 027. The Increasing Availability of Antimicrobial Resistance Determinants: Implications for Pathogen Evolution and Biopreparedness
W. F. FRICKE, Institute for Genome Sciences, Univ. of Maryland Sch. of Med., Baltimore, MD.
Poster Session

07. Monday Poster Session
Location: Grand Ballroom
Session Time: 1:00 PM - 3:00 PM

028 (A) Posterboard 1A
Comparison of Dengue Virus Type 1 Growth Characteristics in Vero and C6/36 Cell Lines
A. OWENS, J. Shufflet, S. Radhakrishnan, K. Langenbach, R. O. Baker; BEI Resources/ATCC, Manassas, VA.

029 (A) Posterboard 2A
Development of Rabbitpox Model for Smallpox
T. BABAS, E. Peters, K. Gong, L. V. Nieves-Duran, S. Lin, D. Hebblewaite, R. Wright, A. Rippeon, D. Golightly, G. Donnelly, L. Rhodes, P. M. Silvera, Southern Research Institute, Birmingham, AL.

030 (B) Posterboard 3A
Characterisation of a Marmoset Model of Inhalational Tularemia
M. NELSON, M. S. Leve, R. E. Dean, V. L. Savage, F. J. Salguero, P. C. Pearce, D. J. Stevens, A. J. Simpson; Dart, Salisbury, UNITED KINGDOM, £VA, Weybridge, UNITED KINGDOM.

031 (B) Posterboard 4A
A Comparative Study of the Cytotoxic Effects of Epsilon Toxin, Epsilon Toxin, and a Recombinant Epsilon Toxin Fragment on Sensitive Cell Lines
M. BOLEY, M. McKee, M. Willis; American Type Culture Collection, Manassas, VA.

032 (B) Posterboard 6A
Dominant-Negative Inhibitors of the Clostridium perfringens E-Toxin
M. MCCLAIN; Vanderbilt Univ., Nashville, TN.

033 (B) Posterboard 7A
In vitro Growth Characterisation Studies of Burkholderia pseudomallei
A. M. BOWN, S. Thomas, A. Atkinson, L. Foster, J. Vipond, S. Funnell; Health Protection Agency, Salisbury, UNITED KINGDOM.

034 (B) Posterboard 8A
Onset of Bacteremia in Mice Following Nose-Only Inhalation of Bacillus anthracis Ames Strains
J. A. BOYDSTON, J. E. Trombley, Z. N. Llewellyn, L. E. Bowen; Southern Research, Birmingham, AL.

035 (B) Posterboard 9A
Determination of the 50% Lethal Dose (LD50) of Bacillus anthracis Ames in Mice Following Nose-Only Inhalation Exposure
L. E. Bowen, Z. N. Llewellyn, J. E. TROMBLEY, J. A. Boydsto; Southern Research Institute, Birmingham, AL.

036 (B) Posterboard 11A
Determination of the 50% Lethal Dose (LD50) of Yersinia pestis in Mice Following Nose-Only Inhalation Exposure
L. E. BOWEN, Z. N. LLEWELLYN, J. A. BOYDSTON, M. B. MINYARD, M. G. BRIDGERS, J. E. TROMBLEY, P. M. SILVERA; Southern Research Institute, Birmingham, AL.

037 (B) Posterboard 12A
Impact of Environmental Factors on Virulence of Aerosolized Francisella tularensis
S. FAITH, D. S. Reed; University of Pittsburgh, Pittsburgh, PA.

038 (B) Posterboard 13A
Effect of N-acetylcysteine or Stimal® on Bacillus anthracis Spore and Spore-Phagocyte Interactions
M. E. BERNU, M. Smith, W. Stone, R. F. Rest; 1Drexel University College of Medicine, Philadelphia, PA, 2Amana Ltd., Melbourne, FL, 3East Tennessee State University, Johnson City, TN.

039 (B) Posterboard 14A
Validated Bacillus Strains as Model for Bacillus anthracis
B. SEGEMANN, M. Fricker, J. A. Agren, M. Ehling-Schulze, R. Knutsson; 1National Veterinary Institute, Sweden, Uppsala, SWEDEN, 2Technical University of Munich, Freising, GERMANY, 3University of Veterinary Medicine Vienna, Vienna, AUSTRIA.

040 (B) Posterboard 15A
Comparison of Francisella tularensis IVS and SCHU S4 Bioaerosols
T. BRASIL, K. Agans, E. Barr, V. Gonzalez, F. Romero, S. Storch, A. Weidier, R. Sherwood; Lovelace Respiratory Research Institute, Albuquerque, NM.

041 (B) Posterboard 16A
Comparative Analysis of the in vitro Stability and Viability of a Panel of Yersinia pestis Strains
M. H. BENDER, J. A. Crawford; 1NIH/NIHBI, Frederick, MD, 2Battelle Memorial Institute, Columbus, OH.

042 (B) Posterboard 17A
Rapid Identification and High Resolution Strain Typing of Francisella tularensis

043 (D) Posterboard 18A
Molecular Identification of Yersinia Pestis as the Cause of an Outbreak of Severe Disease in Afghanistan Following the Consumption of Camel Meat

044 (D) Posterboard 20A
Nanosensors for Bacterial Pathogenesis Identification
C. KAITTANIS, S. Nath, J. Perez; Univ. of Central Florida, Orlando, FL.

045 (D) Posterboard 21A
Development of Strain-Specific Real-Time PCR Assays to Detect Arenaviruses
A. TROMBLEY, J. Jarling, J. Garrison, D. Norwood, L. Hensley, D. Kulesh, USAMRIID, Frederick, MD.

046 (D) Posterboard 21A
Development of Sensitive Rapid Tests Allowing Biological Warfare Agents Detection
H. VOLLAND, N. Khreich, P. Lamroueet, J. Dano, M. Newer, C. Crémion; CEA, IRiTeS, SPI, Gif sur Yvette, FRANCE.

047 (D) Posterboard 23A
Comparison and Development of Magnetic Bead-Based Nucleic Acid Extraction Methods for Biothreat Agents in Clinical Matrices
P. D. CRaw, D. A. Norwood, T. D. MINOGUE, USAMRIID, Frederick, MD.

048 (D) Posterboard 25A
Rapid Antimicrobial Susceptibility Testing of Bacillus anthracis
L. M. WEIGEL, D. Sue, P. A. Michel, B. Kitchel, S. Pillal, CDC, Atlanta, GA, 2Dept. of Homeland Security, Washington, DC.
049 (D) Posterboard 26A
Polyphasic Characterization of Five Different Serotypes of *Salmonella enterica* from a Food-Borne Outbreak

**M. MIRANDA-CLELAND**, S. W. Jones, M. L. McKeen; ATCC/BEI Resources, Manassas, VA.

050 (D) Posterboard 27A
Crimean-Congo Haemorrhagic Fever ELISA Development and Comparison of Results Between Laboratories

**S. D. DOWALL**, K. S. Richards¹, A. Carhan², Y. Uyar¹, J. Chamberlain¹, R. Hewson¹; ¹Centre for Emergency Preparedness and Response, Health Protection Agency, Porton Down, Salisbury, UNITED KINGDOM, ²Refik Saydam Hygiene Centre, Ankara, TURKEY.

051 (D) Posterboard 28A
Pathogen Detection Using a Novel Nanofluidic System


052 (D) Posterboard 29A
Large-scale Automated Detection of Bacterial Pathogens in the Food Chain

S. Ehren², L. A. LUNDIN², P. Ågren¹, S. Garborn², R. Knutsön², C. Dahlberg²; ¹Swedish Veterinary Institute, Uppsala, SWEDEN, ²Swedish Laboratory for Food Safety and Biopreparedness, Uppsala, SWEDEN, ³National Food Administration, Uppsala, SWEDEN.

053 (D) Posterboard 30A
Evaluation and Comparison of the Sherlock® Instant FAME™ Sample Preparation Method and the Standard Preparation Method for Biothreat Agents

**J. CARPENTER**, M. Wolcott; USAMRID, Ft. Detrick, MD.

054 (D) Posterboard 31A
Rapidly Formulated Antigen Capture Assays for Ebola Viruses Based on *In Vitro* Antibody Selections of Semi-Synthetic Llama Antibodies at BSL-4

**L. SHERWOOD**, A. Hayhurst; Southwest Foundation for Biomedical Research, San Antonio, TX.

055 (D) Posterboard 32A
Evaluation of Electrochemiluminescence Detection Capabilities and Toxin Distribution in African Green Monkeys Exposed to Aerosolized Staphylococcal Enterotoxin B


056 (K) Posterboard 34A
ERIC, a Bioinformatics Resource for Entericopathogens

**J. M. GREENE**, D. A. Pot¹, S. Zaremba¹, J. Whitmore¹, P. Shetty¹, J. Fedorko¹, J. Thangiah¹, K. Joshi¹, M. Shaker¹, G. Plunkett¹, III¹, J. Glaser¹, B. Anderson², D. Baumler¹, B. Bielf¹, V. Burland¹, E. Cabot², E. Neemo-Eckwall², B. Mar¹, P. Liss², R. R. Blattner², N. T. Perna²; ¹SRA International, Inc., Rockville, MD, ²Univ. of Wisconsin, Madison, WI.

057 (D) Posterboard 35A
Rapid Sanger Sequencing of Cloned Random RT-PCR Products for Viral RNA Sample Screening

**E. CASTRO**, H. Nordström¹, Z. Hu¹, S. Vene¹, Å. Lundkvist¹, G. Sandström¹; ¹Swedish Institute for Infectious Disease Control, Solna, SWEDEN, ²Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, SWEDEN.

058 (D) Posterboard 36A
Developmental Testing of a Biological Hydrosol Concentrator

**D. S. ALBURY**, A. E. Page¹, P. S. Murochick², Z. A. Packerhing¹; ¹Innovaprep, Drexel, MO, ²AlburtyLab, Inc., Drexel, MO.

059 (E) Posterboard 37A
Studies on Surface Sampling of Anthrax Spore Surrogates from Environmental Surfaces: Effect of Swab Material and Interferants on Spore Recovery


060 (E) Posterboard 39A
Magnetic Bead Based Clean-Up Process for ECL Assays

**A. BAILEY**, Edgewood Chemical Biological Center, Aberdeen Proving Grounds, MD.

061 (E) Posterboard 40A
Shipping Integrity of *Bacillus anthracis* Swab Samples

**H. A. O’CONNELL**, L. Rose, J. Noble-Wang, M. Arduino; Centers for Disease Control and Prevention, Atlanta, GA.

062 (E) Posterboard 41A
Specific Detection of *Bacillus anthracis* Spores by ELISA

**M. PUROHIT**, J. McGovern, W. Y. Shih, W. Shih, R. F. Rest; Drexel University, Philadelphia, PA.

063 (E) Posterboard 42A
Detection and Occurrence of *Enterococcus* in Dairy Ground Waters

**X. LI**, N. Watanabe¹, R. Atwill¹, T. Harker¹; ¹Western Institute for Food Safety and Security, University of California, Davis, Davis, CA, ²Department of Land, Air and Water Resources, University of California, Davis, Davis, CA.

064 (E) Posterboard 43A
Sample Preparation of Biothreat Agents in Complex Matrices Utilizing Two Automated Devices

**B. PASZKIEWICZ**, B. Henry¹, S. Beatty¹, H. He², B. Strobecker¹, E. Ubil¹, J. Rossio¹, J. Meegan¹; ¹In vitrogen Federal Systems, Frederick, MD, ²Adecco, Frederick, MD.

065 (E) Posterboard 44A
Assessment and Suggested Refinement of Sample Analysis Protocols to Support Ongoing Large and Small Scale Sampling Evaluations Related to Building Restoration


066 (E) Posterboard 45A
Method Validation of DNA Extraction and Real-Time PCR for Qualitative Analysis of Environmental Spike Samples for Biological Warfare Agents

**I. FRUCHEY**, J. Bagley¹, A. Dubbs¹, A. Bailey¹, A. Gordon¹, S. Norman¹; ¹Edgewood Chemical Biological Center, Gaithersburg, MD, ²EGG&G Technical Services, Inc., Gaithersburg, MD.

067 (E) Posterboard 46A
Occurrence of Toxicigenic *Vibrio cholerae* and Other Pathogenic Vibrios in the Aquatic Environments of Georgia

**E. J-laiani¹, C. A. Whitehouse¹, N. Janelidze¹, T. Kokashvili¹, M. TEDIASHVILI¹, A. Hugi¹, J. G. Eliava Inst. of Bacteriophage, Microbiol. and Virol., Tbilisi, GEORGIA, ²USAMRID, Frederick, MD, ³Univ. of Maryland, College Park, MD.

068 (E) Posterboard 48A
Brucellosis Exposure Risk Factors in Regions of Uzbekistan

M. D. Akhmedov¹, U. I. NOMALIEV¹, O. S. Kasimov¹, Z. H. Madrimov¹, T. A. Niyazova¹, S. R. Atimov¹, A. V. Khodiev¹, M. K. Butaev¹, S. I. Mavlanov¹, R. G. Yaraev¹, R. A. Ismatova¹, A. R. Gabbasova¹, M. P. Nikich³, D. Clark³, P. Elzer³; ¹Res. Inst. of Epidem., Microbiol. and Infect. Dis. (RIEMID), Tashkent, UZbekistan, ²Uzbekistan Vet. Res. Inst. (UZVRI), Samarqand, UZBEKISTAN, ³Walter Reed Army Inst. of Res. (WRAIR), Silver Spring, MD, ⁴Louisiana StateUniv. AgriculturalCtr., Baton Rouge, LA.
Directed Against 
Corp., Ames, IA.

and Treatment of Smallpox Infection

The Joint Genome Institute and Los Alamos National Laboratory, Los Alamos, NM.

Potential Ebola Therapies from Phenotypic Screening

Centers for Disease Control and Prevention, Atlanta, GA.

The Joint Genome Institute and Los Alamos National Laboratory, Los Alamos, NM.

Development of Hexadecyloxypropyl Cidofovir (CMX001) for the Prophylaxis of Ebola Therapies from Phenotypic Screening

Centers for Disease Control, Fort Detrick, MD.

Stability of Dried Adenovirus Preparations at Elevated Temperature over Time


Comparison of the Complete Genome Sequences of Six More Isolates of 

Burkholderia pseudomallei and Burkholderia pseudomallei with Potential for Broad Spectrum Performance.

K. Matsuyama

Compounds Exerting In Vitro and Cell Based Activity against 
Burkholderia mallei and Burkholderia pseudomallei with Potential for Broad Spectrum Performance.


Construction and Characterization of a Chimeric Neutralizing Antibody Directed Against 
Botulinum Neurotoxin A

S. Simon

Inhibition of Poxvirus and Filovirus Systems by Nanostructured Agents

J. C. Trefry

Sudan Ebolavirus Specific Murine Monoclonal Antibodies

A. Kuehne

Boosting Bioterror Agents Vaccine Efficacies

R. Flick

Comparative Evaluation of Three Types of Anthrax Toxin Neutralization Assays

M. M. Ngundi
088 (I) Posterboard 69A

Cellular and Humoral Immune Response of Cynomolgus Macaques to Francisella tularensis LVS and SChU S4 Antigens

J. A. WILDER, 1 A. Monier, 1 R. L. Sherwood, 1 T. Wu, 1 G. Statum, 2 C. R. Lyons, 2 Lovelace Respiratory Research Institute, Albuquerque, NM, 2 University of New Mexico, Albuquerque, NM.

089 (J) Posterboard 70A

Clorhan Dioxide Disinfection of Biothreat Agents in Drinking Water


090 (J) Posterboard 71A

Evaluation of Inactivation Methods for Orthopoxviruses


091 (J) Posterboard 72A

Inhibitory Effects of Fatty Acids on Bacillus sps.

K. LeForte, 1 A. Raulerson, 1 D. Bowman, M. A. Butkus, 1 M. P. LABARE; 1 United States Military Academy, West Point, NY, 2 Cornell University, Ithaca, NY.

092 (J) Posterboard 73A

Development and Validation of Vaporized Hydrogen Peroxide Decontamination of a Class III Biosafety Cabinet


093 (J) Posterboard 74A

Effectiveness of Commerically Available Antimicrobial Peptides Against Bacillus anthracis Sterne


094 (K) Posterboard 75A

Closing the Gap: Training Biodefense Professionals for the 21st Century

P. J. Cummings, 1, C. M. OBOM; 1 Johns Hopkins Univ., Baltimore, MD, 2 Johns Hopkins Univ., Rockville, MD.

095 (K) Posterboard 76A

Characterization of an Efficient Nebulizer for Generating Bioaerosol Exposures

W. C. LIN, S. Hu, S. Hartsell, A. Wegner, M. Juergensmeyer, B. Gingras, L. Holland; IIT Research Institute, Chicago, IL.

096 (K) Posterboard 77A

Criteria for Addition or Removal of a Biological Agent or Toxin from the Select Agent List

J. F. ALDRICH, J. M. Canaves, B. Sherwin, V. Sutton; Texas Tech University School of Law, Lubbock, TX.

097 (K) Posterboard 78A

The Biosafety & Biosecurity Law Hotline

B. D. SHERWIN, V. Sutton; Texas Tech University School of Law, Lubbock, TX.

098 (K) Posterboard 79A

Development of a Facility-Specific BSL-3 Training Program for the University of Pittsburgh Regional Biocontainment Laboratory


099 (K) Posterboard 80A

The Seattle Structural Genomics Center for Infectious Disease

R. STACY, 1 L. Stewart, 1 W. Van Voorhis, 1 G. Varani, 1 G. Buchlov, 1 P. J. Myler, 1 Seattle Biomedical Research Institute, Seattle, WA; 2 CODE biostructures, Bainbridge Island, WA, 3 University of Washington, Seattle, WA; 4 Pacific Northwest National Laboratories, Richland, WA.

Highlighted Oral Abstract Presentations

08. Proteomic and Genomic Applications

Location: Harborview B
Session Time: 3:00 PM - 4:00 PM

Presentations:

3:00PM 100. High Resolution Francisella tularensis Typing Assay Based on Multi-strain Global SNAP Markers

G. A. PANDYA, 1 M. H. Holmes, 2 J. Petersen, 1 C. Molins-Schneektloth, 2 S. Pradhan, 1 M. Jones, 1 S. Karamycheva, 1 Wolcott, 1 R. D. Fleischmann, 1 S. N. Peterson, 1 JCVI, Rockville, MD, 2 CDC, Fort Collins, CO, 3 USAMRIID, Fort Detrick, MD.

3:12PM 101. Integration of Proteomics and Transcriptomics Results through Metabolic Modeling of Salmonella Pathogenesis: Identification of Knowledge Gaps and Possible Functional States


3:24PM 102. Detection and Identification of RNA Viruses Using Microarrays

M. ATICHOU, S. Strand, A. Raquel, J. Hardick, S. Ibrahim; USAMRIID, Virology, Fort Detrick, MD.

3:36PM 103. Global Analysis of Intact Proteins from Orthopoxviruses

J. N. ADKINS, 1 R. D. Estep, 1 E. Robinson, 1 S. Wu, 1 H. M. Motta, 1, N. Tolic, 1 L. Pasa-Tolic, 1 S. Wong, 2 R. D. Smith, 2 Pacific NW National Lab, Richland, WA, 3 Oregon Health & Science University, Portland, OR.

3:48PM 104. Protein Biomarker for the B. cereus Group in Indoor Air

S. JONES, K. Fox, A. Fox; University of South Carolina, Columbia, SC.

Highlighted Oral Abstract Presentations

09. Therapeutics

Location: Harborview C
Session Time: 3:00 PM - 4:00 PM

Presentations:

3:00PM 106. Non-peptidic Mimics of Host Defense Proteins: Activity against Category A Biopathogens


107. Defining the Therapeutic Window of Opportunity for an Anthrax Therapeutic in New Zealand White Rabbits

J. E. COMER, 1 J. M. Schmidt, E. E. Johnson, A. M. Wasko, J. L. Mango, P. J. Herr-Calomeni, 1 Wickham, F. D. Rabon, G. T. Meister, Battelle Memorial Institute, Columbus, OH.

108. Intravenous Levofloxacin Rescues African Green Monkeys from Lethal Pneumonic Plague

R. C. LAYTON, W. Mega, E. B. Barr, T. L. Brasel, J. D. McDonald, F. T. Koster; Lovelace Respiratory Research Institute, Albuquerque, NM.

109. Susceptibility of Versinia pestis to Broad-Spectrum Nanostructured Therapeutics

R. C. MCRAE, J. C. Trefy, D. P. Wooley; Wright State University, Dayton, OH.
Highlighted Oral Abstract Presentations

10. Animal Models
Location: Harborview D
Session Time: 3:00 PM - 4:00 PM

Presentations:

3:00PM 110. Susceptibility and Suitability of Cynomolgus Macaques of Mauritian Origin in an Aerosol Model of Monkeypox Infection

3:12PM 111. Pharmacokinetics of MDX-1303, an Anthrax Anti-toxin Monoclonal Antibody, in Two Different Non-human Primate Models: Implications for the Animal Rule
M. Meldorf1, D. Blanset2, V. Riddle1; 1PharmAthene, Inc., Annapolis, MD, 2Medarex, Inc., Bloomsbury, NJ.

3:24PM 112. A Novel Small Nonhuman Primate Model for Filovirus Induced Hemorrhagic Fever
R. Carrion, Jr.1, Y. Ko1, A. E. Tieser1, J. A. Montalbo2, K. Mansfield3, K. Brasky3, J. L. Patterson1; 1Southwest Foundation for Biomedical Research, San Antonio, TX, 2Harvard Medical School, Southborough, MA.

3:36PM 113. Aerosol Mouse Model of Inhalational Plague in BALB/c Mice and Disease Progression
K. A. Overheim, T. Brasil, E. Barr, A. P. Gigliotti, J. A. Wilder, R. L. Sherwood; Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM.

3:48PM 114. Imaging of Non-Human Primate Models of Infectious Disease Using PET/CT

Symposium

11. Reversing Pathogenesis
Location: Harborview B
Session Time: 4:15 PM - 6:15 PM

Many of the high-priority pathogens of bioterrorism concern such as Anthrax, tularemia, plague and the hemorrhagic fever viruses have similar pathogenic effects (e.g. disseminated intravascular coagulopathy, immune dysregulation, septic shock). Understanding these general mechanisms of pathogenesis provides host pathways to target for development of therapeutics. This session will discuss virulence mechanisms, pathogenesis and the advancement of broad-spectrum countermeasures in research and clinical care.

Upon completion of this Symposium, participants should be able to:
- Identify major convergent virulence pathways to clinical intervention;
- Describe the advantages of therapeutics that target pathogenic effects in situations of outbreak of emerging disease or potential bioterror; and
- Explain the role of common pathways of disease and the targeting of these pathways as opposed to direct antivirals as therapeutic interventions.

Moderators:
L. Hensley, USAMRIID, Ft. Detrick, MD.
P. B. Jahnling, Integrated Research Facility, NIH/NIAID, Bethesda, MD.

Presentations:

4:15PM 115. Pathway Analysis Prediction Programs
R. Felciano; Research, Ingenium Systems, Inc., Redwood City, CA.

4:45PM 116. Anti-Apoptotic Based Theory of Select Agent Infections
R. S. Hotchkiss; Anesthesiology, Washington Univ., St. Louis, MO.

5:15PM 117. Host Protease-S1PR receptor Signaling Crosstalks as Interventional Target in Sepsis
W. Ruf; Immunology and Microbial Science, The Scripps Res. Inst., La Jolla, CA.

5:45PM 118. Pathogenesis of Severe Dengue Illness and Potential Targets for Intervention
T. P. Endy; Medicine, State Univ. of New York Upstate Med. Univ., Syracuse, NY.

12. Aerosol Biology
Location: Harborview B
Session Time: 4:15 PM - 6:15 PM

This session will focus on the science of bioaerosol generation and characterization. Experts in the field will present talks on the theory and principles of aerosol biology with a focus on practical applications relevant to infectious disease research. The purpose of the session is to provide infectious disease researchers in the field of biodefense an understanding of the practical issues pertaining to the development of aerosol models of infectious disease including the selection of appropriate aerosol generation methods and delivery systems and methods of characterizing aerosols to confirm or troubleshoot performance of aerosol systems.

Upon completion of this symposium, participants should be able to:
- Identify the theoretical and practical issues related to the generation and characterization of biological aerosols;
- Select appropriate systems for the aerosol exposure of animals; and
- Evaluate results generated using bioaerosol systems.

Moderators:
T. J. Merkel; FDA/CBER, Bethesda, MD.
J. M. Hartings; President, Biaera Technologies, LLC, Frederick, MD.

Presentations:

4:15PM 119. Concepts of Aerosol Biology and Methods of Generating Bioaerosols
S. A. Grinshpun; Environmental Health, Univ of Cincinnati, Cincinnati, OH.

4:45PM 120. Methods for Sampling and Characterizing Bioaerosols
G. Mainelis; Environmental Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ.

5:15PM 121. Systems for Performing Animal Aerosol Exposures
R. Barnewall; Director, Inhalation Systems Group, Battelle, Columbus, OH.

5:45PM 122. Studies of Aged Aerosols and Methods for Evaluating the Survival of Microbes in Aerosols over Time
M. T. Hernandez; Civil, Environmental and Architectural Engineering, Univ of Colorado, Boulder, CO.
**Symposium**

13. Arthropod-Borne Infections: Targeting the Messenger

*Location: Harborview C
Session Time: 4:15 PM - 6:15 PM*

Many of the pathogens that comprise the Select Agent and NIAID Priority Pathogen lists reside in arthropod vectors and are transmitted to people by these vectors; the pathogens include numerous viruses, parasites, and bacteria. Therefore, an understanding of the vector biology is key to elucidating the disease processes and to developing effective countermeasures against the infectious agents. This session will detail vector-pathogen interactions with a variety of arthropod vectors, including ticks, fleas, and sand flies, and will also describe the current status of the tick genome project.

*Upon completion of this Symposium, participants should be able to:*

- Define the current status of the project sequencing the *Ixodes scapularis* genome;
- Relate key steps and genes involved in the transmission of *Yersinia pestis* by the flea and how these factors enable to disrupt innate immune responses;
- Describe the salivary components of sandflies that play a role in transmission of *Leishmania*; and
- Characterize the lifecycle of Wolbachia and state why this organism has potential as a tool to modify insect vectors.

**Moderator:**

M. E. BLOOM, Associate Director, DHR/NIAID/NIH, Hamilton, MT.

**Presentations:**

- **4:15PM** 123. Sand Fly Saliva Studies
  J. G. VALENZUELA, Vector Molecular Biology Unit, NIH/NIAID, Rockville, MD.
- **4:45PM** 124. Getting a Jump on Pathogenesis: Transit Through the Flea Vector Preadapts *Yersinia pestis* to Resist Mammalian Innate Immunity
  B. J. HINNEBUSCH, Rocky Mountain Labs, NIH/NIAID, Hamilton, MT.
- **5:15PM** 125. Tick Immunity and Vector-Pathogen-Host Interactions
  S. NARASIMHAN, Internal Medicine – Infectious Disease, Yale Univ., New Haven, CT.
- **5:45PM** 126. Current Status of the *Ixodes scapularis* International Sequencing Project
  S. K. WIKEL, Ctr for Biodefense & Emerging Infectious Diseases, Univ of Texas Med. Branch, Galveston, TX.

**Discussion Roundtable**

14. Diagnostic Testing

*Location: Harborview B
Session Time: 6:30 PM - 7:30 PM*

Rapid identification of biothreat agents from clinical or environmental samples is critical for a rapid public health response. However, the development and validation of diagnostic tests for pathogens that rarely cause human infections in the United States (e.g., *Yersinia pestis*) or that no longer cause human disease (Variola virus) presents unique challenges to diagnostic test developers. This session will discuss the market for these tests as well as different approaches for introducing new diagnostic assays into actual practice. The discussion will include the steps and different routes for FDA approval, how assays are implemented by the Laboratory Response Network, clinical trials of assays, and commercial development.

*Upon completion of this Roundtable Session, participants should be able to:*

- Explain the market for diagnostic assays for biothreat agents;
- State the different routes for FDA approval of diagnostic assays for biothreat agents; and
- Explain how assays are implemented by the Laboratory Response Network.

**Moderator:**

S. A. MORSE, Bioterrorism Preparedness and Response Program, CDC, Atlanta, GA.

**Panel Presentations:**

- **6:30PM** 128. Process To Get an Assay Implemented in the Laboratory Response Network
  S. A. MORSE, Bioterrorism Preparedness and Response Program, CDC, Atlanta, GA.
- **6:40PM** 129. Diagnostic Test Development for Emerging Infectious Diseases: Market Opportunity and Challenges
  M. A. LEWINSKI, Pathology and Lab Medicine, Univ of California, Los Angeles, CA.
- **6:50PM** 130. How To Move a Test from the Lab to General Use
  S. HOJvat, OIVD/CDRH/FDA, Rockville, MD.
- **7:00PM** 131. Panel Discussion
Tuesday, February 24, 2009

Plenary Session

15. The Science Behind the “Anthrax Letter” Attack Investigation

Location: Harborview
Session Time: 8:30:00 AM - 12:00:00 PM

This session will act as a forum for scientists directly involved in the investigation of the anthrax letter attacks of 2001 to present their analyses and conclusions. Innovative science was very important part of the investigation and yet, has been widely mis-represented in the popular press due to secrecy requirements imposed by the FBI. This secrecy veil is now being lifted by allowing the investigative scientists to present their finds.

Upon completion of this Plenary Session, participants should be able to:
• List the principles of forensics investigations;
• Describe the scientific foundations for the criminal investigation of the anthrax letter attacks; and
• Discuss the novel technologies, and their validation, developed from the anthrax letter investigation.

Moderators:
P. KEIM, Northern Arizona University, Flagstaff, AZ.
J. D. BANNAN, Chemical-Biological Sciences Unit, FBI, Quantico, VA.

Presentations:
8:30AM 132. The B. anthracis Ames Strain and the Development of an Investigative Strain Repository
P. KEIM, Northern Arizona Univ., Flagstaff, AZ.
9:00AM 133. The Silicon Content in B. anthracis Spores Is in the Spore Coat, not Exogenously Applied
J. R. MICHAEL, Sandia Natl. Lab., Albuquerque, NM.
9:30AM Coffee Break
10:00AM 135. Comparative Genome Analysis to Identify Minor B. anthracis Mutant Components in the Anthrax-Letters Spores
J. RAVEL, Microbial Genomics, University of Maryland School of Medicine/Institute for Genome Sciences, Baltimore, MD.
10:30AM 136. A1 & A3 Assay Development
T. R. REYNOLDS, Commonwealth Biotechnologies, Inc., Richmond, VA.
11:00AM 137. Morph D Assay Development
V. T. RYAN, Florida Division, Midwest Research Institute, Palm Bay, FL.
11:30AM 138. FBIR Process, Validations and Synopsis of the Results
J. D. BANNAN, Chemical-Biological Sciences Unit, FBI, Quantico, VA.

Poster Session

16. Tuesday Poster Session

Location: Grand Ballroom
Session Time: 1:00 PM - 3:00 PM

139 (A) Posterboard 1B
Rapid Detection of Highly Pathogenic Avian and Seasonal Influenzas using an ELISA-Coupled Cellular Assay
K. N. AGANS, T. Brazel, F. Romero, A. Weldon, D. Cawthon; Lovelace Respiratory Research Institute, Albuquerque, NM.

140 (A) Posterboard 2B
Development of Mouse Models for WEEV and VEEV Infections
T. Babas1, E. PETERS1, A. Rippon1, R. Wright1, G. Donnelly1, L. Rhodes1, N. Richardson-Harman2, P. M. Silvera1; 1Southern Research Institute, Frederick, MD, 2Alpha StatConsult LLC, Damascus, MD.

141 (B) Posterboard 3B
Recent US-Associated Clinical Strains of Mycobacterium tuberculosis including Drug Sensitive and MDR Strains Exhibit an Extreme Range of Virulence in the Guinea Pig Model.
I. ORME, Colorado State Univ., Fort Collins, CO.

142 (B) Posterboard 4B
The Occurrence of Nocardia and Nocardia-like Species Among Sudanese Patients with Pulmonary Infection
M. M. ELHASSAN1, E. Magzoob2, N. S. Saeed3, M. E. Hamid4, M. Goodfellow5; 1Sudan Univ. of Sci. and Tech., Khartoum, SUDAN, 2Univ. of Khartoum, Khartoum, SUDAN, 3Federal Ministry of Hlth., Khartoum, SUDAN, 4King Khalid Univ., Abha, SAUDI ARABIA, 5Newcastle Univ., Newcastle, UNITED KINGDOM.

143 (B) Posterboard 6B
Assessment of the Virulence in Balb/c Mice of Bioreactor Produced Anthrax Spore Stocks, Delivered by the Aerosol Route
G. J. HATCH, D. L. Hopkins, R. W. Fretwell, S. R. Bate, S. G. Funnell, A. D. Roberts; CEPR, HPA, Salisbury, UNITED KINGDOM.

144 (B) Posterboard 7B
Utility of MLVA DNA Profiling for Quality Control and Molecular Tagging of an Archival Bacillus anthracis Collection
S. W. JONES1, K. Stupac, J. Benson, P. Ikonomi, M. L. McKee; American Type Culture Collection, Manassas, VA.

145 (B) Posterboard 8B
Performance of the LRR1 Real-time Rabbit Plethysmography System
E. B. BARR, S. M. Storch, T. Brasel, Lovelace Respiratory Research Institute, Albuquerque, NM.

146 (B) Posterboard 9B
Comparison of Blood Collection Methods in the Early Stages of Anthrax Bacteremia in Mice
Z. LLEWELLYN, J. A. Boydston, J. E. Trombley, L. E. Bowen; Southern Research, Birmingham, AL.

147 (B) Posterboard 11B
Antimicrobial Susceptibilities of Several Burkholderia pseudomallei Isolates to a Range of Antimicrobial Agents
A. CROOK, K. Pearson, G. Vincent, S. Hawkey, R. Godwin, C. Cruttwell, N. Silman; HPA, Salisbury, UNITED KINGDOM.

148 (B) Posterboard 12B
Susceptibilities of Y. pestis Strains to a Range of Antimicrobial Agents

149 (B) Posterboard 13B
Production and Optimum Growth Conditions of Rickettsia Organisms
D. N. SHAHAN, D. R. Merrill, B. Benton, S. Radhakrishnan, K. J. Langenbach, R. O. Baker; ATCC/BEI Resources, Manassas, VA.
150 (B) Posterboard 14B
The Impact of Storage Duration on Critical Bacillus anthracis Ames Spores Characteristics
J. L. AUSTIN, D. J. Guistino, G. V. Stark, R. E. Barnawall, E. S. Syar, R. E. Hunt; Battelle Memorial Institute, Columbus, OH.

151 (B) Posterboard 15B
Determination of the Francisella tularensis Schu S4 aerosol LD50 in Cynomolgus Macaques and Characterization of Tularemia Manifestation
M. W. VALDERAS1, E. Zinter1, T. Brasel1, J. Wilder1, J. Hunt1, R. Sherwood1, C. Lyons2; 1Lovelace Respiratory Research Institute, Albuquerque, NM, 2University of New Mexico, Albuquerque, NM.

152 (B) Posterboard 16B
Natural History Study for Inhalational Bacillus anthracis Exposure in a New Zealand White Rabbit Model: Identification of an Early Biomarker of Infection
S. B. YEE, J. M. Hatkin, D. N. Dyer, M. L. Pitt, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

153 (D) Posterboard 17B
Rapid Real-Time PCR Assays for Detection of Klebsiella pneumoniae with the rmpA or magA Genes Associated with the Hypermucoviscosity Phenotype: Screening of Nonhuman Primates

154 (D) Posterboard 18B
Detection of Ciprofloxacin-Resistant Bacillus anthracis Mutants by Melt Curve Analysis with SimpleProbe Chemistry
B. M. LOVELESS1, A. Yermakova2, D. A. Kulesh1; 1USAMRIID, Frederick, MD, 2Biomedical Sciences Dept., Univ. at Albany, Albany, NY.

155 (D) Posterboard 20B
Simultaneous Detection of Five Biothreat Agents in Powder Samples by a Multiplexed Suspension Array
J. WANG1, Y. Yang2, L. Zhou1, Y. Hou1, J. Wang3, Y. Jiang3, K. Hu1, Z. Zhu1, Z. Gu1, Y. Ding2, R. Yang2; 1Inst. of Hlth. Quarantine, Chinese Academy of Inspec. and Quarantine, Beijing, China, 2Inst. of Microbiol. and Epidemiol., Beijing, China.

156 (D) Posterboard 21B
Comparison of the Promega Maxwell® 16 and the Modified Qiagen DNA Mini Kit for Isolating PCR-amplifiable Nucleic Acid
S. R. COYNE, T. D. Minogue; USAMRIID, Fort Detrick, MD.

157 (D) Posterboard 22B
qPCR Assays for the Detection of Rickettsial Pathogens
S. E. KARPATHY, G. A. Dusch, M. E. Eremeeva, CDC, Atlanta, GA.

158 (D) Posterboard 23B
Forensic Discrimination of Bacillus cereus T-strain Spores Using Fatty Acid Methyl Ester (FAME) Profiles
C. EHRRHARDT, V. Chu1, J. Robertson2, J. Bannari1; 1ORISE, Quantico, VA, 2CFSRU, FBI, Quantico, VA, 3Chemical and Biological Sciences Unit, FBI, Quantico, VA.

159 (D) Posterboard 25B
Multi-Center Development and Evaluation of a Novel Multiplex Real-Time PCR Protocol for Screening of B. Anthracis in Food and Feed Samples
P. WIELINGA1, R. Hamidjaja1, A. de Groot1, J. Bok1, B. Segerman1, J. Ågren2, R. Knutsson2, M. Fricker1, J. Skov1, I. Janse1, B. van Rotterdam2; 1Dutch National Institute of Public Health and Environment (RIVM), Bilthoven, NETHERLANDS, 2National Veterinary Institute (SVL), Uppsala, SWEDEN, 3EL – Abteilung Mikrobiologie (TUM), Freising-Weiterstadt, GERMANY, 4lokchips A/S, Noerresundby, DENMARK.

160 (D) Posterboard 26B
Virus Detection and Virus Discovery: Generic Amplification Combined with Conventional or High Throughput Sequencing
C. UHLENHAUT, S. McGlenahan, S. Tang, A. Sierra-Honigmann, P. Krause; FDA/CBER, Bethesda, MD.

161 (D) Posterboard 27B
Evaluation of Standard Case Definition for Hemorrhagic Fever with Renal Syndrome in Kazakhstan
A. DMITROVSKY1, Y. Zhetenbayeva2, V. Zeman1, G. Sarybayeva2, S. Ajelat1, E. Maes2; 1CDC, Almaty, KAZAKHSTAN, 2CDC, Atlanta, GA.

162 (D) Posterboard 28B
TaqMan Real-Time PCR Assay Development for Rickettsia rickettsii and R. prowazekii
C. Y. KATO, M. E. Eremeeva, L. K. Robinson, S. E. Karpathy, G. A. Dusch; CDC, Atlanta, GA.

163 (D) Posterboard 29B
bcl-Gene Polymorphisms as Basis for Anthrax Detection and Fingerprinting
T. A. Leski1, C. C. Caswell1, D. J. Kline, II1, M. Pawlowski1, J. M. Butnicki1, S. J. Hart1, S. LUKOMSKIJ1; 1Nova Research, Alexandria, VA, 2West Virginia University, Morgantown, WV, 3International Institute of Molecular and Cell Biology, Warsaw, POLAND, 4Naval Research Laboratory, Washington, DC.

164 (D) Posterboard 30B
Llama Single Domain Antibodies Specific for the 7 Serotypes of Botulinum Neurotoxin as Novel Diagnostics and Therapeutics
J. Conway, L. Sherwood, A. HAYHURST; Southwest Foundation for Biomedical Research, San Antonio, TX.

165 (D) Posterboard 31B
Rapid Detection of 18 Bioterrorism Agents Using Multiplex Real Time PCR with Melt Curve Analysis
J. HENAUT, A. Kilani, Clongen Laboratories, LLC, Germantown, MD.

166 (D) Posterboard 32B
Random RT-PCR Protocol for Viral RNA Evaluated by Microarray and QPCR
H. NORDSTRÖM1,2, Z. Hu3, S. Venf1, M. Brytting1, P. Nilsson1, Å. Lundkvist1,2, D. Palm1, 1Swedish Institute for Infectious Disease Control, Solna, SWEDEN, 2Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, SWEDEN, 3Royal Institute of Technology, Department of Biotechnology, Stockholm, SWEDEN.

167 (D) Posterboard 34B
Direct Analysis of Patient Samples for Simultaneous Identification of Respiratory Viruses and Select Agents Using PCR/ESI-MS
L. B. BLYN1, K. F. Chen1, R. Sampath1, H. Matthews1, S. A. Hofstadler1, D. J. Ecker1, J. Holden1, A. Valsamakis1, P. Ramachandran1, J. Wehrlein2, R. Rothman1, C. A. Gaydos1, 1Ibis Biosciences, Carlsbad, CA, 2Johns Hopkins University, Baltimore, MD.
168 (E) Posterboard 35B
Application of Commercial q-dots Fluorescent Conjugates for the Detection of Pathogenic Bacteria by Flow-Cytometry
E. ZAHAVY, V. Heleg-Shabtai, S. Yitzhaki, Israel Institute for Biological Research, Ness Ziona, ISRAEL.

169 (E) Posterboard 36B
Simultaneous Detection of Food-borne Pathogen using 16S rDNA Combinatorial Signature Chip and Pattern Mapping Analysis
B. HWANG, H. Cha, Pohang Unv. of Sci.and Tech. (POSTECH), Pohang, REPUBLIC OF KOREA.

170 (E) Posterboard 37B
Application of Microarrays for Characterization of Emerging, Unknown and Engineered Threats
C. JAIN, S. Gardner, K. McLaughlin, T. Slezak; Lawrence Livermore Natl. Lab, Livermore, CA.

171 (E) Posterboard 39B
Comparison of Ultrafilters for Recovery of Biothreat Agents from 100-L Tap Water Samples
C. M. Smith 1,2, S. R. PAU2, V. R. Hill1; 1CDC, Atlanta, GA, 2Atlanta Research and Education Foundation, Decatur, GA.

172 (E) Posterboard 40B
A Rapid GFP-Based Assay for Comparing Ribosome Inactivating Toxins
J. ALMEIDA, M. Halter, A. Tona, J. Elliott, A. Plant, K. Cole; NIST, Gaithersburg, MD.

173 (E) Posterboard 41B
Isolation, Identification and Antimicrobial Resistance Patterns of Bacterial Isolates from Clinical Environment
G. HE, K. Palladi, M. Landry, C. Zhang; University of Massachusetts, Lowell, MA.

174 (E) Posterboard 42B
Predictive Modeling for Release of Bacillus anthracis Spores in Drinking Water Systems
J. J. CALOMIRIS, U. S. Army, USAHC, Fort Belvoir, VA.

175 (E) Posterboard 43B
Microfluidic Device to Investigate Microbial Response to Chemical Gradients
J. B. MORROW, L. Locascio, J. Attencia; National Institute of Standards and Technology, Gaithersburg, MD.

176 (E) Posterboard 44B
Modifications to LLNL’s Rapid Viability PCR Assay for the Recovery of Bacillus anthracis spores from Environmental Samples Collected from Porous Surfaces

177 (E) Posterboard 45B
Detection and Biological Characterization of Ricin Toxin in Bioforensic Samples
W. V. KALINA1, J. S. Goodrich1, M. Hevey1, L. R. Roberts1, R. B. Harris2, J. P. Burans1; 1Battelle National Biodefense Institute, Frederick, MD, 2Commonwealth Biotechnologies, INC., Richmond, VA.

178 (E) Posterboard 46B
Ecology of Brucella Genotypes in Southern Kazakhstan

179 (F) Posterboard 48B
Detection of Novel Morph A Variants in Bacillus anthracis Samples Discovered during Screening of the FBI Amethrax Ames Repository
G. MEYERS5, M. Wilson6, R. Ostapowicz7, R. Jaffe7, J. Bannon8, R. Langham9, T. Reynolds10; 5Commonwealth Biotechnologies, Inc, Richmond, VA, 6Western Carolina University, Cullowhee, NC, 7NSER, Inc, in support of OSA (CBD & CDP), Washington, DC, 8Federal Bureau of Investigation, Quantico, VA.

180 (G) Posterboard 49B
Sym002 - A Fully Human Polyclonal Antibody against Smallpox
H. S. NIELSEN; Symphogen A/S, Lyngby, Copenhagen, DENMARK.

