Session Time: Poster Session  
Session Number: 003  
Session Title: Poster Session 1  
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm  
Topic: A. Viral Agents  
Poster Presentation Number: 001 (A)  

Chikungunya, a Vector Borne Emerging Threat  

S. Tostenson, W. Dorman, M. Wolcott, T. Clements; USAMRIID, Ft. Detrick, MD  

In 2014, the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) Diagnostic Systems Division (DSD) Special Pathogen Lab (SPL) was contacted to assist with Chikungunya testing by several DOD laboratories. USAMRIID was able to detect over 40 cases of Chikungunya, and validated a Laboratory Developed Test (LDT) for detection of the virus by real-time reverse transcriptase polymerase chain reaction (RT-PCR).(6, 7) The viral RNAs is only detectable by real time RT-PCR a for a short amount of time in the blood or sera after the onset of symptoms. Comparison of both manual and automated extraction methods was also completed. An immune response was detected by ELISA in paired convalescent serum for many of the positive acute samples, as well as in some of the patients with an initial negative PCR result in the acute sample, possibly due to delayed collection after symptom onset. The disease can be mistaken as Dengue Fever, so dual testing for both viruses may be required for suspected cases.

Session Type: Poster Session  
Session Number: 016  
Session Title: Poster Session 2  
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
Topic: A. Viral Agents  
Poster Presentation Number: 002 (A)  

Investigation of a Suspected Diarrhoal Illness Outbreak in Upington - ZF Mgcawu District, Northern Cape, South Africa  

P. Manana¹, N. Page², G. Maupye¹, A. Rakgantso³, T. Mkhencele³, K. Dokubo⁴; ¹Natl. Inst. of Communicable Diseases, Field Epidemiology Training Programme, Johannesburg, South Africa, ²Natl. Inst. of Communicable Diseases, Field Epidemiology Training Programme, Ctr. for Enteric Diseases, Johannesburg, South Africa, ³Div. of Publ. Hlth. Surveillance and Response, Natl. Inst. of Communicable Diseases, Johannesburg, South Africa, ⁴Ctr. for Disease Control and Prevention, Atlanta, GA  

Background: Diarrhoeal diseases are a leading cause of morbidity and mortality in children <5 years. In response to a report about an increased number of diarrhoeal cases in a public hospital in Northern Cape Province, a situational assessment was conducted to confirm the existence of an outbreak, determine cause/s, prevent and control future outbreaks. Methods: We conducted a retrospective review of hospital registers and patients' files between March-July 2015 using a standardized case investigation form. Parents of children admitted to the ward were interviewed using structured questionnaires. Stool samples were screened using the ProSpect Rotavirus ELISA and reverse-transcription polymerase chain reaction (RT-PCR) for genotyping and real-time RT-PCR for virus detection. Results: Between 30 March and 05 July 2015, 638 diarrhoeal cases were identified. Children <5 years accounted for 50% (n=318) and adults ≥45 years for 16 % (n=103) of the cases. Two peaks were identified at epidemiological week 16 (16 cases) and week 24 (18 cases). Of the nine children admitted, one did not receive any dose of rotavirus vaccine. Eight had received one dose of rotavirus vaccine and five of the age-eligible children had received two doses of rotavirus vaccine. Rotavirus was detected in (6/9) stools collected with G9P[8] detected in all cases. Other enteric pathogens detected include sapovirus (n=1), norovirus (n=1) and adenovirus (n=1).  

Conclusion: A seasonal increase in rotavirus is a possible explanation for the observed increase in cases. We recommended strengthening of rotavirus vaccination and diarrhoeal surveillance through routine data collection, analysis and monitoring.
Surveillance and Control of Bluetongue in Kazakhstan

Y. Abduraimov, A. Sansyzbay, N. Sandybayev, M. Orynbayev, Z. Koshemetov; Res. Inst. for Biological Safety Problems (RIBSP), Otar, Kazakhstan

Emergence and re-emergence of infectious diseases may be predicated upon shifting human and animal population profiles, including transportation of animals and animal products. One such emerging disease, bluetongue, is enzootic globally in a band extending between 35 degrees south to 40 degrees north, and is primarily transmitted by vectors of the genus Culicoides (biting midge). Because the virus can persist in blood for weeks, animal migration and importation can lead to epizootics in novel areas, including the Republic of Kazakhstan. Officially, bluetongue is not a registered disease in Kazakhstan. However, biosurveillance conducted by our institute in the nearby countries of Tajikistan (2005-2006) and Kyrgyzstan (2012-2013) identified bluetongue antibodies in sera of sheep and goats. As a result, in 2013 we collected potential vectors along Kazakhstan’s border with Kyrgyzstan and assessed them for the presence of bluetongue nucleic acid by PCR. Approximately 40% were found to be positive. To address the corresponding risk of bluetongue in Kazakhstan, we developed and validated an ELISA test kit that is now used for improved surveillance, as well as an inactivated virus vaccine now approved by the Kazakhstan Ministry of Agriculture for control of the disease. We continue to conduct biosurveillance in high risk transboundary areas, and have proposed a monitoring program for the importation of cattle to Kazakhstan, which from 2011-2014 comprised more than 50,000 head from countries including the USA, the Czech Republic, Canada, France, Australia, Ireland, Austria, the Netherlands and Germany.

Venezuelan Equine Encephalitis Virus Infection Induces Oxidative Stress and Leads to Alterations in Mitochondrial Dynamics

T. Brooks-Faulconer, M. Amaya, F. Keck, C. Bailey, A. Narayanan; George Mason Univ., Manassas, VA

Venezuelan Equine Encephalitis Virus (VEEV) is an Alphavirus of the Togaviridae family. VEEV is classified as a Category B select agent and an emerging infectious agent with the ability to cause significant morbidity and/or mortality in both animal and human populations. Currently, there are no safe and effective, FDA-approved therapeutic treatments available for the treatment of VEEV infections. Our laboratory has been interested in understanding host responses to VEEV infections with the long term goals of developing novel biomarkers of the infectious process and therapeutic candidates. To that end, our recent work has focused on the impact of VEEV infection on mitochondrial dynamics in infected astrocytoma cells (U87MGs). Our data indicate that VEEV infection results in accumulation of Reactive Oxygen Species (ROS) and loss of mitochondrial membrane potential in an infectious dose and time dependent manner. We observed a perinuclear phenotype for mitochondrial distribution in infected cells as determined by TOMM20 distribution. The viral capsid protein localized to mitochondria and displayed co-localization with PINK1 and Parkin. PINK1 and Parkin protein localization in mitochondria is associated with disruption of mitochondrial function and loss of membrane potential, as indicated for other viral infections as well. Ongoing studies are focused on the characterization of mitochondrial structure and the mitochondrial proteome in infected cells.
The First Study for Emerging Zoonotic Coronavirus in Georgian Bats

L. Urushadze¹, G. Babuadze¹, A. Machablishvili¹, M. Dgebuadze¹, I. Natradze¹, P. Imnadze¹, A. Velasco Villa³; ¹Natl. Ctr. for Disease Control and Publ. Hlth., Tbilisi, Georgia, ²Ilia State University, Tbilisi, Georgia, ³Ctr. for Disease Control, Atlanta, GA

Background: The recent implication of a novel beta (β)-Coronavirus (MERS CoV) as the etiologic agent of fatal respiratory disease in the Middle East emphasizes the importance of coronavirus surveillance as these viruses may potentially jump species from bats into humans. Also, the causative agent for newly emergent coronavirus (SARS CoV) may have originated from a similar virus discovered in Chinese horseshoe bats (Rhinolophus spp.). By inferences from other parts of Europe and the world, bats from Caucasus may be implicated in the maintenance of important zoonotic pathogens. Objectives: The aim of this study was to assess the prevalence of coronaviruses in Georgian bats and anticipate possible new coronavirus outbreaks in human population. NCDC Lugar Center in collaboration with CDC, US conducted bat surveillance in Georgia. Methods: Laboratory work was carried out in a BSL3 facility at NCDC Lugar Center. For CoV detection, all samples were tested on PAN coronavirus RT-PCR method based on highly conserved RbRp gene primers. The amplicons were sequenced on ABI 3130 sequence analyzer. The sequences were edited using SEQUENCHER 5.0. Results: Among 150 faecal and anal swabs samples analyzed, 40 were found positive for CoV. The most public health relevant Betacoronaviruses were found in 16 specimens. The species that tested positive with higher frequency were Miotis blity and Rhinolophus eureale. Another four species tested positive (one individual each) Miniopterus and schreibersii, five positive Rhinolophus ferrumequinum and one positive Myotis emarginatus. Conclusion: This study was the first to identify CoV circulating in various bat species in Georgia. This newly implemented testing methodology strengthened laboratory capacity for timely detection and response of emerging CoV outbreaks and will assist to determine pathogen transmission pathways from bats to humans in conjunction with different epidemiological tools.

Bacteriophages Against Bacillus anthracis

L. Leshkasheli², N. Skhirtladze¹, E. Tevdoradze¹, D. Bolkvadze¹, I. Kusradze¹, N. Balarjishvili¹, L. Kvachadze¹, A. Kotorashvili¹, M. Nikolich², J. Farlow³, M. Kutateladze¹; ¹George Eliava Inst. for Bacteriophages, Microbiol. and Virology, Tbilisi, Georgia, ²Walter Reed Army Inst. of Res., Silver Spring, MD, ³Farlow Scientific Consulting Company, Lewiston, UT

Introduction: Phage typing is a reliable method for the identification of various bacterial strains, including especially dangerous pathogens such as Brucella species and Bacillus anthracis. Several well-studied bacteriophage are used as a diagnostic method in the identification of bacterial species. Phage-based methodologies for bacterial strain typing are based on lytic properties of virulent bacteriophages. The aim of this research was to select and study specific phage that reveal lytic activity for B. anthracis and compare their genomes. Materials and Methods: Ten bacteriophages from the Eliava Institute phage collection were selected for a detailed study. B. anthracis Sterne 34F2 was used as the host bacteria for the selected phage. Phage DNA of all ten phage were isolated and restriction analysis was performed using different endonucleases. The genomic size of the phage was estimated by Pulse-Field Gel electrophoresis (PFGE). B. anthracisphage were characterized with polymorphic plaques. Bacteriophages were purified by CsCl gradient density centrifugation. Phage DNA was isolated from purified phage. Ten(10) phage genomes were sequenced using the IlluminaMiseq platform. Results and Discussion: All phage under the study have the same morphology as internationally accepted gamma phage used for identification of B. anthracis strains; the phage have hexagonal heads and long and non-contractile tails. The PFGE pattern of phage genomes showed several bands on the gel. According to preliminary analysis, phages in our analysis are similar to members of the gamma phage
family, as well as an unrelated siphocirus, similar to the Basilisk phage. Detailed comparison of phage genomes and biological properties will provide a new phage typing scheme for the identification of B. anthracis strains.

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: A. Viral Agents
Poster Presentation Number: 007 (A)

Efficacy of Delayed Brincidofovir Treatment in a Randomized, Blinded, Placebo-Controlled Study of Rabbitpox in New Zealand White Rabbits

I. M. Grossi¹, S. Foster¹, M. L. Rose¹, M. R. Gainey², R. T. Krile², J. Khouri¹; ¹Chimerix, Durham, NC, ²Battelle, West Jefferson, OH

Brincidofovir (BCV; CMX001) has advanced in development as a treatment for smallpox under the FDA’s Animal Rule. Previously, in a rabbitpox virus (RPXV) animal model, BCV initiated following the detection of lesions was shown to significantly reduce mortality. The current study assessed the efficacy of BCV in New Zealand White rabbits intradermally infected with a lethal inoculum (300 PFU) of RPXV strain Utrecht, with treatment initiated following the onset of symptomatic disease (ie, fever). Blood samples were obtained throughout the study to assess viral load and infectious virus. At the time of confirmed fever, infected rabbits were randomized to 1 of 5 blinded treatment groups: placebo, immediate BCV, or BCV delayed by 24, 48, or 72 hrs. Animals received either placebo or an initial 20 mg/kg oral dose of BCV, followed by two 5 mg/kg doses at 48-hr intervals; doses were scaled from exposures in BCV Phase 3 clinical trials. A clinically and statistically significant reduction in mortality was observed when BCV was initiated immediately, 24, and 48 hrs following the onset of fever (p<0.05 vs placebo; Fishers exact test). A reduction in mortality was also observed when BCV was initiated 72 hrs after onset of fever, but this was not statistically significant (p=0.091 vs placebo). Furthermore, compared with placebo, BCV treatment was associated with reduced mean peak viral load during the course of the disease, as well as reductions in the number of plaque-positive samples and in mean PFU/mL of positive samples (p<0.05) (measures previously correlated with infectivity). BCV demonstrated clinically and statistically significant efficacy in this lethal orthopox infection model when administered at the time of confirmed fever or at the midpoint of disease progression (48 hrs after the onset of fever in this model). Furthermore, the observed reduction in plaque-positive samples in BCV-treated animals may reflect reduced infectivity and potential additional public health benefits in the event of a smallpox bioterror event.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: A. Viral Agents
Poster Presentation Number: 008 (A)

Serological Research of Acute Febrile Illnesses for Arboviruses in the Republic of Georgia

T. Kutateladze, M. Chubinidze, E. Zangaladze; Natl. Ctr. for Disease Control and Publ. Hlth., Tbilisi, Georgia

Background: Acute febrile illnesses (AFI) form a large disease burden which are not diagnosed in Georgia due to limitations of public health surveillance. This research aims to determine the burden of AFI, particularly those etiologies requiring prompt treatment to avoid or minimize long-term consequences. Methods: ELISA was used to study the sera from patients with febrile illness in Georgia. 186 samples were investigated. Patients aged 4 years or older without diagnosis and with a temperature of ≥38°C for ≥48 hours were enrolled in this study. Travel history, data on clinical manifestation, and information of different exposures was collected. Serum samples were tested for the presence of IgG and IgM antibodies to WNV, dengue and Chikungunya viruses. Results: ELISA assay results were negative for Chikungunya virus. The investigation revealed specific IgM and IgG antibodies against viruses of West Nile (WH) and Dengue Viruses. Patients with antibodies (21) are residents from different
regions of Georgia, 67 % are male. Age distribution of patients with antibodies in the following age groups 20-29, 30-39 and 40-49 are three patients in each, the age group 60+ had nine patients. Most of positive results (8) are in Tbilisi, capital of country. One patient from Kazeti, west Georgia has IgG for both West Nile and dengue viruses. Discussion: The presence of vectors capable of transmitting Arboviruses (Aedes albopictus, Aedes aegypti and Culex pipiens) in Georgia indicates the possibility for these pathogens to infect humans. Results of ELISA assays on samples from patients with AFI confirmed this hypothesis. On the basis of positive IgM antibodies to Dengue Virus and West Nile virus it can be assumed, that these viruses are the causative agents of AFI. A specimen that tests positive for IgG but negative for IgM may indicate the presence of past infection and the possible circulation of Arboviruses in Georgia. Future study and surveillance activities are needed to determine the etiology of acute undifferentiated febrile illness in Georgia.

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: A. Viral Agents
Poster Presentation Number: 009 (A)

Characterization of Equine Monoclonal Antibodies to Influenza Virus H3N8

A. K. Harris, U. Torian, J. R. Gallagher, D. M. McCraw; NIH, Bethesda, MD

Although vaccines and drugs are available, the influenza virus continues to be a major health problem and pandemic threat due to its zoonotic nature and its ability to change its antigenic makeup, most notably amino acid variations of the viral surface glycoprotein, hemagglutinin (HA). While it is appreciated that equine influenza viruses can infect other animals such as domestic dogs, the characterization of antibodies and epitopes of equine influenza HA (H3) has not been studied in great detail, nor has cross-reactivity of equine antibodies to human influenza viruses. We used the methods of hybridoma cell cultures, chromatography, and immunoassays to isolate and characterize a series of equine monoclonal antibodies derived from lymphocytes of ponies immunized with influenza A equine 2 virus (isolate A/Equine/Newmarket/79 (H3N8). Our results indicated well-formed antibody complexes with expected heavy and light chains and molecular weights corresponding to IgG molecules. Interestingly, some antibodies displayed cross-reactivity with human H3 viruses, suggesting that some epitopes may be conserved between human and equine H3 viruses. Sequence alignments and molecular modeling with H3 sequences supported our finding that particular epitopes could be conserved across human and equine HA H3 molecules. Our study suggests that further analyses of equine monoclonal antibodies to H3 might identify novel antibody sequences that could recognize conserved epitopes of influenza H3 hemagglutinin molecules.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: A. Viral Agents
Poster Presentation Number: 010 (A)

The Potential of Andrographis paniculata as Antiviral Herb for Neutralizing Newcastle Disease Virus in Kampong Chicken

I. Firliani; Bogor Agricultural Univ., Bogor, Indonesia

The absence of vaccination program of kampong chickens family poultry farming scale, including Newcastle disease virus vaccine claimed a substituent to control the high degree of mortality and the deficit in small farmers. The high vaccine price and no government subsidy are factors causing the small farmers not to apply the vaccine. The potential development of herbs as the medicine is one of the programs which ASEAN develops this day. The bitter herbs are caused by the high of alkaloid contained of its component structure. Alkaloid has been tested and used to defend the diseases causing by virus, such as AIDS, Hepatitis, and Influenza. Andrographis paniculata is herb containing high alkaloid. The bitter
The potential for differential involvement of DHX9, an RNA helicase, in Rift Valley Fever Virus Infection and Virulence

F. Keck 1, C. de la Fuente 1, C. Pinkham 1, P. Russo 1, A. Garrison 1, C. Schmaljohn 1, K. Kehn-Hall 1, A. Narayanan 1; 1Natl. Ctr. for Biodefense and Infectious Diseases, Manassas, VA, 2Ctr. for Applied Proteomics and Molecular Med., Manassas, VA, 3United States Army Med. Res. Inst. of Infectious Diseases, Frederick, MD, 4DCE Consulting, Vienna, VA

Rift Valley fever virus (RVFV) is a negative stranded RNA virus in the family Bunyaviridae. Several studies demonstrated the involvement of host enzymatic machinery in the establishment of a productive RVFV infection. A Tandem Mass Tag Mass Spectrometry (TMT-MS) approach was used in order to obtain a broad, unbiased, total proteome perspective on important host protein components that play differential roles following infection by either the attenuated MP-12 or virulent ZH548 strains of RVFV. Human small airway lung epithelial cells were infected and lysed at 3, 9, and 18 hours post infection, and the total protein lysates were prepared for MS analysis. Digested peptides were labeled using 6-plex TMT tags to simultaneously obtain relative peptide ratios for identified proteins in each time point, for each infection. Peptide counts were normalized, and fold differences were calculated based on relative label incorporation for every target protein. Pathway analysis of lysates obtained from MP-12 and ZH548 infected cells indicated that these were differentially altered in areas including but not limited to, translational control, cellular oxidative stress, and DNA damage responses. TMT-MS analysis revealed that host enzymatic machineries involved in ribonucleoprotein formation, such as DEAH box protein 9 (DHX9), were upregulated to a higher extent in ZH548 infected cells over MP-12 infected cells. Previous studies have implicated DHX9 as a cytosolic sensor of viral nucleic acids. DHX9 interacting partners, including XRCC5 and PCNA, were also differentially abundant, suggesting that the DHX9 protein interaction network may play an important role in determining virulence. Ongoing studies in our laboratory expand on these data to further elucidate the protein interaction network involving DHX9 in MP-12 and ZH548 infected cells.
2015. All samples were tested for influenza A/B; Parainfluenza viruses (PIV) 1, 2 and 3; Respiratory Syncytial Virus (RSV); Adenovirus (AV) and Human Metapneumovirus (HMPV) by real time PCR using US CDC provided primers/protocols. 290 out of 415 (69.9%) samples tested positive for one or more viruses. Most commonly detected viruses were RSV (n=139), AV (n=85) and influenza B (n=50), followed by PIV 3 (n=24), HMPV (n=19), influenza A/H1p (n=7), PIV 1 (n=6) and PIV 2 (n=5). Mostly found co-infection was RSV with AV (n=16). Influenza related hospitalizations were reported from week 4 to week 18, 2015 reaching its peak in mid of February, 2015 (weeks 6-7). Two fatal cases due to influenza A/H1p were reported in cancer patients. Unlike majority of European countries influenza B was dominant virus for 2014-2015 season in Georgia. AV detection was almost evenly observed in the reported period while RSV circulation coincided with influenza activity but peaked in March, 2015 (weeks 9-13). Incidence rate of RSV was highest (46.9%) in children under 1 year followed by age group 1-4 years (31.7%); AV was mostly found in age groups 1-4 years and 5-14 (24.6% and 19.7% respectively). Our study data demonstrate importance of RSV, AV and influenza viruses in developing severe respiratory infections in young children, sometimes resulting in lethal outcome as in case of influenza. Further monitoring of respiratory viruses is essential for timely implementing appropriate control measures.

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: A. Viral Agents
Poster Presentation Number: 013 (A)

Middle East Respiratory Syndrome Coronavirus Intra-host Populations are Characterized by Numerous High Frequency Variants

M. Borucki¹, V. Lao¹, M. Hwang¹, S. Gardner¹, D. adney², V. Munster³, R. Bowen², J. Allen¹; ¹Lawrence Livermore Ntl Lab, Livermore, CA, ²Colorado State Univ., Fort Collins, CO, ³Rocky Mountain Lab., NIAID/NIH, Hamilton, MT

Middle East respiratory syndrome coronavirus (MERS-CoV) is an emerging human pathogen related to SARS virus. In vitro studies indicate this virus may have a broad host range suggesting an increased pandemic potential. Genetic and epidemiological evidence indicate dromedary camels (Camelus dromedarius) serve as a reservoir for MERS virus but the mechanism of cross species transmission is unclear and many questions remain regarding the susceptibility of humans to infection. Recently Briese et al. (2014) sequenced multiple MERS-CoV samples, and analysis of the sequence data revealed the presence of a high frequency variant genotype, thus leading the authors to speculate about the role of viral quasispecies in cross-species transmission. To fully characterize the genetic variants present in nasal swabs from camels experimentally infected with a low passage human MERS-CoV isolate, we amplified a majority of the MERS genome using multiplexed RT-PCR assays and sequenced the products at high depths of coverage. A majority of the genome was covered and average coverage was greater than 12,000x depth. Although only 5 mutations were detected in the consensus sequences, 473 intrahost single nucleotide variants were identified throughout the genome. Thus infection of camels with a human isolate of MERS-CoV induced numerous high frequency iSNVs upon a single passage in camels. This indicates that MERS-CoV, similar to other coronaviruses, is prone to extreme genetic diversity in intrahost viral populations that may change rapidly in response to a change in host environment. Until patterns of variation are defined for quasispecies present in non-laboratory passaged MERS-CoV samples, design of reagents for detecting MERS CoV infections will be vulnerable to the effects of intrahost genetic diversity on reagent sensitivity.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: A. Viral Agents
Poster Presentation Number: 014 (A)

Development of a Novel In Vitro Model for Analysis of Alphavirus Infection and Persistence in the CNS

M. Borucki, S. Hall, K. Kulp, H. Enright, S. Felix, N. Fischer, J. Osburn, D. Soscia, E. Wheeler; Lawrence Livermore Ntl Lab, Livermore, CA
Numerous viral agents are capable of infecting the human nervous system, often with devastating consequences, however the mechanisms of neurovirulence and persistence are often unclear. Persistence of viral RNA in neurons after an acute infection resolution has been proposed as a mechanism for development of sequelae. Many neurovirulent pathogens lack animal models that accurately recapitulate human infections. Sindbis virus, an alphavirus, is neurovirulent in rodents and is often used as a model for Venezuelan equine encephalitis virus. Rodent models show that Sindbis infection can induce neuron death directly via necrosis or apoptosis, or indirectly via bystander effects of neuron excitotoxicity. Sindbis virus expressing green fluorescent protein was used to infect primary rat neurons seeded on a multi-electrode array (MEA). Data from the 64-electrode MEA show that neuron action potential changes occurred at different stages of Sindbis virus infection. Nonsynonymous changes in the sequence of the viral RNA were detected upon passage of this laboratory-adapted virus in primary rat neurons. Modeling of viral infections and persistence using the MEA platform provides a system for identifying the mechanisms that lead to long lasting neuron damage and for testing of potential treatments for these infections.

**Session Type:** Poster Session

**Session Number:** 003

**Session Title:** Poster Session 1

**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm

**Topic:** A. Viral Agents

**Poster Presentation Number:** 015 (A)

**The Role of Brain Vascular Integrity in Neurotropic Rift Valley Fever**

M. R. Kujawa, A. L. Hartman; Univ. of Pittsburgh, Pittsburgh, PA

**Background:** Rift Valley Fever Virus (RVFV) is a mosquito-borne virus that causes a spectrum of human clinical diseases. A severe outcome in RVFV-infected people is encephalitis. Lewis rats are a small animal model for lethal neurological disease after aerosol infection with RVFV ZH501. Lewis rats develop severe disease within 6 - 8 days after infection. Pathology in the brain during the last 2 days before death is severe, with high levels of infectious virus, many viral antigen-positive neurons, and visible cell death by apoptosis. Prior to 5 d.p.i, virus is scarce, no pathology is present, and the rats display no clinical signs. The aims of this study are to understand the mechanisms behind the neurovirulence of RVFV and to determine the route of neuroinvasion. **Methods:** In vivo imaging (IVIS Spectrum CT) was used to measure loss of integrity of the brain vasculature. Infectious virus and viral RNA were detected in various tissues of the olfactory and CNS systems. Growth of RVFV in the human neuronal cell line SH-SYSY was compared to Vero E6 and BHK-21 cells. Results: Using IVIS, breakdown of the brain vasculature occurred around 5 d.p.i, which coincides with clinical signs, pathology, and high viral load. In rat brain tissues, PCR revealed that viral RNA was detectable by RT-PCR several days before any infectious virus was found or any pathological changes were detected. In vitro growth of RVFV ZH501 on SH-SYSY neuronal cells was exponential and comparable to Vero E6 and BHK-21 cells. **Conclusions:** The detection of low quantities of viral RNA in the brain prior to loss of vasculature integrity suggest that the main route of neuroinvasion is not via the blood brain barrier, but most likely an alternative route such as the olfactory epithelium. Surprisingly, SH-SYSY neuronal cells are as permissive for viral replication as Vero E6 and BHK-21 cells, two cell lines that have various defects in the interferon antiviral response. Our data suggest that infection of neurons and direct cytopathic effect are significant factors in development of neurological disease in rats.
Investigating Genetic Diversity Following Amplification of Low Passage Marburg Virus in Cell Culture


Marburg virus (MARV), a member of the Filoviridae family, is an RNA virus that causes Marburg virus disease (MVD) with high case fatality rates. No approved vaccines or therapies exist for MARV infections and the virus must be handled at Biosafety Level-4 (BSL-4). Typically, RNA viruses have high spontaneous mutation frequencies, which permit rapid adaptation to selection pressures and have other important biological consequences. However, the mutation frequency for MARV is unknown. By obtaining molecular clones and sequencing, we determined the mutation frequency for MARV. The frequency was similar to that found for other RNA viruses. To investigate the consequences of this spontaneous mutation in MARV, we used deep sequencing to assess the genetic changes associated with MARV serial passage in cell culture. As the virus was passaged in cell culture, we detected changes within the quasispecies population. However we only detected one nonsynonymous single nucleotide polymorphism (SNP) at the consensus sequence level and it was found in the signal peptide region of the glycoprotein. These data imply that while the polymerase replicates with poor fidelity, there is low tolerance for change across the genome. These data provide information about the error prone nature of MARV.

The Role of Smad Proteins in Rift Valley Fever Virus Replication


A combinatorial proteomics and transcriptomics analysis was used to identify cellular factors important for the infectivity and replication of Rift Valley fever virus (RVFV). Human small airway epithelial cells were infected with virulent strain, ZH548, or the live attenuated vaccine strain, MP12, and samples were collected at 1, 3, 9, and 18 hours post-infection. Reverse Phase Protein Arrays were utilized to identify changes in phospho-signaling following infection. Many alterations previously observed were confirmed, such as increased phosphorylation of HSP27, p38 MAPK, and p53. The altered signaling profiles were highly conserved between ZH548 and MP12 strains. Novel phospho-signaling events involving Smad1/5/9 and Smad2 transcription factors were identified, with Smad1/5/9 phosphorylation being the most highly upregulated change observed following infection. Smad signaling is a downstream effector of transforming growth factor-β (TGF-β) or bone morphogenetic protein (BMP) type II and type I receptor activation, mediating multiple developmental processes including proliferation, differentiation and apoptosis. Confirmatory experiments indicated that Smad1/5/9 and Smad2 are phosphorylated following infection. Although Smad linker phosphorylation was observed, which can result in the proteasomal degradation of the protein, total Smad1 and Smad2 levels were consistent throughout infection. Phosphorylation changes were found to be dependent on viral replication and the presence of the RVFV protein, Ns. Transcriptomics analysis demonstrated that multiple components of the Smad signaling cascades are altered, including downregulation of negative regulators of the pathway. Ongoing studies are aimed at determining the influence of Smad proteins on gene expression in RVFV-infected cells and how this contributes to viral replication and/or pathogenesis.
Aerosol Infection of Animals With Influenza and Francisella Tularensis; Importance in the Choice of Nebulizer

J. Bowling, D. S. Reed; Univ. of Pittsburgh, Pittsburgh, PA, Ctr. for Vaccine Res., Pittsburgh, PA

Animal studies to demonstrate efficacy of medical countermeasures against respiratory disease or biodefense threats require exposure of animals to aerosolized viruses and bacteria. Prior studies have shown that the choice of culture media and relative humidity in the aerosol chamber can impact the dose of infectious agent delivered to animals. Most infectious aerosol studies have involved the use of Collison jet nebulizers which create a small, relatively monodisperse aerosol that targets the deep lung. Collison nebulizers require a relatively large volume of infectious agent and the jets that create the aerosol may damage the agent being aerosolized. Damage resulting from the nebulizer can impact agent infectivity and virulence as well as study reproducibility. We compared the Aeroneb, a vibrating-mesh nebulizer, to the existing 'gold standard' Collison nebulizer for generation of small particle aerosols containing either a bacterium, F. tularensis, or a virus, influenza. Aerosol performance was assessed by comparing the ratio between the aerosol concentration of an agent and the concentration of the agent in the nebulizer (the spray factor, or SF). Initial aerosols focused on evaluating the Aeroneb and the Collison nebulizer using a nose-only tower. For F. tularensis the Aeroneb achieved a SF of 5.34x10^-6 while the Collison achieved 1.69x10^-6. The Aeroneb similarly achieved a better SF than the Collison with a seasonal influenza virus (6.4x10^-6 vs 1.1x10^-6, respectively). Similar results were obtained with the 2009 pandemic influenza virus (A/Ca/04/09) and an H5N1 highly pathogenic avian influenza virus (A/Vietnam/03/04). The Aeroneb also achieved a higher relative humidity in a whole-body chamber. We have successfully infected cynomolgus macaques with H5N1 using the Aeroneb to achieve a dose of 4.5 x 10^7 pfu using a nebulizer concentration of 7.1x10^8 pfu. This data demonstrates that the Aeroneb is a superior alternative to the Collison for animal efficacy studies especially when a high concentration of an infectious agent is needed to achieve a desired inhaled dose in an animal.

Characterization of a Novel Interaction Between the Arenavirus Nucleoproteins and the Cellular Apoptosis-Inducing Factor 1 (AIF1)

B. Meyer, D. Jackson, H. Ly; Univ. of Minnesota, Twin Cities, MN, Univ. of St. Andrews, St. Andrews, United Kingdom

A number of arenaviruses (BSL4 agents) cause hemorrhagic fever in humans with mortality rates of up to 30%. However, no arenavirus-specific antiviral therapies currently exist. The highly pathogenic New World Arenaviruses (NWAs), such as Junin virus and Machupo viruses, are all group into the same phylogenetic clade (clade B). Clade B also contains viruses that do not cause human disease, such as Tacaribe virus (TCRV), and these therefore represent model systems for studying arenavirus molecular biology in a conventional BSL2 laboratory. The aim of our research, using TCRV as a model system, is to gain a better understanding of arenavirus replication at the molecular level by identifying and characterizing essential arenavirus-host interactions, thereby uncovering potential antiviral drug targets. The multifunctional nucleoprotein (NP) plays important roles in many stages of the viral replication cycle, making it an ideal potential drug target. Using a comprehensive MS/MS mass spectrometry method, we have identified a conserved interaction between multiple clade B arenavirus NPs (including those of pathogenic Junin virus and Machupo virus) and the cellular apoptosis-inducing factor 1 (AIF1), which has been confirmed by GST-pulldown analysis using the recombinant proteins. TCRV infection of AIF1 knockdown cell lines results in a significant reduction in virus yield. This indicates the importance of the NP-AIF1 interaction during viral replication, and of this new interaction as a potential target for the design of antiviral drugs against the pathogenic arenaviruses.
Prevalence of Arboviruses Among Vectors in Armenia


Background: The Armenian environment and the abundance and diversity of sanguinivorous arthropods suggest that arboviruses are in circulation. Earlier work showed the presence of sanguinivorous vectors and arboviruses in Armenia are the basis for further studies to identify arbovirus foci.

Aim: To study the role of sanguinivorous arthropods as potential vectors of arboviruses in various climate-geographical zones of Armenia.

Methods: Mosquitoes and ticks were collected in Armenia from four climate-geographical zones: mountain-steppe, mountain-forest, desert-semidesert, and alpine. Mosquitoes were collected at the places of their day's rest. Ticks were collected from vegetation and cattle. PCR testing was conducted on some mosquito pools for tick-borne encephalitis (TBE) and West Nile Fever virus (WNF). Results and Discussion: A total of 78,429 mosquitoes and 38,190 Ixodidae ticks were collected. The predominant mosquito genus was Anopheles (72.5%), followed by Culex (16.8%), and Aedes (10.7%). Among the collected ticks, Dermacentor (71.0%) was the predominate genus, followed by Rhipicephalus (10.0%), Hyalomma (7.9%), Boophilus annulatus (7.0%), Haemaphysalis (3.0%), and Ixodes ricinus (1.1%). Desert-semidesert and mountain-steppe zones were most densely populated with mosquitoes, followed by mountain-forest, and alpine zones. The most ticks were found in mountain-forest, followed by desert-semidesert, alpine, and mountain-steppe zones. Mosquitos carried different strains of arboviruses than ticks. The range of spread of Batai, Geta, WNF, Sindbis, and Taïnya viruses coincides with that of Anopheles, Culex, and Aedes mosquitoes vectors, which prevail in desert-semidesert and mountain-steppe zones. The range of spread of Dhory, Bhanja, Tamdi, and Crimean-congo Hemorrhagic Fever, and TBE viruses coincides with that of Dermacentor, Hyalomma, Rhipicephalus, and Boophilus ticks, which dominates in mountain-forest and alpine zones. Conclusion: Sanguinivorous arthropods in all climate-geographical zones of Armenia are reservoirs and potential vectors of arboviruses.

Mixed Infection of Emergent Pathogens (Influenza and APMV) Among Wild Birds in the Azov-Black Sea Region of Ukraine

D. Muzyka1, M. Pantin-Jackwood2, B. Stegniľ1, A. Stegniľ1, O. Rula1; 1NSC “Inst. of Experimental and Clinical V, Kharkiv, Ukraine, 2Southeast Poultry Res. Lab., Athens, GA

Introduction. The influenza virus (AIV) and avian paramyxovirus (APMV) are the focus of researchers as pathogens that can cause unpredictable disease in poultry and people. The constant emergence of new strains and variants with new properties and pathogenicity to new hosts necessitates constant monitoring and careful research of new viruses. It is necessary to pay attention to the simultaneous circulation of pathogens - mixed infection, and conduct research on the mutual influence of virus on reproduction, the ability to the mutations and the emergence variants. The purpose is to monitor and study the circulation of AIV and APMV mixed infections in wild waterfowl and shorebirds. Materials and methods. During 2000 - 2011 we sampled the biological material from more than 6,000 wild birds of 66 of Anseriformes and Charadriiformes species. The studies were conducted by conventional techniques recommended by the OIE. Results. According to the results of serological investigations in 2006 - 2010, antibodies to AIV were found in 0,77 - 13,04% of serum samples and in 4,16-8,33% of egg yolks of wild waterfowl. Antibodies to APMV were
detected in 0.77-10.0% of serum, and in 7.14 - 18.18% egg yolks. When studying shorebirds, antibodies to AIV were detected in 13.33 - 23.07% of yolks, and antibodies to APMV in 13.63 - 46.15%. During the autumn migration 34 isolates were identified, of which 20 belonged to AIV, 12 to APMV, and 2 isolates were identified as mixed (H3N8/H4N8, H10/H12). During the wintering, we isolated 51 isolates, including 35 classified as AIV, 11 - as APMV, 5 isolates were mixed (H10/PMV7, H1/H5, APMV-1/APMV-7). During the spring migration, mixed infections were not found.

