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# Frequency of Dengue Virus Serotype 1 in Lahore by In-house assay

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## Abstract

Dengue is an important systemic viral infection that is caused by the dengue virus. Ribonucleic acid (RNA) from dengue NS1 positive samples, collected randomly during dengue epidemic from October 2016 to October 2017 at Chugtai Lab, was extracted for nucleic acid. Both the detection and serotyping of dengue samples were performed using real-time PCR on Rotor Gene Q. From the 70 NS1 positive samples, 57 (81.4%) samples were confirmed to be positive for dengue virus RNA, while the remaining 13 (18.6%) were negative. Serotype 1 (DEN-1) were verified among all samples by in-house assay and using commercial kit FTD (Fast Track Diagnostics) dengue differentiation; it was concluded that our in-house assay is in 100% concordance with commercial kit. Serotype 2 (DEN-2) and serotype 3 (DEN-3) have been documented in Pakistan since 1994. But recent detection of serotype 1 in Pakistan is indicative of more severe dengue haemorrhagic fever in future due to reinfection.

**Keywords:** Dengue, Real-time PCR, Serotype.

## Introduction

Dengue is an important systemic viral infection that is caused by the dengue virus which is a single-stranded RNA virus belonging to the *Family Flaviviridae*. Dengue virus is classified into four antigenically distinct serotypes (DEN-1 to DEN-4).[1] A fifth serotype DEN-5 was identified in December 2013, in a farmer in Malaysia; however, it causes only mild febrile illness.[2] The main transmitting vectors of the virus are *Aedes egypti* and *Aedes albopictus* mosquitoes.[2] The ideal conditions for this mosquito to breed are abundant rainfall and high humidity, during which the temperature of the surrounding environment reaches about 30°C. That is why dengue virus infection is common during September to December. Infection with one serotype of dengue provides lifelong protection against that serotype, but infection with another serotype may result in more serious disease .[3] Currently, more than 125 countries are known to be affected by the dengue viruses.

Dengue infection has been reported across the Americas, South-East Asia and Western Pacific regions, affecting millions of people.[4] After an incubation period of two to seven days, the patient experiences flu-like illness followed by fever, nausea, and vomiting, along with severe frontal and retro-orbital headache and muscle ache. The most severe and serious secondary infection, Dengue Haemorrhagic Fever (DHF) is characterised by fever, or recent history of acute fever with haemorrhagic manifestations and a platelet count of 100,000/mm$^3$ or less along with objective evidence of “leaky capillaries”; the haematocrit is elevated (20% or more over baseline) with low albumin levels.[5] For diagnostic purposes, non-structural protein 1 (NS1) antigen detection and real-time reverse transcriptase PCR (qRT-PCR) are typically used to detect dengue viral genes in the viraemic phase on serum or plasma samples. From the fifth day onwards, detection of IgM and IgG antibodies by ELISA helps in establishing the diagnosis of infection with the dengue virus. There is no specific antiviral treatment for dengue and only supportive treatment with

analgesics and intravenous fluids is recommended.[6] Control of mosquito vectors by the use of insecticides, removal of artificial water containers and mosquito screening of household windows are very effective ways to reduce the outbreaks of dengue.[7] The first dengue vaccine, Dengavaxia by Sanofi Pasteur, was registered in several countries, in 2015-16, for use in people aged 9 to 45 years living in dengue endemic areas. Several other vaccines are in the process of development. The present study was conducted to develop an in-house assay and find the prevalence of dengue serotype in Pakistan.

**Objective:** To develop an in-house assay for detection and serotyping of dengue virus by real-time PCR.

## Patients / Methods and Results

A cross-sectional study was conducted on 70 suspected dengue cases that were sampled during October 2016 to October 2017 at the Molecular Biology and Virology Department, Chugtai Lab, Lahore, on patients belonging to different areas of Lahore.

