Basic Science & Translational Research Category
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Direct RT-PCR Detection of Dengue 1-4 Virus from Crude Samples for Rapid Point-of-care Diagnosis

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Aims: Molecular methods are routinely used for diagnosis of acute dengue infection, on account of its high sensitivity and ability to distinguish serotypes. Conventional Reverse Transcription Polymerase Chain Reaction (RT-PCR) typically takes 3–5 hours and requires purification of viral RNA to remove PCR inhibitors in samples that may otherwise cause false negatives or reduce detection sensitivity. Our aim was to develop a rapid and low cost method to perform RT-PCR directly from blood samples.

Methodology: We have developed an inhibitor-resistant direct protocol and validated it using Dengue virus serotypes 1–4 whole virus spiked into serum and plasma samples. Viral RNA release, RT and PCR were all carried out in the same reaction tube and were able to tolerate 8% (v/v) serum.

Result: Our sensitive and specific one step direct RT-PCR assay can be completed in 28 min (using a QuantStudio™ 6 Flex Real-Time PCR System). The assay enabled the detection of as few as 1 pfu/ml of dengue virus in a quantitative manner. In addition, we demonstrated PCR thermal cycling at a rate of 6-10 sec per cycle, with 35 cycles completed in 6–8 minutes, by using our a novel low-cost ultra-rapid thermocycler, fabricated in-house.

Conclusion: These advances reduce the turn-around-time, manual manipulation, reagents, contamination risk and equipment requirements, and could potentially enable rapid dengue diagnosis in low resource settings and at point-of-care.