Conclusions. The results suggest the simultaneous circulation of AIV and APMV in wild birds of the Azov-Black Sea region. This data support the necessity for constant monitoring of avian influenza in order to timely prevention of highly pathogenic viruses to be a threat to poultry and human health.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: A. Viral Agents
Poster Presentation Number: 022 (A)

Characterisation of Aerosolised Ebola Virus

M. S. Lever, S. Smither; Dstl Porton Down, Salisbury, United Kingdom

Although Ebola virus is not naturally transmitted by the aerosol route, droplet spread may occur during an outbreak. Aerosolised Ebola virus is highly infectious and lethal in different animal models, but the exact amount of virions needed to cause infection or mortality is unknown. The use of Ebola virus as a bioterror or biowarfare agent also remains a possibility. Previous work done by Dstl has shown that the filoviruses are stable in small particle aerosols when tested in a dynamic or captured aerosol. Here we present work that extends our findings by looking at the aerosol stability of more recent Ebola virus strains, Ebola virus Kikwit (1995) or Ebola virus Makona (2014), compared to our historic data with an Ebola virus from the 1976 outbreak. The methods used to evaluate aerosol stability, the Goldberg drum or spiders’ web microthread technology were the same as previously used and described. In addition we present some preliminary data generated using the microthread technology to try and determine the amount of infectious virus in an aerosol. Together this data helps characterise the threat of aerosolised Ebola virus.

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: A. Viral Agents
Poster Presentation Number: 023 (A)

Evaluation of Cell Signaling Pathway Modulation after Rift Valley Fever virus Infection

T. Bell^1, S. Senina^2, V. Espina^2, L. Liotta^2, K. Kehn-Hall^1; ^1George Mason Univ., Natl. Ctr. for Biodefense and Infectious Diseases, Manassas, VA, ^2George Mason Univ., Ctr. for Applied Proteomics and Molecular Med., Manassas, VA

Rift Valley fever virus (RVFV) is an arbovirus transmitted by mosquitoes that commonly infects ruminant livestock with devastating effects including abortion storms and human spillover. The majority of human cases (~90%) result in either no illness or a mild flu-like sickness, but in a subset of infected individuals the disease can be severe and manifest as an acute onset liver disease or a delayed-onset encephalitis or retinitis. Additionally, some cases develop into a hemorrhagic syndrome with a case fatality rate of up to 20% in such patients. We are elucidating host-viral interactions by using reverse phase protein microarrays (RPPA) to determine which cellular pathways are upregulated following RVFV infection. Extensive in vitro work has established that several intracellular signaling pathways are altered following RVFV infection, including the DNA damage response pathway. We hypothesized that similar events occur in vivo. Using an animal model of infection, Balb/c mice, we performed a serial sacrifice study and collected tissues on days 0-8 post infection. Tissues were analyzed by immunohistochemistry, RPPA and western blot to probe for upregulation
of these proteins. Twenty-seven proteins of interest were identified and stained using RPPA in the hopes of capturing critical cellular signaling events during an acute RVFV infection. Immunohistochemical staining, western blot, and RPPA showed an increased level of phosphorylated H2Ax in infected hepatocytes consistent with upregulation of the beginning of the DNA damage response pathway. RPPA also showed upregulation of apoptotic markers (caspase 3 and 6 cleavage), transcription factors (phosphorylated CREB and ATF2), and proteins involved in cell proliferation (phosphorylation of S6 ribosomal protein). The ultimate goal of this research is identification of upregulated host pathways subsequent to viral infection as a means of identifying potential therapeutic targets.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: A. Viral Agents
Poster Presentation Number: 024 (A)

Emergence of Two H5N6 Virus Groups in Vietnam


Introduction: Highly pathogenic avian influenza (HPAI) A (H5N1) viruses have caused a number of outbreaks in poultry in Vietnam and in other South East Asian countries since 2003. In April, 2014, H5N6 virus was first detected in diseased chicken in Lang Son province and later in many other provinces in the North and the Central regions of the country. Methods: A total 10 samples from outbreaks and surveillance programs positive with H5N6 viruses were collected in 2014 and subjected to H5 sequencing. Phylogenetic analysis was performed using MEGA 6 software. Results: According to the phylogenetic analysis of H5 gene, H5N6 viruses of Vietnam belong to the subclade 2.3.4.4 within the clade 2.3.4. However, they are split into 2 groups that meet nomenclature criteria proposed by the WHO/OIE/FAO H5N1 Evolution Working Group to form 2 new subsubclade 2.3.4.4 a that include 9 viruses closely related to the H5N6 virus strain (A/Sichuan/26221/2014 (H5N6)) detected in Sichuan province, China in April, 2014 and the second subsubclade are named 2.3.4.4 b that is closely related the H5N6 viruses from Lao in Mar, 2014. Molecular characterization revealed that the two subsubclade 2.3.4.4 a and 2.3.4.4 b are different at 2 antigenicity associated amino acide. Conclusion: The number of AI outbreaks caused by H5N6 in Vietnam are increasing recently. This may be resulted from the diversity of H5N6 viruses circulating in Vietnam. Therefore, the new emerging H5N6 viruses need to be monitored continuously and evaluated for vaccine efficacy.

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: A. Viral Agents
Poster Presentation Number: 025 (A)

Comparative Transcriptomics for Assessing of Pathogen Specific Host-Signatures of Infection: Human Astrocytes in Response to Alphavirus Infection

J. L. Jacobs1, K. Kehn-Hall2, D. D. Dinman3, A. G. Baer2, L. Lundberg2, N. Shafagatii2; 1MRIGlobal, Rockville, MD, 2George Mason Univ. (NCBID), Manassas, VA, 3Univ. of Maryland, College Park, MD

Eastern, Western and Venezuelan Equine Encephalitis viruses (EEEV, WEEV, and VEEV) are “New World” alphaviruses carried by arthropods that infect mammalian hosts and are implicated in outbreaks of acute encephalomyelitis in the Americas. Although humans are considered a dead-end host for these viruses, they nonetheless cause severe encephalitis and death, with survivors frequently suffering from lifelong sequela. The severity of infection underscores the need for effective treatments and improved diagnostics against these viruses. Nonetheless, little is known about the
virulence and replication mechanisms of encephalitic alphaviruses, presenting significant obstacles to the development of effective therapeutics. In this preliminary work, we carried out a comparative transcriptomics study of mRNA and microRNA expression using RNA sequencing (RNA-Seq) in human astrocytes in response to infection with EEEV, WEEV, or VEEV. This is the first time the host-response to infection by each of these viruses have been compared side-by-side in a single controlled study. Time course interval RNA-Seq experiments were conducted over a period of 16 hours post infection and demonstrate that the host-response to each virus has unique features both in the timing of the response and the host-pathways involved. In addition, we observed both shared and unique cohorts of mRNAs and microRNAs associated with infection for each of the alphaviruses included in the study, opening the door to the development of differential diagnostics or virus-specific host-associated therapeutic targets. In this presentation, we aim to present our initial results from this study, and provide a framework for similar comparative transcriptomics studies that may be carried out in the future.

---

**Session Type:** Poster Session  
**Session Number:** 016  
**Session Title:** Poster Session 2  
**Poster Presentation Time:** Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:** A. Viral Agents  
**Poster Presentation Number:** 026 (A)

**Autonomous Perfusion Platform to Manipulate Viral Evolution**

**E. J. Fong, C. Huang, J. Peña, S. Hall, M. Borucki, M. Shusteff; Lawrence Livermore Natl. Lab., Livermore, CA, CA**

The continuing threats from viral diseases highlight the need for new tools to study viral interactions with host cells. Traditional flask-based culture methods distort cell-virus dynamics, but due to the complexity and expense of in vivo models bridging technologies are urgently needed. Therefore, we have developed a meso-scale continuous perfusion platform that recapitulates more in vivo dynamics than static culture approaches, while offering further aspects of experimental control. Suspension cells are retained using a label-free size-separation device, while used media and viruses are removed. Additional computer-controlled components provide precise control, which allow the platform to automatically resolve problems and operate autonomously for weeks at a time. Aliquots of the culture are collected at experimenter-specified intervals for near-real-time measurement of cell and viral growth rates. As a capability demonstration, we cultured multiple cell types for over one month, and by adjusting operating parameters have stably maintained cells at two different concentrations. Next, we demonstrate controllable removal of Dengue virus while maintaining a stable cell population. This platform combines automation and robustness with the flexibility to adjust parameters and add components, opening the door to a variety of high impact investigations. Acknowledgements: This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-ABS-XXXXXX

Figure 1: A) Cell concentration is controlled using our platform at either high (dark blue) or low (light blue) cell density, while cell population crashes after 5 days in a static flask (red). B) Virus is controllably removed from the high density cell population over 10 days.
Although ebolavirus infections have documented in Equatorial Africa and Southeastern Asia for forty years, the recent Ebola virus (EBOV) outbreak in Western Africa resulted in an unprecedented number of cases. The international response has been primarily focused on halting the virus
spread; however, future countermeasure approval relies on effective model development, where characterization provides an opportunity for understanding of the pathophysiology of the disease and researchers will be able to better inform clinical teams. Several isolates of the EBOV Makona variant have been tested in non-human primates. Although more genetically similar to each other than other ebolaviruses, these isolates may have been collected at different times during the epidemic, from various geographic locations, or from cases with varied clinical presentation. The impact of these variables on the disease course or selection of a virus stock for further model development is unknown. Here we describe the disease course and preliminary biomedical imaging findings following intramuscular (IM) challenge of rhesus macaques with the C05 isolate. EBOV/Mak-C05 is from the earliest virus lineage in Guinea, prior to the emergence of the virus in Sierra Leone or Liberia, and was isolated from a non-lethal case in a 16-yo female who experienced a spontaneous abortion. In contrast to several reports of delayed disease progression with EBOV/Mak, IM exposure of rhesus macaques to either VeroE6p1 or p3 stock in our laboratory has resulted in lethal disease course of expected progression and with similar median survival time to historic EBOV/Kikwit controls. These studies have confirmed the rhesus EBOV/Mak model for testing of potential therapeutics. Additionally, we have acquired the first MRI and CT images of EBOV-infected macaques as initial steps towards correlating Ebola viral disease features using advanced biomedical imaging technology.

**The Impact of Viral Amplification at the Ebola Virus Glycoprotein Editing Site**


Ebola virus (EBOV) is a negative sense RNA virus that can infect humans with a high case fatality rate. For testing countermeasures that may require approval using the Animal Rule, it is critically important to consider the impact of viral amplification on viral genotype and phenotype. Several groups have focused on expansion of the EBOV editing site following amplification in Vero E6 cells; specifically, an additional U is inserted into a run of 7Us. The consequences of this genetic change are currently unclear, but it is reasonable to consider that it will alter the ratio of sGP to GP1,2. We hypothesized that multiplicity of infection (MOI) influences the rate that genetic changes occur following amplification in culture. Interestingly, amplification at higher MOI resulted in reduced expansion of the editing site. This is important because the Filovirus Animal Non-Clinical Group (FANG) recommended low MOI amplification for filoviruses. We next asked if changing infection-conditions affected stability of the genome editing site: passages were performed with daily media change or increased supernatant volume. Our data suggests daily removal of supernatant slows the expansion of EBOV GP editing site and expansion of the EBOV GP editing site occurs more rapidly when supernatant volume is low. This is an important observation as it may affect how future studies are designed to model EBOV disease and test countermeasures.
infections in humans. Some Central African virus strains show a mortality of up to 10%. In Europe the most prominent poxvirus are cowpoxviruses. Originally known as the virus Edward Jenner used for his pioneering vaccination trials, these viruses are today characterized as the orthopoxviruses with the largest genome of all orthopoxviruses, having a broad host range and a usually mild clinical outcome in humans. However, in animal hosts infections can be fatal and rare severe cases in humans are described. The clinical presentation of cowpoxvirus infections is not unique and particularly in an early stage of infection differentiation from other skin diseases may be impossible. Hence, molecular diagnostics is required. we present a selection of clinical presentations of cowpoxvirus infections displaying the broad spectrum of cowpox infections.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: B. Bacterial Agents

Poster Presentation Number: 030 (B)

ASM Biodefense and Emerging Diseases Research

D. ' Rweis; Naratech Labs (Microbiol. Labs), Amman, Jordan

When advancing the science we need to monitor emerging technologies & safeguarding biological materials with guidance which can be provided regarding its use and safeguarding, without impeding future scientific developments, But we cannot exclude the possibility of hostile misuse.

Background: Biosafety and Biosecurity issues have raised many questions for the need of regulations regarding scientific activities which may open an avenue for the potential abuse and misuse of biological infectious agents in microbial labs. The cusp of biotechnology revolution is an example that reflects the need to provide a framework to ensure a certain degree of Biosecurity measures. It’s important to ensure that the work in our laboratories are conducted in safe and secure place in order to balance between the progress of science and security through learning how to analyze potential gaps in biosecurity and policy problems that may arise in biological Laboratories. Plan: Set up of training the trainer’s sessions for Labs workers & operators in handling, storage and disposal of Bio agent to Obligation initiate according to requirements of the CEN 15793:2011 and CEN 16393:2012, in purpose to gather information on the current level of awareness on Biosecurity and dual use biotechnology. Future plans: Broaden the knowledge regarding bio-safety, bio-security and bio-risk management in the microbiological labs and benefit from US strategies and international authorities and organizations via collection of relevant standards and guidelines. Conclusions: It is universally agreed that any ethically unqualified technicians must be prevent from acquiring biological agents. In order to build a strategy for prevention the following strands must be developed How to apply the Laboratory Biosafety and biosecurity Disseminate the ethics of Biosafety & biosecurity in all laboratories workers. Summary: A mapping of legislation as well as practices and initiatives that currently being adopted to improve the levels of biosafety and biosecurity are addressed. analysis and discussion of the critical issues in field for biosafety, biosecurity and biorisk management in the Countries and its relations with the international regulations.

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: B. Bacterial Agents

Poster Presentation Number: 031 (B)

Medical Surge Capacity in the National Capital Region: Modeling a Pneumonic Plague Bioterror Event

M. DeLuca; Georgetown Univ. Sch. of Med., Washington, DC

This research study focuses on the medical surge capacity of the National Capital Region (NCR) using a hypothetical biological terror attack with Yersinia pestis. It examines the capacity of the NCR to manage a substantial increase in critically ill patients associated with a severe public health
emergency. Results obtained from this research can raise awareness regarding limitations of preparedness in the NCR and highlight areas for improvement and investment. The hypothesis was that, despite significant investment in bioterror preparedness and response capabilities, medical facilities in the NCR remain insufficient to effectively provide care for a large scale bioterror event. This research estimated the number of acute care hospital beds in the NCR relative to a hypothetical bioterror event in the NCR Metro system. Medical care demand was estimated using Washington Metropolitan Area Transit Authority ridership data, publicly available data on disease attack rate, infectious dose, reproductive number, incubation period, and clinical severity. Total exposed and infected were calculated. Available beds were calculated using the following sources: DC Hospital Association utilization and occupancy rate data; Maryland Healthcare Commission, Virginia Health Information, Virginia Department of Health, and individual hospital websites. Deficits in available beds during first 6 days of disease spread (primary and secondary infections) were calculated. Results demonstrate a significant shortfall in available acute care beds in the region for a large biological event. Recommendations were made to improve medical surge capacity in NCR using Federal and local resources, including use of health care coalitions and non-traditional care facilities.

<table>
<thead>
<tr>
<th>Session Type:</th>
<th>Poster Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session Number:</td>
<td>016</td>
</tr>
<tr>
<td>Session Title:</td>
<td>Poster Session 2</td>
</tr>
<tr>
<td>Poster Presentation Time:</td>
<td>Tuesday, February 9, 2016, 5:45 pm - 7:00 pm</td>
</tr>
<tr>
<td>Topic:</td>
<td>B. Bacterial Agents</td>
</tr>
<tr>
<td>Poster Presentation Number:</td>
<td>032 (B)</td>
</tr>
</tbody>
</table>

**Site-specific Tyrosine Recombinases as Effective Tools for Genome Engineering in Bacillus anthracis**

**A. P. Pomerantsev, M. Chahoud, S. H. Leppla; NIH/NIAID, Bethesda, MD**

Tyrosine recombinases are polynucleotidyltransferases that catalyze cutting and joining reactions between short specific DNA sequences. These enzymes perform three types of reactions: integration and excision of DNA segments, inversion of DNA segments, and maintenance of the monomeric state of circular DNA molecules. We developed three systems for performing genetic modifications in Bacillus anthracis that use tyrosine recombinases and their cognate target sequences, namely Escherichia coli bacteriophage P1Cre-loxP, Saccharomyces cerevisiae Flp-FRT, and a newly discovered IntXO-PSL system from B. anthracis plasmid pXO1. The first two systems enabled the identification of the pXO1 regions responsible for replication and maintenance of the plasmid. The third system, IntXO-PSL, which presumably prevents intracellular pXO1 dimer catastrophe, was shown to be an important component of a newly-identified, second pXO1 maintenance system. All three tyrosine recombinase systems were used for creation of a B. anthracis sporulation-deficient, plasmid-free strain deleted for ten proteases which had been identified by proteomic analysis as being present in the B. anthracis secretome. This strain was used successfully for production of many different recombinant proteins, including several that are candidates for inclusion in improved anthrax vaccines. The genetic tools developed for DNA manipulation in B. anthracis were also used for construction of strains having chromosomal insertions of 1, 2, or 3 adjacent atxA genes. AtxA is a B. anthracis global transcriptional regulator required for the response of B. anthracis virulence factor genes to bicarbonate. We found a positive correlation between atxA copy number and expression level of several genes placed under control of the anthrax toxin pagApromoter when strains were grown in a carbon dioxide atmosphere. These results demonstrate that tyrosine recombinases provide an approach for genome editing that may be preferred in some cases to alternative methods (e.g., CRISPR/Cas systems).
Comparative Proteomic Studies of Yersinia pestis Strains Isolated from Natural Foci in the Republic of Georgia

R. Solomonia\textsuperscript{1}, M. Nozadze\textsuperscript{1}, E. Zhgenti\textsuperscript{3}, T. Kiguradze\textsuperscript{3}, G. Chanturia\textsuperscript{2}, G. Babuadze\textsuperscript{2}, M. Kekelidze\textsuperscript{2}, L. Bakanidze\textsuperscript{2}, T. Shutkova\textsuperscript{2}, P. Imnadze\textsuperscript{2}, S. Francesconi\textsuperscript{4}, R. Obiso\textsuperscript{5}; 1Ilia State Univ., Tbilisi, Georgia, 2Natl. Ctr. for Disease Control and Publ. Hlth., Tbilisi, Georgia, 3I. Beritashvili Ctr. for Experimental Biomedicine, Tbilisi, Georgia, 4Naval Med. Res. Ctr., Frederick, MD, 5Attimo Res. and Dev., Blacksburg, VA

Yersinia pestis, the causative agent of plague, is a highly virulent bacterium responsible for millions of human deaths throughout history. Dozens of Y. pestis strains have been isolated from two natural plague foci in the Republic of Georgia. Analyses indicate that there are genetic differences between these strains, but it is not known if these differences are also reflected in protein expression. Based on neighbor-joining tree genetic analysis and geographical loci of strain origin we have chosen four strains of Y. pestis (1390, 1853, 2944, and 8787) from the National Center for Disease Control and Public Health collection for proteomic studies. Changes in proteomes were analyzed by two-dimensional gel electrophoresis and mass spectrometry. Select Y. pestis strains were grown under 4 different physiological conditions: 1) 28C without Ca\textsuperscript{2+}; 2) 28C with Ca\textsuperscript{2+}; 3) 37C without Ca\textsuperscript{2+}; and 4) 37C with Ca\textsuperscript{2+} and their proteomes were compared. Candidate proteins were identified and the differences in expression of F1 antigen, tellurium-resistance protein, and outer membrane protein C, porin were validated by Western blotting experiments. The in vitro cytotoxicity activity of these strains was measured by the determination of induced caspase-3 activity in macrophage cell cultures. The results obtained indicate that protein expression and cytotoxic activities differ significantly among these studied strains; these differences could contribute to variations in essential physiological functions in these strains as well as virulence attributes.

Molecular Genotyping of Bacillus anthracis Isolates from Kars province of Turkey

E. Khmaladze\textsuperscript{1}, F. Buyuk\textsuperscript{2}, E. Celibi\textsuperscript{2}, O. Celebi\textsuperscript{2}, A. Saglam\textsuperscript{2}, M. Sahin\textsuperscript{2}, W. Su\textsuperscript{3}, W. Su\textsuperscript{3}, M. Nikolich\textsuperscript{3}, L. Baillie\textsuperscript{4}, P. Imnadze\textsuperscript{1}, A. Kotorashvili\textsuperscript{1}; 1Natl Ctr for Disease Control/Publ. Hlth, Tbilisi, Georgia, 2Faculty of Vet. Med., Dept. of Microbiol., Univ. of Kafkas, Kars, Turkey, 3Walter Reed Army Inst. of Res., Silver Spring, MD, 4Cardiff Sch. of Pharmacy and Pharmaceutical Sci., Cardiff Univ., Cardiff, United Kingdom

Bacillus anthracis causes the acute fatal disease anthrax and is a proven biological weapon. Anthrax is distributed worldwide and is an endemic disease in Kars, a province of Turkey. The aim of the present study was to learn about the genetic diversity of B. anthracis strains from Kars. Two methods, Single Nucleotide Polymorphism (SNP) subtyping and 25 marker Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA 25) were applied for molecular typing of B. anthracis strains isolated in Kars. 30 isolates of different origin and source (animal, soil, human) were genetically characterized in this study. Canonical SNP typing revealed that Turkish strains belonged to A.Br.003 together with Australian 94 lineage. Based on recent evidence, we decided to screen Turkish strains across previously developed Georgian SNP panel (Khmaladze et. al 2014). Results showed little diversity among the Kars strains within Georgian SNP lineage: All 30 strains grouped with A.Br.003 together with Australian 94 lineage. Based on Ba-MLVA-25 genotyping, all 30 B. anthracis strains are divided into two clusters: A and B. Cluster A is more diverse than Cluster B. It might be due to the relatively low sample size and location. These findings suggest that Georgian and Turkish B. anthracis isolates are genetically related what could be explained by close geographic location of both countries. With these analysis techniques established, the response and detection capabilities within the region of Kars will be better able to trace new outbreaks, both natural and man-made.
Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: B. Bacterial Agents
Poster Presentation Number: 035 (B)

**Genetic Diversity of Animal Brucella Isolates in Turkey**

K. Sidamonidze¹, E. Zhgenti¹, F. Buyuk², M. Sahin³, E. Celik³, A. Gulmez³, W. Su⁴, W. Su⁴, L. Baillie⁵, M. Nikolich⁴, P. Imnadze¹, A. Kotorashvili¹; ¹Natl. Ctr. for Disease Control, Tbilisi, Georgia, ²Faculty of Vet. Med., Dept. of Microbiol., Univ. of Kafkas, Kars, Turkey, ³Faculty of Vet. Med., Dept. of Microbiol., Univ. of Kafkas, Kars, Turkey, ⁴Walter Reed Army Inst. of Res., Silver Spring, MD, ⁵Cardiff Sch. of Pharmacy and Pharmaceutical Sci., Cardiff Univ., Cardiff, United Kingdom

Brucellosis is a highly contagious zoonotic disease caused by the genus Brucella that remains endemic in Georgia and Turkey, causing substantial human morbidity and significant agricultural economic loss. In addition, Brucella species are considered potential biological threat agents. Genetic characterization of Brucella isolates by molecular typing methods provides a description of strain diversity, detection of clonal groups, evidence for trace-back to sources of infection and discrimination of naturally occurring outbreaks from a bioterrorist event. Recent studies have demonstrated that multiple-locus variable-number tandem-repeat analysis (MLVA) is a high resolution genetic subtyping tool that can provide valuable information for epidemiological investigations. In the present study, we have studied the genetic variability of 29 cattle and sheep isolates of Brucella isolated in Turkey that were preliminary identified as B. melitensis or B. abortus. Genotypes were revealed by a MLVA approach, using mini satellite markers with discriminatory capabilities based on Huynh et al. 2008. Data obtained were analyzed for construction of a phylogenic tree using BioNumerics Software (Applied Maths). All 29 Brucella strains from Turkey were divided into two clusters according to the MLVA genotyping. One cluster was confirmed as B. abortus based on Bruce-Ladder PCR, another cluster as B. melitensis. The B. abortus cluster is more diverse than the B. melitensis cluster. The B. melitensis cluster was comprised of isolates from four animals from the same geographic location with the same genetic subtype. This suggests a common origin of infection for this group of isolates.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: B. Bacterial Agents
Poster Presentation Number: 036 (B)

**Detection of Clostridial Epsilon Toxin in Biological Matrices by Enzyme Immunoassay and Immunochromatography**

C. Feraudet-Tarisse¹, M. Popoff², C. Mazuet³, C. Lacroix¹, O. Andreoletti³, P. Lamourette¹, C. Creminon¹, H. Volland¹, S. Simon¹; ¹CEA, Gif sur Yvette, France, ²Inst. Pasteur, Paris, France, ³UMR INRA/ENVT, Toulouse, France

Epsilon toxin (ETX) is one of the 4 major toxins of Clostridium perfringens. It is the third most potent clostridial toxin after botulinum and tetanus toxins and is thus considered as a potential biological weapon classified as category B by the Centers for Disease Control and Prevention (CDC). In case of a bioterrorist attack, a rapid, sensitive and specific detection method will be required to monitor food and water contamination by this toxin, as well as a simple human diagnostic test. Very few publications in the literature specifically document the development of detection tests for ETX in food or water and diagnostic methods with human body fluids. We have developed highly-sensitive ETX detection tests (sandwich enzyme immunoassay and immunochromatographic test) reaching a 10 pg/ml (35 pM) sensitivity in buffer. These two tests are also applicable to detect this toxin in different matrices for different purposes: milk and tap water for biological threat detection, serum, stool and intestinal content for human or veterinary diagnostics. Detection limits in these complex matrices are at least 20-fold better than those described in literature (around 1 to 5 ng/ml), reaching 10 to 100 pg/ml using the enzyme immunoassay (performed in 18 h) and 40 to 400 pg/ml using the immunochromatographic test (with results in 20 mn).
Evaluation of Bacillus anthracis UT500 in a Rabbit Model of Infection


Bacillus anthracis UT500, a strain derived by phage transduction of pXO2 from B. anthracis Pasteur 6602 into the Sterne-like strain 7702 and which is phenotypically similar to the wild-type Ames strain, was evaluated in a New Zealand White rabbit subcutaneous model of infection. The median lethal dose (LD50) of UT500 was not statistically different from the Ames strain; however, the mean time between spore challenge and death and between development of a significant increase in body temperature (SIBT) and death were significantly longer in animals infected with UT500. Additionally, blood and splenic bacterial burdens as well as terminal serum protective antigen levels were significantly lower in animals that succumbed to infection with UT500.

Whole genome sequencing single nucleotide polymorphism (SNP) analysis identified several differences between UT500 and the Ames Ancestor published sequence but did not reveal an obvious genetic defect for which the observed disease course attenuation could be attributed. Lastly, it was observed that female rabbits were significantly more susceptible to infection with UT500 compared with male rabbits. These data highlight the differential virulence characteristics of diverse B. anthracis strains that may be observed based on the anthrax model being utilized.

Molecular Detection of Virulence and Resistant Marker on Antibiotic Resistant Staphylococci Using Quantitative Polymerase Chain Reaction (qPCR)


The goal of this study is to detect the virulence sea gene and resistant marker mecA in methicillin-resistant staphylococci and coagulase-negative staphylococci (CoNS). Standard microbiological procedures were employed in the course of this study. Four hundred (400) clinical wound and burn samples were collected, organisms were isolated microbiological standard procedures. The isolates were characterized as Staphylococcus aureus and CoNS. Oxacillin disk susceptibility test with commercially available antibiotics were performed to ascertain methicillin resistance. Quantification of mecA and enterotoxin (sea) genes on the antibiotic resistant staphylococci were performed using duplex quantitative polymerase chain reaction (qPCR) followed by acridine orange curing treatment and agarose gel electrophoresis. The results obtained from this study showed a prevalence rate of 23% (92 of 400) staphylococci while the isolation rate of the characterized staphylococci revealed 80 (87 %) S. aureus and 12 (13 %) CoNS. Oxacillin resistant strains were 21 MRSA and 5 MRCoNS. The multi-drug resistance patterns were: Ampiclox-38(72 %), fluoroquinolones (67-100 %) and aminoglycosides (38-67 %). The acridine orange curing analysis showed that some isolates (19-66 %) were cured (plasmid-borne) while those not cured (chromosomal-borne) were between 34 and 100% among the S. aureus and CoNS strains. The sea genes detected from the antibiotic resistant staphylococci range between 0 and 13551.84 nmoles while the mecA genes range between 0 and 2601.76 nmoles. Amplicon
sizes of mecA and sea genes were detected on agarose gel electrophoresis. This study clearly reveals the isolation of multi-drug resistant methicillin-resistant S. aureus and CoNS with the molecular detection of mecA and sea genes especially among the emerging coagulase negative staphylococci, calling for urgent clinical and pharmaceutical attention.
the measured spore titers as determined by serial dilution and plating on agar media. A notable observation from the spore phenotypic
coloration experiments was the presence of capsular material associated with NBY-produced spores. It was observed following the transfer
of NBY agar plates from 37°C to room temperature that the confluent growth on the plate took on a mucoid appearance. Staining with India ink
and a fluorescein isothiocyanate (FITC) labeled monoclonal antibody confirmed this as poly-gamma-glutamate capsule. Expression of the capsule
under conditions lacking bicarbonate and CO2 is an interesting finding and points to the complex nature of virulence factor expression and
regulation in B. anthracis. Together the data suggest that choice of sporulation medium can influence multiple B. anthracis spore characteristics
including germination rates which can subsequently affect spore titers and resulting calculated LD50 values.

Session Type:  Poster Session
Session Number:  003
Session Title:  Poster Session 1
Poster Presentation Time:  Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic:  B. Bacterial Agents
Poster Presentation Number:  041 (B)

A Comparative Study for Identifying the Presence of Ricin Toxin in Environmental Matrices

M. Perry, D. Centurioni, C. Egan; New York State Dept. of Hlth., Albany, NY

Ricin (RCA-II or RCA60) is a toxic, naturally-occurring glycoprotein found in the seeds of the castor plant (Ricinus communis). Due to the relative
ease of obtaining ricin through castor bean extracts, its ease of dissemination, and its ability to quickly and irreversibly damage cells, ricin is
considered a bioterrorism agent on several threat determinant lists. The current ricin screen approved by the Centers for Disease Control and
Prevention (CDC) for the Laboratory Response Network (LRN) labs is the Time-Resolved Fluorescence (TRF) assay, a type of non-competitive
“sandwich” ELISA. The specific assay employed by the LRN is the Dissociation Enhanced Lanthanide FluoroImmunoAssay (DELFIA). This assay
requires a minimum of 4 hours from start to finish, with additional time needed to analyze results. It involves the use of specialized equipment and
must be performed in a laboratory. However, non-LRN laboratories do not have access to this method and must utilized an alternative means of
detection. Two alternative screening methods capable of rapidly testing for ricin toxin include the Tetracore lateral flow assay (LFA) and the
Luminex MAGPIX with the Biothreat Toxin Panel kit both. The limit of detection (LOD) for both methods was evaluated by spiking environmental
screening buffers with ricin toxin A chain from 0.01 - 100 ng/mL. In addition, twelve environmental powders were spiked with ricin toxin to identify
any potential inhibitors that could cause false negative results, assess the limit of saturation, and verify the impact a powder would have on toxin
detection. It was determined that the LOD for ricin using the LFA varied from 5-10 ng/mL where the LOD using the MAGPIX instrument consistently
detected toxin levels at 0.1-1 ng/mL. Surprisingly, when both flour and a spice seasoning were tested with the LFA, a false positive was observed
with 42% of the tests. Although both methods are relatively simple and quick to perform, there were some great drawbacks including cross-
reactivity, sensitivity of matrices, results interpretation, and instrument consistency that laboratories will have to take into account before adopting
one of these methods.

Prevalence and Characteristics of Clostridium difficile, C. perfringens, and VRE in Chicken Feces Collected from a Large Commercial Farm

M. Alam1, A. Apu1, A. Anu1, J. K. McPherson1, J. Miranda1, L. Q. Le1, C. Cuartero-Crawford1, D. Biswas2, K. W. Garey1; 1Univ. of Houston Coll. of
Pharmacy, Houston, TX, 2Univ. of Maryland-Coll. Park, College Park, MD
**Background:** Commercial chicken farms may be a source for spore-forming bacteria such as C. difficile, and C. perfringens which are capable of persisting on meat and other raw foods contaminated with fecal material of chickens. The objectives of the study are to isolate and characterize C. difficile, C. perfringens and vancomycin-resistant Enterococcus (VRE) from chicken feces of a large commercial farm. **Methods:** We collected 320 chicken fecal samples from a chicken farm and analyzed the samples using anaerobic culture and molecular methods. First we enriched approximately 2g of fecal material in 10 ml of brain heart infusion broth and incubated at 37°C in an anaerobic chamber for 48h and then 1ml of the culture broth was centrifuged, and treated the cell pellet with ethanol. Ethanol-treated culture were then plated on to CCFA and C. perfringens agar plates and incubated 48h anaerobically. Suspected colonies were then identified and characterized by molecular methods. VRE isolates were recovered from enrichment broth culture using vancomycin added mEnterococcus agar. **Results:** Out of a total of 320 chicken fecal samples, C. perfringens was isolated from 201 samples (62.8%) and C. difficile from 157 samples (49.0%). In preliminary analyses, VRE was isolated from 52 of 160 tested samples (32.5%). Eight different ribotypes (001, 002, 014-020, UM8, UM10, UM12, UM18, UM26) of C. difficile were found among 66 strains screened. Toxigenic C. difficile (tcdA and/ tcdB genes) were present in 13.1% (42/320) samples (ribotypes 001, 002, 014-020, UM8, UM12, UM26). Alfa (plc) and enterotoxin (cpe) genes were detected in 54.9 (55/104) and 7.7% (8/104) of isolates of C. perfringens, respectively. **Conclusions:** Overall, chicken fecal samples were frequently positive for the potential human pathogens, C. difficile,C. perfringens, and VRE. Further studies are needed to prevent and control chicken fecal sources of human pathogens in and around our community environs.

