COMMENTARY

Diagnostic Testing for Zika: Observing Rapid Translation During a Public Health Emergency

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INTRODUCTION

Although outbreaks of Zika virus (ZIKV) were previously reported in Africa and Asia, it was not until multiple cases were detected in Brazil in 2015 that ZIKV captured public attention. The association with congenital disease prompted the World Health Organization (WHO) to declare a public health emergency (lifted November 2016). We aim to explore the rapid translation of diagnostics for ZIKV during a public health emergency with the hopes of learning from challenges encountered.

ZIKA VIRUS BIOLOGY AND TRANSMISSION

ZIKV belongs to the Flaviviridae family, which includes other vector-borne viruses such as yellow fever, dengue, and West Nile virus. Flaviviruses consist of a single-stranded RNA genome enclosed in a protein-embedded envelope; these proteins are used to interact with receptors on skin immune cells and neural progenitor cells. Through these protein–receptor interactions, ZIKV gains entry into human cells. Studies suggest that cell receptors involved with the infection process include Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), T-cell Immunoglobulin and Mucin domain (TIM), and Tyro3, Axl, and Mertk (TAM); some of these have been reported to facilitate the entry of dengue virus.

ZIKV is transmitted via infected Aedes aegypti or Aedes albopictus mosquitoes. Although the exact viral incubation period is unknown, experts estimate the timeframe to be 3–12 days. While ∼20% of those infected with ZIKV will develop symptoms (e.g., fever, rash, conjunctivitis, and/or joint pain), most infected individuals remain asymptomatic. Due to rapid viral clearance, the window for diagnostic detection of ZIKV by molecular methods in blood is within 5–7 days of symptom onset; serologic assays for detection of IgM-class antibodies to the virus are most useful 10–14 days following symptom onset.

Unlike other mosquito-borne infections, ZIKV can be transmitted person-to-person through many routes, including sexually and from mother to fetus. A causal relationship between in utero ZIKV infection and congenital disease, including severe microcephaly and excess skin folds, has been established. These skin folds are a result of brain tissue being destroyed and replaced by fluid. A major challenge is that asymptomatic women who are pregnant remain at risk for having infants with birth defects. This striking phenotype caught the attention of Western mainstream media, ushering the outbreak into public consciousness.

Two lineages of ZIKV exist: the African and Asian lineage. The outbreak strain in Brazil shares over 99% of the genetic material associated with the Asian lineage strain responsible for the 2013 outbreak in French Polynesia. A recent investigation reported that the strain in the Brazilian outbreak produced a more robust infection among fetuses by crossing the placenta and targeting cortical progenitor cells. This leads to an increase in apoptosis and autophagy, resulting in impaired neurodevelopment.

EMERGENCY USE AUTHORIZATIONS FOR ZIKA DIAGNOSTICS

After the United States declared a public health emergency in February 2016, 19 in vitro diagnostic (IVD) tests were quickly developed and released for clinical diagnostic purposes (Table 1). The speed of translation of these devices was particularly rapid, due in part to the US Food and Drug Administration’s (FDA) Emergency Use Authorization (EUA) process. The EUA process affords companies developing relevant diagnostic tests the option to temporarily bypass traditional FDA review during an outbreak. This allows tests essential to combating an epidemic to receive FDA clearance in a matter of weeks or even days. EUAs have been issued previously during outbreaks of H7N9 influenza, MERS-Corona virus, Enterovirus D68, and Ebola virus. An EUA-cleared test is treated as “FDA-approved” until the United States’ Department of Health and Human Services (DHHS) officially declares an end to the outbreak; the assay is then reclassified as a laboratory-developed test (LDT) and must be submitted for regulatory review through traditional FDA 510(k) or Pre-Market Approval (PMA) processes. Additionally, clinical laboratories using these assays may have to perform additional internal verification studies to conform to requirements outlined in the Clinical Laboratory Improvement Amendments (CLIA).

