

An Overview of Rapid Detection Methods and Point of Care (POC) in Resource-Limited Settings for Detection of Zika Virus: Current State and Future Trends

Marcia R Silva* and Zahra Gohari

Water Technology Accelerator, University of Wisconsin-Milwaukee, USA

*Corresponding Author: Marcia R Silva, Water Technology Accelerator, University of Wisconsin-Milwaukee, Milwaukee, WI, USA.

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Abstract

Zika virus (ZIKV) is a mosquito-borne *flavivirus* with a single-stranded positive RNA and is transmitted by many *Aedes* spp. mosquitoes. It was first identified in monkeys in Uganda in 1947. The first human case was detected in Nigeria in 1954 and there have been further outbreaks in Africa, South East Asia and the Pacific Islands. Most of these outbreaks were small and Zika has not previously been considered a major threat to human health until May 2015, when it was reported in Brazil and has since spread rapidly, being considered a “pandemic re-emergence” by the US National Institute of Health (NIH). This work will present an overview of the technologies for detection of ZIKV, its limitations and kits available on the market. We also present an overview of the point-of-care (POC) in resource-limited settings, which enhance diagnostic capabilities for its high performance, suitability and affordability, with potential for a strong outbreak management strategy.

Keywords: Zika Virus; Rapid Methods; Sensors; ZIKV; Outbreak Management

Abbreviations

ZIKV: Zika Virus; WHO: World Health Organization; CDC: Center for Disease Control; POC: Point-of-Care; RBS: Ribosome Binding Site; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; Zika MAC-ELISA: Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay; WNV: West Nile Virus; IFA: Immunofluorescent Assay; Mabs: Monoclonal Antibodies; RT-LAMP: Reverse Transcription-Loop Mediated Isothermal Amplification; NAAT: Nucleic Acid Amplification Test; HNB: Hydroxynaphthol Blue; QUASR: Quenching of Unincorporated Amplification Signal Reporters; app: Smartphone Application

Introduction

Infectious disease zika fever and outbreaks

Dengue, Zika and chikungunya are mosquito-borne viral illnesses that are leading causes of illness in tropical and subtropical regions that have also reached northern states like Wisconsin [1]. Low-income countries are particularly vulnerable to the social and economic impacts of these emerging epidemics due to limited resources in the public health sector and at the household-level to prevent and manage the disease [2]. Because there are no vaccines or cures for these viruses there is an increased urgency of those in the public health sector to identify alternative strategies to manage the disease, of which an early warning system is included.

Before 2007, only 14 cases of Zika virus (ZIKV) had been documented in people, according to the World Health Organization (WHO) [3]. Despite the scarcity of symptoms, blood tests showed the virus was actually very common in Africa and had spread to Southeast Asia: from 2% infected in North Vietnam to 75% in Malaysia. Still, ZIKV was not a high international concern until a small island in Micronesia, called Yap, noticed a new illness causing rashes, pinkeye and joint pain. It looked like dengue, but something was different: the fever was

milder and accompanied in some cases by pinkeye. In June 2007, lab samples sent to the Center for Disease Control (CDC) confirmed the disease was Zika fever. In May 2015, eight years after the outbreak on Yap, researchers in Brazil's National Reference Laboratory confirmed ZIKV was circulating in their country [3]. Reports of birth defects like microcephaly that would later be linked to Zika infection began to pour in babies born with small heads and poor eyesight. Zika also was a cause of Guillain-Barre syndrome in adults [4-12]. As of March 2017, 84 countries have reported evidence of ZIKV in native mosquitoes.

Overview of rapid detection methods and limitations

Since 2015, a variety of high quality reviews about ZIKV have been published. Some focus on the incidence and interaction of the virus [13,14], diagnoses and vaccine [15,16], other focus on the transmission of the virus on resource-limited settings [17-20] or on gathering information available about diagnostic testing for ZIKV infection [21,22]. This overview summarizes the rapid detection technologies and the current trend on point-of-care (POC) in resource-limited settings, as this will enable shorter detection times and broader access of the technology. Summary of the rapid detection methods is listed in table 1.