---

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** B. Bacterial Agents  
**Poster Presentation Number:** 043 (B)

**Automation of Antimicrobial Activity Screening**

**S. P. Forry, M. C. Madonna, D. López-Pérez, N. J. Lin, M. D. Pasco; NIST, Gaithersburg, MD**

Manual and automated methods were compared for routine screening of compounds for antimicrobial activity. Automation generally accelerated assays and required less user intervention while producing comparable results. Automated protocols were validated for planktonic, biofilm, and agar cultures of the oral microbe Streptococcus mutans that is associated with tooth decay. Toxicity assays for the antimicrobial compound cetylpyridinium chloride (CPC) were validated against planktonic, biofilm forming, and 24 h biofilm culture conditions, and several commonly reported toxicity/antimicrobial activity measures were evaluated: the 50% inhibitory concentration (IC50), the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC). Using automated methods, three halide salts of cetylpyridinium (CPC, CPB, CPI) were rapidly screened with no detectable effect of the counter ion on antimicrobial activity. This automated assay was completed in 6h of machine time and would have required ≈15h staff time for manual operations.
In many bacteria, the ability to modulate biofilm production relies on specific signaling molecules, either self-produced or made by neighboring microbes within the ecological niche. The microbial ecology of the gram-negative Francisella is of great interest, but is poorly understood. We analyzed the potential interspecies signaling effect of Burkholderia Diffusible Signal Factor (BDSF) on F. novicida, a model organism for F. tularensis and demonstrated that BDSF both inhibits formation and causes dispersion of Francisella biofilm. First, specificity was demonstrated for the cis- vs. trans- form of BDSF. Using RNA-seq, qRT-PCR, and activity assays we found that BDSF significantly altered the expression of many F. novicida genes, including genes involved in biofilm formation, such as chitinases (upregulated ~20 fold), and significantly downregulated expression of genes in the Francisella Pathogenicity island, including virulence factors. We and others have previously shown a significant role for chitinase in F. novicida biofilm production. Using a chitinase inhibitor, the anti-biofilm activity of BDSF was also shown to be chitinase dependent. In addition, BDSF caused an increase in RelA expression and increased levels of (p)ppGpp, leading to decreased biofilm production. These results, combined with confocal microscopy observations, suggest that exposure of F. novicida biofilm to BDSF causes biofilm dispersal. Furthermore, BDSF upregulated (>85 fold) the expression of genes involved in iron acquisition, increasing siderophore production. Finally, in a G. mellonella infection model, treatment with BDSF significantly prolonged survival of those infected. Thus, this study provides the first evidence for the role and mechanism of DSF-signaling in...
the genus Francisella and suggests interspecies signaling between Francisella and other bacteria. Overall, this study suggests that in response to the interspecies DSF signal, F. novicida alters its gene expression and its biofilm production, which may allow it to adapt to the presence of other species of bacteria in its environment.

---

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: B. Bacterial Agents

Poster Presentation Number: 045 (B)

Macrophage Invasion Assay for Virulence Study of F. tularensis From Georgia


Francisella tularensis, the causative agent of tularemia was detected in Georgia seven decades ago. Since that time it has been systematically isolated from rodents and ectoparasites during field work in different regions of the country. More than one hundred strains of F. tularensis became part of an archival collection of NCDC. Even though genotyping of these strains was already performed in the scope of different projects, the virulence of the strains has never been compared. One of the tasks of the DTRA funded project “Epidemiology and Ecology of Tularemia in Georgia” is the implementation of methodology for virulence without using a mouse model. The invasion of J774 macrophage cells with three F. tularensis strains chosen from different genotypes and Live Vaccine Strain (LVS) as a control was performed according to approved SOP at the BSL-3 facility of the NCDC-Lugar Center. Macrophages were lysed and plated on agar media after 0, 24, 48, and 72 hours of incubations. F. tularensis colonies were counted and the number of bacterial doublings between two time points were calculated using the formula ((log of 24 hour counts - log 0 hour counts) x 3.32). The increases in colony numbers were observed at different incubation time points. Wild strains showed higher ability to invade macrophages and grow intracellularly in comparison to LVS. The methodology has been standardized and will be applied to other F. tularensis strains from NCDC archive to compare the virulence of the genetically diverse strains isolated from different regions and various sources.

---

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: B. Bacterial Agents

Poster Presentation Number: 046 (B)

Deletion of MFS Family Transporters Alters the Intracellular Lifecycle of Francisella tularensis

P. M. Balzano, E. M. Barry; Univ. of Maryland Baltimore, Baltimore, MD

Francisella tularensis (Ft) is a Gram negative facultative intracellular coccobacillus, and is the causative agent of the zoonosis tularemia. It is a bioterror concern due to past weaponization efforts, the high level of virulence, and the small inoculum required for infection by the aerosol route. Currently there is no licensed vaccine, but renewed vaccine development efforts are a priority for this select agent pathogen. The targeted deletion of selected genes in Ft to produce candidate live vaccine strains is a strategy we have pursued. We produced 2 vaccine candidates harboring single deletions of major facilitator superfamily (MFS) transporters, fptG, and fptB, an isoleucine transporter, in the Type A strain Schu S4. Based on the attenuating capacity of these deletions in the LVS background, we hypothesized elimination of either transporter, would alter the intracellular replication rate of the virulent Type A strain. Utilizing gentamicin protection assays that eliminate cell to cell spread in human THP-1 macrophage-like cells we have identified modified intracellular replication patterns for both mutants. Intracellular bacterial counts at 24 hours post infection yielded up to ten times more bacteria than wild type for both mutants. Further invasion assays allowing cell to cell spread indicated that
SchuS4ΔfptG replicated at the same rate as wildtype, but appeared delayed in exiting the host cell compared to wildtype. SchuS4ΔfptB likewise appeared delayed in exiting the host cell, but also exhibited a significant growth defect, doubling 1 to 1.5 fewer times in the first 24 hours. SchuS4ΔfptG and SchuS4ΔfptB are the first MFS transporter knockouts to be created in the virulent Schu S4 strain. The mutant’s phenotype of delayed cellular escape is novel and suggests a more active role on the part of the bacteria beyond simply overwhelming the host with high levels of replication. These strains serve as valuable tools to study the intracellular lifecycle of Ft and may be candidates for a live attenuated vaccine.

A Simple Method for Anaerobic Culture of Biodefense Bacteria

H. C. Gelhaus, Jr., M. Krajewski, R. Kelly, C. Nevins; MRIGlobal, Kansas City, MO

During acute infection, bacteria often forms abscesses throughout the body, encountering host microenvironments which lack oxygen and can persist in a hypoxic physiological state. Previous work has shown that four bacteria of biodefense concern (Bacillus anthracis, Burkholderia pseudomallei, Brucella melitensis, and Yersinia pestis) are facultative anaerobes. Furthermore, B. pseudomallei isolated from putatively hypoxic microenvironments are tolerant of many antibiotics used during melioidosis treatment. As such, we believe that antimicrobial testing under anaerobic/hypoxic conditions is valuable. We grew B. anthracis (Ames), Burkholderia pseudomallei (1026b), Brucella melitensis (16M), and Y. pestis (CO92) in sealed Hungate tubes with the assumption that oxygen will be depleted over time to create an anaerobic environment. Cultures were grown aerobically for one day, and subcultured into sealed Hungate tubes in order to facilitate oxygen depletion. Sodium nitrate was added to anaerobic cultures after 48 hours to provide an electron acceptor for anaerobic respiration, and cultures were incubated for additional (2-7) days. Culture samples were taken to determine turbidity (optical density) and viable cell counts after 1, 2, and 7 days of anaerobic conditions. Day 7 tubes were also compared to a control tube that did not receive the additional nitrogen source. After 2 days of anaerobic growth, B. pseudomallei and B. melitensis showed increased cell counts by approximately 1 log. Furthermore, the cultures incubated of 7 days without the nitrate electron acceptor showed no appreciable growth. This suggests that the bacteria are proliferating under anaerobic/hypoxic conditions. We found B. pseudomallei and B. melitensis cultures proliferated under our simple anaerobic conditions. Inhibition of this growth in the presence of increasing concentrations of antibiotics should allow for the determination of minimum inhibitory concentrations under anaerobic conditions. Our anaerobic model should be an excellent way to test antibiotic resistance under conditions found within host organisms.

Descriptive Analysis of the 2014 Cholera outbreak in Kaduna State, Nigeria

I. B. Sule; Nigeria Field Epidemiology and Lab. Training Program, Abuja, Nigeria

Background: Cholera is endemic in Africa with recurrent outbreaks occurring frequently. Africa accounts for 65% of global Cholera deaths in 2013. This study analyses the 2014 cholera outbreak in Kaduna state to determine its magnitude, pattern and trend. Method: We conducted a retrospective analysis of the cholera surveillance and response records. We described the outbreaks in person, place and time and calculated the attack rates (AR), case fatality rates (CFR), and age specific case fatality rates (ASCFR) from all cases. Results: A total of 1,554 cases and 54 deaths...
(CFR 3.47%) were recorded. 55.1% females and 44.9% males. Median age for all cases was 15 years, with age range of 0.04 - 90 years. The ASCFR highest among elderly and under 5yrs (Table 1). Cholera spread was observed to occur across contiguous LGAs. Geographical clustering appear to be in the central and northern senatorial districts of the state. The epidemiologic curve (Fig. 3) shows two peaks in the 10th and 20th epidemic weeks, with highest number of cases (384) seen in the 20th epidemic week (averaging more than 18 cases per day). Conclusion: This study highlights a cholera outbreak that gradually increases in magnitude and lingered through the first half of 2014. With a high CFR (3.47%), which is far above the World Health Organization (WHO) acceptable upper level of 1%. It also highlights poor response to outbreaks and neglect of the vulnerable age groups (elderly and under 5yrs).

Table 1: Age Specific Case-Fatality Rate (ASCFR) of Cholera cases, Kaduna 2014

<table>
<thead>
<tr>
<th>Age Groups (yrs)</th>
<th>ASCFR (%) Male</th>
<th>ASCFR (%) Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>4.47</td>
<td>4.08</td>
</tr>
<tr>
<td>5-19</td>
<td>3.04</td>
<td>0.31</td>
</tr>
<tr>
<td>20-39</td>
<td>6.13</td>
<td>1.14</td>
</tr>
<tr>
<td>40-59</td>
<td>12.50</td>
<td>3.45</td>
</tr>
<tr>
<td>&gt;=60</td>
<td>30.47</td>
<td>5.88</td>
</tr>
</tbody>
</table>

Quantitation of Residual Fatty Acids in Forensic Microbiological Samples Using Direct Analysis in Real Time Mass Spectrometry (DART-MS)

E. M. Childrey, C. E. Stanciu, C. J. Ehrhardt; Virginia Commonwealth Univ., Richmond, VA

The goal of this research was to develop a rapid, quantitative assay for determining the structure and relative abundance of residual fatty acids from forensically-relevant microbiological samples using Direct Analysis in Real Time Mass Spectrometry (DART-MS). Although cellular fatty acids have been used to characterize the taxonomy and growth conditions for an unknown organism, the analytical sensitivity of ambient mass spectrometry techniques and their compatibility with standard forensic collection practices has not been explored. To address this, we developed a fatty acid quantitation assay for Bacillus cereus spores using pentadecanoic acid to analyze membrane-derived, as well as free fatty acids found in different types of forensic samples. These included liquid suspensions of whole cells, dried spore material, spent medium and the supernatant fraction from water wash purification steps of spores. DART-MS profiles of Bc14579 and BcT-strain spores showed five distinct fatty acid biomarkers: 213 m/z, 227 m/z, 241 m/z, 267 m/z and 269 m/z, which correspond to 13:0/13:0iso, 14:0/14:0iso, 15:0/15:0iso, 17:1 w5c, and 17:0/17:0 iso structures, respectively. The limit of detection for the DART-MS method was ~1x10^-5 μg of fatty acid, equivalent to ~100 spore cells. Importantly, the biomarkers from whole cell suspensions were also observed in the culture medium after the spore cells had been removed, as well...
as in the supernatant of subsequent water washing purification steps. Concentrations of fatty acids ranged between 0.002 μg/mL and 0.2 μg/mL for supernatant samples, and averaged at 0.02 μg/mL for spent medium samples. This suggests that Bacillus fatty acids are released into the culture medium and/or the water wash fraction during spore production and persist after the cells have been harvested. This has the potential to enhance forensic collection methods for microbiological samples since residual fatty acids may be used to identify surfaces or locations where biothreat agents were grown, even when cells are not present.

---

Session Type: Poster Session  
Session Number: 016  
Session Title: Poster Session 2  
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
Topic: B. Bacterial Agents  
Poster Presentation Number: 050 (B)

Investigation of Six Fatal Cases of Melioidosis in Yap, 2013-2015  


Since 2013, six cases of fatal melioidosis have been reported on the island of Yap, part of the Federated States of Micronesia. These are the first reported cases in Yap, which raises concern about whether Burkholderia pseudomallei, the bacterium that causes melioidosis, has been previously undetected or may have been recently introduced. Multilocus Sequence Typing (MLST) yielded ST1045 which appears unique to Yap. Whole genome sequencing and multilocus variable number tandem repeat analysis of isolates from five of the patients indicates that the isolates are related, but does not necessarily indicate a point source of inoculation. An investigation into two of the cases looked at a small serosurvey of 34 close contacts and found two individuals with titers above 1:40 by the indirect hemagglutination assay (IHA). Limited environmental sampling (n=39) associated with these two cases failed to detect B. pseudomallei. Testing of a larger historical panel of 500 sera from Yap is in progress and will be presented. Thus far, 144 sera has been tested and demonstrated 12.5% seropositivity. The emergence of this disease paired with the 100% fatality rate in culture confirmed cases in Yap stresses the need for further environmental investigation, heightened clinical awareness and broader testing capacity.

---

Session Type: Poster Session  
Session Number: 003  
Session Title: Poster Session 1  
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm  
Topic: B. Bacterial Agents  
Poster Presentation Number: 051 (B)

American Alligator (Alligator mississippiensis) Derived Novel Peptide Apo6 is Antibacterial Against Francisella  

K. H. Gupta, M. L. van Hoek, B. M. Bishop; George Mason Univ., Manassas, VA  

Francisella tularensis is an important pathogen and is a Category A Select Agent in the USA. We have recently identified novel cationic antimicrobial peptides Apo5 and Apo6 from serum of American alligator (Alligator mississippiensis) and showed antibacterial activity against Gram-positive and/or Gram-negative bacteria. These two native peptides are nested c-terminal fragments of alligator Apolipoprotein C1. Apo5 and Apo6 were able to kill F. tularensis novicida in low salt conditions with EC50 of 5.77 and 2.94 μg/mL and EC50 values of 5.56 and 3.06 μg/mL against F. tularensis LVS strain respectively. Peptides are non-cytotoxic against A549 lung epithelial cells at 330 μg/mL and were able to depolarize and permeabilize bacterial cytoplasmic membranes demonstrating a direct mechanism of antibacterial action against rancisella. Apo6 aggregates DNA at a high concentration. Apo6 is unable to bind to E. coli LPS, however the peptide bound efficiently to Francisella tularensis LVS LPS, suggesting that Apo6 binds to highly anionic outer membrane of Francisella and then disrupt the membrane. When observed by scanning electron microscopy,
the surfaces of the treated Francisella bacteria underwent obvious morphological changes compared with the untreated controls, indicating antimicrobial peptide Apo6 exerts its action by disrupting membranes of this microorganism. When the peptides were exogenously applied to F. novicida-infected A549 alveolar epithelial cells, Apo5 and Apo6 were able to significantly improve cell survival (55 and 40% survival), compared to LL-37 (55% survival) with 30 µM of peptide. G. mellonella was used as an alternative in vivo model of Francisella infection. Apo6 was able to prolong the survival of the Francisella-infected waxworm larvae. Together, these data demonstrate the potential usefulness of Apo6 against the bacterial biodefense pathogen Francisella.

---

**Session Type:** Poster Session  
**Session Number:** 016  
**Session Title:** Poster Session 2  
**Poster Presentation Time:** Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:** B. Bacterial Agents  
**Poster Presentation Number:** 052 (B)

### Mycobacterium tuberculosis drug Resistance is Increasing in Pakistan

**R. Khan, S. Khan; Dow Univ. of Hlth.Sci., Karachi, Pakistan**

Introduction: Tuberculosis is the most life threatening air born bacterial infection. According to the WHO about 2 billion people worldwide have been infected by Mycobacterium tuberculosis complex. PCR is recognized as rapid, specific and sensitive diagnostic technique as compared to the conventional methods. TB can be controlled through early diagnosis followed by effective treatment. Drugs Rifampicin and Isoniazid are commonly used for treatment, but their excessive use has led to the emergence of mutations in the genome of MTB. **Methods:** Total 380 pulmonary and extra pulmonary specimens were collected from different regions of Sindh, Pakistan. Samples were decontaminated using NALC-NaOH protocol followed by DNA extraction. Amplification of extracted MTB DNA was done by real time PCR using primers and fluorescent probes specific for the MTB complex. To check the drug resistant strains, Hybridization of amplified products of positive samples were carried out by MTBDRplus assay. Results: The overall prevalence of MTB infection in this study population was 11%. Prevalence of MTB infection among females and males was 10.14% and 12.13% respectively. Highest positive ratio among different clinical specimens was determined in pus (52%) whereas age group 1-20 years was found most susceptible (13.5%). Among positive samples 33.3% were resistant to Rifampicin and 23.3% were resistant to Isoniazid and 6.6% samples were resistant to both the drugs. **Conclusions:** Real-time assay is rapid diagnostic tool, it can be applied to a wide range of clinical specimens including pus which might show negative AFB smear specially in developing countries like Pakistan where the false negative reporting playing crucial role in the spread of TB. In Pakistan prevalence and drug resistance of TB is on rise due to many socio economical and other issues, determination of drug resistance is important for effective treatment particularly in our region where MDR and XDR TB are not very uncommon.

---

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** B. Bacterial Agents  
**Poster Presentation Number:** 053 (B)

### Sepsis in Inhalational Tularemia

**R. G. Russell¹, L. Westfall², J. Wilder³; ¹Lovelace Respiratory Res. Inst., Albuquerque, NM, ²Southern Res. Inst., Albuquerque, AL**

Objective: Disease progression of inhalational tularemia was studied in cynomolgus macaques to identify key events in mortality. **Methods:** CM were challenged via head-only aerosol inhalation with 201 - 8460 CFU of F. tularensis SCHU S4 (Ft). Hematology, clinical chemistry, serum cytokine
levels, fibrinogen and platelets, coagulation parameters and pathology findings were correlated with outcome and pathology. Results: Lung bacterial counts increased from Day 4 post challenge and overall were similar on each of Days 6 - 9 post challenge, with average counts of approximately 6.4 X 10^8 CFU/g and higher. The plateau in bacterial numbers corresponded to similar number and diameter of granulomas scattered throughout the lung and lack of or limited dissemination within the lung in this time frame post challenge. There was extensive necrosis of lymphoid cells and inflammatory cells in the tracheobronchial lymph node and pronounced bacterial colonization with approximately 1.4 X 10^8 CFU/g. Animals that died on Days 6 - 9 post challenge had bacteremia and early systemic infection that resulted in sepsis, that was a principal cause of mortality, with pronounced pulmonary edema. Animals that did not develop severe sepsis survived longer, to Days 12 - 14, but died due to progressive pneumonia caused by bacterial dissemination in the lung. These animals, and animals that survived maintained white blood cell counts and had lower serum cytokine responses compared to animals that succumbed with sepsis. Animals that died with sepsis had profound utilization of white blood cells parameters and marked elevation in cytokines IFNγ, IL-1β, IL-6, IL-8, IL-ra, IL-15, IL-18, MCP, G-CSF. Conclusions: Inhalational Ft in CM may result in bacteremia and systemic infection that dictates the severity of sepsis. This is the primary determinant of mortality in most animals that succumb on Days 6 - 9 post challenge. Animals with limited or lack of sepsis may survive indefinitely, but some animals develop progressive pneumonia that is fatal. Animals that more effectively constrain bacteremia and sepsis by innate mechanisms have a potential for longer survival.

**Session Type:**  Poster Session  
**Session Number:**  016  
**Session Title:**  Poster Session 2  
**Poster Presentation Time:**  Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:**  B. Bacterial Agents  
**Poster Presentation Number:**  054 (B)  

**Francisella philomiragia Preferentially Directs Proteases to Outer Membrane Vesicles**

**R. E. Pepin**¹, W. Zhou¹, M. L. van Hoek²; ¹George Mason Univ., Manassas, VA, ²Natl. Ctr. for Biodefense and Infectious Diseases, Manassas, VA

Outer membrane vesicles (OMVs) are small non-replicating vesicles produced from the outer membrane of nearly all gram negative species of bacteria that have roles in antibiotic resistance and virulence. Bacteria may be able to direct and enrich specific proteins in OMVs prior to release and can possibly alter the contents of OMVs based on their environment. In general, pathogenic bacteria produce more OMVs than their non-pathogenic cousins, most likely for the dissemination of virulence factors. Francisella tularenis is vector-borne, zoonotic, gram-negative, facultative intracellular bacteria, which has been classified by the CDC as category A agent of bioterrorism. Francisella species lack type III and type IV secretion systems, which are used by many intracellular bacteria as a means of delivering virulence factors. OMVs may provide an alternate mechanism for the secretion of virulence factors used by Francisella. Our previous studies have shown that Francisella novicida produces OMVs that contain many virulence associated proteins and that the protein profiles of the OMVs differ from the secreted proteins. Secreted proteases have been shown to be a mechanism of bacterial virulence. We propose that the related environmental species F. philomiragia preferentially enriches its OMVs with proteases as a mechanism for delivery to target cells. In this work we analyze F. philomiragia for both secreted proteases and those contained within OMVs. The protein content of the OMVs and related supernatant were analyzed by mass spectrometry. The OMVs contained both more and different proteases than those found secreted in the supernatant. One such protease found only in the OMVs is leucine aminopeptidase, which is required for virulence in S. aureus. Protease activity assays indicate that F. philomiragia OMVs have significantly more protease activity than their supernatants. These findings seem to indicate that F. philomiragia is sorting proteases for inclusion in OMVs over direct secretion as a possible mechanism of protection from the host environment.
Impact of Nationwide Antibiotic Administrative Programmes on Antibiotic Usage and Antibacterial Resistance for Clinical Isolates Over a Two-year Period in 14 Chinese and 5 Swedish Tertiary Hospitals

S. P. Sylvan, Working group for Sino-Swedish Bilateral Cooperation on Containment for Antimicrobial Resistance; Uppsala Academic Hosp., Uppsala, Sweden

The aims of this 2-year multicentre study are to systematically evaluate and establish a reference baseline for the resistance of clinically relevant bacterial isolates in 14 tertiary hospitals in China and 5 Swedish hospitals and to characterise the current use of antibiotics in these hospitals. We also aimed to assess the impact of national antibiotic administrative policies on antibiotic use and resistance patterns of the leading nosocomial pathogens in the hospitals. The present study was done at a time when Chinese health authorities launched a campaign to discourage over prescription of antibiotics. At present, it seems their efforts are having a meaningful impact. Following the implementation of the intensive control programme in Chinese tertiary hospitals, we found that the consumption levels of parenteral antimicrobials were reduced and accompanied by a significant increase in susceptibility patterns of both Gram-positive and Gram-negative bacteria, including ESBL-producing organisms. During the 2-year study period, no significant change in the level of resistance to commonly used antibiotic groups could be noted in the clinical isolates from the five Swedish tertiary hospitals.

Anthrax Outbreak Investigation in the Republic of Armenia in 2015

A. Melikyan, H. Batikyan; State Service for Food Safety of the Ministry of Agriculture of the Republic of Armenia, Yerevan, Armenia

Introduction: In August 2015, two human cases of cutaneous anthrax were reported in the Armavir region in Armenia. This abstract describes the investigation conducted by Republican Veterinary-Sanitary and Phytosanitary Laboratory Services Center (RVSPLSC) to identify the source of infection and epidemiology of the outbreak. Materials and Methods: Field teams sent to the Armavir regions conducted interviews with the patients, their family, and other contacts. Meat samples and other food products were collected from their homes and sent to RVSPLSC for microbiological examination. Results: It was determined that beef transported in a privately owned car from the Republic of Georgia was the source of the outbreak. The meat was processed and consumed by 10 individuals from two families. A father and daughter who were involved with cooking the meat, developed ulcers on their hands eight days after handling the meat. Both were diagnosed with cutaneous anthrax by the Ministry of Health. The morbidity ratio was assessed as 20% with consideration of the number of people exposed through processing and consumption of the meat. During interviews, it was revealed that the meat had been stored in the refrigerator of the home and then later the freezer. Visual inspection of the unconsumed meat showed non-coagulated blood and pinkish flesh, typical of anthrax contamination. Testing of the meat samples were positive for Bacillus anthracis. Other food samples collected from the refrigerator were also positive for B. anthracis, revealing cross-contamination. Conclusions: The official confirmation of B. anthracis in the meat was received from RVSPLSC three days after the first human cases were reported. During this time, disinfection and monitoring of the outbreak region was conducted, which contained the spread of disease. This example illustrates how anthrax can spill over into the human population from contaminated meat. Meat should only be purchased from authorized vendors whose meat is subject to veterinary-sanitary practices and testing. This study also reinforces the importance of vaccinating susceptible animals in Armenia.
Poly(3-hydroxybutyrate) Fuels de novo Fatty Acid Biosynthesis During Bacillus anthracis Sporulation


Numerous bacteria accumulate poly(3-hydroxybutyrate) (PHB) as an intracellular reservoir of carbon and energy in response to imbalanced nutritional conditions. In Bacillus sp. accumulation of PHB was found to coincide with acetate catabolism and precede sporulation. The direct link between accumulation of PHB and efficiency of sporulation in the members of the genus Bacillus was observed in multiple studies. Although, the general idea of PHB as an intracellular carbon and energy source fueling sporogenesis was developed several decades ago, the underlying mechanisms of how PHB is involved in sporulation have not been defined. In the current study, we have demonstrated the specific requirement for PHB in maximizing B. anthracis spore formation. Our results demonstrate that disruption of the PHB biosynthesis pathway leads to a significant decrease in the intracellular Ac-CoA, ATP, NAD+, and NADH levels and reduces cellular respiration during sporogenesis. These deficits result in a reduction in both carbon and energy for de novo fatty acid biosynthesis required for new membrane formation during B. anthracis sporulation. In support of this, transmission electron microscopy (TEM) experiments revealed that the lack of PHB during sporulation affects development and composition of the mature endospore. Furthermore, supplementation of the PHB deficient mutant with fatty acids during growth was shown to restore these sporulation defects. Taken together, the results of this study provide important insight into the nutritional requirements for sporogenesis in Bacillus anthracis.

Combating the Emergence and Spread of Antibiotic Resistance Using Evolutionary Forecasting

K. Beabout, A. G. Prater, T. G. Hammerstrom, C. Miller, G. Saxer; Rice Univ., Houston, TX, Rice Univ., Houston, TX, TX

Antibiotic resistant infections are an increasingly worrisome complication of combat wounds in deployed soldiers. To anticipate and deter the emergence and spread of resistance our lab uses a reconnaissance approach, i.e. identifying how resistance to an antibiotic will occur before it has been detected. By predicting what types of resistance mechanisms will arise, these insights can then be applied to develop strategies to minimize and limit resistance, such as determining when a combination of therapies might be more successful than an individual antibiotic. Our lab group has developed a pipeline to determine what mechanisms and genetic networks are most important to resistance. First a pathogen population is established in a bioreactor that maintains a continuous culture at its fastest growth rate using a novel metabolic control loop. The concentration of antibiotic is increased very gradually and over about 3-4 weeks, or about 1,500 generations, we can generate populations that are highly resistant. One nice feature of this system is that we only modestly increase selection pressure and thus maintain a highly polymorphic population of bacteria. Using metagenomics and deep sequencing of the population through time we are able to identify and survey a remarkable range of successful evolutionary trajectories. Once the most important mutations to resistance are identified, biochemical and biophysical experiments are performed to determine the physicochemical basis of resistance. We have successfully used this pipeline to identify mechanisms of resistance against broad-spectrum antibiotics in a range of pathogens. For example, we have identified resistance mechanisms against tigecycline, a translation inhibitor, in
Enterococcus faecalis and Acinetobacter baumannii. We are currently using our pipeline to identify novel resistance mechanisms against daptomycin, a lipopeptide antibiotic, in Enterococcus faecium. By applying this pipeline to clinically relevant antibiotics and pathogens, insights can be gained to take a preemptive approach to combat antibiotic resistance.

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: B. Bacterial Agents
Poster Presentation Number: 059 (B)

Prevalence of Legionella pneumophila in Drinking Water Outlets of a Tertiary Care Hospital

R. Chaudhry, A. Valavane; All India Inst. of Med. Sci., New Delhi, India

Legionella species are ubiquitous in natural and man-made freshwater environments. Both potable and non-potable water can be colonised by the bacterium. Drinking water can act as source of infection, transmitted via microaspiration, which may lead to Legionellosis. In health care settings, given the high number of immunocompromised patients, Legionellosis may cause 15%-50% or higher mortality. A total of 79 Drinking water samples were collected from our hospital campus between July 2012 and May 2014. Temperature of water samples were also noted during collection. The samples were filtered in 0.22µm filters and the filter papers suspended in 5 ml of initial water were further processed for culture according to CDC guidelines. Cultures of the sample were done in BCYE agar at pH 6.9 with GVPC supplements and the plates were incubated at 37°C in a candle jar. After observation for 14 days the suspected colonies were tested biochemically and the colonies were identified as Legionella pneumophila using PCR and Restriction enzyme analysis of 16S rRNA gene. A total of 6.3% (5/79) of drinking water showed the presence of the bacterium by culture followed by PCR and REA. The outlets are drinking water systems installed in OPD area, Radiotherapy ward, Neurosurgery ward and guest house (twice positive). The average temperature of the positive water samples was 24.6 °C (ranging from 20°C to 31°C). Routine monitoring of the sites are underway. Isolation of Legionella pneumophila in hospital settings should mandate routine cleaning and monitoring of high risk sites.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: B. Bacterial Agents
Poster Presentation Number: 060 (B)

The Prevalence Distribution of Diarrheagenic E.coli Categories Their Emerging Resistance to FlouroQuinolones in CrossBorder Counties in Kenya


Background: Diarrhea is one of the main causes of morbidity and mortality among children in sub-Sahara Africa, and one of the main causes of hospital admissions in rural areas of Kenya. In Kenya, antimicrobial resistance surveillance has been conducted only at some private hospitals and at institutional levels, with limited access of data and sharing of information. the actual scale of national antimicrobial drug resistance is not well defined. The aim of the study is to determine the prevalence of common circulating enteric bacterial pathogens and their antimicrobial susceptibility patterns to commonly prescribed antimicrobial agents in Kenya. Methodology: This was a cross sectional hospital based study of which stool samples were collected between 1st February 2013 to 30th September 2015 from a total of 1316 outpatients with diarrhea who were under five years of age from four cross border hospitals in Kenya. Conventional, biochemical methods, multiplex PCR and antimicrobial susceptibility tests were conducted to identify the bacterial causes and virulence factors in the isolates, respectively. Results: Of 1316 patients screened, we identified the causes of 536 cases (40.7%) as follows: Pathogenic E. coli 280(21.3%) Shigatoxigenic 102 (36.4%), enteropathogenic 66
The highest levels of resistance among the E. coli isolates were observed in Ampicillin and Trimethoprim/sulphamethoxazole each at 95% followed by tetracycline at 81%. Shigella isolate levels of resistance ranged from 80% to 100% for Ampicillin, tetracycline and Trimethoprim/sulphamethoxazole. In general, pathogenic E. coli was the most prevalent organism isolated from all the sites as indicated and the highest prevalence of antimicrobial resistance was to Ampicillin followed by Trimethoprim/ sulphamethoxazole and tetracycline. The major concern from our findings is the emerging resistance of enteric pathogens to fluoroquinolones/ciprofloxacin, 3rd generation Cephalosporin’s.
anthrax foci with a history of 40-80 years of repeated outbreaks. Drivers of the persistence of anthrax reservoirs in the environment are still not well understood. It is known that anthrax spores survive best in soil with pH>6.0, rich in humus and calcium. Some researchers emphasize the role of the precipitation, vegetation, heat capacity of the soil, and landscape features for the survival and accumulation of spores in the soil. The objective of this study is to determine the territory in Ukraine where soil conditions have resulted in long term chronic contamination with B.anthracis. Maximum entropy (MaxEnt) algorithm was used as one of the best performing presence-only packages available. For model construction, a sample of the most persistent anthrax locations was chosen. Environmental data includes soil and climatic variables from HWSD, ORNL DAAC, and Bioclim databases. Analysis of variable contribution to the model shows the highest percent for soil total exchangeable bases followed by vegetation index (NDVI), annual precipitation and soil pH. The model characterizes most of Ukraine as suitable for B.anthracis long-term survival. The highest risk is in the forest-steppe zone, the lowest - in the north of the country in marshy woodlands (Polesie), in the west - in the Carpathian mountains, in the south - in the Crimean mountains.

Session Type:       Poster Session
Session Number:    003
Session Title:     Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic:            B. Bacterial Agents
Poster Presentation Number: 063 (B)

Parallel Comparison of Commercially Available Screening Methods for Bacillus anthracis

A. Minor, J. Ferrell, C. Robinson, Z. Kuhl, B. Keavey; WVDA, Charleston, WV

Background: Bacillus anthracis, the etiological agent of anthrax, can cause severe zoonotic disease in herbivores and humans. Anthrax is a disease of antiquity, and has historically been weaponized, due to its lethality, hardiness, and ease of dissemination. While strong cultural detection methods exist, a fast reliable screening method is imperative for ruling out samples in large volumes. This study is based upon a parallel comparison between four commercially available B. anthracis detection kits including the SMART II, BADD, Biothreat Alert, and Tetracore kits and four culture medias; SBA, MYP, ACA, and PLET. Methods: Assay limit of detection studies were conducted in parallel on the Smart II, BADD, Biothreat Alert, and Tetracore kits and select cultural medias with direct fortifications ranging from 1x103 to 1x107 cfu/ml. The manufacturer’s instructions were carried out for each method. Each kit method and fortification level was replicated in triplicate and read at 3, 15, and 30 minute intervals. The ELISA method was read at the endpoint absorbance at 405 nm and the cultural plates were enumerated after a 24 hour incubation. Ten non-target strains were examined for exclusivity at 1x105 cfu/ml. Results:

Performance Characteristics for B. anthracis Screening Methods

<table>
<thead>
<tr>
<th>1x10^5</th>
<th>1x10^6</th>
<th>1x10^7</th>
<th>Exclusivity Strains</th>
</tr>
</thead>
</table>

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Conclusions:** While cultural detection methods for direct B. anthracis growth exhibited a lower limit of detection than the commercially available screening kits examined, due to an overnight incubation period, an additional day is required to achieve a result. The Tetracore ELISA detection kit exhibited 100% sensitivity at an inoculum of 1x10^5 vegetative cfu. The lateral flow devices that performed with the greatest sensitivity were the BADD kits, with a LOD of 1x105 at 56%. All of the kits examined for exclusivity demonstrated 100% specificity, only reacting to Bacillus anthracis.

---

**Session Type:** Poster Session  
**Session Number:** 016  
**Session Title:** Poster Session 2  
**Poster Presentation Time:** Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:** B. Bacterial Agents  
**Poster Presentation Number:** 064 (B)

**Anthrax Toxin Levels in Tissues Related to Progression of Infection**

**M. G. Candela**^1^, J. R. Barr^1^, Z. P. Weiner^2^, J. J. Glomski^2^, Q. P. Conrad^3^, A. E. Boyer^3^; ^1^CDC, Atlanta, GA, ^2^Univ. of Virginia, Charlottesville, VA

**Background:** Bacillus anthracis produces binary toxins. Protective antigen binds to lethal factor (LF) forming lethal toxin (LTx). We previously described a triphasic kinetics of anthrax toxemia using methods to quantify total LF (free LF + LTx) and LTx in serum. However, little is known about the localization of toxin levels during infection. The quantification of LF/LTx in tissues of infected mice were determined during 3 stages of infection and at terminal infection in cynomolgus macaques. **Methods:** Luminescent B. Anthracis Sterne strain was injected into one ear of A/J mice and their luminescence monitored for migration to other organs. Three mice were sacrificed at early, middle and late stages of infection. Serum, brain (BR), infected and control ear (IE, CE) and lymph nodes (IL, CL), heart (H), liver (LV), lungs (LG), kidneys (K) and spleen were collected/analyzed. LF/LTx were analyzed from organs of cynomolgus macaques with inhalation anthrax. Results: In early stage infection in mice, LF was primarily detected in serum (0.4±1.1 ng/mL) and the IE (1.2±7.1 ng/mL). At mid-stage, low levels of LF were measured in most organs but were higher in the IE (21.5±43.4 ng/mL), IL (30.2±18.4 ng/mL) and serum (15.8±31.5 ng/mL). At late stage, LF was elevated in all tissues and was highest in IE (555±220 ng/mL), IL (442±655 ng/mL), LV (636±581 ng/mL) and serum (682±2990 ng/mL). LTx levels were elevated in the same tissues as LF but to a lesser degree. Late stage LTx was highest in IE (430.3±201 ng/mL), IL (574±563 ng/mL), LV (129±432 ng/mL) and serum (102±37 ng/mL). Higher ratios of LTx/LF was observed in tissues compared to serum. Terminal LF levels in an animal that survived 2 days was higher in K, H and plasma compared to an animal that survived 5 days which was higher in LG, LV and SP. **Conclusions:** This study reveals information about the impact toxin levels in tissues. In mice, higher LTx/LF ratios were observed in tissues compared to serum and was 100% in the infected lymph node. Higher LF levels in kidneys and heart of an animal that succumbed early may provide clues to why some animals progress rapidly.
**The Effect of Growth Temperature on the Nanoscale Surface Properties of Yersinia pestis**

C. E. Stanciu, C. Wang, C. J. Ehrhardt, V. Yadavalli; Virginia Commonwealth Univ., Richmond, VA

Yersinia pestis, the causative agent of plague, has been responsible for several recurrent, lethal pandemics in history. Currently, it is an important pathogen to study owing to its virulence, adaptation to different environments during transmission, and potential use in bioterrorism. Here we report on the changes to Y. pestis surfaces in different external microenvironments, specifically culture temperatures of 37, 25, and 6°C, which correspond with the internal temperatures of a warm-blooded mammal host, cold-blooded flea vector, and infected animals during winter hibernation, respectively. Using nanoscale imaging coupled with functional mapping, we illustrate that changes in the surfaces of the bacterium from a morphological and biochemical perspective can be analyzed simultaneously using atomic force microscopy. The results from functional mapping, obtained at the single cell level, show that the density of lipopolysaccharide (measured via terminal N-acetylglucosamine) on Y. pestis grown at 37°C is only slightly higher than cells grown at 25°C, but nearly three times higher than cells maintained at 6°C for an extended period of time, thereby demonstrating that adaptations to different environments can be effectively captured using this technique. The observed differences in LPS was also correlated to changes in the relative proportion of membrane fatty acids, specifically, 14:0-3OH, 16:1 ω7c, 17:0 cyclo, and 18:1 ω7c. This nanoscale evaluation provides a new microscopic approach to study properties of bacterial pathogens and investigate adaptations to different external environments.

