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Sensitivity and Specificity of an All-in-One Cartridge-Based Dengue Real-Time RT-PCR for Point-of-Care Detection and Serotyping of Dengue Virus in Samples From Indonesian Patients

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Background: Dengue is a systemic, viral, mosquito-borne infection that continues to be a major public health issue in endemic regions in tropical and subtropical climates. Accurate tests for rapid diagnosis in point-of-care settings are important to reduce the fatality rates of severe dengue. We evaluated the diagnostic accuracy of the Standard M10 DENV 1-4 system (SD Biosensor, Gyeonggi, Korea), which is a cartridge-based, automated system that integrates nucleic acid extraction, reverse transcription-PCR (RT-PCR) amplification, and detection of dengue virus (DENV) serotypes.

Methods: This was a retrospective diagnostic evaluation study. The index test, Standard M10 DENV 1-4, was evaluated using 320 dengue-positive and 279 dengue-negative archived samples. The reference tests were a combination of Centers for Disease Control and Prevention (CDC) DENV 1-4 real-time RT-PCR, dengue NS1 antigen and IgM antibody detection, and DENV whole-genome sequencing.

Results: The overall sensitivity and specificity of Standard M10 DENV 1-4 were 94% and 100%, respectively. By serotype, the highest sensitivity was 100% for DENV-1, and the lowest was 82% for DENV-4. The overall between the CDC RT-PCR dengue serotyping method and the Standard M10 DENV 1-4 was 95%. Standard M10 DENV 1-4 RT-PCR had comparable sensitivity and specificity to CDC DENV RT-PCR.

Conclusions: Based on its commensurate performance to an established RT-PCR method combined with additional benefits of convenient storage and transport, easy use, and rapid processing, the Standard M10 DENV 1-4 system has potential for DENV detection and serotyping in point-of-care settings.

Key Words: Dengue, Diagnostic accuracy, Point-of-Care testing, Sensitivity, Specificity

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INTRODUCTION

Dengue is a systemic, viral, mosquito-borne infection found mostly in tropical and subtropical climates. It continues to be a major public health issue in endemic regions and has been recently spreading to areas in Europe and the Eastern Mediterranean, leading to 2023 having the largest number of dengue cases reported worldwide [1]. Dengue causes acute illnesses ranging from mild febrile disease to severe or fatal cases with plasma leakage or multiorgan involvement. Fast, easy-to-use, and accurate tests are required for the rapid diagnosis and early management of the disease, particularly in point-of-care settings, for patients to receive prompt medical care to substantially reduce the fatality rates of severe dengue.

In the diagnostic guidelines issued by the WHO, dengue infection is confirmed based on at least one of the following test results: 1) dengue virus (DENV) isolation; 2) a minimum four-fold increase in the anti-dengue IgM or IgG level; 3) DENV antigen detection; and 4) DENV genome detection by reverse transcription (RT-)PCR [2]. However, many of these tests are challenging to implement in point-of-care settings, particularly in peri-urban and rural areas, because of the limited availability and accessibility of advanced equipment, trained personnel, and cold-chain storage.

With technological advancements, molecular diagnostics for infectious diseases are increasingly being developed. Several new dengue diagnostic kits are available on the market; however, their accuracy and performance have not been thoroughly evaluated. DENV is known for its high genome mutation rate [3]; hence, molecular diagnostics must continually be updated to follow the evolution of the virus. DENV genome mutations may occur in the regions targeted by RT-PCR primers, decreasing assay sensitivity. To address this challenge and ensure the accuracy of dengue diagnostics, continuous evaluation of diagnostic assays is important.

The Standard M10 DENV 1-4 system (SD Biosensor, Gyeonggi, Korea) is a multiplex real-time RT-PCR assay that automates and integrates nucleic acid extraction, amplification, and DENV serotype detection. This system utilizes cartridges that contain all the required reagents in lyophilized form and hosts the extraction and RT-PCR processes. The processing time from serum sample loading to result is approximately 1 hr, and the cartridges can be stored at room temperature, providing a fast and convenient kit ideal for point-of-care settings. We aimed to assess the accuracy of this assay on well-characterized dengue-positive and -negative samples.

MATERIALS AND METHODS

Ethics approval

The study protocol for the use of unlinked, anonymized, archived patient samples in this diagnostic evaluation study was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Dr. Cipto Mangunkusumo General Hospital Jakarta, Indonesia (approval No. KET-383/UN2.F1/ETIK/PPM.00.02/2023) and the Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta Indonesia (approval No. KE/FK/0980/EC/2023). The requirement for informed consent was waived by the Ethics Committees, following the International Ethical Guidelines for Health-related Research Involving Humans (CIOMS 2016), because we used anonymized samples.

