Principal Investigator: Barry Alto, PhD, Associate Professor, Florida Medical Entomology Laboratory
Organization: University of Florida
Presentation Title: Point of Sampling Rapid Detection of Zika and Other Mosquito-borne Pathogens. Science, Technology, and Product Delivery.
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: The emergence of Zika virus and other mosquito-borne pathogens threaten public health. The goal of this project is to deliver kits to beta testers (Florida Public Health Services) able to use them immediately to detect Zika and other arboviruses in trapped mosquitoes and human patient samples. The development of this diagnostic tool will improve surveillance and health care services to Floridians. This goal is being accomplished through a collaborative project between Firebird Biomolecular Sciences and University of Florida. Firebird's scientists are leaders in developing chemistry to support human diagnostics. University of Florida provides the expertise on entomology, arbovirology and mosquito-virus interactions. Here we use Firebird's chemical innovations in the field of synthetic biology to develop sensitive, rapid, and cost-effective assays that target DNA and RNA (collectively xNA, nucleic acids) of pathogens. Innovations include: artificially expanded genetic information systems (AEGIS), these allow an xNA-targeted assay to be ultra clean; self-avoiding molecular recognition systems (SAMRS), these allow effortless multiplexing of xNA-targeted assays; biversal nucleotides, these allow an assay to be adaptable as the xNA sequence of the pathogen evolves, something common in RNA viruses like Zika; autodestruct nucleotides which prevent forward contamination to give false positives; universal sterilants that protect an assay user. Using three-dimensional printing a detection device has been constructed that uses LAMP (loop mediated isothermal amplification) in a single tube. Firebird has improved on the design of the detection device from an initial prototype. A distributable prototype is now suitable for use by Public Health Services in Florida for demonstration and testing. We have performed infection studies with Aedes aegypti mosquitoes with Zika virus and tested high temperature and ammonia-detergent mixtures as methods for a universal sterilant. We have prepared lyophilized reagent mixes in tubes that will be used to target Zika virus and other arboviruses. Also, a technique now allows for pixilating oligonucleotides on paper and plastics which may be used as a visual indicator for the presence of the target arbovirus. Pixilated surface readout inside the reaction tube can be used in singleplex and multiplex formats (Zika, chikungunya, and dengue viruses). Test samples consisting of Ae. aegypti infected with Zika, dengue-1 and chikungunya viruses are now being used in further development of the detection device.

Principal Investigator: Waseem Asghar, PhD, Assistant Professor
Organization: Florida Atlantic University
Presentation Title: Development of a Diagnostic Assay for Rapid Detection and Quantification of Zika Virus
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: As Florida is one of the most favorite tourist places for travelers in the World, Floridians are at high risk of ZIKV disease transmission. There is an urgent need to develop a rapid ZIKV detection assay that can be used at airports (Zika detection from saliva), urgent care centers and community health centers. Our goal is to develop a novel, low-cost (using transparency paper and plastic materials, <$2) and automated tool for rapid detection of ZIKV from human serum and saliva samples at POC settings. In 2015, there was a dramatic increase in reports of Zika virus (ZIKV) infection in the Americas. The CDC has reported more than thirty thousand ZIKV disease cases in the US and US territories, with Florida is severely affected. Recent reports suggest the association between ZIKV infection and microcephaly and other neurological disorders among newborns. Current IgM antibody-based ELISA assays cannot reliably distinguish between ZIKV and Dengue Virus (DENV). RT-PCR is a more commonly used method and can distinguish between ZIKV and other flaviviruses with high specificity. However, RT-PCR based methods are complex and time consuming, and require multiple labor-intensive sample preparation and processing steps, hence not suitable for rapid testing at airports, community health centers, urgent care centers, and other point-of-care (POC) settings. To increase access to ZIKV testing and to reduce the disease spread, there is an urgent need to develop a reliable device for rapid ZIKV detection. As of today, we have made significant progress towards the development of POC assay for rapid ZIKV testing. Paper-chip electrodes has been designed, tested, and characterized with various buffers, which are very crucial step to develop a sensitive biosensor. We have obtained various strains of ZIKV and Dengue virus from ATCC and started testing the specificity and capture sensitivity of various commercial anti-envelop antibodies for Zika virus using western blot method. After selection of most sensitive and specific antibody, we will start functionalizing magnetic beads for efficient capture of ZIKV from spiked saliva samples. The captured viruses will be lysed, that will increase the conductivity of the solution. Change in conductivity will be measured using paper-based electrode devices. Using the proposed device, specific travelers showing clinical symptoms (mild fever, rash, joint or muscle pain etc.) can be rapidly tested for ZIKV within 15 minutes by analyzing their saliva samples. Such a rapid and automated assay can also be used at urgent care and community health centers to screen people for ZIKV infection. The availability of rapid ZIKV testing assay will help reducing the transmission of ZIKV in Florida and also in other parts of the world.