---

**Identification of the Zur Regulon in Francisella**

G. B. Moreau, B. J. Mann; Univ. of Virginia, Charlottesville, VA

Francisella tularensis has been classified as a Tier 1 select agent by the Centers for Disease Control because it has a low infectious dose, is easily aerosolized, and infection is potentially lethal. One mechanism host cells use to control bacterial growth is to limit pathogen access to zinc, an essential nutrient required by all cells. Since Francisella grows effectively in host cells it must have efficient mechanisms of acquiring zinc from the intracellular environment, but zinc uptake mechanisms in Francisella have not been characterized. Many bacteria use a zinc-responsive transcription regulator, Zur, to increase expression of genes required for zinc uptake when zinc is limiting. We identified a gene encoding a protein with significant similarity to Zur in Francisella and hypothesized that Francisella Zur acts as the primary regulator of zinc uptake genes. To test this we first identified by quantitative PCR (qPCR) a putative zinc transporter, zupT, which was upregulated in Francisella grown under zinc-limiting conditions. To determine if Francisella Zur regulates genes involved in zinc uptake, we analyzed zupT expression by qPCR in a wildtype and a zur mutant strain of Francisella novicida. Our results showed that zupT was also Zur-regulated, suggesting that Zur acts similarly in Francisella as seen in other bacteria. To identify other Zur-regulated genes in F. novicida, we performed RNAseq with wildtype and zur mutant strains. We identified five protein-encoding genes as upregulated in the zur mutant, including zupT. Ongoing work includes characterizing these genes and their roles in zinc uptake. Interestingly, three of the five identified genes are not thought to encode functional proteins in the virulent F. tularensis Schu S4 strain, indicating that Schu S4 primarily uses zinc importers that are not Zur-regulated. In summary, we have identified a Zur transcriptional regulator in Francisella as well as potential Zur-regulated genes.
Development of an Aerosol Delivery System for Multiple Simultaneous Exposures of Rabbits to Aerosolized Bacillus Anthracis

W. C. Lin, J. Wang, L. Drabek, R. Nevarez, J. Kish, L. Holland, R. Baker, B. Gingras; IIT Res. Inst., Chicago, IL

Objective: An aerosol model of infection for Bacillus anthracis Ames (BA) is being developed to allow multiple simultaneous aerosol exposures of New Zealand White (NZW) rabbits using a modified 64 port inhalation exposure chamber with nebulizers. This development allows for aerosol exposure to multiple rabbits which minimizes the number of runs needed and reducing the variation of exposure concentration for the study.

Methods: BA spores were prepared using the growth parameters of Leighton and Doi (1971). Titer was determined using Trypticase Soy Agar. For the determination of LD50 and spray factor, challenge concentrations of 0, 106, 107, 108, and 109 CFU/mL were prepared to generate a respirable aerosol that was supplied to a modified 64-port nose-only inhalation chamber. Three NZW rabbits were exposed per BA concentration tested. The inhalation chamber was modified for up to eight NZW rabbits for nose only exposure. With 52 ports blocked, remaining ports were enlarged to ¼” dia. for increased flow with 8 ports for rabbits and 4 ports for monitoring (AGI and viable impactor). Four Pari LC Plus nebulizers operated simultaneously at 28 PSI. Results: The spray factor, calculated as a ratio of exposure chamber aerosol conc. to starting concentration, ranged between 7.45E-06 to 2.16E-05 for the concentrations of BA spores tested for an average of 1.28E-05 +/- 7.26E-06. Particle size was between 1-3 μm MMAD. The LD50 in NZW rabbits was 4.19E05 CFU/animal. Conclusions: Results indicate that this developing novel bioaerosol exposure platform allows for multiple simultaneous and consistent exposures of at least three NZW rabbits including peripheral monitoring apparatus. While further testing is needed to test the consistency of exposure with up to eight animals/run, this platform has the capability of consistent delivery of aerosol to each port thereby reducing variation in aerosol concentration and the number of runs needed for a therapeutic or vaccine study.

Proteomics of Bacillus Anthracis Sporulation in Soil Systems

D. Wunschel, B. Kaiser, J. Hutchinson, E. Merkley, M. Warner; Pacific Northwest Natl. Lab., Richland, WA

The survival and infectious cycle of Bacillus anthracis is dependent upon the process of sporulation. The events and molecular changes that occur during sporulation have been studied using organisms cultivated in laboratory medium. However, the impact of environmental conditions in natural reservoirs on measurable phenotypic changes, such as protein expression patterns, is not well understood for this organism. To bridge this knowledge gap, sporulation on two types of agar-immobilized soils was used for comparison to cultures sporulated on two common types of solid laboratory media and one liquid sporulation medium. The cultures produced on laboratory medium had higher overall spore yields and numbers of identified proteins but lower sporulation efficiency. Differences in protein expression were observed related to medium and growth temperature demonstrating that sporulation environment could significantly impact the protein content of the resulting spore. Proteins and pathways were shared between all of the soil-cultivated samples and distinct from the expression profiles in laboratory medium, and vice versa. Proteins involved in thiamine and phosphate metabolism were found in spores produced on soils with a notable increase in expression of ATP binding cassette (ABC) transporters of phosphate and antimicrobial peptides. By contrast, a distinct set of ABC transporters for amino acids, sugars and oligopeptides were found in cultures produced on laboratory media. Sporulated cultures produced in laboratory media also had increases in carbon and amino acid metabolism-related proteins to utilize a nutrient-rich environment. These protein expression changes demonstrate impacts of the sporulation
environment that may be useful in distinguishing naturally-produced from laboratory-generated spores and provide insights into the organism biology.

Critical Toxemia Indices for Inhalation Anthrax in Cynomolgus Macaques

A. Boyer1, M. Gallegos-Candela1, R. Lins2, A. Woolfitt1, M. Solano1, J. Brumlow2, C. Quinn1, D. Sanford3, T. Dreier4, J. Lee4, J. Barr1; 1CDC, Atlanta, GA, 2Battelle, Atlanta, GA, 3Battelle, W Jefferson, OH, 4BARDA, Washington, DC

Background: Inhalation anthrax fatality ratios are high unless antimicrobial treatment (Tx) is started early. Early intervention is usually successful, 90-100% survival, but declines with Tx delays. Biomarkers that define a period for successful intervention have not been characterized. We have previously described the quantification of anthrax toxins using mass spectrometry (MS). We determined critical toxemia indices (CTI’s) below which Tx was successful in non-human primates.

Methods: Cynomolgus macaques randomized into 4 groups were exposed to ~200LD50 Bacillus anthracis spores. Groups 1, 2 and 3 received ciprofloxacin for four days commencing at 36h, 48h, and 72h post-exposure, respectively. Samples were collected prior to and after exposure and Tx. Anthrax toxins, protective antigen (PA), lethal factor (LF), edema factor (EF), lethal toxin (LTx) and edema toxin complex (ETx) were quantified by MS.

Results: Prior to Tx 16% (2/12) animals succumbed at 48h and 33% (6/18) at 72h. Among treated animals, survival was 87.5% (7/8), 60.0% (6/10) and 58.3% (7/12) with Tx at 36h, 48h and 72h, respectively. Geometric mean anthrax toxin levels at the time of Tx were significantly higher in non-survivors than survivors. CTI’s above which later Tx failed were estimated at 30, 10 and 0.5 ng/mL for LF, LTx and EF, respectively. With early Tx (36h), 28.6% (2/7) survived with toxin levels above the CTI’s. Cumulative toxin exposure levels prior to Tx demonstrated a window of success with LF≤20, LTx≤15, and EF≤0.5 ng/mL/h for delayed, 72h Tx.

Conclusions: This study showed that compared to successful Tx, all toxin levels were significantly higher in Tx failures and in animals that succumbed prior to treatment. A toxin-dependent window of treatment success was evident below the defined pre-Tx critical toxemia indices and cumulative exposure levels. Only early treatment, prior to symptom onset, allowed survival above the CTI’s. Toxin levels above these indices indicate the need for advanced therapies.

Clinical Findings After Indoor Microfungal and Trichothecene Exposure

I. H. Grant; Integrative Med. Group, Tarrytown, NY

Background: Trichothecenes (T), cytotoxic mycotoxin (MCT) molecules produced by Stachybotrys (ST), Fusarium and Trichoderma attach to dust and remain active despite disinfection. They directly damage any cell on contact, injuring skin, respiratory and GI tracts, as well as immune and
nervous systems. Animal studies indicate brain penetration occurs via cell-to-cell apoptosis from nasal mucosa to olfactory nerves. **Methods:** Cohort analysis of documented indoor Ts exposure. 1) Spore-trap, microscopy, mold-specific PCR, MCT testing (RealTime Laboratories, Inc.) 2) Medical evaluation: exposure intensity/mold spp, symptom timing/severity, outcomes, immune/genetic parameters, fungal titers, MCT excretion

**Results:**

<table>
<thead>
<tr>
<th>ENVIRONMENTAL MCTs</th>
<th>0-15 Ts-contaminated buildings; none had aflatoxin(a) or Ochratoxin (O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENVIRONMENTAL MOLD CONTAMINATION</td>
<td>minimal, moderate, or high mold/Aspergillus or ST/ST, severe, extreme</td>
</tr>
<tr>
<td>10% Aspergillus, 1% Penicillium, 9% detected 12/10 (92%) &amp; A. niger (55%)</td>
<td></td>
</tr>
<tr>
<td>57% &amp; Ochratoxin 11% (85%)</td>
<td></td>
</tr>
<tr>
<td>Mucor/Rhizopus 10% (77%)</td>
<td></td>
</tr>
<tr>
<td><strong>COHORT DEMOGRAPHICS</strong></td>
<td>82 patients exposed</td>
</tr>
<tr>
<td>42 males, 40 females (4 pregnant), 1 embryo of 12 children, 9 In utero</td>
<td></td>
</tr>
<tr>
<td><strong>MOLD EXPOSURES</strong></td>
<td>26 (81%) exposed to A/P (16 A. niger, 10 P. brevicompactum)</td>
</tr>
<tr>
<td>25 (75%) ST77 strains exposed</td>
<td></td>
</tr>
<tr>
<td>25 (75%) Mucor/Rhizopus 24 (75%) CHI (Barbome), 11 (32%) Trichoderma</td>
<td></td>
</tr>
<tr>
<td><strong>EXPOSURE SEVERITY</strong></td>
<td>(contaminations plus intensity unprotected mold-dusting activities)</td>
</tr>
<tr>
<td>Mild 2, moderate 15, severe 10, extreme 3</td>
<td></td>
</tr>
<tr>
<td><strong>IMMUNE RESPONSE</strong></td>
<td>A. flavus (IgG + 2/328, IgE 6 2.7%)</td>
</tr>
<tr>
<td>P. brevicompactum 21 (93%)</td>
<td></td>
</tr>
<tr>
<td>Cladosporium 19 (95%)</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em> 29 (84%)</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus</em> 11 (65)</td>
<td></td>
</tr>
<tr>
<td>Alternaria 65, Aspergillus 18 (75%)</td>
<td></td>
</tr>
<tr>
<td>Trichoderma 14 (65%)</td>
<td></td>
</tr>
<tr>
<td><strong>URINE MCT</strong></td>
<td>T: Undetectable 2/3, detectable 2/5 (20%) ; humans with 12 ≥ 0.2 ppb, detectable 2/2 dogs</td>
</tr>
<tr>
<td>O: Undetectable 11, detectable 6, elevated 2 ppb, 9/99.85%</td>
<td></td>
</tr>
<tr>
<td>A: Undetectable 2/3, detectable 1/1, elevated in 2</td>
<td></td>
</tr>
<tr>
<td><strong>DISEASE SEVERITY</strong></td>
<td>(illness, disability, permanent damage)</td>
</tr>
<tr>
<td>Minimal 1, mild 9, moderate 20, severe 6, life-threatening 2, fatal 2</td>
<td></td>
</tr>
<tr>
<td><strong>LUNES FREQUENCY</strong></td>
<td>21 (63%)</td>
</tr>
<tr>
<td>Neurological (18 cognitive impairment, 5 toxic encephalopathy)</td>
<td></td>
</tr>
<tr>
<td>1 Mycosphaerella (1 dog) 1 Mycophilus sp. (1 dog)</td>
<td></td>
</tr>
<tr>
<td>15 (45%) Permanent organ damage 9 pulmonary, 7 neurological, 7 dermatologic, 5 severe fatigue (1 renal)</td>
<td></td>
</tr>
<tr>
<td>16 (51) (%) Disabled 8 permanent, 9 lost employment</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions: Mycotoxin testing is an important bio-marker advance in identifying fungal exposures. Matching environmental fungi, specific fungal IgG’s & MCT excretion appear to identify hazardous exposures. Trichothecenes exposure appears highly associated with permanent damage and marks Stachybotrys-exposure. Exposure severity strongly correlates with disease severity and is a more reliable hazard marker than extent of contamination. Genetic MTHFR detoxification defects have an even stronger correlation with disease severity. Further epidemiological studies are urgently needed.
Development and Characterization of an Oro-Nasal Inhalation Plethysmography Mask Exposure System

L. E. Bowen, J. B. Anderson, M. M. Bailey, B. R. Haupt; USAMRIID, Ft. Detrick, MD

An oro-nasal inhalation plethysmography mask exposure system was developed by the Center for Aerobiological Sciences to challenge nonhuman primates and rabbits with biological agents while determining real-time respiratory parameters. The system included novel challenge/plethysmography and sample collection masks that presented aerosol directly to the breathing zone of the animals and sample collection probes. Challenge/plethysmography masks were fitted with a pressure port that interfaced with a differential pressure transducer and signal amplifier to quantify respiratory frequency, tidal volume and minute volume. Challenge/plethysmography masks were calibrated and verified with certified gas-tight syringes. Accuracy was determined from simultaneous comparison tests between the challenge/plethysmograph mask and a head-out plethysmograph using live animals. For cynomolgus macaques, the differences in tidal volume, frequency and minute volume were $5.1 \pm 1.8$ cm$^3$, $8.6 \pm 7.9$ bpm and $67.2 \pm 24.3$ cm$^3$ (n=3). For New Zealand White rabbits, the differences in tidal volume, frequency and minute volume were $1.0 \pm 0.8$ cm$^3$, $1.0 \pm 0.0$ bpm and $208.9 \pm 109.1$ cm$^3$ (n=3). The oro-nasal inhalation plethysmography mask exposure system utilized an aerosol conditioning line and a 12-port flat plenum that directed challenge material to and from the masks. Standardized test were used to characterize the exposure system. The fractional leak rate of the system was 4.17E-05 min$^{-1}$ which was well below the acceptance criteria of 0.001 min$^{-1}$. The theoretical T99 was 1.7 minutes and the observed T99 was 0.6 minutes. Temporal (within mask) variation was 0.51%, total variation was 1.1% and spatial (mask to mask) variation was 0.9%. The mean particle size distribution (MMAD and GSD) of a 25 mg/mL saline solution generated in the system was $1.2 \pm 0.01$ µm and $1.9 \pm 0.2$ (n=8). The oro-nasal inhalation plethysmography mask exposure system reduces dermal contamination and eliminates ocular contamination as compared to contemporary exposure systems. By eliminating space consuming head-out plethysmographs, multiple, simultaneous animal challenges are possible.

Head-Only Inhalation Exposure Chamber Modification

M. M. Bailey$^1$, M. E. Staymates$^2$, J. B. Anderson$^1$, B. R. Haupt$^1$, L. E. Bowen$^1$; $^1$United States Army Military Res. Inst. of Infectious Diseases, Ft. Detrick, MD, $^2$Natl. Inst. of Standards and Technology, Gaithersburg, MD

Nonhuman primates are frequently challenged with infectious aerosols by head-only inhalation. In this study, three chamber modifications were examined that improved chamber efficiency by more closely approximating the theoretical T99 and by eliminating dead space. In traditional head-only inhalation systems, a sedated nonhuman primate is positioned in a supine orientation and its head is inserted inside a rectangular chamber. The head is supported on a wire grid and a flexible piece of dental dam material is sealed around the animal’s neck and the chamber. Aerosol is directed into the top of the chamber, flows past the animal’s head, and is exhausted out of the bottom of the chamber. In examining this traditional method, testing with visible aerosols revealed that the unidirectional presentation of the aerosol resulted in persistent turbulent flow patterns throughout the chamber. Additionally, the asymmetrical exhaust manifold contributed to an abbreviated T99 and increased dead space. In the modified design, three adaptations were made to improve chamber operational efficiency. Aerosol was delivered to the chamber bidirectionally using an opposed normal inlet; a laminar flow element was placed under the inlet nozzles to create a mixing ante-chamber; and a radial exhaust manifold was implemented to improve flow symmetry. Testing with a visible aerosol confirmed a turbulent mixing zone in the ante-chamber and laminar aerosol flow throughout the rest of the chamber. The radial exhaust contributed to less dead space and a T99 that more closely approximated the theoretical T99. In conclusion, these three modifications improved chamber efficiency.
PCR Diagnostic of Glanders in Georgia

N. Vepkhvadze, K. Goginashvili, T. Tigilauri, E. Mamisashvili, M. Ashordia, M. Kokhreidze, M. Donduashvili; The Lab. of the Ministry of Agriculture, Tbilisi, Georgia

Introduction: Glanders is a common infectious disease of horses in certain parts of the world. It is caused by the bacterium Burkholderia mallei. Glanders was widely spread in Georgia until 1924. In this period of time horses had a vital importance in agriculture as well as in the army. In the former Soviet Union, from 1925-1928, 10 million horses were tested by allergic and serological tests; 48 thousand tested positive. Since then, no research on Glanders has been performed in Georgia. It is important to note that we do not have any current information about this disease in Georgia. Recently in Georgia we screened for Glanders within the regions of Guria, Samegrelo-Zemo, Svaneti, Kakheti and Shida Kartli. Materials and Methods: A total of 541 samples from these regions were received at LMA for testing: 100 blood samples from Guria, 242 blood samples from Samegrelo, 162 blood samples from Kakheti and 37 Blood samples from Shida Kartli. DNA was extracted from test samples using DNA Blood&Tissue extraction kit (Tetracore; Rockville, MD). PCR kits were employed according to the manufacturers instructions. PCR was performed on the Roche Light Cycler 2.0. Animal serum samples were tested using Lillidale Diagnostics Lillitest Glanders cELISA kit (BG214 HV, UK). Results And Discussion: The study revealed that all samples were negative for glanders based on serological and molecular studies. Based on obtained results we can hypothesize that Burkholderia mallei is absent or at undetectable levels in the regions surveyed. Importantly, the introduction and implementation of modern diagnostics methods for Glanders will allow the Veterinary Services of Georgia to ensure absence and detection of the disease using OIE prescribed tests, for the goals of disease surveillance and international trade. Geographic Information System (GIS) ArcMap Method (GIS) was used to produce maps of the areas screened. Testing results were also entered into the electronic integrated disease surveillance system (EIDSS).

Substrates for Specific and Quantitative Detection of Anthrax Lethal Factor in Plasma

N. Shine, K. Suryadi; List Biological Lab., Campbell, CA

Bacillus anthracis is regarded as a major biological warfare threat. The inhalation form of Bacillus anthracis infection can kill quickly. While antibiotic treatment can clear the bacterium from the host, if diagnosis is delayed, the toxin, which is rapidly produced, may already be present in lethal amounts. There is a critical need for a rapid, accurate, sensitive and simple assay to determine whether infection has occurred thereby allowing immediate treatment. This report describes a fast, sensitive, specific and accurate detection method to determine active infection by Bacillus anthracis in plasma. Anthrax lethal factor (LF), an endopeptidase, is present in blood early in the infection. The use of peptidic substrates in plasma is problematic due to the presence of other proteases and the likelihood of nonspecific cleavage of the substrate. Fluorescently labeled peptide substrates which are not cleaved by plasma proteases and thus are specific for LF are described here. The LF is enriched by capture from plasma using an LF antibody-coated microtiter plate, and the captured LF is then exposed to the fluorescent substrate. The amount of cleaved peptide substrate is determined by HPLC with fluorescence detection. Concentration of the LF using the antibody-coated plates allows for the detection of 5 pg LF/ml of neat plasma after 2 hours of incubation. Alternately the substrate may be added directly to diluted plasma and cleavage
monitored by an increase in fluorescence as a function of time using a fluorescent microplate reader. The limit of detection by this simpler method is 1 ng LF/ml of plasma after 5 hours of digestion. The results of both methods can be confirmed by analysis of the reaction as a function of time.

Session Type: Poster Session  
Session Number: 016  
Session Title: Poster Session 2  
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
Topic: D. Diagnostics

Poster Presentation Number: 074 (D)  

Early Diagnosis of Ricin Intoxication in a Murine Model

S. Simon, Sr.1, C. Mourton-Gilles, Sr.2, J. Dano, Sr.1, E. Chabert, Jr.2, A. Rouaix, Jr.1, D. Sauvaire, Sr.2; 1CEA, Gif sur Yvette Cedex, France, 2ANSM, Vendargues, France

The Centers for Diseases Control and Prevention have listed the potential bioweapon ricin as a Category B Agent that could be used against civilian and military personnel. Ricin is a so-called A/B toxin produced by plants and is one of the deadliest molecules known. It is easy to prepare and no curative treatment is available. The clinical syndrome resulting from ricin intoxication is dependent upon the route of exposure. In case of a terrorist attack, aerosol exposure is the most likely route of contact to ricin toxin that will result in the most severe toxicity. Early recognition of ricin exposure is essential for taking care of patients, making decision on choice of supportive treatment as well as for limiting number of persons exposed. Diagnostic tests must be fast and easy to use, specific and sensitive. Using an in vivo murine model of intranasal intoxication, we have developed immunoenzymatic and immunochromatographic tests for ricin detection in plasma and bronchoalveolar lavages (BAL). As early as three hours until 48h after administration of 1 LD50 (approximately 40 ng per animal) of ricin, the toxin was detected in 100% of BAL using both tests. In plasma, ricin was detected in 100% of animals 30h after administration of 2.5 LD50 of toxin.

Session Type: Poster Session  
Session Number: 003  
Session Title: Poster Session 1  
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm  
Topic: D. Diagnostics

Poster Presentation Number: 075 (D)  

Strengthening Health and Biosecurity in Tanzania by Biodetection Capacity Building

A. Katz1, Z. Makondo2, J. Masambu2, F. Mramba2, S. Nikkari1; 1Ctr. for Biothreat Preparedness, Finnish Defence Forces, Helsinki, Finland, 2Tanzania Vet. Lab. Agency, Dar es Salaam, Tanzania, United Republic of

The spread of infectious diseases is a global challenge. Epidemics pose a threat not only to local, but potentially even to global health security. Building local laboratory capacity to fight against biothreats - no matter of their origin - strengthens health and biosecurity also globally. Finland has initiated a bilateral collaborative project to strengthen laboratory capacity and to raise awareness in biosecurity and biosafety in Tanzania. This project is funded by the Ministry for Foreign Affairs, Finland, and it is implemented between the Centres for Biothreat Preparedness, Finnish Defence Forces, and Tanzania Veterinary Laboratory Agency, Ministry of Livestock and Fisheries Development. Tanzania is Finland's longest-standing development partner. Many of the emerging and classic endemic infectious diseases burden the public health sectors in Tanzania. In this Project capabilities of the Tanzanian partner are strengthened in rapid field diagnosis. The main objectives are to: - Build local capacity on detection of biothreat pathogens and diagnosis of infectious diseases - Develop a diagnostic system based on local needs, suitable for field use - Train and collaborate with local experts and authorities - Raise local knowledge and awareness in biosecurity, biosafety and biothreat reduction. The achieved expertise and capabilities will strengthen Tanzanian health sectors dealing with biothreat management in concordance with the One Health strategy. This collaboration is one of Finland’s main engagements in the Global Health Security Agenda (GHSA). GHSA is a joint effort of 50 nations and several key international organizations, aiming to improve prevention, detection, and response capabilities for infectious disease
outbreaks. In 2015, Finland was the chair of the GHSA. In addition, this project supports to achieve the goals of e.g. Global Partnership Program (GPP) and the Biological Weapons Convention (BWC).

Rapid Detection Tests for Antibiotics Resistance

**H. Boutal**, T. Naas, K. Devilliers, M. Plaisance, H. Volland; CEA, Inst. of Biology and Technologies of Saclay, Lab. for Immunoanalytical Res.es, Gif sur Yvette, France, ea7361, upsud, Hosp. de Bicetre, APHP, Le Kremlin-Bicetre, France

Antibiotics are for years the main treatment against bacterial diseases. Their use as therapeutic tools put a selective pressure on bacteria and consequently development of bacterial resistances occurred. Beta-lactams are the most prescribed antibiotics, and more and more powerful drugs were administered to tackle the resistances. One way to fight beta-lactams activity for microorganisms is to produce enzymes called beta-lactamases. As a consequence, enzymes able to hydrolyze new drugs emerged (NDM, OXA and KPC like for example) leaving the medical community in a therapeutic impasse. Moreover the increasing prevalence of multidrug resistance strains is a major concern to emerging infectious pathogens and intentional spread out of multidrug resistance bacteria for bioterrorism. Therefore it is crucial to early detect and identify resistant strains after patient’s admission to hospital or bacterial contamination in order to prevent inappropriate treatments and the spread out of resistance genes. Some tests are already commercially available like antibiograms, RT PCR/PCR, selective media. Unfortunately all of them necessitate a preliminary 12 to 48 hours culture step. This delay is not compatible with an efficient crisis management. Our objective is to develop rapid immunodiagnostic tests (lateral flow formats) for the six beta lactamases mostly encountered CTX-M-15, NDM-1, OXA-48, KPC-1, IMP and VIM. The prerequisites of these tests are i) the production of monoclonal antibodies against the six beta-lactamases and ii) the development of an extraction method of the enzymes for their fast detection in clinical samples such as urine, feces, blood, and sputum with the aim of avoiding the time-consuming culture step. We have obtained monoclonal antibodies against CTX-M-15, NDM-1, OXA-48 and KPC-1 and immunization for IMP and VIM are planned. The lateral flow tests are under development and will be presented: antibodies selection, performance (sensitivity, reproducibility, robustness)...

Rapid Detection of Carbapenem Resistance Using Laser Light Scattering

**A. P. Tomaras**, R. Anbazhagan; BacterioScan, Inc., St. Louis, MO

Antibiotics are among the most transformative agents in human health and have had a widespread impact on the practice of medicine, thus the spread of antibiotic resistance threatens effective patient care. The carbapenem class of antibiotics represents the last safe line of defense for serious bacterial infections, but the recent rise in resistance rates poses a serious risk to their clinical utility, which demands the development of rapid diagnostics to accurately predict resistance prior to therapeutic intervention. The BacterioScan laser microbial growth monitor is a compact diagnostic instrument that measures the growth of microorganisms in real-time, with a lower limit of detection of 1x104 CFU/ml. The instrument is commercially available to rapidly determine microbial presence/absence in human specimens, and current efforts have focused on applying this
technology to rapid antibiotic susceptibility testing (AST). Using meropenem as a representative of the carbapenem class, clinical isolates of Klebsiella pneumoniae and Acinetobacter baumannii were evaluated in the BacterioScan instrument using methods compliant with current clinical microbiology standards. Encouragingly, the phenotypic AST outputs provided by the device showed strong correlation with those obtained using standard methods, although the growth kinetics for meropenem-resistant strains appeared to lag considerably relative to meropenem-susceptible strains. Ciprofloxacin AST evaluation of the same strain panel again correlated with standard methods, but failed to demonstrate the same lag in growth observed when meropenem was tested. This suggests that extended incubation times are required to accurately report minimum inhibitory concentrations for meropenem in resistant strains. It should be noted, however, that meropenem CLSI breakpoint-specific resistance/susceptibility was reproducibly detected in 4-6 hours by the BacterioScan device. Taken together, these data support the use of the BacterioScan instrument to provide rapid phenotypic AST for multiple classes of antibiotics, including carbapenems, enabling clinicians to better select appropriate treatment regimens.

Detection of Anthrax Lethal Factor Using an Electrochemiluminescence Assay

P. Maniatis1, M. Epperson1, J. Martin2, A. Sabnis3, C. P. Quinn1, J. Schiffer1, N. Kamal1; 1CDC, Atlanta, GA, 2IHRC Inc, Atlanta, GA

Background: Lethal factor (LF) is the earliest detectable biomarker of Bacillus anthracis infection and therefore an important target for anthrax diagnostics. This study describes an electrochemiluminescence (ECL) sandwich immunoassay with high analytic sensitivity for quantification of LF in serum.

Methods: Two anti-LF monoclonal antibodies (mAb; AVR1675 and AVR3134) were used to capture and detect LF, respectively. AVR1675 capture mAb was spot-coated onto a carbon electrode. AVR3134 detector mAb was sulfo-labelled as the reporter. LF spiked serum samples and negative (un-spiked) sera were tested to assess analytical specificity (SP), analytical sensitivity (SN), accuracy, and precision. Spiked LF samples were created by measuring known quantities of LF antigen into individual and pooled normal human serum. The assay used 25 μL of serum per test. Concentrations were log10 transformed for linear regression analysis. Results: The lower limit of detection (LLOD) in human serum was 20.1 pg/mL. The lower limit of quantification (LLOQ) was set to 60 pg/mL to optimize SP and SN at 86% and 100%, respectively, on a set of 50 negative samples and 22 spiked LF samples (from 2 pg/mL to 250 pg/mL). Assay accuracy and precision was determined by evaluating the agreement between expected values of the spiked LF samples and the observed values generated from the assay. The equation of the best fit line generated by ordinary least squares regression was: log10 (observed) = 0.9217 x log10 (expected) + 0.0604 while the Concordance Correlation Coefficient (ccc) between the two sets of values was 0.9798. Conclusions: The ECL assay had high analytical sensitivity, detecting LF at levels significantly below the observed clinical range (50-300 ng/mL) for symptomatic inhalation anthrax and within the clinical range (5 pg/mL-1264 pg/mL) for cutaneous anthrax. These data show that clinically relevant concentrations of LF can be measured using an ECL assay and a serum matrix. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC.
Rapid Detection and Antibiotic Susceptibility Analysis of Bloodborne Bacillus anthracis Using Phage-mediated Bioluminescence

N. J. Sharp, R. S. Makkar, J. Molineux, D. A. Schofield; Guild BioSci., Inc, Charleston, SC, Univ. of Texas at Austin, Austin, TX

Bacillus anthracis, the etiological agent of anthrax, is a Tier 1 pathogen most likely to be used in a bioterrorism attack. Contraction of anthrax occurs when B. anthracis endospores enter the body through inhalation, ingestion or via cutaneous exposure. Disease progression is fairly rapid, and can be fatal if not treated within the first 24 h of symptom onset. However, early diagnosis of anthrax infection is problematic as inhalational and gastrointestinal anthrax symptoms can be non-specific and are often difficult to differentiate from less serious ailments. Additionally, due to the potential release of deliberately engineered antibiotic resistant strain, rapid detection technologies capable of simultaneously providing antibiotic susceptibility profiles are essential for the timely administration of appropriate antimicrobial therapy. To facilitate rapid detection of B. anthracis, we developed a species-specific ‘light-tagged’ reporter phage capable of transducing bioluminescence to infected cells. This was achieved by integrating the genes encoding bacterial luciferase (luxA and luxB) into the genome of the temperate B. anthracis Wβ phage. Wβ::luxAB displayed 100% inclusivity (37/37 strains) and 96% specificity (6/119 Bacillus spp.; 0/38 non-Bacillus spp.) for B. anthracis. The ability of Wβ::luxAB to detect B. anthracis from positive blood cultures and whole blood was assessed. A signal response was obtained from culture-positive BacT/Alert and Bactec bottles within 60 min. The time to detection from whole blood was longer (5 h); however, a limit of detection of 101 CFU/mL was achieved without the need for extensive sample processing. In addition, Wβ::luxAB was able to provide antibiotic susceptibility information which mirrors the conventional Clinical Laboratory Standards Institute microdilution method in a 5-fold faster timeframe. Thus, the reporter phage technology displays promise for detection and antimicrobial susceptibility analysis of septicemic anthrax at clinically relevant concentrations.

High Through-put Detection of Yersinia pestis Using Reporter Phage

D. J. Wray, D. A. Schofield; Guild BioSci,s, Inc, Charleston, SC

Yersinia pestis, the causative agent of plague and tier 1 select bioterrorism agent, is usually fatal without rapid diagnosis and treatment. The WHO and CDC employ the classical phage assay using phage ΦA1122 in pure culture for identification, as ΦA1122 has shown to lyse ~100% of all Y. pestis strains. Previously, we genetically engineered ΦA1122 with luxAB, the genes encoding bacterial luciferase, to generate a bioluminescent reporter phage and demonstrated its ability to rapidly, specifically, and sensitively detect Y. pestis in pure culture, human serum and enriched whole blood. Here, we investigated the reporter phages functionality in a simulated bioterrorism event requiring the high through-put testing of 500 samples. Y. pestis(attenuated strain) was grown on Sheep Blood Agar plates at 35°C for 48 h. Analysis was performed using a 96 well microtiter plate format, 2 class 2 biological safety cabinets, two technicians, and one microplate luminometer to measure phage-mediated bioluminescence. Six 96 well plates were preloaded with Luria Bertani media harboring the reporter phage. A total of 504 colonies were picked using micropipette tips, along with a blood agar plug to monitor well usage, incubated at room temperature (~25°C) and read 60 min after the initial pick. The reporter phage detected 100% (n=504) of Y. pestis samples in 3.5 h. The combined ‘hands on time’ for both technicians was 4.25 h but, depending on the user, it required 30-40 min to pick 96 colonies into each plate and 22 min to read bioluminescence from the plate. Thus, the rate limiting and time consuming step was the initial picking of the colonies. Although every sample was positively identified as Y. pestis, there was a 1000-fold variation in signal strength. As signal strength is dose dependent, the variability was most likely due to inconsistent colony picking, resulting in different bacterial loads. Nevertheless, the reporter phage displays promise for the detection of Y. pestis, especially in a bioterrorism event when high throughput and ease of use is of paramount importance.
Diagnostic Performance of Smear Microscopy and Incremental Yield of Xpert Test in Detection of Pulmonary Tuberculosis in Rwanda


Background: Tuberculosis control program of Rwanda is currently phasing in light emitting diode-fluorescent microscopy (LED-FM) as an alternative to Ziehl-Neelsen (ZN) smear microscopy for tuberculosis testing. This, alongside the newly introduced Xpert test is expected to improve diagnosis of tuberculosis and detection of Rifampicin resistance. We assessed the accuracy of smear microscopy and the incremental sensitivity of Xpert test at tuberculosis laboratories in Rwanda. Methods: Cross-sectional study involving four laboratories with ZN microscopy and four laboratories with LED-FM. After smear microscopy, the remaining samples, of a single early-morning sputum from 648 participants, were tested using Xpert test and mycobacterial culture as a reference standard. Sensitivity and specificity of each test was compared and the incremental sensitivity of Xpert test after a negative smear was assessed. Results: A total of 96 participants were culture positive for Mycobacterium tuberculosis. The sensitivity (95% CIs) was 55.7% (50% - 61.4%) for ZN, 42.8% (37.4% - 48.2%) for LED-FM and 80% (77% - 83%) for Xpert. The overall incremental sensitivity of Xpert was 31.1% from ZN and 34.2% from LED-FM. The incremental sensitivity of Xpert to either smear methods was statistically significant in peripheral 32.6% p=0.010, 33.3% p=0.006 and not significant in intermediate laboratories 25.0% p=0.125, 37.5% p=0.250 for ZN and LED-FM respectively. Conclusions: Our study findings of the early implementation of LED-FM revealed a significant implementation strategy for Xpert placement at the peripheral laboratories where its diagnostic value is much realized compared to the existing smear microscopy methods. It also supports upward phasing out of smear microscopy such that microscopy experience is maintained at higher levels that can provide refresher training if needed.