Study design and sample sources

In this retrospective diagnostic evaluation study, the design and reporting were performed according to the Standards for Reporting of Diagnostic Accuracy Studies guidelines [4]. We used serum samples from patients with confirmed dengue, according to the WHO-Southeast Asia Regional Office 2011 guidelines [2], and dengue-negative samples collected as part of dengue and zoonotic surveillance studies conducted from January 2018 to February 2020 at hospitals and community health centers in various Indonesian cities. In addition, we used archived serum samples collected during the Applying Wolbachia to Eliminate Dengue study conducted in Yogyakarta between 2016 and 2020. All samples were stored in temperature-monitored freezers at −80°C. Samples were selected from the archive based on the availability of serum volume required for the reference and index tests and randomized for inclusion in the study.

Evaluation laboratories

This study was conducted at EXEINS Health Initiative laboratory, Jakarta, Indonesia, and the Diagnostic Laboratory Yayasan Tahija at Universitas Gadjah Mada, Yogyakarta, Indonesia.

Reference standards

In total, 320 samples confirmed to be positive for dengue, and 279 samples confirmed to be negative for dengue were used. Sample characteristics are described in Table 1. DENV RNA was extracted using QIAamp Viral RNA Kits (Qiagen, Hilden, Germany) according to the manufacturer's protocol. As reference standards for virologically confirmed dengue samples, we used a combination of DENV detection and serotyping using CDC

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Table 1. Characteristics of dengue-confirmed samples used in this study

Variable		N (%)
Age	Children (< 18 yrs of age)	211 (66)
	Adults (≥18 yrs of age)	109 (34)
Sex	Female	153 (48)
	Male	167 (52)
Day of fever onset	≤ 3 days	240 (75)
	> 3 days	80 (25)
Serotype	DENV-1	82 (26)
	DENV-2	86 (27)
	DENV-3	67 (20)
	DENV-4	85 (27)
Immunological status*	Primary infection	226 (74)
	Secondary infection	80 (26)

*Fourteen samples had equivocal IgG ELISA results. Abbreviations: DENV, dengue virus.

DENV 1-4 real-time RT-PCR [7, 8], Platelia Dengue NS1 Ag ELISA (Bio-Rad, Hercules, CA, USA), Ultra Dengue IgM Capture ELISA (SD Biosensor), and MinION whole-genome sequencing (Oxford Nanopore Technologies, UK) [9], based on recommendations for dengue diagnosis in the WHO guidelines [2]. The threshold for a positive result in CDC DENV-1-4 real-time RT-PCR was when the amplification curve crossed the threshold line within 37 cycles (< 37 cycle threshold [Ct]), as specified in the CDC protocol [8]. The threshold for a positive result for Platelia Dengue NS1 Ag ELISA was when the optical density ratio of the sample to the cut-off was > 1.0, as per the manufacturer's instructions. The threshold for a positive result for Ultra Dengue IgM Capture ELISA was when the calculated index value was ≥1.1, as per the manufacturer's instructions.

DENV whole-genome sequencing was performed using MinION (Oxford Nanopore Technologies, Oxford, UK), following the manufacturer's instructions. RNA was reverse transcribed using Superscript III Reverse Transcriptase (Invitrogen/Thermo Fisher Scientific, Waltham, MA, USA) and amplified using multiplex PCR with Q5 High-Fidelity DNA Polymerase (New England Biolabs, Hitchin, UK). The sequencing library was prepared using a Native Barcoding Kit 96 v14 (Oxford Nanopore Technologies) and sequenced using a MinION Mk1B with Flow Cell R v10.4.1 (Oxford Nanopore Technologies). The Pod5 data generated using MinKNOW software v23.07.5 (Oxford Nanopore Technologies) were base-called using Guppy v6.5.7 (Oxford Nanopore Technologies) with a super accurate model. Data QC and sequence assembly were performed using BBDuk v38.84 and Minimap2 on Geneious Prime v2023.2.1. DENV genome sequences were aligned using the MUSCLE program in MEGA Software v11. (www.megasoftware.net).