Principal Investigator: Karin Chumbimuni-Torres, PhD, Assistant Professor
Organization: University of Central Florida
Presentation Title: A Highly Selective Electrochemical Sensor for the Rapid Detection of the Zika Virus
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: The purpose of this project is to develop a new technology for specific and sensitive point of serving testing (POST) platform for the rapid detection of ZIKV. Electrochemical detection will be used along with an amplification technique (Nucleic Acid Sequence Based Amplification, NASBA). This new technology is based on i) high recognition binary probes, that have proven to be able to distinguish single nucleotide substitutions (SNS), ii) a low cost and point of serving amplification technique for viral RNA, such as NASBA, and iii) electrochemical detection that has been proven to allow universal recognition by using a single probe in a re-useable format for RNAs. RNAs are considered key biomarkers for a variety of viral infections. However, these RNAs form folded structures, complicating their detection. To our knowledge, no research has focused on creating a sensor that allows for universal recognition of folded targets. Current approaches, such as NAATs, offer high sensitivity and rapid results, but they are prone to contamination, frequently produce false-positive results, and require technical expertise. Furthermore, these approaches are incapable of differentiating SNS, especially, at ambient temperatures, which is necessary to distinguish among other flaviviruses. The innovative aspect of the proposed platform lies in its capability to distinguish between Zika Virus and other flaviviruses to aid in the control and response of infections. This new technology will be made possible through the use of binary probes that have the capability to differentiate SNS. An additional innovation is the application of electrochemical detection of NASBA products, where the developed sensor is based on one universal probe that offers a re-usable format. To date, the sensor was investigated using synthetic targets fragments as well as MR766 strain RNA products obtained via NASBA amplifications. RNA products of 84 and 141 bases long were obtained using NASBA reaction, purified, and characterized by denaturing polyacrylamide gel electrophoresis. The sensor was optimized in terms of length and concentration of the probe to obtain maximum signal upon target binding. The limit of detection was determined to be around 1 nM for synthetic and NASBA samples. The NASBA-RNA products shown potential to be detected within five minutes, offering rapid detection capability. This sensor will benefit Zika diagnostic test for Floridians as results can be obtained within a few minutes. Furthermore, results show that Zika virus strains can be discriminated by one single base pair promising differentiation with other flaviviruses. Consequently, treatment will be more efficient as false positives caused by other flaviviruses which share similar RNA sequences, will be eliminated. In addition, the components are inexpensive and portable so the technology could be used for POST testing throughout the state, even in remote areas.

Principal Investigator: Sapna Deo, PhD, Associate Professor, Department of Biochemistry and Molecular Biology
Co-Presenters: Sylvia Daunert, PharmD, PhD and David Broyles
Organization: University of Miami
Presentation: Rapid RNA Test for Zika Virus
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: Infection of pregnant women by the Zika virus (ZIKV) carries the potential of serious health effects for the newborn baby and has therefore become the central concern of this viral outbreak. To address these concerns, pregnant women – and women who are planning pregnancies – need a means of obtaining immediate answers in terms of their Zika infection status. Sexual transmission of this virus has added additional complexity to outbreak management and viral safety, since infections can often be asymptomatic or misdiagnosed. However, direct detection of ZIKV RNA from patient samples is both challenging and time consuming. The current strategy for ZIKV RNA detection is reverse transcription polymerase chain reaction (RT-PCR), and requires specialized laboratories and equipment. This results in a significant delay in obtaining information on infection status and may lead to additional anxiety for pregnant women in Florida and around the world who remain at risk of infection. This necessitates the development of testing platforms for ZIKV that are simple, cheap, and rapid; can be easily mass-produced; and easily utilized in locations beyond traditional clinical settings. To solve this significant challenge, we are developing a portable, rapid, and equipment-free detection technique employing rolling circle amplification of ZIKV RNA followed by paper-strip-based visual detection of the amplified product. We have demonstrated the detection of ZIKV RNA analogue in less than two hours, with excellent discrimination of Brazilian Zika from other strains. Our data demonstrates that the technology works at ambient temperature and can identify the presence of ZIKV RNA on a paper strip using gold-nanoparticle conjugated probes that are observable with the naked eye. Moreover, because there is no need for specialized laboratory equipment, the entire test can be performed on-site. Successful detection of ZIKV infections in a rapid manner will meet the substantial need for assisting pregnant women in Florida as well as the general population who currently have to wait several months for results.