Detection of Protein Toxins With LRN-B Mass Spectrometers

S. R. Kalb, A. E. Boyer, J. R. Barr; CDC, Atlanta, GA

Background: Human exposure to protein toxins like botulinum neurotoxins, anthrax lethal factor, ricin, and abrin can cause death. Detection of these protein toxins impacts medical countermeasure use and aids in prevention of future exposure. Detecting the toxin’s enzymatic activity gives insight into toxicity, which is directly related to public health risk. Therefore, detection of enzymatic activity rather than the toxin itself is preferable for LRN laboratories. Recently, two mass spectrometers have been FDA approved for the rapid identification of microorganisms, resulting in movement of mass spectrometers into biological and clinical laboratories. Methods: Our laboratory has developed methods to detect the enzymatic activity of four protein toxins: botulinum neurotoxins, anthrax lethal factor, ricin, and abrin. All methods first utilize antibody-capture with automated washing to purify the toxin from the complex matrix (serum, stool, food) and then involve incubation of the captured toxin with a substrate which mimics the toxin’s natural target. Following incubation, the reaction supernatant containing the substrate (not the toxin) is introduced into a mass spectrometer which records the mass of the substrate and its modification indicating presence of the active toxin. BoNT and anthrax lethal factor utilize peptide substrates, resulting in two cleavage products whereas ricin and abrin use RNA substrates with a single
Depurination of the substrate upon exposure. Results: Using the Bruker Biotyper currently in use in select LRN-B laboratories, the method can achieve a limit of detection of approximately 1 mouse LD50 (estimated to be 10 pg) of BoNT/A, B, E, and F in serum, stool, and select foods. The method can detect approximately 4 pg of anthrax lethal factor in serum or plasma, and 100 pg of ricin or abrin in buffer. Conclusions: Detection of the enzymatic activity of botulinum neurotoxin, anthrax lethal factor, ricin and abrin in complex matrices using mass spectrometers currently in select LRN-B laboratories allows for clinically relevant measurements to accurately assess public health threats.

---

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** D. Diagnostics  
**Poster Presentation Number:** 083 (D)

**High-throughput Screening Assay for Detection of Anthrax Lethal Factor**

M. H. Epperson¹, J. Martin², A. Sabnis¹, C. P. Quinn¹, N. Kamal¹, J. Schiffer¹; ¹CDC, Atlanta, GA, ²IHRC Inc, Atlanta, GA

**Background:** Anthrax lethal factor (LF) is the earliest detectable biomarker of Bacillus anthracis infection and therefore a key target for diagnostics. Currently, there are no laboratory assays for LF detection that can be completed using standard laboratory equipment. This study describes a sandwich ELISA with high analytic sensitivity for LF in serum. The format accommodates 72 samples per plate and can be run in less than four hours on a standard plate reader. **Methods:** Two anti-LF mouse monoclonal antibodies (AVR3139 and AVR3134) were used to capture and detect LF, respectively. AVR3134 was direct-labeled with HRP for detection using an ABTS substrate system. LF spiked sera and negative sera were used to assess assay detection limits, accuracy, and precision. Spiked samples were created by pipetting known amounts of LF antigen into normal human serum. A panel of negative sera from Anthrax Vaccine Adsorbed (AVA) recipients and sera and plasma from subjects with human anti-mouse antibodies (HAMA) was evaluated for assay interference. Results: The lower limit of detection in spiked human samples was approximately 350 pg/mL. The reactivity threshold was optimized to maximize specificity and sensitivity. Each plate can test 72 samples and the assay run time is under four hours. Signal from AVA and HAMA was absent or below the reactivity threshold. **Conclusions:** The ELISA assay had high analytical sensitivity, detecting LF at levels significantly below the observed clinical range (50-300 ng/mL) for symptomatic inhalation anthrax and within the clinical range (5 pg/mL-1264 pg/mL) for cutaneous anthrax. The assay was optimized to maximize sensitivity and specificity and reduce false positives from interfering substances. These data show that clinically relevant concentrations of LF can be measured in high-throughput format using standard lab instrumentation. The assay provides a rapid screening tool for identification of B. anthracis infection. The conclusions in this report do not necessarily represent the official position of the Centers for Disease Control and Prevention.

---

**Session Type:** Poster Session  
**Session Number:** 016  
**Session Title:** Poster Session 2  
**Poster Presentation Time:** Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:** D. Diagnostics  
**Poster Presentation Number:** 084 (D)

**Scrub Typhus and Associated Co-infections: Study of Clinical Epidemiology and Comparative Evaluation of Diagnostic Modalities in a Tertiary Care Hospital in New Delhi, India**

R. Chaudhry, N. Gupta, C. Thakur, B. R. Mirdha, B. K. Das; All India Inst. of Med. Sci., New Delhi, India

**Introduction:** Scrub typhus is a febrile illness caused by Orientia tsutsugamushi. Serology is the preferred diagnostic tool with Indirect Immunofluorescence assay (IFA) as the current gold standard. Various co-infections have been reported in past along with scrub typhus. **Material & Methods:** A total of 229 patients clinically suspected cases of Scrub typhus were enrolled in the study. Clinical data of the patients were collected...
and analysed. The method for diagnosis was IgM Immunofluorescence assay. Other serological assays including IgM ELISA and IgM RFA were also performed to evaluate against the gold standard. IgM ELISA was also performed for Leptospirosis, Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumophila. Peripheral smear and antigen detection was done for Malaria. Results: A total of 33 patients (14.4%) of scrub typhus were detected with 87% of them presenting in the months of September to November. Fever of >39 °C was seen in all the patients. Rash and eschar were seen in 24.2% and 18.2% patients respectively. The calculated sensitivity for IgM ELISA and IgM RFA were 92 and 76% respectively while the specificity was 98.5% and 100 % respectively. 7 patients were additionally positive for Leptospirosis while 4 were positive for Mycoplasma pneumoniae. **Conclusion:** The nonspecific symptoms and lack of classical manifestations makes the diagnosis of scrub typhus difficult. IgM ELISA and IgM RFA appear to be a good alternative to IgM IFA. There is a definite need to increase awareness and heighten the suspicion, especially in the light of increasing number of patients presenting with atypical manifestations.

---

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** D. Diagnostics  
**Poster Presentation Number:** 085 (D)  

**Differential Diagnosis of Swine Influenza by Real Time RT-PCR**

**O. Solodiankin¹, B. Stegniy¹, E. Smolyaninova¹, A. Gerylovich¹, V. Bolotin², M. Bashchenko², A. Buzun¹; ¹Natl. Scientific Ctr. «Inst. of Experimental and Clinical Vet. Med.», Kharkiv, Ukraine, ²Natl. Academy of Agrarian Sci. of Ukraine, Kiev, Ukraine**

**Background:** Any problems associated with health lesions in animals lead to significant economic losses. Swine influenza is the very dangerous problem of swine breeding and this zoonotropone is active disease nowadays. The influenza virus spreads among pigs fast and leads to the depletion of animals, loss of weight, culling of bred pigs and makes animals more susceptible to the other infections. Consider the fact that the influenza virus infects humans, it is very important to differentiate it in a short time from other agent that can cause respiratory disorders such as porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type II (PCV-II). Goal: To develop a multiplex real-time PCR, that will allow differentiating swine influenza virus (SIV) from PCV-II and PRRSV. Results: In our work, we used strain 1024 of PCV-II, PRRS virus strain DV and cDNA from H1N1 strain of SIV as positive controls. DNA and RNA isolation was conducted by affinity adsorption, Homchynsky method, and reverse transcription was performed by commercial kit «First Strand cDNA Synthesis Kit», and amplification was carried out by commercial kit Master Mix «AgPath-ID One-Step RT-PCR Kit». The primers and probes were selected for detection of swine influenza virus – 5'-Joe-AGG ACT GCA GCG TAG ACG CTT TGT-BOQ-1-3, for European PRRSV – Fam-CCT CTG CTT GCA ATC GAT CCA GAC BOQ-1, for circovirus – Rox-AGC AGC AAC ATG CCC AGC AAG AAG BOQ-2, which were described by S. Kleiboeker and modified by authors. For validation of multiplex RT-PCR, we optimized protocol: reverse transcription at 45°C for 10 min, initial denaturation at 95°C for 10 min followed by 40 amplification cycles of 95°C for 15s and 60°C for 30s. Ct values were 14.7±0.5 (SIV), 17.6±0.3 (PRSV) and 20.2±0.7 (PCV-II). **Conclusion:** We developed a multiplex PCR that is sensitive and specific for differential diagnosis of swine influenza. It will allow to timely response to outbreaks and prevent the spread of the disease in Ukraine.
Development and Implementation of real-time PCR for Detection DNA of Animals’ Brucellosis Causative Agent in Ukraine

M. Sytiuk, S. Nychyk, I. Halka, V. Spyrydonov, L. Muzykina; Inst. of Vet. Med. NAAS, Kyiv, Ukraine

Introduction. Brucellosis is the zoonotic disease of agricultural and wild animals caused by different species of Brucella, and develops chronic course of the disease. The goal. Considering the fact that in Ukraine the monitoring on Brucellosis among animals is not fully effective (monitoring diagnosis is made using Rose Bengal test), there is a need to develop more sensitive and specific test systems, such as, real-time PCR to detect pathogen DNA. Methods. Specific primers to detect DNA of two Brucella species (Br. melitensis, Br. ovis) and subspecies primers were used. During real-time PCR the amplifier Rotor Gene 6000 was used. Results. Three real-time PCR kits for Brucellosis detection developed in the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine. Two test kits were developed for the detection of two main species of Brucella genus (Br. melitensis, Br. ovis) and another test kit - for Brucella genus detection. Specific primers were selected, and optimized temperature for annealing (55 °C) and elongation process (15 sec at 95 °C, 20 sec at 55 °C, 30 sec at 72 °C) defined. Frequency of amplification was 40 cycles. The developed kits had the specificity of 99.5 % and sensitivity - 99.1 %. The real-time PCR test kits were successfully used for detection of DNA pathogen (Brucella genus) and for Br. melitensis and Br. ovis as well. Conclusions. Three test kits for real-time PCR for Brucella DNA detection were developed. They are specific and sensitive and can be used for Brucellosis diagnostics.

Experimental Transmission of Bartonella Henselae to Dogs Through Indirect Contact With Bartonella Henselae-infected Cats Infested With Ctenocephalides Felis


Bartonella henselae is transmitted amongst cats by Ctenocephalides felis. Although pet dogs have been shown to be infected by B. henselae, the role of C. felis transmission of this bacterium to dogs has not been evaluated. The purpose of this experimental study was to evaluate the competency of C. felis as a vector for the transmission of B. henselae from bacteremic cats to dogs. Specific pathogen free cats (n = 4) and beagle puppies (n = 16) were housed in three adjoining enclosures, separated by mesh to allow C. felis to move freely amongst the enclosures with no direct contact between the kittens and puppies. Cats were inoculated intravenously with B. henselae; infection was confirmed by seroconversion, culture and polymerase chain reaction (PCR). The cats were housed in a central enclosure flanked by the 8 puppies on each side. Insectary raised Ctenocephalides felis (50 males and 50 females) were placed on each of the 4 cats monthly for 13 applications. Dogs were bled weekly for serology (IFA and Western blotting), 16-23S intergenic transcribed spacer elements (ITS) PCR and Bartonella alpha proteobacteria growth medium (BAPGM) enrichment blood culture. Using indirect immunofluorescent antibody (IFA) assay, 10 of 16 puppies seroconverted to B. henselae between weeks 6 and 14. Western blot analysis revealed, 50-60kDa immunodominant protein band observed in both IFA positive and negative dogs. No correlation was observed between IFA and Western blot results. After BAPGM enrichment blood culture, B. henselae DNA was amplified from 5 puppies between weeks 20 and 26. These results support transmission of C. felis to dogs that are co-housed with B. henselae bacteremic cats. We conclude that C. felis is a competent vector for B. henselae transmission from cats to dogs. However, the results of this experiment cannot be extrapolated to all B. henselae strains, flea populations or dog breeds.
**Detection and Identification of Rickettsia Based on Fluorescence in situ Hybridization**

K. Aistleitner¹, K. Stoecker¹, H. Ge², A. L. Richards³, R. Woelfel¹; ¹Bundeswehr Inst. of Microbiol., Munich, Germany, ²Naval Med. Res. Ctr., Silver Spring, MD

Rickettsia are obligate intracellular bacteria, usually pathogenic to humans. They are found worldwide and are usually transmitted via an arthropod vector. Rickettsia are fastidious to culture and isolation attempts from vectors or human samples are frequently affected by bacterial contamination. The gold standard for the diagnosis of an acute rickettsial illness is qPCR, but this very sensitive method is accompanied by well-known drawbacks such as the lack of multiplex species differentiation. Rickettsia-specific qPCR also provides no information about the presence, quantity and quality of other bacterial contaminations. In order to circumvent these pitfalls and to establish a new method to detect and to differentiate Rickettsia in clinical samples and cultures, we report on the development of a fluorescence in situ hybridization (FISH) based assay for the rapid identification of Rickettsia. Although some published Rickettsia probes exist, a comprehensive probe set for the simultaneous detection and differentiation of all known Rickettsia species is hitherto not available. Thus, we designed group- and species-specific probes for Rickettsia using the arb software package. Probe specificity was evaluated by formamide series and if necessary competitor probes were included in the hybridizations. The developed group-specific probes are used to detect rickettsiae and differentiate between members of the spotted-fever and typhus-group of Rickettsia in a first step. In the following hybridization, species-specific probes are used to identify the human pathogens R. prowazekii, R. typhi and R. rickettsii as well as several other medically-relevant rickettsial species. The development of a FISH-based method is useful in overcoming the drawbacks of serology and qPCR. The ability of this new assay to visualise rickettsiae inside host cells within only four hours and the highly specific nature of the test makes it a valuable tool for the diagnoses of acute rickettsial infection.

**Development and Validation of Novel Multiplex Immunoassay Panels for Detection and Differentiation of Toxins**

N. Venkateswaran¹, J. Walker¹, K. Lubold¹, L. Stanker², S. Diepold¹, T. W. O'Brien¹, W. M. Nelson¹; ¹Tetracore, Rockville, MD, ²USDA, ARS, PWA, WRRC-FTDP, Albany, CA

Multiplexed assays are critical for detection of commonly suspected plant and bacterial toxins that are hazardous to human and animal health. We have developed and validated two different multiplex panels for screening of 6 toxins, Ricin, Abrin, SEB, botulinum neurotoxins (BoNT) A, B and E and confirmation of BoNT A, B and E respectively. While validating the screening panel for 6 toxins we determined that different subtypes of BoNT were not accurately identified. This inspired us to design an additional confirmatory multiplex panel for further detection and differentiation of BoNT A, B and E. These panels were designed using fluorescent microspheres on Luminex multiplex platform. A panel of four different internal controls to assure the quality of assay was also included in each multiplex panel. Design, development and analysis of multiplex panels based on immunoassays are very complex due to presence of cross reactivity of antibodies to closely related targets. Hence, we designed two simple algorithms for analysis of raw data acquired from each of these panels based on pattern of reactivity seen for different analytes in the multiplex panel with different capture and detection reagents. The assays and the algorithms were validated by two different operators, on two different days and on two different machines for reproducibility and LOD confirmation studies. All of the raw data was plugged into the algorithm and accuracy of 95% and above was obtained for screening panel. Sterile culture supernatants for different subtypes and serotypes of BoNT producing...
clostridium strains were tested using BoNT confirmatory panel. 32/32 BoNT A, B or dual toxin producers and 7/7 BoNT E samples were correctly detected and identified. BoNT A and B culture supernatants were also confirmed by real time PCR for presence of toxin genes. We conclude that combination of these two multiplex panels provides rapid, sensitive and specific screening and confirmatory tool for detection and differentiation of six toxins tested in this study.

**Session Type:** Poster Session  
**Session Number:** 016  
**Session Title:** Poster Session 2  
**Poster Presentation Time:** Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:** D. Diagnostics

**Poster Presentation Number:** 090 (D)  
**Rapid Eukaryotic (Fungi / Protozoa) Identification by Next Generation DNA Sequencing**

**D. S. Missan, J. E. Ellis, S. E. Fry; Fry Lab. LLC, Scottsdale, AZ**

The need for rapid identification of unusual parasitic or infectious organisms in a combat theater or in response to a biological threat has become increasingly appreciated. Unbiased molecular methods are a compelling alternative for the rapid identification of organisms. NGS has the potential to address challenges currently faced by the emerging infection response system. Currently, NGS methods require highly trained operators while available bioinformatics analysis methods are excessively time consuming for routine use. We hypothesize that an NGS-based method could be created that is suitable in these scenarios. In order to address NGS challenges, the Rapid Infectious Disease Identification system was utilized to detect and characterize fungi and protozoa of clinical importance. The RIDI™ system is executed with minimal operator guidance, thus minimizing errors during analysis. The system has proven capability with the three major NGS platforms; however, the IonTorrent system is preferred due to speed advantages. Sequence information is characterized utilizing publicly available NCBI databases in conjunction with RIDI™ specific databases. RIDI™ aims to identify high probability sequence identification matches in addition to more divergent sequence matches of various thresholds, thus allowing recognition and differentiation between related species, as well as detecting microorganisms that may represent novel species. The system was challenged using defined ATCC reference DNA and culture standards of more than 28 species individually and in varying combinations. The system accurately and rapidly identifies more than 86.46% ± 17.21% of the DNA sequence reads to the genus level of both reference standards and simulated clinical samples. We were able to use NGS technology in conjunction with RIDI™ to rapidly detect eukaryotic microbes in less than a work shift and with multi-sample throughput. The system is expected to exceed current methods where novel organisms may yield a false negative result or where rapid results are critical for a proper biodefense response.

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** D. Diagnostics

**Poster Presentation Number:** 091 (D)  
**International Genomics Collaboration Development for Enhanced Global Health Security**

**H. Cui, T. Erkkila; Los Alamos Natl. Lab., Los Alamos, NM**

Reducing global health security risk from the spread of dangerous infectious diseases is a shared priority among the worldwide public health communities. It has also become an overarching objective for international cooperative biothreat reduction and scientific engagement efforts. Next Generation Sequencing-enabled genomic research provides a suitable tool with broad global health security applications. In support of the overarching
scientific engagement objectives, the Genome Science Program at the Los Alamos National Laboratory has been leveraging a long history of genomics research and microbial genomic sequencing expertise to support a growing number of partner countries on four continents in developing molecular genomic-based pathogen detection and characterization capabilities. Our approaches to achieving such objective include understanding partner country needs and gaps in building genomics and bioinformatics capacities, followed by scientific and technical training, facility building, and dissemination of pipelines and processes for microorganism genotypic characterization. Continuous subject matter expertise reachback support is provided to the collaborators. While these genomics capabilities are being developed, the collaborators start engaging in scientific collaborations utilizing Next Generation Sequencing and other molecular techniques, such as real-time PCR and immunoassays. By applying these methods and correlating the findings, we aim to better understand emerging and reemerging infectious diseases with global concerns. The collaboration efforts will not only benefit the host countries and regions with state-of-the-art life science methods and technologies, but also build a trusted international network with a shared passion in addressing global emerging infectious disease challenges. Such networks provide the potential for sharing resources, which is essential for approaching the One Health objective and reducing health threats globally. Here we detail our scientific collaboration efforts and highlight the initial research towards pathogen detection and characterization.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: D. Diagnostics
Poster Presentation Number: 092 (D)

International External Quality Assurance Study of Ebola Virus Diagnostic Laboratories


In the current Ebola virus outbreak in West Africa more than 28 000 individuals have been infected claiming over 11 000 deaths. From an index case in December 2013 in the east of Guinea the infection has rapidly spread to the neighboring countries of Liberia and Sierra Leone but individual cases and localized outbreaks were also reported from Mali, Nigeria, Senegal, Europe and USA. Effective outbreak containment measures include the strict isolation of Ebola virus disease (EVD) patients to achieve an efficient interruption of Ebola virus transmission. For this, reliable and rapid laboratory diagnosis of EVD suspected patients is the pre-requisite. In order to survey the performance of diagnostic laboratories involved in or preparing for the detection of Ebola virus RNA an external quality assurance (EQA) study was initiated by World Health Organization and organized by ENIVD in cooperation with Robert Koch Institute, the Bernhard-Nocht-Institute in Hamburg and Philipps University in Marburg. A panel of 11 samples, different strains of Ebola virus - including a recent isolate from Gueckedou, Guinea - , Marburg virus, and negative controls was established to test specificity and sensitivity of the methods used by the participating laboratories. Inactivated samples were freeze-dried and sent out by regular mail or express mail. At present, from 37 countries worldwide more than 60 laboratories, including 30 from Africa, mainly from the outbreak region, participated in the study. Participants were asked to report their results as well as the methods used for sample preparation and for PCR detection. While most results were good or acceptable 11 participants showed a clear need to improve sensitivity and were immediately informed. This lack of sensitivity did not correlate with a particular PCR assay or extraction method.
DNA-directed Antibody Immobilization for Universal Biothreat Agent Detection in the Field

C. Pöhlmann1, L. Bellanger2, B. G. Dorner3, T. Elßner1; 1Bruker Daltonik GmbH, Leipzig, Germany, 2CEA Service de Biochimie et Toxicologie Nucléaire, Bagnols-sur-Céze, France, 3Robert Koch-Inst., Berlin, Germany

Natural outbreaks and the willful use of biothreat agents for acts of terror demonstrate the need to immediately detect and identify biothreat agents. The portable immunoassay based biochip platform pTDi was shown to be well suited for a very sensitive and fully automated detection of biothreat agents. However, one challenging aspect of protein microarray production is printing the proteins onto the sensor surface. Here, we demonstrate an alternative solution for site specific immobilization of antibodies using DNA-directed immobilization (DDI) and the usage of this way generated biochips for sensitive detection of biothreat agents. In DDI, each capture antibody is encoded by a specific DNA sequence covalently attached to it. The antibodies immobilize on their complementary DNA probes immobilized on the gold electrode surface via sequence specific DNA-DNA hybridization. This immobilization approach has several advantages over direct covalent attachment of antibodies, such as increased availability of binding sites for analyte capture by decreasing the steric hindrance and more favorable orientation of antibodies. Exemplary, we present an addressable biochip carrying antibodies specific for F. tularensis, orthopox virus and the plant toxin ricin. Limit of detection (LOD) for F. tularensis and vaccinia virus is approx. 103 CFU mL-1 or 104 PFU mL-1, respectively, whereas LOD for ricin is 0.1 ng mL-1 applying an analysis time of approx. 25 minutes. Furthermore, we demonstrate specificity of the addressable biochip and detection of biothreat agents in food and environmental matrices. These results demonstrate the feasibility of DDI for detection of biothreat agents on a biochip using a portable readout platform. Furthermore, we showed improved assay sensitivity compared to conventional immobilization procedure. Research is funded by the Federal Ministry of Education and Research (BMBF) joint research Project GEFREASE (13N12222).

Use Municipal Wastewater to Assess Community Salmonellosis Disease Burden

T. YAN1, J. M. Shelton1, E. Pagaling1, P. O’Brien2, C. Whelen2; 1Univ. of Hawaii at Manoa, Honolulu, HI, 2Hawaii State Labs, Hawaii Dept. of Hlth., Honolulu, HI

Municipal wastewater systems (MWSs) collect wastewater from households of a community to centralized locations, and hence provide a potentially unparalleled capability in determining community disease burdens. To test this idea, Salmonella concentration in municipal sewage at a wastewater treatment plan in Honolulu, Hawaii was monitored over a 55-week period, and Salmonella isolates from sewage samples were obtained and analyzed to determine their serotypes and PFGE genotypes. The abundance of Salmonella in sewage exhibited a significant rank correlation to clinical salmonellosis case numbers with a two-week lag period. The Salmonella isolates from the wastewater exhibited extensive genotypic difference from those collected from healthcare clinics. However, a Salmonella strain involved in a salmonellosis outbreak in the community was detected as the dominant strain in wastewater during the outbreak period. These results support the feasibility of using municipal wastewater to assess community disease burdens and to detect disease outbreaks.
Next-generation Water-quality Sensors For Defense And Public Health

S. P. Sherchan; Fresno State, Fresno, CA

Water utility treatment failure, as well as intentional or accidental water intrusions can introduce biological and/or chemical contaminants into public drinking water distribution systems. However, recently developed real-time water quality sensors can be implemented to detect such contamination events. The overall objective of this study was to evaluate the potential for real-time monitoring of bacterial contamination of potable water using several different water quality sensors including: the HACH Monitoring Platform; the Instant BioScan, the JMAR BioSentry unit; and the S::CAN spectro::lyser technology. For this, Bacillus thuringiensis spores were used as a surrogate for Bacillus anthracis. The minimum threshold response of sensors to the microbial contaminant was determined by injecting B. thuringiensis spores and E. coli into deionized (DI), filtered or unfiltered tap water. Out of these three evaluated sensors, the BioSentry sensor was capable of detecting introduced spores. In contrast, the HACH and S::CAN units were not capable of direct detection of spores; however, these two sensors can detect changes in water quality parameters such as turbidity, pH, temperature, total organic carbon and conductivity, due to media that may be associated with spores. Thus, these sensors can be integrated into a contaminant warning system for monitoring intrusion events in water distribution systems.

Rapid and Specific Detection of the Five Major Enterohemorrhagic Escherichia coli Serogroups : 026, 0103, O111, O145 and O157

N. Morel1, E. Yang1, K. Desvilliers1, J. Dano1, M. Plaisance1, C. Tadla2, M. Crozier Bouvier2, D. Sergentet1, S. Simon1, E. Loukiadis2, H. Volland1; 1CEA, Gif-sur-Yvette cedex, France, 2VetAgro Sup, Marcy l'Etoile, France

Shiga toxin-producing Escherichia Coli (STEC) are considered as emerging pathogens in public health, causing numerous cases of human infections, ranging from mild diarrhea to severe hemorrhagic colitis and hemolytic uremic syndrome (HUS). The transmission essentially occurs by consumption of contaminated food of animal origin. Although Shiga toxin itself is not considered as a biotreat agent, STEC are listed in category B biological warfare agents of CDC. To specifically detect the major STEC associated with human STEC infection, we have produced and characterized monoclonal antibodies directed against five strains belonging to serotypes 0157:H7, 026:H11, O103:H2, O111:H8 and O145:H28. Using the best combination of capture and detection antibodies specific to each of the five STEC serogroups, we have developed enzyme-immunoassay (ELISA), bead-based assay and immunolateral flow immunoassay (LFIA). The ELISA and bead-based assay recognized STEC with a limit of detection of 1.2x103 to 3x104 cfu/mL depending on the strain tested whereas for the LFIA the limit of detection ranged from 105 to 107 cfu/mL. The applicability of LFIA to detection of STEC in food was tested in 25 g of ground beef or cheese artificially contaminated with 1 to 10 bacteria. After 18-24h post-enrichment all STEC tested were detected positive by LFIA except O111 in cheese.
Detection of Bacillus anthracis Spores from Environmental Samples Using ‘Bioluminescent’ Reporter Bacteriophage

C. Nguyen\textsuperscript{1}, N. J. Sharp\textsuperscript{2}, M. A. Page\textsuperscript{3}, I. J. Molineux\textsuperscript{4}, D. A. Schofield\textsuperscript{2}; \textsuperscript{1}Med. Univ. of South Carolina, Charleston, SC, \textsuperscript{2}Guild BioSci., Charleston, SC, \textsuperscript{3}United States Army Corps of Engineers, Champaign, IL, \textsuperscript{4}Univ. of Texas at Austin, Austin, TX

Anthrax is a serious and rapidly progressive disease caused by Bacillus anthracis. As a Tier 1 select agent, release of B. anthracis spores could potentially lead to a massive anthrax outbreak and public panic. Spores can persist and remain infectious for more than 200 years once released, leaving the affected environment uninhabitable until it is remediated. To ensure public health preparedness for such an event, an efficient and rapid environmental detection system for B. anthracis spores is essential.

B. anthracis can be identified using microbiological methods such as the FDA-approved gamma bacteriophage (phage) lysis assay, or molecular methods such as PCR. However, in general, these assays are not suitable for spore detection from complex environmental samples and do not discriminate between viable and non-viable cells. To address this need, we previously generated a ‘light-tagged’ B. anthracis reporter phage by integrating genes encoding luciferase into the genome of the temperate B. anthracis Wβ phage. Wβ::luxAB was able to rapidly (20 min) confer bioluminescence to viable cells only, an important pre-requisite for environmental detection.

The efficacy of Wβ::luxAB to detect spore-contaminated soil and water from three different water sources (pond, brackish and lake water) was assessed. The presence of endogenous microbial flora, high salt content and poor vegetative cell persistence inhibited detection. Nevertheless, after optimization, a limit of detection of 10\textsuperscript{1}, 10\textsuperscript{2}, and 10\textsuperscript{2} CFU/mL were achieved within 12h from pond, brackish and lake water, respectively. The detection of spores directly from soil samples was more challenging with a limit of detection of 105 CFU/g in 6h. However, spore extraction from samples was not required, which greatly simplified the assay procedure. Collectively, reporter phage displays potential for rapid detection of viable spores from complex contaminated environmental samples.

Development of Multiplex Immunoassays for Staphylococcal Enterotoxins Detection and Diagnosis

C. Goulard-Huet, K. Devilliers, M. Plaisance, S. Fuchs, N. Khreich, H. Volland, S. Simon, C. Féraudet-Tarisse; CEA (Atomic Energy Agency), Gif Sur Yvette, France

Staphylococcal enterotoxins (SE), exotoxins synthesized by enterotoxigenic strains of the gram(+) bacterium Staphylococcus aureus, are a major cause of food poisoning, causing violent vomiting and abdominal cramping with or without diarrhea. Besides SEB, which is considered as a potential bioweapon and classified as category B by the Centers for Disease Control and prevention (CDC) because of its high toxicity, stability, and ease of production and aerosolisation, other SEs might be used. Some of them are well characterized, such as SEA which is the most frequent SE found either alone or in combination with other SE in food poisoning outbreaks (>75%), while no or little informations are available on toxicity and prevalence of other SE like SEG, SEH and SEI whose genes have been frequently detected in clinical strains (Roussel S., et al., 2015), but for which no commercial detection/diagnostic test is available. Thus, there is a need for rapid, sensitive, and specific methods for the detection of these toxins for dual applications (natural and intentional food, air or water poisoning). The objective of our work is to develop a rapid, cheap, accurate and simple immunological method to detect simultaneously several SEs.
Introduction: The high-mountainous regions of Lake Sevan, Gyumri and Sjunik are foci for tularemia. The geographical landscape of these regions support the habitat of both the field mouse, Microtus arvalis, which is the primary animal reservoir, and ticks (Dermacentor marginatus, Hyalomma asiatica), which are the primary vectors of the disease. Human cases of tularemia have been reported and linked to exposure to the field mouse and tick vectors in these regions. Objective: The objective of the study was to conduct continuous monitoring for tularemia in these high-risk regions in order to implement timely measures to prevent morbidity in those areas, with a special emphasis on resorts and tourism sites. Materials and Methods: Annual field collection activities of rodents and ticks are conducted from the beginning of April to the end of December. Collection methods for rodents included excavating burrows, counting the number of rodents by route, and trapping. Ticks were taken off of collected rodents and from rodent burrows. Suspensions of tissue samples from rodents and ticks were cultured. Findings: From 2010 to 2015, about 100 cases of epizootic manifestations of tularemia were recorded; tularemia was isolated from 124 cultures during this time period. In 2015, a culture was isolated from a vector in Jermuk and in Saravan. Conclusions: Epizootic activeness has been of local nature. Although the vectors of tularemia have been reduced due to unfavorable climate conditions, foci of tularemia are still active. Monitoring of tularemia foci is mandatory, especially in recreational areas. This monitoring allows us to take timely measures to prevent morbidity among people, as in the cases in 2015. As a result of early detection in vectors, we were able to implement control measures and no human cases were reported. This study demonstrates the need for further epizootic research of vectors in the natural foci of diseases to control and manage the epizootic situation in a timely manner. It is noteworthy to highlight the necessity of implementing serological monitoring which will enable us to keep potentially dangerous foci under control all year round.

Use of Non-threat Surrogate Materials in Lieu of Attenuated Biothreats in a Functional Training Exercise

A mission capability for on-site biological assessment requires routine training to ensure operators, technologies, and protocols are working properly within the concept of operations. The use of inactivated biological threats is challenging due to safety concerns, public perception, the need for specialized facilities, and potential for equipment contamination leading to false positives. To address this issue, NIST developed a non-threat surrogate: Saccharomyces cerevisiae NE095, a Baker's yeast modified to contain DNA sequence External RNA Control Consortium-00095 for specificity. Herein, we evaluated fitness for purpose of the yeast in a real-time, functional sample collection and laboratory testing exercise, Operation Vigilant Sample (OVS) IV on July 14-16, 2015. Local responders, state public health laboratories, National Guard Civil Support Teams, Environmental Protection Agency, and BioWatch applied state response plans in a biothreat scenario (putative Yersinia pestis). A protocol was developed to affix known numbers of yeast cells onto metal coupons, which were placed throughout the training site (Guardian Centers, Perry, GA). First responders collected samples using a protocol modified from the Bacillus anthracis sampling procedure (Centers for Disease Control and Prevention). Mobile and public health laboratories successfully detected the yeast via quantitative polymerase chain reaction in samples from...
yeast-inoculated but not blank coupons. Overall, \textit{S. cerevisiae} NE095 challenged the entire assessment process, from sample to answer, and enabled exercise participants to demonstrate their ability to assess a biological material in the field while minimizing health and safety risks. The yeast material is expected to enable routine training in local jurisdictions for increased confidence in on-site assessment technologies and user capabilities. Department of Homeland Security Science and Technology Directorate funded this project under Interagency Agreement HSHQPM-14-X-00078 with NIST.

---

**Collection of Viable Airborne Viruses by a Highly Efficient Air Sampler**

\textit{J. Lednicky}\textsuperscript{1}, T. Bonny\textsuperscript{1}, M. Pan\textsuperscript{1}, J. Loeb\textsuperscript{1}, X. Jiang\textsuperscript{1}, A. Eiguren-Fernandez\textsuperscript{2}, S. Hering\textsuperscript{2}, C-Y. Wu\textsuperscript{1}, H. Fan\textsuperscript{1}; \textsuperscript{1}\textit{Univ. of Florida, Gainesville, FL, \textsuperscript{2}Aerosol Dynamics Inc., Berkeley, CA}

Natural, accidental, and intentionally created aerosols containing viable viruses pose significant public health threats. Indeed, respiratory tract infections acquired by the inhalation route of exposure account for a significant proportion of hospital visits. Yet assessments of the biothreat posed by airborne viruses are technically demanding because existing air samplers do not collect virus aerosols with high efficiencies. Furthermore, some air sampling methods inactivate virus particles, necessitating PCR or other techniques to detect viral signatures, and that complicates modeling: is there a biorisk, or were truly non-viable viruses detected? We had previously showed that our \textit{Viable Virus Aerosol Sampler (VIVAS)} significantly outperformed the industrial standard BioSampler for the collection of viable bacteriophage MS2- and Influenza H1N1 virus aerosols by a factor of ten or more. The VIVAS operates using the principle of laminar flow, water-condensational growth, wherein airborne particles as small as 10 nm are enlarged to form micrometer-sized droplets that are gently deposited onto liquid via low-velocity impaction. The condensational growth occurs in a straight, wide-bore, wet-walled tube with two temperature regions, ~8° followed by 40°C. Aerosols are sampled at 7 L/min, and conveniently collected into 1.5 mL of PBS or similar collection media, bypassing the need to concentrate the collected material for down-stream processing. Whereas it is tempting to generalize the outcomes obtained with MS2 and influenza H1N1 viruses, this may not be the case for others, as viruses differ in their dimensions, and their biochemical properties. The VIVAS was thus evaluated for other viruses, with emphasis on the collection of viable viruses from laboratory-created aerosols generated by a bioaerosol nebulizing generator. We conclude that the VIVAS has many potential applications, from assessments of inhalation exposure risks in clinical settings to studies of viruses present within near-atmosphere breathing air.