Blood plasma was used to extract viral RNA using QIAsymphony DSP Virus/Pathogen Midi Kit (Qiagen) on fully automated platform of QIAsymphony SP (Qiagen) by following the manufacturer's instructions. The primers used for genotyping of the positive samples were designed according to the model used by Fatima et al in 2011.[8] The region of C-prM gene junction described by Lanciotti et al [9] was selected for genotyping as this is not very hyper variable and less prone to mutations. The probes for genotyping were designed by using the tool, Primer3, available online.

Detection and genotyping of plasma samples were performed on Rotor-Gene Q instrument (Qiagen) by adopting the one-step qRT-PCR strategy. In this regard, a reaction mixture of 20µl was prepared by adding 5µl of extracted viral RNA, 1µl of forward and reverse primers (each 10 picomol/µl), 0.5µl of probe (10 picomol/µl), 2.5µl of PCR water, and 10µl of master mix (Affymetrix USB

VeriQuest 2x). Furthermore, the thermal profile used was: an incubation at 50°C for 15 minutes, afterward an initial denaturation at 95°C for 10 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds and extension at 72°C for one minute. A final extension was given at 72°C for 10 minutes. Results of four serotypes, DEN 1-4, by in-house assay were further verified through the commercially available FTD dengue differentiation kit (Fast Track Diagnostics) and were analysed by using software SPSS 16.0.

From the analysis of 70 NS1 positive samples, dengue virus RNA was detected in almost 57 (81.3%) of the samples, while in the remaining 13 (18.6%) dengue virus RNA was not detected. (Figure 1). Furthermore, nearly 65 (92.9%) of these positive cases were sampled from Lahore, whereas 5 (7.1%) were from outside Lahore. Out of positive dengue patients 45 (64.28%) were males while 25 (35.72%) were females. All these positive samples were subjected to genotyping by both the in-house assay and the commercially available FTD kit, and results showed 100% co-relation for serotype 1 (DEN-1). All the patients had DEN-1, and no other serotype was detected. Ten random positive samples were sent to the external lab dealing with the viral diagnosis and results were in 100% concordance with the in-house assay.

A one way Anova test of independence was performed to check the relationship between positive cases with gender. The relationship between these variables was significant. The p-value was less than 0.05 which indicates that these variables are not independent of each other and that there is a statistically significant relationship between the categorical real-time PCR output and gender. The significance value was $p = 0.048$ with a confidence interval of 95%, which shows the parameters selected for comparison in this study are relevant and enlighten the significance of this study.

Our country is at high risk of dengue infection due to overcrowding of cities, presence of stagnant water, a large number of refugees and people exposed to

mosquito bite. The first documented outbreaks in Karachi in 1994 and 1995 were reported to be due to dengue serotypes 1 and 2, whereas in 2005 and 2006 outbreaks serotypes 2 and 3 were documented.[10] Whereas, in Lahore serotypes 2, 3 and 4 were reported to cause outbreak in 2008, and later in 2009 only serotypes 2 and 3 were responsible for major outbreaks in Lahore.[11] In the current study, serotype 1 was documented for causing outbreak in Lahore 2016, with a p-value of 0.048, showing relevance of this study. The serotype 1 has re-emerged after 21 years of its first evidence in Karachi, 1994 and then in 1998. DEN-3 and DEN-2 was reported in Karachi in 2006. Drift change in serotype poses a great risk of dengue haemorrhagic fever in population already exposed to other dengue serotype.

## Conclusion

Dengue serotype is changing quite dramatically over the time in Pakistan, because of which greater incidence of dengue haemorrhagic fever is predicted in population previously exposed to other dengue serotype.