181 (G) Posterboard 50B
Synergistic Biothreat Countermeasures from High-Throughput Combination Screening
J. LEHÁR1, L. M. Johansen1, G. Olinger1, T. P. Atkins3, G. R. Zimmermann1; 1CombinatoRx, Inc, Cambridge, MA, 2USAMRIID, Ft. Detrick, MD, 3US Army, USANA, Fort Belvoir, VA.

182 (G) Posterboard 51B
Multi-Drug Resistant Acinetobacter baumannii Drug Combination Evaluation

183 (G) Posterboard 53B
Broad Spectrum Antiviral Therapeutic Based on Iminosugar Derivatives

184 (G) Posterboard 54B
Characterization of Neutralizing Antibodies Directed Against Ricin Toxin
J. PRIGENT, P. Lamourette, C. Crémoinon, H. Volland, S. Simon; CEA, iBiTec-S, Saclay, FRANCE.

185 (G) Posterboard 55B
Efficacy of Cethromycin in a Murine-aerosol Model of Francisella Tularensis (ShuS4)
H. S. Heine1, L. Miller1, J. Bassett1, S. Halasohert1, D. A. Eiznhamer1, M. Leski1, Z. XU2; 1USAMRIID, Fort Detrick, MD, 2Advanced Life Sciences, Woodridge, IL.

186 (G) Posterboard 56B
An Accelerated Path to Safe and Effective Therapeutics (APSET) for Bioterrorism Agents
A. C. SHURTLEFF, L. Gillilan1, J. Mange1, P. Madrid1, T. Keepers1, L. Hokama1, J. Pearson1, S. Samuelsson1, L. Olson1, K. Mortelmans1, S. Bavari2, R. Panchal2, R. Davey3, A. Kolokoltssov4, J. Peterson5, L. Sower5, R. Carrion, Jr.1, J. Patterson5, M. Sojot5, M. Montalbo1, R. L. Burke1; 1SRI International, Menlo Park, CA, 2USAMRIID, Fort Detrick, MD, 3The University of Texas Medical Branch, Galveston, TX, 4Southwest Foundation for Biomedical Research, San Antonio, TX.
187 (H) Posterboard 57B
Identification of Fc Receptors Involved in Antibody-Mediated Fc Receptor-Dependent Anthrax Toxin Neutralization
A. VERMA1, M. M. Ngundi1, B. D. Meade2, D. L. Buras3, FDA, Bethesda, MD, 3Meade Biologics, Hillsborough, NC.

188 (H) Posterboard 58B
Protection in Mice Passively Immunized with Serum from Cynomolgus Macaques Vaccinated with Recombinant Plague Vaccine (rFIV)
P. FELLOWS1, J. Adamovicz2, S. Norris2, W. Blackwelder3, S. Morris3, DynPort Vaccine Co., LLC, Frederick, MD, 2Midwest Research Inst., Frederick, MD, 3USAMRIID, Frederick, MD, 4Biologies Consulting Group, LLC, Alexandria, VA, 5Biomedical Advanced Research and Development Agency, Washington, DC.

189 (H) Posterboard 59B
Electroporation mediated DNA immunization: Application in the Development of Biodefense Vaccines
B. LIVINGSTON, D. Hannaman; Ichor Medical Systems, San Diego, CA.

190 (H) Posterboard 60B
Impact of Concurrent ST-246® Administration on ACAM2000™ Vaccine Efficacy in Cynomolgus Macaques
P. M. SILVER1, E. Peters5, J. Bassler1, G. Gong1, S. Lin1, D. Hebbelwalte1, D. Hruby2, L. Chinsangaram2, R. Jordan2, L. Rhodes5, Southern Research Institute, Frederick, MD, 1SIGA Technologies, Inc., Corvallis, OR.

191 (H) Posterboard 61B
DNA Plasmid and Alphavirus Replicon Based Vaccine Strategies Effectively Elicit Immune Responses against Rift Valley Fever Virus
N. BHARDWAJ1, M. T. Heise4, T. M. Ross4, Univ. of Pittsburgh, Pittsburgh, PA, 4Univ. of North Carolina, Chapel Hill, NC.

192 (H) Posterboard 62B
Lipopolysaccharide from Burkholderia Thailandensis Provides Protection in a Murine Model of Melioidosis
J. PRIOR1, O. Qazi2,3, S. Harding1, S. Ngugi1, B. Klima5, M. Estes2, K. Brown3,4; DSTL, Salisbury, UNITED KINGDOM, 2University of Texas medical branch, Galveston, TX, 3University of Texas at Austin, Austin, TX, 4Imperial College, London, UNITED KINGDOM.

193 (I) Posterboard 63B
Real-time Quantitative Reverse Transcription PCR (qRT-PCR) Assays for Monitoring Cytokine Changes in Mustela putorius furo (Domestic Ferret)
B. M. MINIARD1, E. J. Lubert, R. L. Warren; Battelle Biomedical Res. Ctr., Columbus, OH.

194 (I) Posterboard 64B
Comparative Transcriptional Analysis of the Murine Host Response to Francisella tularensis LVS and Schu4
L. C. KINGRY1, R. Troyer, N. Marlenee, R. Bowen, S. Dow, A. Schenklen, R. A. Slaysk; Colorado State University, Fort Collins, CO.

195 (I) Posterboard 66B
Systems Approaches to Infectious Diseases: A Translational, Infectious Disease Phenome Initiative (TIPP) for the Forward Discovery of Unique and Common Targetable Pathways to Develop Effective Therapeutics
M. KOTR; University of Cincinnati, Cincinnati, OH.

197 (J) Posterboard 67B
Efficacy of Disinfectants Against Pathogenic Viruses
J. STEWARD1, T. Piercy, L. Eastaugh, M. Lever; Dstl, Salisbury, UNITED KINGDOM.

198 (J) Posterboard 68B
Multiplex Cytokine Analysis of Pathogenic Material from Non-Human Primates Using Formaldehyde Fixation
V. A. GRAHAM, S. D. Dowall, K. Steeds, T. Tipton, S. Funnell, M. Finney, J. Vipond; CEPR-Health Protection Agency, Salisbury, UNITED KINGDOM.

199 (J) Posterboard 69B
Standard Method for Dynamic Wipe Efficiency Determination
J. B. MORROW, S. Da Silva; National Institute of Standards and Technology, Gaithersburg, MD.

200 (J) Posterboard 70B
Identification of Test Protocols for EPA Registration of Current and New DoD Decontaminants
B. L. DORSEY, K. M. Weber, A. J. Phipps, L. E. O’Connor; Battelle Memorial Institute, Aberdeen, MD.

201 (K) Posterboard 71B
G. EPSTEIN; Center for Strategic and International Studies, Washington, DC.

202 (K) Posterboard 72B
Great Lakes RCE Animal Research and Immunology Core (ARIC)
V. R. GONZALES, J. Richter, N. A. Cletti, D. Elli, B. Berube, K. Skurasaks, D. Missiakas; The Univ of Chicago, Chicago, IL.

203 (K) Posterboard 73B
One Health: A Concept to Improve Biodefense and Global Health
L. H. KAHN1, C. Blackmore2, L. Conti2, M. Eckols3, B. Kaplan4, Princeton Univ, Princeton, NJ, 2Florida Dept. of Health, Tallahassee, FL, 3Palm Beach County Hlth. Dept., West Palm Beach, FL, 4Retired, Sarasota, FL.

204 (K) Posterboard 74B
Ricin Acute Inhalation Exposures
V. R. GONZALES1, J. Benson, A. Gomez; Lovelace Respiratory Research Institute, Albuquerque, NM.

205 (K) Posterboard 75B
The Swedish Laboratory for Food Safety and Biopreparedness
C. Dahlberg1,2, S. Ehrl1,2, S. Froth1,2, S. GARBOM1,2, R. Knutsson3,2, A. Landin1,2, C. Nilsson1,2, P. Ågren1,2; National Food Administration, Uppsala, SWEDEN, 3Swedish Laboratory for Food Safety and Biopreparedness, Uppsala, SWEDEN, 5Swedish Veterinary Institute, Uppsala, SWEDEN.

206 (K) Posterboard 76B
Aerosol Exposure Results in Increased Deposition and Retention of Fluorescent Microspheres in the Lungs of Balb/c Mice Compared to Intranasal Inoculation
D. REED, S. Faith, A. Hartman, K. S. Cole; University of Pittsburgh, Pittsburgh, PA.

207 (K) Posterboard 77B
Putting More “Sure” in Biosurety
J. J. ADAMOVICZ, K. Wilhelm, R. J. Hawley; Midwest Research Institute, Frederick, MD.
17. Environmental Detection

Location: Harborview B
Session Time: 3:00 PM - 4:00 PM

Presentations:

3:00PM 210. Technology Platform for Rapid Identification of Highly Pathogenic Viruses


3:12PM 211. Multiplex Detection of Biothreat Pathogens by using Suspension Microarrays

I. JANSE, J. M. Bok, R. A. Hamidjaja, B. J. van Rotterdam; National Institute for Public Health and the Environment (RIVM), Bilthoven, NETHERLANDS.

3:24PM 212. Improved Toxin Detection using Single Domain Antibodies

E. GOLDMAN, G. Anderson, J. Liu, M. Swain, R. Bernstein; Naval Research Laboratory, Washington, DC, USA.

3:36PM 213. Prevalence of Coxiella burnetii DNA in Environmental Samples Acquired in the United States between 2006 and 2008


3:48PM 214. Detection of Known and Emerging Vector Borne Pathogens by PCR and ESU/MASS Spectrometry


Highlighted Oral Abstract Presentations

18. Immune Response and Vaccines

Location: Harborview C
Session Time: 3:00 PM - 4:00 PM

Presentations:

3:00PM 215. Elucidation of Protective Immune Responses Using a 1918 Virus-like Particle

B. M. GILES, C. J. Crevar, T. M. Tumpey, T. J. Ross; Or, for Vaccine Res., Univ. of Pittsburgh, Pittsburgh, PA, USA.

3:12PM 216. Ebola VLPs Induce Expression of SOCS1 which Enhances VLP Budding

A. OKUMURA, P. M. Pitha, A. Yoshimura, R. N. Hardy; Univ. of Pennsylvania, School of Vet. Med., Philadelphia, PA, USA, Johns Hopkins University, Baltimore, MD, USA.

3:24PM 217. A Clinical Phase II Study Confirming the Safety of a 3rd Generation Smallpox Vaccine in Vaccinia-naive HIV Infected Subjects

E. T. OVERTON, E. Wagar, A. Von Krempelhuber, G. Virgin, P. Chaplin, and the POX-MIA-09 Study Sites; Washington University School of Medicine, St. Louis, MO, USA.

3:36PM 218. The Bacillus anthracis Toxin, Anthraxolysin O, Stimulates Macrophages through Multiple Receptors Including Tlr4

M. A. WYNOSK-L-DOLFI, M. Bernui, E. M. Mosser, R. F. Rest; Drexel University College of Medicine, Philadelphia, PA.

3:48PM 219. Improved Potency of Codon-optimized Encephalitis Alphavirus DNA Vaccines Delivered by Electroporation

L. C. DUPUY, M. Richards, B. Ellefsen, D. Hannaman, B. Livingston, C. S. Schmaljohn; USA Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, USA.

Highlighted Oral Abstract Presentations

19. Bacterial Basic Science

Location: Harborview D
Session Time: 3:00 PM - 4:00 PM

Presentations:

3:00PM 220. Coordinated Regulation of Virulence Factors during Systemic Infection of Salmonella enterica serovar Typhimurium

H. YOON, J. McDermott, C. Ansong, J. Adkins, S. Forwollife, M. McClelland, R. D. Smith, F. Heffron; Oregon Health & Science University, Portland, OR, USA.

3:12PM 221. How Does the T Domain of Diphtheria Toxin Drive Membrane Binding and Penetration of the G Domain

A. Chassaing, S. Pichard, V. Forge, D. GILLET; CEA, Gif sur Yvette, FRANCE.

3:24PM 222. Cellular Tropism and Characterization of Burkholderia mallei Type III Secretion System in a Respiratory Model of Infection

G. C. Whitlock, B. M. Judy, D. M. Estes, A. G. TORRES; Univ. of Texas Med Branch, Galveston, TX.

3:36PM 223. Analysis of a Novel Regulatory Module Associated with Non-Replicating Persistence in Mycobacterium tuberculosis

L. R. RESPICIO, B. A. Slayden; Rocky Mountain Regional Center of Excellence and Mycobacterial Research Laboratories, Dept. of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO.
Symposium

20. Toxins Structure and Function [CME]
Location: Harborview B
Session Time: 4:15 PM - 6:15 PM
Toxins are significant virulence factors of many bacterial pathogens. The vary greatly in structural diversity and cellular mode of action. In this session, the regulation of synthesis, molecular mechanisms of intoxication, immunological aspects, and countermeasures will be described.

Upon completion of this Symposium, participants should be able to:
• Relate the importance of key biological toxins to bacterial pathogenesis in humans and animals;
• Describe the cellular mechanisms of action of specific bacterial toxins and their relation to important diseases; and
• Recognize promising approaches to the development of countermeasures to combat toxicity.

Moderator: E. A. JOHNSON, Food Research Institute, Univ. of Wisconsin, Madison, WI.

Presentations:
4:15PM 225. Secreted Virulence Factors of Methicillin-Resistant Staphylococcus aureus
F. R. DELEO; Rocky Mountain Laboratories, NIH/NIAID, Hamilton, MT.

226. Treatment of Botulinum Neurotoxin Intoxication: Small Molecule Therapeutic Approaches
K. D. JANDA; Chemistry and Immunology, The Scripps Res. Inst., La Jolla, CA.

5:15PM 227. Anthrax Toxin Pore Structure
M. T. FISHER; Biochemistry and Molecular Biology, Univ. of Kansas Med. Ctr., Kansas City, KS.

5:45PM 228. YadB of Yersinia pestis
S. C. STRALEY; Microbiol., Immuna., and Molec. Genetics, Univ. of Kentucky, Lexington, KY.

Symposium

Location: Harborview C
Session Time: 4:15 PM - 6:15 PM

Moderator: A. G. BARBOUR, Microbiology & Mol. Genetics and Medicine, Univ. of California, Irvine, CA.

Presentation:
4:15PM 229. Genomic Plasticity of Burkholderia pseudomallei: Implications for Disease Acquisition and Bacterial Persistence in the Infected Host
S. J. PEACOCK; Wellcome Unit, Mahidol Univ., Bangkok, Thailand.

4:40PM 230. Burkholderia Pseudomallei Strains That Infect Humans and Animals are Distinct from Environment Strains
D. WAGNER; Biological Sciences, Northern Arizona Univ., Flagstaff, AZ.

5:05PM 231. Animal Model
H. P. SCHWEIZER; Microbiology, Immunology and Pathology, Colorado State Univ., Fort Collins, CO.

5:30PM 232. Burkholderia pseudomallei Type III Secretion System Effectors
J. F. MILLER; Dept. of Microbiology, Immunology & Mol. Genetics, Univ of California Sch. of Med., Los Angeles, CA.

233. Burkholderia pseudomallei and B. mallei: Comparative Genomics and Quorum Sensing
W. C. NIERMAN; Infectious Diseases Program, J. Craig Venter Inst., Rockville, MD.

Symposium

22. Imaging [CME]
Location: Harborview D
Session Time: 4:15 PM - 6:15 PM
New developments in nanotechnology have expanded the applications for imaging in a variety of aspects of infectious diseases including host pathogen interactions, diagnostics and product development. Several different platforms and uses for imaging as applied to basic and translational science will be presented.

Upon completion of this Symposium, the participants should be able to:
• Explain potential roles for nanotechnology and imaging in analyzing different aspects of the interaction of the pathogen with host cells;
• Incorporate some of the uses of imaging in product development efforts; and
• Have a general conceptual understanding of the different methodological approaches used for imaging.

Moderator: R. LYONS; Internal Medicine, Univ. of New Mexico Sch. of Med., Albuquerque, NM.

Presentations:
A. SINGH; Biosystems Research, Sandia National Laboratories, Livermore, CA.

4:45PM 235. Biodefence and Emerging Infectious Disease Imaging: Opportunities and Challenges
K. LI; Radiology, The Methodist Hosp., Houston, TX.

5:15PM 236. Virus Based Nanoparticles as Biomedical Imaging Tools
M. MANCHESTER; Cell Biology, The Scripps Research Institute, La Jolla, CA.

5:45PM 237. Visualizing of Host-Pathogen Interactions with Accuracy
J. A. TIMLIN; Sandia Natl. Lab., Albuquerque, NM.

Discussion Roundtable

23. Select Agent Process [CME]
Location: Harborview C
Session Time: 6:30 PM - 7:30 PM

Moderator: P. B. JAHRLING; Integrated Research Facility, NIAID, Bethesda, MD.

Presentations:
6:30PM 238. Panelist Remarks
V. SUTTON; School of Law, Texas Tech Univ., Lubbock, TX.

6:40PM 239. CDC Select Agent Program
R. S. WEYANT; Meningitis & Special Pathogens Branch, CDC, Atlanta, GA.

6:50PM 240. Panelist Remarks
J. V. LAWLER; NIH/NIAID, Bethesda, MD.

7:00PM 241. Panel Discussion
**Wednesday, February 25, 2009**

**Plenary Session**

**24. Synthetic Genomes**  
Location: Harborview  
Session Time: 8:30 AM - 12:00 PM

Recent advances in molecular biology, virology and genomics have made the construction of genomes to specifications a regular practice. This session will highlight some of the available technologies for construction and modification of synthetic genomes and will discuss the implications and dilemmas raised by the use of these methods.

**Upon completion of this Plenary Session, participants should be able to:**

- Relate different strategies for de novo construction of organisms;
- Differentiate between neutral constructive strategies and those that might increase pathogenicity;
- Understand under what circumstances the application of such technologies is appropriate; and
- Understand and explain the dual use dilemma.

**Moderators:**

K. E. KLOSE, Biology, Univ. of Texas, San Antonio, TX.  
M. J. BUCHMEIER, Molecular Biology and Biochemistry, Univ. of California, Irvine, CA.

**Presentations:**

8:30AM  242. Featured Speaker: Synthetic Virus Assembly  
R. BARIC, Univ. of North Carolina, Haw River, NC.

9:30AM  Coffee Break

10:00AM  243. Synthetic Mycoplasma Genome  
D. GIBSON, J. Craig Venter Inst., Rockville, MD.

10:30AM  244. Polio Virus Synthesis  
E. A. WIMMER, Microbiology, Stony Brook University, Stony Brook, NY.

11:00AM  245. Synthetic Genomes: Research Tool or Regulatory Nightmare  
M. J. BUCHMEIER, Molecular Biology and Biochemistry, Univ. of California, Irvine, CA.

11:30AM  246. Implications  
G. MUKUNDA, Political Science, Massachusetts Inst. of Tech., Cambridge, MA.
028 (A)

Comparison of Dengue Virus Type 1 Growth Characteristics in Vero and C6/36 Cell Lines
A. Owens, J. Shifflett, S. Radhakrishnan, K. Langenbach, R. O. Baker; BEI Resources/ATCC, Manassas, VA

Background: This study compared the growth characteristics of dengue virus type 1 (Strain Hawaii) in Vero and C6/36 cells to determine the optimal temperature and appropriate cell line for propagation. Dengue virus, a NIAID Category A priority pathogen, is a member of the Flaviviridae family and is transmitted by mosquitoes to humans. While classic dengue fever is a self-limited disease, severe forms can lead to shock and hemorrhage and occasionally death. The virus can grow in Vero (from monkey kidney) and C6/36 (from Amblyommia argunmae mosquito clone) cell lines without showing any significant cytopathic effects. While Vero cells grow optimally at 37°C and C6/36 cells grow optimally at 28°C, little information is known about the optimal temperature for dengue virus propagation.

Methods: Dengue virus was propagated at three different temperatures in both cell lines through three passages. Titer was measured by IFA and relative quantitative PCR using SYBR Green. Differences in nucleotide sequences of the viral envelope protein (E) and the non-structural protein 4B (NS4B) were assessed at each passage as the virus adapted to growth in the respective cell line. Results: IFA showed viral titer 1-2 logs higher in Vero cells at 35°C and in C6/36 cells at both 28°C and 35°C compared to the other temperatures tested. Similar trends were seen in relative quantitative PCR. At 35°C virus grew to similar titer in both cell lines. Nucleotide sequence analysis showed differences in five positions in the viral E gene. This difference was seen in virus grown at 28°C in Vero and 37°C in C6/36 cells. There were no mutations in the NS4B gene. Conclusions: This study shows that 33°C is an optimal temperature for growth of dengue virus in Vero or C6/36 cell lines. As the virus is adapted to propagate in these cell lines at extreme temperatures for the cell’s growth, genetic differences become apparent in the envelope gene.

029 (A)

Development of Rabbitpox Model for Smallpox
T. Babas, E. Peters, K. Gong, L. V. Nieves-Duran, S. Lin, D. Hebbelwaite, R. Wright, A. Rippeon, D. Golightly, G. Donnelly, L. Rhodes, P. M. Silvera; Southern Research Institute, Frederick, MD.

Background: The establishment of a reliable and cost effective animal model of smallpox disease is important for antiviral efficacy evaluations. Under NIAID contract N01-AI-30063, we developed a rabbitpox animal model in order to screen drug candidates for smallpox.

Method: Six month old adult New Zealand white rabbits (Charles River) were challenged either intradermally or intranasally with a targeted challenge dose of RPV Utrecht strain ranging between 10⁵ PFU and 10⁴ PFU. The animals were monitored for 14 days after challenge for signs of disease and time to death. Real-time PCR for viral loads, ELISA and PRNT assays were developed to monitor the infection. Results: Three to five days post challenge, rabbits began developing clinical signs of disease including increasing body temperature, decreasing body weight, anorexia, depression followed by mucus membrane discharge in area around the eyes, nostrils, mouth and ano-genital. The rabbits eventually succumbed to severe respiratory distress between days 7-10 of infection. Some rabbits survived the infection by recovering their normal body weight and temperature starting at day 12 post infection. Rabbits that died of rabbitpox disease showed increased viremia and viral loads in the lung compared to survivors. ELISA demonstrated that sera from survivors showed higher anti-RPV titers compared to non-survivors. Furthermore, stronger neutralizing antibody titers (>2,048) were detected by PRNT assay in sera from rabbits that survived by day 28 post challenge compared to non-survivors. Conclusion: The clinical signs of rabbitpox were similar to that of smallpox. There was a direct correlation between viral load in the blood and neutralizing antibody titers. This model will prove to be valuable for testing the efficacy of anti-smallpox drug therapies.

030 (B)

Characterisation of a Marmoset Model of Inhalational Tularemia
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Background. The intracellular gram-negative pathogen, Francisella tularensis, is the causative agent of tularemia and is prevalent in many countries in the northern hemisphere. Animal models are required for future vaccine and therapeutic studies. To determine whether the common marmoset (Callithrix jacchus) would be a suitable non-human primate (NHP) model of inhalational tularemia, a pathophysiology study was undertaken.

Methods. Ten animals were challenged with aerosolised F. tularensis strain SCHU S4. Pairs of animals were culled at 24 hourly intervals between 0 and 96 hours post-challenge. Blood and selected organs were assessed for bacteriology, pathology, haematological and immunological parameters. Results. The challenge dose was 179 ± 17 cfu. The first indication of infection was an increase in the core body temperature, at approximately 3 days post-challenge. This coincided with a number of other factors: a rapid increase in the number of bacteria isolated from all organs, gross organ pathology and evidence of an immune response. As disease progressed higher bacterial counts and cytokine levels were detected. More extensive pathology was observed, with multifocal lesions present in the lungs, liver and spleen. Conclusions. Disease progression in the common marmoset appears consistent with human clinical and pathological features, indicating that this may be a suitable animal model of inhalational tularemia.

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Topic Categories: Below is a list of descriptions of topic categories (letters in parentheses by each presentation number refer to this chart).

A. Viral Agents
B. Bacterial Agents
C. Fungal Agents
D. Diagnostics
E. Environmental Detection
F. Informatics and Genomics
G. Therapeutics
H. Vaccines
I. Immune Responses
J. Decontamination, Biosafety, and Containment
K. Other and Information Only

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**031 (B)**

A Comparative Study of the Cytotoxic Effects of Epsilon Toxin, Epsilon Prototoxin, and a Recombinant Epsilon Toxin Fragment on Sensitive Cell Lines

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**Background:** Epsilon toxin is a pore-forming cytotoxin produced by types B and D *Clostridium perfringens*. This toxin contributes to the pathogenicity of the organism and is a potential agent of bioterrorism. MDCK (ATCC® CCL-34™) and human kidney G-402 (ATCC® CRL-1440™) cell lines are widely used to study Epsilon toxin activity. We tested the sensitivity of these cell lines against three forms of the toxin and identified differences between them.

**Methods:** MDCK and G-402 cells were cultured in complete media up to passages 39 and 19 respectively. For some experiments, MDCK cells were cultured up to passage 89 to obtain high-passage cells. All cells were tested for sensitivity to activated Epsilon toxin, Epsilon prototoxin, and a recombinant Epsilon toxin fragment using cytotoxicity assays in a 96-well plate format. Cells were treated with toxin and the surviving cells were fixed and stained. The percent viability was determined by comparing treated cells to untreated control cells.

**Results:** High passage MDCK cells were 23 times more sensitive to activated Epsilon toxin than low passage cells. Epsilon prototoxin had a similar level of cytotoxicity on the MDCK cells compared to the activated form. The recombinant epsilon toxin fragment was not cytotoxic to G-402 cells and had a large reduction in cytotoxicity to the MDCK cells. G-402 cells showed an intermediate sensitivity to the effects of Epsilon toxin when compared to low passage and high passage MDCK cells. 

**Conclusions:** MDCK and G-402 cells have been evaluated for their sensitivity to 3 forms of Epsilon toxin. MDCK cells that have been cultured to a high passage are the most sensitive to the cytotoxic effects of Epsilon toxin. While G-402 cells were sensitive to activated Epsilon toxin, they were insensitive to the effects of the recombinant epsilon fragment. These results support the use of MDCK and G-402 cell lines for the measurement of Epsilon toxin activity.

**032 (B)**

Dominant-Negative Inhibitors of the *Clostridium perfringens* ε-Toxin

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**Background:** The *Clostridium perfringens* ε-toxin can lead to a fatal enterotoxemia characterized by widespread vascular permeability and edema in the heart, lungs, brain, and kidneys. Due to its extreme potency and the possibility of intoxicating animals and humans, the ε-toxin is considered an overlap select agent. Due to limitations in the effectiveness and applicability of current vaccines and anti-toxins, alternative countermeasures are needed that inhibit the ε-toxin. In this study, we characterized dominant-negative inhibitors of the ε-toxin. 

**Methods:** Site-specific mutations were introduced into the ε-toxin gene and recombinant proteins were expressed in E. coli. The mutations were designed to introduce two cysteines into each mutant protein at locations where the cysteines would be predicted to form a disulfide bond. At least one cysteine of each pair was located within the predicted membrane insertion domain of the toxin. The recombinant mutant proteins were purified and non-cytotoxic mutant proteins were examined for their ability to inhibit the activity of wild-type ε-toxin. 

**Results:** Three mutant proteins lacked detectable cytotoxic activity in a MDCK cell assay. Cytotoxic activity could be reconstituted in one inactive mutant protein by incubation with dithiothreitol, suggesting that the lack of cytotoxic activity was attributable to the formation of a disulfide bond. When mixtures containing equal amounts of mutant and wild-type ε-toxin were added to MDCK cells, the non-cytotoxic mutant proteins each inhibited the activity of wild-type ε-toxin. Significant inhibition of the wild-type toxin was also observed in mixtures containing eight parts wild-type to one part mutant protein. 

**Conclusions:** These results provide further insight into the properties of dominant-negative inhibitors of oligomeric toxins and provide the basis for developing new therapeutics for treating intoxication by ε-toxin.

**033 (B)**

In vitro Growth Characterisation Studies of *Burkholderia pseudomallei*

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**Background:** *For in vitro* aerosolisation studies, *Burkholderia pseudomallei* is traditionally harvested from a mid-exponential logarithmic phase liquid culture. In order to assess suitability of different media types, we compared the growth properties of this BSL3 agent in *Tryptific Soya broth* (TSB) and *Luria Bertani broth* (LBB), and enumerated bacteria from both broths on *Tryptific Soya agar* (TSA) and *Luria Bertani agar* (LBA). We also assessed the optimal time for incubation of agar plates before manual enumeration. 

**Methods:** *B. pseudomallei* strain K96243 was obtained from the National Collection of Type Cultures. Master and working stocks were produced and stored frozen until use. Working stocks were recovered on TSA or LBA. After 48h incubation at 37°C, both TSB and LBB were inoculated to an optical density (OD) at 600nm of 0.09. The broths were incubated with orbital agitation at 57°C and sampled 12 times over a 48h period. Each sample was enumerated onto TSA and LBB, assessed for absorbance at 600nm and smeared onto a slide for Gram stain analysis. Plates were initially counted after 24h but follow up studies included recounting up to 4 days. 

**Results:** The yields of viable bacteria from both broth types were virtually identical. In addition, no drop in viability was observed in late stationary phase up to 48h after inoculation. Agar plates required care in terms of pre-drying before use but when suitably dried, no difference between TSA and LBA was observed. Initial studies were conducted using plates incubated for only 24h but preliminary findings and a follow-up study identified that plates should be incubated for at least 42h before manual enumeration. 

**Conclusions:** The relationship between OD and viable count was found to change with time during planktonic batch growth. Microscopic observations support the notion that the physical properties of *B. pseudomallei* alter between growth on agar. 

**034 (B)**

Onset of Bacteremia in Mice Following Nose-Only Inhalation of *Bacillus anthracis* Ames Spores

J. A. Boydston, J. E. Trombley, Z. N. Llewellyn, L. E. Bowen; Southern Research, Birmingham, AL.

**Background:** *Bacillus anthracis* is the etiological agent of the disease anthrax in humans and animals. The 50% lethal dose (LD<sub>50</sub>) has been well described in several animal species. However, the onset of bacteremia following nose-only inhalation exposure has not been well described. 

**Methods:** Six groups of BALB/c mice, consisting of five males and five females, were exposed by nose-only inhalation to an average inhaled dose of 2.68E5 CFU of *B. anthracis* Ames spores. Mice were euthanized at T=0 (immediately following exposure), and 6, 12, 24, 36, and 48 hours post-exposure. Blood samples were serially diluted, plated, incubated, and enumerated. 

**Results:** Bacteremia was observed in 100% of mice euthanized immediately after exposure. Subsequently, bacteremia rapidly dropped to 0% by 12 hours and increased to 70% at 24 hours. By 48 hours, 100% of mice were positive for *B. anthracis* bacteremia. 

**Conclusions:** These results suggest that initiating anti-microbial treatment may be necessary earlier than 24 hours post-exposure for successful prevention of inhalational anthrax disease.
**035 (B)**

**Determination of the 50% Lethal Dose (LD50) of *Bacillus anthracis* Ames in Mice Following Nose-Only Inhalation Exposure**

L. E. Bowen, Z. N. Llewellyn, J. E. Trombley, J. A. Boydston; Southern Research Institute, Birmingham, AL.

**Background:** The 50% lethal dose of *Bacillus anthracis* Ames was determined in BALB/c mice. Eight groups of mice, consisting of five males and five females each, were exposed to USP Water For Injection or *Bacillus anthracis* Ames by nose-only inhalation. Methods: Varying aerosol concentrations were delivered to the breathing zone of the mice corresponding to inhaled doses of 0 CFU (Group 1), 3.1E2 CFU (Group 2 females), 3.4E2 CFU (Group 2 males), 2.8E3 CFU (Group 3 females), 3.1E3 CFU (Group 3 males), 5.4E3 CFU (Group 4 females), 6.4E3 CFU (Group 4 males), 3.2E4 CFU (Group 5 females), 3.7E4 CFU (Group 5 males), 9.1E4 CFU (Group 6 females), 1.0E5 CFU (Group 6 males), 3.8E5 CFU (Group 7 females), 4.4E5 CFU (Group 7 males), and 1.2E6 CFU (Group 8 females), 1.3E6 CFU (Group 8 males). Animals were observed for up to 15 days, and body weights and temperatures were collected. Blood samples were collected at the time of euthanasia when animals were found dead. Collected sera were analyzed for the presence of *Bacillus anthracis*. Results: Twenty-seven of 80 mice, including 14 males and 13 females, died prior to scheduled euthanasia. No statistical differences were identified in survivability between male and female dose groups; however, comparisons between male and female survivability were not possible for Groups 1, 2, and 4 since 100% survival was the outcome for both samples. Probit analysis projected the LD50 to be 7.5E4 CFU for male mice and 1.3E5 CFU for female mice.

Conclusions: In summary, exposure of mice to *Bacillus anthracis* by inhalation exposure was highly pathogenic and an LD50 similar to that reported in the literature was observed.

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**036 (B)**

**Determination of the 50% Lethal Dose (LD50) of *Yersinia pestis* in Mice Following Nose-Only Inhalation Exposure**

L. E. Bowen, Z. N. Llewellyn, J. A. Boydston, M. B. Minyard, G. Bridges, J. E. Trombley, P. M. Silvera; Southern Research Institute, Birmingham, AL; 2Southern Research Institute, Frederick, MD.

**Background:** The 50% lethal dose of *Yersinia pestis* was determined in BALB/c mice. Eight groups of mice, consisting of five males and five females each, were exposed to USP Water For Injection or *Yersinia pestis* by nose-only inhalation. Methods: Varying aerosol concentrations were delivered to the breathing zone of the mice corresponding to inhaled doses of 0 CFU (Group 1), 3.5E3 CFU (Group 2 females), 6.6E3 CFU (Group 2 males), 1.9E4 CFU (Group 3 females), 2.2E4 CFU (Group 3 males), 6.0E4 CFU (Group 4 females), 7.6E4 CFU (Group 4 males), 1.0E5 CFU (Group 5 females), 1.2E5 CFU (Group 5 males), 4.4E5 CFU (Group 6 females), 5.9E5 CFU (Group 6 males), 4.6E5 CFU (Group 7 females), 5.7E5 CFU (Group 7 males), and 1.8E6 CFU (Group 8 females), 1.9E6 CFU (Group 8 males). Animals were observed for up to 15 days, and body weights and temperatures were collected. Blood samples were collected at the time of euthanasia when animals were found dead. Collected sera were analyzed for the presence of *Yersinia pestis*. Results: Fifty-eight of 80 mice, including 31 males and 29 females, died or were euthanized in moribund condition prior to scheduled euthanasia. No statistical differences were identified in survivability between male and female dose groups; however, comparisons between male and female survivability were not possible for Groups 1, 2, 3, and 4 since 100% survival was the outcome for both samples. Probit analyses projected the LD50 to be 8.3E3 CFU for male mice and 1.8E4 CFU for female mice. The gender neutral LD50 was 1.2E4 CFU. The administration of *Yersinia pestis* resulted in weight losses compared to baseline levels for all dose groups. Conclusion: In summary, exposure of mice to *Yersinia pestis* by inhalation exposure was highly pathogenic and a gender neutral LD50 similar to that reported in the literature was observed.

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**037 (B)**

**Impact of Environmental Factors on Virulence of Aerosolized *Francisella tularensis***

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**Background:** *Francisella tularensis* is a gram negative intracellular bacterial pathogen that is listed as a category A select agent. Animal models of the pulmonary disease caused by *Francisella tularensis* have been used to study the efficacy of candidate vaccines and therapeutics. However, there is no standardization in the methods of preparation or delivery of *F. tularensis* between laboratories and it is possible that environmental factors such as choice of culture media can impact virulence. Methods: Two strains of *F. tularensis*, the attenuated Live Vaccine Strain (LVS) and virulent SchuS4 strain, were grown in Mueller-Hinton (MH) broth or Chamberlain’s Chemically Defined Medium (CCDM) and aerosolized by a Collison nebulizer into rednet nose-only and whole-body exposure chambers controlled by the aerolAB aerosol exposure system (Biareta Technologies, Frederick, MD). Results: The LVS strain of *F. tularensis* grew significantly faster in MH broth than CCDM as measured by both optical density at 600 nm and plating on Cystine Heart Agar (CHA). When both cultures were compared head-to-head with the same starting inoculum, LVS grown in MH broth achieved mid-log phase growth at 18 hours and stationary phase growth at 21 hours, LVS grown in CCDM did not achieve mid-log growth until 24 hours. Conclusions: Choice of culture media alters the growth of LVS in culture; data on the impact of culture media and other environmental factors on aerosolization of LVS and Schu S4 will be presented.

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**038 (B)**

**Effect of N-acetylcysteine or STIMAL® on *Bacillus anthracis* Spore and Spore-Phagocyte Interactions**

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**Background:** Inhalational anthrax is initiated by introduction of *Bacillus anthracis* (BA) spores into the lung and subsequent association with alveolar macrophages (MAGs). Spore germination and bacilli outgrowth are essential to establish infection, and therapeutics inhibiting these key events would prevent anthrax. We hypothesized that N-acetylcysteine (NAC) and STIMAL, liposome-encapsulated NAC, affected BA-phagocyte interactions at different stages during initial infection. Methods: We treated BAa Sterne strain 7702 spores with various concentrations of NAC or STIMAL in vitro in the presence or absence of human MAGs. BA spore germination and viability were measured. Results: STIMAL- or NAC-treated MAGs rapidly killed intracellular germinating BA spores, in contrast to cultures without STIMAL or NAC where BA overgrew MAGs by 8 hr. STIMAL/NAC did not affect MAG viability. Increased intracellular reduced glutathione ([GSH] i) was responsible for this protective effect. Supernatants from NAC-treated MAGs cultured with BA killed vegetative BA but not spores. In the absence of phagocytes, STIMAL or NAC (and GSH and dihydrothiogalactoside) completely but reversibly inhibited BA spore germination but were not bactericidal. This suggested a reversible redox event on the spore surface. Oxidized NAC did not inhibit BA spore germination, confirming that NAC reduces components of the BA germination pathway. Use of different amino acid germinant combinations showed that germination receptors gerk and gerl were specifically targeted. Conclusions: The reducing agents STIMAL and NAC significantly alter BA spore germination. They increase intracellular germination leading to BA death within MAGs, and strongly but reversibly inhibit germination of free spores. STIMAL could be used as an adjunct therapeutic aid following anthrax exposure.
039 (B)

Validated Bacillus Strains as Model for Bacillus anthracis

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Background: One of the research areas of BIOTRACER (www.biotracer.org), an integrated project within the European Union 6th Framework Programme, addresses intentional and accidental contamination scenarios with potential bioterrorist organisms in feed and food chains. As part of this project an analytical chain for the rapid tracing and tracking of B. anthracis in various types of food and feed will be established. To facilitate the development and optimization of such an analytical chain, a model system for the work on B. anthracis spores is needed. In the past, different Bacillus species have been used as a surrogate for B. anthracis, including B. subtilis, B. atrophaeus/B. globigii, and B. thuringiensis. However, since the selected strains do not always behave in the same way as B. anthracis the selection of appropriate strains is very important. As B. anthracis belongs to the B. cereus group, it is most likely that members of this group could serve as valid model organisms for B. anthracis. However, due to the great diversity and strain variability within the B. cereus group the selection of appropriate strains is very important.

Methods: A panel of Bacillus strains has been characterized by MLST, gpdH and analysis and whole genome comparison. In addition to comparison of genotypic characteristics phenotypic spore characteristics have been evaluated. Heat inactivation, germination patterns and hydrophobicity of spores have been measured.

Results: Based on the comparison of genotypic characteristics and phenotypic spore characteristics, a group of B. cereus strains showing high similarity to B. anthracis was identified.

Conclusions: A group of Bacillus cereus strains are now available within the BIOTRACER consortium as validated models for B. anthracis and will be used for the development and optimization of tracing and detection systems for B. anthracis in the food and feed chain.

040 (B)

Comparison of Francisella tularensis LVS and SCHU S4 Bioaerosols

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Background: The demand to test new vaccines and therapeutics against F. tularensis has recently increased. Specifically, the need to evaluate treatments following bioaerosol challenge has become more prevalent. To date, the majority of published studies have incorporated the use of less virulent strains for this task (e.g., F. novicida, F. bolarctica, and LVS) with the assumption that subspecies behave similarly. To test this, we proceeded to compare bioaerosols of F. tularensis SCHU S4, a Type A strain, with LVS.

Methods: All work was performed in a Class III glovebox under ABSL-3 conditions. Bioaerosols were generated across a range of concentrations using disposable single jet nebulizers or a Collison MRE-3. Generator suspensions from 24 or 48 Chamberlain’s broth cultures were prepared fresh. Aerosolized bacteria were sampled from a 16-liter chamber using all glass impingers. Bacterial suspensions were cultured onto blood, yeast extract, chocolate, and agar. Comparison criteria included generator suspension stability, aerosol concentration, spray factor, and particle size. In addition, virulence was confirmed and compared in a BALB/c mouse aerosol challenge model.

Results: Marked differences were observed between the two strains. Bacterial stability was greater with LVS by upwards of 17.5%. LVS aerosol concentrations and spray factors were higher by 0.5 to 1 log10, independent of the nebulizer used. Particle sizes were similar and ranged from 1.5 to 2.2 µm MMAD. Following aerosol challenge, all mice succumbed to low doses of SCHU S4, conversely, survivors were noted following exposure to significantly higher concentrations of LVS.

Conclusions: The hypothesis that subspecies of F. tularensis behave similarly as bioaerosols is incorrect. Our data indicate that caution is warranted when extrapolating data (bioaerosol or otherwise) between strains. This project has been funded in whole or in part with Federal funds from the NIMD, NIH, DHRH, under Contract No. HHSN266200500040C.

041 (B)

Comparative Analysis of the in vitro Stability and Viability of a Panel of Yersinia pestis Strains

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A frequently encountered issue in biodefense and microbial research in general, is the standardization of strains across different lines of experimentation. For the pathogen Yersinia pestis, two strains are primarily utilized as exemplars of the genus for studies involving pathogenesis and the evaluation of countermeasures: C092 (Colorado isolate from 1992) and KIM (Kurdistan Iran Man). C092 is a fully virulent strain and is the predominant isolate utilized as a model for pathogenesis. A virulent original KIM strain is not available to the scientific community, but attenuated KIM strains are commonly used in genetic and biochemical analysis. Because Y. pestis is a globally diverse species, it is unclear whether these two strains are representative of all Y. pestis strains. Our study evaluates a panel of Y. pestis strains in an attempt to cross compare characteristics of genomic stability and their tolerances to environmental stress so that a correlation can be made between a diverse set of isolates. Experiments conducted include the analysis of genomic stability during growth and passage, and the viability of strains after environmental challenges, in an effort to replicate both in vitro and in vivo environments. These studies show that there are differences between strains, even those suggested to be closely related, that could alter assessments of pathogenesis and physiology. These studies will allow for a baseline to be developed against which other strains can be compared with the aim of identifying genotypic markers related to differences in stability, viability, and pathogenesis of Y. pestis.

042 (B)

Rapid Identification and High Resolution Strain Typing of Francisella tularensis


Background: Francisella tularensis is highly pathogenic and has been designated a select agent, necessitating prompt identification in suspected exposures. Identification of species and subspecies of Francisella is critical since they differ in pathogenicity. High resolution strain-resolving genotypes provide more information, enabling epidemiological analysis, comparisons of isolates, and contributing to forensic attribution. Multiple techniques typically are required to span these different levels of identification. We have developed an assay for Francisella that combines these levels of resolution in a single assay format. We describe the validation of the assay using a panel of Francisella strains.

Methods: DNA from cultured Francisella was amplified and PCR products were analyzed by ESI-MS on the Ibis Biosciences T5000 platform. Results: were compared to the existing data associated with the strains. Results DNA from over 100 characterized strains was analyzed. All species identifications were correct, with approximately 70% identified as F. tularensis, and the remainder as F. philomiragia. F. tularensis subspp tularensis, holarctica, novicida, and mediasiatica were correctly identified. In addition the SNP markers parsed subspp tularensis into Groups M001, A002, and AI, and subspp holarctica into Groups B North America/Europe/Asia and B Japan. The twelve VNTR markers provided additional resolution among each of the grouped subspecies. Conclusions: There was 100% concordance in species and subspecies identifications with the PCR assay. Previously we have shown the assay to be sensitive and robust, allowing characterization of environmental samples and tick isolates as well as culture-derived DNA. These results demonstrate the identification and high-resolution typing F. tularensis in a single assay format.
**043 (D)**

**Molecular Identification of Yersinia Pestis as the Cause of an Outbreak of Severe Disease in Afghanistan Following the Consumption of Camel Meat**


**Background:** In late December 2007, an outbreak of severe disease, associated with fever, vomiting, diarrhea, and axillary lymphadenopathy, occurred in the Nimroz Province of Afghanistan. Of the 50 cases, 8 died (16% case fatality rate) and all were known to have consumed camel meat. Due to the gastrointestinal symptoms and the disease severity, the outbreak was initially suspected to be anthrax; however, all diagnostics for Bacillus anthracis were negative. The bis T5000 PCR/electrospray ionization-mass spectrometry (PCR/ESI-MS) system is a novel diagnostic tool capable of unbiased identification of multiple pathogens within a sample. The T5000 system uses base composition data as DNA signatures to provide accurate identification of bacterial and viral pathogens. **Methods:** Whole blood and sera from two patients, as well as tissue samples from the camel were tested using the T5000 PCR/ESI-MS Biodefense assay, which specifically targets 15 bacterial and 4 viral threat agents and their near neighbors. Real-time PCR testing was performed using the Roche LightCycler. **Results:** Whole blood from one patient and camel tissue tested positive for Yersinia pestis, the causative agent of plague, for all three gene targets (inv, pla, and caf genes) in the PCR/ESI-MS assay, and both samples produced identical base composition DNA signatures across all three gene targets. Confirmatory testing included analysis of both the patient whole blood sample and the camel tissue using three separate real-time PCR assays, targeting the inv, pla, and caf genes of Y. pestis. All three real-time Y. pestis-specific assays were strongly positive for both samples. **Conclusions:** Both the broad-range PCR/ESI-MS assay and follow-up real-time PCR testing provided strong evidence that the Afghanistan outbreak was due to plague and that Y. pestis infected camel meat was the source of the infection.

