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Design and evaluation of degenerate primers targeting the NS3 gene for detection of dengue virus by RT-PCR

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ABSTRACT

Background: Dengue fever, caused by the dengue virus, is hyper-endemic in Indonesia. Since no protective vaccine or specific treatments are available, accurate diagnosis is crucial for the early implementation of preventive measures to limit dengue transmission and reduce economic losses. Various diagnostic techniques have been developed, including reverse transcriptase-polymerase chain reaction (RT-PCR) for detecting viral nucleic acids using specific primers.

Objective: This study aimed to design and evaluate the effectiveness of a new primer targeting the NS3 gene of the dengue virus for molecular detection in clinical samples.

Methods: A cross-sectional molecular study was conducted in Banjarnegara, Indonesia. Serum samples were collected from dengue-suspected patients attending hospitals in the city. The diagnosis was initially performed using dengue NS1 antigen and IgG/IgM antibody detection. Dengue virus (DENV) serotyping was conducted using Simplexa Dengue real-time RT-PCR with a newly designed NS3 gene primer. The effectiveness of the new primer was assessed by comparing its performance with a commercial primer.

Results: The primers, DenVNS3F (5'-CGAGTAGGAATGGGWGARGCAGC-3') and DenVNS3R (5'-CTGTCCAGTGAGCRYGGTCTT-3') were able to detect the NS3 gene of the dengue virus. However, the level of agreement in detecting the dengue virus compared to the commercial primer showed a moderate agreement (k = 0.60) with a low confidence level.

Conclusion: The newly designed primers DenVNS3F and DenVNS3R are capable of detecting the NS3 gene. However, the primers may require further optimization to achieve a higher level of accuracy and confidence in detecting the dengue virus, and additional validation through sequencing is necessary to confirm the specificity of the amplified product.

Keywords: Dengue fever, Dengue virus, NS3 gene, primer, RT-PCR

Introduction

Dengue hemorrhagic fever (DHF) is an infection caused by the dengue virus (DENV). This infection is estimated to reach 390 million cases annually worldwide, with the highest prevalence of cases found in Southeast Asia, America, and the West Pacific [1]. The World Health Organization (WHO) reports Indonesia has the highest number of dengue fever cases in Southeast Asia [2]. In 2021, dengue fever was reported in 474 (92.2%) districts/cities in Indonesia. The number of dengue fever cases in 2021 was reported as 73,518 cases with 705 deaths [3].

Dengue virus is a single-stranded RNA molecule that belongs to family Flaviridae, genus Flavivirus. Dengue virus is a type of arbovirus that causes acute febrile illness in humans. The clinical symptoms of dengue infection are difficult to differentiate them from other acute fever infections. These symptoms can manifest into more severe symptoms and even death if not diagnosed correctly [4].

Molecular diagnostics have been widely developed and applied to detect dengue virus. Molecular techniques offer faster, more sensitive, and specific detection to differentiate various DENV serotypes and provide epidemiological data on the distribution of virus serotypes [5]. The molecular method used to detect RNA viruses such as the dengue virus is the Reverse Transcription Polymerase Chain Reaction (RT-PCR) method [6]. RT-PCR is a technique for duplicating cDNA fragments from RNA *in vitro* using a pair of specific primers. One component that plays an important role in the PCR amplification process is the primer. The primer design process is needed to obtain primers with good criteria so that they can detect target genes effectively [7].

Primer design to detect dengue virus from the nonstructural protein 3 (NS3) gene has not been reported. The NS3 protein is a nonstructural protein with important enzymatic functions essential for virus replication. The NS3 gene sequence encodes amino acid residues that are highly conserved within the dengue virus genome [8]. The NS3 protein is approximately 69kDa in size, consisting of a serine protease domain in the N-terminal and three enzymatic domains in the C-terminal (NTPase, RNA helicase, and RTPase) [9]. Due to these conserved features, the NS3 gene sequence can potentially be used as a specific universal PCR primer target for detecting dengue virus.

Therefore, research is needed to design primers to detect the dengue virus from the NS3 gene and its application in dengue fever patient samples using RT-PCR. This research aims to determine a potential PCR primer sequence for detecting the NS3 gene in dengue virus and to determine the effectiveness of the designed primer on dengue clinical samples using the RT-PCR method.

Methods

Study design and sample collection

This study was conducted using a survey method at the Genetics and Molecular Laboratory, Faculty of Biology, Jenderal Soedirman University, and the Balitbangkes Banjarnegara, from July 2023 to April 2024. The research received ethical approval from the Ethics Committee of Health Research Badan Riset Inovasi Nasional (BRIN) and was conducted in accordance with the medical research regulations of the Ministry of Health, Indonesia.