The immunological status of DENV-positive samples was determined based on Ultra Dengue IgG Capture ELISA test results. A positive result, as determined by a calculated index value of ≥1.1 as per the kit manufacturer's instructions, indicated a secondary infection, whereas a negative result (index value of < 0.8) indicated a primary infection. Samples with an index value of 0.8–1.1, interpreted as an equivocal result, were excluded from univariate analysis of the diagnostic parameters and immunological status.

DENV-negative samples were determined based on negative results of CDC DENV-1-4 real-time RT-PCR [7, 8], Platelia Dengue NS1 Ag ELISA (Bio-Rad, USA), and Ultra Dengue IgM Capture ELISA. The DENV-negative panel comprised 50 samples from healthy volunteers, 179 serum samples from patients with fever of non-DENV/unknown etiology, and 50 serum samples from febrile patients confirmed positive for malaria.

Index tests

All samples were tested for DENV 1-4 detection and serotyping using the Standard M10 DENV 1-4 system (Cat. No.: M10-DEN-01) according to the manufacturer's instructions. Standard M10 DENV 1-4 consists of consoles (user interface), modules (cartridge analysis function), and ready-to-use kits (cartridge). The tests were performed under uniform conditions for all samples at both sites by researchers blinded to the demographic, clinical, and reference standard characteristics of the sample. Results were processed through the M10 system, consisting of a touch-screen console that holds the operating system and up to eight cartridge-processing modules per console. The DENV 1-4 test requires 300 μL of serum or plasma, which was dispensed directly into the sample chamber in the cartridge. Subsequently, the cartridge was loaded into the module and processed. The Ct value set by the kit manufacturer was used as a threshold for positive/negative results. In the occurrence of invalid test results, index tests were repeated. When the serum volume was insufficient for retesting, or the retest also produced an invalid result, the sample was excluded from analysis.

Statistical analysis and sample size calculation

R Studio software was used to construct contingency matrices and perform calculations for the diagnostic parameters (sensi-

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Table 2. Comparison of the sensitivities of CDC RT-PCR and Standard M10 DENV 1-4

Serotype	CDC RT-PCR			Standard M10 DENV 1-4		
	Positive (N)	Negative (N)	Sensitivity (%, 95% CI)	Positive (N)	Negative (N)	Sensitivity (%, 95% CI)
DENV-1	78	4	95 (87.3–98.4)	82	0	100 (94.4–100)
DENV-2	80	6	93 (84.9–97.1)	82	4	95 (87.9–98.5)
DENV-3	66	1	99 (90.7–99.9)	66	1	99 (90.7–99.9)
DENV-4	84	1	99 (92.7–99.9)	70	15	82 (72.2–89.5)
Total	308	12	96 (93.4–98.0)	300	20	94 (90.4–96.0)

Abbreviations: DENV, dengue virus; RT-PCR, reverse transcription-PCR; CDC, Centers for Disease Control and Prevention; CI, confidence interval.

tivity and specificity) of the Standard M10 DENV 1-4 test against the reference standards. Z-tests for proportions statistical analysis were also conducted using R Studio software. Statistical significance was set to $P<0.05$. Diagnostic parameters were compared between variables of age, sex, day of fever onset, serotype, and immunological status, using z-tests for proportions, with confidence intervals set at 95%. The sample size was calculated based on the three-step method [5], with a 90% power, 5% precision, and an expected sensitivity of 90% and specificity of 95%, which are slightly more conservative estimates than the sensitivity and specificity of the Standard M10 RT-PCR Assay for Flu/RSV/SARS-CoV-2 determined in a previous study [6]. Using this calculation, a minimum of 177 dengue-positive samples were required for sensitivity evaluation (true-positive dengue cases), and 117 dengue-negative samples were required for specificity evaluation (true-negative dengue cases).

RESULTS

Well-characterized dengue-positive and -negative samples were used in standard tests to evaluate assay sensitivity and specificity. The overall sensitivity and specificity of Standard M10 DENV 1-4 were 94% (95% CI, 90.4–96.0) and 100% (95% CI, 96.9–100), respectively. Regarding infection status, sensitivity was slightly higher in primary infection (96%) than in secondary infection (91%, $P=0.243$). The assay showed a slightly higher sensitivity for the day of fever onset of ≤3 days (95%) than for the day of fever onset of >3 days (91%, $P=0.424$). However, neither of these differences in sensitivity were statistically significant. The progression of M10 sensitivity throughout the day of fever onset is presented in Supplemental Data Fig. S1.

Sensitivity did not significantly differ between age and sex groups. The sensitivity in children (<18 yrs of age) was 94%, whereas that in adults (>19 yrs of age) was 93% ($P=0.738$). The sensitivity in samples from women was 94%, whereas that in samples from men was 93% ($P=0.977$).