**044 (D)**

**Nanosensors for Bacterial Pathogenesis Identification**

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**Background:** Microorganisms quickly develop and acquire resistance mechanisms. Therefore, determination of antibiotic agents and their administration at effective dosages are important. We hypothesized that antibody-conjugated nanoparticles can rapidly quantify bacteria, whereas dextran-coated nanoparticles may quickly assess antimicrobial susceptibility in blood by monitoring the microorganisms’ metabolic activity. **Methods:** Dextran-coated iron oxide nanoparticles were prepared. Antibodies were conjugated to the nanoparticles using Protein G, whereas Concanavalin A was used as the nanoparticle clustering inducer in antimicrobial susceptibility experiments. The solution’s magnetic relaxation was measured using a 0.47T benchtop relaxometer. **Results:** We were able to detect Mycobacterium avium spp. paratuberculosis in milk and blood with high sensitivity (< 20 CFUs) within 45 minutes. Our assay was specific and not susceptible to interference caused by other bacteria. Various clinical samples were tested and the results were confirmed using gold-standard methodology. Our nanoparticle-based method provided results faster without the need of sample purification and amplification. Next, we used dextran-coated iron oxide nanoparticles to assess antimicrobial susceptibility. We found that when the bacteria did not grow, addition of Concanavalin A resulted in the formation of extensive nanoassemblies, allowing MIC determination within 2 hours. When the bacteria grew, there was a decrease in the nanoparticle clusters’ size with smaller shifts in the relaxation times. **Conclusion:** Sensitive bacterial quantification and rapid antimicrobial susceptibility assessment was achieved in complex media using the synthesized nanoparticles. The use of this nanoparticle technology in the clinic and the field is anticipated.

**045 (D)**

**Development of Strain-Specific Real-Time PCR Assays to Detect Arenaviruses**

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**Background:** The family Arenaviridae arenavirus contains five viruses known to cause viral hemorrhagic fever: Lassa, Machupo, Junin, Sabia, and Guanarito. The signs and symptoms of each disease vary, but the initial onslaught begins with headache, fever, and malaise. These diseases can produce vomiting, muscle pains, and petechiae with possible progression to bleeding of the gum, mucus membranes, and mortality rates between 3-30%. The similar clinical manifestations of the five arenaviruses are virtually indistinguishable and can easily resemble that of other diseases indigenous to the virus’s natural distribution. Outbreaks of arenavirus disease are generally confined to specific regions in South America (Machupo: Bolivia, Junin: Argentina, Sabia: Brazil, Guanarito: Venezuela) with the exception of Lassa virus, which has a natural distribution in West Africa. **Methods:** A series of primers and TaqMan®-MGB™ probe sets specific to each virus were developed using Primer Express 2.0 and Allele ID 4/5/6. On the Roche LightCycler 2.0, primer and probe concentrations were optimized and limits of detection (LOD) were determined. Each primer/probe set was tested for cross-reactivity within each virus species. Additional exclusivity data were obtained by analysis against an alphavirus and a viral hemorrhagic fever RNA panel. **Results:** Two primer/probe sets unique to each arenavirus were identified and successfully used in assays to differentiate among them with LODs ranging between 1 and 10^6 PFU. There was no cross-reactivity with either of the RNA panels. **Conclusions:** Fifteen real-time PCR assays were developed to detect and differentiate five arenaviruses. With proper diagnosis and treatment critical to the prevention and spread of the disease, the risk of a potential outbreak should diminish. Real-time PCR assays are a rapid and accurate diagnostic tool for clinicians to identify and treat the emerging hemorrhagic fevers.

**046 (D)**

**Development of Sensitive Rapid Tests Using Biological Warfare Agents Detection**

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**Background:** Biological warfare agents are insidious, their detection is difficult and, within the limit of our current knowledge, far from being instantaneous. This point is even more crucial because the effects of these agents, dramatically effective, are insidious. The development of powerful systems of detection and allowing identification of these agents, as early as their dispersion, is therefore crucial to be efficient against these. These systems will have to be easy to use, because they are susceptible to be used by non specialists. **Methods:** Among the various methods of detection developed, immunochromatography, while preserving the property of specificity of classical immunological assays, presents several advantages: fast, ease of use, adapted to measurements on the ground and very low cost. Monoclonal antibodies directed against different agents: botulinum toxins A and E, Enterotoxin B and ricin were produced in the laboratory. Using these antibodies, we have developed an improved immunochromatographic test using immunoliposomes as label. A new original procedure allowed measuring the fluorescent signal generated by the sulforhodamine B encapsulated into immunoliposomes in an immunochromatographic test. **Results:** A 10 fold increase in sensitivity as compared to visual detection for colored labels (colloidal gold) was observed using the immunoliposome label. The test was completed within 30 minutes, providing a limit of detection below 1 ng/ml for all the agents. **Conclusions:** Since the fluorescent obtained signal is clearly proportional to analyte concentration, the present immunochromatographic test could be possibly used not only as a detection tool but also as a quantification assay applied to field samples. Therefore, using a simple and hand-held commercial fluorimeter device, perfectly suited for in the field measurement, this opportunity is currently under investigation.
**047 (D)**

**Comparison and Development of Magnetic Bead-Based Nucleic Acid Extraction Methods for Biothreat Agents in Clinical Matrices**

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**Background:** Central to clinical diagnostic efforts is extraction of pure nucleic acids from diverse sample matrices. Our current focus is to automate procedures using solid-phase magnetic beads to extract nucleic acids for PCR detection with the evaluation of two magnetic bead-based protocols from Invitrogen™. **Methods:** The first method is Chargeswitch® (CS) technology. This procedure is based on DNA/RNA affinity to magnetic beads coated with a material that is positively charged at low pH. At low pH, nucleic acid is bound, allowing the removal of matrix inhibitory factors with subsequent DNA/RNA elution at high pH. The secondary protocol is Dynabeads® SILANE Genomic DNA and includes chaotropic lysis, binding to Dynabeads® MyOne SILANE beads, washing with alcohol, and eluting with water or Tris buffer. Both technologies were tested with four organisms (*B. anthracis* Sterne (Ba), *Y. pestis* (YP), Vaccinia (V) and Venezuelan equine encephalitis (VEE)) virus in matrices: buffer, sputum, feces, whole blood, and serum. **Results:** An optimized CS Forensic DNA kit provided best results with equivalent nucleic acid purification in buffer, serum, sputum, and feces biological matrices to traditional methods. However, in whole blood, CS Forensic kit yielded poor results with both DNA and RNA being undetectable or detectable only at concentrations of 1x10⁵ or greater compared to conventional QiAamp manual extraction with LODs of 5x10² for better or all organisms. In contrast, the Dynabeads® SILANE method performed well for all organisms in whole blood, buffer, sputum, and serum with limits of detection of 1x10² to 1x10⁵ for all organisms assessed. Yet, preliminary evaluation of Silane beads with Ba in feces yielded no detectable quantities of nucleic acid. **Conclusion:** Current focus is on improvement/automation for both of these nucleic acid purification procedures using solid-phase magnetic beads, as neither method provided an adequate solution for all relevant matrices.

**048 (D)**

**Rapid Antimicrobial Susceptibility Testing of Bacillus anthracis**

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**Background:** In the event of a deliberate release of a biothreat agent, rapid detection of antimicrobial resistance is essential before distribution of antimicrobial agents from the Strategic National Stockpile (SNS) for treatment and prophylaxis. We describe a rapid method of detecting resistance based on a short period of incubation in the presence of any antimicrobial agent followed by qPCR to detect growth. **Methods:** Strips of 12 wells, composed of 11 wells containing two-fold dilutions of ciprofloxacin (CIP, 0.015 - 16 µg/ml) or doxycycline (DOX, 0.06 - 64 µg/ml) and control wells (no drug), were inoculated with *B. anthracis* following CLSI guidelines. After incubation times of 2, 3, 4, 5, or 6 hr, cells were lysed by either heat (100°C for 5, 10, 15, or 20 min) or addition of purified gamma phage lysis, PhG (15 or 30 min). From these cell lysates the relative quantification of *rpoB* by qPCR was compared between each drug dilution well and the growth control well. The assay was evaluated using 14 genetically diverse strains of *B. anthracis* and several derivatives of *B. anthracis* Sterne (resistant to either CIP or DOX). **Results:** A 4-hr incubation period and a 15 min PhG lysis provided consistent, unambiguous MICs for CIP and DOX. qPCR results from heat lysates were inconsistent from well-to-well and varied in reproducibility. Inhibition of growth by CIP or DOX was detected as ΔCt values of 6 to 11 cycles when compared with the no drug control. For each of the 14 *B. anthracis* isolates and resistant control strains tested, rapid assay MIC results were the same as conventional (16-20 hr incubation) MIC results. **Conclusions:** qPCR provides rapid susceptibility test results in <6 hours for *B. anthracis*. The assay is useful for any antimicrobial agent and does not depend on detection of specific genes or mutations associated with resistance. Modifications of this assay (based on species-specific growth parameters) are in development for additional biothreat agents.

**049 (D)**

**Polyphasic Characterization of Five Different Serotypes of Salmonella enterica from a Food-Borne Outbreak**

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**Background:** Salmonella is a common food-borne pathogen causing an estimated 1.4 million cases of salmonellosis each year in the United States. Five *Salmonella enterica* serotypes: Anatum, Javiana, Muenchen, Thompson, and Typhimurium, were isolated by the Pennsylvania Department of Health (PDH) from a food-borne outbreak, which occurred in Pennsylvania and near by states in 2004. This outbreak was associated with the consumption of tomatoes. The PDH reported that pulse field gel electrophoresis (PFGE) played a key role in identifying the outbreak-associated isolates. We characterized forty-one *Salmonella* isolates from the outbreak using a variety of methods. **Methods:** Isolates were characterized using biochemical (API) serotyping (slide agglutination), and molecular methods including rep-PCR (Bacterial Barcodes), Real Time PCR, 16S Ribosomal RNA gene, and ribotyping (Riboprinter). **Results:** By employing the polyphasic approach, we verified that all 41 *Salmonella* strains were *enterica* species. Serotyping, Rep-PCR and ribotyping profiles further confirmed the serological typing results as was previously reported. Moreover, diversity among the isolates was observed by molecular typing and serotyping. Among the molecular authentication methods rep-PCR was less accurate than ribotyping in identifying the serotypes. This may be due to the limited number of serotypes represented in the Bacterial Barcodes library. There was high concordance between serological testing and ribotyping for serotypes, Anatum (group E), Javiana (group D), and Typhimurium (group B or B and D). On the other hand the concordance between serological testing and ribotyping for the serotypes Muenchen (group C2 or C2 and C1) and Thompson (group C1 or C1 and C2) was less stringent. **Conclusion:** Ribotyping was found to be a straightforward technique for molecular confirmation of *Salmonella enterica* serotypes and it has advantages over other molecular method such as PFGE which is more laborious-intensive and time consuming.

**050 (D)**

**Crimean-Congo Haemorrhagic Fever ELISA Development and Comparison of Results Between Laboratories**

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**Background:** Crimean-Congo haemorrhagic fever (CCHF) is a fatal viral disease spread via ticks with a mortality rate of approximately 30% from tick bite and up to 80% in nosocomial circumstances (World Health Organisation). It is the world’s most widespread viral haemorrhagic fever being endemic in many regions including Asia, Europe and Africa. The rapid emergence of CCHF has occurred in Turkey since 2002. Between 2002 and 2007, a total of 1,820 confirmed cases, including 92 deaths, were reported. Cases have risen year-by-year, and in the first-half of 2008 a further 688 cases have been reported with a fatality rate of 5.96%. CCHF virus is a Biosafety Level 4 pathogen. Work with infectious virus including many diagnostic tests requires maximal containment facilities. Using heat-inactivated sera samples in an ELISA assay bypasses the use of live virus. **Methods:** A recombinant baculovirus expression system was developed to produce nucleoprotein from CCHF virus. The CCHF nucleoprotein was purified and used as an antigen in ELISA assays developed at the Health Protection Agency (HPA). Clinical samples from CCHF patients in Turkey were used for assay development. **Results:** The CCHF ELISA assay distinguished between negative and positive samples, detecting all positive Turkish samples (n=20). Methodology has been transferred from the HPA to Turkey via an arranged collaboration under the auspices of the World Health Organisation (WHO), allowing local serological testing. **Conclusion:** The HPA laboratory is a WHO collaborating centre for viral reference and research. Cooperation has enabled the development of a reliable and efficient CCHF assay which utilises heat inactivated sera.
**051 (D)**

Pathogen Detection Using a Novel Nanofluidic System

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The use of capture array technology for the detection of infectious agent nucleic acid has been hampered by poor analytical sensitivity but can be improved by implementing PCR amplification prior to hybridization. However, introducing an initial PCR step necessitates the use of multiplex PCR which can lead to competitive inhibition and cross-assay primer interaction, or PCR product pooling which promotes amplification competition and process challenges. The BioTrove OpenArray®, provides an alternative format that permits the amplification of up to 3072 individual nucleic acid targets within the array. This format eliminates the need to multiplex or pool PCR assays prior to the array step. The OpenArray platform accommodates the use of end-point and real-time PCR technology, which has been documented to provide better analytical sensitivity and specificity over conventional gel-based PCR. We investigated the OpenArray to determine its utility for the detection of rodent infectious agents. An array was produced which contained primer and probes representing 20 common viruses and bacteria. Ten-fold dilutions of each target nucleic acid were evaluated in both the OpenArray and a standard 96-well format (duplicate wells). The analytical sensitivity determined for the OpenArray and 96-well format were similar. The OpenArray was resistant to PCR inhibition associated nucleic acid isolated from feces and also to high concentrations of test nucleic acid (600ng/µl final reaction concentration). Rodent samples, which had previously been determined to be positive for at least one agent by the 96-well format, were positive for the same and sometimes additional agents by the OpenArray. Potential applications for the OpenArray include virus panels for biologics testing and health monitoring panels for post-quarantine or routine health monitoring of sentinel mice.

**052 (D)**

Large-scale Automated Detection of Bacterial Pathogens in the Food Chain

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We have developed and evaluated a high throughput automated bacterial DNA extraction method for real-time PCR analysis to increase the level of biopreparedness in the food chain in Sweden. The method was adapted to a wide range of food and feed matrices with suspected BSL-3 microbial contamination and the method can be executed in a safety cabinet. To maximise the throughput of samples, it is important to develop as general methods as possible. This is a challenging task due to the large heterogeneity of the different food and feed matrices. In total we analysed 50 different food and feed matrices. The matrices were spiked with both the gram positive bacilli Bacillus cereus and the gram negative rod Escherichia coli (EHEC) before extraction. 2 grams of each food matrix was added to 20 ml 0.9% sodium chloride together with glass beads. To disrupt the matrix we shook the sample tube and thereafter we withdrew a portion of the supernatant and added this to the Magnatrix robotic system (Magnetic Biosolutions), for DNA extraction. We evaluated the efficiency of the DNA extraction by real-time PCR. The detection level was found to be 10^3 cfu/g food which corresponds to approximately 1 cfu/PCR reaction in our experimental set up. Some of the matrices contained PCR inhibitors or fluorescent molecules disturbing either the PCR reaction or read-out and some matrices were not possible to solubilise in the sodium chloride (in total 5 out of 50). We also compared several different robotic systems for automatic DNA extraction from a selection of eight matrices. The systems compared were the Magnatrix, the EZ-1 (Igenex) which are based on purification with magnetic beads, and the ABI Prism 6100 (Aplied Biosystems) which is based on silica gel filtration. We found that the ABI Prism 6100 performed best in general for extraction of B. cereus DNA and the Magnatrix performed best for extraction of EHEC DNA.

**053 (D)**

Evaluation and Comparison of the Sherlock® Instant FAME™ Sample Preparation Method and the Standard Preparation Method for Biothreat Agents

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**Background:** The MIDI Standard extraction method is commonly used as the standard in fatty acid extraction in biothreat agent detection. The Instant FAME extraction method is the MIDI’s newer version of identifying biothreat agents. The new extraction procedure cuts down the extraction time to fewer than 3 min and a gas chromatography time of about 10 min per sample, making the system suitable for most diagnostic laboratories. We evaluated the two methods to determine their accuracy in rapidly identifying Yersinia pestis, Francisella tularensis, and Bacillus anthracis. **Methods:** Y. pestis, F. tularensis, and B. anthracis were cultured and processed according to the MIDI standard preparation and Instant FAME preparation method guidelines. **Results:** Y. pestis and B. anthracis were not only comparably identified by both methods, but the Instant FAME produced the results quicker and at times with a greater similarity index than the standard method. With F. tularensis, the results were equivocal for both methods. **Conclusions:** Based on the above results, we found that, the Instant FAME method and the standard method both accurately identified the bacterial agents. The Instant FAME had faster results.

**054 (D)**

Rapidly Formulated Antigen Capture Assays for Ebola Viruses Based on In Vitro Antibody Selections of Semi-Synthetic Llama Antibodies at BSL-4

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**Background:** Previously we had observed that single domain antibodies (sdAb) selected from a semi-synthetic library on live Marburg virus preparations, yielded antigen capture assays capable of detecting 1-10 pfu of virus (Sherwood et al., J. Infect. Dis. S12 213-219). The process yielded sdAb specific for nucleoprotein (NP), known to exist as a highly polymeric antigen, most likely contributing to high assay performance via massively parallel avidity effects. We hypothesized that if viral NP assemblies direct the selection of appropriate sdAb, then closely related viruses should make good candidates for the generation of similar antigen capture assays. **Methods:** Ebola Zaire (Kikwit), Sudan (Boniface), Ivory Coast (IC1) and Reston (Reston) were amplified, concentrated and purified. In vitro selection at BSL4 was used to isolate sdAb. Unique clones were examined for their cross-reactivity between the Ebola species and the most specific clones used to establish lower limits of detection in antigen capture ELISA tests. The antigenic targets of sdAb were indicated by western blotting of virus/virus infected cell preparations, and confirmed by cloning and expression of recombinant antigen genes. **Results:** Populations of sdAb were isolated consisting of species cross-reactive and species specific clones. Lower limits of detection were 10 to 100 pfu with no clones cross-reacting with Marburg virus. All sdAb targeted the NP antigen with each sdAb clone capable of performing as both captor and detector in antigen capture assays, indicating the requirement for polymeric NP. **Conclusions:** In under 3 weeks, unoptimized and sensitive and specific NP antigen capture assays for the Ebola viruses were formulated. The strategy may have applications for the rapid assembly of antigen capture assays to other negative strand viruses, many of which represent emerging and re-emerging threats.
**055 (D)**

**Evaluation of Electrochemiluminescence Detection Capabilities and Toxin Distribution in African Green Monkeys Exposed to Aerosolized Staphylococcal Enterotoxin B**

V. Sanders, L. Chuvala, M. Wolcott, D. Blau; USAMRIID, Ft. Detrick, MD.

**Background:** Staphylococcal enterotoxin B (SEB) is one of several exotoxins produced by Staphylococcus aureus. SEB is a potent superantigen and a potential biological warfare agent. These toxins act as pathogens by over-stimulating the immune response. This study examined the capabilities of detecting the toxin in various clinical specimens post aerosol exposure to SEB. **Methods:** African green monkeys were aerosol exposed to SEB. Blood samples and bronchoalveolar lavage (BAL) were collected at time of euthanasia. Tissues specimens and urine were taken at necropsy at 2, 10, 24, 48, and 54 hours postexposure. In addition, in one set of animals, blood and urine were collected at 15 min; 1 h, 10, 24, 32, and 48 h postexposure. **Results:** M1M® electrochemiluminescence (ECL) immunoassays were used to test samples for antigen levels of SEB. Data suggests that in exposed African green monkeys, SEB was detected as early as 15 min postexposure in urine and serum samples. The highest level of toxin was detected in many of the samples from animals that were exposed 10 h previously. **Conclusion:** ECL data obtained reflect qualitative values of SEB antigen concentrations 15 min to 54 h postaerosol exposure. The toxin detection of SEB throughout various samples showed similar trends related to intoxication over time. High S:B ratios showed that the detection capabilities of ECL were highly specific for SEB.

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**056 (K)**

**ERIC, a Bioinformatics Resource for Enteropathogens**

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ERIC (Enteropathogen Resource Information Center) is one of NIAID's eight Bioinformatics Resource Centers for Biodetection and Emerging/Re-emerging Infectious Disease. ERIC serves as an information resource for five related enteropathogens: diarrheagenic E. coli, Shigella spp., Salmonella spp., Yersinia enterocolitica, and Yersinia pestis. ERIC is a Web-based bioinformatics portal intended to integrate information on the genome sequence, genome annotations, and many types of related biological data for these five organisms to facilitate research into therapeutics, diagnostics, and vaccines. Among our major goals is to provide high-quality annotation of the genomes of these organisms with community contributions, carefully curated using ASAP (A Systematic Annotation Package for community annotation), developed at the University of Wisconsin-Madison. Annotation and curation are in progress using orthology and focusing on genes known to be involved in virulence and pathogenicity. The ERIC system is evolving to provide tools for comparative genomics, genome polymorphisms, antigen prediction, microarray analysis using the NCBI microarray database system (mشد), motif prediction, proteomics data and analysis, and text mining of the PubMed abstracts related to these pathogens for both keywords and relationships such as a gene and its function, or a mutation and its resulting phenotype. The ERIC Portal is online at http://www.ericbrc.org. ERIC provides integrated access to both data and analysis tools. A data warehouse will allow cross-database queries. All tools and data developed under this NIAID contract are freely available in addition. We need to interact often with the enterobacterial research community to meet your needs, and urge your feedback on the system features and your contributions to annotation. ERIC will also focus on providing training and facilitating development of ontologies and controlled vocabularies in collaboration with our sister BRCs.

Please contact info@ericbrc.org for more information.

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**057 (D)**

**Rapid Sanger Sequencing of Cloned Random RT-PCR Products for Viral RNA Sample Screening**

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**Background:** The titers of viral RNA in clinical samples can differ extensively depending on disease, but for many severe viral hemorrhagic fevers the amount of viral RNA will be high early on, >10^4 copies/ml serum. In cases with a need for broader screening or for new or unknown virus strains, methods that investigate the whole nucleic acid population can be valuable. The presented assay is a low cost and accessible alternative to using new broad sequencing methods such as 454 sequencing. **Methods:** Dengue 2 viral RNA was extracted from cell culture and amplified by a random RT-PCR. The mixture of amplicons was purified on agarose gel and introduced into plasmids using standard molecular cloning. From the resulting agar plates, up to 100 positive clones were directly screened for inserts using PCR with vector specific primers. Products visible on agarose gel were then immediately sequenced with a new and rapid Sanger sequencing protocol. **Results:** A dilution series of viral RNA was tested. For 100 or more positive clones picked, a lower limit of detection around 1000 viral genomes in the Random RT-PCR, can be obtained routinely. This was further evaluated by mixing the viral RNA with total RNA from serum and cells. In general, it was found that for serum spiked with higher levels of viral RNA, >10^4 copies/ml, about 1 in 4 clones contains viral sequence. Further work involves pre-treatment of the samples to remove background nucleic acid and tests on other viruses and bacteria. **Conclusion:** This procedure provides a relatively fast and cheap assay to screen for pathogen nucleic acid in a sample from a patient with severe disease, when all other assays are negative. Obviously quite a high amount of viral RNA is needed, but not on levels unrealistic for some early viral disease.

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**058 (D)**

**Developmental Testing of a Biological Hydrosol Concentrator**

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**Background:** Rapid and effective surface and aerosol monitoring has become more important than ever. Highly efficient aerosol collection have been developed that can pull in hundreds of liters of air a minute and capture the particles into a relatively small liquid volume, turning the aerosol into a hydrosol. Many surface samplers, such as the one used here, also collect into a liquid. **Methods:** Most modern aerosol collectors produce a hydrosol sample volume of about 5 to 10 mL. Surface samplers collect into volumes from about 1 mL to 250 mL. A portion of the hydrosol sample is transferred directly to a detector. Great strides have been made in recent years in detector technology making them faster and more accurate than ever. However, these advanced detection systems are only capable of analyzing volumes from around 0.005 mL to 0.1 mL of liquid at a time, and there is a need to concentrate the hydrosol from the collector into a volume matched to the detector analysis volume. An effective hydrosol concentrator accomplishes this task by functioning as a macro-to-micro interface. The final sample size should be settable to quickly reduce the initial sample to the desired analysis volume. **Results:** Known quantities of Bacillus atrophaeus spores were wet-deposit on laminate sampling tickets. They were removed from the surfaces into a liquid using a backpack surface sampler into volumes of approximately 50 mL, and concentrated using a hollow fiber filter hydrosol concentrator. Concentrated and unconcentrated samples were analyzed using lateral flow assays. Concentrated sample volumes were 100 mL. The theoretical concentration factor is thus up to 500X. Results presented here include sampling efficiency, concentration efficiency, concentration factor, and time-to-detect. **Conclusions:** The concentration process is rapid and effective. When coupled with aerosol sampler or surface sampling into a liquid, a concentration step reduces the time needed for detection and allows the use of inexpensive small-volume analytical methods.
**059 (E)**

Studies on Surface Sampling of Anthrax Spore Surrogates from Environmental Surfaces: Effect of Swab Material and Interferants on Spore Recovery


Following a biological agent release, environmental samples are collected and analyzed to provide information on initial agent concentration, location and extent of contamination, and ultimately confirmation that clean-up goals are achieved. Environmental sampling of *Bacillus anthracis* (BA) is critical for determining the contamination level of both indoor and outdoor facilities. Surrogate organisms reported for studying BA spores are *B. subtilis*, *B. atrophaeus* (Bo), *B. cereus* (BC), *B. thuringiensis* (BT), and *B. anthracis* Sterne (BA), although *Bo* has been the most extensively used. Currently, there are insufficient data on recovery efficiency of BA spores from diverse environmental surfaces. Ineffective sampling and recovery methods have the potential for undesired social and economic impact. To address this problem, four different swab materials were evaluated for their recovery efficiency of BA spore surrogates (i.e., *Bo*, *BC*, and *BA*). Dry or premoistened rayon, cotton, polyester, and macrofoam swabs were directly inoculated with a known concentration of spores in suspension. Spores were recovered from swabs by vortexing or sonication, and recoveries of surrogates from different swabs were compared. The highest recoveries of all surrogates were obtained when dry rayon swabs or premoistened macrofoam swabs were inoculated with spores and processed by sonication, revealing differences in interactions between swabs and swab materials. Results on the recovery efficiencies of BA spore surrogates from diverse environmental surfaces using different swab materials and in the presence of interferants is presented. The relationship of recovery of BA spore surrogates from environmental surfaces to their physico-chemical properties is addressed. The results of this research will help in choosing the most efficient swab material for environmental sampling of anthrax spores. Funded by Technical Support Working Group (TSWG).

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**060 (E)**

Magnetic Bead Based Clean-Up Process for ECL Assays

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**Background:** A protocol for the analysis of environmental swipes samples, using BioVeris electrochemiluminescence (ECL) assays, was developed and optimized for screening items for biological contamination. On occasion, swipes samples are comprised of soil and/or other interfering agents which resulted in false positive readings when analyzed using the ECL immunoassay. **Methods:** In an effort to minimize the potential for false positive results, reduce likelihood of inconclusive determinations, and gain an increased confidence in the analysis, the Environmental BioMonitoring Laboratory at the Edgewood Chemical Biological Center, designed an antibody-free bead clean-up method, using magnetic beads not tagged with target antibodies (antibody-free), that mitigates any potential interference from samples prior to analysis. **Results:** By incubating the sample with antibody-free beads prior to standard ECL analysis, it is possible to remove components causing the false positive result. To verify that the clean-up process would eliminate false positive results in “dirty” swipes, but still allow for the detection of the appropriate ECL target, samples were spiked with antigen before incubation with antibody free beads and subsequent ECL analysis. Even in the presence of soil and/or other interfering agents, the process successfully removes the interferent with minimal lose in agent detection. **Conclusion:** The antibody free bead clean-up-step has been shown to be useful in reducing false positive readings and has been incorporated into the laboratory’s concept of operations for “dirty” samples.

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**061 (E)**

Shipping Integrity of *Bacillus anthracis* Swab Samples

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**Background:** Temperature conditions during transportation of surface samples collected during the anthrax mail contamination event of 2001 were not monitored, and it is unknown whether shipping temperatures affected the specimens’ biological integrity. Reports from the Government Accountability Office and a CDC peer review identified transportation methods as a critical gap in the validation of environmental sampling processes. This project was developed to determine optimum conditions for shipping. **Methods:** Macrofoam swabs were premoistened with either neutralizing buffer (NB) or PBS containing 0.02% Tween-80 (PBST) at room temperature, then directly inoculated with ~10^10 *Bacillus anthracis* Sterne spores. Swabs were packaged in primary containers consisting of 15 mL conical tubes sealed with Parafilm and placed in a diagnostic specimen transport bag and stored at either -15ºC, 5ºC, or 21ºC. For each buffer tested, 10 swabs were removed at 0, 1, 2, 4, and 7 days, processed according to Laboratory Response Network protocols, and compared to inoculum controls to establish percent recovery (%R) values. To determine if changes in %R were due to changes in spore numbers rather than viability, total spore counts were performed using a solid phase cytometer. **Results:** Spore recovery declined over 7 days as measured by plate counts. Storage of swabs at 5ºC resulted in 60%R at 2 days and 51%R at 7 days, while storage at -15ºC resulted in 20%R at both 2 and 7 days. %R values for swabs stored at 21ºC were intermediate to those observed for 5ºC and -15ºC. Total spore counts as also declined by 63% at the end of 7 days, factoring into the decrease in culturable spore counts. **Conclusion:** Storage at 5ºC yielded the highest recovery of spores from swabs, while -15ºC yielded the lowest. Swabs should be processed within 48 hours since extended storage may increase drying and spore binding to the swab. Ongoing work will elucidate the impact of storage time and temperature on the limits of detection for this swab processing method.
Detection and Occurrence of Enterococcus in Dairy Ground Waters

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Background: Pathogens in waters have been increasingly of concern for safety and security of food and agriculture. Dairy farms are among the suspect sources of contamination of natural waters. Measuring groundwater occurrence of pathogens impacted by dairy facilities is a significant step towards understanding pathogen transport and for developing agricultural safety and security practices. Enterococcus is being widely accepted as an indicator organism for water contamination. The present study was to develop sampling and analytical methods for detecting Enterococcus and to investigate the occurrence of this bacterium in dairy ground water.

Methods: A stainless steel submersible pump was used to collect groundwater from wells located on six dairy farms in Central California. At least 50 gallons of water was purged prior to collecting a ten liter sample. Samples were filtered using a pressure vessel filtering system and mEI agar plates were used to culture Enterococcus. Colonies on plates were enumerated and concentrations of bacteria were calculated as cfu/100ml of water. Results: In a quality control trial, Enterococcus in spiked water run through the pump rapidly as a pulse and no prolonged tail was observed in breakthrough curve. Purge volume was determined to be 50 gallons as the concentrations of Enterococcus in individual well become stable after this volume. Percentages of Enterococcus positive wells were 93.8% (46/49) and 85.7% (67) respectively for monitoring wells and domestic wells, and the average concentrations were 9.1 and 0.91 cfu/100ml respectively.

Conclusions: The submersible pumping and pressure vessel filtering method can be used for detection of bacteria in dairy monitoring wells. Enterococcus is widely prevalent in dairy groundwater and can be used as indicator organisms for contamination. Differences exist in the occurrence of Enterococcus between different dairy farms.

Sample Preparation of Biothreat Agents in Complex Matrices Utilizing Two Automated Devices

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Background: Samples collected from environments and clinical sources may contain agents at low concentrations and in matrices which are difficult to process. Directly using these samples in PCR and ELISA tests does not reliably indicate the presence of biothreat agents. This research describes the development and testing of two devices that concentrate agents from complex matrices, remove potential assay inhibitors and non-target agents, and purify nucleic acids from captured organisms. Two portable devices are described, one that processes samples in the 0.5-5mL range and one for 10-50mL samples. Target concentration is achieved through immunomagnetic separation (IMS) using antibody coated super-paramagnetic beads. Nucleic acids are purified by organism lysis and DNA binding on magnetic beads. The process is fully automated. We tested the performance of these two devices for automated IMS and nucleic acid extraction of several biothreat agents from a variety of complex matrices.

Methods: The agents tested in this study include: E. coli, Salmonella, Listeria, Legionella, Cryptosporidium, Bacillus, West Nile Virus, Ricin, and SEB. These agents were spiked into the following matrices: water, swab samples, air samples, whole blood, sputum, and stool. These samples were subsequently tested on the two devices developed for this study. Extracted agents were confirmed by culture, ELISA, or microscopy. These captured agents were further processed for nucleic acid extraction. The DNA samples were then tested in PCR assays.

Results: Results show that the two devices used in this study allowed for efficient, fully automated IMS and nucleic acid extraction. Targets were detected by ELISA, culture, PCR, and microscopy, with recovery rates as high as 100%.

Conclusions: These data exemplify and demonstrate the utility of these devices and automated workflow.

Method Validation of DNA Extraction and Real-Time PCR for Qualitative Analysis of Environmental Swipe Samples for Biological Warfare Agents

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Edgewood Chemical Biological Center’s (ECBC) Environmental BioMonitoring Laboratory (EBML) provides high throughput screening of environmental samples using polymerase chain reaction (PCR) and electrochemiluminescent (ECL) immunoassay analysis in support of various government agencies. EBML is pursuing a laboratory accreditation under 17025 guidelines, which specifies the general requirements for the competence to carry out laboratory testing in accordance with laboratory-developed methods. The focus of this study was to design and perform a method validation test plan which will show the effectiveness of our unique laboratory-developed DNA extraction protocol, using two distinct robotic liquid-handling platforms, and also using a non-automated protocol. Validation of this qualitative test method was performed using a commercially available PCR platform, the Applied Biosystems 7900 HT PCR analyzer. Performance characteristics with defined acceptance criteria were established for assays specific to the following biological warfare agents (BWA): Bacillus anthracis, Yersinia pestis, Francisella tularensis, and Orthopoxvirus. EBML’s method validation process focused on assay limit of detection determinations followed by performance tests designed to provide predictive values for true negative and true positive test samples. The PCR performance testing consisted of 60 samples for each BWA assay, analyzed for true negatives and true positives with predictive values in the 95% confidence level. EBML’s performance test results indicate that the validation acceptance criteria were satisfied for both automated and manual DNA extractions and subsequent real-time PCR analysis, signifying that the methods may be deemed fit for use for analyzing environmental biological samples in wipe sample buffer matrix pending review from the accreditation body.
067 (E)

**Occurrence of Toxigenic Vibrio cholerae and Other Pathogenic Vibrios in the Aquatic Environments of Georgia**


**Background:** Vibrios are natural members of marine and freshwater microbial communities. At least 12 Vibrio spp. are considered human pathogens, including *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. The rapid detection of these pathogens is essential for their monitoring and surveillance in the environment. The goals of the current study are to examine the dynamic changes in abundance and composition of Vibrio spp. in Georgian water.

**Methods:** Four sampling sites along the Georgian coast of the Black Sea and three freshwater lakes (Kumisi and Lisi, Tbilisi Sea, near Tbilisi) were sampled monthly for 24 months. All samples were screened for the presence of *Vibrio* spp. using conventional bacteriological, molecular, and immunological methods. In addition, samples were examined by multilocus PCR-electrospray ionization mass spectrometry (PCR/ESI-MS).

**Results:** 278 DNA samples from all sampling sites were analyzed by PCR/ESI-MS. Multiple *Vibrio* spp. including *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. metschnikowii*, *V. alginolyticus*, *V. mimicus* etc. were detected. In the Black Sea samples, *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* were prevalent, whereas the freshwater lake samples were dominated by *V. cholerae*. Some of the freshwater lake DNA samples were positive for ctxA and ctxB genes Water and plankton samples (350) were examined by a direct fluorescent antibody method and revealed *V. cholerae* O1 in Kumisi and Lisi Lakes as well as from the Black Sea samples collected in the summer. In addition, weak positive signals for *V. cholerae* O1 were detected in three Black Sea samples, suggesting the presence of nonculturable forms.

**Conclusion:** The data suggest the existence of toxigenic *V. cholerae* O1 and 9 other pathogenic *Vibrio* spp. in natural aquatic environments in Georgia.

068 (E)

**Brucellosis Exposure Risk Factors in Regions of Uzbekistan**


**Background:** Two Brucella species, *B. abortus* and *B. melitensis*, are known to be endemic throughout the Republic of Georgia, with *Brucella melitensis* as the species responsible for causing human disease. Human infection is predominantly acquired through exposure to infected animals or animal products. During recent years human brucellosis has been widely distributed in eastern Georgia and is related to sheep-farming traditions in the country. Unfortunately, human brucellosis is substantially underreported due to the nonspecific clinical manifestations of the disease, the limitations of current diagnostic tests, and the widespread use of non-prescription antibiotics. Material and Methods: This study will examine serological prevalence of humans and ruminant animals in several areas across Georgia. This study will also attempt to culture *Brucella* from blood (humans and animals) and lactational fluids (animals) from sero-positive animals. *Brucella* isolates will also be subject to genotyping analysis. Discussion: There is clear need to explore additional or alternative assays to improve the surveillance of brucellosis and to allow effective treatment and control of this disease. Previous studies have been aimed at traditional diagnostic testing for *Brucella* based on culture isolation and detection of antibodies in the subject’s serum. The studies, proposed in this project will provide important information leading to the development of a comprehensive control program of brucellosis in Georgia and will also lead to more sensitive assays such as PCR are under development in this project and will provide linkages between human exposure and animal infection.

069 (F)

**Comparison of the Complete Genome Sequences of Six More Isolates of Rickettsia**

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**Background.** The genome sequences for *Rickettsia canadensis* CA10, *R. slovacca* CWPP, *R. massiliae* X7890, *Candidatus R. philippinensis* 364D, and *R. typhi* R991-CWPP and TH1527 were determined in a CDC-JGI-LANL collaborative effort. We investigated the unique and conserved features of these six new genome sequences. Methods. DNAs were extracted from *rickettsia* grown in Vero or irradiated 1329 cells and purified by Renografin density gradient centrifugation. The nucleotide sequences were determined using shotgun Sanger sequencing protocols. Additional information was obtained using 454 pyrosequencing. Sequence comparisons and annotations were made with MAVID, PipMaker, ORF finding programs (Glimmer, GeneMark, GetORF), REPUTER, rRNACan, SE, BLAST and ClustalW. Results. Only one new *rickettsia* plasmid was found. The sequence of the USA *R. massiliae* X7890 isolate plasmid differed from the French Mtn5 plasmid in a number of sites but was highly homologous to it. The California isolate of *R. canadensis* CA10 exhibited numerous single nucleotide polymorphisms (SNPs) in coding sequences as well as insertion/deletion events (INDELS) relative to the 9541 bp large Quebec isolate McKiel. The larger sequence of 364D differed sufficiently from that of *R.ickettsi* to permit its elevation to species status. Conclusions. The characteristics of the six new genome sequences reported here are very consistent with expectations based on the genome sequence previously obtained for 14 species in the genus *Rickettsia* (strong long-range synteny, frequency of INDELS and SNPs, higher plasmid than chromosome sequence diversity). The sites exhibiting sequence differences provide useful markers for molecular epidemiological investigations.

070 (G)

**Potential Ebola Therapies from Phenotypic Screening**

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**Background:** To date, Ebola lacks licensed vaccines or therapeutics for prophylaxis or post-exposure treatment. Phenotypic screening of approved drugs can discover therapies that can quickly be deployed, and which often target host factors with broad-spectrum activity. Methods: We assembled a library of about 3,000 approved drugs and biologically active molecules, and tested them at multiple concentrations for antiviral activity using vero cells infected with an engineered GFP-expressing Ebola virus. To rule out non-specific activities, we counter-screened for cytotoxicity using an ATPlite assay. In both cases, activity was measured using the maximum measured inhibition of expression relative to untreated cultures. Hits were selected using both activity measurements and clinical safety information, and comparisons between agents targeting the same protein were used to distinguish on- vs. off-target effects. Results: We identified ~130 potential therapies with selective antiviral activity on ~50 distinct mechanistic classes. Many of the antiviral activities discovered involve host cell mechanisms with previously unknown relevance to filovirus infection. Moreover, ~60% of the mechanistic classes were active in a similar assay for Hepatitis C virus, suggesting broad-spectrum antiviral potential. We also present preliminary studies testing combinations of these drugs.

**Conclusions:** High throughput phenotypic screening has revealed multiple novel approved drugs that inhibit Ebola virus, appear to have broad antiviral activity and can be rapidly advanced to clinical settings. Testing these drugs in combination can discover antiviral synergies that improve therapeutic selectivity and limit the emergence of resistance.
Development of Hexadecyloxypropyl Cidofovir (CMX001) for the Prophylaxis and Treatment of Smallpox Infection

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Cidofovir (CDV), which is approved for the treatment of cytomegalovirus (CMV) induced retinitis in AIDS patients, is active in vitro against all five families of dsDNA viruses that cause human morbidity and mortality including the orthopoxviruses. However, the clinical utility of CDV is limited by the requirement for administration by intravenous infusion and the frequency of acute nephrotoxicity. Conjugation of a hexadecyloxypropyl moiety to the phosphonate group of cidofovir increases in vitro antiviral activity up to a thousand-fold, promotes high oral bioavailability and has eliminated nephrotoxicity in all preclinical toxicology and clinical safety studies conducted to date. A Phase 1 single and multiple dose clinical study to provide pharmacokinetic, tolerability and safety data to support the smallpox indication has recently been completed in healthy volunteers, and the drug has entered Phase III/b/clinical development for the prevention and treatment of BK polyoma and CMV viral infections that cause major morbidity and graft loss in transplant patients. Pharmacokinetic and safety data gathered in immune suppressed transplant patients will be used in support of the smallpox indication since this population may either be contraindicated for vaccination, or unable to mount a completely protective immune response to smallpox infection despite vaccination. In the Phase 1 study single oral doses of CMX001 up to 2 mg/kg and multiple doses (three doses given on days 0, 6, and 12) of up to 1 mg/kg were administered safely and were well tolerated. No serious adverse events were observed. Gmax and AUC0-9 values increase in a dose proportional manner until the 2 mg/kg dose where there is evidence of saturation. Human plasma levels of CMX001 achieved after the administration of a single 1 mg/kg dose of CMX001 exceed the plasma exposures necessary to completely prevent disease progression in mouse and rabbit models of human smallpox infection (single 20 mg/kg dose).

Compounds Exerting In Vitro and Cell Based Activity against Burkholderia mallei and Burkholderia pseudomallei with Potential for Broad Spectrum Performance.


Background: One hundred seventy three test compounds have been known to exhibit extracellular in vitro activity against select agents. Of these compounds, nine were chosen for initial testing because of their known broad spectrum in vitro activity against Burkholderia mallei (BM), Burkholderia pseudomallei (BP), Bacillus anthracis, Yersinia pestis, Francisella tularensis, and Brucella abortus. Method: SRI International has initiated screening of these compounds for intracellular activity against BM and BP using murine macrophages. Results: Seven compounds have demonstrated activity at a minimum inhibitory concentration (MIC) of 16 µg/ml or lower. Activity was not found against BM; however, two compounds were found to be active against BP at an MIC of 0.5-2 µg/ml. The MICs of another four compounds were 2-8 µg/ml, while two compounds exhibited activity at 8-16 µg/ml. Conclusions: Data collected from drug screening will allow SRI to deduce possible mechanisms of action and identify structure-activity relationships that can be responsible for broad spectrum activity at several molecular levels. The work was supported under NIH contract HHSN2662004000051/N01-AI-40005, with Dr. Kristin DuBord as Project Officer.

