



Validation of the Pockit Dengue Virus Reagent Set for Rapid Detection of Dengue Virus in Human Serum on a Field-Deployable PCR System

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ABSTRACT Dengue virus (DENV) infection, a mosquito-borne disease, is a major public health problem in tropical countries. Point-of-care DENV detection with good sensitivity and specificity enables timely early diagnosis of DENV infection, facilitating effective disease management and control, particularly in regions of low resources. The Pockit dengue virus reagent set (GeneReach Biotech), a reverse transcription insulated isothermal PCR (RT-iiPCR), is available to detect all four serotypes of DENV on the field-deployable Pockit system, which is ready for on-site applications. In this study, analytical and clinical performances of the assay were evaluated. The index assay did not react with 14 non-DENV human viruses, indicating good specificity. Compared to the U.S. CDC DENV-1–4 real-time quantitative RT-PCR (qRT-PCR) assay, testing with serial dilutions of virus-spiked human sera demonstrated that the index assay had detection endpoints that were separately comparable with the 4 serotypes. Excellent reproducibility was observed among repeat tests done by six operators at three sites. In clinical performance, 195 clinical sera collected around Kaohsiung city in 2012 and 21 DENV-4-spiked sera were tested with the RT-iiPCR and qRT-PCR assays in parallel. The 121 (11 DENV-1, 78 DENV-2, 11 DENV-3, and 21 DENV-4) qRT-PCR-positive and 95 qRT-PCR-negative samples were all positive and negative by the RT-iiPCR reagent results, respectively, demonstrating high (100%) interrater agreement (95% confidence interval [CI_{95%}], ~98.81% to 100%; $\kappa = 1$). With analytical and clinical performance equivalent to those of the reference qRT-PCR assay, the index PCR assay on the field-deployable system can serve as a highly sensitive and specific on-site tool for DENV detection.

KEYWORDS dengue virus, RT-PCR, field-deployable, on-site detection

Dengue virus (DENV) infection causes a wide range of clinical signs in humans, from asymptomatic to acute febrile illness (dengue fever [DF]) or severe hemorrhagic fever/dengue shock syndromes (DHF/DSS) (1). It is a major public health problem in developing tropical countries and has been continuously spreading to new geographical areas (2–5). Transmission of the virus occurs mainly by the mosquito species *Aedes aegypti* and, less efficiently, by *A. albopictus* (6). Moreover, frequent international travel to regions of dengue endemicity or epidemicity has contributed to the escalating numbers of imported dengue cases in temperate regions (7, 8). DENV, belonging to the

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genus *Flavivirus* in the family *Flaviviridae* (9), has a single-stranded, positive-sense RNA genome (10, 11); DENV is comprised of four distinct serotypes (DENV-1, -2, -3, and -4), with distinct genotypes within each serotype (12, 13). Since the late nineteenth century, Taiwan has had dengue epidemics in its southern region, especially in the city of Kaohsiung (14–16). Recently, relatively large outbreaks occurred, in 2014 and 2015, with DENV-1 and -2 being the major serotypes, respectively (17).

Diagnosis of DENV infections is important since serious manifestations such as DF and DHF/DSS may lead to death if not managed properly. However, clinical presentations of acute DENV infection are nonspecific and resemble those of other diseases such as malaria, leptospirosis, typhoid, typhus, chikungunya, and Zika disease, making diagnosis of dengue solely from clinical symptoms a difficult task (18). Early and rapid diagnosis of DENV infection is crucial to prevent fatal cases and serves as an alert to initiate timely community and vector control programs to help mitigate the spread of infection. Current laboratory methods to aid diagnosis of DENV infection include virus isolation and detection of viral nucleic acid (e.g., reverse transcriptase PCR [RT-PCR]), NS1 antigen, or virus-specific antibodies (e.g., neutralization test, IgM capture enzyme-linked immunosorbent assay [MAC-ELISA], and indirect IgG ELISA) (19–21). However, those methods require relatively expensive equipment and specialized personnel, limiting their use to only well-equipped laboratories. For remote regions with limited resources, timely on-site confirmation of suspected cases requires simple, rapid, accurate, and affordable diagnostic tools for DENV detection. Fast and easy immunological DENV NS1 and IgM/IgG rapid tests are available for these purposes (22–24). However, the specificity and sensitivity of these methods were shown to be lower in general for some sample types and virus serotypes (25–29). The Pockit dengue virus reagent set, an RT-PCR method, has potential to meet these needs.

