Dear Sir/ Madam,

Dengue is a mosquito-borne viral disease caused by dengue virus serotype 1 to 4 (DENV 1 to 4). Dengue outbreaks are common in both east and west Malaysia. Between July 2016 and December 2017, 200 serum samples were found to be positive for dengue by rapid test (Panbio Dengue Duo Cassette and Panbio Dengue Early rapid test kits; Abbott, Australia) of samples collected from patients in Sandakan and Kudat of Sabah state during dengue outbreaks. Serotypes of the dengue viruses were determined by reverse transcriptase polymerase chain reaction (RT-PCR) followed by nested PCR. C-prM primers designed by Lanciotti et al.\(^1\) and later redesigned by Chien et al.\(^2\) were used for the purpose. All the four dengue serotypes were detected with PCR products with specific sizes in gel electrophoresis. However, in four samples, no serotype-specific band was amplified by the nested PCR, while they were dengue-positive in RT-PCR showing 511 base pair (bp) amplicon. It was first presumed that these DENV might belong to a new DENV serotype, accordingly nucleotide sequences of the 511 bp amplicons of the four samples were determined. As a result, all the four samples (K35, K81, S23, and K75) (K and S stand for Kudat and Sandakan respectively) were found to belong to DENV4. Then the sequences of these samples were aligned with that of DENV 4 reverse primer (rTS4) (Figure 1).

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**LETTER TO THE EDITOR**

**Detection of Four Mismatched Nucleotides between DENV4 Specific C-prM Primer (rTS4) and Current DENV 4 Sequences in Sabah**

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The DENV4 specific primer rTS4 was found to have four mismatched nucleotides to the current DENV4 sequences in Sabah leading to the absence of DENV4 specific bands (260 bp) in nested PCR.

<table>
<thead>
<tr>
<th>Primer</th>
<th>5' to 3' sequence</th>
</tr>
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<tbody>
<tr>
<td>K35</td>
<td>TCAATATGCTGAAACGGGAGAAGACCC</td>
</tr>
<tr>
<td>K81</td>
<td>TTCAATATGCTGAAACGGGAGAAGACCC</td>
</tr>
<tr>
<td>S23</td>
<td>-TCAATATGCTGAAACGGGAGAAGACCC</td>
</tr>
<tr>
<td>K75</td>
<td>-AACGGGAGAAGACCC</td>
</tr>
<tr>
<td>rTS4-reverse</td>
<td>TCTCAACGGAAGACCC</td>
</tr>
</tbody>
</table>

**Figure 1** Alignment of DENV4 sequences of samples (K35, K81, S23, and K75) and rTS4 primer sequence. Primer mD1 sequence is shaded and reverse complement sequence of rTS4 primer is bolded. Asterisk indicates identical nucleotide.
REFERENCES

