



Potential Point-of-Care Testing for Dengue Virus in the Field

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ABSTRACT The four serotypes of dengue virus (DENV) cause one of the most important and rapidly emerging mosquito-borne viral diseases in humans. Of the currently available diagnostic tests for dengue, the reverse transcription-PCR (RT-PCR) assay is the most sensitive and specific, and so it is commonly used as the gold standard. However, the requirement of a sophisticated and expensive thermal cycler makes it very difficult to use as a point-of-care diagnostic test in resource-limited regions where dengue is endemic. Tsai et al. (*J Clin Microbiol* 56:e01865-17, 2018, <https://doi.org/10.1128/JCM.01865-17>) report the analytical and clinical performances of a reverse transcription-insulated isothermal PCR (RT-iiPCR) assay with a portable nucleic acid analyzer for rapid detection of the four DENV serotypes; its reproducibility and complete agreement on clinical samples with the multiplex RT-PCR assay developed by the Centers for Disease Control and Prevention suggest that the dengue RT-iiPCR is a potential point-of-care test. Compared with other DENV RNA detection methods, the unique isothermal PCR design of RT-iiPCR, together with further improvements, would represent a promising new type of field-deployable diagnostic test for dengue.

With an estimated 390 million new infections per year, the four dengue virus (DENV) serotypes are the leading causes of mosquito-borne viral infection and disease in humans (1, 2). While many DENV infections are clinically mild or inapparent, clinical disease ranges from a self-limited illness, dengue fever, to severe and potentially life-threatening disease, dengue hemorrhagic fever/dengue shock syndrome (1–3). The forms of the disease are classified as dengue, dengue with warning signs, and severe dengue according to the 2009 WHO revised case definition (3). There are approximately 3 billion people in more than 120 countries who are at risk of DENV infection (1, 2). Currently, no FDA-approved antiviral drug is available to treat dengue; the only licensed dengue vaccine, Dengvaxia, is recommended only for persons who have experienced previous DENV infection and not for dengue-naïve individuals (4–6).

Accurate and rapid laboratory diagnosis of dengue is critical for confirmation of clinically suspected cases to ensure timely management of severe dengue disease, especially in urban areas of resource-poor developing countries. Laboratory diagnosis for DENV infection includes virus isolation, reverse transcription-PCR (RT-PCR), non-structural protein 1 (NS1) antigen detection, and serological tests such as IgM and IgG enzyme-linked immunosorbent assay (ELISA) and neutralization tests (2, 3). Each diagnostic method has its advantages and limitations; overall RT-PCR is the most sensitive and specific diagnostic test and has been commonly used as a gold standard for comparisons with other tests (reviewed in reference 7).

The report by Tsai and colleagues in this month's issue of the *Journal of Clinical Microbiology* suggests that the reverse transcription-insulated isothermal PCR (RT-iiPCR) assay with a portable Pockit nucleic acid analyzer (GeneReach, Lexington, MA) is a promising point-of-care (POC) test for rapid detection of four serotypes of DENV (8). The

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TABLE 1 Comparison of dengue viral RNA detection assays^a

Parameter	Description		
	RT-PCR test	RT-iiPCR test	RT-LAMP test
Principle	Sample + primers and/or probe + enzymes; one-step RT followed by PCR; denaturation, annealing, and extension	Sample + primers/TaqMan probe + enzymes; RT followed by insulated isothermal PCR; a single heating source driving fluid convection through a temp gradient and Rayleigh-Bernard convective PCR (denaturation, annealing, and extension in 3 zones)	Sample + primers + enzymes; RT followed by loop-mediated isothermal amplification; self-recurring strand displacement synthesis primed by 6 primers targeting distinct sequences
Signal detection	TaqMan probe or fluorescent dye, built-in detection	TaqMan probe, built-in detection module	Fluorescent dye or turbidity, visual detection or fluorometer or turbidimeter
Equipment	Thermal cycler	No thermal cycler; Pockit nucleic acid analyzer	No thermal cycler
Stability of reagents	Cold chain for enzymes	Lyophilized premix	Cold chain for enzymes
Time ^b	~150 min (65–90 min)	<60 min (40 min)	~70 min (30–45 min)
Targeting genes	3' UTR, NS5/prM/E	3' UTR conserved region	3' UTR, C/prM, NS1
Detection of different serotypes	Yes; multiplex RT-PCR (9)	Not yet; pan-DENV (current format) (8, 17)	Yes; multiplex (12–14)
Detection of other arboviruses (ZIKV, CHIKV)	Yes; multiplex RT-PCR (18–21)	Not yet	Yes; multiplex (15, 16)

^aRT-PCR, reverse transcription-PCR; RT-iiPCR, reverse transcription-insulated isothermal PCR; RT-LAMP, reverse transcription-loop-mediated isothermal amplification; ZIKV, Zika virus; CHIKV, chikungunya virus; UTR, untranslated region; NS, nonstructural protein; C, capsid; prM, premembrane; E, envelope.