PCR primer design for dengue virus detection

For primer design, complete genome sequences of DENV serotypes 1-4 were obtained from the NCBI GenBank database. The NS3 gene was identified from these sequences, and a consensus sequence was generated through multiple sequence alignment using the ClustalW algorithm in BioEdit software. This alignment enabled the identification of conserved regions within the NS3 gene that could serve as targets for universal primer design. Using Primer3web version 4.1.0, primers were designed based on these conserved regions, with their quality evaluated using IDT OligoAnalyzer for parameters such as melting temperature, GC content, and potential secondary structures (hairpin, self-dimer, and cross-dimer). Primer specificity was assessed through BLASTn analysis against the NCBI database to confirm selective binding to dengue virus sequences.

To enhance detection sensitivity across all serotypes, degenerate primers were developed by aligning the selected forward and reverse primers with the NS3 gene consensus sequence. Nucleotide positions showing variability across serotypes were modified using the IUPAC nucleotide code to accommodate these differences. The designed degenerate primers were validated in silico using SnapGene software to simulate the amplification process, including primer binding sites and amplicon size prediction. Primers meeting the specificity and target gene compatibility criteria were subsequently synthesized in Singapore for experimental validation.

RNA extraction from clinical samples

Total RNA was extracted from blood samples (in tubes containing anticoagulants) of suspected dengue fever patients using the Total Mini RNA Kit (Blood/Cultured Cell) (Geneaid) following the

manufacturer's protocol. Briefly, 300 µL of each sample was mixed with 1 mL RBC lysis buffer, vortexed, and incubated on ice for 10 minutes with intermittent vortexing. After centrifugation at 3,000 rpm for 5 minutes, the supernatant was discarded, and the pellet was resuspended in 400 µL RB buffer with 4 µL β-mercaptoethanol. The mixture was centrifuged at 3,000 rpm for 5 minutes, and the supernatant was discarded. The pellet was resuspended in 400 µL RB buffer with 4 µL β-mercaptoethanol, incubated at room temperature for 5 minutes, and mixed with 400 µL ethanol. The mixture was transferred to an RB column in a 2 mL collection tube and centrifuged at 14,000 rpm for 1 minute. The column was washed sequentially with W1 buffer and wash buffer, each followed by centrifugation at 14,000 rpm. The purified RNA was eluted by adding 50 µL of RNase-free water to the column matrix and centrifuging at 14,000 rpm for 1 minute.

Screening of clinical samples using real-time RT-PCR [10]

Blood samples were screened using the Novaplex™ Tropical Fever Virus Assay kit (Seegene) following the manufacturer's protocol. RNA extraction was performed as described above. The RT-qPCR master mix (total volume 15 µL) consisted of 5 µL TFV MOM, 5 µL EM5, and 5 µL EM5 buffer, homogenized by vortexing and spun down. A 5 µL aliquot of RNA was added to the qPCR tube containing the master mix, and the mixture was subjected to real-time PCR. The thermal cycling program included cDNA synthesis at 50°C for 20 minutes, pre-denaturation at 95°C for 15 minutes, followed by 45 cycles of amplification (denaturation at 95°C for 10 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 10 seconds). Positive and negative DENV controls were included as references for accurate interpretation.

Validation of designed primers using conventional RT-PCR

To evaluate the designed primers, RNA samples confirmed as positive or negative for DENV through real-time RT-PCR were tested using conventional RT-PCR. The MyTaq™ One-Step PCR Kit (Bioline) was used with a reaction volume of 25 µL, containing 12.5 µL 2X MyTaq One-Step Mix, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 0.25 µL reverse transcriptase enzyme, 0.5 µL RNase inhibitor (10 U/µL), 7.75 µL DEPC-treated water, and 2 µL RNA template. The reaction was vortexed, spun down, and subjected to PCR amplification in a thermocycler with the following conditions: cDNA synthesis at 60°C for 1 hour, pre-denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 59.5°C for 1 minute, extension at 68°C for 2 minutes, and a final extension at 68°C for 5 minutes.

Electrophoresis and visualization of RT-PCR products

RT-PCR products were analyzed using 1.2% agarose gel electrophoresis and visualized under a UV transilluminator.

Data analysis

Data analysis combined descriptive and statistical approaches. In silico primer design results were analyzed descriptively using software including BioEdit, Primer3, IDT OligoAnalyzer, BLASTn, and SnapGene. The effectiveness of the designed primers was assessed by comparing their performance to commercial real-time RT-PCR primers in detecting DENV in clinical samples. Statistical analysis was performed using the Cohen's Kappa test in SPSS software to evaluate the level of agreement between the two methods, with significance determined at $p < 0.05$.