Principal Investigator: Nazira El-Hage, PhD, Associate Professor
Organization: Florida International University
Co-Presenters: Chet Ojha, MS, Myosotys Rodriguez, PhD, Michal Toborek, MD, PhD, and Madhavan Nair, PhD
Presentation Title: Understanding the role of autophagy in Zika Virus Infection Using Autophagy Deficient Animal Model
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: Zika virus (ZIKV) is a mosquito-borne flavivirus that has emerged as a public health threat in the state of Florida as well as other parts of the United States, because of its association with neurodevelopmental complications like microcephaly in infant and Guillain-Barre Syndromes in adult. The factors that contribute to the emergence, spread and change in the neuro-pathogenesis of ZIKV are not understood. In this study, we demonstrate that glial cells (microglia and astrocytes) from autophagy deficient mice (Beclin1+/-) are highly susceptible to ZIKV infection and recapitulate virus infection in human astrocytes. Further, we exploit these two *in vitro* culture model to compare ZIKV neuro-pathogenesis caused by a panel of ZIKV strains of a range of spatiotemporal history of isolation and representing African (Uganda_1947) and Asian (Puerto Rico_2015, Honduras_2016) lineages. Compared to Uganda and Honduras, the Puerto Rican strain (PRVABC59) caused a significant increase in the release of MCP-1 (3-fold) and IL-6 (14- fold) in human astrocytes, which correlated with a significant increase in neuronal cell death. In comparison, Asian strains induced higher and prolonged levels of inflammatory chemokines in wild-type murine glial cells that were significantly down-regulated in glia from Beclin1+/- deficient mice, while the African strain induced higher and prolonged levels of inflammatory chemokines in glia derived from Beclin1+/- deficient mice. Interestingly, viral protein levels in human astrocytes cells did not correlate with the pathogenicity of the different strains. Taken together, we have established a new murine model that supports ZIKV infection and demonstrate its utility in highlighting intrinsic differences in the inflammatory response induced by different ZIKV strains potentially leading to neuronal injury. This study paves the way for the future investigation of strain-specific changes in the ZIKV genome and their contribution to viral neuro-pathogenesis.

Presented by: Allen Caobi, Arti Vashist, PhD, Asahi Tomitaka, PhD, Adriana Yndart-Arias, BS, Madhavan Nair, PhD and Andrea Raymond, PhD
Principal Investigator: Nazira El-Hage, PhD, Associate Professor
Organization: Florida International University
Presentation Title: ZIKV infection of human placental cells modulates gene expression to promote cell survival and anti-viral immunity
Research Priority Area: Health Effects of Zika Virus

Abstract: The placental barrier (PB) protects the fetus from the maternal immune invasion and prevents inter-mingling of fetal and maternal blood while allowing for the exchange of oxygen, carbon dioxide, red blood cells antigens, antibodies, some viruses, and nutrients. Zika Virus (ZIKV) infection during early pregnancy results in placental insufficiency a complication in some pregnancies in which the placenta does not function well leading to reduced oxygen supply for the baby, poor baby growth/fetal death and more difficult labor for the mother. ZIKV-associated placental insufficiency clearly suggests a direct impact of the virus on the placenta. Here we show that ZIKV (strain MR766-ZIKVMR766) infects primary human placental trophoblasts (HPTs) cells that comprise the PB. Traditional plaque assays and digital droplet polymerase chain reaction (ddPCR) were used to titer ZIKVMR766 infection in HPTs. The ddPCR techniques proved to be much more sensitive than the plaque assay. ZIKVMR766 genes were detected in HPTs as early as 24 hours post infection. Gene expression profiles of ZIKV MR766-infected and control HPTs were compared using a quantitative polymerase chain reaction (qPCR) array. The qPCR array data show that expression of genes such as WNT1 and LFNG1 important for fetal development pathways were significantly down-regulated in ZIKVMR766- infected HPTs. Other genes that exhibited reduced expression include BCL2L1 and IRF important for cell survival and antiviral immunity, respectively. We confirmed that the lowered expression of aforementioned genes resulted in reduced protein expression of BCL21, IRF1, WNT1, and LFNG in HPTs via western blot. Together, these changes in HPT gene expression could lead to an environment that not only promotes ZIKVMR766 growth in placental cells and but hinders the neurological development of the fetus. This study identified molecular pathways in placental cells altered by ZIKVMR766 and thereby revealed potential therapeutic targets. Future studies investigating the therapeutic potential of IRF1- or BCL2L1-targeting agents in nanomedicine bases approaches to block ZIKV placental pathogenesis are now in progress. Overall, findings from this study could lead to the development of anti-ZIKV agents for use in early in pregnancy that prevent placental insufficiency and fetal neuronal abnormalities associated with ZIKV infection.