The DENV reagent set works on a portable and compact PCR device, the Pockit nucleic acid analyzer (Pockit; GeneReach Biotech, Taichung, Taiwan), which can generate simple qualitative PCR/RT-PCR results with a single program within 1 h. The sensitivity, specificity, and clinical performance of the Pockit system have been demonstrated to be comparable to those of reference methods (real-time PCR, nested PCR, virus isolation) for various important microbial pathogens of humans, animals, and plants in different clinical sample types (30–40). The methodology is fluorescent probe hydrolysis-based insulated isothermal PCR (iiPCR) performed in a capillary tube (R-tube; GeneReach Biotech) heated at the bottom by a single heating source (41, 42). Once Rayleigh-Bénard convection is established, the fluids are cycled naturally through temperature zones to allow the denaturation, annealing, and extension steps to be completed sequentially, eliminating the need for an expensive thermocycler. Signals from the probe are processed and interpreted automatically (41). Moreover, a mobile Pockit PCR laboratory can be easily established with several simple and easy-to-perform field-deployable nucleic acid extraction methods, including an automatic nucleic acid extraction device, i.e., the taco mini automatic nucleic acid extraction system (taco mini; GeneReach Biotech).

The POTKIT dengue virus reagent set, targeting the 3' untranslated region of the DENV RNA genome, was derived from the pan-DENV RT-iiPCR system reported previously (43). In this study, clinical performance of the assay was assessed with serum samples collected from dengue-suspected patients in Taiwan. Analytical sensitivity and specificity, reproducibility, and interference of the reagent were also evaluated.

MATERIALS AND METHODS

Cells and viruses. Four DENV isolates, DENV-1 (Hawaii strain; 2.46×10^6 PFU/ml), DENV-2 (New Guinea-C strain; 2.07×10^6 PFU/ml), DENV-3 (DN8700829A strain; 1×10^6 PFU/ml), and DENV-4 (DN9000475A strain; 1.9×10^6 PFU/ml), were propagated in the mosquito C6/36 cell line (*A. albopictus*). The virus was diluted in RPMI medium (Gibco-Life Technologies, Grand Island, NY, USA) containing 1% fetal calf serum (FCS) and was added to the cell at a multiplicity of infection of 0.01. After incubation at 28°C in 5% CO₂ for 4 to 7 days (until the cytopathic effect was observed), titers of DENV stocks were determined by plaque assay. Briefly, 10-fold serial dilutions of the DENV stock were made in maintenance medium (minimum essential medium [MEM]; Gibco-Life Technologies) and added to BHK-21 cells in 6-well plates (at about 1×10^6 cells per well). Maintenance medium was used in mock infections. Each

dilution was done in duplicate. Adhesion was allowed at 37°C in 5% CO₂ for 2 h before addition of 3 ml of overlay medium containing 1.2% methylcellulose. Cells were incubated for a further 5 to 10 days (until plaques became visually apparent by microscopy), fixed, and stained with crystal violet. Plaques were counted manually, and PFU counts per milliliter were determined with the plaque quantification program (44, 45).

Fourteen virus species known to cause febrile illness or skin rash illness were tested for analytical specificity. West Nile virus (WNV; synthetic RNA, VR-31985D) and Zika virus (MR766; PRVABC59 strain) were from the American Type Culture Collection, Manassas, VA, USA. Chikungunya virus (CK9500004 strain) was from the Taiwan Center of Disease Control, Taipei, Taiwan. The Japanese encephalitis virus, hepatitis C virus, influenza virus A, influenza virus B (Yamagata strain), human coronavirus NL-63, enterovirus 71, coxsackievirus A16, herpes simplex virus 1, herpes simplex virus 2, cytomegalovirus, and hepatitis B virus samples tested were local clinical samples identified and provided by the Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung, Taiwan.