Results

PCR primer design for dengue virus

We used the NS3 gene sequence from the National Center for Biotechnology Information (NCBI) for primer design. Our search of the NCBI database yielded 5,040 entries for complete DENV 1-4 genome sequences ranging from 10,605-10,736

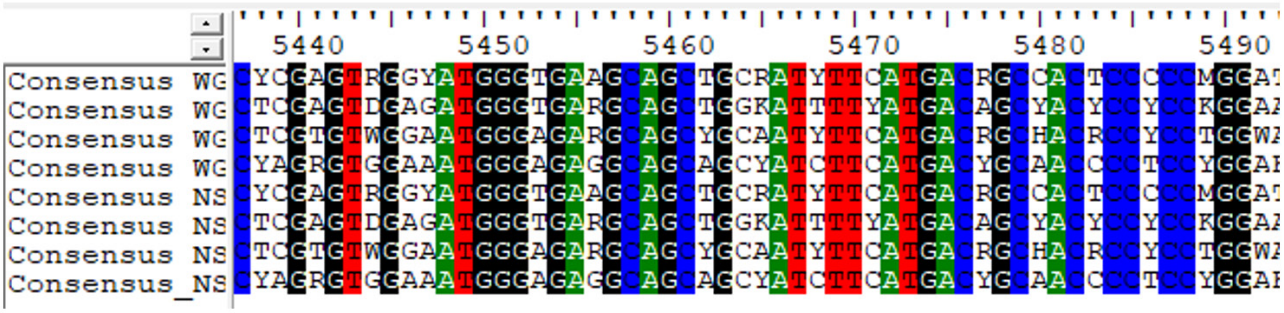

Figure 1. Multiple sequence alignment of the DENV NS3 gene showing conserved regions across all four serotypes

Table 1. Sequences and characteristics of NS3 gene primers designed using Primer3

No.	Primer code	Nucleotide sequences (5→3)	Base lenght	GC (%)	Tm (°C)	Product size
1.	NS3F470	AGAATGGTGGCTACGTCAGTGG	22	54.55	63.13	645
	NS3R1114	TGTCATTCCCGGCTTTGATGCT	22	50.00	63.45	
2.	NS3F910	CGTGTAGGAATGGGAGAAGCAGC	23	56.52	64.22	446
	NS3R1355	ACGGTGACTGGCATTGGTCC	20	60.00	63.84	
3.	NS3F1334	CAGGACCGATGCCAGTGACT	20	60.00	63.01	138
	NS3R1471	CCGTCCAGTGAGCGTGATCTT	21	57.14	63.24	
4.	NS3F1516	ATCCCAGCCCTCTTCGAGCC	20	65.00	65.39	227
	NS3R1742	TCCACGTCCATGTTCTCCTCCA	22	54.55	64.06	

Table 2. Analysis of the degenerate primer properties

Criteria	Forward primer (DenVNS3F910)	Reverse primer (DenVNS3R1471)
Primer length	23	21
GC content (%)	58.7	57.1
Melting temperature (°C)	59.4-61.6	57.0-61.9
Hairpin (kcal/mol)	0.55	0.62
Self-dimer (kcal/mol)	-3.61	-7.75
Cross-dimer (kcal/mol)	-5.02	-5.02

bp. For this study, we selected 44 complete genome sequences representing all four DENV serotypes from various countries between 2019-2022.

NCBI identification revealed that the DENV genome encodes three structural proteins: capsid (C), pre-membrane/membrane (prM/M), and envelope (E), as well as seven nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. Multiple sequence alignment demonstrated that NS3 gene contains well-conserved regions across all four serotypes (Figure 1).

The primer design results using the Primer3 program yielded four primer candidate pairs, each consisting of one forward (NS3F) and one reverse primer (NS3R) (Table 1). Based on primer properties and quality analysis, we selected NS3F910 and NS3R1471 as the optimal candidates. Specificity analysis revealed that both primers exhibited 100% query coverage, low E-values, and 86-100% sequence identity against dengue virus sequences.

To enhance detection sensitivity and specificity across all dengue serotypes [11], we designed degenerate versions of the selected primers. The resulting forward primer (DenVNS3F: 5'-CGAGTAGGAATGGGWGARGCAGC-3') and reverse primer (DenVNS3R: 5'-CTGTCCAGTGAGCRYGGTCTT-3')

Table 3. Comparison of dengue clinical sample testing results using commercial real-time RT-PCR and conventional RT-PCR with our designed primers

Sample code	Type	Commercial primer		NS3 gene primer	Cohen's Kappa test
		DENV	Ct value		
NC	Negative control	-	N/D		
1	Sample	-	N/D	+	
2	Sample	+	33.30	+	
3	Sample	+	39.06	+	
4	Sample	+	24.96	+	
5	Sample	+	25.65	+	
6	Sample	+	32.51	-	k = 0.600 (p = 0.058)
7	Sample	-	N/D	-	
8	Sample	-	N/D	-	
9	Sample	-	N/D	-	
10	Sample	-	N/D	-	
PC	Positive control	+	21.16		

Note: N/D = not detected (no amplification curve observed in real-time PCR)

meet standard primer design criteria with lengths between 18-30 bp, GC content of 40-60%, and melting temperatures between 50-65°C. Although secondary structures such as hairpins and dimers may form, their low ΔG values suggest minimal impact on amplification efficiency (Table 2).

