Rapid Direct Detection of Dengue Virus in Blood Samples by Real Time-Polymerase Chain Reaction (RT-PCR) using a Portable Point-of-Device Care

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ABSTRACT

Introduction: Dengue virus (DENV) in a mosquito borne human virus causing both asymptomatic and severe infection in tropical and subtropical regions of the world. A rapid detection of the virus is critical to patient management and for surveillance of diseases. A dry format quantitative reverse transcriptase polymerase chain reaction assay was developed for field use and systemically evaluated previously using Cepheid SmartCycler platform for rapid detection of DENV. In this current study we optimized this assay for use with T-COR 8™ integrated system that includes a portable thermocycler, simple sample collection and processing devices to detect DENV in whole blood samples directly. The objective of the study was to develop an assay suitable for not only the laboratory settings but also for point-of-care settings, low resource settings and austere environments.

Methods: The assay specificity was previously tested by using a panel of related flaviviruses. Eighty one confirmed dengue positive samples and 25 negative samples were tested to validate the assay on Cepheid SmartCycler. Titration of spiked samples showed a wide dynamic range with 5 logs of dilution when T-COR 8 integrated system was used. Comparison of results of samples and direct testing from blood samples using novel RDBA buffer show similar results. Test performed on T-COR 8 integrated system gave similar or better results than a traditional bench top thermal cyclers.

Conclusions: The data from the study shows that using this novel assay system presence of DENV can be detected rapidly in blood samples without the need for RNA extraction and other traditional lab supplies. This novel system can be used equally effectively in labs, in field, in austere environments, on running cart or at the bed side for detection of DENV. This system includes T-COR 8™ device, simple sample collection and processing system with the dried down test reagents for DENV and software that provide test results both locally and remotely.

INTRODUCTION

Dengue has re-emerged worldwide due to urbanization, increase in travel and climate change, becoming a major global public health concern. Various methods used currently for laboratory detection of DENV infection include virus culture, viral antigen detection, immunohistochemistry, serological methods, and viral RNA detection. Although there are many challenges to these dengue detection and diagnostic methods. Detection of DENV antigens such as NS1 is not very sensitive. Serological methods, in which antibodies to DENV are detected, may be more cumbersome and time consuming, requiring expertise and an appropriate laboratory facility. Although there is a lack of availability of specific antiviral drugs for treatment of these diseases yet timely intervention can avoid development of more severe symptoms like Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). Thus, a significant need exists for the development of diagnostic methods that can be used in low resource settings and with minimal training at the point-of-care. This study was designed to develop a fully integrated system for rapid and direct detection of DENV in whole blood that can be used equally effectively in laboratories and in low resource settings.

Materials and Methods

To establish the equivalence of a traditional thermocycler with our portable thermocycler T-COR 8™ we used 10 fold serial dilutions of DENV 2 procured from ZellBioTech in TE buffer. Testing of these samples was done using a PAN assay that targeted conserved sequences for all four serotypes of DENV with two forward and two reverse primers. Probe is FAM (fluorescent) labeled and BHQ is used as quencher. For internal control (IC) one forward and one reverse primer were used and probe is labeled with ATTO647N and quencher is BHQ. The Ct values were generated by Smart CT™ analysis software on T-COR 8™ portable point of care system with the dried down test reagents for DENV, and software that provides test results both locally and remotely.

Results: Using the set of 81 confirmed positive and 25 negative samples the assay was found to be 98.77% sensitive and 100 % specific on Cepheid SmartCycler. Titration of spiked samples showed a wide dynamic range with 5 logs of dilution when T-COR 8 integrated system was used. Comparison of results of samples and direct testing from blood samples using novel RDBA buffer show similar results. Test performed on T-COR 8 integrated system gave similar or better results than a traditional bench top thermal cyclers.

Conclusions: The data from this study shows that using this novel assay system presence of DENV can be detected rapidly in blood samples without the need for RNA extraction and other traditional lab supplies. This novel system can be used equally effectively in labs, in field, in austere environments, on running cart or at the bed side for detection of DENV. This system includes T-COR 8™ device, simple sample collection and processing system with the dried down test reagents for DENV and software that provide test results both locally and remotely.

Results and Discussion

The PAN DENV assay was previously validated using Cepheid SmartCycler. SmartCycler has been considered as closed to a portable system traditionally. The assay was found to be 98.77% sensitive and 100% specific when 81 confirmed positive and 25 negative samples were tested in this test. We evaluated T-COR 8 portable thermocycler for detection of spiked blood samples. Various hardware and software features for the T-COR 8 thermocycler and described in Table 1 and Table 2 respectively. Efficiency of PCR by integrated sample preparation and amplification on T-COR 8 instrument was compared with traditional methods on lab based thermocycler ABI 7500. A comparison of standard curves obtained from dilution of DENV 2 in TE buffer and Ct values obtained from T-COR 8 portable thermocycler were compared with ABI 7500. Efficiency of PCR reaction on T-COR 8 over a 5 log range of dilution was found to be better than ABI 7500 for the reaction DENV 2 mix used in this test. The slope of the curve for T-COR 8 is 3.54 as opposed to that obtained on ABI 7500 at 4.56 (Figure 3). DENV 2 spiked blood samples were processed through the blood collection device and tested on T-COR 8 using C2T cartridge. Traditional RNA extraction was also done with each dilution. The spiked samples were also tested directly without sample processing or RNA extraction. A comparison of amplification curves from T-COR 8 for this study is shown in Figure 4. Ct values for DENV 2 were obtained two dilutions tested for novel sample processing and traditional RNA extraction methods were comparable. There is no pipetting or centrifugation required for novel sample processing method making it very simple and rapid for use with minimal training and equipment requirements. At least four replicates of each of the 5 logs of dilution of DENV 2 in blood were tested by 2 performers. Table 2 shows that method was very reproducible with a coefficient of variance (CV%) below 5% for Ct values. A higher CV% was seen as the highest dilution expected. The amplification curves for 4 replicates at limit of detection of 1/100000 dilution shown in Figure 5.

Conclusions

Despite the lack of needed diagnostic tools that will be very useful for rapid detection of DENV in low resource settings, the diagnostics industry has not prioritized this need. Major limitation of RT-PCR based assays being the need of extraction of RNA from samples using accurate pipetting, centrifugation and other methods for a good quality RNA template generation. In this study we have shown that a simple sample processing protocol integrated with nucleic acid amplification using a portable thermocycler can generate quality template for DENV 2 from blood samples that can be effectively detected with our portable thermocycler T-COR 8™. Further investigations are being done to evaluate this system with more serotypes of DENV.