Clinical specimens. Research protocols involving retrospective clinical specimens were approved by the Kaohsiung Medical University Hospital Institutional Review Board (KMUH-DC-101-0301). Waiver of informed consent was obtained. A total of 195 archived serum specimens (100 DENV positive and 95 DENV negative) from clinically suspected dengue patients were collected at the Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, in 2012 for routine diagnosis using RT-PCR methods (46). These included 94 male and 101 female subjects. For DENV-4 samples, 21 of the DENV-negative serum samples were spiked with tissue culture fluids of a DENV strain (DN9000475A). In total, 216 samples were tested for comparisons between the Pockit dengue virus reagent set and reference qRT-PCR with respect to performance. In addition, the blood samples used in the analytical sensitivity and reproducibility studies were from another set of donors recruited with informed consent in 2016 to 2017. All serum samples were stored at -20°C until nucleic acid extraction was performed.

Nucleic acid extraction. For analytical sensitivity analysis, tissue culture fluids containing DENV-1, -2, -3, or -4 were subjected to 10-fold serial dilutions in DENV-negative human sera before nucleic acid extraction was performed. Samples for specificity testing were subjected directly to nucleic acid extraction. The archived human serum samples in the clinical performance study were subjected directly to extraction. Nucleic acid extraction was done on a taco mini system (GeneReach Biotech) using a Taco preloaded DNA/RNA extraction kit (GeneReach Biotech) according to the manufacturer's instructions. Briefly, 200 μ l of the sample was added into the first well of the extraction plate before the automatic extraction steps were performed. All nucleic acids were collected individually and maintained at -80°C until further use.

DENV RT-iiPCR assay. The Pockit dengue virus reagent set (GeneReach Biotech), which was derived from the previously published pan-DENV RT-iiPCR system (43), was used as described in the user manual. Briefly, R-tubes were labeled with sample identifiers. Lyophilized dengue virus premix was reconstituted in 50 μ l of premix buffer, and 5 μ l of test nucleic acid extract was added. A 50- μ l volume of the premix/sample mixture was transferred into the correspondingly labeled R-tube, which was sealed subsequently with a cap, spun briefly in a microcentrifuge (cubee; GeneReach Biotech), and placed into a Pockit device. The default program, including an RT step at 50°C for 10 min and an iiPCR step at 95°C for about 30 min, was completed in less than 1 h. Signal-to-noise (S/N) ratios, i.e., ratios calculated as levels of light signals collected after iiPCR/levels of fluorescent signals collected before iiPCR (41), were converted automatically to "+," "-", or "?" data according to the default S/N thresholds used by the built-in algorithm. The results were shown on the display screen at the end of the program.

CDC DENV-1-4 real-time RT-PCR assay. The multiplex U.S. CDC DENV-1-4 real-time RT-PCR assay (47) contained 4 sets of oligonucleotide primers and 4 dually labeled 5' fluorescent TaqMan probes (see Supplementary Table 1 in <http://www.cdc.gov/dengue/clinicalLab/realTime.html>). The reaction was performed with a SuperScript III Platinum One-Step qRT-PCR kit (Invitrogen, Carlsbad, CA, USA) without 6-carboxy-X-rhodamine in a model 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). Signals were collected using the 6-carboxyfluorescein, hexachlorofluorescein, Texas Red, and Cy5 channels for the detection of fluorescence generated from the DENV-1, -2, -3, and -4 probes, respectively. The thermocycling program was set up as follows: an RT step at 50°C for 30 min, followed by 95°C for 2 min and 45 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 1 min. Samples generating a threshold cycle (C_T) value of >37 were considered negative. Each run included negative controls spiked with water and positive controls with *in vitro* transcribed RNA containing the target sequences of known copy numbers.