In-silico PCR testing confirmed that our degenerate primers could amplify the NS3 gene from all four DENV serotypes (Figure 2). Using complete genome sequences as templates, the predicted RT-PCR product for each serotype was 562 bp.

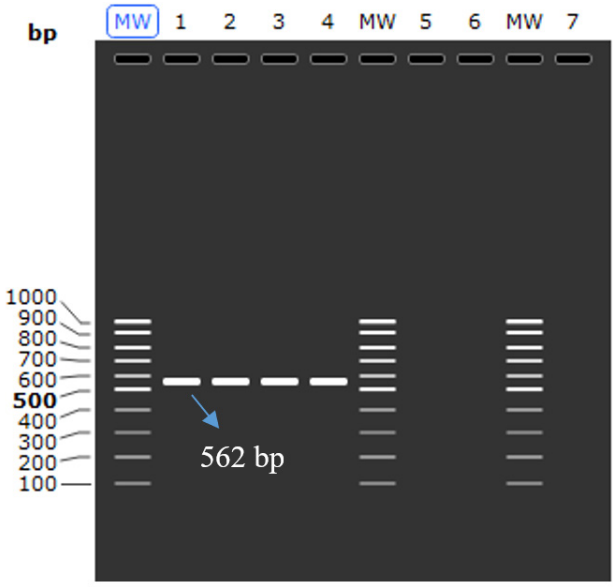

Figure 2. In-silico visualization of RT-PCR products (562 bp) on a 1.2% agarose gel using SnapGene software, demonstrating expected amplification from all four DENV serotypes

Testing primer effectiveness on clinical samples

We evaluated our designed primers using clinical samples that had been previously screened with commercial real-time RT-PCR primers. In the commercial screening, five samples tested positive (samples 2, 3, 4, 5, and 6) and five tested negative (samples 1, 7, 8, 9, and 10) (Table 3).

We then tested these same samples using conventional RT-PCR with our designed primers. The RT-PCR products were visualized on 1.2% agarose gel under a UV transilluminator. Our primers successfully detected the Dengue virus NS3 gene, with five samples testing positive (samples 1-5) and five negative (samples 6-10). Positive samples displayed a clear 562 bp amplicon, while negative samples showed no visible bands (Figure 3).

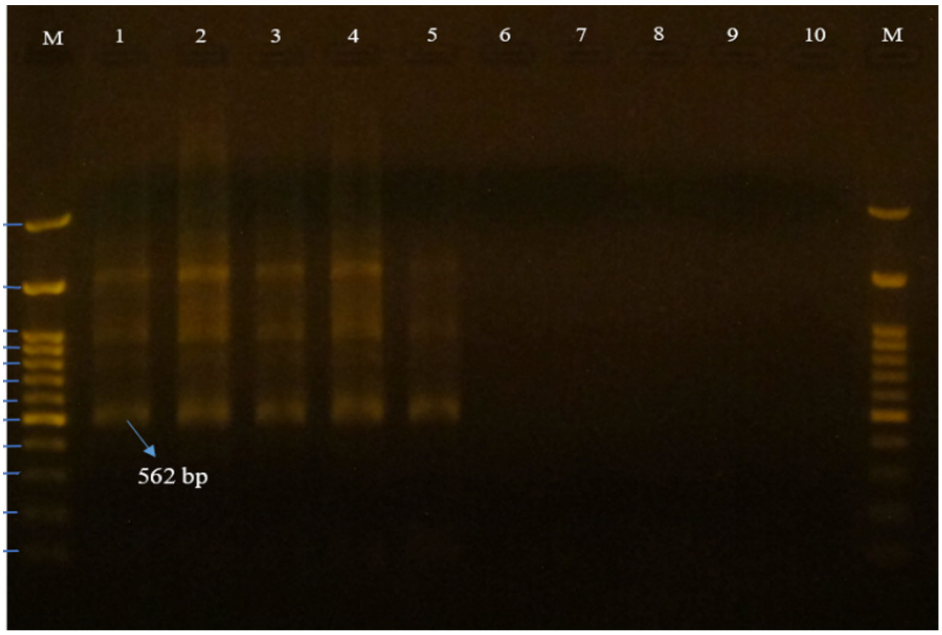

Figure 3. Visualization of RT-PCR products from clinical samples on 1.2% agarose gel, showing positive samples (1-5) with the expected 562 bp band and negative samples (6-10) with no amplification

To assess the agreement between our conventional RT-PCR method and the commercial real-time RT-PCR screening, we performed Cohen's Kappa statistical analysis. The resulting Kappa value was 0.60 ($p = 0.058$) (Table 3), indicating moderate agreement between the two methods. According to established interpretation criteria, a value of $0.40 < k \leq 0.60$ represents moderate concordance in detecting Dengue virus [12].