Construction and Characterization of a Chimeric Neutralizing Antibody Directed Against Botulinum Neurotoxin A

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Background: Potential use of biological agents for terrorism purpose should reinforce the interest for immunotherapeutic approaches, involving antibodies attenuating or neutralizing the effects of these agents. Indeed for several agents of biological threat, antibodies remain the only effective therapy for in vivo neutralization, despite possible side effects. The present work aims at preparing chimeric immunoglobulins against botulinum neurotoxin type A (BoNT/A), a class A agent of bioterrorism. Methods: Mouse monoclonal antibodies (mAbs) were produced against BoNT/A and tested for their in vitro neutralizing effect. The most efficient one was selected to produce a chimeric antibody useful for human therapy. The variable regions of this mAb were cloned and fused with the constant counterparts of human IgG1 (light and heavy chains). Chimeric antibody production was evaluated in mammalian myeloma cells (SP2/0-Ag14) and insect cells (Sf9). After purifying the recombinant Ab by affinity chromatography, the biochemical properties of chimeric and murine Ab were compared. Results: Concentrated recombinant chimeric antibody has been produced in mouse ascitic fluid. The chimeric antibody and its murine relative exhibited the same dissociation constant (kd) of 10^-12 M as determined using Biacore. Moreover, the chimeric antibody retained the same in vitro neutralizing capacity as the murine one, and its pharmacokinetic properties are under evaluation in mice. Conclusions: The strong affinity and the high neutralizing efficiency make the present chimeric antibody interesting for immunotherapy in humans in case of intoxication with the most dangerous Botulinum neurotoxin, further supported by the probable limitation of immunological side effects classically observed using heterologous immunotherapeutic sera.
075 (G)  
**Inhibition of Poxvirus and Filovirus Systems by Nanostructured Agents**  
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**Background:** Broad-spectrum antimicrobial agents for key biodefense threats such as smallpox virus or filoviruses have been identified as a high priority for development of medical countermeasures by the Department of Health and Human Services. The present study seeks to develop and examine such new countermeasures based on emerging nanotechnologies. **Methods:** Enginnered silver nanoparticles (AgNPs) were used to inhibit two viral systems based on identified biodefense threats. The AgNPs were acquired commercially or synthesized in-house using reduction methods. TEM and UV-Vis were used to evaluate the physiochemical properties of the AgNPs. The AgNPs were tested against a smallpox analog and select agent viral pseudotype system capable of expressing a variety of glycoproteins responsible for wild type viral entry. High throughput antiviral assays were developed for this project to facilitate the screening of the AgNP formulations. **Results:** Results to date showed complete viral inhibition with doses as low as 15 micrograms per mL for some AgNPs in the absence of cell toxicity. Partial viral inhibition was observed in the 2-15 micrograms per mL range for both viral systems. Protection from viral infection was dependent on AgNP size and distribution in solution; small and monodispense particles demonstrated greater broad-spectrum antiviral activity. **Conclusions:** Thus, AgNPs have the potential to serve as a promising broad-spectrum antiviral agent with a high therapeutic index. Additional preclinical and mechanistic studies are underway and the results will be presented. The AgNPs under study are highly stable and capable of being stockpiled in the event of a natural or man-made disaster. Such stability, combined with the broad-spectrum antiviral nature of the AgNPs, would allow for immediate short-term treatment in the field before an accurate diagnosis and specific treatment could be determined.

076 (G)  
**Sudan Ebolavirus Specific Murine Monoclonal Antibodies**  

Viruses of the genus *Ebolavirus* (EBOV) cause hemorrhagic fever that is 50-90% fatal for humans. The majority of research in the filovirus community has focused on the *Zaire* virus species of EBOV; however the *Sudan ebolavirus* (SEBOV) species is of similar public health concern. Given this deficiency, we chose to direct efforts to developing reagents and therapeutic options for the Boniface and Gulu strains of SEBOV. We evaluated murine monoclonal antibodies directed against the glycoprotein (GP) of Boniface that were generated through vaccination with Venezuelan equine encephalitis virus replicon (VRP). Thus far we have identified thirteen IgG1 monoclonal specific for Boniface and Gulu strains by ELISA and western blot. The majority of these monoclonals are IgG1 isotype; however, we also identified three IgG2a and one IgG2b isotypes. After infecting VERO cells with VRP expressing the GP of Boniface or Gulu, we showed the utility of three of these antibodies by flow cytometry. We determined the sequences of the GP to which these antibodies were binding utilizing SPOTS membranes. Additionally, we assessed the capacity of these monoclonals to neutralize Sudan viral infection *in vitro* with and without complement. The development and characterization of these reagents will help expand the scope of filovirus research. These monoclonals may be beneficial as a viable therapeutic option for individuals infected with Ebola Sudan.

077 (H)  
**Boosting Bioterror Agents Vaccine Efficacies**  

We will demonstrate the efficacy and broad applicability of the immune-modulating HyperAcute (R) alphaGal Technology. **Background:** The alphaGal Technology is the only platform that exploits the natural zoonotic barrier against infection by pathogens from lower mammals. This defence system is based on anti-alphaGal epitope antibodies that rapidly recognize and target foreign pathogens to antigen-presenting cells via Fc receptors; this leads to a robust humoral and cellular immune response. Vaccines modified with alphaGal epitopes are substantially more potent compared to their unmodified counterparts. The alphaGal epitope functions like an adjuvant in that it enhances the robustness of an immune response toward a modified antigen. **Methods:** alphaGal epitopes can be applied to vaccines by biological, enzymatic or chemical means, and this step can be readily integrated into existing cGMP manufacturing processes. To demonstrate proof of concept, we applied our proprietary alphaGal Technology to the development and evaluation of viral vaccine candidates for Ebolavirus (EBOV), Influenza (Flu), Lassa virus (LV) and Rift Valley fever virus (RVFV) using peptides, pseudotyped virus and virus-like particles (VLPs). **Results:** 1. AlphaGal-modified EBOV and LV GP-pseudoviruses are preferentially neutralized by normal human serum, which illustrates the robustness of alphaGal immunity. 2. AlphaGal modification of Flu HA leads to a 100-fold increase in antibody titers, indicating that alphaGal modification can enhance the T_{b2} response. 3. Cytokine secretion is greater from PBMCs isolated from mice immunized with alphaGal-modified EBOV GP-pseudoviruses compared to unmodified vaccine. Profiles suggest a balanced T_{b1}/T_{b2} (T_{b0}) response. 4. alphaGal-modified vaccine candidates are more efficacious than unmodified vaccines in a lethal challenge model. **Conclusion:** Taken together, alphaGal modification enhances the immunogenicity of antiviral vaccines, leads to the development of a balanced T_{b1} and T_{b2} immune response, and to superior protection in challenge models.

078 (H)  
**Comparative Evaluation of Three Types of Anthrax Toxin Neutralization Assays**  
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**Background:** Anthrax toxin neutralization assays (TNA) have been used in clinical studies to measure the ability of antibodies against protective antigen (PA) to neutralize the action of anthrax toxin on cells. The three commonly used TNAs are a J774A.1 cell-based lethal toxin (LT)-TNA, a RAW 264.7 cell-based LT-TNA, both of which measure the ability of antibodies to neutralize the cytotoxicity of LT, and a CHO cell-based edema toxin (ET)-TNA which measures the ability of antibodies to neutralize ET-induced increases in intracellular cAMP. We have investigated the three TNAs with the goal of establishing whether they yield comparable estimates of neutralizing activity. **Methods:** Rabbits were immunized with rPA (10 μg/dose) adsorbed to Alhydrogel at days 0, 28 and 56, and bled on days 0, 14, 28, 42, 56 and 70. Using each of the TNAs, serial dilutions of test sera and a rabbit anti-rPA reference serum were assayed. The reference serum was a pool of sera from 190 rabbits immunized with rPA at weeks 0, 4 and 8, and bled at weeks 6, 8, 10 and 11. Cell viability was estimated in UT-LTNAs whereas cAMP was estimated in the ET-TNA. A 4-parameter logistic regression analysis was employed to estimate ED_{50} values for both test and reference sera. Relative activity was calculated by dividing the ED_{50} for test serum by that of reference run on the same plate. **Results:** The estimates of ED_{50} for a given serum sample were significantly different for the three assays, as might be expected. However, the calculated relative activities were similar for all three assays. For later bleeds, the quantitative agreement between assays was extremely good but early bleeds showed some heterogeneity in relative estimates. Thus, even though the mechanisms contributing to toxin neutralization may differ for the three assays, when toxin neutralizing activities are determined relative to an appropriately-selected common reference, our results indicate that the assays yield comparable estimates of neutralization.
079 (H)

Long-term Protective Efficacy and Antibody Response Induced by a Single Dose of Attenuated Smallpox Vaccine LC16m8

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Background: The attenuated smallpox vaccine LC16m8 was developed in Japan and is known to have less adverse events compared to conventional smallpox vaccines. It is believed that LC16m8 should be an effective counter medicine against bio-terrorism. In this study, we investigated both the long-term protective efficacy and the antibody response induced by a single dose of LC16m8 in animal models.

Methods: BALB/c mice were vaccinated with a single dose of LC16m8 or Lister by multiple-puncture with a bifurcated needle. Among them, a certain number of mice were challenged intranasally with a lethal dose of WR virus and the same number of mice was left unchallenged and used for the WR-specific PRNT and WR-specific proteome microarray analysis at each evaluation point up to 1.5 years post immunization.

Results: All of the non-immunized mice died from a lethal intranasal WR challenge at all the evaluation points, while LC16m8 vaccinated mice exhibited neither death nor continuous weight losses, and they also showed a high protection up to 1.5 years post immunization. Also, LC16m8 immunized mice maintained a high level of neutralizing antibody titers (NTs) as well as the Lister immunized mice up to 1.5 years post immunization. Further, in both immunized mice, WR challenge boosted NTs at 1.5 years post immunization as well as 7 weeks. In the proteome microarray analysis, both LC16m8 and Lister immunized mice showed a high reactivity against two EEV antigens (A56R and A33R), three IMV antigens (D13L, H3L, and D8L) and one core antigen (IL1). Further, the reactivity against EEV and IMV antigens remained high up to 1 year post immunization.

Conclusions: This study showed that the efficacy lasted over a long term, and the level of efficacy and the induced antibody response were similar to those of the Lister.

080 (H)

Characteristics of Antibiotic and Vaccine Therapy and Relapse of Brucellosis in Georgia - Chart Review Analysis

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Background: The treatment of brucellosis in Georgia has changed substantially over the last decades. We conducted a review of the treatment of patients with a confirmed diagnosis of brucellosis at the Institute of Parasitology and Tropical Medicine (IP) in Tbilisi. One particular interest was the use of intravenous Brucella vaccine therapy, which was in practice before 1980.

Methods: Data were abstracted from patient records of the IP. In total, 300 records were reviewed from three time points: 1970-73, 1980-89, and 2004-2008. Results: Routine antibiotic treatment at the Institute of Parasitology was three courses of different antibiotics with 10 days of treatment interruption between each course. A total of 19.1% of patients had received antibiotic treatment before admitting to the IP. The most commonly used antibiotics was chloramphenicol. Generally, vaccine therapy was applied to 50 patients (17.1%). In the 1970-75 period, most of the patients with relapse were treated by the vaccine and no antibiotics. Each administration of vaccine typically induced a temperature reaction of more than 38°C. In 22.9% of patients, vaccine therapy was stopped because of side effects. The frequency of relapsed infection was 14.7% - 44 cases. The percentage of treated cases with relapsed infection declined in the three analyzed time periods.

Conclusions: These data on brucellosis treatment outcomes in Georgia are consistent with reports from other countries. The occurrence of relapsed brucellosis infection after initial treatment was most common during 1970-1973, which was also the period of widespread use of vaccine therapy exclusively. Establishing this correlation requires further investigation. The observations provide insight for efforts to develop a human brucellosis vaccine.

081 (H)

Stability of Dried Adenovirus Preparations at Elevated Temperature over Time

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Background: Thermal stability of adenovirus is an issue which will have an impact on the widespread use of adenovirus vectors for prophylaxis or therapeutic use. Currently, strict temperature control is essential to preserve the infectivity of the vector, which limits the application of adenovectored technologies. Lyophilized or aqueous formulations of viral preparations usually require refrigeration during storage and distribution to avoid loss of infectivity of the preparation. Maintaining correct storage requirements can be both logistically challenging and expensive. Stabilitech Ltd have developed a proprietary stabiliser which, when mixed with virus supernatants, prevents thermal damage enabling the product to be lyophilized and stored at elevated temperatures. Furthermore, upon reconstitution, the stabilizer product shows no observable loss of infectivity based on the titre of the preparation as measured by plaque assay. Stabilitech’s approach is based on mimicking of biochemical events occurring during the maturation of seeds. During this process, seeds are rendered desiccation and thermo-tolerant.

Methods: 50µl of virus/vaccine solution was mixed with 250µl of stabilizer. The mixture was frozen and lyophilized over the course of 2 days. Accelerated thermal challenge studies were performed by holding the samples at 57°C for up to 68 days. At intervals during this period virus infectivity assays were carried out. Further longer term studies are ongoing. Results: Adenovirus was rendered thermally stable to a high level over the timeframe examined. Infectivity losses observed during processing were negligible (consistently less than 1 log10). In the absence of excipients, or removal of any one excipient component, a significant loss of infectivity occurred. Conclusions: Our results demonstrate that Stabilitech’s technology has overcome a major hurdle affecting vaccine storage and distribution. This technology also has the potential for expanded application to other biologic products.

082 (H)

A Double-Blind, Parallel-Group Study of the Safety, Tolerability and Immunogenicity of a Recombinant Protective Antigen (rPA) Anthrax Vaccine in Healthy Subjects

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Background: The study aim was to evaluate safety, tolerability and immunogenicity of SparVax (an rPA vaccine). 2 different dose levels and 2 different schedules were tested. Subjects received 3 priming doses and a challenge dose, in order to assess immunological recall.

Methods: 415 healthy volunteers were enrolled into 4 groups in the priming phase of the study. Two 3 dose schedules were examined at 2 different dose levels. At day 182, subjects were re-randomized to receive a challenge dose of SparVax at the original dose level, on either day 182 or 364. Results: Safety: Overall, there were no differences in the incidence of subjects reporting at least one possibly associated treatment-emergent AE and no vaccine related serious AEs were recorded. This was true for both the priming and challenge phases of the study. There were no significant differences among the four dose groups regarding safety results. Immunogenicity: Geometric mean (GM) anti-rPA antibody concentrations were measured by ELISA on day 14 after the third dose. The data showed that the titers were significantly higher on one regimen (by almost 5x) than those on the other regimen. No significant difference in GM titer was seen between groups that received different dose levels. An antigenic challenge dose was given at either day 182 or day 365. A significant response was seen in all groups that received an antigenic challenge, but no significant difference in the level of that response was seen across these groups. Conclusions: The rPA vaccine is safe and well tolerated. The immunogenicity data reported confirm that the rPA vaccine is immunogenic for both doses as well as both dosing schedules. However, the data suggest that the dosing schedule has a clinically and statistically significant effect on anti-rPA antibody concentration, independent of rPA vaccine dose. The challenge phase data suggest that the rPA vaccine promotes good immunological recall.
Immune Response and Side Effects to the Live, Attenuated Plague Vaccine Utilized to Protect Laboratory Workers in Georgia

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Introduction: The live, attenuated plague vaccine has been employed throughout the countries of the former Soviet Union to protect laboratory workers against potential exposure. There are minimal data in the English-language literature regarding the side effects and immune response to this vaccination. Methods: Laboratory workers receiving the live, attenuated plague vaccine at the NCDP were recruited to provide blood samples before and after primary and booster vaccination. Samples were obtained at the following time points: pre-vaccination, day 8, 14, 28, 64 and 6 months, and tested for antibodies to F1 and V antigens using ELISA. Vaccine recipients were assessed for local and systemic symptoms at day 1, 2, and 8 after vaccination. Results: Sixteen volunteers provided blood samples before and after initial vaccination, with only one volunteer developing a low-level anti-F1 antibody response. Three volunteers had low-level baseline anti-V antibodies and one volunteer developed anti-V antibodies after vaccination. Twenty volunteers provided blood samples before and after booster vaccination. Of these, 8 developed anti-F1 antibodies (15.6±9.3 ug/ml among responders). Eight of 22 receiving the booster had anti-V antibodies at baseline. One of these volunteers developed a booster antibody response. Side effects of the primary and booster vaccination were minimal. Conclusions: The live, attenuated plague vaccine is well-tolerated, but does not produce a strong serological response to F1 and V antibodies after primary vaccination. Some volunteers developed an immune response after booster vaccination. More study is needed to characterize the immune response to this vaccination.

Secretory Expression of K88 Fimbrial Adhesin FaEG by Lactococcus lactis and its Protective Immune Response in Mice

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Background: Enterotoxigenic Escherichia coli (ETEC) strains that produce K88 (F4) fimbriae on their surfaces commonly induce diarrhea in piglets, which is an important cause of both mortality and reduced growth rate. FaEG is the major subunit of K88 fimbriae and plays an important role in the pathogenesis of ETEC infections. As a GRAS organism, L. lactis is considered to be a good candidate to develop safe oral vaccine. The purpose of this study was to determine the protective effect of L. lactis-FaeG recombinant strain in C57Bl/6 mice challenged by ETEC. Methods: L. lactis-FaeG was used for challenge and the L. lactis subsp. Cremoris NZ9800, NZ9700 as the hosts. The recombinant plasmid pNZ8048-FaeG was constructed, then introduced into the host Cremoris NZ9800, NZ9700, respectively. The raisin controlled expression of FaEG was tested by Western blotting. The L. lactis-FaeG strain and control were delivered orally to C57Bl/6 mice. The F4-specific antibody production was checked by ELISA and the interaction between immunized mice and FaEG in NZ9800 was detected by slide agglutination test. The protective effects were demonstrated after challenge with ETEC C85549. Results: L. lactis-FaeG could successfully induce the F4-specific antibody in immunized mice. The mortality rate of mice immunized with the L. lactis-FaeG (10%) was lower than that of two control groups inoculated with the L. lactis (50%) or PBS (66%) after challenge with virulent ETEC. Moreover, most mice in control groups developed diarrhea after challenge, together with evident lesions in viscera. The slide agglutination test showed the sera from immunized mice could induce a macroscopic agglutination of ETEC. In contrast, the sera from unvaccinated mice evidenced no agglutination. Conclusions: The L. lactis-FaeG could induce protective immune response against ETEC infection in mice. Expression of FaEG in food-grade bacterium, L. lactis, may lead to a promising use in prevention of piglet’s diarrhea and has potential for widespread application.

Proteomics Investigation of Salmonella-infected RAW 264.7 Macrophages

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Macrophages play central roles in Salmonella-mediated systemic infection. To investigate the global responses of macrophages to Salmonella infection, we infected RAW 264.7 macrophages with Salmonella enterica serotype Typhimurium (STM) and then performed a comparative liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics analyses of the infected macrophages. A total of 1006 macrophage and 115 STM proteins were identified with high confidence from this study. Most of the STM proteins were found in the late stage of the infection time course, consistent with the fact that STM proliferates inside RAW 264.7 macrophages. The peptide abundances of the most identified macrophage proteins, including housekeeping proteins glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and cytoplasmic superoxide dismutase 1 (SOD1), were relatively constant during the time course of infection. Compared to those in the non-infection control, the peptide abundances of 244 macrophage proteins (or 24% of total identified macrophage proteins) changed significantly after STM infection. The functions of these STM infection-affected macrophage proteins were diverse and ranged from production of antibacterial nitric oxide (i.e., inducible nitric oxide synthase (iNOS) or production of prostaglandin H2 (i.e., cycloxygenase-2 or COX-2) to regulation of intracellular traffic [e.g., sorting nexins (SNX) 5, 6 and 9], demonstrating a global impact of STM infection on the macrophage proteome. Western blot analysis not only confirmed the LC-MS/MS results of GAPDH, SOD1, COX2, iNOS and SNX6, but also revealed that the protein abundance of mitochondrial SOD2 increased after STM infection, indicating an infection-induced oxidative stress in mitochondria.

An Antigen-Specific B-1a Antibody Response Induced by Francisella tularensis Lipopolysaccharide Is Required for Protection against Francisella tularensis Challenge

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Background: Francisella tularensis (FT) is the causative agent of tularemia. Infection of mice with <10 FT Live Vaccine Strain (Ft LVS) organisms i.p. causes a lethal infection that resembles human tularemia. Ft is classified as a Category A agent due to its ability to be disseminated via the aerosol route, low infectious dose, and potential to cause severe morbidity and mortality. Methods: Animal survival studies, Hi-Dimensional FACS analysis, ELISA assays. Results: Immunization with as little as 0.1 ng Ft LVS lipopolysaccharide (Ft-LPS), but not Ft lipid A, generates a rapid antibody response that protects wild-type mice against lethal Ft LVS challenge 2-3 days later. Protection is not induced in Ft-LPS immunized B cell-deficient mice (µMT or JhD), male xid mice, or IgG transgenic mice engineered to produce only a single IgG. Focusing on the cellular mechanisms that underlie this protective antibody response, we show that: 1) Ft-LPS immunization specifically stimulates proliferation of B-1a lymphocytes and plasma cells that bind fluorochrome-labeled Ft-LPS; and, 2) these plasma cells secrete anti-Ft-LPS antibodies that are detectable in serum. This B-1a cell response, which peaks 5 days after immunization with Ft-LPS, is not dependent on T cells or typical inflammatory processes as it is normal in T-deficient (TCRαβ -/-, TCRγδ -/-) and Toll-like receptor 4 (TLR4)-deficient (TLR4-/-) mice. Conclusions: Immunization with soluble Ft-LPS activates a small, distinct, antigen-specific subset of B-1a cells and this induced response prevents fatal infection in the absence of inflammation or T cell help.
Towards Multiplex Immunoassays for Non-Human Primate Cytokines

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Background: Non-human primate (NHP) models of infectious disease are often the means to analyze efficacy of vaccines and therapeutics before entry into clinical trials. To understand the successes and failures of candidate treatments a full analysis of host responses including cytokine profiling is warranted. Although antibodies for multiplex immunoassay analysis of human cytokines are available, they are often poorly cross-reactive with the equivalent NHP cytokines. Using the Marmoset as a starting place, we aim to develop panels of cytokine specific antibodies to fill this technology gap. Methods: A range of cytokine genes were PCR amplified based upon degenerate primers designed using human cytokine gene sequences. Recombinant proteins for immunization were purified to homogeneity from material secreted to the E. coli periplasm. Llamas were immunized with proteins, immune repertoires cloned and displayed on phage and single domain antibodies (sdAbs) selected and screened on recombinant proteins purified from 293T cell supernatants. Bioplex/Luminex was used to find pairs of non-competing sdAbs and determine lower limits of detection (LOD) on recombinant cytokines and on cytokines present in marmoset plasma or supernatants of stimulated marmoset PBMC. Results: Genes encoding IL4, IL6, IL10, IL18, and TNF-α were cloned, sequenced, expressed and purified. Seroreconversions were observed in all llamas immunized with the recombinant cytokines. A panel of four sdAbs so far isolated against IL6 indicates a lower LOD towards 1 ng/mL in an unoptimized screening assay. Conclusions: Sensitivity of the initial Marmoset IL6 assay indicates the approach may be feasible for these and perhaps broader panels of cytokines with some optimization. Since the methods are based upon recombinant protein and recombinant antibody technology, we should be able to provide unlimited yet defined monoclonal NHP cytokine resources to other non-profit investigators.

Cellular and Humoral Immune Response of Cynomolgus Macaques to Francisella tularensis LVS and SCHU S4 Antigens

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Background: and Methods: In order to aid in the development of novel vaccine candidates for Francisella tularensis, we have developed assays to detect humoral and cellular immune responses in both non-vaccinated and LVS-vaccinated non-human primates (NHP, cynomolgus macaques of Vietnamese origin). Results: Surprisingly, we have found that approximately 10% of all NHPs tested have detectable IgG anti-LVS antibodies in their plasma prior to LVS vaccination. In addition, when peripheral blood mononuclear cells (PBMCs) were purified from non-LVS-vaccinated NHPs, greater than 50% of such preparations responded to either formalin-fixed (FF) or heat-killed (HK) LVS by secreting IFNγ as measured by ELISPOT assay. Formalin fixed preparations of LVS stimulated the highest percentage of PBMC preparations from non-LVS vaccinated NHPs to secrete IFNγ and did so in a dose-dependent manner. When proliferation was measured, approximately 40% of non-LVS vaccinated NHPs responded to either FF- or HK-LVS or -SCHU S4. Patterns of IFNγ secretion to FF- or HK-LVS and SCHU S4 were reproducible upon subsequent testing of independent PBMC preparations from individual NHPs. Upon vaccination of NHPs with LVS, both IgG anti-LVS titers and the responses of PBMCs to FF- and HK-LVS or SCHU S4 were increased above the levels observed in non-vaccinated NHPs in all assays. Conclusions: These data suggest that pre-screening of NHPs is advisable prior to LVS vaccination such that vaccination-specific responses can be differentiated from baseline responses when using FF- or HK-LVS or SCHU S4 to test such responses ex vivo.

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Chlorine Dioxide Disinfection of Biothreat Agents in Drinking Water


Background: Concern for the security of our drinking water has led to collection of data regarding the efficacy of disinfectants on biothreat agents in water. While chlorine dioxide (ClO2) is only used by 8% of water treatment plants for disinfection of drinking water, public health officials may benefit by understanding the efficacy of ClO2 against a select group of biothreat agents. This study investigated the efficacy of ClO2 to inactivate 3 species of biothreat bacteria. Methods: The efficacy of ClO2 was tested on 2 strains each of Bacillus anthracis (BA) spores (Sterne and Ames), Yersinia pestis (YP; A1122 and Harbin) and Francisella tularensis (FT, LVS and NY98). Tests were performed in KH2PO4 buffer at pH 7, 8 and 5 and 25°C, 2 mg/L ClO2 for BA spores and 0.25 mg/L for YP and FT. Contact time (CT) values (ClO2 concentration in mg/L x time in min) were calculated for a 3-log reduction (CT 99.9) in viability. Results: >20,000 ppm available chlorine, 4% formalin in PBS, 2% paraformaldehydesolution, and 4% formalin in PBS with less than 2 hours contact time were unsuccessful. Conclusion: Several protocols for inactivation of a variety of samples that originated from Monkeypoxvirus-infected primates were assessed and satisfactory inactivation was achieved.
Inhibitory Effects of Fatty Acids on Bacillus sps.
K. LeFevre1, A. Raulerson1, D. D. Bowman2, M. A. Butkus1, M. P. Labare1; 1United States Military Academy, West Point, NY, 2Cornell University, Ithaca, NY.

The minimum inhibitory concentrations (MIC) of short chain fatty acids for vegetative cells of Bacillus subtilis and B. anthracis Sterne’s strain were determined. Seven fatty acids (FA), acetic through octanoic, were tested at a pH of 4.0 and 7.0. Of those tested pentanoic and hexanoic acids were the most toxic, with MIC of 6.3 mM and 0.95 mM, respectively at pH 4.0. The MIC for all the FA at pH 7.0 were at least one order of magnitude greater than at pH 4.0. The toxicity decreases as the carbon chain increases; octanoic acid does not inhibit growth of vegetative cells at the highest concentration tested. The germination of B. subtilis spores was also inhibited by the FA at pH 4.0 with and without 200 mM Malanine added as a germinant. The exception was octanoic acid, which in the presence of palmitic, appeared to enhance germination. At pH 7.0, acetic, propanoic and butanoic acids all reduced B. subtilis alanine-induced spore germination. However, the FA, pentanoic, hexanoic and heptanoic acid (without alanine) did not significantly inhibit germination to the extent as seen at pH 4.0. These same FA, amended with alanine, appeared to enhance germination when compared to the NaCl plus alanine treatment. Again, octanoic acid yielded different results (i.e. germination was reduced).

Development and Validation of Vaporized Hydrogen Peroxide Decontamination of a Class III Biosafety Cabinet

Background: The University of Pittsburgh Regional Biocontainment Laboratory (RBL) is a large, multi-user BSL-3 enhanced facility. The RBL houses an Aerobiology Core that contains a Class III biological safety cabinet (BSC) for aerosol exposure of animals. Given the need for exposure of multiple animal species to different BSL-3 pathogens, vaporized hydrogen peroxide (VHP) technology was purchased for rapid, safe, and effective decontamination of the Class III BSC. This study will present the cycle development and validation for VHP decontamination of the Class III BSC and its mobile transfer cart. Methods: The VHP 100P portable units (Steris Corp.) were purchased to enable gaseous/vaporous decontamination of spaces and equipment within the RBL. The cycle development guide furnished with the unit was used to develop parameters for each of the four stages of the VHP process. Biological indicators consisting of bacterial spores and chemical indicators (color-change strips) were used for determination VHP treatment efficacy. Real-time levels of VHP were monitored using a two-wire gas transmitter located inside the Class III BSC. Cycles were developed and validated for the Class III BSC (with aerosol exposure equipment) with the docked mobile transfer cart. Results: VHP inactivated biological indicators placed throughout the Class III BSC and mobile transfer cart, and validation data will be presented. Engineering control changes which contained VHP followed by rapid flushing and removal of VHP will also be reviewed. A relatively short but validated, 2 hour cycle time will allow for rapid turnover of the Class III BSC in between pathogens. Conclusions: Development and validation of a gaseous decontamination method for a Class III BSC used in aerosolization studies is essential to reduce cross-contamination between studies. Furthermore, the short decontamination time increases the capacity of a multi-user aerobiology Core facility.

Effectiveness of Commercially Available Antimicrobial Peptides Against Bacillus anthracis Sterne
R. Quizon, E. Seay, S. Kim, C. G. Young; The Johns Hopkins University Applied Physics Laboratory, Laurel, MD.

Background: Antimicrobial peptides with recurrent structural and functional themes have been isolated throughout microbial and eukaryotic phyla. Therefore, they are believed to represent an ancient host defense mechanism that is just now beginning to be understood as an inexpensive approach for a broad range of applications in the fields of medicine, therapeutics, veterinary health, decontamination, and protective materials. We investigated the ability of a number of commercially available antimicrobial peptides to kill growing cultures of Bacillus anthracis Sterne strain in order to identify candidates for further research in decontamination approaches. Methods: Three different commercially available antimicrobial peptides were tested in this study including Parasin 1 (Quality Controlled Biochemicals), Indolicidin (Quality Controlled Biochemicals) and Theromacin (Anspec). Two-fold serial dilutions of each peptide were added to tubes containing trypticase soy broth (TSB) or to trypticase soy agar (TSA) plates to reach final concentrations of peptide between 1 and 128 pg/mL. Liquid and solid media containing each peptide as well as controls that did not contain antimicrobial peptide were then inoculated with standard concentrations of a B. anthracis Sterne overnight broth culture and incubated for 18 hours at 37°C prior to determining cell numbers by spread plating or observing solid media for growth. Results: The results of this study showed that neither Parasin 1 nor Theromacin had any significant affect on B. anthracis Sterne growth at the concentrations tested. However, Indolicidin did inhibit B. anthracis Sterne growth with a calculated minimum inhibitory concentration (MIC) of 32 µg/mL. Conclusions: Indolicidin is an effective antimicrobial peptide against growing cultures of B. anthracis Sterne and will be used in further studies to investigate the potential use of antimicrobial peptides as a decontamination strategy.

Closing the Gap: Training Biodefense Professionals for the 21st Century
P.J. Cummings1, K. M. Obom2; 1Johns Hopkins Univ., Baltimore, MD, 2Johns Hopkins Univ., Rockville, MD.

Responding to a biological threat requires a highly skilled workforce. Johns Hopkins University (JHU) and the United States Army Medical and Research Institute of Infectious Disease (USAMRIID) have partnered to develop a Master of Science degree in Biotechnology with a concentration in Biodefense. Students who pursue this program receive a strong foundation in Biochemistry, Cell Biology, Molecular Biology and Bioinformatics on which to build their understanding of biodefense research and application. Students take required courses in Biological & Chemical Threat Response and Forensics, Medicine & Policy in Biodefense; and can choose electives such as Vaccinology, Radiation Biology, Biodefense Lab Methods, Immunology, Emerging Infectious Diseases and policy courses. As part of the curriculum, students may elect to do wet lab or computer lab research through an Independent Research Project course with USAMRIID investigators. A new educational opportunity recently established between JHU and USAMRIID provides students in depth training in biodefense studies through a fellowship program. The competitive fellowship program gives students the opportunity to work at USAMRIID on projects in the field of biodefense for up to two years, while completing a M.S. graduate program at JHU’s Montgomery County Campus in Rockville, MD. Graduates of this fellowship program will have an in-depth knowledge and practical laboratory skills in the field of biodefense, and they will fill a growing need for trained professionals for this essential discipline within the science community and for homeland security. This program serves as a model for developing the trained workforce necessary to protect the nation’s security in the 21st century.

Characterization of an Efficient Nebulizer for Generating Bioaerosol Exposures

Background: Key difficulties in aerosol generation of living biological materials, such as a bacterial suspension, are maintaining the viability of the organism during the aerosolization process as well as maximizing the efficiency of delivering the aerosolized material to the target. While the Collison nebulizer is the industry standard, it requires high shear forces resulting in lowered viability of aerosolized bacterial cells. Several nebulizers were evaluated as alternatives to the Collison nebulizer for use with a 64-port Canon-style nose-only bioaerosol system. We show here that the Pari LC Plus with a nose-only exposure chamber, is an effective method for bioaerosolization studies and a suitable alternative to the Collison nebulizer. Methods and Results: The Pari LC Plus nebulizer is a jet nebulizer which depends on pressure to drive the nebulization process. For operation within the bioaerosol system, the Pari LC Plus nebulizer was modified to spray upwards, rather than sideways as originally designed. With Escherichia coli as the test organism, six Pari LC Plus nebulizers were operated simultaneously to evaluate the effect of pressure on viability. When operated at 20 psi for 10 min with a 9.8 x 10^6 CFU/ml culture, the E. coli cells withstood the shear forces generated by the nebulizer and yielded 4.6 x 10^6 total CFU and 5.1 x 10^5 CFU E. coli/L air with a spray factor of 8 x 10^-5. Using an Anderson viable-count 6-stage impactor, the average particle size generated by the Pari LC Plus was within respirable range with a MMAD of 1.86 µm and a GSD of 1.55. The complete bioaerosol system was also measured for spatial port output homogeneity and found with viable counting methods to be within 15% coefficient of variance. Conclusions: These results indicate that the Pari LC Plus nebulizer in conjunction with our bioaerosol system is an effective bioaerosol generation method and demonstrates that our bioaerosol system can maintain a high degree of viability as well as consistent generation of bacterial cells.
**Criteria for Addition or Removal of a Biological Agent or Toxin from the Select Agent List**

**J. F. Aldrich, J. M. Canaves, B. Sherwin, V. Sutton; Texas Tech University School of Law, Lubbock, TX.**

**Background:** In 2005, the United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) established final rules regarding the possession, sale, and transfer of select agents, as required by the Public Health Security and Bioterrorism Preparedness Act of 2002. These select agent rules, set forth in 42 CFR part 73 (HHS), 7 CFR part 331 (USDA), and 9 CFR part 121 (USDA), list a total of 52 select agents. Select agents, mainly pathogenic bacteria, viruses, fungi, and toxins, are regulated because they potentially pose a severe threat to the health and safety of humans, cattle, and crops. Furthermore, these agents have the potential to be used to develop biological weapons. However, the stringency of the rules may burden the progress of biodendence research. The precise biological characteristics and the risk assessments used to decide the inclusion of an agent in the list or its exclusion from the list are not apparent. The administrative process applied by the responsible agencies, the Center for Disease Control (CDC) and Animal and Plant Health Inspection Service (APHIS), is equally nebulous. Therefore, our research focuses on attempting to clarify these issues, such that microbiologists and other scientists working with select agents can better scrutinize the appropriateness of select agent inclusions or exclusions by CDC and APHIS. **Methods:** First, we have analyzed trends in the exclusion of attenuated strains of select agents. Secondly, we have submitted Freedom of Information Act (FOIA) requests to the CDC and APHIS to gain access to records regarding previous petitions to include or exclude select agents. **Results:** Our results indicate that approximately 64 percent of excluded attenuated strains were generated via deletion mutagenesis. **Conclusions:** We expect that our analysis will provide scientists with clear insights and criteria to apply when drafting a petition to include or exclude a biological agent or a toxin from the select agent lists.

**The Biosafety & Biosecurity Law Hotline**

**B. D. Sherwin, V. Sutton; Texas Tech University School of Law, Lubbock, TX.**

**Background:** The Select Agent Program and Biosafety Improvement Act of 2008 was introduced to reauthorize and improve the Select Agent Program. Section 205 of the Act proposes that a “Biological Laboratory Incident Reporting System” for “voluntary reporting of biosafety or biosecurity incidents of concern” be established and that statistics on these reports “may” be collected and analyzed to determine where trends exist in order to make improvements in biosafety. The Center for Biodefense, Law & Public Policy at Texas Tech University School of Law (the “Center”) established a toll-free hotline in October 2008 for biological safety officers, biodefense researchers, and attorneys, intended to answer select agent rule compliance questions. The goal of the hotline is to help identify questions that arise under the current Select Agent Program. The hotline is open to callers 24 hours a day, all year round. The hypothesis in this ongoing project is: “What specific areas of the regulation do the callers have questions about and, can any trends or patterns be seen from the questions?” **Methods:** A toll-free number was created by the Center and advertised through presentations, posters and mailers. Calls not directly answered by the Center are directed to a call answering service which relays the message back to the Center. The Center then immediately returns then call to answer caller questions. All calls to the hotline are confidential and callers are given the option to allow their question to be included in ongoing Center research, where the data collected is checked for purposes of assuring anonymity, categorized and then analyzed for patterns to further determine ongoing “areas of concern.” **Results:** Due to the recent creation of the hotline, results will be forthcoming. **Conclusions:** The hotline was created by the Center to provide answers and guidance on select agent rule compliance questions. In creating the hotline, the Center hopes to continue to assist the lawmakers and the regulated community in bridging the gap in knowledge and understanding of regulating select agents.
16 - Tuesday Poster Session

Tuesday, February 24, 2009, 1:00 pm - 3:00 pm  ■ Location: Grand Ballroom

139 (A)

Rapid Detection of Highly Pathogenic Avian and Seasonal Influenzas using an ELISA-Coupled Cellular Assay

K. N. Agans, T. Brasel, F. Romero, A. Weldon, D. Cavathorn; Lovelace Respiratory Research Institute, Albuquerque, NM.

Background: Highly pathogenic avian influenza (HPAI) is an emerging virus that has caused several economically devastating outbreaks in poultry. Erupting outbreaks in humans have prompted the need for rapid and reliable methods of detection. We coupled an indirect ELISA with a cellular based assay to detect HPAI (A/Vietnam/1235/04) and a seasonal strain of influenza (A/New Caledonia/20/1999).

Methods: Virus serially diluted from 5000 FFU/ml in a 96-well cell culture plate. Madine Darby Canine Kidney (MDCK) cells were harvested, diluted to 1.5 x 10^5 cells/ml, and added to all wells. Sealed plates were incubated for 19-21 hours at 37.0°C and 5.0% CO₂. Upon virus removal, wells were washed and fixed. Optimal primary (monoclonal) and secondary (polyclonal-HRP) antibodies were determined by titration.

Results: All results were optimized for strain specific viral detection.

<table>
<thead>
<tr>
<th>Virus Strain</th>
<th>Blocking Buffer</th>
<th>Primary Antibody (Dilution)</th>
<th>Secondary Antibody (Dilution)</th>
<th>Substrate Incubation</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Vietnam/1235/04</td>
<td>Skim Milk</td>
<td>Mouse NP anti-Influenza A (1:1000)</td>
<td>Goat anti-mouse IgG-HRP (1:4000)</td>
<td>2 minutes</td>
<td>Yes</td>
</tr>
<tr>
<td>A/New Caledonia/20/1999</td>
<td>Skim Milk</td>
<td>Mouse NP anti-Influenza A (1:1000)</td>
<td>Goat anti-mouse IgG-HRP (1:4000)</td>
<td>6 minutes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Conclusions: Our data show that within 24 hours, viral detection can be objectively obtained for HPAI and seasonal influenza strains using an indirect ELISA-coupled cellular assay.

140 (A)

Development of Mouse Models for WEEV and VEEV Infections

T. Babas1, E. Peters1, A. Rippoon1, R. Wright1, G. Donnelly1, L. Rhodes1, N. Richardson-Harman2, P. M. Silvera1;1 Southern Research Institute, Frederick, MD, 2Alpha StatConsult LLC, Danvers, MD.

Background: Western and Venezuelan Equine Encephalitis viruses (WEEV and VEEV) belong to Togaviridae represent a group of severely neuropathogenic emerging pathogens in many domestic animals and humans. Additionally, the rising concerns of potential use of these viruses as biological warfare prompted public health concerns aggravated by the lack of a safer and efficacious vaccine. Under NIAID contract N01-AI-30063 we developed and characterized mouse models for WEEV and VEEV infections that will be valuable for evaluating the efficacy of antiviral drugs.

Method: Groups of mice were challenged intranasally with serial dilutions ranging from 10^5 pfu to 10 pfu for Trinidad donkey (TrD) strain and TC83 attenuated strain of VEEV, and California and CB487 strains of WEEV. The C57/HeN mouse strain was used for TC83 and the Balb/C mouse strain was used for all the other virus strains. Mice were monitored for 14 days post challenge for clinical signs of disease progression and time to death to determine the respective 50% lethal doses (LD50).

Result: Challenge dose dependent morbidity and mortality were observed in the WEEV and VEEV models. 100% mortality was observed at 10^3 pfu and 10^2 pfu of WEEV California and VEEV CB487 strains, respectively. As expected, 10^2 pfu of VEEV TrD was sufficient to induce 100% mortality; however up to 2 x 10^5 pfu of VEEV TC83 strain was necessary to induce 100% mortality. Within a few days of challenge animals experienced a steady body weight loss. The body weight loss was detected as early as day 2 of challenge with the virulent VEE strain, TrD compared to day 5 to 6 of challenge with the attenuated VEE strain, TC83. The mean LD50 values were 2.20x10^2 pfu, 12 pfu, 8 pfu and 2.89x10^3 pfu for California, CB487, TrD and TC83 strains, respectively, as determined by Probit and Reed-Muench methods.

Conclusion: we have developed and characterized consistent mouse models for both WEEV and VEEV strains to evaluate antiviral drug candidates.

141 (B)

Recent US-Associated Clinical Strains of Mycobacterium tuberculosis including Drug Sensitive and MDR Strains Exhibit an Extreme Range of Virulence in the Guinea Pig Model.

I. Orme; Colorado State Univ., Fort Collins, CO.

Whereas virtually all new vaccine and drug candidates for tuberculosis are tested against the laboratory strains H37Rv or Erdman, in the real world patients are infected by a large number of different isolates, including the particularly worrisome W-Beijing family of strains. Very little is currently known about these organisms in terms of basic biology including virulence and pathogenicity. We have characterized several in the guinea pig model. These consisted of both drug sensitive and MDR strains of both Beijing and non-Beijing varieties. They exhibited an extraordinary range of lung and lymph node pathology. This ranged from strains that generated minimal necrosis in the lungs, at least initially, to strains that gave moderate lung damage similar to the laboratory strain H37Rv, to highly damaging lung pathology with extensive necrosis, to strains giving very severe necrosis and rapid lung consolidation. In addition, several caused clear cavitation of draining lymph nodes. This extreme range of pathology in this model is a new and unexpected variable that will have to be addressed in the context of the efficacy testing of new vaccine and drug candidates.
**142 (B)**

**The Occurrence of Nocardia and Nocardia-like Species Among Sudanese Patients with Pulmonary Infection**

M. M. Elhassan1, E. Magzoob1, N. S. Saed1, M. E. Hamid1, M. Goodfellow2,3; 1Sudan Univ. of Sci. and Tech., Khartoum, SUDAN, 2Univ. of Khartoum, Khartoum, SUDAN, 3Federal Ministry of Hth., Khartoum, SUDAN, 4King Khalid Univ., Abha, SAUDI ARABIA, 5Newcastle Univ., Newcastle, UNITED KINGDOM.