Interference test. The target sequences of the Pockit dengue virus reagent set in the 3' untranslated region were highly conserved among the available sequences of DENV-1, -2, and -3. Therefore, the interference and reproducibility analysis tests were done with DENV-2 (representing DENV-1, -2, and -3) and DENV-4. For interference testing, laboratory-grade human endogenous interference biomolecules were diluted in DENV-negative human serum to the physiologically relevant pathological concentrations (hemoglobin [Sigma-Aldrich, St. Louis, MO, USA], 2 g/liter; cholesterol [Sigma-Aldrich], 13 mmol/liter; EDTA [Sigma-Aldrich], 292 μ mol/liter; heparin [Sigma-Aldrich], 3,000 U/liter; bilirubin [Alfa Aesar, Ward Hill, MA, USA] and human genomic DNA, 4 mg/ml) suggested by the National Committee for Clinical Laboratory Standards (NCCLS), EP7-A2 (2005). Virus stocks were diluted in these samples to moderately positive, low positive, and high negative concentrations; each sample was subjected to nucleic acid extraction in triplicate by the taco mini method. Nucleic acids were tested with the Pockit dengue virus reagent set.

Statistical analysis. The degree of agreement between two assays was assessed by calculating Cohen's kappa values. Sensitivity was calculated as follows: number of true positives/(number of true

TABLE 1 Analytical sensitivity of Pockit dengue virus reagent set^a

Strain	Titer (PFU ml ⁻¹)	Detection rate (no. of assays with positive result/total no. of assays)	
		qRT-PCR	RT-iiPCR
DENV-1	10 ²	5/5	5/5
DENV-2		5/5	5/5
DENV-3		5/5	5/5
DENV-4		5/5	5/5
DENV-1	10 ¹	5/5	5/5
DENV-2		5/5	5/5
DENV-3		5/5	5/5
DENV-4		5/5	5/5
DENV-1	1	0/5	3/5
DENV-2		5/5	5/5
DENV-3		5/5	5/5
DENV-4		5/5	4/5
DENV-1	10 ⁻¹	0/5	0/5
DENV-2		0/5	2/5
DENV-3		1/5	1/5
DENV-4		3/5	1/5
DENV-1	10 ⁻²	0/5	0/5
DENV-2		0/5	0/5
DENV-3		0/5	0/5
DENV-4		0/5	0/5

^aDENV, dengue virus; qRT-PCR, real-time reverse transcription-quantitative PCR; RT-iiPCR, reverse transcription insulated isothermal PCR. Boldface data indicate a 100% detection endpoint.

positives + number of false negatives). Specificity was calculated as follows: number of true negatives / (number of true negatives + number of false positives).

RESULTS

Analytical sensitivity. To test analytical sensitivity, 10-fold serial dilutions (100, 10, 1, 0.1, and 0.01 PFU/ml) of DENV-1, -2, -3, and -4 isolates were made in DENV-negative human serum samples and each was subjected to nucleic acid extraction in 5 replicates, followed by DENV viral detection with the Pockit DENV assay and real-time RT-PCR (qRT-PCR). The 100% detection endpoints were 10 PFU/ml for DENV-1 and 1 PFU/ml for DENV-2 and -3 with both PCR assays (Table 1). For DENV-4, the 100% detection endpoints were 10 and 1 PFU/ml with the Pockit and qRT-PCR assays, respectively (Table 1). The Pockit assay had detection endpoints with the four serotypes comparable to those seen with the U.S. CDC DENV-1–4 real-time RT-PCR assay (47).

Analytical specificity. The Pockit dengue virus reagent set detected the four DENV serotypes (DENV-1, -2, -3, and -4) and did not react with the 14 viruses in the exclusivity test panel, indicating that the assay had excellent specificity for DENV (Table 2).

Interference. Significant levels of residual biological components derived from sample collection tools and sample matrix and, when present, nucleic acid extracts can result in inhibition of RT-PCR. In order to ensure that the endogenous biological chemicals in human serum did not interfere with the performance of the Pockit dengue virus reagent set, DENV-negative human serum samples spiked with biological concentrations of EDTA, hemoglobin, cholesterol, heparin, bilirubin, or human genomic DNA were tested. Aliquots of each preparation was spiked with four different titers, i.e., moderately positive (10× the limit of detection [LoD] [100% LoD]), low positive (1× LoD), high negative (0.1× LoD), and mock (negative), of different DENV serotypes in triplicate and subjected to nucleic acid extraction; the nucleic acid extracts were tested by the DENV Pockit assay. DENV-2 and -4 were tested, as the PCR target regions were highly conserved among DENV-1, -2, and -3. The detection rates were all 3/3 for the moderately and low-positive samples, and some were 2/3 for the high-negative samples, as expected (Table 3), indicating that the tested materials did not interfere with