---

**Stand-Alone NGS Biothreat Detection and Characterization**


Rapid, accurate, and comprehensive characterization of potential biothreat agents from environmental samples or isolates is desirable so that high-consequence/low-regret decisions can be made. Samples may be obtained virtually anywhere on the globe and transportation to fixed laboratories can be slow, expensive, and risk degradation. Rapid assays are incapable of deep characterization and may have sensitivity and specificity issues.
We have built bioinformatics hardware and software platforms capable of rapidly and comprehensively analyzing DNA sequence data from pure or metagenomic samples without requiring network connectivity; delivering actionable information and confidence data to non-expert end-users. The Microbial Threat Characterization Pipeline (MTCP) leverages Open Source software and tools previously built at LLNL. It accepts paired-end FASTQ input from desktop NGS sequencers, performs an in silico microarray probe analysis to determine if any key threat agents are present, and attempts assembly. At the same time, the reads are examined by the LMAT (Livermore Metagenomics Analysis Toolkit) software: a scalable K-mer analysis that utilizes separate reference “marker” libraries for genus, species, and plasmids. This provides 3 rapid metagenomic views, even on a small-memory compute platform (a 2U Linux server with 128GB RAM and 4 cores.) MTCP also runs on a 4U Linux server with 1TB RAM and 40 cores; “full” LMAT genus, species, and plasmid libraries are supplied that utilize all available full and draft genome sequence data. BLAST comparisons and phylogenetic analyses are performed for key threat agents when genome coverage is sufficient. An Executive Summary presents stoplights for key threat agents and sequencing confidence via a web interface that summarizes all results and has a one-click PDF generation. Analysis times range from 2-6 hours depending on sample complexity and platform.

**Development and Validation of Metagenomics Sequencing Pipelines for Biosurveillance and Diagnostics**


Next generation sequencing (NGS) has the potential to allow unbiased detection and characterization of biothreat agents and emerging pathogens from a variety of clinical and environmental samples. This capability would greatly benefit multiple applications, including microbial forensics, biosurveillance, and clinical diagnostics. However, current sample to sequence pipelines are complex, and there is a growing need for them to be simplified, standardized, and validated before results can be made comparable across multiple laboratories. Specific needs include standard reference materials, simplified sample and library preparation, trusted reference databases, robust bioinformatics pipelines, and clear regulatory pathway. MRIGlobal is developing and validating methods for accelerating the use of NGS as a powerful tool for biosurveillance and clinical diagnostics, and in this presentation will present our progress and initial recommendations for the community.

**Droplet Microfluidics Based Bioaerosol Detector**

B. Damit; The Johns Hopkins Univ. Applied Physics Lab., Laurel, MD

Current bioaerosol detectors are limited by lengthy response time, etc. and these weaknesses have compelled development of new detectors. In this work, a detector was developed which applies the principles of droplet microfluidics to bioaerosol detection. Droplet microfluidics is based on the creation of nanoliter/femtoliter droplets with compartmentalized reagents and boasts enhanced assays and easy fluidic manipulations. This
A proposed bioaerosol detector operates by finely focusing aerosols directly into these droplet microreactors. By concentrating aerosol into the microfluidic volumes, the assay kinetics can be improved (i.e. decreased detection time) while conserving expensive reagents. A prototype detector, which consisted of an aerodynamic focusing lens, aerosol-focusing capillary, custom microfluidic chip, and optical microscope, was constructed. As achieving fine aerosol focusing was crucial, computational Fluid Dynamic simulations and Lagrangian particle tracking modeling were completed to identify the optimal conditions for focusing. Aerosol deposition experiments showed that the aerosol focusing system achieved sub 200-µm deposition spot diameters (on solid substrates) for aerosol sizes of 2-5 µm. For aerosol impingement experiments, the microfluidic chip was positioned below the capillary, a pinned air-liquid interface was created at the chip surface, and aerosols were focused into the collection liquid. Microscopic inspection confirmed aerosol capture at the interface. Finally, to prove the robustness of the prototype, a simple droplet Propidium Iodide (PI) assay was performed. The detector was able to differentiate between E. coli and non-biological aerosols collected in the droplet within 20 s of PI incubation. Overall, this research was successful in showing that the detector concept can be a promising improvement to current detectors.

---

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** E. Environmental Detection  
**Poster Presentation Number:** 105 (E)  

**Current Status of EPA’s Biothreat Agent Sampling and Analysis Program**

T. Nichols¹, T. Smith¹, L. Mapp¹, F. Cruz¹, E. Rhodes², W. Calfee³, S. Shah¹; ¹US EPA, Washington, DC, ²US EPA, Cincinnati, OH, ³US EPA, Raleigh, NC

The field of sampling and analysis for biothreat agents is constantly evolving due to intentional and unintentional outbreaks, new technologies, and the identification of scientific and implementation gaps in the current methodologies and strategies for sampling and analysis. Generally, during a response to a biothreat contamination incident, EPA conducts sampling to determine the extent of contamination to inform remediation activities and then after decontamination to determine decon efficacy. To address these response needs, EPA has established the Environmental Response Laboratory Network (ERLN). As the Federal Water Sector Lead, EPA has also established the Water Laboratory Alliance (WLA) to focus solely on drinking water and wastewater sample analysis. The goal of both the ERLN and the WLA is to provide consistent analytical capabilities, capacities, and quality data in a systematic, coordinated response by integrating capabilities of existing Federal laboratories, and public and private sector laboratories during an incident. This presentation will provide information on the scope and activities of member laboratories. Ensuring the availability of appropriate sampling and analysis methods is critical so that timely decisions can be made during each phase of response to an incident. To address this need for the ERLN and WLA, EPA’s Homeland Security Research Program (HSRP) works to develop and provide uniform biothreat sampling and analysis methods through evaluation and modification of existing methods or developing innovative methods. On a routine basis, EPA convenes SME panels to evaluate and select methods for incorporation into a compendium, Selected Analytical Methods for Environmental Remediation and Recovery (SAM), which pairs the sample matrix and biothreat agent to the most relevant, scientifically-defensible, and potentially field-deployable method(s). This presentation will provide examples of research such as the Rapid Viability PCR and the jointly developed EPA-CDC vacuum sampling methods.
Proteomics for Microbial Forensics


Bacterial proteomics has evolved tremendously over the past 20 years and is now a powerful tool for many scientific disciplines including microbiology and biomarker discovery. Proteomics complements genome analysis and can provide information about the character of both organisms and specific proteins of interest, making it applicable in several areas of microbial forensics. We focus herein on the most promising applications of proteomics in microbial forensics, and give examples of experimentation in the areas of organism characterization, detection of specific proteins of concern, and characterization of proteins of concern. Within these areas, proteomics can begin to address questions such as: whether the organism was a naturally-occurring or laboratory-adapted strain, the growth conditions in which an organism was cultivated, the growth environment itself (as environmental peptides and proteins often remain associated with microbial biomass), the presence of a toxin or other protein of interest in a sample (even if the protein had been inactivated), and characteristics of a protein of interest such as serotype or variant.

A Microbial Genome Population Graph Annotated With Protein Structure Data to Predict Antibiotic Resistance


Antibiotics are often applied pro-actively to prevent infection with resistance recognized retrospectively through the identification of an individual gene or nucleotide polymorphism. Selective pressure on microbial communities and ongoing use of antibiotics means the resistance mechanisms evolve over time, requiring genetic detection assays to be updated. A vast number of microbial genes and genomes with resistance profiles are now being sequenced, which opens the possibility to identify key genetic features associated with antibiotic resistance. However, for any newly sequenced sample, accurately prioritizing efficacious antibiotic use remains a fundamental challenge. In this study, previously sequenced microbial genomes and genes associated with antimicrobial resistance are collected and stored in a novel de Bruijn genome population graph. Homology based protein structure prediction is conducted for the antibiotic resistance genes and added to the graph as meta-data. A novel graph traversal algorithm is developed to identify sequence-structure motifs associated with unique antibiotic resistance function. Results show how the annotated “resistance” population graph is efficiently searched with unassembled next-gen metagenomic sequence data to predict antibiotic resistance potential in organisms extracted from a complex metagenomic sample. Genome population graphs annotated with functional data provide efficient storage and retrieval of the growing knowledge base of antibiotic resistance genotypes to improve recognition of mechanisms of resistance.
Computational Simulation Of The Evolution Of RNA Single Stranded Viruses In Cell Culture

T. Kostova-Vassilevska, J-S. Yeom, P. D. Barnes Jr.; Lawrence Livermore Natl. Lab., Livermore, CA

RNA virus infections present significant challenges for biodefense because of the high mutation rate and fast adaptation of the virus to antiviral treatments and vaccines. Many factors play a role in the within-host and between-host evolutionary processes: the amount of the initial infection and its genotype composition, the intrinsic mutation rate and the plasticity of the viral quasispecies, the characteristics of the host cells. We are developing two computational models of virus evolution in serial cell culture passages. These are multi-scale models, at the molecular, cellular and cell culture levels. Our models include virus replication/mutation realism not present in other models and are extendable to within-host evolution. Our current goal is to experiment with DENV2 evolution in silico, mimicking the lab experiment of serial passage in order to understand the interplay between the above-mentioned factors. Our two models reflect the same process but are based on different simulation paradigms. The first is a serial, individual-based model allowing more restricted simulations with small cell cultures (up to 5000 cells). The second model is a parallel discrete-event simulation, where the goal is to enable scalable computations of the evolution of virus variants in real size cell cultures (of 100,000-500,000 cells). Both model individual mutant virions represented by their full RNA sequences and involve parameters that include the factors mentioned above. We will present our findings with the two simulation approaches, will compare their capabilities and performance and will relate them to real data.

Antimicrobial And Phytochemical Screening Of Stem Bark Extracts Of Lovoa Trichiliodes (harm) And Trichilia Heudelotii Planc (harm)

B. O. Opawale¹, A. K. Onifade², A. O. Ogundare²; ¹Rufus Giwa Polytechnic, Owo, Nigeria, ²Federal Univ. of Technology, Akure, Nigeria

The antimicrobial properties of stem bark extracts (cold water, ethanol and acetone) of Lovoa trichiliodes and Trichilia heudelotii were investigated using agar well diffusion method. The percentage yield of the extracts ranged from 3.90 to 6.53% and 9.63 to 10.20% respectively for the plant materials. Phytochemical screening of the plant materials revealed the presence of alkaloids, saponins, tannins, phlobatansins, phenols, anthraquinones and glycosides. Terpenes, cardenolides and flavonoids were absent in the two plants. However, all the extracts remarkably inhibited the growth of Bacillus subtilis, S. aureus, E. coli, E. faecalis, K. pneumoniae, S. typhi, A. flavus, C. albicans and C. glabrata. The mean diameter of the zone of inhibition exhibited by the extracts was between 8.00 and 22.33mm while the minimum inhibitory concentration (MIC) was between 2.5 and 200mg/ml. However, the cold water extracts of L. trichiliodes stem bark exhibited no inhibitory activity against the organisms. The results of this investigation confirmed the folkloric uses of these plants for the treatment of various infectious diseases.
The Use of Meta-analysis Approach to Demonstrate Added Benefit of Obiltoxaximab (ETI-204) in Treatment of Inhalational Anthrax

N. Serbina¹, G. Stark², A. Fisher³, L. Casey¹, R. Slay⁴, R. Conrad¹; ¹Elusys Therapeutics, Inc., Pine Brook, NJ, ²Battelle BioMed. Res. Ctr, Columbus, OH, ³BDM Consulting, Inc, Somerset, NJ, ⁴OBRRTR/DMID/NIAID, Bethesda, MD

Background: Monoclonal antibody ETI-204 binds protective antigen (PA) of Bacillus anthracis and is being developed under FDA’s Animal Rule for treatment of inhalational anthrax in combination with antibiotics. FDA required evaluation of added benefit of ETI-204 with antibiotics as a prerequisite to licensure to show that ETI-204 does not interfere and potentially improves antibiotic efficacy. Here we discuss the approach taken with FDA to address this requirement. Methodology: We considered 2 approaches. In the 1st approach, a delayed 84 hr treatment study in rabbits was proposed to demonstrate statistically significant improvement in survival with ETI-204 co-treatment. Power calculations assumed 40% survival to treatment and required 280-750 animals to achieve statistically significant difference between antibiotic and combination arms. 2nd approach included a meta-analysis of data from 6 model development studies conducted with ETI-204. The studies were conducted in rabbits and primates and explored different treatment times and 3 antibiotics (levofloxacin, doxycycline, and ciprofloxacin) given at the human equivalent dose and lower. All studies were randomized, controlled, and open label with modest numbers of animals. Statistical significance was seen in 1 study. Meta-analysis included Mantel-Haenszel test and an exact stratified conditional logistic regression and concluded a low probability that all 6 studies would be positive by random chance (p = 0.0015 and 0.0024). Differences in survival outcomes and lack of interference were displayed by forest plot. Lack of interference was confirmed by antibiotic concentration analysis and added benefit was supported by analysis of serum PA. Conclusions: Meta-analysis was successfully applied to demonstrate that ETI-204 co-treatment results in higher survival outcomes than antimicrobial therapy alone, negating the need for a large rabbit study. Federal funding from ASPR/BARDA and NIAID.
The approach presented here may be applicable to human dose selection for other therapeutic agents being developed under the Animal Rule. Supported by Federal funds from ASPR/BARDA under Contract No. HHSO100201000026C

Safety, Pharmacokinetics and Immunogenicity of Intramuscular (IM) Administration of Obiltoxaximab (ETI-204) to Healthy Humans

C. Nagy¹, J. Mondick², A. Czech¹, A. King³, R. Guttendorf⁴; ¹Elusys Therapeutics Inc., Pine Brook, NJ, ²Metrum Res. Group LLC, Tariffville, CT, ³Covance Clinical Res. Unit, Inc., Dallas, TX, ⁴Aclairo Pharmaceutical Dev. Group Inc., Vienna, VA

Background: Obiltoxaximab (OBIL), a high affinity monoclonal antibody that neutralizes protective antigen, was developed for treatment and is being investigated for prophylaxis of inhalational anthrax under FDA’s Animal Rule guidance. The recommended intravenous (IV) dose for treatment in adult humans is 16 mg/kg. The efficacious IM dose for pre- and post-exposure prophylaxis of inhalational anthrax in monkeys is 16 mg/kg.

Methods: Safety, pharmacokinetics (PK) and immunogenicity were evaluated in 36 healthy adults after IM administration of 4, 8, 16, 20, and 24 mg/kg OBIL or placebo into the Vastus lateralis in a double-blind, randomized, placebo controlled trial. Exposures in humans after IM administration were compared to those in monkeys at the efficacious IM dose for prophylaxis using observed data and PK/pharmacodynamic (PK/PD) modeling and simulation. Results: Administration of OBIL IM to healthy humans was well tolerated. No injection site abscesses or hypersensitivity reactions occurred; no subjects developed treatment emergent antitherapeutic antibodies. Local tolerability was acceptable up to 24 mg/kg IM, up to 6 injections per dose, and up to 5 mL per injection. Systemic exposures were approximately dose proportional, maximum serum concentrations were observed at 6 - 9 days and terminal half-life was 16 - 23 days. Absolute IM bioavailability was 64%. The relationship between observed and simulated exposures in humans at 20 and 24 mg/kg OBIL and those in monkey models of pre- and post-exposure prophylaxis at the efficacious IM dose of 16 mg/kg was evaluated. Conclusions: IM administration of OBIL to adult humans was well tolerated and may provide a valuable option for prophylaxis of systemic anthrax in emergency settings when alternative therapies are not available or appropriate and IV administration is not available or feasible. Supported by Federal funds from ASPR/BARDA under Contract No. HHSO100201100034C

Novel Antibiotic Gepotidacin is Active in vitro and Efficacious in a Rabbit Aerosol Challenge Treatment Model of Bacillus anthracis

R. Gruber¹, K. O'Dwyer¹, L. Qian¹, L. Henning², S. Demons³, C. Jakielaszek¹; ¹GlaxoSmithKline, Collegeville, PA, ²Battelle, Columbus, OH, ³US Army Med. Res. Inst. of Infectious Diseases, Fort Detrick, MD

Background: Gepotidacin, a novel bacterial topoisomerase inhibitor in clinical development, is currently being evaluated for use against biothreat and conventional pathogens. Gepotidacin selectively inhibits bacterial DNA replication via a unique binding mode different from other antibacterials, thereby providing activity against drug resistant target pathogens. Gepotidacin was evaluated against Bacillus anthracis (BA) in vitro and in an inhalational New Zealand White rabbit treatment model. Methods: Minimum inhibitory concentrations (MICs) were determined on 130 BA isolates using CLSI methods. Male rabbits were exposed to aerosolized BA (Ames strain, gepotidacin MIC 0.5 μg/mL) targeting 50 LD50s. Gepotidacin (70 mg/kg/dose; 2 or 3 times/day for 5 days as 3 or 6 hr IV infusions), saline (IV infusion) or levofloxacin (50 mg/kg oral; once daily for 5 days) was initiated based on the presence of circulating protective antigen (PA). Samples were evaluated for PA, PK and bacteriology (blood and
tissues). Body temperature and survival were monitored for 28 days post challenge. This study was conducted in accordance with GSK and Battelle (IACUC) Policies on the Care, Welfare and Treatment of Laboratory Animals. Results: The gepotidacin MIC50/90 against 130 BA isolates was 0.5 and 1 μg/ml, respectively. All animals were bacteremic prior to treatment. Gepotidacin treatment resulted in 88-100% survival and 100% clearance of bacteria from the blood of surviving animals. All saline control animals succumbed (0% survival), while there was 100% survival in the levofloxacin control group. **Conclusions:** Gepotidacin was active in vitro against 130 isolates of BA and efficacious in the rabbit anthrax treatment model. These results suggest that gepotidacin should be further evaluated in a pivotal anthrax model. This project has been funded in whole or in part with Federal funds awarded by the Defense Threat Reduction Agency under Agreement No. HDTRA1-07-9-0002.

---

**Session Type:** Poster Session  
**Session Number:** 016  
**Session Title:** Poster Session 2  
**Poster Presentation Time:** Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:** G. Therapeutics  
**Poster Presentation Number:** 114 (G)  
**Cathelicidin Antimicrobial Peptide From Alligator mississippiensis Has Antimicrobial Activity Against MDR Gram Negative Bacteria**  

**S. M. Barksdale, E. J. Hrifko, M. L. van Hoek; George Mason Univ., Manassas, VA**

Alligator mississippiensis (American alligator), as a member of the order Crocodilia, is more closely related to dinosaurs than other modern reptiles and appear to have robust immunity. Their serum has been shown to have antibacterial activity beyond that of human serum, and it is believed that this activity is partially due to antimicrobial peptides (AMPs), which are produced as part of the innate immune system. AMPs are attractive possible platforms against multi-drug resistant bacteria, such as those found in biofilm-infected wounds, because they seldom cause genetic resistance in bacteria and are effective against antibiotic resistant bacteria. One conserved AMP type is the cathelicidin, which can have direct antimicrobial activity and modulate the host immune system. In this work, we identified, synthesized, and characterized a cathelicidin from the American alligator named AM-CATH. We discovered this AMP by comparing American alligator ESTs with propeptide cathelicidins of other reptiles. We analyzed the structure and found that AM-CATH has a mixed structure, with an N-terminal alpha-helix and a center Pro hinge. In MIC assays, AM-CATH has strong activity against Gram-negative bacteria, including Pseudomonas aeruginosa and multi-drug resistant (MDR) Acinetobacter baumanii. It was found that AM-CATH forms pores in the bacterial membrane as its mechanism of action, and is less sensitive to salt than many other AMPs. AM-CATH was also tested for its cytotoxicity against A549 human lung epithelial cells and sheep red blood cells. AM-CATH was not hemolytic against red blood cells at 300 µg/ml, and was not significantly cytotoxic against A549 cells after 24 h exposure. AM-CATH has activity similar to other AMPs from reptiles such as NA-CATH. It is possible that AM-CATH plays an important role in the innate immunity of A. mississippiensis, similar to LL-37 in humans. In addition, due to its strong against MDR bacteria and its lack of cytotoxicity, AM-CATH could be a strong basis for a clinical product.

---

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** G. Therapeutics  
**Poster Presentation Number:** 115 (G)  
**Komodo Dragon-inspired Peptide Drgn-1 Promotes Clearance and Healing of Polymicrobial Biofilm-infected Wounds**

**M. L. van Hoek, M-C. Chung, S. N. Dean, C. N. Propst, B. M. Bishop; George Mason Univ., Manassas, VA**

Cationic antimicrobial peptides (CAMPs) are multifunctional molecules from the innate immune system that have a high potential as novel therapeutic agents. Reptiles are promising sources of potential CAMPs; however, their isolation and identification is labor-intensive. Recently, we
developed a novel approach for the discovery of new CAMPs from reptile blood involving the capture of peptides by nanoparticles and de novo sequencing by mass spectrometry. We identified a histone H1-derived peptide from the Komodo dragon (Varanus komodoensis), called VK25, as a potential CAMP. Using this peptide as inspiration, we designed a synthetic peptide DRGN-1, which contains two reversed amino acids at the N-terminus from the original protein sequence (VK25), and evaluated the antimicrobial and antibiofilm activity of both peptides against P. aeruginosa and S. aureus. DRGN-1, but not VK25, exhibited potent antimicrobial and antibiofilm activity, permeabilized bacterial membranes, and bound to DNA. A circular dichroism study suggested that DRGN-1 has a random structure. Wound healing was significantly enhanced by DRGN-1 in both uninfected and mixed biofilm (P. aeruginosa and S. aureus)-infected murine wounds. In a scratch wound closure assay used to elucidate the wound healing mechanism, the peptide promoted migration of HEKa keratinocyte cells, which was inhibited by mitomycin C (proliferation inhibitor) and AG1478 (EGFR inhibitor). DRGN-1 also transactivated the EGFR-STAT1/3 pathway. Thus, DRGN-1 is a strong candidate for use as an alternative to antibiotics, especially for mediating the innate immune response.

Session Type: Poster Session 2
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: G. Therapeutics
Poster Presentation Number: 116 (G)

Design of a D-peptide Universal Ebolavirus Entry Inhibitor

S. E. Apple1, F. G. Whitby1, T. R. Clinton1, R. McKinnon1, C. P. Hill1, B. D. Welch3, D. M. Eckert1, M. S. Kay1; 1Dept. of Biochemistry, Univ. of Utah, Salt Lake City, UT, 3D-peptide Res. Div., Navigen, Inc., Salt Lake City, UT

There are five species of Ebolavirus in the Filoviridae family. These viruses result in high mortality in humans and non-human primates, and there is currently no approved treatment or preventative. A conserved region of the surface glycoprotein (GP), the N-trimer, plays a key role in membrane fusion with the host cell endosome and is exposed and vulnerable to inhibition during viral fusion. N-terminal residues of the N-trimer are identical in all known species and therefore serve as an ideal “universal” target for discovering drugs to prevent entry of all Ebolaviruses. We have used mirror-image phage display to identify D-peptides that bind to this region and are developing them into effective pan-Ebolavirus inhibitors. D-peptides cannot be broken down by natural L-proteases, allowing them to persist longer in the body with reduced immunogenicity and enabling greatly reduced dosing and cost. Trimeric versions of these D-peptides exploit avidity (binding to the N-trimer) to improve inhibitor affinity and potency. We have structurally characterized our D-peptides bound to the Ebola N-trimer using X-ray crystallography to identify the binding interface and inform D-peptide inhibitor design. These methods allow us to optimize inhibitors as we aim to develop a universal cost-effective Ebolavirus entry inhibitor suitable for stockpiling.

Session Type: Poster Session 1
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: G. Therapeutics
Poster Presentation Number: 117 (G)

A Biologically Contained (BSL-3) Ebolavirus Plaque Reduction Neutralization Assay

J. Long, S. Hamilton, D. Audley, A. Cook, K. Lohman, C. Gelhaus, D. Read; MRIGlobal, Kansas City, MO

Background: There have been many experimental prophylactics and treatments administered in West Africa in attempt to contain and ultimately eradicate the Ebola virus (EBV). However, the requirement to perform testing in a BSL-4 laboratory limits the throughput for new Ebola medical countermeasure discovery. Using reverse genetics, an EBV that lacks the essential transcription factor VP30 gene (termed ΔVP30) was developed.
This virus is biologically contained to a cell line stably expressing VP30 and can be handled safely in a BSL-3 laboratory. This system abrogates the need for BSL-4 testing conditions and greatly expedites evaluation of new products. **Methods:** A plaque reduction neutralization test (PRNT) was developed and optimized in Vero cells expressing VP30 using the ΔVP30 in vitro testing system. Antibodies specific for the EBV envelop glycoprotein were tested (mouse monoclonal antibodies AB-EB-MAB1 and AB-EB-MAB2; human mAb KZ52).

EBV was diluted to a concentration of 500 PFU/mL in Eagle's minimum essential medium supplemented with 2% heat-inactivated fetal bovine serum. Antibody samples were serially diluted and tested in triplicate (100 - 0.0001 micrograms/mL). The virus/mAb mixture was incubated for 1 h at 37°C followed by removal of the inoculum and addition of the primary overlay. The cells were incubated for 2 days, at which time a secondary overlay containing a vital dye was added. On Days 8-10, plaques were counted and the PRNT50 values determined. Results: The mAb AB-EB-MAB1 failed to neutralize VP30 virus. The mouse mAb AB-EB-MAB2 and the KZ52 human mAb showed EBV neutralizing activity with PRNT50 values of 4.8 mg/mL and 0.875 µg/mL, respectively. **Conclusions:** We have demonstrated that the human KZ52 mAb and mouse AB-EB-MAB2 mAb inhibit EBV plaque formation, and that the KZ52 mAb may be a candidate for further development. Furthermore, transfer and application of the EBV ΔVP30 in vitro model may provide a more rapid and lower cost screening process to ease the backlog on testing Ebola MCMs in BSL-4 laboratories.

---

**Poster Presentation Time:** Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:** G. Therapeutics

**Poster Presentation Number:** 118 (G)

**Potentiators Enhance Antimicrobial Activity to Overcome Bacterial Resistance**

S. Arcidiacono¹, V. Kurker², J. Soares³, S. Sarangapani⁴; ¹US Army Natick RD&E Ctr., Natick, MA, ²Univeristy of Massachusetts Lowell, Lowell, MA, ³US Army Natick RD&E Ctr., Natick, MA, ⁴ICET Inc, Norwood, MA

The prevalence of antibiotic resistant microorganisms highlights the need for new therapeutic strategies. Here proprietary formulations of prospective potentiators and the plant antimicrobial berberine were evaluated against organisms with known efflux pumps that would provide efficacy against a range of resistant organisms. Activity was determined by a kinetic microplate assay to monitor cell optical density in the presence of berberine plus each potentiator candidate; growth inhibition of was indicative of activity. A checkerboard assay was used to screen the potentiators in combination with varying ratios and concentrations of berberine against clinical and biothreat surrogates and was optimized through a series of formulation iterations. Berberine alone had no activity at the concentrations tested; however, in the presence of several potentiators, berberine had broad spectrum activity against all surrogate organisms. Results against surrogates were validated when broad spectrum activity was demonstrated against of BSL-3 organisms including genuine biothreat agents (Bacillus anthracis, Burkholderia pseudomallei, Francisella tularensis and Yersinia pestis) and multiple strains of bacterial (Staphylococcus aureus, Enterococcus faecium, Pseudomonas aeruginosa, Acinetobacter baumannii) and fungal (Candida albicans) clinical isolates. These results demonstrate the efficacy of potentiator formulations using berberine as the proof-of-concept antimicrobial compound. In addition, potentiators combined with antibiotics overcame resistance of Methicillin Resistant S. aureus (MRSA), Vancomycin Resistant S. aureus (VRSA), and Vancomycin Resistant Enterococcus (VRE). Formulations of the select potentiators identified here, in combination with other antimicrobials including traditional antibiotics, may represent additional opportunities for overcoming antimicrobial resistance of clinical and biothreat organisms.
Therapeutic Potential of Predatory Bdellovibrio bacteriovorus against Antibiotic-Resistant Gram-Negative Pathogens in Serum

D. Negus, D. Raghunathan, P. Radford, J. Tyson, L. Sockett; Univ. of Nottingham, Nottingham, United Kingdom

Background: Antibiotic resistance in Gram-negative bacteria (GNB) has been identified as a serious threat to global public health. Moreover, multi-drug resistant (MDR) forms of these pathogens are a cause of substantial morbidity and mortality in hospitalised patients and are of particular concern in the intensive care unit setting. Novel therapeutic paradigms to combat these recalcitrant strains are badly needed and those with a broad-spectrum of activity against GNB are highly desirable. Bdellovibrio bacteriovorus are predatory bacteria with the capacity to prey on a wide range of GNB in vitro and therefore represent a potential candidate as a therapeutic agent active against MDR strains of these pathogens.

Methods: We examined the capacity of B. bacteriovorus to prey upon and kill clinical isolates of GNB with a range of important drug resistances (Klebsiella pneumoniae: NDM-1 & KPC, Acinetobacter baumannii: OXA-23, Pseudomonas aeruginosa: VIM, Enterobacter aerogenes: NDM-1). Prey pathogens (approximately 1x10^9 cfu/ml) were incubated in the presence of B. bacteriovorus HD100 in biologically relevant fluids (bovine fetal calf serum and human serum) at either 30°C or 37°C. Killing of prey strains was enumerated by viable counts, and also immune interactions between human sera and predatory bacteria were monitored. Results: All GNB examined were found to be susceptible to predation by B. bacteriovorus. Typically, a 1 to 3-log reduction in viable prey numbers was observed by 48 hours incubation time, coinciding with an increase in B. bacteriovorus numbers. Importantly, predation was observed at 37°C in human serum.

Conclusion: Predation by B. bacteriovorus on clinically relevant GNB results in a significant reduction of viable prey cells in serum. These preliminary findings provide the potential to develop a broad-spectrum therapeutic active against medically important drug-resistant strains of GNB.

Antimicrobial and Antibiofilm Activity of Antimicrobial Peptides Against Yersinia pestis

R. M. Schoenemann, M. L. van Hoek; George Mason Univ., Manassas, VA

Yersinia pestis is the agent of the disease plague, and is classified by the CDC and NIAID as a Category A pathogen. Plague has caused several epidemics over the centuries and has the potential to continue to be a major health threat. Plague infects several hosts such as rodents and fleas, along with humans. There is no current vaccine for plague and understanding how this pathogen is affected by cationic antimicrobial peptides (CAMPs) can lead to a better understanding of the host innate immunity against plague. In this work, we tested the activity of several previously identified CAMPs, including SMAP-29, NA-CATH, and LL-37 against multiple strains of Yersiniae. We tested these CAMPs against 3 attenuated (BSL-2) strains of Y. pestis: A1122, which is of the biovar Orientalis, Kim10+, which is of the biovar Medialis, and CO92, which is of the biovar Orientalis and lacks the the pgm and pst loci. CAMPs were first tested for their antimicrobial activity in low salt buffer. We found that the sheep cathelicidin SMAP-29 and the elapid snake cathelicidin NA-CATH were the most effective against the 3 strains grown at 37°C, all with extremely low EC50 values. SMAP-29 (EC50= 0.01 µg/ml), NA-CATH (EC50= 0.09 µg/ml), and Ch-CATH F2/5/12W (EC50= 0.12 µg/ml) were chosen for further study. We then determined the minimal inhibitory concentration (MICs) and found that NA-CATH was still effective at moderate concentrations of peptide against these Yersiniae strains. To determine the potential mechanism of action of these peptides, we used DiSc3(5) and ethidium bromide dyes to study the bacterial membrane perturbation as a result of peptide treatment. Scanning electron microscopy was performed to examine the membrane disrupting effect of the most effective peptides. These results give a broader understanding of the efficacy that CAMPs have against this pathogen, which could lead to novel treatments and a better understanding of the host innate immune response to Y. pestis infection.
Formulation and Functionalization of Surfactant Vesicle Drug Delivery Systems

M. Hurley, N. Soto, L. Robinson, S. Zhu, D. Moncelet, L-X. Wang, V. Briken, P. DeShong; Univ. of Maryland, Coll. Park, College Park, MD

Cationic vesicles spontaneously form when sodium dodecylbenzenesulfonate (SDBS) and cetyltrimethylammonium p-toluenesulfonate (CTAT) are mixed in the appropriate ratio in aqueous solutions. When a 3:1 molar ratio of SDBS:CTAT is mixed, the resulting vesicles are anionic and spherical with an average diameter of 130 nm. The vesicles are more stable than their phospholipid liposomal counterparts, and small hydrophobic molecules can be readily incorporated into them. Their ease of preparation, stability, and loading capacity make them ideal drug delivery vehicles. Here, the stability of the vesicles in different formulation conditions is evaluated, and methods to incorporate targeting molecules into the vesicles are highlighted. To determine the limitations of the vesicles in various formulation mixtures, dynamic light scattering was used to assess the effect of different cosolvents and additives on the formation and stability of vesicles. Vesicles form in PBS solutions containing up to 25% by volume ethanol, and up to 50% by volume DMSO and DMF. They also form in PBS solutions containing up to 70% by weight glycerol and 50% by weight sucrose. The sizes of the vesicles increase as the amount of cosolvent or additive increases. The difference in size within a series is substantial for unpurified vesicles. However, after SEC purification, the size difference is dramatically lowered. These findings have lead to vesicle formulations with increased drug loading and shelf life. To prepare vesicles with targeting capabilities, the vesicles must be functionalized with biomolecules that act as targeting agents. Vesicles functionalized with folic acid, carbohydrates, peptides, and proteins have been prepared. Methods to maximize biomolecule incorporation and develop vesicles with high binding selectivity will be discussed.