Discussion

In this study, we successfully designed degenerate primers targeting the NS3 gene of the dengue virus for molecular detection. The designed primers, DenVNS3F (5'-CGAGTAGGAATGGGWGARGCAGC-3') and DenVNS3R (5'-CTGTCCAGTGAGCRYGGTCTT-3'), demonstrated the ability to detect the NS3 gene across all four dengue serotypes, as confirmed through in-silico PCR analysis. When evaluated against clinical samples, our primers showed moderate agreement ($k = 0.60$) with commercial real-time RT-PCR primers, indicating promising potential for dengue virus detection. The ability to amplify a specific 562 bp product from clinical samples provides preliminary evidence for the utility of these primers in laboratory settings, though further optimization is clearly needed to enhance their performance.

Several real-time PCR-based methods for detecting DENV have targeted various genomic regions, including the 3'UTR, NS5, core, and envelope genes [10]. However, our strategy deliberately focused on the NS3 gene due to its highly conserved nature across all four dengue serotypes. The NS3 protein is a multifunctional protein with critical roles in viral replication, containing a serine protease domain at the N-terminal for polyprotein processing and three enzyme domains (NTPase, RNA helicase, and RTPase) at the C-terminal for RNA replication and capping [13]. This functional importance has likely contributed to the evolutionary conservation of NS3, making it an ideal target for universal dengue detection.

The conserved regions within NS3 provide advantages for universal detection compared to more variable regions like the envelope gene, which often requires serotype-specific primers. By targeting these conserved regions, our primers can potentially detect all serotypes with a single PCR reaction, offering a more efficient screening approach. A group-specific PCR targeting the NS3 gene would be a valuable tool for initial screening,

with positive samples subsequently subjected to serotyping.

Our degenerate primer strategy incorporated nucleotide variations at specific positions based on multiple sequence alignment of the NS3 gene across serotypes. Degenerate primers have variations in the bases of the same nucleotide sequence [11], enhancing detection sensitivity across all serotypes without sacrificing specificity. These primers were created by aligning the forward and reverse primers with the consensus nucleotide sequence of the target gene, with several bases whose sequence did not match the conserved region in the NS3 gene consensus sequence having their notations replaced according to the IUPAC nucleotide code [14]. This approach provides advantages over non-degenerate primers that might miss variants containing polymorphisms at critical binding sites.

The results of primer length analysis obtained from forward and reverse primers are between 20-23 base pairs. The ideal primer length is around 18-30 base pairs. A primer length of less than 18 bases will cause mispriming, while a primer length of more than 30 bases will cause a hybridization with another primer [15]. The results of the GC content analysis obtained from the forward and reverse primers are from 50-65%. A good primer has a GC percentage of around 40-60% [16]. A GC content that is too high causes the double strands in the primer and template DNA to be challenging to separate, while a GC content that is too low causes the primer to be unable to attach effectively to the target [15]. The melting temperature (Tm) obtained ranged from 63-65°C. The optimal Tm value ranges from 50-65°C, with the difference in Tm between the forward and reverse primers being no more than 5°C [17]. A Tm value that is too high makes it difficult for the primer and DNA template to bind to each other, while a Tm value that is too low causes the sensitivity of the primer. A difference in Tm of more than 5°C will cause a decrease in the efficiency of the amplification process [18].

Primer specificity analysis functions to compare the primer sequence with a collection of sequences from various organisms contained in the NCBI database. Primer specificity must be considered to determine the suitability of the primer to the desired target gene [13]. The high level of similarity of the candidate primers to the dengue virus indicates that the primers are specific enough to attach to the dengue virus sequences from the database. A query cover value of 100% means that all primary bases are the same as those contained in a particular base sequence in the dengue virus sequence from the database. The E-value is the mismatch between the primer and the sequence from the database. A lower E-value indicates a lower level of mismatch between the primer and the target sequence. A high percentage identity value suggests that the primer base sequence is similar to the dengue virus sequence from the database [19].

Testing the designed primer was conducted using blood samples from patients suspected of dengue collected from several hospitals in Banjarnegara, Central Java. The real-time RT-PCR screening stage is used to confirm positive and negative dengue virus sample results. The RT-PCR method is the gold standard for detecting the Dengue virus due to its higher sensitivity and ability to differentiate DENV serotypes [20]. The real-time RT-PCR method is commonly employed for dengue virus detection because it can quantify RNA molecules produced through the Ct value. A low Ct value indicates a high viral load level, while a high Ct value indicates a low one. Ct values can also be influenced by the kit used, the skill of the laboratory technician, and the method or timing of sample collection. Patients with mild symptoms have lower CT values than patients with severe symptoms. This could be due to samples collected before symptom onset having high viral load levels, resulting in lower CT values [21].

