



**COLLEGE OF ENGINEERING
AND COMPUTER SCIENCE**
FLORIDA ATLANTIC UNIVERSITY

Announces the Ph.D. Dissertation Defense of

Md Alamgir Kabir

for the degree of Doctor of Philosophy (Ph.D.)

“Development of Point-Of-Care Assays for Zika Virus Diagnostic”

November 12, 2021, 12:00 p.m.

EE-96, Room 405

777 Glades Road

Boca Raton, FL

DEPARTMENT:

Electrical Engineering and Computer Science

ADVISOR:

Waseem Asghar, Ph.D.

PH.D. SUPERVISORY COMMITTEE:

Waseem Asghar, Ph.D., Chair

Massimo Caputi, Ph.D.

Imadeldin Mahgoub, Ph.D.

Mirjana D. Pavlovic, Ph.D.

Kevin Yunqing Kang, Ph.D.

ABSTRACT OF DISSERTATION

Development of Point-Of-Care Assays for Zika Virus Diagnostic

Zika virus (ZIKV) is an emerging flavivirus transmitted to humans by Aedes mosquitoes. ZIKV can be transmitted from mother to fetus during pregnancy and can cause microcephaly and other birth defects. Effective vaccines for Zika are yet to be approved. Detection of the ZIKV is based on serological testing that often shows cross-reactivity with the Dengue virus (DENV) and other flaviviruses. Currently, identification of ZIKV infection is usually done by i) testing the patient's serum sample to detect ZIKV RNA using reverse transcriptase-polymerase chain reaction (RT-PCR), ii) testing patient's serum sample for the presence of the NS1 protein antigen or iii) serological assays to determine the presence of virus-specific immunoglobulin antibodies (IgG and IgM) by the use of ELISA assay. But ELISA-based assays show cross-reactivity and poor sensitivity. The gold standard for ZIKV RNA detection is RT-PCR, involves expensive medical facilities and skillful technicians. However, the plaque reduction neutralization test are executed to quantify neutralizing antibodies of the virus-but show high accuracy only after day 7 of the disease onset. Therefore, the development of POC assays which has the ASSURED (affordability, sensitivity, specificity, user-friendly, rapid and robust, equipment-free and deliverable) criteria defined by the World Health Organization are topmost priority. The core objective of this thesis is to find inexpensive, sensitive, precise, and fast assays for the specific diagnosis of ZIKV suitable for resource-constrained settings.

We aimed to assemble a highly specific anti-Zika antibody panel to be utilized in the development of a highly specific and cost-effective ZIKV rapid quantification assay for viral load monitoring at point-of-care settings. To this end, we tested the affinity and specificity of twenty-one commercially available monoclonal and polyclonal antibodies against ZIKV and DENV envelope proteins utilizing nine ZIKV and twelve DENV strains. We finalized and tested a panel of five antibodies for the specific detection and differentiation of ZIKV and DENV infected samples. Next, the panel of selected antibodies were tested with deidentified ZIKV and DENV viral culture lysates and their lower limit of detection was defined by western blot. Investigation of the viral samples correctly identified 5.0×10^6 FFU/mL of the DENV and 4.0×10^4 PFU/mL of the ZIKV. Then the selected antibody was also used to functionalize microbeads to capture the virions and bound on the surface of the microchips. The lensless imaging setup has numerous advantageous features including wide-field imaging of the micrometer sized magnetic beads immobilized inside the microfluidic chip. When the emanated light from the LED passes through immobilized beads, diffraction shapes are documented using a CMOS image sensor positioned under the microfluidic chip. The recorded raw image is then analyzed by an computational image processing algorithm that reverses the diffraction shapes into particles image on the surface modified microchip. A counting algorithm is used to evaluate the reverse diffracted image to count the number of beads captured. The performance of this method is compared with gold standard RT-PCR method which ensures the efficacy of our

developed method.

We also report an automated flow free POC assay for detecting the NS1 antigen of ZIKV. The developed assay replicates the conventional sandwich ELISA-based colorimetric detection inside a microfluidic chip with the help of antibody-magnetic bead conjugation. To automatize the detection process we have utilized a in house built 3D printed platform and an arduino controlled unit operates a stepper motor to manipulate the movement of the beads inside the microchip. The assay requires a total of 9 min to automatically control the post-capture washing, horseradish peroxidase (HRP) conjugated secondary antibody probing, washing again, and, finally, color development. By measuring the saturation intensity of the developed color from the smartphone captured video, the presented assay provides high sensitivity with a sensitivity of 62.5 ng/mL in whole plasma. These results advocate a great promise that the platform would be useful for the POC diagnosis of ZIKV infection in patients and can be used in resource-limited settings.