Developing Peptide Mimotope Vaccines for Burkholderia

R. C. Bernhards\footnote{1}, P. Guo\footnote{2}, J. Zhang\footnote{2}, B. Li\footnote{2}, C. H. Weaver\footnote{1}, S-C. Lo\footnote{2}, S. L. Welkos\footnote{1}; \footnote{1}USAMRIID, Fort Detrick, MD, \footnote{2}FDA, Silver Spring, MD

Burkholderia pseudomallei and Burkholderia mallei represent bacterial biothreats and are classified as Tier 1 select agents. B. pseudomallei causes melioidosis which is endemic to Southeast Asia and Northern Australia, while B. mallei causes glanders primarily in horses but is also capable of infecting humans. Both of these diseases have high mortality rates and there are currently no vaccines available for either of them. The capsular polysaccharide (CPS) and lipopolysaccharide (LPS) expressed by these pathogens are important virulence factors and have been evaluated as potential protective vaccine antigens in animals. However, such polysaccharides usually induce short term immune responses but not longer-term immune memory, and are often associated with significant toxicity in the vaccinated host. Therefore, development of safer and more defined and immunogenic derivatives of CPS and LPS antigens is a priority. Peptides or other small analogs which mimic the immunogenic epitopes of CPS or LPS are possible candidates for safer and more effective vaccines. Phage display panning was used to discover peptide mimotopes of LPS and CPS using highly specific and protective monoclonal antibodies. The peptides were then tested for specific binding to their respective monoclonal antibodies using ELISA and Bio-Layer Interferometry (BLI). Peptides showing highly specific binding will be conjugated to carrier proteins, subjected to BLI, and tested for protection in mice and nonhuman primates. The goal is to develop a multivalent CPS/LPS vaccine that is highly protective against both B. pseudomallei and B. mallei.
Burkholderia mallei CLH001 Attenuated Vaccine Strain Is Immunogenic and Demonstrates Full Protection Against Acute Respiratory Glanders

C. L. Hatcher, T. M. Mott, L. A. Muruato, A. G. Torres; Univ. of Texas Med. Branch, Galveston, TX

Burkholderia mallei is an HHS Tier 1 Select Agent and the causative agent of glanders, an incapacitating disease with high mortality rates in respiratory cases among animals and humans. It’s endemcity in certain parts of the world and lack of effective treatment emphasize its public health threat and highlight the need for a vaccine. In this study, we constructed a B. malleiΔtonB Δhcp1 strain (CLH001) deficient in iron uptake and type VI secretion and investigated its ability to protect against acute respiratory Burkholderia murine infections. Intranasal (i.n.) administration of CLH001 (1.5x10⁴ CFU) to BALB/c and immunodeficient NSG mice resulted in 100% survival with no detectable colonization or abnormal histopathology in the lungs, liver or spleen at day 21. BALB/c mice vaccinated i.n. with 1.5x10⁵ CFU of CLH001 in a prime/boost regimen, showed full protection at 35 days post challenge with either 5 LD50 of B. mallei ATCC 23344 or 22 LD50 of B. mallei lux report strain CSM001. All organs analyzed from surviving mice were clear of bacterial colonization and any histopathological abnormalities. Serum samples taken prior to challenge, show high B. mallei-specific IgG serum titers as well as a Th1 biased immune response (IgG2a:IgG1 ratio = 5.9), both of which are used as predictors of protection. In a cross protection study using the same prime/boost regimen, BALB/c mice were only partially protected at 35 days post challenge with 3 LD50 B. pseudomallei K96243. Overall, our studies showed CLH001 to be attenuated and safe, and when administered in a prime/boost regimen, effective at providing complete protection against lethal B. mallei challenge. This work, combined with an improbability for reversion to wild-type, has not only made CLH001 the first B. mallei strain to be excluded from Select Agent regulation but also demonstrate its potential as a viable vaccine platform for further advancement into pre-clinical studies.

A Clinical Phase III Trial Demonstrating Lot Consistency and Confirming Cardiac and Overall Safety of the Non-replicating Smallpox Vaccine

E. T. Overton¹, S. J. Lawrence², E. Wagner³, K. Nopora³, S. Rösch³, P. Young³, D. Schmidt³, T. P. Meyer³, H. Weidenthaler³, N. Samy³, P. Chaplin²; ¹Univ. of Alabama at Birmingham, Birmingham, AL, ²Washington Univ. Sch. of Med., St. Louis, MO, ³Bavarian Nordic, Martinsried, Germany

Background: Modified Vaccinia Ankara (MVA BN; invented name IMVANEX in the EU, trade name IMVAMUNE outside the EU) is a live, highly attenuated, non-replicating viral vaccine under advanced development as a non-replicating smallpox vaccine. Methods: In this randomized, double-blind, placebo controlled, multicenter Phase III trial healthy, 18 to 40 year old vaccinia-naïve subjects received 2 injections of either MVA from 1 of 3 consecutively produced lots or placebo 4 weeks apart. To meet the objective of establishing lot consistency in regards to safety and immunogenicity, subjects were monitored for adverse events, particularly cardiac events. Immune responses were assessed using a plaque reduction neutralization test (PRNT) and an enzyme-linked immunosorbent assay (ELISA). Results: 4,005 subjects received at least 1 injection (3,003 MVA, 1,002 Placebo). Vaccination was well tolerated by all subjects without any safety concerns, in particular with regards to the development of cardiac diseases. A total of 25/3,003 subjects (0.8%) vaccinated with MVA reported 27 Serious Adverse Events. A total of 8/3,003 subjects (0.3%) in the MVA groups experienced an Adverse Event of Special Interest. MVA induced a strong immune response measured by PRNT
and ELISA 2 weeks after the second vaccination, with seroconversion rates of 99.8% (PRNT) and 99.7% (ELISA). All MVA groups were found to be equivalent per PRNT GMTs equivalence analysis. **Conclusions:** In this large phase III trial, MVA elicited consistently robust humoral immunity across 3 separate production lots, and demonstrated an excellent safety and tolerability profile comparable to previous trials, clearly differentiating MVA from replicating smallpox vaccines. Supported by Contract No.: HHSO100200700034C

**Background:** The development of a major cutaneous reaction (take) after scarification is used as a surrogate parameter for efficacy of a smallpox vaccine. Since the non replicating MVA does not induce a take, alternative strategies to provide indicators of protective efficacy were agreed upon with the FDA. Indicators of Efficacy: Neutralizing antibodies are acknowledged as a biological marker correlated with protection against smallpox. In preclinical studies, MVA has been shown to induce equivalent immune responses and protection compared to replicating smallpox vaccines like Dryvax or ACAM2000 (ACAM). A correlation between vaccine dose and antibody responses was demonstrated in mice, non human primates and humans, translating into a vaccine dose-related protection in a lethal monkeypox challenge model. In humans, antibody responses to MVA were comparable to responses to Dryvax. In addition, prior vaccination with MVA attenuated the take following a subsequent vaccination with Dryvax, which is associated with pre-existing immunity against smallpox infections. Therefore, the design of the ongoing pivotal Phase 3 trial includes a co-primary endpoint to demonstrate: 1. MVA induces non inferior immune responses to ACAM 2. Attenuation of the take following ACAM in subjects pre-vaccinated with MVA

**Methods:** Vaccinia-naïve subjects 18-42 years old receive either (a) two injections of MVA (days 0 and 28) followed by scarification with ACAM (day 56) or (b) vaccination by scarification with a single ACAM dose. Subjects are monitored for adverse events with a special focus on cardiac safety. Antibody immune responses are assessed using PRNT and ELISA. Maximum lesion area of the take after scarification with ACAM is measured with and without prior vaccination with MVA. Status: This trial is currently enrolling subjects at the BAACH. Supported by Contract No.: HHSO100200700034C

**Background:** Cryo-electron Microscopy and Image Analyses of Influenza Vaccine Nanoparticles Indicates Full Occupancy of Displayed Epitopes is Facilitated by Particle Design.

**Methods:** Vaccines based upon recombinantly engineered proteins are revolutionizing the process of vaccine design. Presentation of multiple copies of antigens on nanoparticles substantially increases the immune response to vaccination when compared to unitary monovalent counterparts, in part due to multivalent binding sites on immunogens facilitating B-cell receptor activation. To determine structural and geometrical parameters for
multivalent nanoparticle design, we determined the structure of a ferritin-based nanoparticle displaying conserved influenza hemaglutinin (HA) stem epitopes in complex with ScFv and Fab fragments of antibodies. Using cryo-electron microscopy, image processing and molecular modeling, we describe the molecular organization of the nanoparticle complex. We observe that a full complement of ligands can simultaneously bind the nanoparticle. HA spikes on the nanoparticle surface are in close enough proximity that steric clash could disrupt binding to adjacent epitopes, but the angular orientation of the spikes directs symmetry related positions away from steric clash. Fabs passed next to each without steric overlap, which was facilitated by the nanoparticle design. Guidelines for nanoparticle based vaccine design based upon validated structural data such as ferritin-HA promise to lead to improved immune responses to nanoparticle based vaccine candidates and a rapid development process for more efficacious influenza vaccines.

---

Programmed -1 Ribosomal Frameshifting in the Encephalitis Family of Alphaviruses

J. D. Dinman¹, K. A. Joseph¹, d. Cynthia², K. Kehn-Hall², J. L. Jacobs³; ¹Univ. of Maryland, College Park, MD, ²George Mason Univ., Manassas, VA, ³MRIGlobal, Rockville, MD

The new world encephalitic alphaviruses - Eastern, Western and Venezuelan Equine Encephalitis viruses (EEEV, WEEV, and VEEV) are implicated in outbreaks of acute encephalomyelitis. Little is known about the virulence and replication mechanisms of these viruses, presenting significant obstacles to the development of effective therapeutics. A programmed -1 ribosomal frameshift (-1 PRF) signal has been described in the alphavirus structural gene, 6K, whose frameshift product is thought to play a role in viral egress. Here, we have characterized the -1 PRF signals of these three viruses, and probed the importance of frameshifting with regard to viral replication and virulence. The ability of computationally predicted -1 PRF sequences from EEEV, VEEV and WEEV to promote efficient frameshifting was assayed in HeLa and U8-MG astrocyte cell lines using standard dual reporter vectors. Frameshifting was ablated by mutation of the canonical “slippery sites” present in these sequences. Additional controls to probe for cryptic promoters, splice site, or IRES elements in these sequences were all negative, consistent with the hypothesis that these are true -1 PRF signals. While a recombinant live VEEV strain TC83 virus harboring slippery site mutations resulted in only a small delay in viral replication kinetics in both BHK and C3/36 mosquito cells, the frameshift mutant almost completely abrogated TC83 lethality in mouse infection models. These results suggest that, while frameshifting does not exert a significant influence on alphavirus replication, it has a strong effect on virulence. This latter observation suggests the potential utility of -1 PRF abrogation for the production of live attenuated vaccine strains. To better understand these targets, the -1 PRF stimulating RNA structures of EEEV, VEEV and WEEV have been resolved using chemical probing and primer extension methods. Ongoing studies are employing a mutagenesis-based approach to destabilize these key elements of the -1 PRF signals, the results of which could potentially lay the foundation for the development of live attenuated vaccines.

---

Virus-like Particles (VLPs) Co-localize Four Influenza HA Subtypes Within a VLP Structure

P. Pushko¹, R. Hidajat¹, B. Nickols¹, R. O. Prather¹, I. Tretyakova¹, T. M. Tumpey²; ¹Medigen Inc, Frederick, MD, ²CDC, Frederick, MD
Influenza VLPs comprised of hemagglutinin (HA), neuraminidase (NA), and matrix (M1) proteins have been previously used for immunological, virological and vaccine studies. We have demonstrated that influenza VLPs can be made in Sf9 cells by using influenza M1 protein or the bovine immunodeficiency virus gag protein in place of M1 protein. VLPs were prepared for several influenza subtypes including H1N1, H5N1 and H10N8. Furthermore, we prepared the first quadri-subtype VLPs, which co-expressed within the VLP the four HA subtypes derived from avian-origin, human isolates of H5N1, H7N9, H9N2 and H10N8 viruses. VLPs showed hemagglutination and neuraminidase activities and reacted with specific antisera. The content and co-localization of each HA subtype within the quadri-subtype VLP were determined. Electron microscopy showed that quadri-subtype VLPs resembled influenza virions with the diameter of 150-200 nm. Immunogenicity and efficacy of quadri-subtype VLPs will be discussed. We conclude that this innovative quadri-subtype design of influenza VLP can be used for preparation of influenza vaccines with broad protective capabilities.

---

**Poster Presentation Number: 129 (H) WITHDRAWN**

---

**Session Type:** Poster Session  
**Session Number:** 016  
**Session Title:** Poster Session 2  
**Poster Presentation Time:** Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:** H. Vaccines  

**Poster Presentation Number: 130 (H)**

**Structural Informatics of Influenza Hemagglutinin Antibody Engagement**

**D. M. McCraw, A. K. Harris; NIH, Bethesda, MD**

Hemagglutinin (HA) is the major surface glycoprotein to which neutralizing antibodies are elicited for influenza virus. Neutralizing epitopes reside in both the head region composed of HA1 globular domains and the stem region composed of HA2 domains. Highly conserved epitopes within the stem region of HA2 are the targets of a number of broadly neutralizing antibodies and hold promise for the design a universal influenza vaccine. However, it is still unclear what strategies will prove successful in engineering a vaccine that promotes broadly neutralizing antibodies to conserved HA epitopes. Here, we used structural informatics approaches in which we combined structural, biochemical, and immunological analyses in order to understand HA antigen-antibody interactions and we identified factors that influence hemagglutinin-antibody engagement.

---

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** H. Vaccines  

**Poster Presentation Number: 131 (H)**

**DNA-Launched Vaccines for Venezuelan Equine Encephalitis and Chikungunya Viruses**

**I. Tretyakova¹, B. Nickols¹, I. Lukashevich², S. Weaver³, P. Pushko¹; ¹Medigen, Inc., Frederick, MD, ²Univ. of Louisville, Louisville, KY, ³Inst. for Human Infections and Immunity, Galveston, TX**

Live-attenuated vaccines have the advantage of rapidly inducing long-term immunity after a single dose vaccination, while DNA vaccines have remarkable genetic stability. We have combined advantages of live and DNA vaccines in the novel iDNA® technology of DNA-launched live-attenuated vaccines. We applied this method for vaccine development for two biodefense-relevant viruses, Venezuelan equine encephalitis virus
(VEEV) and Chikungunya fever virus (CHIKV). The iDNA consists of a plasmid that encodes the full-length RNA genome of the live-attenuated vaccine virus downstream from a eukaryotic promoter. The VEEV iDNA vaccine was designed to transcribe the full-length viral RNA in vivo and to launch live-attenuated VEEV vaccine virus. Likewise, the CHIKV iDNA vaccine was designed to launch live-attenuated CHIKV vaccine virus. Both VEEV and CHIKV vaccines were characterized in vitro and in vivo. BALB/c mice were vaccinated with a single dose of VEEV iDNA. After vaccination, all mice seroconverted with no adverse reactions. Four weeks after immunization, animals were challenged with the lethal epidemic strain of VEEV. All iDNA-vaccinated mice were protected from fatal disease, while all unvaccinated controls succumbed to infection and died. Similarly, protection against challenge with CHIKV was observed when mice were vaccinated with CHIKV iDNA vaccine. Therefore, vaccinations with VEEV and CHIKV DNA-launched iDNA vaccines resulted in efficient protection against challenges with respective wild type pathogenic viruses. We conclude that our DNA-launched vaccine approach combines characteristics of DNA and live attenuated vaccines and represents a promising vaccination strategy for VEEV and CHIKV viruses.

Live Attenuated Francisella novicida Vaccine Protects Against Francisella tularensis Pulmonary Challenge in Rats and Non-Human Primates

J. Q. Nguyen1, P. Chu1, A. Cunningham1, J-J. Yu1, J. Barker1, C. R. Lyons2, J. Wilder2, M. Valderas2, R. L. Sherwood2, B. P. Arulanandam1, K. E. Klose1; 1Univ. of Texas-San Antonio, San Antonio, TX, 2Lovelace Respiratory Res. Inst., Albuquerque, NM

Francisella tularensis causes the disease tularemia. Human pulmonary exposure to the most virulent form, F. tularensis subsp. tularensis (Ftt), leads to high morbidity and mortality, resulting in this bacterium being classified as a potential biothreat agent. However, a closely-related species, F. novicida, is avirulent in healthy humans. No tularemia vaccine is currently approved for human use. We demonstrate that a single dose vaccine of a live attenuated F. novicida strain (Fn iglD) protects against subsequent pulmonary challenge with Ftt using two different animal models, Fischer 344 rats and cynomolgus macaques (NHP). The Fn iglD vaccine showed protective efficacy in rats, as did a Ftt iglD vaccine, suggesting no disadvantage to utilizing the low human virulent Francisella species to induce protective immunity. This is the first report of a defined live attenuated vaccine that demonstrates efficacy against pulmonary tularemia in a NHP. Comparison of specific antibody profiles in vaccinated rat and NHP sera by proteome array identified a core set of immunodominant antigens in vaccinated animals. However, there was a notable lack of humoral response in Fn iglD-vaccinated animals to the LPS of Ftt, emphasizing the lack of cross-reactivity between the Fn and Ftt OAg. Our results indicate that the low human virulence F. novicida functions as an effective tularemia vaccine platform.

Antigen Identification Using Catanionic Vesicle Extraction: A Francisella tularensis Vaccine

P. DeShong1, L. Stocker1, A. Horn1, M. Hurley1, A. P. Dhabaria1, C. Fenselau1, R. K. Ernst2, K. Richard2, B. J. Mann3, S. N. Vogel2, D. C. Stein1; 1Univ. of Maryland, Coll. Park, College Park, MD, 2Univ. of Maryland, Sch. of Med., Baltimore, MD, 3Univ. of Virginia, Charlottesville, VA

Catanionic surfactant vesicles (CSVs) are unilamellar vesicles that form spontaneously from mixtures of cationic and anionic surfactants. The size of the vesicle and its surface charge can be controlled by choice of surfactants, and the external leaflet of the vesicle can be decorated with non-ionic surfactants without affecting the stability of the vesicle in biological media. CSVs are stable at high ionic strength (1M NaCl buffer), have a wide pH range, and have been used in the development of vaccines against hepatitis B, rabies, and tetanus.
tolerance (pH 2-12), and can be pasteurized and lyophilized without significant alteration of their morphology. Extraction of Gram-negative bacterial pathogens yields CSVs that have cell surface antigens incorporated into the vesicle leaflet (fCSVs). Characterization of the bacterial components incorporated into the fCSVs by mass spectrometry indicated that the vesicles were highly enriched in cell surface glycopeptides, lipoproteins, and lipopolysaccharide. Immunization with fCSVs prepared from extraction of the Francisella tularensis LVS strain protected mice against intraperitoneal Ft LVS challenge, while immunization with either LVS-vesicles or Schu S4-vesicles partially protected mice against an intranasal (i.n.) Ft Schu S4 challenge. LVS-CSV-immunization elicited high levels of IgG against non-LPS epitopes and antisera conferred passive protection against challenge with Ft LVS. These results demonstrate that fCSVs are an excellent vaccine platform due to the ability to rapidly and stably formulate epitopes and their capacity to elicit a strong adaptive immune response.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: H. Vaccines
Poster Presentation Number: 134 (H)

Assessment of Serotype-independent Immunity Elicited by Shigella T3SS Proteins

F. J. Martinez Becerra, M. Pressnall, O. Arizmendi Perez, W. Picking, W. Picking; Univ. of Kansas, Lawrence, KS

Shigellosis is a gastrointestinal disease of worldwide public health importance for which there is no licensed vaccine. It is also classified as a category B bioterrorism agent. Our group has developed a serotype-independent vaccine based on two proteins of the Shigella type three secretion system (T3SS). These proteins have important roles in pathogenesis and are conserved among virulent Shigella strains. We generated a fusion protein (DB fusion) that comprises the T3SS tip proteins IpaB and IpaD. This vaccine has been shown to be protective in the mouse pulmonary model. We propose to use the T3SS vaccine as a model to identify the host immune responses that confer protection against Shigella infection. We hypothesize that using vaccine/infection models we will identify correlates of protection that can be measured in future clinical trials with this vaccine. We determined the type of immunity involved in protection by vaccinating animals using different administration routes. Afterwards, mice are challenged with S. flexneri. We also stimulated dendritic cells with IpaB, IpaD and the combination in the presence of dmLT and measure cytokine release and up-regulation of activation markers. In combining an identification of the correlates of protection with the use of biophysical methods for optimizing vaccine formulation, we hope to establish the basis for evaluating the likely efficacy of this vaccine in protecting humans against shigellosis.

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: H. Vaccines
Poster Presentation Number: 135 (H)

Antibody as a Correlate of Protection for Vaccination Against Pneumonic Tularemia

D. Reed1, E. Stinson1, K. Willett1, N. Grant1, K. Hazlett1, K. Hazlett1, E. Barry3; 1Univ. of Pittsburgh, Pittsburgh, PA, 2Albany Med. Coll., Albany, NY, 3Univ. of Maryland, Baltimore, MD

Francisella tularensis is the causative agent of tularemia, a severe zoonotic infection in humans with a 30% fatality rate in untreated subjects when inhaled. Vaccines are urgently needed that can protect the Warfighter against the threat posed by the intentional release of aerosolized F. tularensis. Type A strains of F. tularensis are highly virulent but the current live vaccine strain (LVS) is based on a type B strain and is only partially protective against aerosol challenge with SCHU S4, a type A strain. We have previously shown that by scarification genetically modified derivatives
of SCHU S4 are attenuated in rabbits and 27-40% of rabbits survived high dose aerosol challenge with virulent SCHU S4. Prime-boost vaccination resulted in 50-83% protection against challenge depending upon the route of vaccination and vaccine used. ELISA assays demonstrated a strong serum anti-hkFt IgM, IgG, and IgA response to vaccination. Further analysis indicated that the majority of the humoral response was directed at the endotoxin that is a major component of the bacterial membrane. Pre-challenge IgM and IgG titers correlated with the survival seen after aerosol exposure to SCHU S4. A single passive immunization of naïve rabbits with hyperimmune serum extended time to death by 2 days after challenge with SCHU S4. These data demonstrate that antibody can serve as an immune correlate of protection against aerosol challenge with F. tularensis.

**Poster Presentation Number:** 136 (H)  *WITHDRAWN*

---

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** I. Immune Response

**Poster Presentation Number:** 137 (I)

**Gene Expression Profiling of Host Defence Responses to Identify Pre-symptomatic Biomarkers in Early Stages of Acute Infection**

*S. Sheibani, M. Christopher, N. Barabé, G. Fisher, G. Schnell, L. McLaws; Defence Res. and Dev. Canada Suffield Res. Ctr., Box 4000, Station Main, T1A 8K6, Medicine Hat, AB, Canada*

An attack with a biological agent poses a serious threat to military personnel, first responders and civilians. Early diagnosis of infection is crucial for successful therapy. In many cases, disease specific symptoms appear in the later stages of diseases, thus underlying the importance of identifying infected individuals presenting with non-specific symptoms. Different classes of pathogens trigger specific pattern-recognition receptor (PRRs) within an infected individual; thus, assessment of the host response following exposure to a specific pathogen can provide information on biomarkers that could be used as a surrogate marker of infection. The host immune response to three Biosafety level two agents were evaluated: Bacillus anthracis Sterne (Gram (+) bacteria), Francisella tularensis Live Vaccine Strain (LVS) (Gram (-) bacteria) and, influenza A/PR/8/34 (RNA virus). In vivo (mouse) and in vitro (human peripheral blood mononuclear cells (PMBCs)) studies were performed in parallel. Blood from infected mice was collected before infection, prior to and following symptom development. PMBCs were sampled at various times following infection; control samples were mock-infected using culture media. To determine whether messenger RNAs (mRNAs) could be used as biomarkers of acute infection, samples were analyzed using microarray technology. As the long-term goal of this project is the development of assays that can be used for rapid diagnosis of infection using a hand-held field-deployable device, the focus of this study was on genes that were up-regulated by infection. Genes up-regulated in mice and PMBCs were compared and those that had overlap were selected for further analysis. A subset of genes was subjected to Reverse Transcription quantitative PCR (RT-qPCR) to corroborate the microarray data. This work suggests that changes in human host responses following pathogen exposure may have use for development of diagnostic assays for early detection of infectious diseases.
Assessment of the Human Immune Response to Anthrax Infection


Anthrax affects humans via contact with infected animals or their products, or from deliberate release of spores. Vaccines licensed for human use include Anthrax Vaccine Adsorbed (USA) and Anthrax Vaccine Precipitated (UK), which are cell free preparations of Protective Antigen (PA), and a live attenuated anthrax vaccine used in FSU countries. These vaccines all have limitations and replacements are being sought. Anthrax is endemic in Georgia and Turkey, with hundreds of human cases registered annually. This provides a unique opportunity to study human immune responses to anthrax and generate data relevant to next generation vaccine development. We screened 153 serum samples from 100 patients with cutaneous anthrax in 4 regions of Turkey: Erzurum (27), Va (13), Malatya (38) and Kayseri (22). Samples were tested for IgG antibodies to PA, Lethal Factor (LF) and Edema Factor (EF). Only half (77/154) exhibited anti-PA antibody; 26.7% (41/154) and 2.6% (4/154) were positive for anti-LF and anti-EF IgG, respectively. The magnitude and overall pattern of toxin specific IgG responses were similar to those reported previously in 92 samples from 46 patients from East Georgia, with IgG antibodies to PA in 85%, LF in 60% and EF in 10%. Toxin specific antibody responses were seen in 47% of Turkish and 59% Georgian samples. These data suggest that PA is the principal immunogen in the context of infection, supporting its role as the main component of human vaccines. Further studies will provide information to inform development of next generation vaccines.

Francisella is Susceptible to Mosquito Antimicrobial Peptide Cecropin

A. Kaushal¹, K. Gupta², R. Shah³, M. L. van Hoek²; ¹Dept. of Biology, George Mason Univ., Manassas, VA, ²Natl. Ctr. for Biodefense and Infectious Diseases, George Mason Univ., Manassas, VA, ³Thomas Jefferson Sch. of Sci. and Technology, Alexandria, VA

Francisella tularensis, a gram negative intracellular coccobacillus, causes the zoonotic disease tularemia. Arthropods such as ticks have been implicated in transmission of Type A tularemia to humans in outbreaks of tularemia in the USA. Epidemiological studies from Sweden implicate mosquitoes in the transmission of Type B tularemia. Recently it has been shown that mosquitoes can retain Francisella as larvae but do not transmit the pathogen to mice through actual bites. We have hypothesized that mosquitoes may produce antimicrobial peptides that could be instrumental in killing Francisella. These are small cationic, amphipathic proteins that are less than 5 kDa and are produced in response to infections as part of innate immunity. These peptides have host defense functions that may include direct antimicrobial activity. Here, we have investigated two classes of insect antimicrobial peptides, defensins and cecropins, from the Asian tiger mosquito (Aedes albopictus), a vector of many significant viruses such as dengue and yellow fever, and tested them against BSL-2 model strain F. tularensissubspecies novicida. The antimicrobial activity of synthesized peptides was measured in low salt buffer against F. novicida, and we found that mosquito cecropins were antimicrobial against F. novicida with EC50 values below 8 µM. Mosquito defensin had an EC50 of >20 µM. We assessed membrane permeabilization and cytoplasmic depolarization using the ethidium bromide uptake assay and DiSC3(5) release assay. We concluded that mosquito cecropin acts by disrupting membrane potential and eventually forming pores in the membrane of the pathogen. The expression of these
peptides in response to Francisella infection of mosquito cells was examined by qRT-PCR. In conclusion, the mosquito genome encodes at least one host-defense antimicrobial peptide that may have antibacterial activity against Francisella.

**Session Type:** Poster Session  
**Session Number:** 016  
**Session Title:** Poster Session 2  
**Poster Presentation Time:** Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:** I. Immune Response  
**Poster Presentation Number:** 140 (I)

**Rapid Throughput Characterization of Ebola Neutralizing Antibodies**

**D. P. Langan, M. Slojadinovic, S. Stronsky, B. Eaton, J. Nuss, S. Bavari, C. L. Cooper; United States Army Res. Inst. of Infectious Disease, Frederick, MD**

Neutralizing antibodies against viral pathogens is a hallmark signature of protective humoral immune responses established during natural infection and vaccination. While characterization of neutralizing antibody titers using live-viral plaque reduction (PRNT) assays have been established for many viral models, these systems are neither readily amendable nor transferable with use of highly pathogenic biosafety level 4 (BSL-4) viruses such as, Ebola (EBOV). Causative of hemorrhagic fever and the 2014 West-African epidemic with ~30,000 confirmed infections and ~10,000 fatalities; EBOV neutralization by antibody-mediated mechanisms has been previously demonstrated. However, current characterization of EBOV antibody neutralization depends upon live-viral PRNT as the gold standard with few available BSL-2 surrogate systems. To this end, we have developed a flow cytometry and high content imaging (HCI) based BSL-2 surrogate system that allows for rapid characterization of EBOV antibody neutralization. Using a recombinant vesicular stomatitis virus expressing eGFP and the EBOV glycoprotein (rVSV-eGFP/EBOV), we demonstrate reproducible characterization of EBOV neutralization with 24 hours across both flow cytometry and HCI assays. Further, using EBOV therapeutic antibodies, MB-003 and Z-Mapp, we illustrate similar neutralizing properties across the BSL-2 surrogate system and live-viral PRNT. Given the importance of neutralizing antibodies and the lack of available assays for EBOV serological studies, this assay offers a safe BSL-2 surrogate with increased sensitivity, speed and reproducibility over current EBOV PRNT assays. Collectively, this assay provides an additional tool for characterization of EBOV neutralizing titers during natural infection or within a vaccine setting.

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** I. Immune Response  
**Poster Presentation Number:** 141 (I)

**Genetic Changes in Hiv Genome and Its Effect on Immune Response**

**S. Khan; Dow Univ. of Hlth.Sci., Karachi, Pakistan**

**Background:** Pakistan has witnessed a transition from low prevalence to a country with concentrated epidemic in high risk groups. Pakistan has an overall HIV prevalence of 0.1% but within certain high risk groups such as IDUs and MSM, concentrated HIV epidemics exist. Cytotoxic T lymphocytes (CTLs) play an important role in controlling HIV infection while the virus encounters and evade the CTL response through a variety of escape mutations. Sequencing and Bioinformatics is a very useful tool to study genetic diversity and to predict HIV drug resistance and its mutation which can assist physicians in selecting effective combination of drugs. Objective: 1-To analyse drug resistance, and Mutations in gag and pol gene of HIV-1 and their effect on immune response. **Methodology:** A total of 90 HIV-1 infected plasma specimens were collected from the patients. DNA was extracted using Qiagen DNA Extraction kit and was subjected to nested Polymerase Chain Reaction to amplify the HIV gag and pol gene using specific primers. PCR products were sequenced using sequencing primers. Further 196 HIV sequences retrieved from Los Alamos database were
included in the study. Nucleotide sequence alignment and phylogenetic analysis was executed using MEGA5. Results: Sequence alignment and phylogenetic analysis showed (72.5%) isolates to be subtype A1, (2.5%) subtype D, (12.5%) subtype C, (12.5%) subtype G. Phylogenetic analysis revealed that Pakistani HIV sequences are closely clustered with Afghani, Indian and African sequences. We identified T303V mutation in gag region in most of the Pakistani isolates which is a stronger proteosomal degradation sites as compared to T303T in majority of the sequences that we retrieved from Los Alamos HIV sequence database. **Conclusion:** Our findings suggest that HIV-1 in Pakistan is dominated by subtype A-1. Frequency of male patients was higher than female and patients were relatively younger in age. The HIV infection in the region is relatively new and the virus is evolving according to the local environment and immune pressure by the patients. We have identified T303V mutation in locally spread virus.

**Cellular and Humoral Immunity Response and Distribution of Viral Antigens in Chickens After Infection with a Low Pathogenic Avian Influenza Virus (LPAIV) Isolated From Wild Ducks**

A. Stegniy¹, D. Muzyka¹, B. Stegniy², A. Gerilovych², M. Bashchenko³, M. Mandygra³; ¹NSC “Inst. of Experimental and Clinical V, Kharkiv, Ukraine, ²Natl. Academy of Agrarian Sciencies, Kyiv, Ukraine, ³Natl. Academy of Agrarian Sci., Kyiv, Ukraine

Introduction. Influenza is a serious problem for the health of people, animals and birds, therefore comprehensive studying of the influenza virus, its natural reservoir, pathogenesis and immune response will provide further opportunity to ensure protection for animals, birds and people from this infection. Aim. To study the immune response in poultry on the administration of LPAIV isolated from the natural reservoir. Materials and methods. Four-week-old commercial chickens were intranasally inoculated with a H4N6 LPAIV isolated from a garganey. Cecum, spleen, lung, and trachea samples were collected from infected chickens on 1 - 14 dpi and examined by immunohistochemical techniques. The studies were done with an IACUC. Results. The virus did not cause disease in chickens and no pathological changes were observed in inoculated birds. No virus was isolated from internal organs, but 3 chickens of 5 showed antibodies to AIV at intranasal infection. Between 7 to 14 dpi, a sharp increase in the number of cells bearing CD4, IgM, IgG, and IgA was observed. In the spleen, the number of CD4 T lymphocytes and macrophages was increased by immunohistochemical staining when compared to controls. In the lung, B lymphocytes expressing IgM (6.8±0.5%), IgG (9.4±1.3%), and IgA (8.6±0.1%) were detected in higher numbers than in controls. High levels of IFN-γ, IL-2, IL-15 were present on 7 dpi. We also found LPAIV NP staining in the trachea observed on 10 dpi (2.7±0.4% of antibody-stained areas) as well as in the spleen on 5 dpi (3.3±0.2%). There was no NP antigen in other organs. Conclusion. Although infection with a LPAIV did not cause obvious clinical disease, viral replication was detected in the trachea and spleen and both local and systemic cellular and humoral immune responses were elicited in these LPAIV-infected chickens.

**Protection Against a Neuroinvasive Virus is Dependent on CNS Resident Immune Memory**

S. A. Garcia, C. Portocarrero, R. Kean, D. Hooper; Thomas Jefferson Univ., Philadelphia, PA

Rabies virus (RABV) is a neuroinvasive Lyssavirus which is nearly 100% fatal in humans once clinical symptoms appear. RABV can be effectively aerosolized and infect through inhalation without any indication of exposure until it is too late for current treatments. The virus can only be cleared
from host CNS tissues by the local production of cytokines and virus neutralizing antibody (VNA) by infiltrating CD4 T and B cells. This response occurs during the immune response to live attenuated RABV vaccine virus but is not induced by wild-type RABV infection or vaccination with killed virus. However, if confined to the periphery even an appropriate immune response cannot ensure protection from an aerosol challenge. In this case, RABV can enter CNS tissues with minimal exposure to VNA. In this scenario, only a vaccination regimen that delivers RABV-specific immune effectors to the CNS is fully protective. Here we show that CNS-targeted immunization is significantly better than conventional peripheral immunization in protecting mice against intranasal challenge with wild-type RABV.

Adoptive transfer experiments confirm that primed immune cells are not capable of migrating from the periphery into the brain to confer protection against a CNS challenge. We conclude that the difference between vaccination regimens is likely to be due to the increased formation of immune memory cells that become resident in brain tissues. This results in a more robust and effective local response during a CNS infection. The requirement for the safe delivery of immune effectors and memory cells into CNS tissues is likely to hold for a wide variety of neuroinvasive viruses. The implication for the establishment of immune defense against neurotrophic biological agents suitable for weaponization is that novel CNS-targeted vaccination strategies are required.

---

Do Virulence Factors Contribute to Host Range Expansion and Disease Differences Between Toxoplasma gondii and Closely Related Species?

R. S. Coombs, E. D. English, J. P. Boyle; Univ. of Pittsburgh, Pittsburgh, PA

The parasite Toxoplasma gondii is a class-B biodefense pathogen and can infect large numbers of people by contaminating food and water supplies. Simple genetic modifications increase virulence and drug resistance, it is the causative agent of the human disease Toxoplasmosis, and can be lethal in utero. In addition, T. gondii has the unique ability infect most warm-blood animals on the planet. This expanded host range makes T. gondii one of the most successful eukaryotic parasites and prime agent for bioterrorism. Genomic comparisons between T. gondii and its close relative Neospora caninum have revealed a highly syntenic relationship (over 90% orthologous). Despite genetic similarities, T. gondii varies greatly in virulence and host range. T. gondii causes severe disease in mice, unlike N. caninum. Both parasites grow similarly in the first 24h of but the mouse immune system curtails N. caninum infections and T. gondii continues to grow. To understand how host-pathogen interactions contribute to the emergence of new diseases we use this genetically tractable, comparative system to investigate the genetic and molecular basis for virulence and host range expansion of T. gondii. Specialized organelles secrete hundreds of proteins in a T. gondii infection. Secreted proteins ROP5 and ROP18 have been identified as key virulence factors in T. gondii mouse infections. Here, we investigate the role of T. gondii virulence factors in the host immune response by transfecting N. caninum parasites with T. gondii ROP5, ROP18, or ROP18/5 alleles. Mice were infected with transgenic N. caninum parasites and burden was monitored. In Nc:TgROP5, Nc:TgROP18, and Nc:TgROP5/18 infected mice, parasite burden increases initially but failed to bypass the critical 24h point where burden declines. However, in TgROP18/5 infected mice we observe a delay in burden decline. This shows that ROP18 or ROP5 alone are not sufficient to induce T. gondii like virulence in N. caninum. But TgROP18/5 infections result in a small delay in parasite decline indicating a possible change in infection success and timing.
Repertoire-scale Analysis of Immunosequencing Data From the B Cell Response to An Ebola Antigen Under Different Adjuvant Conditions

S. Chaudhury1, I. Khavrutskii1, C. L. Cooper2, S. Bavari2, A. Wallqvist1; 1BHSAI, U.S. Army Med. Res. and Materiel Command, Frederick, MD, 2U.S. Army Res. Inst. of Infectious Diseases, Ft Detrick, MD

Repertoire-scale sequencing of B cell receptors (BCR), or immunosequencing, is a powerful tool for characterizing humoral immune responses. However, quantitative analysis of the immunosequencing data has a number of challenges. The BCR CDR3 loop, responsible for antigen specificity, has tremendous genetic diversity as a result of germline VDJ recombination and somatic mutations, making accurate annotation challenging. Standard analysis methods treat unique sequences in the BCR repertoire independently and ignore the underlying lineage relationships, which can lead to errors in VDJ gene annotation and distortions of repertoire-scale properties such as the distribution of CDR3 loops or enrichment of certain BCR sequences. In order to analyze immunosequencing data while taking into account lineage relationships within the repertoire, we developed a method that identifies clonally related sequences, selects an exemplar ‘parent’ sequence for each clonally related family, and assembles a database of independent, ‘parental’ and non-independent ‘clonally-expanded’ BCR sequences. We applied this approach to immunosequencing data from lymph node B cells of mice that were immunized with an Ebola vaccine with and without adjuvant. We generated a database of parental sequences and clonally expanded sequences and found that the clonally expanded sequences contained repertoire features unique to the adjuvant vaccine condition, such as differences in CDR3 length distributions and V gene family usage that were not found in parental sequences. These results suggest that the adjuvant does not affect the initial selection of the naïve B cells, but instead alters their clonal expansion and hypermutation during affinity maturation. Importantly, this method is able to evaluate the statistical significance of differences between BCR repertoires and provide valuable insight into how the underlying structure of these repertoires shifts under different vaccine conditions.