The results of amplifying the NS3 gene using degenerate primers show multiple bands, suggests non-specific amplification. A good RT-PCR product

typically yields a single band of the expected size [22]. Multiple bands can occur when the designed primer has low sensitivity, leading to amplification of non-specific regions. Another factor contributing to the occurrence of various bands in RT-PCR products is suboptimal PCR conditions. High primer concentration, high template DNA concentration, and suboptimal annealing temperature can cause the primer to bind to non-specific sites or amplify sequences other than the target gene, resulting in multiple bands [23,24].

The moderate agreement (k = 0.60) between our conventional RT-PCR with designed primers and commercial real-time RT-PCR requires careful interpretation. According to McHugh, the acceptable level of conformity is at least 80%, and a discrepancy rate of more than 40% would be a severe problem for a clinical laboratory, requiring further evaluation [12]. This suggests that while our primers show promise, they require further refinement before implementation in diagnostic settings.

Analysis of the discrepancies revealed intriguing patterns, particularly with samples 1 and 6. Sample 1 tested negative with commercial primers but positive with our designed primers, while sample 6 showed the opposite pattern (positive with commercial primers, negative with our designed primers). These contradictory results could stem from several factors, including false positive and false negative results in the RT-PCR method.

False negative results can be caused by several factors, including poor sample quality, samples having a low level of virulence, the presence of inhibitors, and mutations in the target area [25]. The whole blood samples used in this study were stored for approximately 5-8 months after sampling, which may have reduced RNA quality. Storing samples for an extended period at inappropriate temperatures can reduce the quality of RNA obtained [26]. Fresh blood samples generally yield better RNA quality [27].

Blood samples can contain various natural inhibitory compounds, such as hemoglobin and immunoglobulin G, which can interfere with the PCR process. Hemoglobin can affect DNA polymerase activity, thereby disrupting the amplification process, while immunoglobulin G will bind to genomic DNA, potentially inhibiting the DNA polymerization process [26].

False positive results can occur due to several factors, including sample contamination, non-optimal PCR conditions, and low primer specificity [28]. Sample contamination, both during the extraction and PCR mixing processes, is the leading cause of false positive results [29]. RNA contamination with genomic DNA can cause the formation of DNA amplification bands in negative samples, even without the reverse transcriptase step [30].

Our study faced several limitations that warrant consideration when interpreting the results. The relatively small sample size (10 clinical samples) limits the statistical power of our findings and prevents comprehensive assessment of primer performance across diverse patient populations and viral loads. A larger sample set would provide more robust evidence for primer efficacy and better characterize sensitivity and specificity parameters. Additionally, our study was geographically limited to samples from Banjarnegara, Central Java, Indonesia, potentially restricting the diversity of dengue virus strains evaluated. Geographic variation in dengue virus sequences could affect primer performance in other regions.

Several strategies could enhance the performance of our designed primers. Modification of primer sequences based on more comprehensive sequence alignments, potentially incorporating more recent isolates or geographically diverse strains, could improve detection across variants. Additionally, further refinement of the degenerate positions might balance inclusivity and specificity more effectively.

PCR condition optimization presents significant opportunities for improvement. Systematic evaluation of annealing temperatures could enhance specificity and reduce non-specific amplification. Annealing temperature significantly affects the PCR amplification process [28]. Suboptimal annealing temperature can cause the primer to amplify sequences other than the target gene,

producing non-specific bands. Similarly, optimization of cycle number, primer concentration, and $MgCl_2$ concentration could enhance both sensitivity and specificity. The PCR amplification process for 38 cycles showed the presence of a band in the negative control, while amplification for 28 cycles showed the absence of a band in the negative control [30], suggesting that optimizing cycle number could reduce false positives.

Strategies to reduce secondary structure formation might improve amplification efficiency. The primer secondary structures analyzed in our study include hairpin, self-dimer, and cross-dimer. The ΔG and Tm values influence the hairpin structure. A low hairpin Tm value far below the annealing temperature can prevent hairpin structure formation [31]. The hairpin structure can be broken down if it has a ΔG value greater than -3 kcal/mol [32]. For self-dimer and cross-dimer structures, the ΔG value required for breakdown is greater than -6 kcal/mol [17]. While our hairpin structures had acceptable ΔG values, the self-dimer and cross-dimer ΔG values were somewhat below ideal thresholds, potentially affecting primer performance.

Conclusion

Our study successfully developed degenerate primers (DenVNS3F: 5'-CGAGTAGGAATGGGWGAR GCAGC-3' and DenVNS3R: 5'-CTGTCCAGTGAGCRYGG TCTT-3') targeting the conserved NS3 gene region of dengue virus, demonstrating the ability to detect the virus across all four serotypes. The moderate agreement (k = 0.60) with commercial primers indicates promising potential, though further optimization is needed for clinical implementation.

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Author contributions

SA: Conceptualization, Methodology, Writing – Original Draft. RCS, SA: Data Curation, Formal Analysis, Visualization. RCS, DP, TR: Investigation, Resources, Validation. SA, RCS, DP, TR: Supervision, Writing – Review & Editing.