^bTime varies depending on different assays; parentheses indicate setup time for reaction.

high specificity of the assay using pan-DENV primers and probe was demonstrated by the lack of detection of 14 non-DENV human viruses; the reproducibility was supported by consistent results from repeat testing by six operators at three sites. Compared with the Centers for Disease Control and Prevention (CDC) reference test, the RT-iiPCR assay showed comparable detection endpoints with four DENV serotypes (8, 9). In testing performed with 256 clinical samples, the RT-iiPCR assay showed complete agreement (100%; 95% confidence interval [CI_{95%}], ~98.81% to 100%; κ = 1) with the CDC quantitative RT-PCR assay.

In the field of molecular diagnosis, several isothermal amplification modalities such as loop-mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), thermophilic helicase-dependent amplification (tHDA), and recombinase polymerase amplification (RPA) have been developed for the detection of different DNA or RNA microorganisms, including those causing malaria (10, 11). For molecular diagnosis of dengue, reverse transcription-LAMP (RT-LAMP) has been reported previously as a potential field-deployable POC test (12–16). Results of comparisons of the RT-PCR, RT-iiPCR, and RT-LAMP tests for dengue regarding the principles, equipment, primers/probes, reagent stability, time, and detection capability with respect to different DENV serotypes or related viruses are summarized in Table 1.

The iiPCR system is unique in its employment of a thermally baffled device and a single heating source to drive Rayleigh-Bernard convection to allow the three steps of PCR (denaturation, annealing, and extension) to occur in different temperature zones (22, 23). Since the first report of the assay in 2012, the RT-iiPCR or iiPCR system (via the use of a Pockit nucleic acid analyzer) has been widely applied for rapid detection of various parasitic, bacterial, and viral pathogens in animals and humans, including *Plasmodium*, *Salmonella*, bluetongue virus, foot-and-mouth disease virus, classical swine fever virus, influenza A virus, and coronavirus (24–27). A previous study of RT-iiPCR for dengue samples reported sensitivity of 90.5% and specificity of 98.3% compared with a composite 3-assay reference, including the CDC quantitative RT-PCR (qRT-PCR) assay (17). The study by Tsai et al. further validated the RT-iiPCR test for dengue diagnosis with higher sensitivity and specificity (8). The rapid, simple, convenient, and user-friendly protocol of the Pockit nucleic acid analyzer, together with its small size, portable design, rechargeable battery, and the option of an automatic RNA extraction system, makes it a promising POC diagnostic modality for dengue. The

RT-iiPCR assay for dengue has successfully completed two independent clinical evaluations (8, 17) and has shown very encouraging results. Since both studies were carried out in research laboratories, however, studies in different regions or countries of dengue endemicity are needed to further validate its performance as a POC test.

There are several limitations of the RT-iiPCR test. First, it cannot distinguish different DENV serotypes. Given that multiple DENV serotypes have been cocirculating in regions of endemicity and that two or more DENV serotypes were reported increasingly during the same outbreak, several RT-PCR or RT-LAMP tests have been developed in multiplex format to detect and distinguish different DENV serotypes (9, 12–14). Future development of multiplex RT-iiPCR for dengue would greatly improve its clinical applications to better understand the extent and serotypes of infecting virus in the field sites. Second, the dengue RT-iiPCR test can test only 8 samples at the maximum; future design enhancements to increase its detection capacity would make it more useful during outbreak investigations. Additionally, it cannot detect other arboviral infections with similar clinical presentations such as Zika virus (ZIKV) and chikungunya virus (CHIKV). The recent spread of CHIKV followed by ZIKV to regions of the Americas where DENV is endemic highlights the importance of distinguishing these three arboviruses in regions of dengue endemicity. Several triplex RT-PCR or RT-LAMP tests have been developed to detect DENV, ZIKV, and CHIKV simultaneously (15, 16, 18–21). Future development of the RT-iiPCR test into a multiplex format to cover ZIKV and CHIKV would significantly strengthen the utility of this potential POC test in the field.

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