Activation of the Autophagy Pathway After Infection With Australian Bat Lyssavirus in Cells From the Natural (Bat) and Accidental (Human) Host

E. D. Laing, D. L. Weir, B. C. Schaefer, C. C. Broder; Uniformed Services Univ., Bethesda, MD

The black flying fox (Pteropus alecto), a species of bat, is the natural reservoir of both Australian bat lyssavirus (ABLV) and Hendra virus. Unlike other terrestrial mammals, such as humans that are accidental hosts of these pathogenic viruses, bats do not uniformly develop symptomatic disease. We hypothesize that unique immune responses in bats might account for the observed disparities in disease outcome. Autophagy, a cellular process involved in protein homeostasis, has been implicated to function as an antiviral mechanism during infection with several pathogens. Proper function of autophagy is of particular importance in post-mitotic cells such as neurons where maintenance of cellular homeostasis is critical to avoid cell death and pathogenesis. The role of autophagy during infection with neurotropic viruses such as ABLV and other rabies virus-related viruses is unknown. We hypothesize that autophagy will function as an antiviral mechanism during ABLV infection of cells derived from black flying fox tissue. Black flying fox brain tissue cell lines (PaBrH) and kidney tissue cell lines (PaKiT) were infected with reporter ABLV that expresses a green fluorescent protein (GFP) open reading frame. A human neuroblastoma cell line (NBF-L) was used for comparative investigation of the autophagy pathway. Autophagy was activated by ABLV infection in both bat and human cell lines. To elucidate the potential
benefit of therapeutic induction of autophagy during lyssavirus infection, human NBF-L cells were treated with BEZ235, a known pharmacologic inducer of autophagy. We examined ABLV levels harvested from NBF-L cells treated with BEZ235. Preliminary data suggests that activation of autophagy by BEZ235 resulted in decreased levels of ABLV GFP expression in human cells. Elucidation of the anti- or pro-viral role of autophagy during ABLV infection in both bat and human cells will be assisted by future genetic and pharmacologic studies.

---

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** J. Decontamination, Biosafety, and Containment  
**Poster Presentation Number:** 147 (J)

**Adapting biosecurity to meet the demands of emerging infectious disease: applications for Global Health Security**

R. N. Burnette; AT-RISK Intl., LLC, Chantilly, VA

The Global Health Security Agenda (GHSA) is a multinational operation aimed at enhancing global capabilities to prevent, detect and respond to infectious diseases, emerging both from natural and intentional sources. A major component of GHSA is promoting development of robust biosafety and biosecurity (BS&S) infrastructure and capacity in many parts of the world where these elements are currently lacking. While public health efforts have dominated donor activity in the developing world (e.g. PEPFAR) for some time, our world is faced with a new paradigm comprised of both public health and defense. In this sense, GHSA is somewhat unique: it is equally focused on health and security. The proliferation of clinical and diagnostic laboratories in the developing world have resulted in greater capacity to detect and diagnose infectious diseases. However, lacking biosecurity policies and capabilities result in inadvertent stockpiles of pathogens and toxins of international security concern. Therefore, it is imperative that biosecurity measures be applied in conjunction with public health activities to ensure well-rounded global health network operating in a secure manner, particularly in regions of the world with limited resources to adequately address security. Today, a major gap exists between public health efforts and biosecurity. Threat assessment and vulnerability analysis are two common tools in any security culture, including biosecurity, where access to dangerous biological agents is limited. Both of these tools have yet to be adapted and scaled to meet the demands of global pandemics and international biological incidents. This presentation will review the fundamentals of biosecurity, threat assessment and vulnerability analyses, and expand these concepts to the operating magnitude of the GHSA. This resulting model of biosecurity will be applied against the objectives of the GHSA, indicating areas of potential weakness, and opportunities for improvement.

---

**Session Type:** Poster Session  
**Session Number:** 016  
**Session Title:** Poster Session 2  
**Poster Presentation Time:** Tuesday, February 9, 2016, 5:45 pm - 7:00 pm  
**Topic:** J. Decontamination, Biosafety, and Containment  
**Poster Presentation Number:** 148 (J)

**Biofilm Removal Enables Surface Decontamination**

M. J. Bruno, C. K. Burzell; Aequor, Inc., Oceanside, CA

Contamination, spores and radioactive particles may firmly attach to surfaces of equipment, structures, materials, fabrics, and tissues due to bacterial biofilm - the glue-like matrix that most bacteria form in order to protect the colony. Biofilm is everywhere, even on the surface of water itself. Traditional removal remedies include mechanical scraping, heat, and the use of biocides (antimicrobials, disinfectants, antiseptics). But biofilm was recorded on a titanium plate within 30 seconds after sterilization, and many surfaces cannot be scraped. As validated by Lonza, no
known biocide or antibiotic can remove biofilm at non-lethal doses. The chemicals in biofilm’s top layers can neutralize the harshest biocides. Some surface modification coatings, and novel biobased antibiofilm chemical agents effectively inhibit biofilm formation in many contexts, but their efficacy in a spore or bio-attack has not been verified. Every pathogen on the CDC list of potential bioterrorist threats (anthrax, botulism), pandemics (cholera, Listeria, plague, etc.), and antimicrobial resistant strains (MRSA, MDR-TB, etc.) is a biofilm-former. Biofilm is not only found on Earth, but is also in space, where it grows thicker and more virulently on surfaces, instruments, and in air and water systems. Biofilm on satellite optical sensors can skew readings. A comprehensive biosafety program should include enhanced antibiofilm remedies to be deployed as a rapid countermeasure in a wash or spray to remove surface biofilms and residues in the event of a detonation, bio attack or pandemic, or to inhibit the ability of target pathogens to colonize. For longer-term protection, antibiofilm agents can be incorporated in gels, pastes, coatings, paints, and materials to inhibit biofilm formation on critical surfaces. Ideally, these antibiofilm/antifouling agents are effective and benign to the user and the environment, avoiding further cumulative and persistent impacts on soils, waters, and surfaces, as well as human and animal health.

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: J. Decontamination, Biosafety, and Containment

Chemical Warfare Remediation Utilizing "Cell Free" Bacterial Nanobioreactors

N. J. Alves, K. B. Turner, S. A. Walper; Naval Res. Lab., Washington, DC

All bacteria release outer membrane vesicles (OMVs) packaging a diverse array of small molecule, protein, and genetic cargo for a variety of functions. We sought to hijack this bacterial cell export pathway to simultaneously produce, package, and stabilize an active enzyme within an OMV. Phosphotriesterase (PTE) was selected as a representative enzyme with applications for the environmental remediation of organophosphate contaminated regions. PTE is a highly promiscuous enzyme capable of hydrolyzing a broad range of organophosphate based pesticides (primary substrate: paraoxon) as well as V and G type nerve agents. To drive packaging of PTE within the OMVs the SpyCatcher/SpyTag (SC/ST) bioconjugation system was utilized to create a PTE-SpyCatcher (PTE-SC) fusion protein and SpyTag fusion to an abundant transmembrane protein known to be present within OMVs. When the SpyTag and SpyCatcher domains interact with one another they form a covalent isopeptide bond tethering the PTE within forming OMVs. The enzyme loaded OMVs were then characterized for size distribution, number of vesicles produced, cell viability, packaged PTE enzyme kinetics, OMV loading efficiency, and packaged enzyme stability. The PTE-loaded OMVs exhibited native-like enzyme kinetics in addition to greatly improving the overall PTE production levels by providing the bacteria with a mechanism to mitigate toxicity through exporting the recombinant product. Additionally, greatly enhanced enzyme stability was observed as PTE-filled OMVs maintained activity post lyophilization and iterative cycles of freeze-thaw compared to free PTE.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: J. Decontamination, Biosafety, and Containment

Inadequate Disposal of Laboratory Waste in Karachi, Pakistan

N. Maqsood, S. Khan; Dow Univ. of Hlth.Sci., Karachi, Pakistan

Background: Clinical labs are considered as a major source of infectious and hazardous waste that requires strictly regulated disposal procedures. These waste streams are often overlooked or not considered a serious health hazard. In recent years Karachi has witnessed rapid mushrooming of
clinical labs that has led to rapid production of infectious waste. However due to lack of resources, awareness and improper practices could become a serious threat to public health. Objective: Our aim is to carry out a survey to evaluate the current level of waste disposal procedure and management in clinical labs in Karachi, Pakistan. **Methodology:** Questionnaire, based on standard guidelines of waste management and handling were prepared and distributed to 12 public and private sector labs in Karachi. Results: Waste management: According to the results only 34% of the labs have posted waste disposal instructions, warning signs and labels accordingly. Where as 75% labs liquid waste disposal sinks are directly connected to public sewerage lines. 58% of the labs dispose their infectious waste in red bags. Proper waste treatment facilities available only in 50% labs. Waste Handling and decontamination: Question regarding waste containers were filled to brim before disposal, 50% participants said yes. 58% labs agreed that they carried out microorganism specific decontamination, Reporting of accident and spill of waste only 75% people followed this practice. 58% labs have biosafety officers whereas 67% labs running without waste management policy. **Conclusion:** Our results indicate that waste disposal guidelines are not being strictly followed and regulated in most of the clinical labs in Karachi. Few labs were applying proper waste management practices. Important requirements regarding decontamination, waste management and spill reporting are being overlooked and not checked upon regularly. Our study suggest that waste management program should be developed and clearly defined. All lab personals should have extensive basic training. It would minimize the risk of accident and injuries and also prevent disease transmission.

---

**Session Type:** Poster Session  
**Session Number:** 003  
**Session Title:** Poster Session 1  
**Poster Presentation Time:** Monday, February 8, 2016, 11:30 am - 1:00 pm  
**Topic:** J. Decontamination, Biosafety, and Containment  
**Poster Presentation Number:** 151 (J)  

**Impact Of Biosafety Training On The Practices Of Laboratory Workers**

S. Khan¹, N. maqsood¹, M. Zahid¹, B. A. Khan²; ¹Dow Univ. of Hlth.Sci., Karachi, Pakistan, ²Quaid-i-Azam Univ., Islamabad, Pakistan

**Background:** Concern regarding incident reporting and biosafety training in clinical lab in Pakistan has increased remarkably in the last few years due to rapid increase in diagnosis and research on infectious organisms. In order to ensure the safety of employees, this issue needs to be addressed immediately. Biosafety training sessions and lectures are necessary for the protection of lab workers which also help to minimize the count of incident reporting in the lab. Objective: To carry out an inter-departmental surveys in lab regarding the awareness of biosafety practices before and after biosafety training sessions during last three years. **Methodology:** We conducted a 36 questions survey in 2013 (before training session) to gather information about biosafety awareness. Questionnaire consist on basic questions related to general laboratory practices, Biosafety levels and waste management. Afterwards, we conducted surveys after training sessions and workshops in 2014 and 2015. Result: The results showed that before training session 32% of the participants were aware of biosafety level being used in their lab whereas after the session this percentage increased to 72% in 2014 and 80.9% in 2015. Awareness regarding proper management of hazardous waste increased from 32% to 64% and in 2015 it increase to 71%. The incident reporting, proper disinfection, usage of PPE and hand hygiene was previously reported to be 40%, 65%, 48% and 52% that increased to 80%, 85%, 76% and 88% in 2014 and 95%, 86%, 90%, 90% in 2015 respectively after the training sessions. **Conclusion:** The first survey results showed lack of awareness that suggest nearly all employees are required to have biosafety trainings, and inspection at least twice a year by a biosafety officer. After the training session, significant changes in awareness level and attitude of the employees were observed in 2014 and then it has been further improved in 2015. The results indicate that increasing in workshop and trainings have improved the quality of biosafety practices. Therefore, biosafety sessions should be carried out regularly in clinical labs.
Evaluation of Radio Frequency Identification (RFID) Technology in Biological Specimens Stored at Ultra-low Temperatures

M. Rahman; US Army Med Res Inst Infect Dis Res, Fort Detrick, MD

Radio frequency identification (RFID) technology is being used extensively in both government and private sectors for inventory management. Application of RFID technology in the identification of biological specimen can have a significant impact on inventory management. RFID has been used extensively in many applications at various temperatures, e.g., ambient (~22°C), 4°C, -20°C and -80°C. However, testing of this technology on biological specimen labels stored at ultralow temperatures matching the boiling point for liquid nitrogen (LN2; -196°C) is limited and remains inconclusive. In an effort to optimize work flow and biological specimen identification and management, we evaluated the performance of three RFID systems (BoxMapper, handheld reader, and brickyard antenna) in biological samples stored at ultra-low temperatures (-80°C and LN2). Preliminary findings suggest that these RFID devices maintain high accuracy of detection (>99.5%) and could withstand ultra-low temperature conditions. The potential applications of RFID technology for specimen management system for conducting research, internal audits, and regulatory inspections will be discussed.

The German Partnership Program Supports Biorisk Management in Kazakhstan

S. Frey1, Z. Shapiyeva2, S. Essbauer1; 1Bundeswehr Inst. of Microbiol., Munich, Germany, 2Scientific and Practical Ctr. of Sanitary and Epidemiological Expertise and Monitoring, Almaty, Kazakhstan

Due to the various environmental conditions Kazakhstan offers a wide range of natural foci for epidemiologically significant diseases like plague, tularemia, tick-borne encephalitis and Crimean-Congo hemorrhagic fever. All are potential pathogenic B-agents and can be a threat to the public health, be it by appearing accidentally in the laboratory, by being intentionally misused or in a terrorist attack. One aim of the German Partnership Program for excellence in Biological and Health Security is to identify biorisks while handling relevant pathogens in biological laboratories (BL). To reduce the possibility of any misuse or accidental release of highly dangerous pathogens in BL’s a good Biorisk Management System is necessary. Herein we report how the WHO Biorisk Management System can be adapted in Kazakhstan and how it will help to increase biosafety and minimize the potential risk of human infection by dangerous pathogens. In our Risk Assessment we identified several risks in the BL; i.e. crushing potentially infected ticks without carrying personal protective equipment (PPE) and without using a biosafety cabinet (BSC). As Mitigation Measures the laboratory was equipped with new furniture, a BSC and a tissue lyser that allows homogenization of ticks free of aerosol. Standard Operating Procedures were implemented for all necessary procedures to be carried out in the BL including PPE. To minimize the risk of laboratory infections we started to educate the laboratory staff in several tabletop exercises to raise their awareness. Effective Performance was achieved by setting up a laboratory quality system in which proper procedures are embedded. Briefings and internal audits are given to the laboratory on a regular basis. The establishment of a Biorisk Management System has proven to effectively increase the risk awareness in the local BL’s. The laboratory setting is a good example of how Biorisk Management becomes a necessity in those environments and how easily biosafety and biosecurity can be carried out once a good management plan is in place.
Development of a Portable Decontamination Suit

H. Truong\textsuperscript{1}, D. Izadjoo P.hD\textsuperscript{2}; \textsuperscript{1}ChiScan LLC, Tempe, AZ, \textsuperscript{2}Trideum, Frederick, MD

There is a significant potential for biological threats to both civilians and military personnel in a domestic or hostile environments. Currently, there are no effective protective measures against these infectious agents particularly in situations where an extremity or a face of the warfighters are exposed. To address this critical need, our team has developed five energy platforms to test against these microorganisms. Prototypes have been built that will allow the robust testing of micro-organisms within plasma fields, electrical fields, radio frequency, magnetics, and light energies. The first, most promising platform is the use of a novel next generation plasma energy technology using ambient oxygen as the plasma source. Our approach has been to develop microarrays that create each form of energy. These microarrays are driven by custom electronics that allow modulation of the energy flow to allow optimization of the energy delivered. We have developed a proprietary method to create gas plasma ("non-thermal plasma") on a thin membrane using ambient oxygen. This design can be easily modified to create a portable, biological “decontamination” suit or fabric, very lightweight and using very low power. Our laboratory testing demonstrated efficacy of this novel technology against both bacterial and fungal agents. This technology has significant potential as a countermeasure for multidrug-resistant and hard-to-treat infectious agents in both clinical and field settings.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Treatments (frequency/ time)} & None & I & II \\
\hline
\textbf{Microorganism} & Candida albicans V286 (10\(^7\) CFU/ml) & & \\
\hline
\textbf{Conditions} & None & 241 Hz /1 min & 482 Hz /1 min & 964 Hz /10 min \\
\hline
\textbf{Results (CFU/ml)} & Grey & Grey & <100 & <100 \\
\hline
\end{tabular}
\caption{Effect of ChiScan Cold Plasma Technology against Candida albicans, a Fungal Pathogen. Validated by our biosciences laboratory.}
\end{table}

\textbf{Experimental Procedure:}
1. Fungal cultures were spread onto agar plates and air dried for 5-10 min.
2. Plasma Array pad (ChiScan) was placed on the plate and applied plasma.
3. Incubated the agar plates at 37°C for 18-24 hours.
4. Plates were examined for fungal growth.
Strengthening Biosafety and Biosecurity in Our Nation’s Laboratories

R. Goswami, C. Mangal; Association of Publ. Hlth.Lab., Silver Spring, MD

Public health laboratories serve in an integral role in responding to public health emergencies and keeping our nation safe. Recent lapses in biological safety were observed in the domestic response to Ebola, finding of smallpox vials at a federal laboratory and in the inappropriate shipping of live Anthrax. In response to these issues, the Association of Public Health Laboratories (APHL) engaged with the U.S. Centers for Disease Control and Prevention (CDC), to develop recommendations, provide tools guidance to assist public health laboratories with strengthening their quality management systems. CDC provided funds to 62 jurisdictions to facilitate hiring specialized biosafety officers, perform outreach to sentinel clinical laboratories, and enhance Biosafety and Biosecurity practices. APHL is developing and distributing training tools for officers as well as serving as subject matter experts for the laboratories to be successful in the implementation of their biosafety and biosecurity programs. Risk assessment tools, mitigation guidance, and clinical laboratory communication resources are being distributed. The efficacy of this initiative was and continues to be measured via calls and surveys with each lab individually and improvements in laboratory biosafety and biosecurity were noted within the first quarter of implementation. This session will focus on APHL’s partnership with CDC to strengthen Biosafety and Biosecurity across U.S. public health and sentinel clinical laboratories.

Poster Presentation Number: 156 (J) WITHDRAWN

Removal of Soil and Bacteria From Stainless Steel

E. A. Gonzalez¹, P. Nandy¹, A. D. Lucas¹, K. Kelly², V. M. Hitchins¹; ¹FDA, Silver Spring, MD, ²Univ. of Maryland, College Park, MD

Stainless steel is a commonly used material in reusable medical devices (RMDs). RMDs can become contaminated with human-derived soil and select agent pathogens when being used to treat infected patients. We used wipes soaked with water, 70% alcohol, or 0.5% bleach to clean and disinfect stainless steel (316L) coupons that were contaminated with bacteria and artificial blood or fecal test soil. We investigated whether the presence of soil or material roughness affected the ability of wipes to remove bacteria from the stainless steel coupons. After cleaning, residual bacteria, protein, and total organic carbon (TOC) were measured on the coupons. The presence of bacteria did not prevent removal of the soil. However, the presence of either blood or fecal test soil inhibited the removal of bacteria. While we previously reported that rough plastic materials retained more soil than smoother ones, we did not see the same trend with the stainless steel coupons. There was no clear advantage in using either 70% alcohol or 0.5% bleach compared to water as all three cleaning agents removed soil and bacteria to comparable levels. The residual soil was measured using two quantitative assays: microBCA and TOC, which had very similar results. Overall, we conclude that of the following variables: cleaning agent, surface roughness, presence of soil, and type of soil, only the presence of soil affected our ability to remove bacteria from stainless steel surfaces.
Texas DSHS LRN Laboratory Response to the 2015 Department of Defense Anthrax Incident

E. Swaney; Texas Dept of State Hlth. Services Lab, Austin, TX

Over Memorial Day weekend in 2015, multiple state public health departments around the US were contacted by the CDC, alerting them that the Department of Defense (DoD) inadvertently shipped potentially live Bacillus anthracis material to laboratories in their jurisdictions. They requested cooperation in reaching out to the affected labs to discover the current status of the material, the nature of the work that had been performed with it, containment techniques used during handling especially aerosol generating procedures, and the health status of their employees. As the incident unfolded, it was learned that a larger number of laboratories than originally thought were involved, and that some laboratories had also shared this material with other facilities. Testing proved that the material was not properly inactivated, and viable material was recovered from the samples. Over the next four weeks, the Texas Department of State Health Services assisted the DoD, CDC, and EPA with response to this incident. In Austin, the LRN laboratory aided the military Civil Support team, the BioWatch laboratory, and a local company with securing the agent, packaging and shipping samples to the CDC, and destroying material. As part of the CDC’s Laboratory Response Network, the DSHS Laboratory was well suited to assist and perform their mission of responding to emerging threats of public health significance.

Threats and Realities of Synthetic Biology

J. M. Marioneaux; George Mason Univ., Fairfax, VA

There is widespread misinterpretation of synthetic biology and with misunderstanding comes fear and mistrust. The basic applications of synthetic biology are strictly peaceful and for academic, medical, and industrial use to better the world around us in diverse ways. Therefore, it is important to dispel the widespread belief that biologists, chemists, and others working with designer microbes are out to build something to destroy the world. As some authors note, the expanding field of synthetic biology has been used on numerous occasions to help better understand the inner workings of biology, such as leading to a deeper understanding of the workings of the human biome, the creation of designer organisms for insulin production, and environmental remediation. Does the possibility exist that this research could be subverted and used for malicious purposes? Of course, but the benefits are substantial and, fortunately, the level of technology that is required for that level of synthetic biology is well out of reach for all but the most determined groups or institutions.
Studies in the Medicinal Potential of Tridax procumbens

N. O. Orakwelu; Nnamdi Azikiwe Univ., Awka, Nigeria

Background/Aim: In spite of great advances observed in modern medicines, plants still make contribution to health care (Shariff, 2001). This aims at determining the phytochemical constituents and its antimicrobial effect on some of the clinical isolates. **Methods:** Phytochemical test was carried out on the extracts to ascertain the presence of some bioactive components as described by Sofowora, (1993); Edeoja, et al., (2005). The effect of the extracts were tested on Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella spp, Aspergillus flavus and Candida albicans using agar disc and agar well diffusion methods of Garrod, et al., (1981) and Irobi (1992). Results: Both the methanolic and aqueous extracts of the leaves showed the presence of saponins, tannins, flavonoids, anthracine glycosides and cyanogenic glycosides. The methanolic extract of Tridax procumbens showed antimicrobial activity against some of the test organisms (bacteria) with the highest activity on E. coli and the least with P. aeruginosa while there was no zone of inhibition on the fungal isolates with both extracts. **Conclusion:** The leaves of Tridax procumbens can be used as herb because of its inhibitory capacity on some bacteria. The combination of the root, stem and the leaf will be more effective.

Survey of Awareness and Attitudes of American and Pakistani Graduate Students Towards Dual Use Research

S. Sarwar, k. Lobo, M. DeLuca; 1Hlth.Security Partners and Young Ambassador of American Society for Microbiol. to Pakistan, Lahore, Pakistan, 2Cornell Univ., Ithaca, NY, 3Georgetown Univ. Sch. of Med., Washington, DC

Dual use research poses a significant challenge for scientists operating in the biomedical field and for global health security in general. Prevention of the misuse of facilities, equipment, agents and scientific knowledge requires high levels of awareness of the concept of dual-use research, starting with early-career scientists and graduate students. There is limited data on the differences in awareness and understanding of the concept of responsible conduct of research among young scientists from different countries and at early stages of their careers. In this study, the attitudes and level of awareness of graduate students in the US and Pakistan towards the issues surrounding dual use research was assessed through a survey containing both quantitative and qualitative questions adapted from an NRC/AAAS survey on dual-use research and validated by subject-matter experts. A web-based survey was distributed to all students pursuing a PhD in microbiology at Cornell University in the US and the University of the Punjab, in Lahore, Pakistan. The authors hypothesized that there would be limited awareness of the concept of dual-use research among students and that significant differences would exist between students from the US and Pakistan regarding their understanding and interpretation of issues related to dual-use research. Preliminary data from this survey supports these hypotheses. The findings of this survey will be valuable to understanding country-specific gaps in awareness and training related to dual-use research among early-career scientists. The findings will inform the future development of training workshops on dual-use research for graduate students.
Protein Catalyzed Capture (PCC) Agents as Synthetic Antibody Alternatives with Tunable Properties for In Vitro and In Vivo Applications


Protein Catalyzed Capture (PCC) agent technology allows the adaptation and optimization of peptide-based antibody alternatives for a wide range of in vivo and in vitro applications through iterative click chemistry screening steps. The combination of tailored affinity, selectivity, size, thermal stability, and chemical/biological stability matured from peptide candidates discovered via phage and bacterial display panning methods will be described for the protein targets Protective Antigen (PA) of Bacillus anthracis and Vascular Endothelial Growth Factor (VEGF). Each PCC agent screen iteration results in necessary improvements in affinity, selectivity, size, and stability for the application of interest. The capture agent matured against PA exhibits impressive thermal stability with no loss in performance after 1 hour at 70°C and retains greater than 81% binding activity after heating at 90°C for 1 hour. The anti-VEGF reagent shows less than 20% degradation in human plasma and mouse liver microsomes over a period of 1 hour at 37°C and exhibits a mean elimination half-life (T1/2) of 36 minutes. The breadth of properties achieved through the PCC agent screening process can aid in the discovery, maturation, and modification of next generation peptide-based antibody alternatives for numerous application spaces.

Survey to Assess the Awareness about Biosecurity, Bioethics & Dual use concerns among the PhD students of Life Sciences in Pakistan

B. A. Khan1, S. Khan2; 1Quaid-i-Azam Univ., Karachi, Pakistan, 2Dow Univ. of Hlth.Sci., Karachi, Pakistan

Background: Life sciences research is crucial to make scientific advances for public health and safety. Contrary to its benefits certain types of research can be utilized for harmful purposes called “dual use research.” Many regulations and guidelines are there which address risks associated with bioterrorism, biosecurity, bioethics, personnel reliability and risks related to dual use research. Objective: To undertake a gap analysis to elucidate the extent of biosecurity, bioethics and dual use research awareness among the PhD students in Pakistan. Methodology: We distributed a 40 questionnaire to PhD students of different universities of Pakistan which consist of basic questions related to biosecurity, bioethics, bioterrorism & dual use research. Result: The results showed that 75% of the participants were aware of the term bioethics, bioterrorism, bioweapon, biosecurity whereas only 17.5% have heard about Dual use research concerns & codes of conduct. 92.5% of the participants have never learned about CWC, OPCW, BTWC and declaration of Helsinki in their curriculum. 65% of the participants did not know about any national/international organization/law regulating and prohibiting the non-peaceful use of life science research. 72.5% of the participants did not know if there lab setup or field of study involves techniques that have dual use potential. However 95% of the participants agreed that aforementioned topics are important & should be taught to life sciences students. Conclusion: The results indicated the awareness about bioethics, code of conduct, dual use research concern & laws prohibiting the non-peaceful use of life sciences research are extremely low among the PhD students of life sciences in Pakistan. There is urgent need for workshops and training to make them aware about these issues to avoid any potential misuse. On positive note most of the surveyed participants were in favor of teaching & including bioethics, biosecurity and dual use research concerns related education in their curriculum.
Prions as a Potential Biological Weapon

L. F. Roberge; Laboure Coll., Milton, MA

Prions are defined as protein based infectious particles. Prions have been found to cause a variety of neurological disorders including variant Creutzfeldt-Jakob (vCJ) disease, Kuru, Gerstmann-Straussler-Scheinker (GSS) disease in humans and Scrapie in sheep and goats, Bovine Spongiform Encephalopathy (BSE) in cattle, and Chronic Wasting Disease (CWD) in deer and elk. The pathways of infection include either consumption of prion infected tissue, by contact with contaminated (i.e. iatrogenic infection) surgical instruments, and recent research indicated airborne transmission via prion particles inhaled and absorbed by nasal epithelial tissue. Studies have demonstrated that prions have been found to persist for years as infectious agents in soils as well as aqueous extracts derived from prion contaminated soils. Cattle and wildlife (e.g. elk) would become infected by grazing on land contaminated by the prions and consumption of prion absorbed soil particles. Therefore, analysis of the data indicated that prions would be a serious bioweapon threat either as a strategic weapon intent on massive damage to agricultural commodities (e.g. cattle, meat) or infecting civilians as well as a weapon for terror by bioterrorists. Since the dispersal of prion infected material would remain infectious for years, agricultural land contaminated by prions would reduce agricultural productivity and render the farmland useless. Furthermore, since no present treatment exists for prion based diseases and this agent could be considered an emerging disease with potential for massive production, prion agents would be considered a Class C bioterrorism agent by CDC bioterrorism agent classification (i.e. high infectious, emerging disease, potential for massive production, and no treatment available). One scenario discussed would be the airborne dispersal of prions by bioterrorists which would result in eliciting a high degree of anxiety and social disruption in the civilian population; create severe disruption of cattle based commodity markets and international trade; as well as overload the resources of the public health sector. Potential for possible countermeasures and detection of a prion attack is also discussed.

Next Generation Teaching: Bioterrorism, Biodefense and Authentic, Course-Based Research

M. J. Lee-Brown, A. T. Barbour, D. J. Hulburt; Guilford Coll., Greensboro, NC

Teaching the next generation about bioterrorism and biodefense is critical for building an informed citizenry and part of the ethos of Guilford College; to develop critical thinkers who are able and willing to tackle real world issues. BIOL 291- Introduction to Scientific Inquiry: Bioterrorism, is an undergraduate ‘science by immersion’ course where students model discipline-specific modes of communication, including reading, writing and oral presentations. The students learn the value of communication as they work together to overcome the challenges, frustrations and exhilarations inherent to research. This course addresses the difficult concepts of government sovereignty, ethics, the difference between bioreadiness and biodefense, and the difference between the mindset of bioterrorists and bioreponse personnel. As part of their course work, the students work in small groups to develop a plausible dissemination method, vehicle or exploitable weakness using Bacillus thuringiensis as the model for Bacillus anthracis. In this authentic, student-generated research, students are responsible for experimental design, execution, data analysis and reporting. At the end of the semester the research groups present their findings to the Director of the Guilford County Regional Response Team to link their research to the biodefense community. Classroom Undergraduate Research Experience (CURE) survey results for this course exceeded the national mean for both research related learning gains and active-learning, course element gains. Peer analysis reports and
post-course evaluations also indicated a positive intellectual experience in this course. After presenting the learning outcomes and assessment data for this course, two BIOL 291 students will present their original dissemination research projects using loose tobacco and paper money.

Session Type: Poster Session
Session Number: 016
Session Title: Poster Session 2
Poster Presentation Time: Tuesday, February 9, 2016, 5:45 pm - 7:00 pm
Topic: K. Other and Information Only

Poster Presentation Number: 166 (K)

**The Influence of Globalization and Climate Factors on the Emergence and Recrudescence of Mosquito-Borne Diseases**

**A. Owsenek; CSC, Falls Church, VA**

With globalization and climate change comes disruption of natural ecosystems which can lead to emergence and recrudescence of vector-borne diseases, including mosquito-borne diseases. West Nile virus, first identified in New York City in 1999, has become ubiquitous across the United States, and has been detected in more than 65 different mosquito species, making it the most widely distributed arbovirus (1). Additionally, nearly 40% of the world’s population live in geographic areas favorable for dengue virus transmission (2). While it is well documented that human built and natural environments influence the prevalence of mosquito-borne diseases (3), analyses on the complex interactions of globalization and climate change and their respective influences on mosquito-borne diseases are limited. Presented here is a review and analyses of select factors of globalization and climate change and their cumulative effects and interactions influencing the prevalence of select mosquito-borne diseases. By determining a factor’s or set of factors’ favorability towards mosquito breeding environments the results indicate trends for mosquito-borne disease emergence or recrudescence, but also indicate wide knowledge gaps in our understanding of these complex relationships. References: 1.Centers for Disease Control and Prevention. 2013. West Nile virus in the United States: Guidelines for surveillance, prevention, and control. Centers for Disease Control and Prevention (CDC), Division of Vector-Borne Diseases. U.S. Department of Health and Human Services Public Health Service. 2. Centers for Disease Control and Prevention. 2011. A CDC framework for preventing infectious diseases: Sustaining the essentials and innovating for the future. (CDC), Division of Vector-Borne Diseases. U.S. Department of Health and Human Services Public Health Service. 3. Portier CJ, Tart KT, Carter SR, Dilworth CH, Grambsch AE, Gohlke J, Hess J, Howard SN, Luber G, Lutz JT, et al., 2013. A human health perspective on climate change: a report outlining the research needs on the human health effects of climate change. Journal of Current Issues in Globalization, 6:4: 621-710.

Session Type: Poster Session
Session Number: 003
Session Title: Poster Session 1
Poster Presentation Time: Monday, February 8, 2016, 11:30 am - 1:00 pm
Topic: A. Viral Agents

Poster Presentation Number: 167 (A)

**Stability, Persistence, and Disinfection of Ebola Virus in Human Blood**


We investigated the persistence and disinfection of two Zaire ebolavirus (EBOV) variants (EBOV/Makona-C05 and EBOV/Yam-May) in clinical matrices (human blood, simulated vomit, or feces). Tests were performed on non-porous surfaces (stainless steel, TyChemTM QC, nitrile, polyvinylchloride, polypropylene and aluminum) representative of hospitals, patient air-transport units, and personal protective equipment utilized by individuals treating Ebola patients. During the persistence study, both EBOV/Makona-C05 and EBOV/Yam-May persisted longest in dried blood in an environment representative of exterior West Africa, with short persistence in simulated vomit, and no persistence in feces. Persistence was
not affected by non-porous surface type. EBOV/Makona-C05 was more resistant to the effects of drying in blood than EBOV/Yam-May, and therefore may persist longer in dried blood when starting virus titers are the same. We also examined the efficacy of common disinfectants (bleach, acidified bleach, Micro-Chem PlusTM, SteriplexTM SD, EASYDECON DF200TM, PurellTM Advanced, and peracetic acid) against EBOV in human blood and cell culture media matrices. Although all of the disinfectants reduced EBOV/Makona-C05 below detection levels in cell culture media dried on surfaces, virus was still detected in some disinfected samples containing wet or dried blood, with the methods used. Only peracetic acid reduced EBOV/Makona-C05 titers below the limit of detection in dried blood. The resistance of EBOV/Makona-C05 to the stresses of drying in blood and the observed difficulty of disinfecting EBOV in dried blood may partially explain the magnitude of the 2014 outbreak in West Africa. This knowledge can be used to inform risk assessments and outbreak control procedures in future EBOV outbreaks.