TABLE 2 Specificity of Pockit dengue virus reagent set

Pathogen	Detection rate (no. of assays with positive result/total no. of assays)
Dengue virus-1	3/3
Dengue virus-2	3/3
Dengue virus-3	3/3
Dengue virus-4	3/3
West Nile virus	0/3
Chikungunya virus	0/3
Zika virus	0/3
Japanese encephalitis virus	0/3
Hepatitis C virus	0/3
Influenza virus A	0/3
Influenza virus B	0/3
Coronavirus	0/3
Enterovirus 71	0/3
Coxsackievirus A16	0/3
Herpes simplex virus 1	0/3
Herpes simplex virus 2	0/3
Cytomegalovirus	0/3
Hepatitis B virus	0/3

the DENV detection system comprising the taco mini nucleic acid extraction test and Pockit PCR test.

Reproducibility. Reproducibility tests were also done with DENV-2 (representing DENV-1, -2, and -3) and DENV-4. Operator-to-operator, run-to-run, and site-to-site reproducibility was evaluated using a panel of serum samples spiked with DENV to four titers, i.e., negative, high negative, low positive, and moderately positive. The tests were done at three laboratories in Taiwan (Department of Pathology & Laboratory Medicine, Taichung Veterans General Hospital, Taichung; Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung; and GeneReach Biotechnology, Taichung). All samples were processed in a blind manner during the tests. Two operators tested all samples in triplicate once a day for 5 days at each site. A total of 720 tests were performed, and detection rates were as expected, at a 100% detection rate with the moderately positive samples; >96.7% (87/90 samples) and >34.4% (31/90) with the low-positive and high-negative samples, respectively; and 0% with the negative samples (Table 4), indicating that this DENV detection system has excellent reproducibility among operators, test runs, and test sites.

Clinical performance. To evaluate the clinical performance of the Pockit dengue virus reagent set, 195 serum samples collected from dengue-suspected patients in Taiwan in 2012 and 21 DENV-negative human serum samples spiked with different

TABLE 3 DENV detection by Pockit dengue virus reagent set in the presence of potentially interfering substances^a

Virus	Detection rate (no. of assays with indicated result/total no. of assays)					
	DENV-2			DENV-4		
	Moderately positive titer	Low positive titer	High negative titer	Moderately positive titer	Low positive titer	High negative titer
Hemoglobin	3/3	3/3	2/3	3/3	3/3	2/3
Cholesterol	3/3	3/3	2/3	3/3	3/3	2/3
EDTA	3/3	3/3	1/3	3/3	3/3	2/3
Heparin	3/3	3/3	1/3	3/3	3/3	3/3
Bilirubin	3/3	3/3	2/3	3/3	3/3	3/3
Human genomic DNA	3/3	3/3	2/3	3/3	3/3	3/3

^aThe concentrations used were as follows: for hemoglobin, 2 g/liter; for cholesterol, 13 mmol/liter; for EDTA, 292 μmol/liter; for heparin, 3,000 U/liter; for bilirubin, 342 μmol/liter; for 7.6 human genomic DNA, 4 mg/ml. DENV, dengue virus.

TABLE 4 Summary of reproducibility test results for Pockit dengue virus reagent set^a

Virus	Titer	Detection rate (no. of samples with indicated result/total no. of samples) (%)
DENV-2	Moderately positive	90/90 (100)
	Low Positive	87/90 (96.7)
	High negative	31/90 (34.4)
	Negative	0/90 (0)
DENV-4	Moderately positive	90/90 (100)
	Low Positive	90/90 (100)
	High negative	68/90 (75.6)
	Negative	0/90 (0)

^aDENV, dengue virus.

concentrations of a DENV-4 isolate (6 replicates at 1.9×10^4 PFU/ml, 8 at 1.9×10^2 PFU/ml, 4 at 1.9 PFU/ml, and 3 at 0.19 PFU/ml) were tested. Nucleic acids extracts were tested by the Pockit assay and the U.S. CDC DENV-1–4 real-time RT-PCR assay in parallel. Among the samples, 121 were positive and 95 were negative by both PCR assays (Table 5). Compared to the reference qRT-PCR assay, the Pockit assay had 100% positive agreement (95% confidence interval [CI_{95%}], 97.81% to 100%) and 100% negative agreement (CI_{95%}, 97.22% to 100%). The overall agreement between the two assays was 100% (CI_{95%}, 98.76% to 100%; $\kappa = 1.0$).