**Background:** The aim of the present study was to determine the prevalence of *Nocardia* and *Nocardia*-like spp. **Methods:** Three hundred Sudanese patients, presented with pulmonary infection were included in this study. Those patients were examined for the presence of acid-fast bacilli. They were suspected to have tuberculosis infection according to the symptoms. Thin bacterial smears were prepared and stained by Zielh Neelsen (ZN) stain. Lowenstein-Jensen (LJ) medium was inoculated with 20 µl of the neutralized sputum sample. All cultures were incubated at 37° C for 8 weeks before being discarded. Growth was monitored daily during the first week to observe the presence of rapid growers which if encountered will show growth within 7 days, and then the growth was observed weekly up to the 8th week. Phenotypic characterization was performed by using different biochemical tests. Genotypic characterization was done by using 16 rDNA. **Results:** Ten isolates showed rapid growth pattern within 2-3 days after inoculation. Further conventional methods suggested that all these isolates were belonging to the family Nocardia. More confirmation was obtained by 16fDNA which revealed 100% similarity with *Nocardia africana*. The new isolates were assigned as SD1001-SD1010. **Conclusions:** Nocardia and Nocardia-like spp revealed significant occurrence among patients with pulmonary infections (3.3%). This finding suggested that pulmonary nocardiosis might occur in a substantial proportion of patients who exhibit chronic lung disease in Sudan. It is important, therefore, that clinicians in Chest Units, particularly those in teaching governmental hospitals, should consider this condition, especially when patients with respiratory infections fail to respond to antitubercular therapy.

**143 (B)**

**Assessment of the Virulence in Balb/c Mice of Bioreactor Produced Anthrax Spore Stocks, Delivered by the Aerosol Route**

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**Background:** The requirement to evaluate new vaccines and therapeutics for licensure against Select Agents has led to the expansion of aerosol generation research at HPA, CEPR. A lethal aerosol model of *Bacillus anthracis* in Balb/c mice was developed. However, production of the required volume of high titre spore stocks was prohibitive. A bioreactor production method was developed and the virulence of these spore stocks assessed. **Methods:** Anthrax: NR3088 (*B. anthracis* Ames, BEI) was obtained and grown in bioreactor fed batch culture. Following sporulation, these stocks were washed by centrifugation and stored in distilled water. Aerosolisation: Ten-fold serial dilutions of bioreactor produced anthrax spores were aerosolised using the AeroMP-Henderson apparatus, in conjunction with a 6-jet Collision nebuliser and delivered to groups of 10 mice. Retained dose. The level of spore retention compared to the presented dose was assessed by enumeration of the anthrax load in the lung tissue immediately post-challenge in the high dose group. **Results:** Bioreactor grown anthrax spores delivered using the AeroMP-Henderson apparatus produced a lethal infection in Balb/c mice. The estimated LD50 (9.1E+04 CFU), level of lung retention (20%) and the pattern of disease progression were all consistent with previous findings using anthrax spores grown on solid agar. **Conclusions:** Bioreactor-produced anthrax spores are virulent and are suitable for infection studies. The production of large volume, high titre anthrax spores will enable consistency of challenge agent across a large series of experiments.

**144 (B)**

**Utility of MLVA DNA Profiling for Quality Control and Molecular Tagging of an Archival Bacillus anthracis Collection**

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**Background:** The usefulness of the Multi-locus Variable Number of Tandem Repeats Assay (MLVA) for DNA profiling was examined using extracted DNA from archival cultures of *Bacillus anthracis* (BA), deposited in the BEI Repository in 2005. This sensitive fluorescent assay was hypothesized to be a potentially discriminatory test for molecular “tagging” of each BA “number” designation by using the molecular weights of the amplified DNA fragments. **Methods:** Desiccated archival cultures from the BEI collection were revived in broth, subcultured onto Tryptic Soy Agar with 5% Sheep Blood and PLET agar to assess differential colony types under nonselective and selective culture conditions, respectively. DNA was extracted, quantitated, and subsequently amplified using a published MLVA assay containing 15 different genetic loci on the BA chromosome, as well as, on the plasmids, pX01 and pX02. The labeled amplified products were then resolved by capillary electrophoresis (CE). DNA from BA Ames SR-411 was used as an independently verifiable positive control. The DNA extraction process reagent blanks and PCR no-template reactions were used as controls in the extraction and amplification, respective-ly. Precision of the CE instrument was verified for the ability to reproducibly detect and size MLVA alleles. **Results:** DNA profiles from Sterne, Vollum, and Ames strains, from different laboratory sources were tested and compared and found to have null alleles or allelic variation. Mixture BA template DNA containing various ratios of input DNA from samples with different alleles yielded readily distinguishable mixture profiles. **Conclusion:** The *B. anthracis* MLVA DNA profiling assay is useful for molecular tagging of strains and when included in a QC algorithm of microbiological and molecular testing raises confidence that the microbiological purity and genetic consistency of these strains is excellent.

**145 (B)**

**Performance of the LRRI Real-time Rabbit Plethysmography System**

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**Background:** Select agent studies using rabbits typically determine dose delivered to the animal by measuring chamber concentration of the aerosolized agent and estimating respiratory minute volume (MV) of the animal using Guyton’s formula MV, cc/min = 2.1(weight, g)0.75. LRRI employs a system that measures actual inhaled volume using real-time whole-body plethysmography. The LRRI plethysmography system is designed to contain an unanesthetized rabbit in a uniquely designed chamber for nose-only exposures. The plethysmograph system is composed of the plethysmograph chamber, screen pneumotachograph, pressure transducer, Buxco MaximX preamplifier, and BioSystem XA for Windows software. **Methods:** The plethysmograph chamber is calibrated prior to each use by injecting a 20 mL bolus of air into the sealed chamber. Pressure drop is generated across a series of fine mesh screens (pneumotachograph) that are connected to the chamber. The pressure drop is detected by a transducer and a signal is sent to a preamplifier. The BioSystem XA software correlates the signal to flow rate. The rabbit is placed in the chamber and movement of air caused by the rise and fall of the thoracic cavity is detected by the pneumotachograph and frequency (F), tidal volume (TV), and MV are calculated by the software. The chamber is connected to a nose-only exposure system after which select agent aerosol is delivered. Concentration of the agent in the generator suspension is calculated to deliver the target inhaled dose in a predetermined inhaled volume. **Results:** 104 rabbits weighing between 2.4 and 3.8 kg were exposed to a select agent dose determined by chamber aerosol concentration and a target total inhaled volume of 10 L or 20 L. Average P was 181 +/- 73 hpm, TV was 11 +/- 5 mL, and average MV was 1707 +/- 727 mL/min. **Conclusions:** The LRRI rabbit plethysmography system demonstrates that inhaled volume can be quantified accurately. Results show that the F, TV, and MV of similar weight animals can differ significantly.
**146 (B)**

**Comparison of Blood Collection Methods in the Early Stages of Anthrax Bacteremia in Mice**

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**Background:** *Bacillus anthracis* (*B. anthracis*) is the causative agent of the disease anthrax in humans and animals. The route of blood collection could affect bacterial counts from potential ocular and dermal contamination during a nose-only inhalation exposure. The comparison of different blood collection routes to determine bacterial load during the early stages of bacteremia has not been well described. **Methods:** In this study we compared retro orbital and cardiac puncture blood collection routes during the early stages of the onset of bacteremia. Five groups of BALB/c mice, consisting of five males and five females, were exposed by nose-only inhalation to an average inhaled dose of 3.3E9 CFU of *B. anthracis* Ames spores. Mice were euthanized at T=0 (immediately following exposure), 6 and 12 hours post-exposure. Blood samples were collected via retro-orbital bleeding or cardiac puncture at 0 and 6 hours and by retro-orbital bleeding at 12 hours. Blood was serially diluted, plated, incubated, and enumerated. **Results:** For retro-orbital blood collection, bacteremia was observed in 100% of mice at T=0 and 90% at 6 hours. The average bacterial counts were 1.1E+03 CFU/mL and 77 CFU/mL, respectively. For cardiac puncture blood collection, bacteremia was observed in 90% of mice at T=0 and 40% at 6 hours. The average bacterial counts were 5.9E+03 CFU/mL and 323 CFU/mL, respectively. By 12 hours, 100% of mice were negative for *B. anthracis* in the blood. **Conclusions:** These results suggest there was no effect on the determination of bacteremia at time zero with either collection method. At 6 hours there was a difference in the percent of bacteremia between collection methods, but the differences between average bacterial loads were insignificant.

**147 (B)**

**Antimicrobial Susceptibilities of Several Burkholderia pseudomallei Isolates to a Range of Antimicrobial Agents**

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**Background:** *Burkholderia pseudomallei* is the causative agent of melioidosis, which is endemic in South-East Asia and Northern Australia; a few cases are detected annually in the UK. There are a range of patient treatments available, depending on the country and a body of literature is being built up describing the in vitro sensitivity of clinical panels and culture collection strains. **Methods:** Thirteen *B. pseudomallei* isolates obtained from the National Collection of Type Cultures (NCTC), HPA, UK and seventeen clinical isolates obtained from the Centre for Infections, HPA, UK were tested using the broth microdilution assay, performed according to CLSI guidelines. All assays were performed using 96-well plates with a final volume of 20μl/well using a test inoculum in Cation Adjusted Mueller Hinton Broth (CAMHB). Plates were incubated for 18-24 hours at 37°C and then turbidity read at 620nm. Initial experiments did not yield acceptable growth in the control wells so Luria broth (LB) was also used to determine MIC values. **Results:** All thirteen NCTC isolates tested in CAMHB were susceptible to the six drugs tested, with the MIC values falling within the CLSI recommended or within ranges published in the literature. Of the seventeen clinical isolates tested in CAMHB five were resistant to cefazidime and seven showed resistance to doxycycline. One clinical isolate was resistant to all six antibiotics tested. **Conclusions:** The isolates from the NCTC are all susceptible to the two antibiotics in the guidelines and fall within the expected & published ranges. This is in comparison to the clinical panel which showed a greater range of sensitivities to the antibiotics. There appears to be a difference in antibiotic susceptibility between the clinical panel and the culture collection isolates which will be investigated using molecular typing techniques.

**148 (B)**

**Susceptibilities of Y. pestis Strains to a Range of Antimicrobial Agents**


**Background:** *Yersinia pestis* is the causative agent of plague, a disease primarily of rodents but which manifests in bubonic, pneumonic or septicemic forms in humans. Plague is endemic in many parts of the world with transmission to humans most commonly occurring via bites of fleas from infected animals. Pneumonic plague is less common but this form of the disease is invariably fatal if left untreated. Due to its high pathogenicity and ability to cause disease by the aerosol route *Y. pestis* is considered to be a potential bioterrorism agent. We have tested the susceptibility of twelve strains of *Y. pestis* to the antimicrobial agents gentamicin, doxycycline and ciprofloxacin which are the current recommended choice of treatments in contained or mass casualty situations. In addition we determined the susceptibility of the same strains to the broad spectrum antibiotics levofloxacin and meropenem, the use of which are becoming more common to treat infections caused by other *Enterobacteriaceae*. **Methods:** The minimum inhibitory concentration (MIC) of each drug was determined using the broth microdilution method described in the guidelines published by the Clinical and Laboratory Standards Institute (CLSI). 100 μl bacterial suspension was added to each well of a 96-well microtitre plate containing 100 μl antibiotic dilution. Plates were incubated for 48 hours at 37°C after which the optical density was measured at 620nm. **Results:** All twelve strains were susceptible to the five drugs tested, with the MIC values falling within the CLSI recommended range where available. No published data was available for meropenem and levofloxacin but their efficacy was comparable with the other drugs. **Conclusions:** Testing a panel of *Y. pestis* strains has shown that in addition to the currently recommended prophylactic treatments, levofloxacin and meropenem show similar efficacy in MIC testing and could potentially be used for treatment of *Y. pestis* infections.

**149 (B)**

**Production and Optimum Growth Conditions of Rickettsia Organisms**

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**Background:** The members of the genus *Rickettsia* are small, aerobic, gram negative intracellular parasites. Many of these species have a high level of pathogenicity and are included on the HHS Select Agent List and NIAID Priority Pathogen List. The objective of our work was to identify optimal growth conditions for various *Rickettsia* spp. in order to provide the scientific community with an abundant and diverse *Rickettsia* collection. **Methods:** Growth of 7 *R. prowazekii* isolates and 7 other *Rickettsia* species in Vero cells were assessed qualitatively by observation of cytopathic effect (CPE), and quantitatively by qPCR at various time points post-infection under temperature conditions ranging from 30°C-37°C. Samples were checked frequently for CPE and harvested when CPE reached 75% to 100%. If no CPE was visible, the samples were harvested when the mock-infected sample began to degrade. Nucleic acids extracted from all samples were evaluated using a pan-*Rickettsia* qPCR assay. In order to provide an additional growth platform, two *Rickettsia* species were grown in the yolk sac of embryonated chicken eggs. **Results:** Among the 7 *R. prowazekii* strains tested, 4 showed optimal growths at 35°C, 2 at 30°C, and the remaining strain at 32°C. Of the 7 non-*prowazekii* *Rickettsia* species tested, 3 had an optimal growth temperature of 35°C, 3 at 32°C, and the remaining strain at 30°C. Preliminary results comparing growth platforms suggest that growing *Rickettsia* in the yolk sac of a 5-day old embryonated chicken egg yields similar titers to that of *Rickettsia* grown in Vero cells. **Conclusions:** Our study demonstrates that different species of *Rickettsia* grow optimally at different temperatures. Further, growth of *Rickettsia* in eggs gives another possible host, which may be important for animal challenge and vaccine studies. This study continues our effort to provide the scientific community with a high quality and diverse *Rickettsia* collection available through the BEI Resources program.
150 (B)
The Impact of Storage Duration on Critical Bacillus anthracis Ames Spores Characteristics

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The Battelle Biomedical Research Center (BBRC) has been producing and storing Bacillus anthracis Ames and Vollum strain spores in support of vaccine and therapeutic testing programs against anthrax challenges for over six years. Currently we have two ongoing long-term storage studies: a stability study examining the current storage conditions and a storage matrix study which places spores into 8 different storage media at 3 different temperatures. Both studies are designed to examine the impact of storage media, temperature, and duration on spore viability, microscopic characteristics, virulence and aerosolization. The goal of this research was to determine the most appropriate long-term storage conditions of B. anthracis spore suspensions. Data from the stability study indicates that the current BBRC method of storing B. anthracis spores suspensions at 2-8°C in 1% phenol or ≤70°C in 1% phenol with 10% glycerol maintains the viable spore counts within ±0.25 log of the original concentration for six years. However, some storage media and temperatures cause a greater than 50% loss of viable spore counts within 180 days. Any change to the microscopic characterization of the spore suspensions over time has been attributed to the use of purification gradients prior to storage and not to the storage media, temperature, or duration. The various storage conditions did not impact the aerosol characteristics of spore suspensions; however some storage media and conditions were not conducive to effective spray factor testing. Virulence of the spore suspensions was tested via guinea pig intradermal median LD₅₀ assays. There was no change in the virulence in spores stored at 2-8°C in 1% phenol over time. Spores stored in water, 66% ethanol, 1% phenol with 10% glycerol, and 0.015% Tween-80 at various temperature, selected from the storage matrix study for virulence testing, indicated that there was no change in the virulence of these spore suspensions after two years of storage.

151 (B)
Determination of the Francisella tularensis Schu S4 aerosol LD₅₀ in Cynomolgus Macaques and Characterization of Tularemia Manifestation

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F. tularensis, the causative agent of tularemia, is a highly virulent bacterium capable of infecting and proliferating within macrophages and other cell types. Human infection occurs via several routes with strain type and route of infection governing severity. The deadliness manifestation is pneumonic tularemia, which results from inhalation of bacteria or secondary to bacteremia. Subspecies tularensis is the most virulent and is often associated with lethal pulmonary infections. To determine the 50% LD₅₀ and to characterize tularemia in non-human primates, we infected 30 cynomolgus macaques with varying doses of Schu S4 using a Collison nebulizer. The final wave is in progress and the LD₅₀ will be calculated in 4 weeks. Two forms of disease are observed dependent on aerosol dose. Primary pneumonic disease leading to acute death was observed in animals presented with ~1000 cfu or greater, while animals receiving a presented dose of ~100 cfu or less developed a protracted disease that culminated with death resulting from dissemination. Mortality (75%) was observed in animals presented with ~1 cfu; higher doses were 100% lethal. High respiration rates and hypothermia typified the primary pneumonic disease, while anorexia, weight loss, and malaise typified the protracted disease. Initial bacteremia corresponded with presented dose. High numbers of Schu S4 were recovered from lungs and tracheobronchial lymph nodes despite dose; burden in other organs was more variable. Results indicate that F. tularensis is highly pathogenic bacterium that manifests disease in a variety of ways depending on aerosol dose. Determination of the LD₅₀ and disease characterization will support testing of vaccines and therapeutics in the interest of public health and biodefense. This project was funded in whole or in part with Federal funds from NIAID, NIH, DHHS, Contract No. HHSN266200500040C.

152 (B)
Natural History Study for Inhalational Bacillus anthracis Exposure in a New Zealand White Rabbit Model: Identification of an Early Biomarker of Infection

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Background: Without aggressive prophylaxis or intervention, inhalational anthrax infection results in high mortality rates. Rapid and early diagnosis is therefore necessary to successfully treat the disease. In this study, we developed a natural history portrait for inhalational Bacillus anthracis exposure in a New Zealand white (NZW) rabbit model to better identify potential, early biomarkers of anthrax.

Methods: Accordingly, 10 NZW rabbits were exposed to aerosolized B. anthracis spores (Ames; ~200 LD₅₀) and clinical signs, physiological parameters (including body temperature, complete blood count, and bacteremia), and presence of protective antigen in the blood (i.e., antigenemia) were determined. Following B. anthracis exposure, rabbits appeared to be normal until several hours before death. Antigenemia, as determined by electrochemiluminescence immunoassay (ECLI), almost consistently occurred at the same time as bacteremia and correlated well with bacterial loads in the blood. However, ECLI determination of antigenemia proved to be much faster (i.e., completed within 1 to 2 h) compared to the more time-intensive, 24-h culture required for traditional determination of bacteremia. Additionally, pyrexia and modest changes to hematologic parameters occurred after antigenemia and bacteremia were first observed.

Conclusions: Taken together, these results indicate that antigenemia is a viable, early biomarker for B. anthracis infection. Consequently, antigenemia determined by ECLI permits the rapid diagnosis of anthrax, leading to timely therapeutic intervention against this highly pathogenic disease.

153 (D)
Rapid Real-Time PCR Assays for Detection of Klebsiella pneumoniae with the rmpA or magA Genes Associated with the Hypermucoviscosity Phenotype: Screening of Nonhuman Primates


The relationship of the mucoviscosity-associated (magA) and/or regulator of the mucoid phenotype (rmpA) genes to the Klebsiella pneumoniae hypermucoviscosity (HMV) phenotype has been reported. We previously demonstrated that rmpA+ K. pneumoniae can cause serious disease in African green monkeys (AGM). We have also isolated rmpA+ and magA+ HMV K. pneumoniae from other species of nonhuman primates (NHP). The NHP are imperative to research done at USAMRIID for the development of vaccines, therapeutics, and diagnostics for biowarfare agents. To rapidly screen the colony of NHP for these infections, we developed three real-time PCR assays. The first was K. pneumoniae-specific, targeting the kbe gene while the other two targeted rmpA and magA. Primer Express 2 was used with the K. pneumoniae kbe, rmpA, and magA genes to generate sequence-specific primers and TaqMan®/TaqMan®-MGB probes. Dual oral and rectal swabs and necropsy samples were collected and processed for routine microbiology culture and DNA extraction. K. pneumoniae colonies were identified on the Vtek 2, and DNA eluates were tested with the K. pneumoniae-specific assays. Initial testing of 45 AGM resulted in 19 kbe+ samples from 14 animals, with none testing positive for either rmpA or magA. Subsequent testing of 307 NHP resulted in 64 HMV K. pneumoniae, of which 42 were rmpA+ and 15 were magA+. NHP testing at USAMRIID demonstrated the ability to screen both live and necropsied animals for the presence of K. pneumoniae by culture and to determine the HMV genotype by real-time PCR.
Detection of Ciprofloxacin-Resistant \textit{Bacillus anthracis} Mutants by Melt Curve Analysis with SimpleProbe Chemistry

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\textbf{Background:} The potential for genetic modification of biological warfare agents makes rapid identification of antibiotic resistant strains critical. Ciprofloxacin, a fluorinated quinolone, is an antibiotic effective for treating bacterial infections by inhibiting DNA garses (\textit{gyrA}, \textit{gyrB} \& \textit{parC}) activity. Hotspots within the quinolone resistance-determining region (QRDR) of \textit{gyrA}, \textit{gyrB} and \textit{parC} genes give rise to mutations that cause resistance to the antibiotic. SimpleProbes\textsuperscript{™} are a single probe system designed for mutation detection by melting temperature change. \textbf{Methods:} Wild type (wt) and ciprofloxacin-resistant (cipR) mutants of \textit{Bacillus anthracis} \textit{ANR} strain (pXO1- \& pXO2-) were amplified by primers flanking a 156-bp (\textit{gyrA}), 292-bp (\textit{gyrB}) and 157-bp (parC) segment of the QRDR region of \textit{ANR} genes. SimpleProbes\textsuperscript{™} designed with sequences complementary to \textit{ANR} wt spanned known hotspots within the QRDR region. Cycling conditions on the LightCycler 2.0 were 45 cycles of 95°C for 5 sec and 55°C for 45 sec. Reaction conditions followed manufacturers recommended concentrations for SimpleProbe\textsuperscript{™} with KlenTaq LA used as the polymerase. \textbf{Results:} Ten \textit{B. anthracis} \textit{ANR} Isolates containing one or more of eight different point mutations in the three genes were successfully differentiated from \textit{ANR} wt with a 4°C-12°C shift in probe melting temperature. Nine additional non-characterized clones were also tested and found to have the same Tm difference from the wild type. \textbf{Conclusions:} We developed a SimpleProbe\textsuperscript{™} assays that can easily and exclusively detect eight different point mutations in the \textit{B. anthracis} \textit{ANR} \textit{gyrA}, \textit{gyrB} and \textit{parC} genes by melting peak analysis. Follow-up of positive \textit{B. anthracis} results by rapid PCR with mutation detection analysis would determine the best course of antibiotic treatment.

Comparison of the Promega Maxwell\textsuperscript{®} 16 and the Modified Qiagen DNA Mini Kit for Isolating PCR-amplifiable Nucleic Acid

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\textbf{Background:} Isolation of high-quality nucleic acid is essential to the successful PCR-based molecular detection of biological threat agents. DNA and RNA extraction methods should be simple, reproducible, and able to remove sample matrix-derived inhibitory substances. Although a modified Qiagen DNA Mini kit protocol, our laboratory gold standard, is robust regardless of matrix, it is labor and time intensive and utilizes reagents and equipment that are not readily compatible with field laboratories. In an attempt to find faster, more reproducible, and safer alternatives, we investigated use of the automated Promega Maxwell\textsuperscript{®} 16 low-elution volume (LEV) kit for preparing nucleic acids from various biothreat agents. \textbf{Methods:} Serial dilutions of \textit{Bacillus anthracis} Sterne vegetative cells, gamma-irradiated \textit{Vermis pestis}, vaccinia and Vesnerman horse encephalitis (VEE) viruses were prepared in Dulbecco's phosphate-buffered saline (dPBS) or human whole blood. Triplicate 100-µl samples were then extracted using either the Qiagen DNA Mini kit or Promega's LEV kit with or without heat pretreatment the Qiagen DNA Mini kit. Purified nucleic acid was analyzed by real-time PCR using the Idaho Technology, Inc. R.A.P.I.D. \textbf{Results:} Equivalent limits of detection (LOD) were obtained between the Qiagen and Promega system when the heat pretreatment was included in the sample preparation. The LEV kit without pretreatment produced LODs at least half of a log higher. \textbf{Conclusion:} The automated LEV kit combined with a 10-min heating step is an excellent option for preparing purified nucleic acid for downstream PCR analysis. The capacity of the instrument to extract DNA or RNA from up to 16 samples at a time, using prefiltered reagent cartridges, makes this system a safe and rapid alternative to the modified Qiagen DNA Mini kit protocol while minimizing risk for human error.

Simultaneous Detection of Five Biothreat Agents in Powder Samples by a Multiplexed Suspension Array

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\textbf{Background:} The threat of bioterrorism has attracted great attention after the letter containing anthrax spore terrified the U.S.A. and the letter with “white powder” flimed all over the world. Dozens of techniques, including microbiological, immunological and nucleic acid-based methods, have been developed for detecting and identifying biothreat agents. Here we described multiplexed immunoassays for the simultaneous detection of five biothreat agents (\textit{B. anthracis} spores, \textit{Y. pestis}, SARS-CoV, Staphylococcal enterotoxin B (SEB), and ricin) from a single powder sample (milk powder, corn starch, wheat flour, or instant fruit-flavored drink mix powder). Methods: The 5-plexed immunoassays using sets of 9-plexed coupled fluorescent beads were employed to simultaneously detect five representative biothreat agents, including \textit{B. anthracis} spore, \textit{Y. pestis}, SARS-CoV, SEB, and ricin, from a single powder sample and the feasibility for field samples was demonstrated by both blinded and standard laboratory trials. \textbf{Results:} The detection sensitivity and dynamic range for the five biothreat agents from different powders might be varied depending on the nature of the powder and the feature of the contaminating agent. The limit of detection for \textit{Y. pestis}, \textit{B. anthracis} spores, SEB, ricin, SARS-CoV N protein in milk powder was 400 cfu/mL, 2.22×10\textsuperscript{3} cfu/mL, 2.2 ng/mL, 108 ng/mL, and 40.2 ng/mL, respectively. Compared to conventional ELISA method, the suspension array has a higher sensitive ability, and can detect five biothreat agents simultaneously with high reproducibility. \textbf{Conclusions:} A suspension array-based multiplexed immunoassay was developed for rapid, sensitive, specific, and simultaneous detection of multiple biothreat-associated agents in powder samples.

	extbf{qPCR Assays for the Detection of Rickettsial Pathogens}

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\textbf{Background:} 	extit{Rickettsia} are obligate intracellular bacteria that depend on arthropod vectors for their maintenance and transmission. Numerous rickettsial agents pose human public health risks including \textit{R. typhi}, \textit{R. prowazekii}, \textit{R. rickettsii}, and \textit{R. felis}. \textit{R. rickettsii} and \textit{R. prowazekii} are Category B Bioterrorism agents and Select Agents because they can cause severe aerosol infections with potential mortality. Serology is commonly used for the diagnosis of rickettsial diseases. However, rickettsial antigenic determinants are highly conserved, resulting in significant cross-reactions that make species identification difficult. Additionally, antibodies may not be detectable early in infection so treatment with appropriate antibiotics may be delayed. \textbf{Methods:} A series of novel multiplex Taqman PCR assays has been developed to allow for the simultaneous detection and identification of four rickettsial agents: \textit{R. prowazekii}, \textit{R. rickettsii}, \textit{R. typhi}, and \textit{R. felis}. These assays employ a single probe primer pair that targets a homologous region of the citrate synthase gene (\textit{glt}) from all \textit{Rickettsia} and utilizes species specific Taqman probes to identify the specific rickettsial agents present. Locked nucleic acid bases were incorporated to improve probe binding. \textbf{Results:} Three assays have been developed that allow for the detection and identification of: 1) \textit{R. typhi} and \textit{R. felis}, 2) \textit{R. prowazekii} and \textit{R. rickettsii}, and 3) \textit{R. prowazekii} and \textit{R. typhi}. A fifth probe has been developed to be used in conjunction with the above assays to allow for the detection of all \textit{Rickettsia}, thus ensuring detection of any rickettsial agent for which there is no specific probe. \textbf{Conclusions:} Early diagnosis is an important factor contributing to a favorable outcome in many rickettsial diseases. Multiplexed assays allow for the concurrent detection and identification of clinically relevant \textit{Rickettsia} in natural or bioterrorism events. This allows for the rapid diagnosis of rickettsioses while decreasing both the time and amount of sample needed if each agent was screened independently.
158 (D)

Forensic Discrimination of Bacillus cereus T-strain Spores Using Fatty Acid Methyl Ester (FAME) Profiles

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In forensic microbiology, few phenotypic markers exist that provide information on the culturing conditions or growth substrates used to cultivate bacterial organisms. In this study, cellular Fatty Acid Methyl Ester (FAME) profiling was examined as a potential forensic tool for discriminating among sporulating cultures of Bacillus cereus T-strain bacteria grown on 12 different media formulations. To test for statistical differences between FAME profiles, the relative abundances of both individual FAME biomarkers and the four structure classes of Bacillus fatty acids (iso-odd, iso-even, anteiso, and straight-chained) were analyzed with a variety of techniques including non-metric multidimensional scaling (nMDS), analysis of similarities (ANOSIM), and non-parametric multivariate analysis of variance (NPANOVA). The relative impact of each FAME variable on the dissimilarities between media cultures was assessed with Discriminant Function Analysis (DFA). Results indicated that growth on different media induced shifts in the relative ratios of fatty acids that produced bacterial cultures with statistically distinct FAME signatures for each of the media types surveyed. While the number of detectable differences were found in the relative proportions of fatty acids, one FAME biomarker, oelic acid (18:10oe), was only found in cultures grown on Columbia Agar supplemented with sheep’s blood. Comparisons between individual-based and structure-based FAME profiling suggest that both types of analyses have promise as microbial attribution tools. Lastly, DFA identified a subset of FAME variables that accounted for a majority of the variation among media. This suggests that targeted studies may reduce the intrinsic complexity of FAME profiles and help this technique become a viable tool in forensic microbiology.

159 (D)

Multi-Center Development and Evaluation of a Novel Multiplex Real-Time PCR Protocol for Screening of B. Anthracis in Food and Feed Samples

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The current work is part of BIOTRACER, which is an Integrated Project within the EU 6th Framework Program under the research area of Food Quality and Safety. BIOTRACER has 46 partners from 24 countries and started January 2007. The results presented here were generated in work package 15 aiming to develop methods to rapidly screen and identify important food and feed pathogens. Many real-time PCR (qPCR) assays have been developed for B. anthracis but earlier assays often showed cross-reactivity with close members of the B. cereus family or detected chromosomal and plasmid markers separately. To overcome this problem RIVM, SVL and TUM collaborated by 1) compiling a set of 15 B. cereus strains sharing major characteristics with B. anthracis, 2) identifying specific genetic B. anthracis biomarkers by the use of whole genome comparison of B. cereus, B. thuringiensis and B. anthracis strains, and 3) generating a set primers and probes with optimal characteristics for the multiplex detection of the B. anthracis chromosome, pX01 and pX02 and a B. thuringiensis internal control. Using this approach we set up a set of highly specific primers and Taqman probes for the detection of B. anthracis in food and feed samples. The qPCR assay was shown to identify Bacillus organisms either directly from biological specimen or from cell culture. The ability of the DOP-PCR to amplify parts of viral genomes without any prior knowledge of those sequences makes this a very promising assay not only for detection and discovery of clinically relevant viruses but also in bioterrorism preparedness and response.

160 (D)

Virus Detection and Virus Discovery: Generic Amplification Combined with Conventional or High Throughput Sequencing

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Background: Bioterrorism preparedness requires reliable, sensitive, and broad detection capabilities not only for viral agents specified in traditional lists of bioterror agents but also for unexpected, newly emerging, or genetically altered agents. Testing for a plethora of viruses usually requires a multitude of assays, which may nonetheless not detect or identify all viruses directly. The degenerate oligo primer PCR (DOP-PCR) has the potential to detect and identify all viral agents using only one aliquot of a given sample in one assay. Method: Our DOP-PCR is a truly generic, highly degenerate PCR, used in combination with physical and biochemical purification. It is not based on pathogen sequence information. The purification step combines targeted digestion of host nucleic acids and purification of virus capsids. Viral nucleic acids are amplified with DOP-PCR and PCR products are analyzed either by cloning and sequencing or by high throughput sequencing. Results: The DOP assay was shown to detect members from RNA virus families, e.g. retroviridae, coronaviridae, caliciviridae, flaviviridae, picornaviridae, orthomyxoviridae, and DNA virus families such as herpesviridae, paroviridae, adenooviridae, circoviridae, and polyomaviridae. A single DOP PCR analyzing infected cell culture supernatant revealed about 45% of the viral genome of a new type of veterinary virus. Multiple viruses can be identified within a single sample. High throughput sequencing allows deeper analyses of complex samples and further improves the detection limit. Discussion: The DOP assay was used to identify viruses either directly from biological specimens or from cell culture. The ability of the DOP-PCR to amplify parts of viral genomes without any prior knowledge of those sequences makes this a very promising assay not only for detection and discovery of clinically relevant viruses but also in bioterrorism preparedness and response.

161 (D)

Evaluation of Standard Case Definition for Hemorrhagic Fever with Renal Syndrome in Kazakhstan

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Background: Hemorrhagic Fever with Renal Syndrome (HFRS) has not been reported in Kazakhstan until 2000 when first 5 cases were identified in its Western region. A standard case definition for HFRS surveillance did not exist in the country. In 2005 CDC with funding support from DTRA’s Threat Agent Detection and Response (TADR) Program developed a surveillance case definition for persons suspected to have HFRS. This study was conducted to evaluate the surveillance case definition. Methods: Cases were identified from recently diagnosed cases and by admission record review at two hospitals in Burlinskiy and Zelenovskiy districts of Western Kazakhstan region between November 1, 2005 through February 3, 2006. A suspect case was defined as a person with acute severe illness with fever and signs of either renal failure or hemorrhagic syndrome. Controls were selected randomly among persons living in vicinity to cases. Controls did not have history of HFRS and did not meet HFRS case definition at time of data collection. Blood samples were collected from cases and controls. Presence of either IgM or IgG to HFRS found by ELISA (FOCUS Diagnostics) defined subject as true positive HFRS case. Results: The study included 80 HFRS cases (determined by positive ELISA) and 142 controls. Fifty seven subjects from case group had positive ELISA results and 125 from control group had negative ELISA results. Seventy one percent sensitivity and eighty eight percent specificity were calculated for HFRS case definition. Conclusions: The study found a good level of sensitivity and specificity of HFRS case definition. Further studies on case definition application need to be done.
162 (D)

TaqMan Real-Time PCR Assay Development for Rickettsia rickettsii and R. prowazekii

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Background: Rickettsia rickettsii and R. prowazekii, the etiological agents of Rocky Mountain spotted fever and epidemic typhus, respectively, are class B bioterrorism threat agents because of their efficiency in causing infection by aerosol route and the high degree of morbidity and mortality in untreated individuals. Detection of these agents and the differentiation of them from other exanthematic diseases are necessary for determining the causation of severe disease events. Methods: Specific signature sequences for both R. rickettsii and R. prowazekii were derived from computational analysis of 11 spotted fever and typhus group rickettsial genome sequences. Primers and probes for TagMan qPCR were designed in relation to the signature sites to maximize species specificity. Primers were tested for specificity and efficiency by SYBR Green PCR, and TaqMan probes were designed and tested for performance and specificity. Results: Of the 4165 signature sequence sites analyzed for R. rickettsii, 6 candidates were identified for assay design, of which two suitable primer/probe sets were evaluated. Of the 1985 signature sequences obtained for R. prowazekii, primers were developed from 37 sites and screened by SYBR Green PCR for specificity, of these 27 were screened for relative performance. 24 complementary probes were designed and tested; six suitable sites were evaluated in detail. Conclusions: Detailed comparative characterization of R. rickettsii and R. prowazekii genome sequences is crucial for understanding the intra species genetic variation. The stable genomic differences represented in the unique signatures derived from computational analyses provide detailed attributes for the development of highly specific qPCR assays.

163 (D)

bcl-Gene Polymorphisms as Basis for Anthrax Detection and Fingerprinting

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Background: Bacillus cereus group includes three closely related species of B. anthracis, B. cereus, and B. thuringiensis that form a highly homogeneous subdivision of the genus Bacillus. A major challenge to defend against anthrax is the inability to rapidly and accurately distinguish infection by this agent from more benign infections with other members of the B. cereus group. Methods: Here, we use bioinformatic analyses, mathematical modeling, and PCR to evaluate sequence and length polymorphisms of the Bacillus collagen-like genes, bcl, as a basis for anthrax detection and fingerprinting. Results: PSI-BLAST searches identified 236 proteins with collagen-like sequences that were annotated to the Bacillus genus. The Bcl proteins were classified using the Cluster Analysis of Sequences into 10 main (sub)families or classes I-10. A total of five genes, designated bcl4-E, encoding putative spore-associated proteins are found in B. anthracis strains. Examination of multiple sequence alignments of bcl4-E identified sequence polymorphisms within bcl4 allelles of the Bacillus cereus group organisms. These sequence polymorphisms allowed for the specific detection of B. anthracis strains by PCR using both purified DNA and spores as templates. By exploiting length variation of the bcl alleles we demonstrated that the combined bcl4-E PCR products generate markedly different fingerprints for the B. anthracis Ames and Sterne. Moreover, we predicted that bcl4-E length polymorphisms create unique signatures for B. anthracis strains, using a linear model that relates the theoretical and experimental fragment lengths. Conclusions: Altogether, we present a new diagnostic concept for anthrax detection and fingerprinting, which can be used alone or in combination with already established typing platforms.

164 (D)

Llama Single Domain Antibodies Specific for the 7 Serotypes of Botulinum Neurotoxin as Novel Diagnostics and Therapeutics

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Background: Botulinum neurotoxins (BoNT) represent a challenge for the development of diagnostics and therapeutics since they are all exceptionally potent, existing as 7 different serotypes (A-G) often with several subtypes (e.g. A1-A4). Since single domain antibodies (sdAb or nanobodies) are small, rugged and yet can be high affinity, we hypothesize that they may offer new routes of BoNT recognition and inhibition. Methods: A llama was immunized with BoNT toxins A and the immune repertoire cloned for phage display. We pooled on each of the biotinylated seven serotypes in solution, in the presence of excess unbiotinylated non-target serotypes. Unique sdAb were expressed, purified and assayed for their ability to recognize toxin and toxin complexes using micro-bead arrays. The neuro-2a in vitro cell culture assay was used to indicate which sdAb could inhibit BoNT activity. Results: Populations of sdAb reactive to serotypes A-G were isolated. Characterization of 18 unique anti-A clones revealed absolute specificity for serotype A(1) toxin and complex in solution with 5 of the clones exhibiting 20-30% cross-reactivity with subtype A2 complex. An unoptimised assay established a lower limit of detection of A1 toxin in milk of between 10 and 100 pg. At least 2 of the clones mediated a clear reduction in the amount of SNAP-25 cleaved in the western blot assay of BoNT A intoxicated neuroblastoma cells. Conclusions: Our ability to subtructively select antibodies on live botulinum in solution appears to be fruitful in yielding primarily serotype specific antibodies, some of which have neutralizing ability. Although limits of detection and Kd may need to be improved, the isolation of sdAb, especially to the rarer forms of BoNT (e.g. G) will provide an effective starting point to close this biosecurity gap while expanding our knowledge of BoNT structure function.

165 (D)

Rapid Detection of 18 Bioterrorism Agents Using Multiplex Real Time PCR with Melt Curve Analysis

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There has been a growing demand for rapid assays that can detect biowarfare agents in the battlefield, the environment, enclosed spaces, physical articles, and a variety of food and water sources. In this study, we describe detection assays based on real time Polymerase Chain Reaction (PCR) detection (or reverse transcription followed by real time PCR) of highly conserved regions of select agents. The studies describe several multiplexing options based on differences in melt curve temperatures. The assays described herein can be adapted to molecular Beacon or Taqman probe detection. We describe assays aimed towards the detection of the following 18 bacterial and viral select agents listed by the Centers for Disease Control and Prevention: Bacillus anthracis, Clostridium botulinum, Brucella species, Burkholderia mallei, Burkholderia pseudomallei, Chlamydia psittaci, Vibrio cholera, Clostridium perfringens, Coccidioides immitis, Escherichia coli O157:H7, Francisella tularensis, Ricin toxin, Rickettsia prowazekii, Salmonella species, Salmonella typhi, Shigella species, Variola major (Smallpox), and Yersinia pestis. The availability of such assays would facilitate a quick response to potential bioterrorism threats should they take place. Assays for the 18 described agents can be completed in less than 24 hours. The assays utilize positive control sequences cloned in multiple plasmids. The studies are performed using Cepheid’s SmartCyler and Stratagene’s MX4000 model real time PCR instruments.
Random RT-PCR Protocol for Viral RNA Evaluated by Microarray and qPCR

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Background: For broad nucleic acid screening of clinical samples, a random amplification is essential, before microarray hybridisation or random sequencing. Several different random amplification methods exist, but PCR based methods have the advantages of being widely used and relatively fast, adaptable and suitable for RNA viruses with small genome segments. Here we present the evaluation of a modified random RT-PCR protocol on a model system with 3 RNA viruses. Methods: Three single stranded RNA viruses with different genome organisation were selected, including Dengue 2 New Guinea C with one genome segment, Lassa virus strain Josiah with two segments, and Human Influenza A H1N2/H3N2 with eight segments. The genome segments were cloned into plasmids and used as references for Microarray and qPCR assays. A microarray was designed for investigating the distribution of the random amplicons, with 50mer oligonucleotides, distributed 500-1000 nucleotides apart over the genome segments. In addition, 1-3 qPCR assays were designed for each segment. Results: The Random RT-PCR was optimised to reduce problems with background and produce a uniform and random amplification as possible. The amplification of viral RNA was several million times for cellcultured Dengue 2 virus spiked in serum. The distribution of amplicons over the virus genomes was relatively uniform, for 1000 virus copies of viral RNA or more, with exception for the genome ends. Further work involves evaluation of methods to remove background nucleic acid. Conclusion: The developed protocol showed a good distribution and amplification of the viral RNA useful for microarray detection and identification as well as for whole genome sequencing of viruses.

Direct Analysis of Patient Samples for Simultaneous Identification of Respiratory Viruses and Select Agents Using PCR/ESI-MS

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Background: Accurate diagnosis of viral respiratory infections is critical to providing treatment to patients. Many bio-threat (BT) infections would share symptoms with respiratory infections in the early stages. The ability to provide additional detection of BT select agents during routine patient care could provide a powerful early warning system. Methods: Over 190 nasopharyngeal aspirates were collected from patients during the 2007-8 respiratory season and analyzed by PCR/ESI-MS to detect respiratory viruses from seven major families. Concurrently, throat swabs taken from these patients were screened with a biodefense assay capable of detecting a wide range of BT pathogens. Results: The PCR/ESI-MS respiratory assay performed with a sensitivity of 88.3% and a specificity of 92.3% for the identification of respiratory viruses from seven families. Viruses that are not detectable with conventional methods were also identified. Associated throat samples were also evaluated in the biodefense assay by spiking nucleic acids from appropriate agents/surrogates to simulate infection with BT agents. Detection using the combined viral and bacterial assay format will be presented. Viral load was quantified where appropriate and ranged from 15 - >2000 copies/well. Time to first detection was less than 8 hours. Conclusion: The PCR/ESI-MS technology accurately detected respiratory viruses. Concurrent testing of samples for BT agents was shown to be a viable and practical method for early monitoring of a biological attack. Monitoring patients for BT agents as part of routine infectious disease diagnostics could provide biodefense surveillance on a national scale.

Application of Commercial q-dots Fluorescent Conjugates for the Detection of Pathogenic Bacteria by Flow-Cytometry

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Background: Fluorescent semiconductor nanocrystals (q-dots) benefit from practical features such as high fluorescence intensity, broad excitation band and emission diameter dependency. These unique spectroscopic characteristics make q-dots excellent candidates for new fluorescent labels in multi-chromatic analysis, such as Flow-Cytometry (FCM). Methods: In this work we new possibilities of multi-labeling and multiplex analysis of pathogenic bacteria were demonstrated, by Flow-Cytometry (FCM) analysis and new specific IgG - q-dots conjugates. We have prepared specific conjugates against B. anthracis spores (q-dots585-IgGα B. anthracis and q-dots655-IgGα B. anthracis) Results: The new prepared q-dots conjugates combined with the bacterial FCM analysis enabled us to achieve double staining of B. anthracis spores which improve the FCM analysis specificity toward control Bacillus spores. Moreover, when specific conjugates toward the B. anthracis spores (q-dots585-IgGα B. anthracis) and Y. pestis bacteria (q-dots655-IgGα Y. pestis), were labeled with different q-dots, each characterized by its own emission peak as a marker, a specific and sensitive multiplex analysis for both pathogens where achieved, down to 10^3 bacteria per ml in the sample. Conclusion: Utilizing the unique fluorescent q-dots conjugates with FCM analysis can increase selectivity in bacterial pathogen detection (figure 1A) and can be use for multiplex analysis (figure 1B) as well.