BIOGRAPHICAL SKETCH

Born in Dinajpur, Bangladesh

B.S., Rajshahi University of Engineering and Technology (RUET), Rajshahi, Bangladesh, 2013

M.S., Florida Atlantic University, Boca Raton, Florida, 2018

Ph.D., Florida Atlantic University, Boca Raton, Florida, 2021

CONCERNING PERIOD OF PREPARATION & QUALIFYING EXAMINATION

Time in Preparation: 2017 – 2021

Qualifying Examination Passed: Fall 2017

Published Papers:

Kabir, M. A., Zilouchian, H., Younas, M. A., & Asghar, W. (2021). Dengue Detection: Advances in Diagnostic Tools from Conventional Technology to Point of Care. *Biosensors*, 11(7), 206.

Kabir, M. A., Ahmed, R., Chowdhury, R., Iqbal, S. M. A., Paulmurugan, R., Demirci, U., & Asghar, W. (2021). Management of COVID-19: Current Status and Future Prospects. *Microbes and infection*, 104832.

Kabir, M. A., Ahmed, R., Iqbal, S. M. A., Chowdhury, R., Paulmurugan, R., Demirci, U., & Asghar, W. (2021). Diagnosis for COVID-19: current status and future prospects. *Expert Review of Molecular Diagnostics*, 21(3), 269-288.

Kabir, M. A., Soto-Acosta, R., Sharma, S., Bradrick, S. S., Garcia-Blanco, M. A., Caputi, M., & Asghar, W. (2020). An antibody panel for highly specific detection and differentiation of Zika virus. *Scientific reports*, 10(1), 1-8.

Sharma, S., Kabir, M.A. and Asghar, W., (2020). Lab-on-a-Chip Zika Detection With Reverse Transcription Loop-Mediated Isothermal Amplification-Based Assay for Point-of-Care Settings. *Archives of Pathology & Laboratory Medicine.*, 144(11), 1335-1343.

Kabir, M. A., Zilouchian, H., Caputi, M., & Asghar, W. (2020). Advances in HIV diagnosis and monitoring. *Critical reviews in biotechnology*, 40(5), 623-638.

Kabir, M. A., Zilouchian, H., Sher, M., & Asghar, W. (2020). Development of a flow-free automated colorimetric detection assay integrated with smartphone for Zika NS1. *Diagnostics*, 10(1), 42.

Coleman, B., Coarsey, C., Kabir, M. A., & Asghar, W. (2019). Point-of-care colorimetric analysis through smartphone video. *Sensors and Actuators B: Chemical*, 282, 225-231.

Herrada, C. A., Kabir, M. A., Altamirano, R., & Asghar, W. (2018). Advances in Diagnostic Methods for Zika Virus Infection. *Journal of medical devices*, 12(4)

Coarsey, C., Coleman, B., Kabir, M. A., Sher, M., & Asghar, W. (2019). Development of a flow-free magnetic actuation platform for an automated microfluidic ELISA. *RSC advances*, 9(15), 8159-8168.

Afrouz Ataei, Kabir, M. A., Andy WC Lau, Asghar, W. (2021) "A novel rheotaxis-based microfluidic device for selecting sperm contaminated with virus in assisted reproductive technology". *Fertility and Sterility Science* (Accepted)

Sharma, S., Kabir, M.A. and Asghar, W., "Selection of healthy sperm based on positive rheotaxis using a microfluidic device" *Lab on chip* (Under Rev.)

Kabir, M.A., Caputi, M. and Asghar, W., "Development of a highly specific point of care diagnostic assay for Zika virus using a lensless imaging system" *Manuscript in preparation*

Chowdhury, R.A, Kabir, M. A., Meeroff, D., Asghar, W. "Surveillance of COVID-19 in the highly populated community by detecting SARS-CoV-2 RNA in untreated wastewater" *Manuscript in preparation*

Thomas Kent, Mazhar Sher, Esmail Ahmed Elbassa, Kabir, M. A., Asghar, W. , Deguo Du "Electrical Impedance Spectroscopy Based Detection of

amyloid-beta (1-40)." Manuscript in preparation