PUBLICATION HIGHLIGHT: *10x More Sensitive than BLI or ELISA*

Details taken from: Afsahi, S., et al. **Towards Novel Graphene-Enabled Diagnostic Assays with Improved Signal-to-Noise Ratio**. *MRS Advances*. 2017. doi:10.1557/adv.2017.431. Read the full pre-print paper on our site.

Agile™ R100

Find your needle of signal in a haystack of noise.

Agile R100 is a graphene-based personal assay system that has drastically lowered the limit of detection of biomolecular interactions, letting you develop and validate your tight binders with a reliable label-free method. The proprietary Field Effect Biosensing (FEB) technology on which Agile R100 is based is an electrical technique that enables cost-effective, portable form factors that can be applied in life science research, drug discovery applications, and diagnostic platforms.

PAPER SUMMARY

- Zika viral antigen is measured using an Octet RED96, a Bio-layer Interferometry (BLI) platform, and Agile R100, an FEB platform, both of which provide kinetic binding data. Agile R100 **shows detection at a concentration 10x lower than on the Octet**, while providing kinetic analysis in line with the BLI instrument.
- Inflammatory protein IL-6 is measured with an Invitrogen ELISA kit and with Agile R100. Agile R100 **lowered the limit of detection (LLOD) for IL-6 by 60x** over the ELISA**. No assay adjustment was made between ELISA and Agile R100.**
- Agile R100 is an easy-to-use assay system that **increases signal-to-noise (SNR) at ultra-low concentrations by 10x** over BLI, a typical kinetic characterization platform, and ELISA, a conventional tool for clinical diagnostics.

OVERVIEW

While BLI and ELISA are widely used in drug discovery and disease diagnostics, the platforms suffer noise interference at ultra-low concentrations, which reduces measurement sensitivity. Highly-sensitive measurements are necessary to develop pharmaceuticals at therapeutically-relevant levels and to gain accurate early clinical results.

In this study, **Agile R100 showed 10x sensitivity over BLI and 60x over ELISA**, representing a significant advancement for drug development and highlights potential clinical capabilities.

MATERIALS AND METHODS

Zika Viral Antigen Measurement

Anti-Zika NS1 mouse monoclonal was diluted to a working concentration of 14.6 nM in 1X PBS pH 7.4 and applied to an NHS Agile biosensor chip surface. Further antibody immobilization followed the standard procedures listed below. ZIKV NS1 recombinant antigen was diluted in 1X PBS pH 7.4 to concentrations of 0.4 nM, 4.0 nM, and 11.9 nM.

Label-free BLI analysis on Octet RED96 was performed in kinetic buffer (PBS/0.05% BSA/0.02% Tween20), using anti-mouse Fc (AMC) biosensors. Biosensors were equilibrated for 10 minutes in kinetic buffer. Microplates were then filled with 200 µl of sample in kinetic buffer and agitated at 1000 rpm. A 6-point titration curve of binding to ZIKV NS1 with subtracted buffer reference was performed for 1000 s and buffer dissociated for 2000 s. Data was analyzed in Octet Data Analysis 9.0 software.

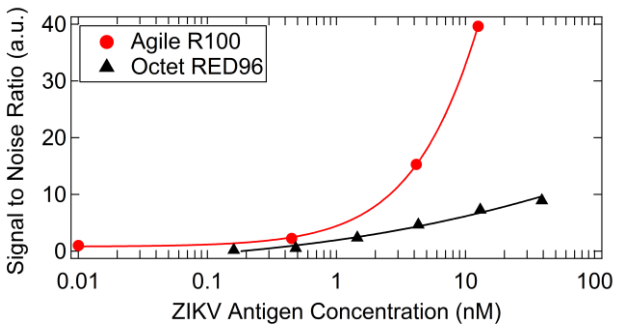
IL-6 Measurement

A solution containing 5 mL 50 mM MES pH 6.0, 2 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and 6 mg N-hydroxysulfosuccinimide (sNHS) was mixed and immediately added to COOH Agile biosensors for 15 minutes. The chips were rinsed in 50 mM MES pH 6.0. Human monoclonal antibody to Interleukin 6 (anti-IL6) was diluted to 14.6 nM in 1X phosphate buffered saline (PBS) pH 7.4 and immobilized on the surface over a period of 15 minutes. Residual amine-reactive sNHS ester groups were subsequently quenched using 3 mM amino-PEG5-alcohol solution pH 7.4 and 1 M Ethanolamine pH 8.5 for 15 minutes. Sensitivity of IL6 detection on graphene biosensors was determined by running multiple concentrations of the analyte to determine the LLOD. Each analyte concentration was prepared in 1X PBS pH 7.4 and applied for 10 minutes to detect analyte association.

IL-6 ELISA binding data is reported from the Invitrogen Human IL-6 ELISA Kit Product Information Sheet.

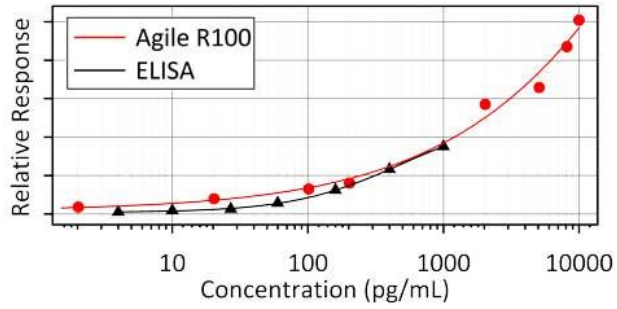
RESULTS AND DISCUSSION

Sensitivity of ZIKV NS1 detection with Agile R100 and Octet RED96 was determined by running several concentrations of viral antigen to determine LLOD and gather binding kinetics. Octet RED96's LLOD was 1.4 nM, while Agile R100's LLOD was 0.45 nM, indicating higher sensitivity with Agile R100. The figure below shows a plot of SNR versus Zika antigen concentration. Over a range of concentrations, Agile R100 biosensor chips have greater SNRs due to low electrical noise in the graphene material. This indicates FEB signals are more robust and easier to detect than BLI signals.



The Agile R100 measurement generated kinetic binding data comparable to that gathered on the more costly and laborious Octet RED96. On both platforms, association of ZIKV NS1 to immobilized anti-Zika NS1 was quantified using the Hill-Langmuir model of saturable kinetic binding. The resulting association rates (ka) were 2.97×104 Ms-1 on the Octet RED96 and 9.27×104 Ms-1 on Agile R100. These association rates agree within error.

The below figure shows the relative response of IL-6 with a commercial IL-6 ELISA assay and with Agile R100 over a wide range of concentrations. **No assay adjustment was needed in the transition from ELISA to Agile R100.** ELISA'S LLOD is 120 pg/mL and Agile R100's LLOD is 2 pg/mL, **60 times lower than that of ELISA,** indicating an increase in sensitivity of 60x.



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