Synthetic biology has led to new opportunities for molecular diagnostics [23-25], allowing reduction of cost and technical barriers to the development of synthetic biology-based diagnostics. For example, Programmable RNA sensors called toehold switches, can be rationally designed to bind and sense virtually any RNA sequence [26-28]. This sensing portion of the toehold is placed upstream of a ribosome binding site (RBS) to create a RBS-locking hairpin structure, which block RNA binding to ribosome and thereby prevents the translation of a downstream reporter protein. When targeted, "trigger" RNA binds the toehold, peeling the hairpin and opening the blockade, allowing the RNA to bind to the ribosome and subsequent translation of the reporter protein (LacZ enzyme). This converts a yellow substrate to a purple product, indicating the presence of the virus.

Another technology, a freeze-dried, paper-based, cell-free protein expression platform, allows for the deployment of these toehold switch sensors outside of a research laboratory by providing a sterile and abiotic method for the storage and distribution of genetic circuits at room temperature [29]. The combination of these technologies created a platform for rapidly and inexpensively developing and deploying diagnostic sensors [30-32]. This diagnostic tool does not require highly skilled personnel or expensive equipment to operate and can be used in low resource areas [33]. Another rapid detection method used is the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 genotyping, where by employing a (CRISPR)/Cas9-based module that can cut preprogrammed sequences in isothermal amplification method for nucleic acids, NASBA, amplified product and thereby prevent reporting the selected target sequences, the researchers were able to discriminate different genotypes of ZIKV [32].

There are other ZIKV detecting methods that are not as new and have some limitations. They include: (a) PCR-Based Methods, a technique commonly to detect RNA expression [34]; (b) Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA) is used for the qualitative detection of ZIKV IgM antibodies in serum or cerebrospinal fluid [35-38]; (c) cell culture, where for virus isolation, both Vero and Vero E6 cells are used, following the procedures described for West Nile Virus (WNV) isolation, with slight modifications [39], (d) another method is NASBA, which has the potential for broad applications in the field of RNA amplification and detection [38,40]; (e) Immunofluorescent assay, where anti-ZIKV IFA (IgM or IgG) utilizes ZIKV-infected cells as the antigenic substrate. Positive and negative results are evaluated by fluorescence microscopy [41]; (f) protein antigen detection method where the applicability of the Monoclonal Antibodies or MAbs for the development of antigen detection ELISA was evaluated using YF-17D and recombinant YFV-E protein. Several MAb combinations were assessed, and MAb 4C9 (capture antibody) and MAb 3F4 (labeled antibody) were selected based on the results of the previous IgG indirect ELISA [22].

Type of assay	Specimen	Limitations	Reference
Cell culture	Serum, urine and saliva	Since this method is based on viral isolation, test can only give accurate results if the virus is still in the patient's body and will give false negative if the viral cytoplasm or nuclei is not available.	[39,42]
PCR-based methods	Blood and saliva	Take a long time to get results and expensive to do for patients.	[37]
Sequencing based method	Serum/Blood/Amniotic fluid/ New born babies	Since viral genome is constantly going under mutation, it might be challenging to use this method over long periods of time.	[40]
Immunofluorescent assay	Human serum	No limitation has been mentioned, but can be improved for faster and cheaper results.	[41]
Zika MAC-ELISA	Virus cell culture/Human serum	Cross reactivity with other types of Flavivirus	[43]
Protein Antigen Detection	Virus cell culture/Human serum	This test can only give accurate results if the virus is still in the patient's body and will give false negative if the viral cytoplasm or nuclei is not available.	[44]
Rapid detection methods	Virus cell culture (ZIKV RNA genome)/Plasma samples	The sample must contain a minimum amount of viral genome	[30-32]

Table 1: Short description of detection methods used for ZIKV.

Commercial kits and limitations

To our knowledge, there are a few commercially available test kit for detection of ZIKV in a short time [45-51]. Detection kits can be a good tool to get results in a short amount of time (about 20 - 40 minutes), they have a limit of detection, and since they are mostly based on RT-PCR or ELISA methods they might have cross reactivity with viruses similar to Zika.