Presented by: Ana Leda, Ibolya Andras, Diana Avilla, Madhavan Nair, PhD, and Michal Toborek, MD, PhD
Principal Investigator: Nazira El-Hage, PhD, Associate Professor
Organization: University of Miami
Presentation Title: The Role of the Blood-Brain Barrier in ZIKA Infection
Research Priority Area: Health Effects of Zika Virus

Abstract: The blood-brain barrier (BBB) selectively regulates the cellular exchange of macromolecules between the circulation and the central nervous system. The BBB is formed by brain microvascular endothelial cells (BMEC) joined by tight junctions (TJs). Here, we hypothesize that ZIKV infects the brain via disrupted BBB and alterations of TJ proteins. To assess his hypothesis, ECs were infected with a Honduras strain of ZIKV (R103451) at MOI 0.01 and MOI 0.1 and assessed for TJ protein levels. Immunoblotting assays revealed a statistically significant downregulation of claudin-5 ($p=0.036$) and upregulation of ZO-1 ($p=0.039$) protein levels at MOI of 0.01, compared to uninfected controls. The levels of occludin did not change significantly between groups. The evoked changes demonstrate a finely tuned response of brain endothelial cell TJs to ZIKV exposure. In addition, the activity of P-glycoprotein (P-gp), a major efflux transporter, was assessed in BMEC using the fluorescent dye rhodamine 123 uptake assay. Cells infected with ZIKV at MOI of 0.01 had a tendency of larger rhodamine uptake compared to infection at MOI of 0.1 and uninfected controls. In addition, cells infected at MOI of 0.01 tend to present lower P-gp expression levels, compared to cells infected at MOI of 0.1 and uninfected controls; however, there were no statistical significance between the groups. Overall, these results suggest that ZIKV may cause BBB disturbances by altering TJ expression. These events may contribute to the dissemination of the viral infection in the CNS.

Presented by: Madhaven Nair, PhD, Andrea Raymond, PhD, Allen Caobi, Arti Vashist, PhD, Asahi Tomitaka, PhD, and Adriana Yndart-Arias, BS
Principal Investigator: Nazira El-Hage, PhD, Associate Professor
Organization: Florida International University
Presentation Title: Magneto-electric Nanoparticles as a Novel Delivery System of siRNA Targeting ZIKV Receptors Across an *in vitro* Fetal Blood-Brain-Barrier Model
Research Priority Area: Health Effects of Zika Virus

Abstract: The fetal blood-brain barrier (BBB) similar to the placenta regulates the transport of molecules into the developing brain and serves as the second line of defense against neurotropic viruses. There is evidence that ZIKA virus (ZIKV) not only crosses the placenta but also has the capacity to cross the fetal BBB. ZIKV has been shown to infect neuronal progenitor cells slowing down growth and inducing apoptosis. ZIKV strains from Asia have been associated with abnormal neuronal development/decreased brain development head size (microcephaly). Therapeutic strategies targeting the fetal brain are needed to prevent ZIKV-associated neuropathology. In order to infect neuronal cells ZIKV must interact with one of the TAM receptors (Tyro3, AXL, or Mer). Preventing this interaction would eliminate ZIKV infection in the fetal brain. The purpose of this study was to develop a novel nanotherapeutic that can inhibit ZIKV interaction with TAM receptors. Magneto-electric nanoparticles (MENPs) have been used in our laboratory to deliver the anti-HIV drug Tenofovir across an artificial BBB model. Using a similar method, an in vitro model of the fetal blood brain barrier (Ft-BBB) was developed in our laboratory and used to demonstrate MENPdelivery of siRNA targeting the ZIKV-TAM receptor, AXL across the barrier. The MENP-siAXL formulation was first characterized, and then applied to the blood-side of the Ft-BBB-model. The MENP-siAXL- treated model was placed on a magnet for 18 hours. An electrical charge was later applied to release the siAXL in brain side of the model. Release, transport efficiency and therapeutic efficacy of the MENP-siAXL was assessed. Preliminary findings demonstrate that greater than 10% of the MENP-siRNA crosses the Ft-BBB and can significantly reduce expression of AXL on SH-SY5Y neuronal cells. Taken together, we provide a proof-of-concept of an innovative approach in which to treat ZIKV infections in the brain suggesting that nano-medicine based therapeutics may be a promising mode of drug delivery for future anti-viral therapeutics.