Declaration of interest

The authors have no conflicts of interest to declare.

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References

1. Nagar PK, Savargaonkar D, Anvikar AR. Detection of Dengue Virus-Specific IgM and IgG Antibodies through Peptide Sequences of Envelope and NS1 Proteins for Serological Identification. J Immunol Res. 2020;2020: 1-8. https://doi.org/10.1155/2020/1820325
2. Candra K, Asnia Z, Asriati A. Evaluasi Pelaksanaan Program Pencegahan dan Penanggulangan Penyakit Demam Berdarah Dengue di Kota Kendari. Jurnal Ilmiah Obsgin. 2022;14: 226-241. doi:10.36089/job.v14i3.835
3. Kementerian Kesehatan RI. Profil Kesehatan Indonesia Tahun 2021. Jakarta: Kementrian Kesehatan Republik Indonesia; 2021.
4. Rahman NAA, Djamil FM, Balasubramaniam VR, Hassan SS, Yap WB. Dengue Virus Non-structural (NS) 1 Gene as A Molecular Marker for Early Detection of in vitro Dengue Virus Infection. Sains Malays. 2021;50: 3035-3043. https://doi.org/10.17576/jsm-2021-5010-16
5. Sasmono RT, Aryati A, Wardhani P, Yohan B, Trimarsanto H, Fahri S, et al. Performance of Simplexa dengue molecular assay compared to conventional and SYBR Green RT-PCR for detection of dengue infection in Indonesia. PLoS One. 2014;9. https://doi.org/10.1371/journal.pone.0103815
6. Prayitno PA, Kusmintarsih ES, Wahyono DJ. Deteksi molekuler virus Dengue serotipe 3 pada nyamuk aedes aegypti di wilayah Purwokerto Timur. BioEksakta Jurnal Ilmiah Biologi Unsoed. 2020;2: 215. https://doi.org/10.20884/1.bioe.2020.2.2.1826
7. Messe Y, Budiarsa IM, Laenggeng AH. Desain Primer Polymerase Chain Reaction (PCR) secara In Silico

untuk Amplifikasi Gen gyrA Extensively Drug Resistant Tuberculosis (XDR-TB). jurnalfkipuntad.com. 2020. doi:10.22487/jbse.v8i2.1169