Simultaneous Detection of Food-borne Pathogen using 16S rDNA Combinatorial Signature Chip and Pattern Mapping Analysis

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Background: There have been many attempts to develop sensitive and accurate techniques for the detection and diagnosis of pathogenic bacteria using nucleic acid-based technology. Here, to achieve efficient simultaneous detection of eleven selected pathogens, we propose the combinatorial oligonucleotide microarray containing double specific capture probes with pattern mapping analysis. Methods: After probe selection, two specific capture probes were selected from the total variable region of 16S rDNA for precise detection. To discriminate very close subtypes having nonspecific binding, the pattern mapping model was established by the neural network algorithm and repeated experimental data. Results: This microarray system harboring double capture probes and pattern mappings showed successful subtype discrimination between two closely related species having nonspecific hybridization. Conclusions: Therefore, using the proposed oligonucleotide microarray total solution, we could classify species and even subtypes of pathogens easily, promptly, and simultaneously.
170 (E)

Application of Microarrays for Characterization of Emerging, Unknown and Engineered Threats


Background: Defense against emerging, unknown and engineered biological threats is needed for national security. A comprehensive strategy for dealing with these threats is to provide the nation with a biodefense capability that goes beyond identifying a small handful of traditional high-threat agents. In this study, we report our development of several high-throughput microarrays designed to detect bacterial and viral pathogens.

Methods: We have developed a Virulence Array to detect virulence and drug resistance genes from known and unknown organisms; a Microbial Discovery Array to characterize species in complex bacterial and viral mixtures, and a whole genome SNP array to discriminate strains of high-profile bacterial threat agents. We have tested our arrays with various bacterial and viral pathogens, genetic engineering vectors, engineered strains, and clinical samples. We have also developed a sensitive and specific algorithm to analyze the results.

Results: The Virulence and Discovery arrays detected most tested pathogens at the species or strain level. The Virulence Array detected genetic engineering vectors both as pure samples and as remnants integrated into engineered chromosomes. The Microbial Discovery Array correctly identified multiple viral and bacterial infections in feline, fowlpox and fowl pox (prions and prion-like) samples that we later confirmed independently. Conclusions: Our microarrays are efficient and cost-effective tools to rapidly characterize emerging, unknown and engineered pathogens. They will enable informed responses to novel biological threats and provide a complement to high-throughput sequencing data.

171 (E)

Comparison of Ultrafilters for Recovery of Biothreat Agents from 100-L Tap Water Samples

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Background: Hollow fiber ultrafiltration (UF) can be an effective procedure for recovery of diverse waterborne microbes, including viral, bacterial and parasitic biothreat agents, during water-related emergency response events. Hollow-fiber UF is often performed using dialysis filters, for which there are numerous vendors but little data regarding comparative performance for microbe recovery. Methods: Using an established UF procedure, five single-use ultrafilters (Fresenius F200NR, Asahi REXEEDE-2S, Baxter Eceltra Plus 210, Baxter DICEA 210, and Minntech HPH 1400) were investigated to assess their performance differences and their ability to concentrate microbes from seeded 100-L tap water samples. The DICEA 210 filter was found to have insufficient hydraulic flux performance for large-volume water sampling. For the other four ultrafilters, at least five replicate recovery experiments were performed for the following microbes: Bacillus anthracis (Sterne) spores, Yersinia pestis, Francisella tularensis, Salmonella enterica serovar Typhimurium, Clostridium perfringens spores, vaccinia virus, MS2 bacteriophage, phi X174 bacteriophage, and Cryptosporidium parvum oocysts. Microbes were quantified in ultradilute concentrates and tap water controls to calculate recovery efficiencies. Results: In general, the ultrafilters associated with the highest recovery efficiencies were the F200NR and REXEEDE-2SS filters. These recovery percentages were greater than 50% for the following microbes: B. anthracis spores (89 and 90%, respectively). Y. pestis (193 and 242%, respectively), MS2 bacteriophage (61 and 66%, respectively), and C. parvum oocysts (76 and 88%, respectively). Conclusion: Data from this study demonstrate that UF can be effective for recovering diverse microbes in 100-L drinking water samples. These data indicate that recovery of some microbes was significantly higher when using F200NR and REXEEDE-2SS ultrafilters.

172 (E)

A Rapid GFP-Based Assay for Comparing Ribosome Inactivating Toxins

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Background: Ricin has been used as an assassination weapon and is currently listed as a category B agent. Detection devices for this agent have been developed, however there are no standard materials available to compare the sensitivity and robustness between the various instruments. As part of characterizing toxin standards, the activity of the protein needs to be determined. Conventional assays for cytotoxicity require additional reagents, are time consuming, and require cell death as an end product. We are using a rapid green fluorescent protein (GFP) degradation assay to specifically measure protein synthesis inhibition for quantifying the potency of ribosome inactivating proteins. Methods: Vero cells (African green monkey kidney cells) were stably transfected with enhanced GFP driven by a CMV promoter. Single clones were sorted and the cells were expanded and characterized by quantitative PCR and flow cytometry. The protein synthesis inhibition assays for toxin potency were conducted in 96-well plates using fluorescence microscopy to monitor the loss of GFP intensity. Conventional cytotoxicity assays were also performed by the addition of MTT reagent to the indicator cells treated with toxins after 24 hours. The GFP intensity after 6 hours was compared to the MTT cytotoxicity data at 24 hours. Results: The Vero cell lines described here strongly and stably produces GFP over many passages. For ricin and shiga toxins, the loss of GFP intensity is easily detected at 6 hours and is well correlated with the cytotoxicity measured by the MTT assay at 24 hours. Conclusions: The GFP-based assay is convenient, requires no additional reagents for detection, and is specific for ribosome inhibition. The degradation of GFP due to protein synthesis inhibition is rapidly detected and can be normalized to cell number using a nuclear stain. In contrast, conventional assays such as MTT are time consuming and dependent upon cell number. After only 6 hours of contact time with the toxins, the GFP-based assay predicts the cytotoxicity determined by MTT.

173 (E)

Isolation, Identification and Antimicrobial Resistance Patterns of Bacterial Isolates from Clinical Environment

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Disinfectants such as Benzalkonium chloride, Hexidine gluconate, and Triclosan are used very frequently in hospitals and are suspected to be a selective pressure for the development of antibiotic resistance. However, how these antibacterial agents associate with antibiotic resistance in clinical environment has not been understood well. To further understand the antibiotic resistance mechanism, investigating the occurrence of disinfectant resistance and the resistance rate are very important. Here, we report the occurrence of disinfectant resistance in clinical environment. In this study, twenty one clinical environmental samples were collected from different clinical laboratories at Massachusetts between January and March of 2008. Individual colonies were selected from nutrient agar plate containing variable antibacterial agent after 24 to 48 hours aerobic incubation at 37°C. Identification was conducted through biochemical tests and the DNA sequencing of 16S rRNA. Antimicrobial susceptibility was determined by Kirby–Bauer's disc diffusion method. Overall, eighteen hexidine Glucanate resistant isolates, ten Triclosan resistant isolates, and one Benzalkonium chloride resistant isolate were obtained. Biochemical tests and DNA sequencing suggested one isolate is Staphylococcus pyogenes, one is Enterococcus faecalis, four are Staphylococcus aureus, seventeen are Staphylococcus saprophyticus, and six are undetermined. Results of antimicrobial susceptibility demonstrated one Triclosan resistant S. aureus has across resistance to Ampicillin and Penicillin, and one Benzalkonium chloride resistant S. aureus has intermediate resistance to Ciprofloxacin, and Kanamycin. These data revealed that bacterial cells have developed ability to against disinfectants.
**174 (E)**

**Predictive Modeling for Release of Bacillus anthracis Spores in Drinking Water Systems**

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Predictive models offer value for formulating emergency response and consequence management strategies addressing release of biological agents into drinking water systems of municipalities and military installations. Utility of current water models (such as ICWater and PipeNet) may be limited due to insufficient scientific data on biological agents in water systems. For this reason, experimental trials were conducted to elucidate the fate of Bacillus anthracis spores during pipe distribution under defined conditions of a municipal chlorinated drinking water system. Dormant B. anthracis spores tolerated typical chlorine disinfection (1 mg/liter free available chlorine) and maintained viability for extended periods (at least 15 h) while traveling through pipe loop systems. Spore attachment to pipe surfaces during laminar flow was a function of pipe material and was enhanced by biofilm. Association of spores with pipe surface biofilm increased spore survival upon dosing with elevated chlorine residual. Experimental data are being evaluated to determine how variables, such as pipe diameter, could influence spore deposition and pipe system contamination. Findings of this study could provide the basis for improving predictive models and establishing more effective operations that guard against the threat of B. anthracis release in drinking water systems.

**175 (E)**

**Microfluidic Device to Investigate Microbial Response to Chemical Gradients**

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**Background:** Chemical gradients play an important role in microbial response and cellular function. Understanding the role of gradients in microbial function may lead to advanced detection and separation strategies as well as new drug development. A new device, a microfluidic palette capable of generating multiple diffusive chemical gradients simultaneously inside a microfluidic chamber was utilized to investigate the temporal and spatial response of Bacillus spores to germinants and the chemotactic response of Pseudomonas aeruginosa PA01 to glucose. **Methods:** The response to a two dimensional concentration gradient of stimulants of B. thuringiensis spores, B. anthracis Sterne spores, measured using membrane permeable fluorescent dyes, and the direct observation of chemotactic response of P. aeruginosa PA01 (GFP labeled) was monitored by fluorescent microscopy. Microbial response stimulants included spore germinants, 1 mM inosine, 8 mM L-alanine and glucose was observed in both the palette design as well as a control chamber with individual channels. **Results:** Here we demonstrate the use of a microfluidic device that generates overlapping diffusive gradients that can be maintained static or modified dynamically in a controlled way. When chemotaxis was monitored, stimulant concentration gradients formed gradually and reached steady state after ~40 minutes. Bacteria and spores were introduced into the chamber after gradients had fully developed. Spore germination was 99% complete after 2 hr. For motile bacteria, higher bacterial accumulation was recorded in the regions with the highest glucose concentration and cell motility was maintained and motility persisted for over 16 hours. **Conclusions:** The ability to measure microbial response to a stable gradient of chemical signal provides insight into stimulant synergy as well as provides a means to monitor microbial response to gradients similar to their native and host cell environment.

**176 (E)**

**Modifications to LLNL's Rapid Viability PCR Assay for the Recovery of Bacillus atrophaeus from Environmental Samples Collected from Porous Surfaces**


**Background:** The rapid viability protocol (RV-PCR) developed by Lawrence Livermore National Laboratory (LLNL) utilizing a combination of culturing and time-point PCR was compared with traditional plating methods on the ease of use and performance on samples collected from various porous and non-porous materials. Modifications were made to address discrepancies in viability data between culture and RV-PCR analysis caused by high particulate loads from HEPA sock samples collected from porous materials, particularly ceiling tile. **Method:** Ceiling tile coupons were deposited with Bg via aerosolization followed by sample collection via HEPA sock vacuum to represent the collection method and sample material that would yield the most particularites. Samples were extracted from the HEPA socks according to “CDC/NCID Protocol for the Quantitative Testing of Bacillus anthracis (Ba) spores” with modifications to accommodate the use of Bg. The extracted samples were then processed via LLNL’s RV-PCR protocol with two added steps after sample lysis. The first was a quick centrifugation step to pellet particulates and the second was a 1:10 dilution of the suspected inhibitor. **Results:** Modifications yielded improved agreement between RV-PCR and plating-based culture from 50% to >99% in laboratory testing. **Conclusions:** The 1:10 dilution of samples containing high particulate loads successfully addressed potential inhibitors of PCR.
**178 (E)**

**Ecology of Brucella Genotypes in Southern Kazakhstan**

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**Introduction:** *Brucella* species cause severe and chronic human diseases and are a Category B bioterrorism agent. Brucellosis is endemic in Kazakhstan and has remained a critical disease for the last 20 years with more than 2,000 new human cases of brucellosis registered each year. In this project we studied the ecological and molecular characteristics of *Brucella* species in two oblasts of Kazakhstan. We studied biotypes and genotypes of *Brucella* species in relation to epidemiology and clinical signs of brucellosis in humans and animals. **Methods:** A system was developed for sample collection from animals and humans with suspect brucellosis infections. All samples were subject to serological testing, microbiology, and PCR for detection and genotyping *Brucella*. Samples were mapped for assessment of geographical distribution in this endemic region of isolates obtained from animals and humans and their genetic types, and for entering the data on those isolates into a GIS database. **Results:** In 2007-2008, a total of 2,095 samples were collected from animals and 1,066 from humans referred to hospitals with suspected brucellosis. All samples were tested by serology and positive samples were cultured for *Brucella*. 71.3% of veterinary samples were positive; 82.6% of human samples were positive. 54 *Brucella* strains were isolated from veterinary samples and 275 *Brucella* strains were isolated from human samples. All bacterial isolates were genotyped confirmed by PCR to be *B. melitensis*. **Conclusion:** There is a significant distribution of *Brucellae* in Kazakhstan. This project has significantly improved the quality of brucellosis diagnostics in KZ.

**179 (F)**

**Detection of Novel Morph A Variants in Bacillus anthracis Ames Samples Discovered during Screening of the FBI Amerithrax Repository**

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Shortly after the start of the Amerithrax investigation, scientists from CBI worked with the FBI to find suitable forensic markers which could differentiate Ames isolates from the Anthrax used in the 2001 mailings. Sequence analysis of morphologically distinct colonies isolated from the Anthrax evidentiary samples uncovered genetic differences that could differentiate the evidence mix from other laboratory isolates. CBI developed two assays to target specific genetic rearrangements found in the 16s rRNA gene of one of the morphologically variant (known as Morph A). After validation, the assays were selected by the FBI for use in screening samples to help in determining the source of the Ames used in the mailings. CBI discovered 26 variants of the original Morph A rearrangements during the process of screening samples collected by the FBI for source attribution. CBI’s Morph A assays were able to pick up additional genetic variations in the 16s rRNA gene region in a small number of Ames isolates while absent in the evidentiary samples. Data presented here includes a comparison of the wild type *B. anthracis* Ames sequence to the previously undetected variants and to the original Morph A rearrangements. The complete set of variants obtained from screening the FBI Anthrax samples will allow for the creation of a genetic database of *B. anthracis* Ames samples similar to ODDES used in human forensics. This will provide a starting point for forensic analysis of any potential future *B. anthracis* Ames outbreaks or acts of terrorism. Further, the discovery of additional variation in the Morph A 16s rRNA gene region highlights a potential new hypervariable site in Anthrax.

**180 (G)**

**Sym002 - A Fully Human Polyclonal Antibody against Smallpox**

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The threat of smallpox outbreaks as a result of bioterrorism and the emergence of related viruses such as monkeypox have revived the need for anti-orthopoxvirus products which can be used therapeutically and prophylactically. Symphogen has employed the Symplex™ technology to copy the humoral anti-vaccinia virus antibody repertoire with the purpose of developing a fully human recombinant polyclonal antibody product as a biodefense agent against smallpox virus. Using our Symplex™ technology, natural cognate pairs of antibody genes were isolated directly from plasma blasts from recently vaccinia-immunized donors. Maintaining the original antibody heavy and light chain pairing ensures that the configuration and specificity of the resulting antibodies are preserved in an unaltered form. The resulting antibody repertoires were screened for virus and antigen reactivity revealing a large panel of anti-vaccinia virus antibodies reactive against multiple antigens on both the IMV and EEV particles. This strongly indicates that the isolated antibody repertoire mirrors the natural humoral anti-vaccinia virus response. As such, Sym002 is a polyclonal antibody product containing a number of potentially neutralizing, high-affinity antibodies against distinct and biologically relevant antigens on both the IMV and EEV. In vitro Sym002 exerts significantly improved neutralizing activity against a broad range of orthopoxviruses as well as high EEV-specific activity, when compared to the commercial available VIG product (Cangene). Likewise, Sym002 has proved superior antiviral activity in vivo in virus-challenge models. We here present a novel approach to the development of antibody-based therapeutics against Smallpox infections.

**181 (G)**

**Synergistic Biothreat Countermeasures from High-Throughput Combination Screening**

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**Background:** Biological threats are diverse and can emerge with little warning, so rapid and flexible countermeasure discovery is essential. Screening approved drugs with cell-based phenotypes can discover therapies that can be quickly deployed, and combining drugs dramatically increases such opportunities. Drug synergies can also overcome resistance, and are likely to target host factors with broad-spectrum activity. **Methods:** We are systematically exploring combinations of ~3,000 drugs and other bioactive compounds in phenotypic assays, using a high throughput robotic platform. Phenotypes are tested in matrices across many combined doses, and volumetric or isobologram scores measure synergy over single agent effects. Discovered synergies are prioritized on known drug properties, confirmed in animals, and advanced to the clinic. We have applied our platform to many disease areas, resulting in 5 current clinical trials.

**Results:** We present biodefense-relevant discoveries from screening in viral, bacterial and toxin survival assays. Examples include a cidal synergy against multidrug-resistant *Staph. aureus* between two non-antibacterial drugs, a combination that rescues cells from anthrax toxin via host signaling pathways, and an anti-viral synergy that acts through novel host factors. Several of these examples’ activities are confirmed in animals. We also discuss preliminary results from our collaborative projects focused on hemorrhagic fevers and Burkholderia. **Conclusion:** Systematic drug combination screening in biodefense-relevant assays is a promising strategy for countering existing and emerging bioterrorisms.
182 (G)
Multi-Drug Resistant Acinetobacter baumannii Drug Combination Evaluation
Background. Acinetobacter species are usually associated with nosocomial infections. The incidence of A. baumannii infections in US military hospitals has increased since 2003, particularly amongst wounded troops in Southwest Asia. The multi-drug resistant strain in this study was isolated from a wounded soldier (A. baumannii WR12G, Walter Reed Army Med. Cent.). A current goal of the NIH Biodefense program is to identify therapies that can be used to treat emerging infectious diseases. The purpose of this NIH-supported contract study was to evaluate drug combinations that might be useful to treat A. baumannii. Methods. Following CLSI guidelines, a colorimetric 96-well broth microdilution method was used. A. baumannii WR12G was screened against 36 commercial drugs. MICs were determined and used to design drug combination assays. Assays included combinations of the following drugs: colistin, doxycycline, levofloxacin, novoblocin, polymyxin B, rifampin and tetracycline. Combination assays were interpreted by calculating the fractional inhibitory concentration (FIC). A checkerboard pattern was prepared in seven, two-fold dilutions, with the highest concentration being two-fold above the MIC. Plates were read and data were directly incorporated into a spreadsheet. From this spreadsheet, data were compiled and analyzed using a tracking database. Results. Synergism (FIC ≤ 0.5) was observed with rifampin in combination with either colistin or polymyxin B. Additive effects (FIC >0.5 ≤1.0) were seen with the combination of colistin and novoblocin. Indifference (FIC >1.0 – ≤4.0) occurred with all other drug combinations. No combination showed antagonism (FIC >4.0). Conclusions. These results show promise as possible multidrug therapies for infections with the multi-drug resistant A. baumannii. These types of assays should be useful in determining other possible combination therapies with other multi-drug resistant emerging infectious agents.

184 (G)
Characterization of Neutralizing Antibodies Directed Against Ricin Toxin
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Background: Among the agents considered as biological threat, ricin is listed as a class B bioterrorism agent. Moreover, no curative treatment is available for this easy to prepare toxin. Then an immunotherapeutic approach could be of interest, assuming the characterization of antibodies attenuating or neutralizing the effects of the toxin. The present work aims at preparing neutralizing monoclonal antibodies against ricin, to further develop an effective therapy for in vivo neutralization, despite possible side effects which would be reduced by chimerisation of the antibodies. Methods: Mice monoclonal antibodies (mAbs) were produced against the two subunits of ricin toxin (RTA and RTB) and their neutralizing capacity in vitro tested using a viability test on immortalized T lymphocytes cells. Apparent affinities were evaluated using a competition enzyme immunoassay. Cloning of the variable regions is ongoing to construct chimeric antibodies. The mechanism of inhibition of the mAbs is under evaluation by immunofluorescence and flow cytometry. Results: Two of the 16 anti-RTB and two of the 5 anti RTA mAbs proved to neutralize efficiently the toxin effect in vitro. Moreover a combination of three antibodies (one anti-RTA and the 2 anti-RTB) enhanced significantly the protective effect. Apparent affinity of each of these mAbs was determined for ricin in the 10⁻⁹ M to 5 10⁻¹⁰ M range, similarly to other non-neutralizing mAbs showing the absence of correlation between protection and affinity. Anti-RTB neutralizing mAbs seem to prevent toxins from entering the cells whereas anti-RTA neutralizing mAbs would preferentially inhibit catalytic activity of the toxin. Conclusions: The strong neutralizing efficiency provided by the three associated mAbs makes them potentially useful in case of ricin intoxication. Further chimerisation, if protection is retained, would limit the immunological side effects classically observed using heterologous immunotherapeutic sera.

183 (G)
Broad Spectrum Antiviral Therapeutic Based on Iminosugar Derivatives
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Introduction: Iminosugars are glucose analogues which inhibit processing of viral glycoproteins and effectively impair the production of infectious virus, iminosugar antivirals limit the emergence of drug-resistant viral mutants
Efficacy of Cethromycin in a Murine-aerosol Model of Francisella Tularensis (ShuS4)
H. S. Heine1; L. Miller1; J. Bassett1; S. Halasohoris1; D. A. Eiznhamer2; M. T Flavin2; M. Lesk2; Z. Xu3; 1USAMRIID, Fort Detrick, MD, 2Advanced Life Sciences, Woodridge, IL.
Background: Francisella tularensis (FT) is the causative agent of tularemia, normally a zoonosis producing flu-like symptoms in humans. The low numbers of organisms required to cause infection makes FT a potentially effective agent for a terrorist attack. Previously determined FT in vitro susceptibilities for cethromycin showed great potential as a therapy for tularemia infections. Given the added possibility of resistance to current treatments through genetic engineering or natural emergence, identifying effective antibiotics with novel mechanisms of action is critical. Methods: BALB/c mice were challenged with aerosolized ShuS4 strain of FT. Animals were treated 24 hr postchallenge by oral intubations with cethromycin QD at 50 mg/kg, 75 mg/kg, or 100 mg/kg, or Q12 with 100 mg/kg. Gentamycin 24 mg/kg i.p. Q12 or saline were included as controls. Duration of treatment was for 14 days. An additional experiment of 75 mg/kg of cethromycin QD for 21 days was also performed. Results: Survival analysis suggested that the 100-mg/kg cethromycin doses were toxic while the 50-mg/kg dose was only partially effective in protection. The 14-day treatment with the 75-mg/kg dose was effective, but animals relapsed shortly after treatment was terminated. Extension of the 75-mg/kg treatment to 21 days did offer 100% protection from disease. Conclusions: Previous, duration of treatment, bactericidal activity, and tissue penetration were shown to play a role in the therapeutic outcome of tularemia infection. Cethromycin has shown significant in vitro activity against FT. Cethromycin preferentially accumulates in the alveolar cells of the lungs, which may enhance efficacy and is indicated by the murine data. These properties, combined with the oral bioavailability and once-a-day dosing make cethromycin an attractive candidate for use in tularemia therapy.
An Accelerated Path to Safe and Effective Therapeutics (APSET) for Bioterrorism Agents

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The rapid development of effective Medical Countermeasures against potential bioweapons is vital to the defense of the United States. The APSET program addresses this important need by developing a platform for rapidly screening and repurposing FDA-approved drugs as broad-spectrum Medical Countermeasures. The objective of APSET is to screen all FDA approved drugs for off-label broad spectrum efficacy against B. anthracis, F. tularensis, and C. burnetii bacteria and Ebola, Marburg, and Lassa hemorrhagic fever viruses. To date, the APSET program has screened 1302 FDA approved compounds against these 6 biothreat pathogens using in vitro cell culture assays, and the team has found a variety of hit drugs against 1 or more of the biothreat pathogens, indicating that some FDA approved drugs may be good broad spectrum therapeutics for these diseases. In vivo efficacy studies are planned next. All compounds evaluated in the program already have well-established safety and pharmacokinetic profiles. Once efficacy has been confirmed, SRI will work with the FDA to identify an accelerated path to approval for the new indication. SRI will also publish the information so new treatment options could be considered in case of a national emergency.

Identification of Fc Receptors Involved in Antibody-Mediated Fc Receptor-Dependent Anthrax Toxin Neutralization

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Background: The anthrax lethal toxin neutralization assay (TNA), which measures antibody-mediated protection of macrophages from the cytotoxic activity of anthrax lethal toxin (LT), has been used in clinical studies to assess the immunogenicity of anthrax vaccines. A clear understanding of mechanisms of toxin neutralization is essential for proper interpretation of TNA output. Both Fc receptor-dependent and -independent mechanisms of neutralization have been described (Vitale et al., Infect. Immun. 74: 5840, 2006). Mouse macrophages express four different Fc receptor types that bind the most prevalent antibody isotype, IgG. These are FcγRI, FcγRII, FcγRIII, and FcγRIV. In this study, we examined the role of two of these receptor types, FcγRII and FcγRIII, in the TNA in order to better understand Fc receptor-dependent neutralization. Methods: Bone marrow-derived macrophages (BMDM) were isolated from wild type mice, FcγRII-deficient (FcγRII-/-) mice, and FcγRIγ-chain-deficient mice (FcγRI/III/IV-/-). Polyclonal antisera generated by immunization with anthrax vaccine were assayed in TNA performed without and with an FcR-blocking antibody (mAb 2.4G2) using BMDM from each of the three strains of mice. Serum neutralizing antibody titers, expressed as EL IgGt, were determined. Results and Conclusions: We found that mAb 2.4G2, which specifically blocks FcγRII and FcγRIII, significantly decreased neutralization titers of sera when BMDM from either wild-type, FcγRI/III/IV-/-, or FcγRII-/- mice were used. Since FcγRI/III/IV-/- mice express only FcγRIIB, the component of neutralization blocked by mAb 2.4G2 is due to FcγRIIB. Similarly, since FcγRII-/- mice express FcγRII but not FcγRIIB, the component of neutralization blocked by mAb 2.4G2 is due to FcγRII. These results demonstrate that both FcγRIIB and FcγRIII can contribute to FcR-dependent neutralization of LT.

Protection in Mice Passively Immunized with Serum from Cynomolgus Macaques Vaccinated with Recombinant Plague Vaccine (rF1V)

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Introduction: A passive transfer assay was developed to evaluate the ability of rF1V antibodies generated in vaccinated cynomolgus macaques (CM) to protect naive Swiss Webster mice against pneumonic plague. Methods: A pharmacokinetic (PK) study was performed to establish the uptake, plateau and decay of circulating levels of rF1V antibodies following passive transfer to determine the appropriate time to challenge and monitor the mice post-challenge. To evaluate protection, 0.5 mL of whole serum from vaccinated CMs with known rF1V antibody titers was transferred into naive Swiss Webster mice via the intraperitoneal route on Day 0. Mice were bled 2-3 hours prior to challenge on Day 1 to determine the level of circulating rF1V antibody. Mice were then exposed to an aerosol challenge of 25 LD_{50} Y. pestis CO92 and survival monitored for 14 days. Results: The PK study demonstrated that peak circulating levels of rF1V antibodies occurred 4 hours following passive transfer into mice, were approximately ten-fold lower than levels in donor serum and were detectable in recipient mice for 8 days. Passive transfer studies demonstrated that mouse survival was positively correlated with anti-rF1, anti-F1 and anti-rF1V antibody levels in the CM donor serum and the circulating levels of these antibodies in the recipient mice. Conclusions: The results of the CM-to-mouse passive transfer assay demonstrate that humoral immunity is associated with protection against pneumonic plague. Although an immunological correlate of protection has not been identified for pneumonic plague, the passive transfer assay can be used to evaluate the functional role of rF1V antibodies in protection against aerosol exposure.

Electroporation mediated DNA immunization: Application in the Development of Biodefense Vaccines

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Background: In vivo electroporation (EP) enhances the uptake of DNA through the application of brief electrical fields at the site of administration. When compared to administration by conventional injection, EP enhances the magnitude, breadth, and functionality of DNA vaccines by several orders of magnitude. Methods: In separate studies, an integrated electroporation delivery system was used to immunize nonhuman primates with DNA vaccines encoding antigens from Venezuelan equine encephalitis virus and Bacillus anthracis. Antigen-specific responses were measured using standard methods. Results: EP delivery of 50μg VEEV DNA vaccine induced significant viral neutralizing titers after a single immunization, challenge of the nonhuman primates after a second immunization found that this low dose was capable of conferring protection from viral infection. Two EP mediated immunizations with a plasmid encoding anthrax protective antigen induced antigen-specific antibody responses that were capable of conferring protection from lethal challenge with anthrax spores nearly a year after the boost. Overall the humoral responses in both studies were comparable those induced by approved VEEV and anthrax vaccines utilizing attenuated/inactivated pathogens. The electroporation device used in these studies is suitable for human use and is currently being utilized in multiple Phase 1 clinical studies, including the first use of EP in a prophylactic clinical trial with healthy adults. To date, no vaccine related serious adverse events have been reported with all subjects in compliance with the vaccination schedule. Data from tolerability surveys suggest general acceptance of the procedure, even for preventative immunization. Conclusions: Collectively, these data demonstrate the potency and clinical applicability of EP in the development of DNA vaccines.
190 (H)

Impact of Concurrent ST-246® Administration on ACAM2000™ Vaccine Efficacy in Cynomolgus Macaques

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Background: ST-246®, a small-molecule inhibitor of poxviruses has demonstrated safety and efficacy profiles in various animal model systems. Therefore, it could potentially be used in combination with the current licensed smallpox vaccines to improve their safety profiles with the proviso that vaccine efficacy is not compromised. Here, we investigated the impact of concurrent ST-246 administration on ACAM2000™ vaccine efficacy in the non-human primate monkeypox virus (MPXV) model. Methods: Cynomolgus macaques were vaccinated with ACAM2000™ followed by daily administration of 10 mg/kg ST-246 or vehicle alone by oral gavage for 14 days. Animals given either ST-246 or vehicle alone by the same dose and route served as controls. Forty-five days post-vaccination, all animals were challenged with 5×10⁸ PFU MPXV Zaire strain via IV route, and monitored for clinical signs typical of MPXV disease. Results: Vaccination followed by ST-246 treatment reduced lesion size formation at the site of injection (p < 0.05). Following challenge with MPXV, four of seven animals given ACAM2000™ plus ST-246 developed no or low pox lesions and similar viral loads (p < 0.05) compared to animals given vaccine plus vehicle. However, all vaccinated animals whether treated with ST-246 or vehicle were protected from severe MPX disease and demonstrated significantly lower viral loads compared to controls (p < 0.05). By contrast, all control animals rapidly developed typical signs of MPXV disease with viral loads between 7 – 8 logs at time of euthanasia. Conclusion: Our findings demonstrate that ST-246 appears to reduce vaccine reactogenicity and concurrent administration with ACAM2000™ conferred similar levels of protection in 4/7 animals compared to animals given vaccine alone. Pending immunological evaluations will shed further light on the overall impact of concomitant ST-246 treatment on vaccine efficacy.

191 (H)

DNA Plasmid and Alphavirus Replicon Based Vaccine Strategies Effectively Elicit Immune Responses against Rift Valley Fever Virus

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Background: Rift Valley fever virus (RVFV) is an arthropod-borne Phlebovirus (family Bunyaviridae) associated with abortion storms, neonatal mortality in livestock and hemorrhagic fever or fatal encephalitis in a proportion of infected humans. The ability of this emerging virus to cross national and international boundaries poses a significant agro-biological threat to both livestock and humans. Although, the inactivated RVFV vaccines have been used in livestock, there is no licensed vaccine available to protect the human population. Therefore, there is an urgent need for developing safe and effective vaccines that rapidly elicits protective immunity against RVFV infection. Methodology/Principal Findings: To address this, DNA plasmids expressing ectodomain of RVFV Gn glycoprotein in conjunction with three copies of molecular adjuvant C3d were constructed and analyzed along with Gn expressing Sindbis virus replicon for their ability to act as potent vaccine candidates against RVFV in mice. An experimental live-attenuated vaccine (MP-12) was used as a benchmark for comparison. These vaccines were able to express RVFV Gn in vitro and elicited anti-RVFV antibody responses in immunized mice, as determined by RVFV specific ELISA, IgG isotype ELISA, and production of a neutralizing antibody response. Interestingly, these vaccines were able to elicit cellular immune responses as determined by Gn specific ELISPOT assay. Conclusion/Significance: This work was supported by an NIH award R01AI074946 to T.M.R. and the CVR
Comparative Transcriptional Analysis of the Murine Host Response to Francisella tularensis LVS and Schu4
L. C. Kingry, R. Troyer, N. Marlenee, R. Bowen, S. Dow, A. Schenkel, R. A. Slayden; Colorado State University, Fort Collins, CO.

Francisella tularensis, a gram negative coccobacillus, is classified as a class A select agent and is the cause of deadly pneumatic tularemia. Mechanisms into the ability of F. tularensis to cause deadly disseminated disease upon inhalation of as few as 10 organisms continue to elude researchers. Our study aims to characterize the global transcriptional response differences between Type A F. tularensis Schu4 and the Type B vaccine strain (LVS) using a lethal pulmonary model of disseminated infection in C57Bl/6 mice. In order to further investigate the rapid dissemination associated with F. tularensis pulmonary infection our group has utilized a systematic analysis of the disease process, including transcriptional profiling of host response, rate and extent of dissemination to secondary sites in the body, and determination of bacterial burden in vital organs. C57Bl/6 mice infected intranasal or via low dose aerosol with F tularensis LVS or Schu4 were examined for changes in global and targeted transcriptional response, histopathology and bacterial burden in the lung and spleen at 12, 24, 48, and 120 hours post infection. Transcriptional profiling of the host response to both LVS and Schu4 revealed molecular biomarkers that represent the initial and late stages of dissemination that will help guide future therapeutic and prophylactic studies. Furthermore, targeted transcriptional analysis of the host response has led to a better understanding of the subtle differences in pathogenesis between Type A and Type B Francisella.

Efficacy of Disinfectants Against Pathogenic Viruses
J. Stewart, T. Piercy, L. Eastaugh, M. Lever, Dstl, Salisbury, UNITED KINGDOM.

Background: Effective disinfectants are critical for the safe laboratory handling of highly pathogenic viruses such as Vaccinia, VEEV, Ebola virus or Marburg virus. Many of these disinfectants, however, contain one or more substances which are hazardous to human health; therefore, there is a requirement to test the efficacy of alternative less hazardous disinfectants for use within the high containment laboratories. Within the BSL 3 and 4 laboratories at Dstl Porton Down, an aqueous solution of aldehydes and quaternary ammonium compounds is used as a disinfectant. The objective of this study was to compare the efficacy of a range of commercially available solutions, against select agents including filoviruses.

Methods: The efficacy of the disinfectants was assessed by the reduction in virus titre, by the use of a modified microtitre assay to determine the TCID50 of the respective viruses based on BS EN 14476:2005. A contact time of 5 minutes was used for the disinfectants at varying concentrations as per manufacturers’ guidelines. Results: The halogenated tertiary amine TriGene Advance was effective at reducing the titres of Vaccinia WR and Venezuelan Equine Encephalitis virus (VEEV) by at least 4 logarithms. Ebola Zaire and Marburg virus however were more resistant to the disinfectant and viral titres were only reduced by 2 logarithms.

Conclusion: TriGene Advance is an effective chemical against Vaccinia WR and Venezuelan Equine Encephalitis virus but not against the Filoviruses. A disinfectant may not be appropriate for all virological agents. This indicates that there is a need to validate each disinfectant against the specific agent to be used to determine activity.

Systems Approaches to Infectious Diseases: A Translational, Infectious Disease Phenome Initiative (TIPI) for the Forward Discovery of Unique and Common Targetable Pathways to Develop Effective Therapeutics
M. Koth; University of Cincinnati, Cincinnati, OH.

The pathogenesis of most infectious diseases involves complex interactions of many different host systems and pathways. Identifying networks of pathways that modulate susceptibility to several pathogens affords a means to develop therapeutics for a number of infectious diseases. We have developed and optimized a forward systems genetics approach that allows for unbiased discovery of disease interactive pathways. Our approach takes advantage of a large genetically distinct population of mice, where each member of this population is represented by an inbred line. Because these mice are extensively genotyped, we have been able to correlate variations in susceptibility and the various infection phenotypes to the genotypic variation across the panel. In doing so, we have been able to map gene loci that harbor genes involved in modulating infection outcomes. By parsing those genes into pathways, we have been able to tease out disease interactive pathways and generate roadmaps for different infections, and this allows us to identify pathways that are unique or common to more than one pathogen. Translation from mouse to human is more feasible using this approach because it involves pathway to pathway comparisons rather than gene to gene correlations. We have been successful in applying this approach to a number of infectious diseases that will be presented. We believe we have generated a robust platform for the forward discovery of disease pathways, biomarkers and immune correlates that will inform the design of preventive and protective measures.

Multiplex Cytokine Analysis of Pathogenic Material from Non-Human Primates Using Formaldehyde Fixation
V. A. Graham, S. D. Dowall, K. Steeds, T. Tipton, S. Funnell, M. Finney, J. Vipond; CEPR-Health Protection Agency, Salisbury, UNITED KINGDOM.

Due to ethical limitations of research involving Biodefense and Emerging Pathogens, it is necessary to develop non-human primate (NHP) models of these types of infectious diseases. To obtain as much information as possible from NHP studies, whilst minimising sample draw volumes, there is a need to multiplex assays. However, the acquisition equipment required for multiplexing assays, e.g., Lumines200TM, can prove sensitive to BS3L containment conditions. In order to avoid decontamination of the Lumines200TM, we have investigated formaldehyde treatment as a means of decontamination of the sample.

Methods: To test the suitability of formaldehyde treatment as a decontaminant, we first demonstrated that the technique was suitable for material from cynomolgus macaques (Macaca fascularis) using standards from a single IFNγ plex. The assay was performed as per manufacturers’ guidelines with the addition of pre-treating the stained micropheres with various concentrations (10, 5, 2, 1, and 0%) of formaldehyde overnight, before acquisition on a Lumines200TM. Results: We have shown that IFNγ micropheres can be treated with up to 10% formaldehyde overnight without loss of specificity. However, there was a decrease in mean fluorescence intensity, which was proportional to the increasing concentration of formaldehyde. With the recent introduction of a NHP 23-multiplex, results from all of these analytes, with and without formaldehyde treatment, will be presented.

Conclusion: Our results show that by using formaldehyde fixation, acquisition of multiplexed assays can be performed with highly pathogenic material at a lower level of containment. This avoids the need to put the Lumines200TM at risk of formaldehyde vapour damage. Data from cytokine analysis may lead to additional interpretations in the study of pathogenesis and vaccination against disease, and has the potential to identify novel strategies against these causative agents.
**Abstracts**

**Tuesday Poster Session**

### 199 (F)

**Standard Method for Dynamic Wipe Efficiency Determination**

**J. B. Morrow**, S. Da Silva; National Institute of Standards and Technology, Gaithersburg, MD.

**Background:** We are developing a standard sampling method for the removal of spores on nonporous surfaces using wipes to evaluate new sampling materials as they become available in the marketplace, and to determine the fundamental impacts of environmental parameters including relative humidity, sampling speed and pressure on sampling efficiency for spores on nonporous. Both nonwoven and woven wipe materials were evaluated for their ability to remove spores and for recovery from wipe material surfaces. **Methods:** *B. anthracis* Sterne spores with a GFP label are deposited on nonporous control substrata (glass, stainless steel, copper, Teflon and aluminum). Adhesion forces due to deposition method are determined by imaging in contact mode at increased force with atomic force microscopy. Wipe extraction procedures were characterized by viable plate counts for wetting agents (PBS, PBST, water and Tween 80). Both nonwoven and woven wipe materials were investigated for the efficiency of removal of spores on nonporous glass surfaces. Spores deposited on surfaces were quantified, before and after wipe procedures, by fluorescent microscopy and residual spores were quantified by viable growth on contact plates. **Results:** We have developed a method to perform wipe surface sampling of spores on nonporous surfaces under controlled environmental conditions. Wetting agents, including the addition of a surfactant were found to significantly impact the extraction efficiency. Tween 80 addition increased wipe extraction when wipes of nonwoven and woven materials were investigated. The strength of spores association with wipe material surfaces was dependent on the interaction of the wetting agent and the wipe material. **Conclusions:** Developing a standard wipe method for spores deposited on surfaces is critical to accounting for efficiency perturbations on sampling capabilities as well as advancing wipe surface sampling technology for both biodefense as well as clean room technology.

### 200 (F)

**Identification of Test Protocols for EPA Registration of Current and New DoD Decontaminants**


**Background:** In order for a Department of Defense (DoD) decontaminant to be considered a sporicide it must meet the data requirements for registration by the United States Environmental Protection Agency (EPA). By developing at least one method to satisfy both DoD’s efficacy needs and EPA’s product registration needs, sporicides can be tested, reviewed, approved and made available for use in war and peace time situations. **Method:** The three assays that were utilized were the ISOP/DoD method, the modified three step method (mTSM), and the quantitative carrier test-2 (QCT-2). The three decontaminants that were tested were unamended bleach, amended bleach (pH 7.0 ± 0.5), and DEF200. **Results:** The initial test results with unamended bleach at 6000 ± 300 ppm with a contact time (CT) of 30 minutes showed nearly a complete reduction in *B. subtilis* spores on the carriers for all methods. The initial log reductions in colony forming units (CFU) were roughly equivalent to the CFU/carrier of the mock treated/neutralized control carriers, effectively limiting the range of the assays to a maximum determined by the recovered CFU per mock treated and neutralized carrier(s). Therefore, the concentration of the bleach was adjusted to 3,000 ± 300 ppm and the CT was reduced to 15 minutes successfully achieve a partial reduction in *B. subtilis* spores recovered from the treated carriers to facilitate a comparison between the assays. The majority of decontamination agents tested in these assays at various concentrations and CTs exhibited a complete log reduction in *B. subtilis* spores. **Conclusions:** Future research will involve the comparison of these methods using less effective decontamination agents at various concentrations/CTs to determine which assays are optimal for the study of novel decontamination technologies to be used with biodefense etiologic agents.

### 201 (K)


**G. Epstein; Center for Strategic and International Studies, Washington, DC.**

The Global Forum on Biorisks is premised on the fact that many different professional communities — including not only scientific research but others such as public health, human and veterinary medicine, law enforcement, disaster management, counterterrorism, and industry — play vital roles in managing biological risks, particularly those that are deliberately induced. These communities all share two characteristics. First, addressing deliberate biological risks is not their highest priority; they all have “day jobs” that have greater claim on their time and attention. Second, they typically do not interact with one another in the course of their everyday activities. Nevertheless, they will all have to work together to reduce, mitigate, or respond to natural, accidental, or deliberate sources of disease. At the same time, health risks transcend national boundaries, and so must any efforts to plan for or respond to them. This Forum is a novel approach to foster and empower the partnerships among professional communities around the world that are needed to anticipate, mitigate, and respond to sources of biological risk. It offers a new model for global, “bottom-up,” decentralized governance that will operate by socializing and advancing biological risk management within each of the relevant professional communities, and by creating a venue in which these communities can interact, understand each other’s rules, share best practices, assess each other’s progress, and pursue joint efforts. It offers an approach that is appropriate for a decentralized problem involving non-state actors as well as states, and in which there is little consensus about the magnitude or the likelihood of the problem.

**GLRCE ARIC acquired CDC registration and certification to sustain a flourishing select agent program that already encompasses several different A-C agents: *Y. pestis* (plague), *B. anthracis* (anthrax), *R. typhi* (typhus), *R. rickettsii* (Rocky Mountain Spotted Fever), *R. conorii* (Mediterranean Spotted Fever), and MRSA (drug resistant S. aureus).** The GLRCE ARIC is the mission of the Great Lakes Regional Center for Biodefense and Emerging Infectious Diseases (GLRCE) Animal Research and Immunology Core (ARIC) to provide professional research services in support of the development of vaccines, diagnostics and therapeutics against NIAID Category A, B and C agents and to support GLRCE under emergency situations, marshalling wet laboratory infrastructures and biodefense expertise for the RCE network. The GLRCE ARIC acquired CDC registration and certification to sustain a flourishing select agent program that already encompasses several different A-C agents: *Y. pestis* (plague), *B. anthracis* (anthrax), *R. typhi* (typhus), *R. rickettsii* (Rocky Mountain Spotted Fever), *R. conorii* (Mediterranean Spotted Fever), and MRSA (drug resistant S. aureus). The GLRCE ARIC provides standardized, high quality animal model systems featuring mice, rats and guinea pigs. Highly trained Select Agent professionals perform study design, manipulation, management, and post-infection analysis on all the animal models. The Core also provides immunological tests, such as ELISA for specific antibody titers, antibody isotyping, ELISPOT and T cell proliferations assays as well as FACS sorting for the analysis of immune cells. We also prepare rabbit polyclonal antibodies and monoclonal antibodies. Our most recent research frontier is the development of an aerobiology laboratory for Select Agents and the continuous development of the Howard Taylor Ricketts Laboratory as the Regional Biocontainment Laboratory for the GLRCE.
One Health: A Concept to Improve Biodefense and Global Health

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Background: One Health is a growing international movement of physicians, veterinarians, public health professionals, and scientists who recognize the need to join forces to meet the global health challenges of the 21st century. Many of the agents of bioterrorism and nearly 75% of the newly emerging infectious agents are zoonotic diseases. Examples of such diseases include HIV/AIDS, Ebola, SARS, and Nipah and Hendra viruses. Almost 20 professional organizations including the American Medical Association (AMA), the American Veterinary Medical Association (AVMA), the American Society for Microbiology (ASM), and the American Society of Tropical Medicine and Hygiene (ASTMH) have endorsed One Health and the importance of understanding cross-species disease transmission. Cross-cutting educational programs and funding to support a multidisciplinary approach to diagnostics, vaccine and antibiotic resistance research are also needed. A new “One Health Initiative” website has been established to serve as a repository of information for all One Health efforts. This website is available at: http://www.onehealthinitiative.com. The goal of this website is to facilitate communication and collaboration between professionals of different disciplines. Two additional valuable sources of One Health information are ProMed (http://www.promedmail.org) and the One Health Newsletter (http://www.doh.state.fl.us/Environment/community/One_Health/OneHealth.html).