Principal Investigator: Yulia Gerasimova, PhD, Assistant Professor
Organization: University of Central Florida
Presentation Title: Visual Point-of-Care Compatible Assay for Diagnostics of Zika Virus Infection
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: With no vaccine available to prevent Zika virus (ZIKV) infection, no specific treatment, and a strong link between the infection and the development of neurological diseases, accurate diagnosis becomes crucial to confine the spread of the infection and to avoid the outbreak, as well as to allow for more efficient case management and epidemiological monitoring. The CDC recommended ZIKV testing methods require highly trained personnel and sophisticated equipment and are limited to several qualified laboratories. Therefore, only a limited number of patients can be tested for ZIKV infection. At the same time people not meeting the criteria for ZIKV detection can still be infected, which may result in the spread of the infection to others. To improve surveillance, case management, and control of ZIKV infection, molecular tests, which can be used at or near the point of care or even at home are needed. Here we propose a novel diagnostic platform for detection of ZIKV, which requires minimal to no equipment or user expertise. The platform takes advantage of isothermal amplification of viral RNA followed by the detection of the amplified fragments using highly specific split deoxyribozyme (sDz) sensors. The signal read-out is a color change generated in response to the presence of ZIKV RNA, which can be monitored and analyzed by the naked eye. The intensity of the color is proportional to the amount of RNA in the sample, so quantification or semi-quantification of the viral load is possible. In the frame of the project, 50 sequences of complete genome or polyprotein gene for ZIKV isolates and strains circulating in the Americas available in the ZikaVR database were aligned using ZBLAST tool. Based on the alignment results, as well as on the literature data, three fragments of the viral genome - in the genes encoding Envelope (E) and NS5 proteins – were selected as targets for vsDz probes. The probes were first tested using synthetic DNA analytes corresponding to the targeted fragments. Next, the probe-targeted fragments of ZIKV RNA were amplified with Nucleic Acid Sequence Based Amplification (NASBA) to produce 84-92-nucleotide fragments of the viral RNA. Alternatively, Reverse-Transcriptase Loop-Mediated Amplification (RT-LAMP) producing double-stranded DNA products was used. The sensitivity of NASBA method was sufficient to generate enough RNA for vsDz analysis starting from as low as 0.01 pg/uL of viral RNA, which corresponds to the detectable concentration of viral nucleic acids in clinical samples. Next, the selectivity of vsDz assay combined with either NASBA or RT-LAMP amplification was tested using RNA from Dengue virus (DENV) as a non-specific control. In both cases, the signal in the presence of the non-specific amplification product was at the background level. Currently, the range of ZIKV strains and non-specific controls is expanding. In addition, further optimization of the amplification and detection stages of the assay is in progress.

Principal Investigator: Charles Lockwood, MD, MHCM, Senior Vice President USF Health, Dean, Morsani College of Medicine
Co-Presenters: Ozlem Guzeloglu-Kayisli, MSc, PhD, Umit A. Kayisli, MSc, PhD, and Fred Schatz, PhD
Organization: University of South Florida
Presentation Title: Uterine Decidual Cells Express Molecules Required for ZIKV Cell Entry; Implications for Perinatal Infection
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: Zika Virus (ZIKV) infection has little impact on the mother, but can cause severe fetal developmental problems ranging from growth restriction to abnormal brain development (microcephaly). While these fetal effects occur in 10-20% pregnancies, the mechanism(s) for selective perinatal transmission is/are unknown. The placenta is attached to the uterine decidua, the sole site of maternal and fetal cell-cell interactions (aka the maternal-fetal interface). This maternal specialized tissue is composed of equal numbers of decidual cells (50%) and decidua-specific white blood cells including uterine natural killer cells (60-80%), macrophages (20-25%), T-lymphocytes and dendritic cells (1-2%). Since syncytiotrophoblast, the placental cell type in contact with maternal blood, resists ZIKV infection, we propose that placental transmission occurs following viral infection of the decidua. Identification of molecules required for ZIKV attachment to and replication in decidual cells will permit development of agents that block perinatal transmission. Using whole genome analysis, RT-PCR and immunohistochemistry, we will identify levels of expression of known mediators of ZIKV attachment in decidual (maternal) cells compared with ZIKV resistant placental trophoblast (fetal) cells. Our whole genome analysis found that compared to primary cultures of cytotrophoblasts (n=3) and syncytiotrophoblasts (n=3), primary term decidual cell cultures (n=3) express significantly higher levels of several mediators of ZIKV attachment, Gas 6, PROS1 and AXL (29.8, 25.5 and 2.74-fold increase, respectively). Our comparative RT-PCR analysis of these molecules in endometrial stromal cells (HESCs) from non-pregnant uterus, pregnancy-derived first and third trimester decidual cells as well as cytotrophoblasts and syncytiotrophoblasts revealed that HESCs and first trimester decidual cells exhibited the highest levels of Gas 6, AXL and PROS1 expression with negligible expression in cytotrophoblasts and syncytiotrophoblasts (Fig. 1A-C). Moreover, our immunohistochemical analysis confirmed in situ expression of these molecules in decidual cells at the maternal-fetal interface obtained from first and third trimester specimens. In 2016, Florida accounted for 1076 of 5102 (21%) travel associated ZIKV cases in the U.S. as well as 276 locally acquired cases. Our current results suggest that decidual cells are highly susceptible to ZIKV entry/replication and may serve as a reservoir for placental transmission and fetal infection. Agents that efficiently block ZIKV entry into decidual cells may prevent congenital ZIKV infection, providing immense public health benefits. Ongoing experiments are testing drugs and biological agents that block ZIKV infection of decidual cells.