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Received 18 October 2017; accepted 11 November 2017; published online on 1 December 2017. doi:10.1111/cts.12529
Table 1 Summary of Zika in vitro diagnostic tests brought to practice through the EUA, including date of authorization, assay name, and method of detection

| Date of EUA       | In vitro diagnostic                          | IgM captureELISA | RT-PCR | Transcription mediated amplification |
|-------------------|----------------------------------------------|------------------|--------|-------------------------------------|
| 26 February 2016  | CDC Zika MAC-ELISA                           | X                | X      |                                     |
| 17 March 2016     | CDC Triplex                                  |                  | X      |                                     |
| 28 April 2016     | Quest Diagnostics Zika Virus RNA Assay       |                  | X      |                                     |
| 13 May 2016       | RealStar Zika Virus Kit                      |                  | X      |                                     |
| 17 June 2016      | Aptima Zika Virus Assay                      |                  |        | X                                   |
| 19 June 2016      | Viracor-IBT Laboratories Inc. Zika Virus test|                  |        |                                     |
| 29 July 2016      | VERSANT Zika RA 1.0 Assay Kit                |                  |        |                                     |
| 4 August 2016     | Luminex xMAP MultiFLEX Zika RNA Assay        |                  | X      |                                     |
| 17 August 2016    | In Bios ZIKV Detect IgM Capture ELISA        |                  |        |                                     |
| 23 September 2016 | Sentosa SA ZIKV Test                         |                  | X      |                                     |
| 28 September 2016 | ARUP Zika Virus                              |                  | X      |                                     |
| 21 November 2016  | Abbott RealTime Zika                         |                  |        | X                                   |
| 9 December 2016   | ELI Tech Group Zika ELITE MGB Kit            |                  |        |                                     |
| 20 March 2017     | Nanobiosym Gene-RADAR Zika Virus Test        |                  | X      |                                     |
| 5 April 2017      | DiaSorin Liaison XL Zika Capture IgM Assay   |                  | X      |                                     |
| 2 August 2017     | Thermo Fisher TaqPath Zika Virus Kit         |                  | X      |                                     |
| 11 April 2017     | Columbia Cil-ArboViro Plex rRT-PCR           |                  |        |                                     |
| 18 September 2017 | Siemens ADVIA Centaur Zika Test              |                  | X      |                                     |
| 27 April 2017     | Chembio DPP Zika IgM Assay System            |                  | X      |                                     |

The IVDs created to address the ZIKV epidemic employ a variety of established scientific techniques, including reverse-transcription real-time polymerase chain reaction (rRT-PCR), transcription-mediated amplification (TMA), and IgM antibody capture enzyme-linked immunosorbent assays (MAC-ELISAs). These assays can be performed on a variety of patient samples, including plasma, urine, serum, and/or cerebrospinal fluid. The Centers for Disease Control and Prevention (CDC) has developed two assays for the detection of ZIKV: the ZIKV MAC-ELISA and the Triplex RT-PCR. The ZIKV MAC-ELISA utilizes an IgM capture antibody, a ZIKV-specific antigen, and an IgM antibody to detect humoral response to infection. A colorimetric output is generated based on interaction of the enzyme and chromogenic substrate. As dengue virus and other flaviviruses cocirculate in the same regions where ZIKV is now endemic, crossreactivity between antibodies to flavivirus may occur; this may result in false-positive ZIKV MAC-ELISA values. Therefore, the ZIKV MAC-ELISA is considered a screening assay, after which all samples with reactive results require confirmatory testing by a plaque reduction neutralization test (PRNT). The CDC RT-PCR Triplex is designed for qualitative detection of RNA from ZIKV, dengue virus, and Chikungunya virus, and is able to differentiate between these viruses using specific nucleic acid probes.