DISCUSSION

Good clinical diagnosis and management can improve the recognition of severe dengue warning signs, such as impending intravascular leakages (48). Point-of-care DENV detection could help bring health services to local levels, reducing costs and turnaround time while achieving early disease diagnosis to facilitate disease management, surveillance, and control. The use of NS1 antigen ELISA in the diagnostic laboratory has been recommended for diagnosis of dengue in the acute phase of illness. Similarly, the rapid NS1 antigen test allows early DENV detection at points of care. However, the sensitivity of the rapid NS1 antigen detection test was relatively low on days 1 and 2 and after day 5 postsymptomatic onset compared to that seen with the RT-PCR methods (25–27). In addition, rapid NS1 antigen detection had lower sensitivity in secondary infections, probably due to the interference presented by anti-NS1 antibodies in the patient (49, 50); the rapid NS1 antigen detection methods also appeared to have lower sensitivity for certain serotypes, e.g., DENV-2 and -4 (28, 29). DENV RNA can be reliably detected during viremia (5 days after the onset of illness) (51) and up to 10 days after the onset of clinical symptoms in some clinical cases by qRT-PCR (48). To aid laboratory confirmation of DENV infection during the first 5 to 6 days after symptomatic onset, detection methods for DENV RNA have been recommended by WHO (16).

Currently, nucleic acid-based methods for DENV detection are more widely available in referral hospitals than in clinics or local hospitals, leaving the majority of the patients without access to early diagnosis in resource-limited regions (51). For these regions,

TABLE 5 Agreement between Pockit dengue virus reagent set and reference DENV-1–4 real-time RT-PCR for DENV detection in serum from dengue-suspected patients

Pockit dengue virus reagent set result	No. of samples with indicated U.S. CDC DENV-1–4 real-time RT-PCR assay result ^a		
	Positive	Negative	Total
Positive	121	0	121
Negative	0	95	95
Total	121	95	216

^aDENV-1–4 real-time RT-PCR data are from reference 47. Agreement, 100% (95% confidence interval [CI_{95%}] = 98.7% to 100%).

highly sensitive and specific detection tools that are rapid and affordable for the detection of DENV RNA are needed in primary care facilities. A variety of isothermal amplification methods, such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification, have also been developed for these purposes (1, 14, 25, 31, 32, 40, 42, 47, 52, 53). However, since LAMP methodology relies on the use of generic fluorescent dyes or turbidity, its limitations included the propensity to result in false-positive reactions and variations in visual interpretations of LAMP signals among different observers (54). The results seen in this study demonstrated that the Pockit dengue virus detection set in the portable and compact Pockit device had excellent analytical sensitivity, specificity, and reproducibility; it also showed high agreement with the qRT-PCR to detect DENV RNA in serum samples from dengue-suspected patients (100%; $CI_{95\%}$ ~98.81% to 100%; $\kappa = 1$; $n = 216$). Hence, the index assay can serve as a relatively inexpensive, rapid, and simple point-of-need (PON) tool for early detection of viremic dengue patients in routine dengue diagnosis, improving clinical management in underserved communities. The pan-DENV RT-iiPCR described previously also had excellent analytical sensitivity, specificity, and reproducibility (43). Overall, similar results in clinical performance were observed in these two studies. The clinical samples tested for the previous report were plasma samples collected in Sri Lanka and included mainly DENV-1 ($n = 145$) and a few with DENV-4 ($n = 5$) but no DENV-2 or -3. In this study, the clinical samples were collected in Taiwan and included 11, 87, and 11 DENV-1, -2, and -3, respectively.