Presented by: Sara B. York, Graduate Research Assistant
Principal Investigator: David G. Meckes, PhD, Assistant Professor, Department of Biomedical Sciences
Organization: Florida State University
Presentation Title: Fetal Brain Exosomes in the Maternal Circulation for the Detection of Zika Virus Infected Fetuses
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: Zika virus is an emerging infectious disease that has spread rapidly across the Caribbean and South America with over 200 confirmed cases of locally-acquired Zika in the state of Florida. Infection of pregnant women during the first trimester has been linked to microcephaly, a neurological condition where babies are born with significantly smaller heads due to abnormal brain development. Babies born with microcephaly can develop convulsions and suffer physical and learning disabilities as they grow older. Despite the significance of Zika virus, little is known about how the virus infects the fetus or causes disease. Furthermore, there currently is no non-invasive test available to detect fetal infection and specifically, determine whether this infection will lead to microcephaly or other birth defects. Extracellular vesicles (EVs) are membrane-encapsulated structures released by cells that are present in all biological fluids. EVs carry signaling factors, proteins and microRNAs that mediate intercellular communication, and there is recent evidence that EVs may play important roles in the progression of neurological conditions, infectious diseases, and fetal development. Recently, Bukong and colleagues demonstrated that EVs obtained from Hepatitis C virus (HCV) infected individuals and cells contain replicative-competent viral RNA and are capable of infecting hepatocytes. Being a member of the same viral family, it is likely that Zika virus also hijacks EV pathways to package viral components and secrete vesicles that are potentially infectious but less immunogenic. It is additionally possible that EVs may be a means by which the virus can alter cellular environments and perhaps cross barriers to infection like the placenta or blood-brain barrier. Therefore, gaining a greater understanding of how Zika affects EV cargo may aid in the development of novel therapeutics to combat the virus. Recent evidence suggests that placental- and fetal-derived EVs are present in the maternal circulation at high levels and changes in EV cargo may reflect fetal health or development. Therefore, as part of this study we aim to characterize circulating EVs as a means to detect Zika virus infection of the fetus. To begin to assess infectivity as well as EV protein and RNA cargo, EVs and virions were separated from Zika-infected and uninfected cells by differential centrifugation and density gradient ultracentrifugation. Preliminary results suggest that Zika may alter protein cargo, density, and the quantity of secreted vesicles. Now that we have developed a new method to separate Zika virus from EVs, we plan to measure infectivity and further assess EV cargo with real-time RT-PCR, mass spectrometry and deep sequencing approaches. Findings from our in vitro data will be translated to the study of EVs isolated from the plasma of healthy pregnant women or those that have been infected with Zika virus to assess the utility of EVs for the detection of fetal infection.

Presented by: Gabriela M. Blohm, PhD, Postdoctoral Research Associate
Principal Investigator: John Glenn Morris, MD, MPH, TM, Professor, Emerging Pathogens Institute
Organization: University of Florida
Presentation Title: Rapid Diagnostic Test for Zika Virus in Dried Blood Spots with Low Demands on Instrumentation
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: Diagnosis of Zika virus (ZIKV) infection typically involves the collection of blood, saliva or urine from the patient, followed by RT-PCR tests for ZIKV genomic RNA. Based on our previous work in Haiti and in Venezuela, we have found that blood is a crucial specimen for differential diagnostic testing of ZIKV and other mosquito-transmitted viruses that cause similar symptoms. As RT-PCR tests require expensive instrumentation and trained laboratory scientists to perform the tests, there are no point-of care tests currently available. Most often, the specimens must be shipped refrigerated or frozen to the testing laboratory; without cold storage, sample integrity deteriorates, leading to false negative results. Furthermore, this requirement for cold-storage adds increased costs related to shipping and handling of cold, infectious material, as well as delays in diagnosis. Alternative low-cost methods for shipping blood have been tested and validated for the shipment of virus-containing blood, such as blotting a drop of patient's blood on sterile filter paper followed by dry storage at room temperature. Dry storage on filter paper facilitates shipment at greatly reduced costs while preserving the integrity of the virus for diagnostic tests. Elimination of cold-chain storage alone substantially reduces costs; however, follow-up RT-PCR tests are time-consuming and expensive to perform. Recent advances in the design of diagnostic molecular tests include utilization of RT-LAMP, an innovative, sensitive and effective testing method that can be performed using minimal, inexpensive instrumentation. In this study, we show that ZIKV in dried blood spots can be rapidly detected using RT-LAMP in a manner that does not require expensive instrumentation. We have implemented a very robust RT-LAMP system that relies on previously described primers and a relatively new commercially available enzyme system. Our results indicate that we can rapidly detect ZIKV in blood and other specimens at a sensitivity that matches or surpasses current procedures. We have also determined that, when compared to other types of filter paper, high-quality chromatography paper is a superior substrate for the preparation of dried blood spots containing ZIKV intended for RT-PCR or RT-LAMP tests. Finally, we show that the suitability of virus nucleic acid extracted from dried blood spots for RT-LAMP or RT-PCR is dependent on the purification method, where purification with magnetic silica beads is more effective than traditional column-based methods for RNA purification. Lessons learned from this study lead the way to cost-effective, reliable, and fast methods that do not require complex and expensive instruments for the diagnosis of ZIKV infections. Moreover, future developments will lead to systems that may be performed point-of-care by minimally trained personnel.