Due to the scale of the epidemic, the high influx of specimens submitted for ZIKV testing to the CDC, and the long turnaround time for confirmatory PRNT (7–10 days), reporting of results was significantly delayed—often 3–4 weeks following specimen submission. The CDC worked with multiple national reference laboratories, including ARUP, LabCorp, Mayo Medical Laboratories, and Quest Diagnostics, to build surge capacity for ZIKV testing, reducing the turnaround time, particularly for ZIKV MAC-ELISA negative results, to 2–3 days. During this time, multiple commercial laboratories were developing molecular assays for detection of ZIKV RNA. Quest Diagnostics was the first to successfully develop and receive an EUA from the FDA for their ZIKV rRT-PCR assay. Notably, meeting the demand for ZIKV diagnostic testing in a timely manner came at a hefty cost—unlike the $100 tests used during the Ebola outbreak, Quest placed a hefty $500 price tag on each ZIKV molecular test.
the patient. This service, however, as discussed above, was met with a significant delay in turnaround time.

**CLINICAL TRIALS FOR ZIKA VACCINES**

At least two major ZIKV vaccine clinical trials are under way. The Zika purified inactivated vaccine (ZIPV) trial, led by Walter Reed Army Institute of Research in Bethesda, MD, is currently in a phase I clinical trial.\(^8\) The proposed vaccine employs the same technology as the Japanese Encephalitis virus vaccine, with inactivated whole ZIKV particles used in place of the encephalitis virus.\(^8\) Initial nonhuman primate safety studies in rhesus macaques demonstrated that the vaccine induced production of host antibodies capable of neutralizing ZIKV.\(^6\) A second DNA-based vaccine is in development by the National Institute of Allergy and Infectious Disease (NIAID), and is moving into phase II/IIb clinical trials.\(^9\) The NIAID’s vaccine platform is based on the strategy previously used to develop a candidate vaccine for the West Nile virus. The vaccine is injected intramuscularly and contains a plasmid encoding the precursor membrane and envelope proteins found on the surface of the virus.\(^9\) The primary objective is to generate an immune response to the particles formed by the vaccine without placing subjects at risk for an active ZIKV infection.

**BARRIERS TO AND FACILITATORS OF RAPID TRANSLATION**

There were several barriers that prevented the successful and rapid translation of IVDs for Zika virus in the United States. As is the case with translation of any new discovery, buy-in from all stakeholders is critical. One challenge that delayed translation of Zika diagnostic tests was the difficulty in securing congressional funding and support. The US Senate was unable to pass an emergency funding bill for ZIKV before reaching the recess period in 2016.\(^10\) This is believed to have contributed to the substantial delay in the development and release of in vitro diagnostic tests.\(^10\)

An additional challenge was ensuring patients received timely ZIKV test results. Given the virus’s asymptomatic presentation and fast viral clearance, physicians had to act in a timely manner to detect ZIKV in patient samples. However, due to a large demand for testing, there was a backlog of tests in laboratories providing ZIKV diagnostics; this meant that many pregnant women had to wait up to 5 weeks before receiving their results. This created a serious ethical issue for women living in states that do not allow termination of pregnancy after 24 weeks of gestation. Thus, women who were tested after 20 weeks of pregnancy were up against a ticking clock.

Nevertheless, the FDA’s EUA served as a key facilitator for translation of ZIKV diagnostic assays as IVDs. The fast clearance granted by the EUA significantly incentivized companies to develop assays and submit their applications given that the average approval time for 510(k) medical device submissions is typically 5 months.\(^5\)

**LESSONS LEARNED**

The aim of this narrative is to identify challenges encountered during rapid translation of much-needed diagnostic tests during an international public health crisis. Through the Zika crisis, three lessons on rapid translation during an acute health emergency can be gleaned. First, sufficient resources need to be in place in case of an emergency in order to ensure rapid, timely, and safe translation of a needed product to the wider population. Second, it is crucial to ensure that all key stakeholders, including funding sources and politicians, are supportive of quick and efficient development and delivery of needed testing during an emergency situation. Third, further attention should be drawn to developing an infrastructure that can support the demand generated by a global outbreak. Possible routes by which this can be accomplished include effective communication between the public and private laboratory sectors, enhanced collaboration between assay manufacturers and regulatory bodies, and finally, through the establishment and adherence to clinical, diagnostic, and treatment guidelines for patient care.

**Acknowledgments.** This publication was supported by Grant Number U1 TR001135 from the National Center for Advancing Translational Sciences (NCATS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

**Conflict of Interest.** The authors declared conflicts of interest.

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