8. Ayub A, Ashfaq UA, Idrees S, Haque A. Global Consensus Sequence Development and Analysis of Dengue NS3 conserved domains. Biores Open Access. 2013;2: 392-396. https://doi.org/10.1089/biores.2013.0022
9. Costa SM, Yorio AP, Ajs G, Vidale MM, Ecb C, Mohana-Borges R, et al. Induction of a protective response in mice by the dengue virus NS3 protein using DNA vaccines. PLoS One. 2011;6: e25685. https://doi.org/10.1371/journal.pone.0025685
10. Gurukumar K, Priyadarshini D, Patil J, Bhagat A, Singh A, Shah P, et al. Development of real time PCR for detection and quantitation of Dengue Viruses. Virol J. 2009;6. https://doi.org/10.1186/1743-422X-6-10
11. Johnson BW, Russell BJ, Lanciotti RS. Serotype-Specific detection of dengue viruses in a fourplex Real-Time Reverse Transcriptase PCR assay. J Clin Microbiol. 2005;43: 4977-4983. https://doi.org/10.1128/JCM.43.10.4977-4983.2005
12. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb). 2012;22: 276-282. https://doi.org/10.11613/BM.2012.031
13. Sariyanti M, Dewi BE, Sudiro TM. Pengklonaan Gen Nonstruktural 3 Virus Dengue Serotipe 2 (NS3 DENV-2) Strain Indonesia ke dalam Plasmid UMVC4A. Medical Journal of the Christian University of Indonesia. 2014;30: 2-7.
14. Sasongko ND, Aziz S, Sulisyono Y. Degenerated primers for the KCS enzyme gene encoding erucic acid content in winged bean seeds. IOP Conference Series Earth and Environmental Science 2020;550. 2020. https://doi.org/10.1088/1755-1315/550/1/012032
15. Purwakasih DB, Achyar A. Desain Primer dan PCR In Silico untuk Deteksi Shigella sp. pada Sampel Air Minum Isi Ulang. Serambi Biologi. 2021;6: 1-6.
16. Nugraha R, Dewi PS, Nurilmala M. Evaluasi Primer Gen COI sebagai Biomarker Ketertelusuran Ikan menggunakan Bioinformatika. J Pengolah Has Perikan Indones. 2022;25: 67-79. https://doi.org/10.17844/jphpi.v25i1.36501
17. Fakih TM, Wijaya S, Priani SE. Desain Primer Gen 12S sRNA dari DNA Mitrokondria Babi (Sus scrofa) secara In Silico sebagai Kandidat Primer dalam Analisis Molekuler Kehalalan Produk. Jurnal Sains Farmasi & Klinis. 2021;8: 316. https://doi.org/10.25077/jsfk.8.3.316-322.2021
18. Yunita R, Rosadi FN, Jamsari N, Azizah A. Optimization of annealing temperature for amplification of the exon one region of the HPPD gene in HA1 accession sunflowers. IOP Conference Series Earth and Environmental Science 2023;1160. 2023. https://doi.org/10.1088/1755-1315/1160/1/012002
19. Trianom B, Arwiyanto T, Joko T. Perancangan Primer Spesifik Subspesies Berbasis Gen Endoglukanase untuk Deteksi Ralstonia syzygii subsp. syzygii. Jurnal Perlindungan Tanaman Indonesia. 2018;22: 124. https://doi.org/10.22146/jpti.32217
20. Kesuma S. Uji Diagnosis NS 1. IgG dan IgM Dengue Metode Immunokromatografi dan Elisa. Jurnal Analis Laboratorium Medik. 2022;7: 72-85. https://doi.org/10.51544/jalm.v7i2.3374
21. Tonglolangi OS, Pratiningrum M, Yadi H. Gejala Klinis dengan Nilai CT pada Pemeriksaan Realtime PCR SARS-CoV-2. Jurnal Kedokteran. 2021;8: 89-99. https://doi.org/10.30872/j.ked.mulawarman.v8i3.6559
22. Yuenleni. Langkah-Langkah Optimasi PCR. Indonesian Journal of Laboratory. 2019;1: 51-56. https://doi.org/10.22146/ijl.v1i3.48723
23. Jannah M. Optimalisasi Kondisi PCR untuk Amplifikasi Sekuen Gen HBB. Jurnal Pendidikan Biologi. 2023;12: 36-42. https://doi.org/10.33627/oz.v12i1.1057
24. Setyawati R, Zubaidah S. Optimasi Konsentrasi Primer dan Suhu Annealing dalam Mendeteksi Gen Leptin pada Sapi Peranakan Ongole (PO) Menggunakan Polymerase Chain Reaction (PCR). Indonesian Journal of Laboratory. 2021;4: 36-40. https://doi.org/10.22146/ijl.v4i1.65550
25. Mollaei HR, Afshar AA, Neyestanaki DK, Fazlalipour M, Aflatoonian B. Comparison Five Primer Sets from Different Genome Region Of COVID-19 for Detection of Virus Infection by Conventional RT-PCR. Iran J Microbiol. 2020;12: 185-193. https://doi.org/10.18502/ijm.v12i3.3234
26. Ardiyanto D, Wuryastuty H, Wasito R. Deteksi Brucella abortus dari Sampel Darah-Utuh dengan Uji Polymerase Chain Reaction Tanpa Ekstraksi DNA. Jurnal Sain Veteriner. 2020;38: 222. doi:10.22146/jsv.53506 https://doi.org/10.22146/jsv.53506
27. Vilahur N, Baccarelli AA, Bustamante M, Agramunt S, Byun H-M, Fernandez MF, et al. Storage conditions and stability of global DNA methylation in placental tissue. Epigenomics. 2013;5: 341-348. https://doi.org/10.2217/epi.13.29
28. Damo NY, Porotu?o JP, Rambert GI, Disease RFDC. (COVID-19) dengan Pemeriksaan Laboratorium Mikrobiologi Klinik. Jurnal e-Biomedik. 2019;2021: 9. https://doi.org/10.35790/ebm.v9i1.31899
29. Sulistyowatiningsih WCDW, Wijaya H. Evaluasi Penyebab Hasil Invalid pada Pemeriksaan RT-PCR Pasien COVID-19. Jurnal SainHealth. 2022;6: 1-7. https://doi.org/10.51804/jsh.v6i1.1727.1-7
30. Martel F, Grundemann D, Schomig E. A simple method for elimination of false positive results in RT-PCR. BMB Rep. 2002;35: 248-250. https://doi.org/10.5483/BMBRep.2002.35.2.248
31. Merdekawati F, Nurhayati B. Desain primer gen pengkode RNA Dependent RNA Polimerase (RdRp) untuk deteksi SARSCOV2 dengan menggunakan real time polymerase chain reaction. Jurnal Riset Kesehatan Poltekkes Depkes Bandung. 2023;15: 30-36. https://doi.org/10.34011/juriskesbdg.v15i1.2179
32. Mawarnursavira K, Sari R, Apridamayanti P. Optimasi Melting Temperature Primer Degenerate pada Suhu 60?C Gen ERM(T) (Erythromycin Ribosome Methylase) yang Resistensi terhadap Bakteri Streptococcus pyogenes. Jurnal Mahasiswa Farmasi Fakultas Kedokteran dan Ilmu Kesehatan UNTAN. 2019;4: 1-12.