Point-of-care (POC) in resource-limited settings

Reverse transcription-loop mediated isothermal amplification (RT-LAMP) [52] has emerged as a popular isothermal Nucleic Acid Amplification Test or NAAT for viral detection due to simplified thermal management and high sensitivity and specificity towards targeted sequences [53-56]. Furthermore, they are affordable as the reactions are conducted in an inexpensive and portable "LAMP box" supplemented with a consumer class smartphone. In addition, it can detect ZIKV directly from crude human sample matrices and show the potential for widespread clinical deployment. Isothermal NAATs eliminate the need for thermal cyclers, significantly lowering the complexity of hardware required for these tests. RT-LAMP detection of dengue [57-60], chikungunya [61], and other RNA viruses has been demonstrated previously, and at least one recent report has demonstrated RT-LAMP detection of ZIKV [55]. In most cases, these reports detect amplification with non-specific indicators of total DNA synthesis, such as monitoring turbidity [62], observing the color-change indicator hydroxynaphthol blue (HNB) [59], performing a post-reaction analysis involving addition of an indicator dye (e.g. SYBR Green) [63], or performing gel electrophoresis to analyze the banding pattern [54]. Post-reaction analysis that requires opening the tube is undesirable, both for reasons of complexity and the risk of amplicon contamination, whereas non-specific indicators such as turbidity or HNB may be prone to detection of false-positive amplification events. These issues can be addressed by implementing RT-LAMP for Zika, dengue, and chikungunya in a closed-tube, multiplexable, target-specific format. The Zika and chikungunya assays utilize a technique that does quenching of unincorporated amplification signal reporters (QUASR). QUASR offers very bright signals, greatly reduces the detection of false-positive amplifications, and offers the ability to multiplex two or more targets per reaction. These features reduce reagent

costs and dilution when sample volume is limiting. QUASR is also fully compatible with complex sample matrices such as blood. The use of QUASR in turn enables us to carry out the assays on a modular, wireless smartphone-operated platform powered by a 5 V USB charger. A custom smartphone application (app) controls a low-powered isothermal heating module and a multicolor LED excitation module via Bluetooth. The app also acquires images from the phone camera and processes them through a novel color and luminance-based detection algorithm capable of detecting multiplexed QUASR assay signals [64-66].

Conclusion

Since the 2015 ZIKV outbreak in Brazil, the development of rapid detection methods has been evolving quickly. Even though there are detection kits on the market, there are still challenges that need to be overcome such as cross-reactivity, false negatives, long detection time, complexity in operation, portability and cost. Therefore, the POC in resource-limited settings seem to be the trend to become a potential outbreak management strategy. The next generation of sensors need to bring the detection time to nearly real time. Future studies could also explore other potential ZIKV reservoirs and further investigate the ZIKV pathogenesis pathways and host cellular response to aid the development of a robust detection assay, ZIKV vaccine, and antiviral therapeutics.

Bibliography

1. Shastri D. "Mosquito capable of spreading Zika found in Wisconsin for the first time". *Milwaukee Journal Sentinel* (2017).
2. Mark Johnson and Devi Shastri. "Pathway to peril". *Milwaukee Journal Sentinel* (2017).
3. Mary Kay Kindhauser, *et al* "Zika: the origin and spread of a mosquito-borne virus". *Bulletin of the World Health Organization* 94.9 (2016): 675-686C.
4. Galindo-Fraga A., *et al*. "Zika virus: A new epidemic on our doorstep". *Revista de Investigación Clínica* 67.6 (2015): 329-332.
5. Oehler E., *et al*. "Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013". *Euro Surveillance* 19.9 (2014): 20720.
6. Smith DW and J Mackenzie. "Zika virus and Guillain-Barré syndrome: another viral cause to add to the list". *The Lancet* 387.10027 (2016): 1486-1488.
7. Victora CG., *et al*. "Microcephaly in Brazil: how to interpret reported numbers?" *The Lancet* 387.10019 (2016): 621-624.
8. Martines RB. "Notes from the field: evidence of Zika virus infection in brain and placental tissues from two congenitally infected newborns and two fetal losses—Brazil, 2015". *MMWR: Morbidity and Mortality Weekly Report* 65.6 (2016): 159-160.
9. Oduyebo T. "Update: interim guidelines for health care providers caring for pregnant women and women of reproductive age with possible Zika virus exposure—United States, 2016". *MMWR: Morbidity and Mortality Weekly Report* 65.5 (2016): 122-127.
10. Mohammed H., *et al*. "Prevalence of anti-dengue immunoglobulin G antibodies among American Red Cross blood donors in Puerto Rico, 2006". *Transfusion* 52.8 (2012): 1652-1656.
11. Kutsuna S., *et al*. "Two cases of Zika fever imported from French Polynesia to Japan, December 2013 to January 2014". *Euro Surveillance* 19.4 (2014): 20683.
12. Korhonen EM., *et al*. "Zika virus infection in a traveller returning from the Maldives, June 2015". *Euro Surveillance* 21.2 (2016).