Table 3. Agreement between CDC RT-PCR and Standard M10 RT-PCR results

CDC RT-PCR	Standard M10 DENV 1-4	
	Positive	Negative
Positive	288	20
Negative	12	279

Abbreviations: DENV, dengue virus; RT-PCR, reverse transcription-PCR; CDC, Centers for Disease Control and Prevention.

The results of the comparison of the sensitivities of CDC RT-PCR and Standard M10 DENV 1-4, in total and by serotype, are presented in Table 2. CDC RT-PCR and Standard M10 performed similarly, and the difference in overall sensitivity was not significant ($P=0.204$). CDC RT-PCR had the lowest sensitivity for DENV-2 infections, whereas Standard M10 had the lowest sensitivity for DENV-4 infections.

The overall agreement between dengue serotyping with CDC RT-PCR and Standard M10 DENV 1-4 was 95% (Table 3). All samples that tested positive by the two methods also had serotyping agreement. To confirm the identity of samples with discrepant results between the two methods, we sequenced the DENV genomes. Out of 32 samples with discrepant results, 16 were successfully sequenced. Of the 16 sequences, 10 were from CDC RT-PCR-positive but Standard M10-negative samples (one DENV-3 and nine DENV-4), whereas seven were from CDC RT-PR-negative but Standard M10-positive samples (two DENV-1, three DENV-2, and one DENV-4).

The sequences of the primers used in CDC RT-PCR were aligned with the sequences of samples that had negative CDC RT-PCR results (Supplemental Data Fig. S2).

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DISCUSSION

To our knowledge, this was the first study to evaluate the SD Biosensor Standard M10 DENV 1-4 RT-PCR test. Other automated, cartridge-based dengue RT-PCR methods include the POCKIT Dengue Nucleic Acid Analyzer (GeneReach, Taichung, Taiwan) [10, 11], the Convergys POC RT-PCR Dengue Virus Typing Kit (Convergent Technologies, Cölbe, Germany), and the FlashDetect Dengue Assay (Coyote Bioscience, Beijing, China). Given its relative novelty, peer-reviewed studies on this type of molecular diagnostic technology are limited or lacking.

The Standard M10 DENV 1-4 RT-PCR test had comparable sensitivity (95%) and specificity (100%) to the CDC DENV RT-PCR method (96% and 100%, respectively). Disagreements between the two methods may be attributed to differences in primer/probe sequences. The CDC RT-PCR primers and probes were developed to detect various global DENV strains. However, validation was performed mostly using isolates from the American continent and prototype strains from the African and Asia-Pacific regions [7]. Using our panel of Indonesian DENV strains isolated between 2016 and 2020, we found that CDC RT-PCR had the lowest sensitivity for DENV-2. Continued virus evolution from annual DENV outbreaks in endemic areas may lead to divergence from these diagnostic strains and lowered serotype-specific sensitivities. The CDC DENV RT-PCR primers and probes have been found to have mismatches against DENV-2 genomes isolated from the 2017 Burkina Faso dengue outbreak [12]. Diagnostic methods that consider the genetic variations in more contemporary DENV strains must be developed to maintain accurate detection.

The Standard M10 DENV 1-4 test was able to detect 12 DENV infections that the CDC RT-PCR did not, although it missed 20 DENV infections that the CDC RT-PCR was able to detect. Out of the 20 samples that the Standard M10 missed, 15 were DENV-4 infections. DENV-4 is the serotype with the least number of published whole-genome sequences worldwide and, in many countries, is the infecting serotype with the lowest prevalence [13]. In recent years, DENV-4 prevalence in Malaysia [14] and some areas in Indonesia [15] has increased. This highlights the need for continued genomic surveillance in affected areas to develop highly sensitive detection methods for this serotype.

The strengths of this study are the inclusion of a relatively high proportion of DENV-4 and the relatively high number of total samples tested. In addition, our study provides important diagnostic performance data for real-time testing in field conditions, which may help validate the feasibility and reliability of the test in actual point-of-care settings. A limitation of this study is that the samples included were all strains from Indonesia, which were all of Genotype I for DENV-1, Cosmopolitan genotype for DENV-2, Genotype I for DENV-3, and Genotype II for DENV-4. Different genotypes may circulate in other dengue-endemic areas, such as in the African and American continents and the Pacific islands, and the diagnostic kit may perform differently in those regions. The retrospective nature of the study and lack of broad demographic variety in the sample population might limit the applicability and generalizability of the findings across different age groups, genetic backgrounds, and health conditions. Another limitation is that non-DENV flavivirus clinical specimens were not available for specificity testing.

