LAMP assay for specific detection of Asian and African lineage Zika virus: will it meet the expectations?

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The current Zika virus (ZIKV) outbreak started in 2015 in Brazil and since then has spread to over 70 countries in South and Central America, Africa and Asia (1). One of the major issues of concern with respect to ZIKV infection is the increased risk, especially during the first trimester of pregnancy, of congenital central nervous system malformation including microcephaly (2,3). Since sexual transmission of ZIKV has been reported (3) in addition to the primary route of infection through mosquitoes (4), there is significant importance to accurately and rapidly diagnose ZIKV infections not only in pregnant women who are at risk of bearing children with severe birth defects, but also in their male partners.

ZIKV is a mosquito-borne positive stranded RNA virus belonging to the genus flavivirus (family Flaviviridae) (1,4). Clinical diagnosis of ZIKV infection is difficult due to similar clinical symptoms of other human pathogenic Arboviruses such as Dengue and Chikungunya which are co-circulating in many ZIKV endemic countries (1). Serological laboratory diagnosis is also challenging due to high cross-reactivity of ZIKV with other flavivirus antibodies (5,6). Therefore, molecular diagnosis by nucleic acid amplification tests (NAATs), primarily quantitative real-time (qRT) PCR, to detect viral RNA in plasma, is currently considered the "gold standard" to confirm ZIKV infection, although the window of detection of ZIKV RNA is relatively short (7-9). Zika virus is currently spreading primarily in developing countries lacking modern laboratory infrastructure and expensive instruments required for the multi-step assay (qRT-PCR). Moreover, the time for transportation of the samples to a remote, well equipped laboratory may reduce sample quality due to RNA degradation and hamper preventive efforts.

The recent study by Chotiwan *et al.* (10), describes the development of a Loop Mediated Isothermal Amplification (LAMP) for specific detection of the Asian and African lineages of ZIKV RNA. The main advantage of the LAMP technology, which was developed in 2000 (11), compared to qRT-PCR is that it is performed in one constant temperature using simple inexpensive equipment (heat block for the reaction and visual observation of turbidity for detection), and therefore can be conducted in the field. Specifically in this study the assay was shortened to one step by using Bst DNA polymerase for reverse transcription of viral RNA during amplification instead of using a dedicated reverse transcription step and body fluids were directly tested without RNA isolation. This resulted in an assay which is both rapid and simple for use. In addition, Chotiwan *et al.* (10) reported the development of two LAMP assays to distinguish between Asian- and African-lineage Zika viruses. These African and American ZIKV assays are suggested to be used for two purposes: mosquito surveillance and clinical diagnosis. While under clinical setting there seems to be no advantage in lineage specific

identification, especially since the ZIKV lineages are currently circulating in different regions, it is important for mosquito surveillance and for research purposes particularly if co-circulation of these lineages occur.

Impressively, this study includes a thorough examination of all parameters and possible applications of the LAMP assay including directly comparing it to qRT-PCR using ZIKV infected cell culture and by testing whole and dissected ZIKV orally infected mosquitoes, ZIKV spiked samples of human blood, plasma, saliva, urine and semen and clinical samples from ZIKV infected persons. In the case of ZIKV laboratory infected mosquitoes the assay performed as expected under the experimental conditions: direct detection in mosquito homogenates without prior RNA extraction was comparable to tissue culture isolation, as all infected mosquitoes were identified and non-infected mosquitoes were negative. While this is very promising, it still remains to prove that this assay will be as sensitive as qRT-PCR for detection of ZIKV in mosquitoes captured in nature. Direct detection of ZIKV from ZIKV infected cell culture using the LAMP assay was comparable to qRT-PCR from RNA extracted ZIKV infected cell culture, However, since body fluids needed to be diluted 1:100 when taken directly to the LAMP assay, direct detection of clinical samples (without RNA extraction with the LAMP assay) was significantly less sensitive than qRT-PCR and in addition, yielded some false positive results. Indeed RNA extraction of the samples before LAMP, substantially improved the sensitivity of detection of the LAMP assay. This result seems to weaken the potential of the LAMP assay as a useful rapid and sensitive field test for clinical diagnosis. Given the limitations of this part of the study: low quality of the clinical samples (frozen samples), testing by two different laboratories in two different countries and unidentified reference methods, more controlled studies seem to be required to further elucidate the true potential of this test for clinical diagnosis.

Lastly, the use of the LAMP assay as a field test in remote areas and developing countries would still require transportation and maintenance of highly sensitive reagents (primers, nucleotides, enzymes etc.) keeping a cold chain throughout. Experiments with pre-dried reagents that will allow less stringent transportation conditions as described by Ganguli *et al.* (12) would be very useful.

Molecular detection of ZIKV infection by LAMP assay was previously developed (13), however, the sensitivity of the assay was only compared to conventional reverse transcriptase PCR with gel detection and no clinical samples from ZIKV infected patients were assessed. Following the study by Chotiwan *et al.* (10), two other studies developed a LAMP assay for ZIKV RNA detection which is preceded by both RNA extraction and an external reverse transcriptase step (RT-LAMP) (14,15). Both studies concluded that the assay has high specificity and sensitivity and despite the fact that the RT-LAMP is still approximately 1 log less sensitive than qRT-PCR, it could be used for clinical diagnosis. This is a very challenging conclusion by the authors as patient samples can have low level of ZIKV RNA and therefore detection can be missed, thus mandating a secondary confirmation test to be used to complement the result of the RT-LAMP assay.

In conclusion, qRT-PCR is currently the most sensitive method for detection of ZIKV RNA from clinical samples and should continue to be used as the molecular "gold standard" tool in a diagnostic laboratory. The studies by Chotiwan *et al.* as well as others (10,13-15) demonstrate that LAMP can be used as a frontline screening tool of ZIKV in areas where expensive and sophisticated instruments are limited—currently many places where ZIKV is circulating.

The development of ZIKV LAMP assays are relatively new and once improvement in the assay specificity and sensitivity is achieved and well established, its ease of use and inexpensive price has the potential to revolutionize molecular detection and bring high standard clinical diagnosis everywhere.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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