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**Conflict of Interest:** None

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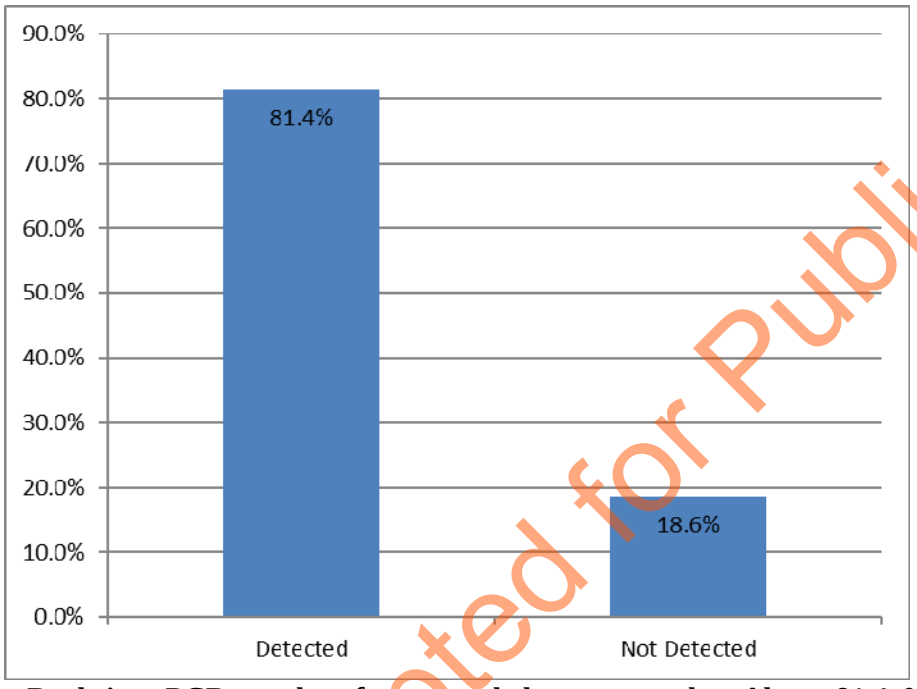


**Figure 1: Real-time PCR results of suspected dengue samples. About 81.4 % of the samples were found to be RNA positive for dengue virus.**

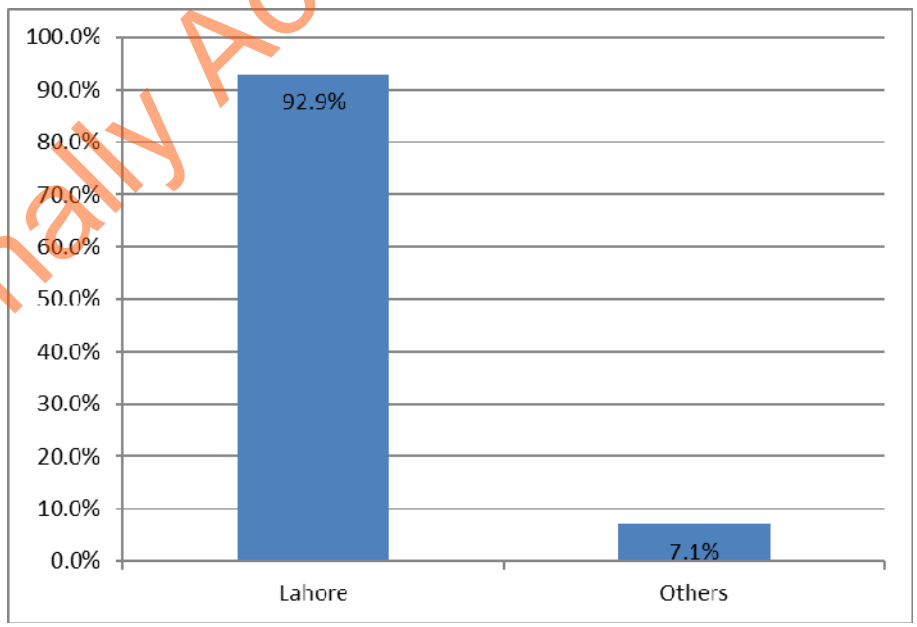


**Figure 2: Area wise distribution of RNA positive dengue cases. Almost 93% of cases sampled were from the Lahore region.**