

# INCREASE IN THE SENSITIVITY OF DENGUE DIAGNOSIS BY COMBINATION OF REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION AND PASSAGE ON CELL CULTURES

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**Abstract.** We passaged 52 serum samples from dengue patients on C6/36 cells for 7 days and checked the culture fluids by RT-PCR. Two serum samples, which were negative by direct RT-PCR, became positive. One sample was collected on fever day 1 and the other on fever day 2. Results indicate that combination of reverse transcriptase-polymerase chain reaction (RT-PCR) with passage of serum samples on C6/36 cells increases the sensitivity of dengue diagnosis.

## INTRODUCTION

Dengue virus infections are a serious cause of morbidity and mortality in many areas of the world : Southeast and South Asia, Central and South America and the Caribbean (Monath, 1994). The clinical manifestations of dengue virus infections range from asymptomatic infection to two forms of illness (World Health Organization, 1997). Dengue fever (DF) is a self-limited febrile illness. Some patients with dengue virus infection develop a severe, life-threatening syndrome called dengue hemorrhagic fever (DHF). It is estimated that 100 million cases of dengue fever and 250,000 cases of DHF occur annually (Halstead, 1988). Thus, DF/DHF is one of the most important infectious diseases in the world.

We previously reported that reverse transcriptase-polymerase chain reaction (RT-PCR) is a useful diagnostic method for dengue virus infection (Yamada *et al*, 1999a). When patients had fever, detection of dengue genome by RT-PCR was usually positive. On the other hand, detection of dengue genome by RT-PCR was negative, once fever subsided. In the present study, we attempted to increase the sensitivity of diagnosis by combining RT-PCR and pas-

sage of serum samples on C6/36 cells.

## MATERIALS AND METHODS

Serum specimens were obtained from dengue-suspected cases in clinics and hospitals in Japan from 1996 to 1999, and sent to Department of Virology 1, National Institute of Infectious Diseases for laboratory diagnosis of dengue. In the present analysis, fever day 0 is the day of defervescence, that is, the day when the temperature falls below 37°C without further significant temperature elevation. Days prior to fever day 0 are designated fever day 1 (1 day before defervescence), fever day 2, etc. The day after defervescence is fever day 1.

RT-PCR was performed as previously reported (Yamada *et al*, 1999a,b). Briefly, RNA was isolated from 0.05 ml of serum specimen using 0.2 ml of Isogen-LS (Nippon gene, Tokyo) and 0.04 ml of chloroform. RT and PCR were done in a single tube. The tubes were set in an oil bath thermal programmer (Iwaki, Co, Tokyo) and subjected to programmed incubation: at 53°C for 10 minutes for reverse transcription, followed by 30-40 PCR cycles of amplification. PCR products were then subjected to agarose gel electrophoresis. Amplified DNA fragments were visualized by ethidium bromide staining. The primer sequences used to amplify each serotype of dengue viruses and target size were previously reported (Yamada *et al*, 1999a,b).

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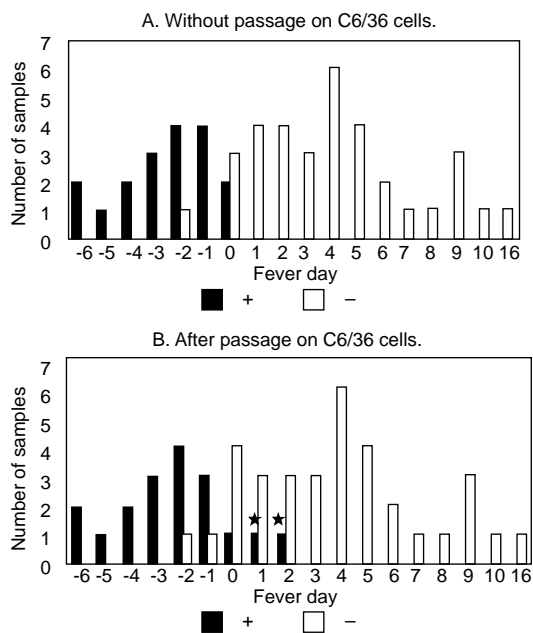


Fig 1—Detection of dengue viral genome by RT-PCR.  
 A: Results of direct RT-PCR without passage on C6/36 cells.  
 B: Results of RT-PCR after passage of serum samples on C6/36 cells.  
 ★ These two samples were PCR-positive after passage on C6/36 cells.

## RESULTS

We examined 52 serum specimens from 27 dengue-suspected cases by RT-PCR with or without passage on C6/36 cells. All the samples but one that were collected before fever day 0 were positive by RT-PCR, and 2 of the 5 samples collected on fever day 0 were positive. None of the samples collected on fever day 1 or later were positive by RT-PCR (Fig 1A).

We passaged these serum samples on C6/36 cells for 7 days, and checked the culture fluids by RT-PCR. Two samples, which were determined negative by direct RT-PCR, turned out to be positive (Fig 1B). One sample was collected on fever day 1 and the other on fever day 2. Presence of dengue viruses in the culture supernatants was also confirmed by plaque formation assays (data not shown).

## DISCUSSION

We previously reported that (i) when patients had fever, detection of dengue virus ge-

nome by RT-PCR was usually positive, although IgM-capture ELISA may still be negative, and that (ii) once fever subsides, detection of viral genome by RT-PCR was usually negative, but IgM-ELISA was positive (Yamada *et al.*, 1999a). However, in the present study we could demonstrate the presence of dengue virus in the serum samples collected on fever days 1 and 2 by combining RT-PCR and passage on C6/36 cells. Some serum samples, which were determined negative by direct RT-PCR, became positive after passage on C6/36 cells. Passage of serum samples on C6/36 cells is not a complicated, laborious or time-consuming procedure. We, therefore, recommend that one includes this procedure to increase the sensitivity of the detection of dengue viral genome by RT-PCR.

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