13. Afolabi LO., *et al.* "A Review on the Incidence, Interaction, and Future Perspective on Zika Virus". *Journal of Basic and Clinical Reproductive Sciences* 5 (2016): 61-74.
14. Plourde AR and EM Bloch. "A literature review of Zika virus". *Emerging Infectious Diseases* 22.7 (2016): 1185.
15. Shan C., *et al.* "Zika Virus: Diagnosis, Therapeutics, and Vaccine". *ACS Infectious Diseases* 2.3 (2016): 170-172.
16. Thomas SJ. "Zika Virus Vaccines — A Full Field and Looking for the Closers". *The New England Journal of Medicine* 376.19 (2017): 1883-1886.
17. Bogoch II., *et al.* "Potential for Zika virus introduction and transmission in resource-limited countries in Africa and the Asia-Pacific region: a modelling study". *Lancet Infectious Diseases* 16.11 (2016): 1237-1245.
18. Kaushik A., *et al.* "Electrochemical biosensors for early stage Zika diagnostics". *Trends in Biotechnology* 35.4 (2017): 308-317.
19. Chua A., *et al.* "Update on Zika diagnostic tests and WHO's related activities". *PLoS Neglected Tropical Diseases* 11.2 (2017): e0005269.
20. Chan K., *et al.* "Rapid, affordable and portable medium-throughput molecular device for Zika virus". *Scientific Reports* 6 (2016): 38223.
21. Charrel RN., *et al.* "Background review for diagnostic test development for Zika virus infection". *Bulletin of the World Health Organization* 94 (2016): 574D-584D.
22. Shukla S., *et al.* "Rapid Detection Strategies for the Global Threat of Zika Virus: Current State, New Hypotheses, and Limitations". *Frontiers in Microbiology* 7 (2016): 1685.
23. Lu TK., *et al.* "Advancing bacteriophage-based microbial diagnostics with synthetic biology". *Trends in Biotechnology* 31.6 (2013): 325-327.
24. Kotula JW., *et al.* "Programmable bacteria detect and record an environmental signal in the mammalian gut". *Proceedings of the National Academy of Sciences* 111.13 (2014): 4838-4843.
25. Slomovic S., *et al.* "Synthetic biology devices for in vitro and in vivo diagnostics". *Proceedings of the National Academy of Sciences* 112.47 (2015): 14429-14435.
26. Green AA., *et al.* "Toehold switches: de-novo-designed regulators of gene expression". *Cell* 159.4 (2014): 925-939.
27. "Detecting Zika with Synthetic Biology". *Twist Bioscience* (2016).
28. McAlpine KJ. "Paper disc can quickly detect Zika virus in the field". *Harvard Gazette* (2016).
29. Pardee K., *et al.* "Based synthetic gene networks". *Cell* 159.4 (2014): 940-954.
30. Heater B. "A \$1 paper-based test can detect the Zika virus in around two hours". *Tech Crunch* (2016).
31. Ubelacker S. "Zika portable test can detect virus". *Canadian Press* (2016).
32. Pardee K., *et al.* "Rapid, low-cost detection of Zika virus using programmable biomolecular components". *Cell* 165.5 (2016): 1255-1266.