A nucleic acid extraction step to remove PCR inhibitors in the sample matrix is critical to ensure the proper working of the enzymatic reaction (55). Accordingly, nucleic acid extraction is key for the performance of RT-iiPCR. Automatic extraction methods, such as the taco mini method, can greatly reduce hands-on time and minimize inconsistencies in extraction efficiency due to human errors. In addition, health hazards to the personnel were minimized in the protocols of taco mini by adding clinical samples directly to the lysis buffer in the first step, which can inactivate all pathogens immediately.

For PON applications, the combination of the field-deployable taco mini system (30 by 26.5 by 26 cm [width by depth by height {W by D by H}]; 5 kg) and the PCR device (Pockit; 31 by 26 by 15 cm [W by D by H]; 2.1 kg) is available in a durable suitcase (Pockit combo; GeneReach Biotech). All of the instruments can be operated using a car battery or a rechargeable battery. Only a few simple steps from sample to results are needed with this mobile PCR system. The PCR reagent is provided in a ready-to-use lyophilized format for easy shipping at ambient temperatures and storage (tolerating at least 2 years in a refrigerator). Automatic nucleic acid extraction is followed by simple PCR assembly protocols: (i) rehydration of the lyophilized reagents, (ii) addition of sample nucleic acids, and (iii) transfer of reaction mixture into reaction tubes and placing of the tubes on the Pockit device. Interpretation of PCR results automatically generates simple results on the display screen after the 1-h reaction, eliminating data processing and analysis by the user. A hand-held Pockit model, namely, the Pockit Micro Plus (GeneReach Biotech) (throughput, 4 tests; 6.3 by 15.2 by 5.0 cm [W by D by H]; 0.3 kg), is also available. It is a small, lightweight, stand-alone device operated with a rechargeable battery and also can generate and display qualitative results on the monitor in 45 min.

DENV can be transmitted through mosquito bites, particularly during the viremic phase of the disease in which high levels of DENV viremia are present in the bloodstream. For dengue disease control, it is important to extend DENV surveillance in the human and mosquito populations to points of care, generating early alert signals to prevent or control DENV epidemics and reducing the spread of DENV infection. Mosquitoes can be infected by DENV through a blood meal obtained on a viremic individual. The 50% infectious doses of DENV-2 for female *A. aegypti* mosquitoes were found at $>10^4$ PFU/ml blood meal (56). The 100% detection endpoints of the Pockit reagent on the taco mini/Pockit system were about 1 to 10 PFU/ml serum for the four serotypes (Table 1); therefore, the DENV RT-iiPCR test on the taco mini/Pockit system could potentially detect patients whose infection is contagious. Once infected by DENV,

the mosquitos could transmit the virus for life after a 4- to 14-day extrinsic incubation period (52, 53). In the absence of a vaccine for DENV infection, surveillance and control of mosquito vectors are important measures for dengue prevention. In the case of WNV, surveillance of the pathogen in the vector populations has been used to follow virus circulation and/or emergence (57, 58), helping in allocating control and prevention efforts to the geographical areas most in need. The DENV Pockit dengue virus reagent set/Pockit system is ready in a mobile and user-friendly format to help implement DENV surveillance in mosquitos in resource-limited regions. Evaluation of the performance of the platform with mosquito samples is currently ongoing.

The Pockit dengue virus reagent set with the field-deployable Pockit combo platform can serve as an easy-to-use tool to aid dengue diagnosis at points of care. It also has the potential to serve as a flexible mobile tool for DENV surveillance in mosquito populations in various settings. Furthermore, as serotyping of DENV currently relies mainly on RT-PCR methods (59), development of RT-iiPCR reagents for DENV serotyping is under way to allow on-site serotype identification.

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J.-J.T. participated in designing and executing the project and analyzing the results; L.-T.L. in executing the experiment and interpreting the results; P.-C.L. and C.-Y.T. in executing the experiments; P.-H.C. in designing and executing the experiments and analyzing the results; Y.-L.T. in designing the experiments and interpreting the results; H.-F.G.C. in revising and approving the article; and P.-Y.A.L. in interpretation of the results and drafting and revising the article.

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