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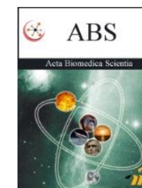


# A PILOT STUDY FOR THE IDENTIFICATION OF DENGUE VIRUS USING REAL TIME-PCR

**Shiju Mathew***

Department of Medical Lab Technology (Genetics Lab) College of Applied Medical Sciences, Jazan University P.O. Box No. 114, Jazan, Kingdom of Saudi Arabia.

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**ABSTRACT**

Dengue hemorrhagic fever (DHF) is brought about by a mosquito-borne flavivirus with four unique serotypes (dengue sorts 1–4: Den1–4). Every one of the four serotypes, are endemic in the greater part of the nations in the tropical and sub- tropical locales. Due to the high affectability and specificity of PCR analytic procedures, a few PCR-based methods for the identification of dengue have been produced as of late. In this study intended to create an one-stage reverse translation (RT)-PCR measure utilizing general preparation for fast discovery of viral RNA of all dengue serotypes. The study is a quick system, helpful and gives precise conclusion in suspected dengue.

## INTRODUCTION

Dengue fever, a mosquito borne disease. The strategies are of expanding imperativeness in pathogen recognition, and are slowly supplanting serology and culturing in numerous applications. PCR is especially generally utilized on account of its incredible scientific affectability, yet obliges first stages with immaculate or close succession match to the pathogen genome. In spite of the fact that it is frequently not hard to outline preparations particular to an individual strain of a pathogen, hereditary float and determination creates a mixture of arrangement variations that can be hard to target viably [1-2].

Dengue fever (DF) and dengue hemorrhagic fever (DHF) have been broadcasted as worldwide general wellbeing issues since first development in the eighteenth century. These days, the territories endemic for dengue incorporate Southeast Asia, the Western Pacific, Africa, the Americas and the Eastern Mediterranean. DF and DHF are brought on by a solitary strand RNA infection of the family Flavivirus, transmitted to people by Aedes mosquitoes (*Aedes aegyptii* and *A. albopictus*).

Corresponding Author

**Shiju Mathew**
Email: - sjohn@jazanu.edu.sa

The progressive to conclusive conclusion by serological testing or infection detachment if necessary. A few PCR-based techniques, which are quick and sensitive, have as of late been produced for the identification of dengue infection in clinical specimens [3-5]. In this study, it was meant to create one-stage reverse interpretation (RT)-PCR examine utilizing an all-inclusive preparation for the fast identification of the viral RNA of all dengue serotypes. First stages were planned taking into account the genome of dengue infection gathered in Thailand. Moreover, it was explored whether fringe blood mononuclear cells (PBMC) give higher affectability than plasma to viral RNA detection.

## MATERIALS AND METHODS

The Eight Sample was collected from XYZ, Kerala prick blood samples were collected from doubtful patients with a provisional diagnosis of dengue infection. After all the samples had been routinely tested for hematological values, they were used for this study, and also tested for antibodies against dengue using IgG/IgM ELISA. Serum was collected from the blood sample.

### RNA extraction and RT-PCR

RNA was extracted using (QIAamp Viral RNA

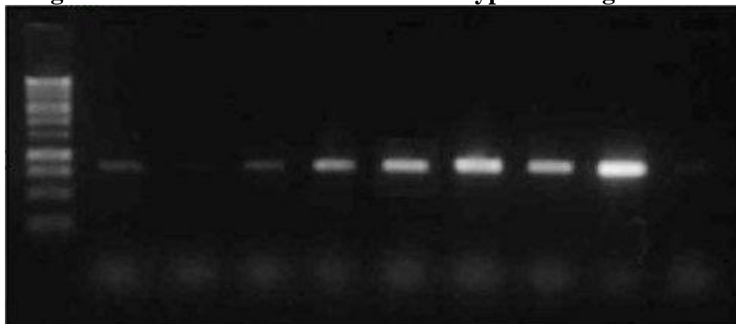
Mini kit, Germany) as per the manufacturer's recommendations from the serum using kit manual and later aliquot to preserve in -80°C. RNA was reverse transcribed into complimentary DNA (cDNA). RNA was reverse transcribed into complimentary DNA (cDNA) copy in a 25µL reverse transcription (RT) reaction mixture using 20ng of random primers, 1mM dNTPs, 5 µL of rRNAsin and 6 µL of Virus reverse transcriptase (Bangalore Genei, India) and 13.75µL RNA. The RT reaction mixture was incubated at 37°C for 90 minutes followed by enzyme inactivation at 65°C for 15 minutes. The PCR was carried out in a 25µL reaction using 1X Taq buffer, 200µM dNTPs, 0.4µM primers (D1 and D2), 1.5U of Taq DNA polymerase (Bangalore Genei, India) and 3 µL cDNA. The PCR was carried out as follows: 94°C for 2 minutes followed by 35 cycles of 94°C for 30s, 52°C for 30s and 72°C for 60s and a final extension at 72°C for 10 minutes. The segment specific to all 4 dengue serotypes was amplified using RT- PCR.

**RESULTS**

Dengue virus specific RT-PCR was conducted on all samples. DENV was detected in dengue samples using universal degenerate primers specific to the NS1 region of dengue genome (serotype 1–4). The evaluation of newly synthesized primers and investigation for appropriate assay conditions were conducted by RT amplification. The results demonstrated that RT-PCR provides a DNA band of the expected size as shown in figure 1.

Detection of dengue RNA in clinical specimens was performed using the conditions established above. The RT-PCR negative samples were samples with ELISA kit. However, there was no amplification product detected in some of the RNA.

**Figure 1. RT-PCR of RNA from 4 serotypes of dengue viruses**



**DISCUSSION**

This study demonstrated the development of a rapid and simple method for dengue detection by RT amplification of viral RNA. The use of degenerate primers enabled the detection of 4 serotypes of dengue in a single step. This technique is different from earlier methods because it can be used with a small amount of blood, previous reports have also suggested that higher sensitivity was obtained when using samples instead of plasma for viral RNA detection. According to the serological pattern in this study, the negative results of RT-PCR in samples with positive IgG might be caused by the low level of viruses in later days of the disease. For these reasons the conditions of RT-PCR testing need to be modified to obtain higher potency. This study was also limited by the lack of negative control specimens carrying other related viral infections to evaluate the specificity of the method. In spite of these limitations, on the basis of this study to the researchers propose this technique as a simple alternative in the diagnosis of dengue infection by using finger prick blood samples at the time of provisional diagnosis. Further studies with increased numbers of clinical samples would be necessary to confirm such a particular advantage (Lanciotti *et al.,* 1992; Wu W).

Additionally, there are some valuable observations arising from this study. It can be seen that there is a discrepancy in the diagnosis of dengue infection. Some serologically positive cases have a negative PCR result while some PCR positive cases have a negative serological result. This can be explained by the fact that in the latter situation the usefulness of the PCR testing for early diagnosis at the time of presentation to the physician is confirmed. This indicates the pitfalls of PCR and serology testing if used singly. Indeed, patients in endemic areas usually give no clear-cut history of illness, which makes it hard to estimate the exact timing of the disease. A combination of finger prick blood PCR and serology testing can be particularly helpful for diagnosis in such cases.

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