In conclusion, the Standard M10 DENV 1-4 RT-PCR test has comparable sensitivity and specificity to the CDC DENV RT-PCR method. Therefore, and because of the additional benefits of convenient storage and transport, easy use, and rapid processing, the Standard M10 test has the potential for DENV detection and serotyping in point-of-care settings.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2024.0558

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AUTHOR CONTRIBUTIONS

Sasmono RT, Santoso MS, and Arguni E contributed to the conception and design of the study; Santoso MS, Arguni E, Rana B, Adiniko ME, Denis D, Supriyati E, and Indriani C were involved in diagnostic evaluation; Santoso MS, Arguni E, Ahmad RA, Trianty L, and Noviyanti R provided resources and interpreted the results; Santoso MS performed the statistical analysis; Santoso MS, Arguni E, and Sasmono RT drafted the manuscript; and Sasmono RT supervised the study. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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REFERENCES

1. WHO. Dengue and severe dengue. 2024. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue (Updated on Aug 2024).
2. WHO-SEARO. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Revised and expanded. New Delhi, India: World Health Organization, 2011.
3. Twiddy SS, Holmes EC, Rambaut A. Inferring the rate and time-scale of dengue virus evolution. Mol Biol Evol 2003;20:122-9.
4. Cohen JF, Korevaar DA, Altman DG, Bruns DE, Gatsonis CA, Hooft L, et al. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. BMJ Open 2016;6:e012799.
5. Hess AS, Shardell M, Johnson JK, Thom KA, Strassle P, Netzer G, et al. Methods and recommendations for evaluating and reporting a new diagnostic test. Eur J Clin Microbiol Infect Dis 2012;31:2111–6.
6. Domnich A, Bruzzone B, Trombetta CS, De Pace V, Ricucci V, Varesano S, et al. Rapid differential diagnosis of SARS-CoV-2, influenza A/B and respiratory syncytial viruses: validation of a novel RT-PCR assay. J Clin Virol 2023;161:105402.
7. Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, et al. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. PLoS Negl Trop Dis 2013;7:e2311.
8. Centers for Disease Control and Prevention. CDC DENV-1-4 Real-Time RT-PCR assay for detection and serotype identification of dengue virus. 2013. https://www.cdc.gov/dengue/resources/cdc-usapackageinsert.pdf (Updated on Jun 2023).
9. Stubbs SCB, Blacklaws BA, Yohan B, Yudhaputri FA, Hayati RF, Schwem B, et al. Assessment of a multiplex PCR and Nanopore-based method for dengue virus sequencing in Indonesia. Virol J 2020;17:24.
10. Stittleburg V, Rojas A, Cardozo F, Muñoz FM, Asturias EJ, Olson D, et al. Dengue virus and yellow fever virus detection using reverse transcription-insulated isothermal PCR and comparison with real-time RT-PCR. Am J Trop Med Hyg 2020;103:157–9.
11. Tsai JJ, Liu WL, Lin PC, Huang BY, Tsai CY, Lee PA, et al. A fully automated sample-to-answer PCR system for easy and sensitive detection of dengue virus in human serum and mosquitos. PLoS One 2019;14:e0218139.
12. Letizia AG, Pratt CB, Wiley MR, Fox AT, Mosore M, Agbodzi B, et al. Retrospective Genomic Characterization of a 2017 Dengue Virus Outbreak, Burkina Faso. Emerg Infect Dis 2022;28:1198–210.
13. Tsheten T, Gray DJ, Clements ACA, Wangdi K. Epidemiology and challenges of dengue surveillance in the WHO South-East Asia Region. Trans R Soc Trop Med Hyg 2021;115:583–99.
14. Suppiah J, Ali EZ, Mohd Khalid MKN, Mohd Ghazali S, Tee KK, Zulkifli MMS, et al. Resurgence of dengue virus serotype 4 in Malaysia: a comprehensive clinicodemographic and genomic analysis. Trop Med Infect Dis 2023;8:409.
15. Aryati A, Wrahatnala BJ, Yohan B, Fanny M, Hakim FKN, Sunari EP, et al. Dengue virus serotype 4 is responsible for the outbreak of dengue in East Java City of Jember, Indonesia. Viruses 2020;12:913.

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