33. Research M. "Low-Cost Paper-Based Diagnostic for Zika Virus". *Global BioDefense* (2016).
34. Freeman WM., *et al.* "Quantitative RT-PCR: pitfalls and potential". *Biotechniques* 26.1 (1999): 112-125.
35. Buathong R., *et al.* "Detection of Zika virus infection in Thailand, 2012–2014". *The American Journal of Tropical Medicine and Hygiene* 93.2 (2015): 380-383.
36. Zanoluca C and CND dos Santos. "Zika virus—an overview". *Microbes and Infection* 18.5 (2016): 295-301.
37. Lanciotti RS., *et al.* "Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007". *Emerging Infectious Diseases* 14.8 (2008): 1232-1239.
38. Calvet G., *et al.* "Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study". *The Lancet Infectious Diseases* 16.6 (2016): 653-660.
39. Barzon L., *et al.* "Isolation of West Nile virus from urine samples of patients with acute infection". *Journal of Clinical Microbiology* 52.9 (2014): 3411-3413.
40. Faria NR., *et al.* "Zika virus in the Americas: early epidemiological and genetic findings". *Science* 352.6283 (2016): 345-349.
41. Tappe D., *et al.* "First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013". *Euro Surveillance* 19.4 (2014): 20685.
42. Tiwari SK., *et al.* "Zika virus infection reprograms global transcription of host cells to allow sustained infection". *Emerging Microbes and Infections* 6.4 (2017): e24.
43. CDC, Zika MAC-ELISA (2017).
44. Adungo F., *et al.* "Development and characterization of monoclonal antibodies to yellow fever virus and application in antigen detection and IgM capture enzyme-linked immunosorbent assay". *Clinical and Vaccine Immunology* 23.8 (2016): 689-697.
45. diagnostics Ft. "Fast-track diagnostics develops kit to detect Zika virus" (2016).
46. Diagnostics A. "02.09.2016 - altona Diagnostics' RealStar® Zika Virus RT-PCR Kit 1.0 receives EUA for Korea" (2016).
47. Diagnostics Q. Quest Diagnostics Launches Zika Antibody Test Service Based on the CDC MAC-ELISA Antibody Test (2016).
48. InBios. Detect™NS1 ELISA Kit.
49. MyBioSource.com. ZIKV reagent:: Zika Virus (ZIKV) Reagent.
50. MyBioSource.com. IgG elisa kit :: Zika Virus IgG ELISA Kit.
51. Tanaka. "TANAKA develops ZIKA virus detection kit" (2016).
52. Lee D., *et al.* "Simple and Highly Sensitive Molecular Diagnosis of Zika Virus by Lateral Flow Assays". *Analytical Chemistry* 88.24 (2016): 12272-12278.

53. Notomi T, *et al.* "Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects". *Journal of Microbiology* 53.1 (2015): 1-5.
54. Notomi T, *et al.* "Loop-mediated isothermal amplification of DNA". *Nucleic Acids Research* 28.12 (2000): E63.
55. Song J., *et al.* "Instrument-free point-of-care molecular detection of Zika virus". *Analytical Chemistry* 88.14 (2016): 7289-7294.
56. Tian B., *et al.* "Attomolar Zika virus oligonucleotide detection based on loop-mediated isothermal amplification and AC susceptometry". *Biosensors and Bioelectronics* 86 (2016): 420-425.
57. Teoh BT, *et al.* "Detection of dengue viruses using reverse transcription-loop-mediated isothermal amplification". *BMC Infectious Diseases* 13.1 (2013): 387.
58. Parida M., *et al.* "Rapid detection and differentiation of dengue virus serotypes by a real-time reverse transcription-loop-mediated isothermal amplification assay". *Journal of Clinical Microbiology* 43.6 (2005): 2895-2903.
59. Lau YL., *et al.* "Colorimetric detection of dengue by single tube reverse-transcription-loop-mediated isothermal amplification". *PloS one* 10.9 (2015): e0138694.
60. Dauner AL., *et al.* "Development of a pan-serotype reverse transcription loop-mediated isothermal amplification assay for the detection of dengue virus". *Diagnostic Microbiology and Infectious Disease* 83.1 (2015): 30-36.
61. Parida M., *et al.* "Rapid and real-time detection of Chikungunya virus by reverse transcription loop-mediated isothermal amplification assay". *Journal of Clinical Microbiology* 45.2 (2007): 351-357.
62. Mori Y., *et al.* "Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation". *Biochemical and Biophysical Research Communications* 289.1 (2001): 150-154.
63. Iwamoto T., *et al.* "Loop-mediated isothermal amplification for direct detection of Mycobacterium tuberculosis complex, M. avium, and M. intracellulare in sputum samples". *Journal of Clinical Microbiology* 41.6 (2003): 2616-2622.
64. Priye A., *et al.* "A smartphone-based diagnostic platform for rapid detection of Zika, chikungunya, and dengue viruses". *Scientific Reports* 7 (2017).
65. Reese O. "Tracking and Detection Of Zika Virus Outbreaks and Symptoms Made Easy By Smartphone App Kidenga". *Parent Herald* (2016).
66. Hales L. "Smartphone testing for Zika virus (2016).

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