Principal Investigator: Cuong Nguyen, PhD, Assistant Professor
Organization: University of Florida
Presentation Title: Massively Parallel On-Chip Detection of Zika Virus and Antibodies Using Subnanolitre Wells
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: Zika virus (ZIKV) infection is often asymptomatic during an incubation period that can range from 3 to 12 days. The serological detection of ZIKV is even more complex due to extensive cross-reactivity between antibodies triggered by various flavivirus infections. The current methods for detecting ZIKV are limited by need for relatively large sample volumes together with low sensitivity and specificity. This hinders both experimental and diagnostic sensitivity for a disease that has low viremia levels, a short incubation period, and a particularly detrimental characteristic of rapid infection via multiple mechanisms. We have developed a nanowell-based method to detect copies of ZIKV transcripts directly from minimal amounts of sample (nanoliter volume) by one-step, single-cell, reverse transcription polymerase chain reaction (RT-PCR). This simple method, when combined with microengraving, identifies ZIKV-specific antibodies in the same nanowell. To test this technology, samples were deposited in a nanochip containing 248,832 wells with 30μm dimension. A slide was incubated with the nanochip to capture only antibodies present in the samples. A fluorescent-labeled ZIKV envelop protein was used to detect ZIKV-specific antibodies. In parallel, RT-PCR master mix containing ZIKV probes and primers were added into the nanochip wells and placed onto a specialize thermocycler for denaturation, annealing, and elongation. The fluorescent intensity of the PCR product was detected via fluorescent microscopy. Microarrays of the ZIKV-specific antibodies were scanned using GenePix 4400A Microarray scanner and custom-made software was used to correlate data obtained from ZIKV RT-PCR imaging and microarray micrographs. The preliminary data indicate that on-chip detection of ZIKV and ZIKV-specific antibodies is feasible in a high-throughput and nanoliter volume. Further work will be performed to demonstrate reproducibility, specificity, and accuracy of the assay. Our technology will detect ZIKV infection at the early stage when viremia is low. At the same time, it can identify ZIKV-specific antibodies at the later stage of infection. The process requires minimal amount of sample for the diagnosis. The rapid viral and serological detection will help patients be more informed and counseled with their daily activities and travels. Microcephaly in fetus is one of the most devastating consequences of Zika infection, therefore having a timely infection status will better prepare the patients. Our research will provide a necessary and temporary relief to the families and public health concern in the state. We have recently awarded a R21 grant ($417,897.00) from the National Institute of Health as the result of the support from the FDOH.

