

Development of a rapid and cost effective assay for Zika Virus infection.

Mario Stevenson PhD.

Zika virus (ZIKV) was first isolated in 1947 in Uganda but remained an obscure and infrequent mosquito-borne human pathogen causing a mild, self-limited viral syndrome confined to a few regions of Africa and Southeast Asia for close to 60 years. It was not until 2013-14 that an epidemic in French Polynesia led to the first descriptions of an association between Guillain-Barre syndrome and ZIKV infection. This was followed in 2015-16 by a rapidly expanding epidemic in South and Central America, the Caribbean, South Florida, and a burgeoning number of cases of microcephaly and other neural tube malformations in infants born to women who contracted the infection during pregnancy as well as additional reports of post-infection Guillain-Barre syndrome. While viremia is short-lived (typically less than one week), it has now become apparent that ZIKV may persist for longer periods of time in fluids such as urine and semen (6 months or more), and there have been reports of sexual transmission of infection weeks after the initial infection in an index patient. Thus, the ability to diagnose the infection in different body fluids is critical for transmission prevention and for reproductive health decisions.

However, diagnosis of ZIKV is not as simple as it could and should be. Serologic diagnosis of the infection is not reliable because of: 1) antibody cross reactivity to related flaviviruses causing similar acute febrile illnesses but not associated with ZIKV-type sequelae, and; 2) the inability to distinguish between clearance and persistence of ZIKV infection. 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. This model is challenging anywhere and particularly in the resource-limited settings that are hardest hit by ZIKV

The solution to this unmet clinical need is a simple, inexpensive assay suitable for use anywhere including resource-limited settings. We have exploited a novel polymerase to design a rapid, point-of-care ZIKV assay. The merits of our assay compared to the currently available options are as follows:

- Use of a novel enzyme with both RNA-dependent and DNA-dependent polymerase activities with the capability to amplify ZIKV RNA directly from clinical samples without RNA purification and with exquisite sensitivity (single copy) and specificity.
- Use of standard PCR equipment available in most regional hospitals and not expensive as opposed to complex RT-qPCR that requires extensive operator experience.
- Immediate visualization of amplification products through the use of a fluorescent probe and an inexpensive, handheld blue light.

We believe this platform, which can be adapted to many viral pathogens, has the attributes required for implementation in any health-care setting.