Ricin Acute Inhalation Exposures

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Background: Ricin is a toxic protein product of the castor plant. It is a CDC Category B bioterror threat and considered an agent that can be utilized by terrorists. At LRRMI, ricin inhalation studies were performed to compare acute toxicity, lung clearance and histopathology in rats and mice. Information obtained may also be useful in later assessments of the efficacy of countermeasures developed to treat ricin exposure in people. Method: Female Sprague Dawley Rats and BALB/c mice were exposed to a known ricin aerosol concentration to achieve dose levels determined by exposure duration. A nose-only inhalation exposure system consisting of a flow past Intox chamber, modified continuous flow Aerotech III nebulizer, all glass impinger sampler, and low flow cascade impactor for particle size analysis were used. Target deposited doses for the acute toxicity studies were 0.12 - 1.2 µg/kg. The observation period was 7 days, with tissues harvested for histopathological evaluation at time of euthanasia or death. Lung clearance and tissue distribution were assessed at 6 time points, up to 72 hrs post exposure to approximately 95 ng ricin/L air for 20 and 40 min. Generator and impinger samples were stabilized in milk solution and gel electrophoresis used to assess protein integrity before and after generation. Results: Rats and mice were exposed to ricin aerosol (1.06 µM MMAD). The calculated median lethal doses were 0.13 µg/kg for rats and 0.56 µg/kg for mice. For rats, initial lung burdens following 20 and 40 min exposures were 52 ± 7.7 and 145 ± 25 ng/lung (45 ± 10, and 110 ± 25.2 ng/g lung) respectively. Initial lung burden in mice following a 40 min exposure was 12.6 ± 7.7 ng/lung (88.5 ± 65.9 ng/g lung). Pulmonary lesions included hemorrhaging, vasculitis, and bronchiolitis. The half times of ricin elimination from the lung were less than 24 hrs for both species. Conclusion: Results indicate that ricin is extremely toxic following inhalation, that ricin is eliminated from the lung rapidly, and that responses of rats and mice are similar.

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The Swedish Laboratory for Food Safety and Biopreparedness

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The Swedish Laboratory for Food Safety and Biopreparedness is a collaborative effort between the National Food Administration and the National Veterinary Institute in Sweden. The purpose of this collaboration is to increase the diagnostic capability for extraordinary events of suspected microbial contamination in the food chain in Sweden. The food chain runs from “farm to fork” and includes for example feed used in food production — as well as the food products ready for consumption. Within this collaboration we have access to BSL-3 laboratories where we develop new methods as well as train our capacity to process large number of samples with bacterial contaminants. We also work with model organisms in a BSL-2 environment where we can develop the methods and then implement them in the BSL-3 lab. One major focus of the work is to develop methods for efficient and automated DNA extraction from a wide variety of food and feed matrices, to achieve this we have access to several different robotic platforms. To complete the diagnostic circle we are developing specific real-time PCR assays for a number of food pathogens. We are including pathogens that are not BSL-3 classified, but unusual in Sweden today, such as Vibrio spp., Shigella spp., Clostridium botulinum, as well as BSL-3 pathogens such as Bacillus anthracis. We are also part of a larger national biopreparedness network, FBD (Forum for Biopreparedness Diagnostics) where, in addition to the food and veterinary disciplines, human and environmental diagnostics of pathogens also are represented through the Swedish Institute for Infectious Disease Control and the Swedish Defence Research Agency. This network is primarily focused on harmonisation of methods and equipment between the participating laboratories to increase the biopreparedness.

Aerosol Exposure Results in Increased Deposition and Retention of Fluorescent Microspheres in the Lungs of Balb/c Mice Compared to Intranasal Inoculation

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Background: Intranasal inoculation has been posited as a “poor-man’s” method for aerosol challenge of animals. However, there is very little data comparing intranasal inoculation head-to-head with a “true” aerosol exposure. Methods: Balb/c Mice were exposed by either intranasal inoculation or aerosol exposure in a whole-body exposure chamber to fluorescent microspheres. Aerosol exposures were conducted with a Collison 3-jet nebulizer controlled by the AeroMP aerosol exposure system (Biaera Technologies, Frederick, MD). At various timepoints after exposure, mice from each group were euthanized and the lungs removed and analyzed by flow cytometric analysis for the initial deposition and retention of microspheres in lung. Results: Two hours after exposure, only 1% of the microspheres were retained in the lungs of mice that were exposed by intranasal inoculation to 105±106 microspheres. In contrast, 13.5% of the microspheres were retained in the lungs of mice aerosol exposed to an inhaled dose of 3.8±105 microspheres. Forty-eight hours after exposure microspheres were no longer detectable in the lungs of intranasally inoculated mice but aerosol exposed mice retained 5.8% of the inhaled dose of microspheres. Conclusion: These data demonstrate that aerosol exposure resulted in an increased deposition and retention of fluorescent microspheres in the lungs of Balb/c mice compared to that of intranasal inoculation. It will be necessary to perform further comparisons of intranasal inoculation with true aerosol exposures using pathogens as these differences in deposition and retention are likely to alter the virulence or pathogenesis of viruses, bacteria and toxins entering through the respiratory tract.
207 (K)

Putting More “Sure” in Biosurety

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Background: The United States Department of Defense (DoD) has implemented a new regulation to govern the access of personnel to biological select agents. This regulation, Army Regulation 50-1 (AR-50-1) published on the 28th of July, 2008, has broad implications for DoD employees and non-DoD contractors. The set of rules that comprise the regulation form a program referred to as Biological Surety or Biosurety Methods Literature review and comparison of current and pending surety programs. Results: The philosophical underpinnings of the DoD Biosurety program were derived from the related programs for nuclear material and chemical material. They are also different than the Biosurety program adopted by NIH and those currently proposed by others. The Biosurety program as currently written places a heavy burden on the individual to “self-report” any potentially disqualifying information. This may be a concern when coupled with the perceived current lack of a standard methodology for determining mental fitness for the program. In addition, the criteria for enrollment or suspension from the program are sometimes subjective. The decisions to enroll or disenroll individuals in the program rest solely with an appointed certifying official and it is essential that the guidelines for these decisions be applied consistently within and across organizations. We intend to highlight these and other philosophical underpinnings and focus on the perceived strengths and weaknesses of this program and offer alternatives for consideration. Conclusions: Because this regulation has far reaching implications outside of the DoD, we believe that a deliberate effort should be made to establish a single national Biosurety standard. Such a standard should provide for consistency across all organizations and funding agencies working with select agents and a strong emphasis should be placed on a tiered, risk-based approach to all aspects of the biosurety program. The implications for the scientific enterprise are great and we hope to induce careful consideration of this nascent aspect of biosurety management.

208 (K)

Integration of High Frequency Stability and Particle Size Measurements into a Whole-Body Exposure System

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In a real-world situation, the most relevant route of exposure to potential bioterror agents is inhalation of an agent-containing aerosol. Therefore it is important that laboratory studies aimed at modeling diseases or evaluating therapeutics against these bioterror agents utilize inhalation of agent-containing aerosols as the route of exposure. In a laboratory scale exposure system, it is necessary to accurately and reproducibly generate and characterize the experimental aerosol. Previous exposure systems have utilized sampling devices that collect one sample over an exposure period and yield an average concentration or particle size over that period. Since only one measurement is made, spikes and/or troughs in aerosol concentration that occur during the exposure period cannot be detected. The goal of the present study was to integrate a higher frequency sampling device, namely a Model 3321Aerodynamic Particle Sizer (APS) capable to monitoring both particle size and concentration, into the existing Automated Bio-aerosol Exposure System (ABES), a computer controlled platform for controlling and recording relevant parameters during an aerosol exposure. The aerosol characteristics obtained while running the APS continuously were compared to those obtained when a 30-s APS sample was taken after the aerosol had theoretically reached a steady state concentration. A single 30-s APS sample resulted in perturbation in the chamber concentration profile due to time necessary for the APS pump flow and ABES exhaust flows to equilibrate. However, when the APS was run continuously throughout the exposure period, no perturbations in chamber stability were seen since the pumps did not switch on and off. Furthermore, by integrating a continuously sampling APS into the ABES, aerosol stability and particle size in the chamber can be monitored throughout the exposure period, allowing for error detection/correction and future feedback-based dynamic dosing control.

209 (K)

The Center for Structural Genomics of Infectious Diseases

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The NIAMD funded Center for Structural Genomics of Infectious Diseases (CSGID) has been established to apply structural genomics approaches to potential drug targets from NIAMD priority microbial pathogens for biodefense and emerging diseases. The CSGID provides a high throughput pipeline for structure determination that carries out all steps of the project, from target selection through structure deposition. The CSGID emphasizes selection of targets with biomedical relevance and potential therapeutic benefits. Target proteins include drug targets, essential enzymes, virulence factors, vaccine candidates and proteins requested by the broader scientific community. One focus of the CSGID is determining the structures of complexes of the target proteins with small molecule ligands such as natural substrates, cofactors and drug candidates. The resulting protein expression systems, purified proteins, ligand screens and protein structures will provide valuable data and reagents for the scientific community. These products of the CSGID are essential groundwork that will benefit future research and drug discovery. Target proteins are screened using a denaturation thermal shift assay to identify possible small molecule ligands. The change in fluorescence of a dye bound to denatured protein that occurs upon denaturation and aggregation is measured as a function of temperature in the presence of libraries of small molecule ligands. The structures of target proteins and their complexes with ligands that have been determined will be presented.

This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases National Institute of Health, Department of Health and Human Services, under Contract No. HHSN272200700058C.

209-2 (H)

Thermal Stabilisation of Influenza Haemagglutinin Using Proprietary Stabilisers

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Background: A thermally stable pandemic influenza vaccine would have a significant impact in any future stockpiling plans. Currently, any such stockpile is bound by the need for strict temperature control which is essential to preserve the efficacy of the vaccine, and brings with it huge logistical implications, significant cost, and complications surrounding deployment. Stabilitech Ltd is a UK-based company which has overcome live virus thermostability issues using its proprietary stabilizing formulations, and more recently has demonstrated the utility of the technology for stabilizing isolated proteins and sub-units. We have developed a proprietary stabilizer which, when mixed with haemagglutinin suspensions enables the product to be lyophilized and prevents thermal damage when stored at elevated temperatures. Upon reconstitution it no change in protein levels as measured by ELISA and SRID assays could be detected. Stabilitech’s approach was based on mimicry of the biochemical events occurring during the maturation of seeds. During this process, seeds are rendered desiccation and thermo-tolerant. Methods: Protein solution was mixed at a ratio of 1:5 with stabilizer. The mixture was frozen and lyophilized over the course of 2 days. Accelerated thermal challenge studies were performed by holding the samples at 50°C for several days or 80 °C for several hours. At the end of this period the remaining haemagglutinin was determined by ELISA and SRID. Results: Haemagglutinin was rendered thermally stable to a very high level over the timeframe examined. Protein losses observed during processing were negligible (consistently less than 10%). In the absence of excipients almost total loss occurred. Conclusions: Stabilitech’s technology has overcome a major hurdle affecting Influenza vaccine storage and distribution, which will significantly simplify any stockpiling plans for the vaccine.
High Resolution *Francisella tularensis* Typing Assay Based on Multi-strain Global SNP Markers

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**Background:** Multi strain whole genome sequencing can provide genotyping methods for *F. tularensis*, a causative agent of tularemia and select A agent. Earlier, we reported collection of whole genome sequence and global SNP data from 40 *F. tularensis* strains using an array based approach. We now report SNP markers and diagnostic PCR assays for the discrimination of *F. tularensis* subspecies, clades and subclasses using these data. **Methods:** A phylogenetic tree was generated from the forty compressed sequences representing positions at which a SNP was called in one or more of the whole-genomes of resequenced strains. Node pairings which discriminated between subspecies or within subspecies were selected for the development of SNP typing assays. PCR primers for the 32 discriminating SNP markers identified *in silico* were designed. Real-time PCR assays were developed based on the set of discriminating locations identified to type subspecies, clades and subclasses. **Results:** DNA from 25 type A and 20 type B strains was used for validation of the SNP diagnostic assays. **Conclusion:** The 40 resequenced strains were distributed into 64 phylogenetic nodes and revealed clustering of type A and type B strains with discrimination of strains within cluster. Diagnostic SNP assays clearly distinguished between the 25 type A and 20 type B strains. The 25 type A strains were subdivided into 16 A1 and 9 A2 strains. The 16 A1 strains were distinguished into 8 A1a and 8 A1b strains. Type B isolates were further discriminated as 11 B1 and 9 B2 based on a single SNP. **Results**

**Results**

The whole-genome SNP based clustering shows the potential for selecting discriminating markers for high-resolution typing of *F. tularensis* strains.

**Integration of Proteomics and Transcriptomics Results through Metabolic Modeling of Salmonella Pathogenesis: Identification of Knowledge Gaps and Possible Functional States**

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**Background:** A major challenge in studying bacterial pathogenesis is to understand the mechanisms of how multiple environmental cues are processed and integrated into a logical and coordinated cellular response. Salmonella is a model Gram-negative bacterial pathogen that infects a variety of hosts, is a major public health problem with millions of cases per year, and possesses potential as a bioweapon. Salmonella must be able to survive transit through the host’s digestive system to invade the intestinal epithelium where it provokes an inflammatory response and colonizes normally microbicidal macrophages.

**Methods:** To identify the changes in Salmonella’s metabolic network that allow it to survive and transition through these diverse environments, we employed a systems-biology approach whereby our genome-scale reconstruction of Salmonella metabolism was utilized to integrate and analyze proteomics and transcriptomics data from knock-out mutants of 15 essential virulence regulators grown in pathogenesis-relevant conditions. **Results:** Results from this analysis have identified knowledge gaps in the metabolic network, thus pointing to areas in need of further experimental studies and model refinement. They also identified functional states that might explain how Salmonella uses its metabolic network during pathogenesis. **Conclusion:** This approach possesses potential as a means to delineate novel mechanisms involved in pathogenesis and to identify possible novel therapeutic targets. This work is supported by the NIH NIAID through IAA Y1-AI-8401-01.

**Detection and Identification of RNA Viruses Using Microarrays**

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Numerous molecular diagnostic methods have been developed for detecting RNA viral pathogens. While these methods are highly sensitive and specific, they are designed to detect a single or a limited number of sequence targets within the genome and may not be able to identify variants. We developed a resequencing microarray for definitive identification of multiple species and strains in the viral families Arenaviridae, Bunyaviridae, Filoviridae, Flaviridae, and togaviridae. The array was designed to identify viral sequences representing 205 strains or isolates representing 37 species, eight genera and five families. Targeting multiple sequence regions within the same species enabled multiple strain identification. The method was evaluated with 34 viruses representing all these families. Each unknown sample was interrogated against all 205 viral strains or isolates in a single array hybridization experiment, and results showed that it was possible to correctly identify the viral species or strain in an unknown sample with 100% accuracy. This approach has a potential not only for rapid and accurate identification of known viral pathogens, but also for emerging or uncharacterized viruses.
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**Global Analysis of Intact Proteins from Orthopoxviruses**


**Background:** Orthopoxviruses are among the largest and most complex of the animal viruses. Recent outbreaks of lethal strains and concerns for possible weaponization of these viruses have resulted in the need for new biological studies. Proteomics technologies used to study intact proteins offer scientists the ability to rapidly discover protein post-translational modifications and processing. **Methods:** Two Orthopoxviruses (vaccinia and human monkeypox) were both grown in HeLa cells and the intracellular mature virion particles were purified. Intact viral proteins are separated using reversed-phase chromatography and ionized directly into a 12 Tesla FTICR mass spectrometer to offer superior mass measurement. During the chromatography step the flow is split and fractionated to perform secondary analysis of the eluting proteins in a targeted fashion. The secondary analysis includes digestion with trypsin then analyzing the resulting peptides with “shotgun” proteomic techniques including the use of LC-MS/MS for a qualitative analysis and LC-high resolution and high mass accuracy MS for more quantitative analysis. **Results:** Analysis of intact proteins (a.k.a. Top-down proteomes) is a complementary technology to the more common shotgun approach to proteomics. There are substantial challenges, but this overall approach has led to the discovery of novel post-translational modifications that appear to have important roles in virogenesis including membrane anchoring. Validation efforts are currently on-going. **Conclusion:** Utilizing intact proteomics technologies to increase our understanding of pathogens isolated from host systems may lead to novel approaches for combating infectious diseases. We will demonstrate the use of intact proteomics methods towards that goal for two Orthopoxviruses. This work is supported by the NIH NIAID through IAA-Y1-Al-4894-01.

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**Protein Biomarker for the B. cereus Group in Indoor Air**

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**Background:** Bacilli are commonly found in indoor air and their presence may interfere with technology designed to detect Bacillus anthracis, a member of the B. cereus group, released during a bioterrorist attack. Protein biomarkers for the B. cereus group could be useful in establishing baseline levels and also serve as specific markers for B. anthracis. Our current research employs proteomics to identify such biomarkers. **Methods:** Airborne dust samples were collected from occupied and unoccupied rooms using an Ionic Breeze. The samples were cultured at 37°C for ~24h on nutrient agar plates, and pure colonies were isolated. Based upon Gram stain characteristics, 8 colonies of gram-positive, rod-shaped, filamentous bacilli were selected. Proteins were extracted from the isolates and separated using SDS-PAGE. Trypsin digestion was performed on selected protein bands, and the released peptides were analyzed by MALDI TOF-TOF tandem mass spectrometry (MS/MS). **Results:** Six isolates had a similar SDS-PAGE protein profile. A ~25 kDa band was observed for each of the 6 isolates. This band was selected for trypsin digestion and analysis by MS/MS. A product-ion peak was identified that corresponded to a Mr-superoxide dismutase found in B. cereus and B. thuringiensis. The amino acid sequence of the peptide was YQQGQQGQH. Further analysis using a protein BLAST search algorithm demonstrated this sequence is highly conserved in the B. cereus group with the exception of B. mycoides. The sequence in B. cereus, B. anthracis, and B. thuringiensis was identical whereas B. weihenstephanensis showed a single amino acid substitution of lysine to threonine. This sequence is also common among other Firmicutes; however, there is considerable variability in the last 4 amino acids of the sequence. **Conclusion:** Our results demonstrate that members of the B. cereus group are common in indoor air. Furthermore, Mn-superoxide dismutase may serve as a biomarker for detection of the B. cereus group and, in conjunction with other protein markers, might serve as part of a rapid biodetection scheme for anthrax.

**09—Therapeutics**

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**Non-peptidic Mimics of Host Defense Proteins: Activity against Category A Biopathogens**


**Background:** To address some of the greatest concerns to US security, it is critical to develop new treatments for emerging diseases or the threat of biological weapons. We have developed non-peptidic mimics that capture the activity and mechanism of host defense proteins. The synthetic analogs have many advantages over peptides because of their small size (MW < 1,000), which increases their stability and tissue penetration, and ability to fine tune their structures for optimization of potency and safety. Furthermore, because these compounds target the bacterial cell membrane, there is a lower risk for the development of resistance. Several of the more broadly active compounds in the library were screened against Category A biopathogens. One compound, PMX-30063, is in Phase 1 clinical trials, intended to treat Staphylococcus infections. **Methods:** All susceptibility assays were performed under standard CLSI conditions in broth microcultures using clinical or field isolates of B. anthracis, Y. pestis, and F. tularensis (30 strains/organisms). Cytotoxicity was evaluated in bioassay experiments using human liver (HepG2) and mouse (NIH/3T3) cell lines. Hemolysis of human red blood cells (RBCs) was evaluated by measuring release of hemoglobin. **Results:** PMX30057 was the most potent and broadly active compound in the screening set, having MIC90 values of 2 µg/ml for B. anthracis, and 4 µg/ml for Y. pestis and F. tularensis. PMX30016, PMX30024 and PMX70004 were active against B. anthracis (MIC90 = 2 µg/ml) and F. tularensis (MIC90 = 4 µg/ml) but less active against Y. pestis. Cytotoxic EC50 values for PMX30057 were 178 and 480 ug/ml in 3T3 and HepG2 cells, respectively, and 167 ug/ml in human RBCs. Selectivity indices for PMX30057 (EC50/MIC) were at least > 40 for each of the Category A pathogens. **Conclusion:** These antimicrobial and selectivity properties warrant animal testing of the current active compounds and further medicinal chemistry for optimization of efficacy and safety.

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**Defining the Therapeutic Window of Opportunity for an Anthrax Therapeutic in New Zealand White Rabbits**

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**Background:** The risk of another anthrax attack has prompted development of an appropriate animal therapeutic model to assess the efficacy of therapeutics in the event of a Bacillus anthracis exposure. **Methods:** Twenty four pathogen free New Zealand White (NZW) rabbits were challenged with B. anthracis (Ames strain) spores via the inhalation exposure route and monitored for clinical and physiological changes following challenge. Three groups of six animals were treated at various times [0, 6, or 12 hours (h)] post-exposure of a significant increase in body temperature (SIBT) with a monoclonal antibody (10 mg/kg) against B. anthracis protective antigen (PA). One group of 6 animals was not treated and served as control rabbits. **Results:** All control animals succumbed to infection with a median time to death of 96 hours. There was no statistical difference in survival rates between the SIBT and the SIBT + 6 h groups. Eighty three percent of animals treated at SIBT survived and 100% of the rabbits in the SIBT + 6 h group survived the spore challenge. Survival rates decreased to 66.6% in the SIBT +12 h group with one of the animals dying before treatment. Measurement of physiological parameters such as body temperature, hematology, CRP levels, bacteremia, PA levels in the blood (assessed via electrochemiluminescence (ECL) and ELISA) confirmed that the treated rabbits were administered the test material in a therapeutic fashion. The median time to SIBT and positive PA ECL was 27 and 24 hours, respectively. **Conclusion:** Treatment at SIBT or 6 hours post-exposure of SIBT provided significant protection against a lethal aerosol anthrax spore challenge. Moreover, the close relationship between the time to SIBT and time to the positive PA ECL suggested that either parameter may be used as a trigger for treatment in therapeutic models of inhalational anthrax.
Intravenous Levofloxacin Rescues African Green Monkeys from Lethal Pneumonic Plague

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Background: Evaluating vaccines and therapies for pneumonic plague are neither feasible nor ethical in humans. Therefore, evaluations rely on animal, e.g., nonhuman primate (NHP), models to test efficacy. Under Good Laboratory Practice regulations, the ability of post-exposure intravenous levofloxacin to prevent death after aerosol challenge with Yersinia pestis was tested. Methods: African green monkeys (Chlorocebus aethiops) were randomized into either antibiotic treatment (9 animals) or mock treatment (5 animals) and into two exposure cohorts having representatives from each. Animals were exposed to a mean of 96 ± 35.9 LD₅₀ aerosolized Y. pestis C929 in a head-only chamber. Telemetry using Integrated Telemetry Services equipment was used to determine onset of IV treatment (within 6 hr of an hour-average temperature ≥ 39 °C), as well as heart and respiration rate used for disease progress. A syringe pump to deliver twice daily levofloxacin (or saline mock) at 8 mg/kg and 2 mg/kg, alternating (Q12), for a total of twenty infusions. Blood samples were collected to determine bacterial load, chemistries, and antibiotic levels. Moribund and surviving animals were necropsied to determine bacterial load within tissues. Results: Fever began 65.20 ± 7.18 hr post exposure (excluding two animals). Bacteremia was detected prior to infection for half of the subjects. All monkeys receiving levofloxacin were rescued from disease, as determined by physiological measurements (return to baseline), behavioral characteristics, bacteremia (not detected in blood or tissue), and survival to 14 days after completion of the final infusion. All mock treated control monkeys succumbed to pneumonic plague. Conclusions: Post-exposure treatment with intravenous levofloxacin for ten days beginning at the onset of fever completely prevented mortality from an approximate Y. pestis aerosol dose of 100 LD₅₀. Future studies should refine the optimal route, and duration of levofloxacin treatment.

Susceptibility of Yersinia pestis to Broad-Spectrum Nanostructured Therapeutics

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Background: Broad-spectrum antimicrobials against Y. pestis have been identified as a high priority medical countermeasure by the Department of Health and Human Services. To fulfill this need, the present study seeks to develop such new drugs based in the emerging, cutting edge field of nanotechnology. Methods: Novel silver nanoparticles (AgNPs) were used in the present study to inhibit Y. pestis. The minimum inhibitory concentrations (MIC) for specific sized nanoparticles were determined by agar and broth dilution techniques following Clinical and Laboratory Standards Institute guidelines. Techniques in transmission electron microscopy were used to evaluate the physiochemical properties of the AgNPs. Control experiments were conducted to determine the ability of assay cells to survive at specific AgNP concentrations. Results: Results to date with the agar dilution method showed complete bacterial inhibition at concentrations as low as 20 micrograms per ml for nanoparticles in the 25-nm size category with no toxicity on assay cells at effective concentrations. Y. pestis was most sensitive to AgNPs. Unexpectedly, the MIC for Y. pestis (gram-negative) was more similar to the gram-positive control (S. aureus) as compared to the gram-negative control (E. coli). Conclusion: AgNPs have the potential to serve as a promising broad-spectrum antibacterial agent with a high therapeutic index. Macrophage neutralization studies are in progress to determine whether the AgNPs are able to inhibit infection by Y. pestis, thus preventing the cytotoxic effects on these cells. The AgNPs under study are highly stable and capable of being stockpiled in the event of a natural or man-made epidemic. Such stability, combined with the broad-spectrum nature of the particles, would allow for immediate short-term treatment in the field before an accurate diagnosis and specific treatment could be determined.
A Novel Small Nonhuman Primate Model for Filovirus Induced Hemorrhagic Fever

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Members of the filoviridae family cause severe hemorrhagic fever with a case fatality of up to 90% for humans. We provide evidence that the common marmoset (Callithrix jacchus) is a promising model of Ebola and Marburg hemorrhagic fever.Groups of two animals were inoculated with 10 PFU or 1000 PFU of Ebola Zaire (EBOV) or MarburgMusoke (MARV) and were monitored daily for weight loss, fever, anorexia, depression, and neurologic signs. Infected animals appeared normal until 2 dpi when clinical abnormalities could be seen and overall lethargy was noted while low dose groups experienced the onset of clinical symptoms at 4 dpi. All animals became moribund by 7 days post infection. The viral load in tissue at time of lethargy was noted while low dose groups experienced the onset of clinical symptoms at 4 dpi. All animals had evidence of a severe disseminated viral infection characterized principally by multifocal to coalescing hepatic necrosis. This process was accompanied by infiltration by small numbers of neutrophils and varying degrees of hepatocellular dissociation. The other striking finding in these animals was lymphoid necrosis and lymphoctic depletion observed in spleen of the high dose animals. These changes were accompanied by fibrin deposition in the medullary cords. Significant differences in histopathology were observed between animals inoculated with EBOV and MARV. EBOV infection was associated with widespread intravascular coagulation that was apparent in multiple organs including the spleen, adrenal gland, kidney and lung. While high dose MARV inoculated animals had moderate fibrin deposition in the spleen, widespread intravascular coagulation was not observed suggesting a significant difference in pathogenesis between the two agents in the NHP species. These findings provide preliminary evidence that the Common Marmoset can be used as an alternative NHP model for filovirus induced Hemorrhagic Fever.

Aerosol Mouse Model of Inhalational Plague in BALB/c Mice and Disease Progression

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Background: Yersinia pestis is the causative agent of plague, a disease that can manifest as pneumonic or bubonic forms. Pneumonic plague is the deadliest manifestation, due to rapid progression in the host. This progression is believed to be a result of the ability of Y. pestis to evade or suppress the immune system. Disease progression of aerosol induced pneumonic plague has not been fully described. Methods: Therefore, we set out to characterize pneumonic plague in the BALB/c mouse model of disease by determining a 50% lethal (LD50) dose, bacterial dissemination, cytokine and chemokine production, bronchoalveolar lavage fluid (BALF) cytology, hematology and histopathology over a 48 to 72 hour period following aerosol challenge with Y. pestis CO92. Mice were exposed (nose-only) to varying doses of Y. pestis CO92 aerosolized using a Collison nebulizer. Results: The LD50 inhaled dose was identified to be 1.2 x 10^4 cfu. In subsequent studies we infected mice with approximately 50 LD50 (6 x 10^5 cfu) and harvested organs, blood and BALF at 1, 24, 48 and 72 hours post-infection. Analyses revealed that there was not a strong inflammatory response to aerosolized plaque until 48 hours post challenge, with most animals succumbing by 72 hours. Conclusion: Histopathological changes, bacterial load in tissues, chemokine/cytokine levels in BALF and white blood cell counts did not increase until 48 hours after exposure, suggesting that Y. pestis does evade or suppress the immune system, until it is too late for the host to respond, thus resulting in lethal disease.

Imaging of Non-Human Primate Models of Infectious Disease Using PET/CT


Background. Small animal hybrid imaging systems have been available for several years while imaging of larger research animals has relied on the use of clinical imaging instruments. This lack of a single imaging solution for animals ranging in size from rodents to non-human primates motivated the development of a preclinical PET/CT imaging platform based on the state-of-the-art in PET solutions. Methods. The microPET Focus 220 (Siemens Molecular Imaging) and the mobile CereTom CT scanner (Neurologica Corp.) were installed in an animal biosafety level 3 (ABSL3) environment at the University of Pittsburgh Regional Biosafety Laboratory. An in-line hybrid arrangement provides intrinsically co-registered CT and PET images acquired sequentially with the subject on a common imaging pallet and accommodates species ranging in size from the mouse to the adult rhesus macaque monkey. Results. The instruments were aligned and spatial co-registration of sequentially-acquired microPET and CT images validated using a Ge-68 cylinder phantom. Once validated, cynomolgous macaques experimentally infected with Mycobacterium tuberculosis were sedated, intubated and placed on a respirator, injected with 5mCi of [18-F]FDG, and subjected to whole body scans as frequently as every two weeks post infection, and at baseline. Results from these studies demonstrated the ability to visualize increased lymph node metabolic activity and the formation of small tuberculosis lesions in the primate lung as early as two weeks post infection, which was significantly earlier than the onset of symptoms and detection using CT alone. Conclusion. The demonstration of a highly sensitive preclinical imaging system provides a novel mechanism for modeling the pathogenesis and disease profile without the need for serial sacrifice of animals. Future goals include adaptation of this preclinical imaging system to study the pathogenesis and development of vaccine and therapeutic strategies for other emerging infections.
210 Technology Platform for Rapid Identification of Highly Pathogenic Viruses


Background: Three Swedish Government Agencies have invested 1 M Euro to develop a technology platform for the rapid identification of highly pathogenic viruses. Methods: The project builds on technology that enables image analysis of virus structures in Transmission Electron Microscopy (TEM) images, developing algorithms to screen viruses in TEM images. Combined with patented automated image analysis software, it allows rapid and objective identification of highly pathogenic viruses in various types of biological samples. An added advantage in a biosecurity is the ability to analyze chemically inactivated non-infectious samples. Unlike other virus detection methods, it is open-view, able to detect all species of viruses present in a sample. After detection, morphological comparison to a database of previously characterized viruses is made and the virus particles are classified corresponding to virus family. Results: Viruses under investigation include Crimean Congo Hemorrhagic Fever, Lassa, Rift Valley Fever, Parapox, Orthopox, Influenza A, Dengue and Ebola. Once complete the technology will identify a virus (including presence and identity of multiple viruses) within minutes from taking a digital picture of the sample. Conclusion: The software has successfully been used to detect and classify gastroenteroviruses; Adenovirus, Rotavirus, Astrovirus and Norovirus in fecal samples. The easy and safe sample handling and automated image analysis makes this a valuable analysis method in TEM images. Developing algorithm to screen viruses for rapid and reliable diagnostic tools are needed for the detection of agents with a major public health impact. Molecular detection methods can achieve the required sensitivity, specificity and speed. An essential prerequisite for molecular detection methods developed for screening purposes is the ability to simultaneously detect multiple genetic markers. For rapid recognition of 4 highly pathogenic bacteria: B. anthracis, F. tularensis, Y. pestis and C. burnetii we have developed multiplex real-time PCR assays. Real-time PCR provides speed, sensitivity and specificity, while multiplexing enhances reliability through detection of multiple markers and incorporation of internal controls. However, real-time PCR permits only limited multiplexing while we aim to detect many pathogens simultaneously. Therefore, we are expanding our multiplexing capabilities by developing DNA microarrays. In order to maintain speed in the detection, we use bead-based suspension arrays which allow rapid measurements of 100 different color-labeled nanobeads coated with specific probes by using flow-cytometry. We have identified several discriminating targets (phylogenet- ic as wells as virulence markers) for selected biothreat agents. Specialized software was used to design oligonucleotide probes and primers for specific recognition of these target sequences under specific conditions. Oligonucleotides were designed for application in direct hybridization assays, as well as for an alternative assay format using target-specific primer extension for labeling and incorporation of address labels, combined with hybridization to universal arrays. To achieve acceptable sensitivity for the microarrays, multiplex PCRs are being developed for unbiased amplification of a maximum number of diverse markers. The performance of the different DNA microarrays with respect to sensitivity, specificity and speed was investigated by using panels of target pathogens and control organisms.

212 Improved Toxin Detection using Single Domain Antibodies

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Background: Llamas possess both conventional IgG and unique forms of antibody that contain only heavy chains. Antigen binding in these unconventional heavy-chain homodimers, is mediated through a single variable domain which can be expressed recombinant- ly and are termed single domain antibodies (sdAb). sdAb possess good solubility, thermal sta- bility, and can re-fold after heat and chemical denaturation. We have previously isolated sdAb binders towards botulinum A (BoNT A) complex and ricin. We hypothesize that direc- tional immobilization and methods to increase the amount of label per sdAb will further enhance toxin detection when using these unique recognition elements. Methods: We have isolated unique binders towards both ricin and BoNT A complex from immune-based llama libraries of sdAb displayed on phage. Targets engineered on the c-terminus of the sdAb were used to provide directional immobilization of the sdAb capture reagents. In addition, we have dye-labeled both the soluble sdAb as well as phage-displayed sdAb for use as recogni- tion elements. Results: We incorporated directionally immobilized sdAb capture into sand- wich assays for the detection of BoNT A complex and ricin. Limits of detection were compared using the directional capture surfaces and surfaces where capture was immobi- lized through chemistry that did not orient the sdAb. Although the small size of the sdAb reporters can be advantageous, it limits the number of labels that can be incorporated for signaling. We explored the use of dye-labeled phage displayed sdAb as reporter elements in sandwich assays. These strategies enabled our limits of detection to be improved. Conclusions: The unique properties of sdAb may give them an advantage as diagnostic and detection agents over conventional IgG. Taking advantage of the ability to add c-termi- nal tags as well as using the whole phage-displayed sdAb as a reagent are strategies to opti- mize limits of detection in sdAb-based assays.

213 Prevalence of Coxiella burnetii DNA in Environmental Samples Acquired in the United States between 2006 and 2008


Background: Coxiella burnetii is an obligate intracellular bacterium that causes the human zoonotic disease Q fever. Q fever is acquired by the inhalation of aerosols containing as few as 1-10 bacteria, and most patients recover after experiencing a flu-like illness. Because C. burnetii is so highly infectious, can survive under a variety of environmental conditions, and has been weaponized in the past, it is classified as a select agent and is con- sidered a potential bioweapon. The agent is known to be present in domestic livestock and in wild animal populations, but the background levels of C. burnetii in the environment have not been reported. Without this knowledge, it may be difficult to determine if organisms detected after an intentional release are higher than the normal background levels. Methods: To better understand the amount of C. burnetii present in the environment of the U.S., between 2006 and 2008 we obtained greater than 1,600 environmental samples from 6 different states. The samples included sponge wipes, vacuum samples, and bulk soil from diverse locations. DNA was purified from these samples, and after it was shown to be free of PCR inhibitors, the presence of C. burnetii DNA was evaluated by quantitative PCR of the IS1111 repetitive element. Results: Coxiella burnetii DNA was readily detectable in all six of the states sampled. The percentage of positive samples in individual states ranged from 6 to 30 percent, and DNA was detected in both urban areas and rural areas near live- stock. Conclusion: This study demonstrates that C. burnetii is fairly common in the envi- ronment in the U.S., and any analysis of C. burnetii after a suspected intentional release should be interpreted in light of these background levels. It also suggests that human ex-posure to C. burnetii may be more common than what is suggested by the number of report- ed cases of Q fever.
Detection of Known and Emerging Vector Borne Pathogens by PCR and ESI/Mass Spectrometry

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Background: A large number of biowarfare agents are vector-borne in the natural environment. For example ticks can transmit Coxiella burnetii, Rickettsia rickettii and Franciscella tularensis. Mosquitoes can transmit Alphaviruses and fleas can transmit Yersinia pestis. Effective monitoring of emerging pathogens requires the monitoring of these important vectors of transmission. Using broad-range PCR and electrospray ionization mass spectrometry we identified a number of known and potentially emerging pathogens in field collected ticks and mosquitoes. Methods: A multi-focus PCR assay targeting known pathogen families as well as broad range targets was developed. PCR/ESI-MS performed on the Biis TS9000 biosensor platform was used to detect and identify microorganisms in ticks and mosquitoes. Base composition signatures determined from these PCR amplicons were used to identify the organisms found in the vectors. Results: We examined the microorganisms (pathogens) from >1200 field collected ticks from diverse parts of the US and compared them to farm raised ticks of the same species. From this study we identified a number of known pathogens in addition to genetic near neighbors to known pathogens, which may represent potential human pathogens. We also looked at field collect mosquitoes and detected a novel alphavirus isolate in addition to numerous known alphaviruses. Conclusions: We demonstrated broad-range detection of vector-borne pathogens in a single assay. Our study has shown that we can detect both pathogenic bacteria and viruses without prior knowledge of the organisms in the sample. Furthermore we demonstrate the detection of mixtures of pathogens from samples as small as a single nymphal tick.

Ebola VLPs Induce Expression of SOCS1 which Enhances VLP Budding

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Ebola virus initially targets monocytes/macrophages and dendritic cells which leads to the induction of proinflammatory cytokines and chemokines, which contribute to the pathogenesis of Ebola virus infection. Thus the development of circulatory shock seen in fatal EBOV infections is assumed to be mediated by the released inflammatory cytokines. Assembly and budding of Ebola virus-like-particles (VLPs) mediated by the VP40 matrix protein can be targeted by components of the host innate immune response. Here we report that suppression of cytokine signaling 1 (SOCS1), an inducible negative-feedback regulator of the cytokine response, enhances egress of VP40 VLPs. Our findings suggest that ubiquitination of VP40 by mediated by SOCS1 alone, or in combination with Nedd4 E3 ubiquitin ligase, results in more efficient budding of VP40 VLPs. In addition, we found that in human monocytic cell line (THP-1) VLPs containing the EBOV VP40 and GP (glycoprotein) induce transcription of both SOCS1 RNA as well as proinflammatory cytokines. These data identify SOCS1 as a novel host innate immune protein that may play a critical role in budding, pathogenesis, and immune evasion of Ebola virus.
The Bacillus anthracis Toxin, Anthrolysin O, Stimulates Macrophages through Multiple Receptors Including Tlr4

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Background: Bacillus anthracis (BA) produces virulence factors that alter host cellular signaling and immune functions and lead to the disease anthrax. One such factor, whose role has yet to be fully elucidated, is the cholesterol-dependent cytolsin, Anthrolysin O (ALO). In mouse bone marrow-derived macrophages (mBMDM), ALO signals through Toll-Like Receptor 4 (TLR4) (J Exp Med [2004] 200:1647). To determine if ALO signals exclusively through TLR4 or whether additional receptors or signaling mechanisms are involved, we investigated the effects of ALO on macrophages and epithelial cells. We used full-length recombinant ALO (rALO) that can bind, oligomerize and form pores in the cell membrane, and a mutated rALO (W477A) that can bind and oligomerize, but cannot form lethal pores.

Methods: We examined the effects of 500ng/ml of rALO or W477A on chemotaxis of RAW 264.7 macrophage-like cells, TLR4 knockout mBMDM, and 2Sacr TLR4 knockout mouse macrophages towards the chemotactrant FMLP. Results: rALO and W477A both caused ~50% inhibition of chemotaxis of TLR4 knockout macrophages while causing complete inhibition of chemotaxis in macrophages that express TLR4. We also examined the activation of signaling pathways in macrophages by rALO or W477A, using Western blot analysis. rALO and W477A each activated p58-MAPK and NF-κB pathways regardless of TLR4 expression; however, the dose and time needed to induce this response in knockout cells differ when compared to cells that express TLR4. Conclusions: Our data indicate that ALO signals through TLR4 and through additional receptors or mechanisms. The additional receptor or mechanism may help ALO cause additional pathologies.

Coordinated Regulation of Virulence Factors during Systemic Infection of Salmonella enterica serovar Typhimurium

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Salmonella must respond to a myriad of environmental cues during infection of a mouse and express specific subsets of genes in a temporal and spatial manner to subvert the host defense mechanisms but these regulatory pathways are poorly established. To unravel how micro-environmental signals are processed and integrated into coordinated action, we constructed in-frame non-polar deletions of 84 regulators inferred to play a role in Salmonella enterica serovar Typhimurium virulence and tested them in three virulence assays (intraperitoneal and intragastric in BALB/c mice, with persistence in SfJ129 mice). Overall 36 regulators were identified that were less virulent in at least one assay, and of those, 15 regulators were required for systemic mouse infection. Transcriptional and proteomics profiles were obtained for each of these 15 regulators from strains grown under four different environmental conditions. These results with publicly available transcriptional data were used to produce a network inference-based model of the regulatory network of these regulators. The resulting regulatory network suggests how the 15 regulators integrate signals to control the genes necessary for Salmonella to cause systemic infection. We validated this model by expressing a subset of the regulators in trans while observing expression of 7 known virulence factors. These experiments validated the model and demonstrating regulators SsrF with SsA are critical regulators that integrate signals for virulence. This work is supported by the NIH NIAID through IAAY1-AI-8401-01.

Improved Potency of Codon-Optimized Encephalitic Alphavirus DNA Vaccines Delivered by Electroporation

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Venezuelan, eastern, and western equine encephalitis viruses (VEEV, EEEV, and WEEV) are alphaviruses that are recognized for their potential use as agents of biowarfare or bioterrorism due to their pathogenicity in humans, relative ease of production, considerable stability, and high infectivity in aerosols. The limitations associated with the existing VEEV, EEEV, and WEEV IND vaccines have prompted the development of a new generation of vaccines to protect against encephalitic alphavirus infections. We found that codon optimization of the E1 and E2 envelope glycoprotein genes of VEEV, EEEV, and WEEV IND vaccines have prompted the development of a new generation of vaccines to protect against encephalitic alphavirus infections. We found that codon optimization of the E1 and E2 envelope glycoprotein genes of VEEV, EEEV, and WEEV resulted in significant increases in the in vitro expression of these antigens relative to that of the wild-type viral genes in mammalian cells. We further determined that intramuscular electroporation (EP) delivery of the individual codon-optimized alphavirus DNA vaccines using the Ichor Medical Systems TriGrid Delivery System (TDS) elicited robust immune responses, including high levels of neutralizing antibodies, against the three different viruses in mice. Importantly, additional mouse immunogenicity studies demonstrated that these antibody responses were not significantly altered when the three DNA vaccines were administered in combination using this delivery technology. Moreover, strong neutralizing antibody responses were detectable in nonhuman primates vaccinated with the codon-optimized VEEV construct administered by Ichor EP, and these animals were protected from aerosol challenge with VEEV. The improved potency of codon-optimized VEEV, EEEV, and WEEV DNA vaccines delivered by EP supports the development of the individual and combination encephalitic alphavirus DNA vaccines into mature vaccine candidates suitable for clinical testing.