Principal Investigator: Gaurav Saigal, MD, Assistant Director, Diagnostic Radiology Residency Program, Professor, Clinical Radiology
Organization: University of Miami
Presentation Title: Longitudinal Brain MRI Characterization of Zika-positive and Exposed Children Using Advanced MRI Techniques and Correlations with Neurodevelopmental Outcomes
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: The impact of congenital Zika virus (ZIKV) infection on the developing brain of children who were prenatally infected ("ZIKV-infected") is just beginning to unravel. Microcephaly and other macro-anatomical brain abnormalities in ZIKV-infected group have been confirmed using ultrasound, CTand conventional MR imaging. However, a significant percentage of infants (71-99%) who were prenatally exposed to ZIKV ("ZIKV-exposed" due to maternal ZIKV infection) may not show any clinical abnormality at birth, have a normal head circumference, and demonstrate no macro-anatomical brain abnormalities. Recent reports suggest that these ZIKV-exposed children may develop a redued head circumference mant months after birth. Extensive brain malformations and neurological abnormalities are being seen in these children which are felt to be secondary to of microstructural damage caused by ZIKV on the developing fetal brain.
The purpose of this study is to use advanced brain imaging MR techniques such as Diffusion Kurtosis Imaging (DKI) and MR Spectroscopy (MRS) to examine the status of the brain tissue microstructures and metabolite levels. Furthermore, 3D MR volumetric imaging techniques will be utilized in both the subject groups to measure brain volume changes longitudinally over a 3-year period. Tissue structural and metabolite changes in the brain will be correlated with outcomes of neurodevelopmental and behavioral tests performed at the same time points as the MR imaging. Imaging of ZIKV-infected babies is necessary to assess the extent of brain and other organ damages in the context of determination of their burden on health care. It has been recognized that children who have congenital Zika syndrome will need continuous health care for the rest of their lives. . In addition, it is equally important to follow the ZIKV-exposed children who do not show any clinical or neurological symptoms at birth over long periods to ensure that no brain malformations and neurological abnormalities develop as they grow up. Though WHO recently declared that Zika emergency is over, it intends to continue studying ZIKV as a serious infectious disease, which would require years of research. To date we have performed MRI scans and neurodevelopmental tests on two infants, one is ZIKV-infected (images below) and the other ZIKV-exposed. It is critical that we have a complete understanding of the extent of damage on the brains of both the Zika-infected and -exposed babies who are born in hospitals in Florida. This information will serve three purposes. First, it will help clinicians (and others) to come up with definite guidelines for the prevention, management and treatment of ZIKV syndrome. Second, it will help determine resources needed to take care of these patients. If the ZIKV-exposed group shows significant brain morphometric changes together with concomitant deficits in neurodevelopmental outcomes, these findings may warrant revision of the State health policy so that appropriate funding and other resources are made available to manage the ZIKV-affected people in Florida. Third, this information will help to establish an epidemiological database in the State.

Principal Investigator: Mark Sharkey, PhD, Research Assistant Professor
Organization: University of Miami
Presentation Title: Development of a Rapid Diagnostic Assay for Zika Virus Infection
Research Priority Area: Innovative Diagnostic Testing or Therapeutics

Abstract: The overall goal of this project is to develop a simple, rapid, low cost, point-of-care assay to detect ZIKV infection. The assay will be optimized to efficiently and specifically amplify ZIKV RNA from unprocessed biological fluids, including urine, saliva, semen and cervicovaginal fluids. A key element of our approach is the use of a novel enzyme that possesses both RNA-dependent and DNA-dependent polymerase activity that is specially formulated for direct from cell RNA detection. This approach eliminates two substantial steps normally required for ZIKV diagnosis which significantly reduces cost, effort and time for sample analysis. Diagnosis of ZIKV is not as simple as it could and should be. The best current diagnostic modalities are based on relatively expensive real-time quantitative PCR (RT-qPCR) techniques that require shipment of samples to central laboratories with turnaround times of several days to weeks in clinical practice. In contrast, the attributes of our approach are listed below: direct detection of ZIKV RNA from biological samples with a novel polymerase and unique methodology, ability to perform the assay in most regional hospital laboratories and clinics with inexpensive standard PCR machines, low reagent costs, immediate detection of amplification products using a fluorescent probe and an inexpensive, handheld blue light source, detection of ZIKV RNA in approximately one hour, point-of-care potential for this platform to diagnose ZIKV infection. In its final form, this assay would provide the means for widespread, economical testing ideally suited for basic testing, epidemiological surveillance and longitudinal analyses needed to guide pregnancy decisions. In contrast to last year, very few ZIKV infections have been identified this year and we were only able to recruit one subject into the study. A single individual who acquired a travel-related ZIKV infection was enrolled and provided samples. The participant underwent follow up visits at baseline, week1, week2, one month, and two months. Additional samples at different time-points were collected through a separate approved study. The presence of ZIKV RNA in urine, blood, plasma, saliva and vaginal fluid was assessed longitudinally for 31 days by quantitative PCR using purified RNA. ZIKV was detected in blood, plasma, urine, and saliva on D1, with the highest levels detected in saliva. After day 1, ZIKV RNA levels gradually decreased until they became undetectable. Results of processed samples versus unprocessed samples were similar, although reduced sensitivity was observed when using unprocessed samples. Modifications to the protocol are being evaluated to improve the sensitivity of detecting low copy number targets in biological samples and the use of PCR additives to overcome the presence of inhibitory compounds commonly found in biological fluids, such as, blood are being tested.