Novel Graphene-based Biosensor for Early Detection of Zika Virus Infection

B.R. Goldsmith[1], F. Barron[1], S. Afsahi[1], A. Walker[1], L. Locascio[1], D. Pan[1], J.M. Goldstein[2], J. Lee[2], X. Tang[2], D.A. Bagarozzi, Jr. [2]

[1]Nanomedical Diagnostics

[2]Reagent and Diagnostic Services Branch, Division of Scientific Resources,
National Center for Emerging Zoonotic Infectious Diseases, Centers for Disease Control and Prevention

ABSTRACT

Agile R100 is an assay platform we have used to develop a portable, cost-effective, and label-free biosensor to detect targets such as Zika virus when used with highly specific immobilized monoclonal antibodies. The new technology Field Effect Biosensing (FEB) uses mAbs or equivalent capture molecules covalently linked to graphene sensors for direct, real-time, quantitative detection of target antigens or biomarkers. The speed, size, sensitivity, and specificity of this first-of-its-kind graphene-enabled Zika biosensor make it an ideal candidate for development as a medical diagnostic test.

MATERIAL AND METHODS

Graphene biosensor chips were read using the Agile R100 system. The system components are shown in Platform Description. The biosensor chips were functionalized as shown below with an anti-Zika NS1 mouse mAb (6B1) developed by the CDC. Anti-Zika NS1 was diluted to a working concentration of 14.6 nM in 1X phosphate buffered saline (PBS) pH 7.4. During immobilization and measurement, 75 µL of all solutions were used on the biosensor chips and incubation steps took place at room temperature.

The software was calibrated to baseline in 1X PBS. mAbs were immobilized on the pre-NHS activated sensor surface by incubativng for 15 minutes. Polyethylene glycol and ethanolamine were used to block and quench before wash.

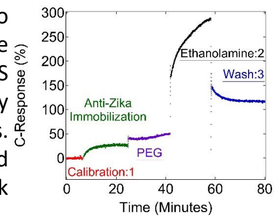
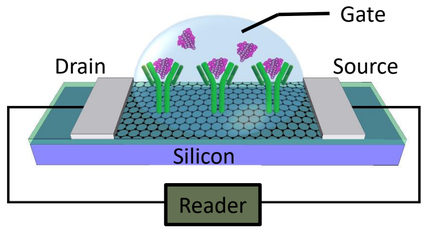


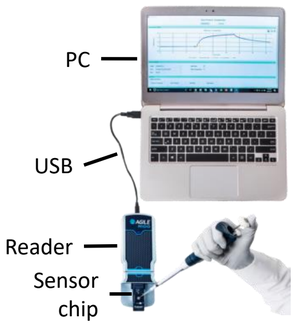
Figure 1

PLATFORM DESCRIPTION



Field Effect Biosensing is a breakthrough label-free technology for measuring biomolecular interactions. It is an electrical technique that measures the current across a graphene biosensor surface functionalized with immobilized biomolecular targets. Any interaction or binding that occurs on the surface causes a change in conductance of the biosensor. FEB is a unique orthogonal technology to optical and labeled systems that can be used via the Agile R100 system.

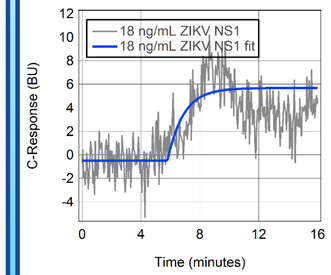
There are 2 read-out channels available: the I-Response (current through the electrical sensor chip) and the C-Response (capacitance of biosensor to the liquid).



The Agile R100 is the only portable biosensor of its kind. It uses disposable biosensor chips that can be tailored to detect different biomarkers.

The low weight, low power requirements, and lack of reagents required make this a flexible platform for laboratory research and a promising platform for fieldable assay development.

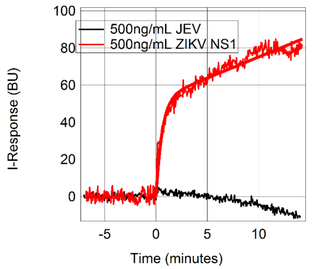
ZIKA PROTEIN DETECTION



18 ng/mL sensitivity

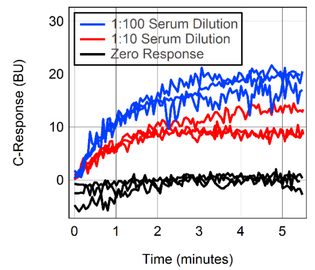
A simple measurement and fit of sensing Zika NS1 protein in buffer using chips functionalized with anti-Zika NS1 antibodies. Measurement starts with calibration in 1X PBS, followed by addition of 18 ng/mL NS1 in 1X PBS at about 5 minutes. Roughly 1/10 the lower limit of detection compared to BLI.

Data shown here is from Afsahi, *et. al.,* Biosensors and Bioelectronics, 100, 85-88



Target Specificity

To demonstrate the selectivity of anti-Zika NS1 immobilized on graphene based biosensor chips, 500 ng/mL ZIKV NS1 and JEV NS1 was applied to biosensor chips coated with anti-ZIKV NS1. JEV NS1 shares 56.5% amino acid sequence identity with ZIKV NS1. JEV NS1 did not elicit a measurable sensor response when exposed to anti-ZIKV NS1, demonstrating high selectivity for ZIKV NS1 antigen.



Function in Serum

Spike-in measurements in simulated serum were performed at 2 dilutions in triplicate to demonstrate the potential for optimization of this assay for diagnostic purposes. Dilution of serum improved the coefficient of variation percentage (CV%) from 19.9% to 9.2% despite lowering the overall sensor response. This suggests the possibility of a diagnostic value in a clinical setting.

Nanomedical DIAGNOSTICS