How Does the T Domain of Diphtheria Toxin Drive Membrane Binding and Penetration of the C Domain

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Background: During cell intoxication by diphtheria toxin, endosome acidification triggers the translocation of the catalytic (C) domain into the cytoplasm. This event is mediated by the translocation (T) domain of the toxin. The T domain has been proposed to act as a chaperone for the C domain, to induce its insertion in the membrane. We describe such a role here. Method: Using partitioning experiments with lipid vesicles, fluorescence spectroscopy, and lipid vesicle leakage assay, we characterized the successive steps by which the C and T domains of a CT protein (the toxin lacking the receptor-binding domain) interact with an anionic membrane as a function of pH. Results: The protein binds to the membrane and partially unfolds from pH 7 to 5, starting with T followed by C about 0.5 pH unit lower. The protein penetrates into the lipid bilayer and adopts a second conformational change, from pH 6 to 3.5, T preceding C again by about 0.5 pH unit. In contrast with T, however, the conformational changes of C do not strictly overlap membrane binding and penetration. The behavior of T is marginally modified by the presence or absence of C while that of C is greatly affected by the presence of T. All the steps leading to membrane insertion of the C domain are triggered at higher pH by the T domain, by 0.5 to 1.6 pH unit. Conclusion: T interacts with C, stabilizing partially folded states of C at each step of membrane penetration. This demonstrates that T acts as a specialized pH-dependent chaperone for C. Interestingly, this chaperone activity acts on very different states of the protein: in solution, membrane-bound and membrane inserted.
**Cellular Tropism and Characterization of Burkholderia mallei Type III Secretion System in a Respiratory Model of Infection**

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*Burkholderia mallei*, the causative agent of Glanders disease, is a facultative intracellular pathogen capable of intracellular survival and replication in phagocytic cell lines. To address the capability of *B. mallei* to invade nonprofessional phagocytes we have utilized the human alveolar type II (ATII) epithelial cell line A549 and murine respiratory epithelial line LA-4. Bacterial burden recovered from naïve BALB/c mice infected by intranasal delivery indicate a high tropism to the lower respiratory system. Employing cell invasion assays, light microscopy and electron microscopy, we have demonstrated that *B. mallei* possesses the capacity to adhere but not invade these cultured cell lines in vitro. Furthermore, murine alveolar macrophages (MH-S) were capable of efficient phagocytosis of *B. mallei* following serum coating of bacteria, suggestive of complement and FCR mediated uptake. MH-S cells infected with wild-type *B. mallei* resulted in a reduced recovery of intracellular organisms, although a type three secretion system effector mutant (BopA) demonstrated increased survival within MH-S cells. Our data suggest that *B. mallei* induces cytotoxicity (apoptosis) in mouse macrophages, leading to bacterial escape. These data support our in vitro experiments, in which the bopA mutant showed attenuation of virulence as evidenced by survival following intranasal challenge of BALB/c mice. Our data demonstrate that BopA is critical to intracellular survival in alveolar macrophages by allowing for cellular escape. Additionally, we evaluated the protective ability of bopA DNA using a genetic vaccination strategy. Our results suggest that a DNA delivery mechanism may be sub-optimal for efficient protection, while second generation sub-unit vaccination provided enhanced protection.

**Analysis of a Novel Regulatory Module Associated with Non-Replicating Persistence in Mycobacterium tuberculosis**

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A clinical hallmark and unique feature of *Mycobacterium tuberculosis* (*M.tb*) is the organism’s ability to maintain a latent infection in the host after apparently effective drug treatment. Currently, the complex regulatory and physiological changes occurring during natural infection which allow for bacterial persistence and drug tolerance are poorly understood. Studies to define these molecular mechanisms may allow for insights into pathogenesis as well as new strategies to combat latent *M.tb* infection. In the current study, we examine a novel bacterial mechanism involved in growth phase regulation in *M.tb*. This system, encoded by a unique operon, consists of transcriptional regulator, SgpR1, and endoribonuclease, SgpE1. Interestingly, SgpE1 is orthologous to the endoribonuclease MauE; a protein previously described to have regulatory roles in bacteriostasis and growth arrest in other prokaryotes. Transcriptional analysis of *sgpR1* and *sgpE1* revealed both genes to be upregulated during mid-exponential growth and under hypoxic conditions, coincident with an observed reduction in growth rate. Additional analyses revealed overexpression of SgpR1 led to an induction of ORFs involved in metabolic stability and a repression of several genes involved in the cell cycle. Furthermore, SgpE1 expression conferred drug tolerance to frontline chemotherapeutics isoniazid and ethambutol, but not rifampin, consistent with the reduction of cell growth. In addition to these findings, animal studies demonstrated an increase in *sgpR1*/*sgpE1* transcripts during late stages of infection. We propose a model where SgpR1 and SgpE1 are involved with controlling non-replicating persistence leading to the associated phenomenon of drug tolerance observed in latent *M. tuberculosis* infection.
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Science-Based Support to Attribution and Awareness in Biodefense
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The National Biodefense Analysis and Countermeasures Center (NBACC) is a Department of Homeland Security Center that is managed and operated by BMBI. The NBACC mission is to provide the nation with the scientific basis for awareness of biological threats and bioforensic analysis to support attribution of their use against the American public. The NBACC programs in bioforensic and threat awareness will be described.

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Correlates of Protection against Anthrax and Extrapolation to Humans
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Bioterrorism concerns since 2001 and finalization of the Food and Drug Administration (FDA) “Animal Rule” in 2002 has resulted in a convergence of interest, funding and activities regarding development of medical countermeasures whose efficacy must be demonstrated in animal models. Nonetheless, demonstration of product efficacy using animal data rather than clinical data remains a challenge for developers of new biodefense products because the pathway for doing so represents a new paradigm. The Division of Microbiology and Infectious Diseases (DMID) at NIAID/NHI has been engaged in development of animal models and assays to support development of products that will be licensed using the Animal Rule; in particular, the animal models and assays to support second generation anthrax vaccines. This presentation will discuss several topics and concepts including types of animal studies associated with different stages of product maturity, terminology, a brief summary of the Animal Rule with regard to importance of human data, determination of the “humanized” dose, correlation of protection data from animal efficacy studies with human immunogenicity data, and, ultimately, extrapolation of these data to human efficacy. The presentation will demonstrate how correlates of protection might be used in the application of the Animal Rule to anthrax vaccines. The presentation will also illustrate why implementation of the Animal Rule is product-specific, and why universal application of all aspects unique to one model is precluded.

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Establishment and Operational Capacity of the Zonal Diagnostics Laboratory at Kutaisi, Republic of Georgia for the U.S. Department of Defense/Defense Threat Reduction Agency (DTRA) / Threat Assessment Detection Response (TADR) Program
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Background: Human and animal migration has facilitated the dissemination of zoonotic and human diseases across Georgia resulting in the establishment of endemic foci for these pathogens. The area is known to have large outbreaks of zoonotic and insect-borne pathogens such as anthrax and tularemia. As a result, little is known about the potential pathogens that regionally deployed U.S. soldiers are at risk for and how to reduce this risk. Methods: Under the DTRA TADR program, a clinical diagnostic laboratory for Especially Dangerous Pathogens (Select Agents) has been established at the Zonal Diagnostic Laboratory in Kutaisi, Republic of Georgia. Through a coordinated and integrated system utilizing clinicians, epidemiologists and public health facilities, clinical cases of human disease caused by EDPs can be rapidly identified and clinical samples can then be collected and sent to the regional laboratory for diagnostic testing. Results: The results of this functional evaluation have revealed that the Kutaisi laboratory has the operational capacity to isolate and identify these pathogens using the equipment, Standard Operating Procedures and microbiological and molecular biology techniques provided by the DTRA TADR program and that this laboratory conforms to the international standards of biosafety and biosecurity established for Select Agents. Conclusions: We have demonstrated successful implementation of detection and identification of nonpathogenic surrogates for anthrax and tularemia for these regional laboratories.

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Hazard Identification: From Environmental Sampling to the Laboratory
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Recent events have caused the environmental sampling community to scrutinize and reconsider the conventional approaches taken for surface sampling and analysis in the context of biological contamination in buildings and public spaces. In 2007, and again in 2008 a series of multi-agency exercises were conducted to investigate the efficacy and operational utility of a variety of sample collection methods, strategies, and analytical techniques for assessing the microbial risk during building decontamination events. Conventional analytical techniques coupled to specific sampling methods (swabs, wipes, vacuum socks) associated with biological surface sampling were tested in a small scale (single room, 400 ft^2) and large scale (Location: Idaho National Laboratory, Abandoned Office Building, 2 levels, 15 rooms per floor, 4025 ft^2 per floor), study and they were assessed for overall recovery, throughput, consumables use, and their potential for introducing contamination to the analytical process. In all cases spore preparation of dry Bacillus atrophaeus supplied by the U.S. Army Dugway Proving Grounds Life Sciences Facility was used as a surrogate for sporulated biological threats. CDC-recommended extraction protocols for recovering material from Bacron swabs, cotton-based wipes, and vacuum socks were tested, and modified. In preparation for the larger scale study, an assessment of the sample extraction and analysis protocols was conducted to identify potential modifications for improving reproducibility of results, increasing throughput, and minimizing contamination. The value of conducting a small scale decontamination exercise coupled to a larger scale exercise was realized through the fact that it led to protocol improvements, as well as the identification of numerous additional process improvements that could ultimately be incorporated into future sampling and analysis strategies. Finally, the involvement the multi-agency involvement will hopefully lead to a more common set of protocols for sampling and sample analysis that can be used in government-led biohazard sampling events.
Hazard Identification: Validation of Laboratory Methods

S. A. Morse; Bioterroism Preparedness and Response Program, CDC, Atlanta, GA.

Validation is essential in the development of methods that are used to identify threat agents as well as to generate reliable and defensible data for the assessment of microbial risk. The response to a biological attack needs to be rapid and based on the best available scientific data. Accurate and credible results are needed because the interpretation of such results could have serious consequences. Therefore, the methods for the collection, extraction, and analysis of clinical and environmental samples that could be used to generate key results need to be as scientifically robust as possible. Proper interpretation of results relies substantially on understanding the performance and limitations of the methods of collection, sample transport, sample processing, and analysis. Validation should be considered a dynamic process in order to periodically assess the impact of new knowledge and findings. This is particularly true for some of the real-time PCR assays used for threat agent detection and identification. The BioWatch program is a nationwide surveillance system for sampling air for the presence of selected bioterrorism agents. DNA extracted from filter-collected air samples is extracted and analyzed by real-time PCR with primers for certain bioterrorism agents. In October 2001 in the Houston, Texas, area, several BioWatch filters tested positive for Francisella tularensis. Because the pattern of positive results was inconsistent with that of a deliberate release of F. tularensis, the results were interpreted as being due to a natural event. Soil and water samples collected proximal to the positive BioWatch collectors were extensively characterized and found to contain DNA sequences from a wide variety of Francisella-related species, some of which appeared to be previously unknown. These findings impact the specificity of the PCR-based assays used to detect the presence of a threat agent and suggest that, as we gain additional knowledge of the diversity of microbial populations, further optimization of the assay may be indicated.

Dose-Response: Use of Animal Models to Determine Doses in Humans


Microbial dose-response assessments determine the relationship between the magnitude of exposure (dose) to a biological agent and the frequency of adverse health effects (response). Animal models of infection and disease are essential for dose-response assessments of biothreat agents because these agents are potentially deadly or severely disabling, and therefore cannot be tested on human subjects. Furthermore, since naturally occurring human cases are extremely rare for most of the agents, these animal models provide the sole sources of information for quantifying human exposures. Ideally, an appropriate animal model: 1) has the same mechanism of pathogenicity; 2) has similar physiological and immunological responses; and 3) shows comparable quantitative relationships between infectivity, morbidity, and mortality, as the human disease being modeled. In the absence of conclusive data supporting the possible species-related differences in response to the biothreat agent, an extrapolation default approach similar to that applied for chemical risk assessments could be considered. Currently, the EPA’s National Homeland Security Research Center (NHSRC) and other collaborating Agencies are developing Bacillus anthracis-specific kinetic and dynamic models to decrease the uncertainty in interspecies extrapolation. Data from published studies and from controlled experiments performed using a rabbit model are utilized to construct these complex models. To adjust for kinetic differences, detailed deposition and physiologically-based biokinetic modeling are employed, while anthrax toxin toxicity models are used to correlate the dynamic differences between the host species. Results from the recent B. anthracis Ames strain rabbit studies, the kinetic and dynamic modeling approaches, and future microbiological data needs will be presented.

Exposure Assessment: Comparing the Physical Properties of Anthrax Spore Surrogates

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The physical properties anthrax spores that relate to dispersion and sampling is lacking although the biological properties of pathogenic B. anthracis (BA) are generally understood. Spore dispersion models based on studies using B. atrophaeus (BG) spores do not accurately represent BA spores because of anatomic differences between both. This limits the development of appropriate models to represent the dispersion, agent transport, deposition on surfaces, and estimation of the resultant hazard. A better understanding of the physical properties of BA surrogates will fill the existing gaps in these areas. The goal of this study was to determine and compare the physico-chemical properties of spores of three BA surrogate species, namely BG, B. cereus (BC), and B. anthracis Sterne (BAst) that were sporulated and processed under identical conditions. The properties of purified BG, BC, and BAst spores were compared using several physico-chemical methods. Electron microscopic studies of surrogate candidates revealed significant differences in length, width and other computed parameters between species. The surface area of all three surrogates determined by methylene blue adsorption assay was significantly larger than computed values obtained from physical measurements revealing complexity of spore surface. Comparison of dimensions of BG, BC and BAst spores to BA Ames (BAa) revealed several similarities; however, dimensions of BG spores, the most commonly used anthrax surrogate spore were significantly different from those of BAa spores. Surface charge of spores revealed the highest charge for BC spores, followed by BG spores. The hydrophobicity of BC and BAst spores were comparable while BG spores had the lowest hydrophobicity. Thus, the study reveals significant differences in physicochemical properties between BG, the most commonly used anthrax spore surrogate and members of the BC group. Due to lack of knowledge on surface charges and hydrophobicity of BAa spores, the data obtained with surrogate spores from this study is a logical approach to selection of appropriate BAa spore surrogates for future studies.

Keynote Address: Studying Bacterial Transcription Factors Involved in Virulence and Antibiotic Resistance: Dual Use Research of Concern?

S. B. Levy; Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA.

Two major factors contribute to the emergence and treatment of infections: virulence and drug resistance. While the former is intrinsic to the organism, the latter may arise from chromosomal gene mutation or resistance gene acquisition. Work in our laboratory has defined a family of endogenous transcriptional regulators which control both virulence and antibiotic resistance in different types of bacteria. The discovery of these transcription factors was initially made in E. coli where a “multiple antibiotic resistance/multiple adaptational response” gene (MarA) was identified. MarA regulates more than 80 other genes in the cell and is involved not only in resistance to multiple antibiotics, but also virulence, as demonstrated in a mouse model of kidney infection. In V. harveyi, MarA also controls virulence (in a pneumonia model) and drug resistance. Medicinal chemistry work at Paratek Pharmaceuticals has identified small molecules which inhibit MarA proteins, thereby offering alternative ways to address infectious diseases by targeting virulence, not growth. Does identifying and studying these proteins constitute “dual use research of concern”? Knowing what proteins are involved in virulence and drug resistance and how they affect pathogenesis offers important insights into the infection process and a novel means of controlling these infectious disease agents.
Waiting for the Next Flu to Drop

B. R. Levin, Dept. of Biology, Emory Univ., Atlanta, GA

Flu caused by the influenza A viruses H3N2 and H1N1 has been with us for years and continues to be a significant source of morbidity for virtually everybody, and for the very young and very old, mortality as well. Nevertheless we have learned to live with and deal with endemic influenza cause by these viruses. Did you get your shot?

On the other side, we continue to be aroused and some nearly panicked by the threat of a flu pandemic cause by the avian influenza virus, H5N1. Is this anxiety justified? In the more than 15 years since it was first recognized, this bird flu virus has yet cause very much mortality in humans or to be readily transmitted between people. Nevertheless, because of the high case mortality of humans infected with H5N1 (sometimes exceeding 90%), pandemic influenza cause by this avian virus has, appropriately stimulated a great deal of research on the microbiology, immunology, pathology, virulence, epidemiology and evolution of influenza. It has also contributed to a renaissance of interest in the “great influenza” of 1918. This pandemic of more than 90 years ago continues to loom over us as a worst-case scenario, killing as many as 50 million people worldwide. Although the H5N1 virus has become the object of much planning and preparation for the next pandemic, it may not be the virus responsible for the next pandemic. We can say with almost certainly that within the next few decades the human population will encounter another influenza pandemic. This next pandemic could well have the potential to kill as many or more people than that in 1918, and as illustrated by the SARS epidemic of a few years ago, even if it is only modestly lethal, a new influenza pandemic would be disastrous to the world economy.

At one level it would seem that we are far better prepared to deal with the next influenza pandemic than we were that of 1918. Unlike now, in 1918; (i) it was not clear that a virus was responsible for the pandemic, (ii) there were no vaccines or even ways to develop vaccines to prevent the disease, (iii) there were no antiviral drugs to mitigate the course of this disease and reduce the rate of transmission, and (iv) there were no antibiotics to treat secondary bacterial infections that evidence suggests was the major cause of mortality influenza infected patients or vaccines to prevent invasive infection with the normally commensal bacteria responsible. We also have vastly better communication networks now than we did then and, hopefully, there will be no masing of great densities of vast numbers of people associated with mobilization for war as there was in 1918. But are we better prepared for pandemic influenza now than we were then?

Bacterial Utilization of Antibiotics

G. Dantas, Genetics, Harvard Medical School, Boston, MA

The increasing levels of multi-drug resistance in human pathogenic bacteria are compromising our ability to treat infectious disease. Since antibiotic resistance determinants, often encoded on mobilizable elements, can be readily transferred between bacteria, we must understand the relative abundance and diversity of reservoirs of resistance genes encoded within microbial communities from different environments and their accessibility to clinically relevant pathogens. We recently isolated hundreds of soil bacteria with the capacity to grow on antibiotics as a sole carbon source, highlighting a previously unappreciated reservoir of antibiotic resistance (Dantas, Sommer et al., Science, 2008). Of 18 antibiotics tested, representing 8 major classes of natural and synthetic origin, 13-17 antibiotics supported growth of clonal bacteria from each of 11 diverse soils (Fig 1A). Bacteria subsisting on antibiotics are surprisingly phylogenetically diverse and many are closely related to human pathogens. Furthermore, each antibiotic consuming isolate is resistant to multiple antibiotics at extremely high concentrations (Fig 1B,C). We have subsequently applied a high-throughput functional genomic approach to identify and characterize hundreds of antibiotic resistance genes from bacteria subsisting on antibiotics. We find that many of these genes are identical or highly similar to resistance genes from many clinically relevant multi-drug resistant human pathogens. These findings have opened up a new area of research, coupling antibiotic resistance with metabolism, and emphasize the importance of environmental reservoirs of antibiotic resistance.

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Host Protease-S1P Receptor Signaling Crosstalks as Interventional Target in Sepsis

W. Ruf

Immunology and Microbial Science, The Scripps Res. Inst., La Jolla, CA

Common host pathogenic pathways represent potential targets for diverse infectious and systemic inflammatory response syndromes. The activation of the host coagulation system by tissue factor (TF) in bacterial sepsis is causal for sepsis lethality due to innate immune cell activation and vascular dysfunction. Recent progress points to a crucial role of coagulation signaling pathways in promoting dendritic cell (DC) mediated inflammation that causes vascular dysfunction, persistent systemic inflammation, and death from multiple organ failure. The specificity of coagulation protease signaling through the protease activated receptor (PAR) family of G-protein coupled receptors (GPCRs) is dependent on coupling to another GPCR class, the sphingosine 1 phosphate (SIP) receptors. Inhibition of thrombin signaling through PAR1 expressed by DCs prevents severe systemic inflammation and rescues mice from sepsis lethality. PAR1 coupled to SIP receptor 3 promotes enhanced DC migration and sustains late stage inflammation in severe sepsis. Novel, signaling selective variants of activated protein C (aPC), a natural anticoagulant already approved for sepsis therapy, further show pronounced sepsis protective activities without an increased risk of causing bleeding. These data indicate that it is feasible to target coagulation-related signaling without compromising the safety of patients with bleeding disorders. In addition to bacterial sepsis, coagulation abnormalities, bleeding and vascular leakage are frequent in viral hemorrhagic fevers. The pathogenic pathways identified in sepsis may similarly operate in other infectious diseases and provide opportunities for effective therapeutic interventions at crucial nodal points of the coagulation signaling networks.

Pathogenesis of Severe Dengue Illness and Potential Targets for Intervention

T. P. Eady
Medicine, State Univ. of New York Upstate Med. Univ., Syracuse, NY.

Dengue disease is a spectrum of illness that ranges in clinical manifestation from the mild febrile illness to its more severe form, dengue hemorrhagic fever (DHF). It is caused by the four serotypes of mosquito-borne dengue virus (DENV-1-4), a positive-sense RNA virus belonging to the genus Flavivirus. DENV is the most common arboviral infection of humans in the subtropical and tropical regions of the world. The World Health Organization estimates that 2.5 billion people are at risk from dengue with 50 million dengue infections worldwide every year. Approximately 500,000 people with DHF require hospitalization each year, of which 2.5% die. Dengue is one of the most studied of the viral hemorrhagic fever viruses yet the reasons for severe dengue illness are still unknown. The pathogenesis of this global health problem will be reviewed with particular emphasis on identifying potential targets for intervention.

Methods for Sampling and Characterizing Bioaerosols

G. Mainelis
Environmental Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ.

The presence of bioaerosols in various air environments is determined by collecting air samples followed by their analysis. This presentation will provide an overview of the commonly used as well as the novel bioaerosol sampling methods and will discuss their advantages and disadvantages of. In addition, the commonly used and emerging sample analysis methods will also be described and discussed. Briefly, the common bioaerosol samplers employ techniques such as filtration, impaction, and impingement. Recently, application of electrostatic collection method for collection of bioaerosols has received increased attention due to its low power consumption and lower mechanical stress to sensitive organisms. The physical performance of bioaerosol sampling devices is characterized by their ability to aspirate biological particles into the sampler’s inlet, to transmit them through the sampler’s interior and to collect them onto the collection surface. The physical performance of samplers based on impaction and impingement can be characterized by their cut-off size, i.e., size of the particles at which the sampler achieves 50% collection efficiency. A collected sample can be analyzed for the presence of viable or total (viable and non-viable) bioaerosol particles. To determine viable particles, they could be either directly collected on agar by using impactors, such as Andersen impactor or similar, or be transferred to agar from liquid samples, such as impingers and wetted-wall cyclones. However, viable airborne particles cannot, in general, be recovered without some inactivation or loss during or after sampling. The presence of total bioaerosols could be determined by analyzing liquid or filter samples using traditional techniques such as microscopy or by using advanced analysis techniques, such as PCR, flow cytometry, and ATP-based analysis, which provide more comprehensive and accurate information about the bioaerosol samples.

Getting a Jump on Pathogenesis: Transit Through the Flea

V. J. Hinnebusch
Rocky Mountain Labs, NIH/NIAID, Hamilton, MT.

Yersinia pestis, the agent of plague, is transmitted to mammals primarily by infected fleas. Y. pestis exhibits a distinct life stage in the flea, where it grows in the form of a cohesive biofilm that promotes transmission. After transmission, many known virulence factors of Y. pestis, such as the antiphagocytic Type III secretion system and the F1 capsule, are induced that confer resistance to innate immunity. However, these factors are not produced in the low-temperature environment of the flea, suggesting that Y. pestis is vulnerable to the initial encounter with innate immune cells at the flea bite site. We used whole-genome microarrays to compare the Y. pestis in vitro transcriptome in infective fleas to in vitro transcriptomes in temperature-matched biofilm and planktonic cultures, and to the previously characterized in vitro gene expression profile in the rat bubo. The results characterize a specific phenotype in the flea vector, implicate Y. pestis genes involved in biofilm formation, and show that Y. pestis differentially expresses distinct subsets of genes during its life cycle to establish a productive infection in its two disparate hosts. Furthermore, several genes with known or predicted roles in resistance to innate immunity and pathogenicity in the mammal were upregulated in the flea, and Y. pestis from infected fleas had increased resistance to phagocytosis. The results indicate that the Y. pestis transcriptome in the flea is not merely a reflection of the mammalian innate immune response, but that the vector-specific phenotype of Y. pestis as it exits the flea and enters the mammal enhances infectivity.
Current Status of the *Ixodes scapularis* International Sequencing Project

S. K. Wikel; Ctr. for Biodefense & Emerging Infectious Diseases, Univ. of Texas Med. Branch, Galveston, TX.

Tick genomics is being advanced rapidly due to projects generating large databases of expressed sequence tags (ESTs) of tissues of several tick species; the first whole genome sequencing project for a chelicerate, *Ixodes scapularis*; and, the ability to suppress expression of specific tick genes by RNA interference. Tick genomes are large and contain numerous repetitive sequences. Reported sizes of Ixodid tick genomes are 1.04 to 7.1 Gbp with genome size of *Ixodes scapularis* approximately 2.1 Gbp. NIAID Microbial Sequencing Centers recently announced the annotation release 1.0 of the *Ixodes scapularis* genome. The 1.0 release contains 20,486 high confidence protein-coding genes. Emerging genomic data will help address important areas of tick research, especially those topics previously not readily amenable to study. Tick genomics is advancing understanding of arthropod genome organization, evolutionary relationships, host selection, vector competence, modulation of the host environment, immune defenses, neurobiology, endocrinology, pathogen development and transmission. EST projects are generating a wealth of information. Salivary gland transcriptome analyses provide novel insights into tick-host-pathogen interactions and convergent evolutionary strategies for blood feeding. Integrated analyses of tick, host and pathogen genomic information are facilitating development and use of tools such as microarrays to pursue focused systems biology approaches to the study of these interactions.

Treatment of Botulinum Neurotoxin Intoxication: Small Molecule Therapeutic Approaches

K. D. Janda; Chemistry and Immunology, The Scripps Res. Inst., La Jolla, CA.

Botulinum neurotoxins (BoNT) are the etiological agents responsible for botulism, a disease characterized by peripheral neuromuscular blockade and a characteristic flaccid paralysis of humans. With the current warfare and terrorist activities, potential bioterrorist agents are a high priority. Due to the long paralysis and necessity for mechanical respiration, the numbers of medical care units capable of proving supportive care for recovery are limited. Countermeasures are urgently needed, in this context. We have concentrated our research efforts on ways to uncover small molecule inhibitors for BoNTs. To accomplish these tasks we are engaged in multiple approaches. The first is based on inhibition of the protease of BoNT using a series of small molecule inhibitors that ultimately have the correct characteristics for potential drug development. Our second tact examines Toosendanin, a triterpenoid that has been used in traditional Chinese medicine. We have performed studies on the natural product toosendanin using both cell and mouse models, key features that are central to the antibotulinism properties of toosendanin, and its mechanism of action will be detailed. Our third strategy engages discovery of molecules that when administered will promote release of the neurotransmitter acetylcholine, in other words poly-functional intervention. Against this backdrop we believe it is important to embrace methods that would provide both immediate and possible long-term relief from these neuroparalytic effects of the BoNTs. As such the overarching goal of this aspect of our research is to uncover molecules that can act within an intoxicated cell to provide symptomatic relief to BoNT/A.

*YadBC* of *Yersinia pestis*

S. C. Straley; Microbiol., Immuno., and Molec. Genetics, Univ. of Kentucky, Lexington, KY.

*Yersinia pestis* is the causative agent of plague in humans and is a concern as a potential bioterrorist weapon. A pair of putative trimeric autotransporter proteins called YadB and YadC (*YadBC*), encoded by the chromosomal *yadBC* operon, is crucial for lethality of bubonic plague in mice. YadB-C has lesser impact on lethality in the lung infection; however YadC can protect against pneumonic plague. There are no significant similarities between the putative virulence-related “head” regions of YadB or YadC and proteins in any organisms other than *Y. pestis* and *Y. pseudotuberculosis* and unlike many other members of the trimeric autotransporter family, YadB-C appears not to be an adhesin. However, YadB-C does promote invasion of epithelial or macrophage-like cells. This talk will present our recent findings about the YadB-C structure and function.

Genomic Plasticity of *Burkholderia pseudomallei*: Implications for Disease Acquisition and Bacterial Persistence in the Infected Host

S. J. Peacock; Wellcome Unit, Mahidol Univ., Bangkok, THAILAND

Meliodosis, the serious bacterial infection caused by the Gram-negative soil saprophyte *B. pseudomallei*, is a major cause of sepsis across SE Asia and Northern Australia. The genome of this bacterium appears to be highly dynamic, with large regions of genomic difference identified between isolates. A lower estimate of the proportion of the accessory genome that is variably present is 14%, much of which is localised to genomic islands (GIs). Gene function of this variable portion is diverse and includes a range of determinants that would be predicted to alter interactions with its environment. The broad host range infected by *B. pseudomallei* combined with marked variability in clinical features during human melioidosis has led to the hypothesis that genomic diversity of *B. pseudomallei* may influence host specificity and clinical manifestations. This will be discussed and the current evidence reviewed. *B. pseudomallei* has the ability to persist in around 10% of infected humans despite the combined efforts of the immune response and appropriate antimicrobial therapy. We have defined large-scale genomic deletion as a cause of persistence in some patients. The size of the deletion ranged from 145kbp to 309kbp in different isolates, with an average deletion size of 227kbp. Although none of the regions of deletions were identical, all led to the deletion of a common region containing 49 genes. The genetic basis for persistence in these cases will be discussed.

*Burkholderia pseudomallei* Strains That Infect Humans and Animals are Distinct from Environment Strains

D. Wagner; Biological Sciences, Northern Arizona Univ., Flagstaff, AZ.

*Burkholderia pseudomallei*, causative agent of melioidosis, is a significant cause of mortality and morbidity in endemic regions and is widespread in the environment. Our environmental studies in both Australia and Thailand identified *B. pseudomallei* in a majority of soils samples collected from geographically diverse sites. Given that *B. pseudomallei* appears to be ubiquitous in endemic regions, it is surprising that even more human cases are not reported. These patterns suggest that only some *B. pseudomallei* strains are capable of infecting humans and other vertebrates. We tested the hypothesis that *B. pseudomallei* strains that infect humans and other vertebrates are a distinct subset of the strains actually available in the environment. To do this, we utilized human and environmental isolates (250+) collected from Ubon Ratchathani province in northeast Thailand and goat and environmental isolates (150+) collected from the Northern territory of Australia. We found that isolates from humans and goat isolates were distinct from strains isolated from their respective environmental background locations, possessing genomic elements associated with increased virulence. We conclude that in the natural environment only some *B. pseudomallei* strains appear to be infective.
Animal Model

H. P. Schweizer, Microbiology, Immunology and Pathology, Colorado State Univ., Fort Collins, CO.

Background: Melioidosis is caused by *Burkholderia pseudomallei* (Bp), an emerging pathogen of biodefense interest. Because of its Select Agent (SA) listing Bp must be handled in approved BSL3 laboratories. Treatment of melioidosis is confined to a few antibiotics and new drug discovery efforts are hampered by the need for SA compliant BSL3 facilities. Exempt attenuated Bp strains would accelerate in vitro drug discovery efforts and facilitate other studies with this bacterium. Methods: A ΔpurM mutant of Bp strain 1026b was constructed using SA compliant methods, and its virulence evaluated in murine and hamster melioidosis inoculation models. Intranasally (IN) infected BALB/c mice were used to assess efficacy of a new monoactam, BAL30072, in treated (intraperitoneal [IP] injection) and untreated animals. Results: At infectious doses of 10^6-10^8 bacteria the ΔpurM mutant was avirulent in various mouse strains, including susceptible (BALB/c and SCID) and hyper-susceptible (IPNg K/0 and 1206s/Ev) strains, as well as Syrian hamsters. In animals infected with 1026b, bacteria rapidly dispersed into and at time of death were present in large numbers in lung, liver and spleen. In contrast, the ΔpurM mutant was rapidly cleared from infected animals and organs were sterile 30 to 35 days post-infection. The in vitro activity of BAL30072 was assessed with a panel of 60 clinical and environmental Bp isolates. Minimal inhibitory concentration (MICs) were in the 0.004-0.016 mg/mL range for >93% of all strains. In initial experiments, IP administered BAL30072 afforded excellent protection on BALB/c mice that were infected IN with 5000 bacteria. Conclusions: A Bp ΔpurM mutant was found to be avirulent in mice and hamsters. After completion of control experiments, we plan to file a request with CDC for exemption of the avirulent strain as an investigational/experimental product. BAL30072 exhibited excellent in vitro and in vivo activity against Bp. Its in vivo MIC is vastly superior to current clinically employed b-lactams and this compound may thus be a useful new melioidosis drug.

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*B. pseudomallei* Type III Secretion System Effectors

J. E. Miller, Dept. of Microbiology, Immunology & Mol. Genetics, Univ. of California Sch. of Med., Los Angeles, CA.

*B. pseudomallei*, which causes melioidosis and is considered to be a potential biological weapon, employs three Type III secretion systems (T3SS) to deliver protein effectors into host cells. Intracellular invasion, survival and cell to cell spread in vitro, and virulence in vivo, are TSSS-dependent phenotypes. We have devised bioinformatic screens that allow the identification of *B. pseudomallei* T3SS effector genes by the presence of conserved eukaryotic functional domains, similarity to other T3SS effectors, and co-localization with class IA chaperones. We have examined phenotypes associated with four *Burkholderia* effector protein (Bep) candidates - BepB, BepD, BepE, and BepF. BepD has a chimeric structure in which T3SS localization and chaperone-binding sequences are fused to a C-terminal domain found in T3SS family insecticidal toxins. BepE localizes throughout the cytosol and expression results in cytotoxicity and morphological alterations characterized by the formation of tubular structures that remain anchored to the substratum. BepF contains a P-loop ATP/GTP hydrolysis and nucleotide binding domain fused to an a-actinin-type actin binding motif, and expression leads to the disruption of stress fibers and actin reorganization to the cell periphery. We hypothesize this factor is a novel actin-binding protein that controls cytoskeletal dynamics. BepF contains partial homology to Cif, an EPEC effector that functions as a cyclomodulin that arrests cell cycle progression at the G2-M transition. BepF also has a predicted P-loop ATP/GTP hydrolysis domain and the ORF is flanked by transposable, suggesting recent horizontal acquisition. BepF expression results in membrane ruffling, alterations in actin distribution and an increase in the size of focal adhesions. These phenotypes are similar to those associat ed with Cif and may represent downstream effects of alterations in cell cycle control. BepD, which does not display sequence similarity to other known effectors, displays a striking pattern of colocalization with cytoplasmic microtubules which is eliminated by treatment with nocodazole. BepD also localizes to the spindle apparatus of mitotic cells and it can be secreted through surrogate T3SSs. Experiments focused on defining the roles of these and other effectors in *B. pseudomallei*-host interactions are currently underway.

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A Microfluidic Platform for Imaging and Proteomic Analysis of Host-Pathogen Interactions with Single-Cell Resolution

A. Singh, Biosystem Research, Sandia National Laboratories, Livermore, CA.

Innate immunity is our first line of defense against a pathogenic bacteria or virus. A comprehensive “system-level” understanding of innate immunity pathways is the key to deciphering mechanisms of pathogenesis and may lead to improvements in early diagnosis or developing improved therapeutics. Current methods for studying signaling focus on measurements of a limited number of components in a pathway and hence, fail to provide a systems-level understanding. Current methods also lack single-cell resolution necessary for accurate quantification of concentration and kinetics. We have developed a systems biology approach to decipher toll-like receptor 4 (TLR4) pathways in murine macrophages in response to pathogenic bacteria and their lipopolysaccharide (LPS). Our approach integrates a microfluidic cell handling and analysis platform with high-resolution imaging to provide spatially- and temporally-resolved measurement of signaling pathways. The integrated microfluidic platform is capable of imaging single cells to obtain dynamic translocation data as well as high-throughput acquisition of quantitative protein expression and phosphorylation information of selected cell populations. The platform consists of multiple modules such as single-cell array, cell sorter, and phosphoflow chip to provide confocal imaging, cell sorting, and flow cytometry-based phosphorylation assays. The single-cell array module contains fluidic constructions designed to trap and monitor up to 100 single cells for hours (cells viable for >18hrs), enabling detailed statistically-significant measurements. The module was used to analyze translocation behavior of transcription factor NF-kB in macrophages upon activation by E. coli and E. coli LPS. Activation of NF-kB is preceded by phosphorylation of many kinases and to correlate the kinase activity with translocation, we performed flow cytometry-based phosphorylation assays in the Phosph°Chip module. To allow further downstream analysis on selected cells, we also implemented an optical-trapping based sorting of cells. This has allowed us to sort macrophages infected with bacteria from uninfected cells with the goal of obtaining data only on the infected (the desired) population. The various microfluidic chip modules have been integrated into a portable package that can be mounted on a typical inverted microscope. The accessories required to operate the microfluidic platform such as pumps, heaters, and electronic control have been assembled in a bench-top automated controller. The data generated is being utilized to refine existing TLR pathway model by adding kinetic rate constants and concentration information. The microfluidic platform allows high-resolution imaging as well as quantitative proteomic measurements with high sensitivity (pg/mL) and time-resolution (~15 s) in the same population of cells, a feat not achievable by current techniques. Furthermore, our systems approach combining the microfluidic platform with high-resolution imaging, biological reagents and computational modeling, will significantly improve our ability to study cell signaling involved in infectious diseases research.

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Biodefence and Emerging Infectious Disease Imaging: Opportunities and Challenges

K. Li, Radiology, The Methodist Hospital, Houston, TX.

Biodefence and emerging infectious disease imaging requires the development of both imaging equipment that are specially designed for these applications and adaptations of both morphologic and molecular imaging technologies. This requires intense collaboration of researchers with expertise in multiple disciplines. In this presentation I will illustrate how an interdisciplinary team tackles the problem of designing imaging facilities that can be used for biodefence and emerging infectious diseases and how advanced imaging techniques can be applied to studying these diseases. Using an interdisciplinary approach we can explore many opportunities in biomedical research that are difficult to tackle otherwise. The challenge is to effectively integrate scientists with multiple different backgrounds to work on common goals and create synergy.
Virus Based Nanoparticles as Biomedical Imaging Tools
M. Manchester: Cell Biology, The Scripps Research Institute, La Jolla, CA.

We are developing virus-based nanoparticles for therapeutic purposes, primarily focusing on structures based on phage or plant pathogens. The plant virus cowpea mosaic virus (CPMV) has emerged as a nanoparticle platform for biomedical and pharmaceutical applications. CPMV has an especially stable structure of icosahedral symmetry, highly suitable for multivalent display of peptides and chemical bioconjugation of a variety of molecular substrates including proteins, fluorescent labels, metals, and carbon nanostructures. The capacity for genetic peptide display and the ability to chemically attach certain molecules expands CPMV technology for a variety of applications such as vaccines, antivirals, antitoxins, targeting and treatment of tumor cells, and microsurgically delivered therapeutics. Recently we showed that CPMV particles are highly effective nanoparticles for live-animal vascular imaging and tissue-specific targeting in vivo. CPMV particles appeared to specifically interact with mammalian endothelial cells in the vasculature of live animals. The goal of our studies is to learn how we can use these nanoparticles to selectively target and image sites of inflammation and infection in vivo, by designing specialized ‘smart’ nanoparticles that can highlight the sites of disease.

Visualizing of Host-Pathogen Interactions with Accuracy
J. A. Timlin, Sandia Natl. Lab., Albuquerque, NM.

We report our development and use of multicolor fluorescent protein fusions and a custom confocal spectral imaging microscope to visualize spatio-temporal protein expression during host-pathogen interaction with Francisella novicida (F. novicida). F. novicida is a surrogate for the highly virulent Francisella tularensis, a gram-negative bacterium that causes tularemia and is a potential bioterrorist agent. In experiments where murine macrophages are infected with F. novicida we use confocal spectral imaging to visualize the expression of IgA and IgG, two cytoplasmic virulence proteins required for growth of F. novicida in a host macrophage cell. We will present results that demonstrate the importance of using advanced spectral unmixing methods to isolate and remove the confounding effect of the overlapping host cell autofluorescence spectrum in order to accurately detect the naturally weak expression levels of the fluorescent proteins present in vivo. Using this sensitive analytical imaging technology, we will map protein expression within the host cell with high spatial resolution at various timespoints post-infection. We will detail the spectroscopic imaging and multivariate analysis approach, including its potential for wide-spread application in biodefense. We will present examples of additional host-pathogen systems currently being studied to elucidate fundamental information about the interactions. The spectroscopic imaging and multivariate analysis approach is unique because it allows direct visualization of the spatial location of multiple species (the pathogen, the host, and labeled proteins for example) in intact cell systems - a feat which is not possible with current biological assays such as western blots.

*Sandia is a multi-program laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under Contract DE-AC04-94AL85000.

Panelist
V. Sutton; School of Law, Texas Tech. Univ., Lubbock, TX.

A nationwide survey of biodefense researchers about their opinions concerning the select agent regulations, 42 CFR §73, and the effectiveness of these rules in achieving their regulatory goals of national security and protecting public health, was conducted in the first two weeks of September 2007, and the first three weeks of August 2008. The survey had a return rate of approximately 54%, and addressed aspects of the select agent program regulations including, registration, recordkeeping, inventories, background security checks of researchers, laboratory biosecurity, training and education, and career impacts. The results of the survey clearly indicate a review of the select agent program is warranted to address some of the major findings in the survey, which indicate that the goals of national security and protecting public health could be more effectively achieved by other means. Further findings in the survey which indicated a need for a center for neutral regulatory compliance advice for select agent matters, led to the establishment in August 2008 of the National Hotline for Biosecurity and Biosafety Law at the Center for Biodefense, Law and Public Policy, Texas Tech University School of Law.

This research was supported in part, by a grant from NIH to the Law, Policy and Ethics Core through the Western Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research, NIH Grant Number U54 AI057156, the Center for Biodefense, Law and Public Policy and the Center for Public Service, Texas Tech University.

Featured Speaker: Synthetic Virus Assembly
R. Baric, Univ. of North Carolina, Haw River, NC.

Synthetic Biology (SB) combines new technologies for large-scale DNA synthesis with an emphasis on the creation of standardized, modular biological parts and the de-skilling of the techniques of genetic engineering. We conducted the first comprehensive survey of leading practitioners in the field to gather their views on its implications for biosecurity and combined them with existing research on biosecurity. SB is likely to have significant biosecurity implications in the medium and long term. It may both increase the absolute ability to modify living systems and broaden the pool of people able to do so. It is likely to vastly increase uncertainty, although it is most probable to advantage offense initially and defense eventually. A combination of policy approaches involving community-based efforts, regulation and surveillance, further research, and designing security into the fundamentals of the technology may minimize Synthetic Biology’s implications for offense and maximize its contributions to defense.

Synthetic Genomes: Research Tool or Regulatory Nightmare
M. J. Buchmeier; Molecular Biology and Biochemistry, Univ. of California, Irvine, CA.

The promise of de novo synthesis of microbes and viruses may have seemed like an Orwellian fantasy a decade or two ago, but with the generational advances in molecular biology and genetics the practice has become routine in some disciplines. As a tool of research and production these practices afford unparalleled flexibility and precision. To a regulatory agency charged with keeping the biosecurity of the Nation in check these new methods bring unprecedented challenges. Clearly there is a period of exploration and review currently underway that challenges our notions of biotechnology regulation and review, and achieving a new consensus will be necessary to achieve the full potential of this new science.
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2009 ASM Conferences

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3rd ASM Conference on DNA Repair and Mutagenesis
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3rd ASM Conference on Prokaryotic Development
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Abstract Submission Deadline – Friday, March 6, 2009
Pre Registration Deadline – Friday, May 8, 2009

Bacillus-ACT 2009: The International Bacillus anthracis, B. cereus, and B. thuringiensis Conference, an ASM Conference
August 30 – September 3, 2009, Santa Fe, New Mexico
Abstract Submission Deadline – Friday, May 1, 2009
Pre Registration Deadline – Friday, July 10, 2009

ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications
Abstract Submission Deadline – Wednesday, June 10, 2009
Pre Registration Deadline – Friday, July 24, 2009

3rd ASM Conference on Salmonella: Biology, Pathogenesis & Prevention
October 5 – 9, 2009, Aix-en Provence, France
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Pre Registration Deadline – Friday, May 1, 2009

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Pre Registration Deadline – Monday, September 14, 2009
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110th General Meeting
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