# Development of RT-PCR for rapid detection of dengue virus type 1-4 from clinical specimens and a preliminary phylogenetic study of dengue virus isolates from Sri Lanka.

By

V.G.N.S. Velathanthiri



Thesis submitted to the University of Sri Jayawardenapura for the award of the Degree of Master of Philosophy in Microbiology

On

01.12.2004

Development of RT-PCR for rapid detection of dengue virus type 1- 4 from clinical specimens and a preliminary phylogenetic study of dengue virus isolates from Sri Lanka.

By

V.G.N.S. Velathanthiri

## ABSTRACT

Dengue virus is the causative agent of dengue fever (DF) and its complications; dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). It has four serotypes (Dengue 1-4) and is a member of the family flaviridae. It is one of the important causes of morbidity and mortality through out the subtropical and tropical regions, including Sri Lanka.

i. Patients with dengue need to be closely monitored for evidence of haemorrhage and shock. In order to do this, it is necessary to differentiate patients with dengue from non dengue patients. This however, is a challenge since dengue often presents with non specific symptoms such as fever, headache and body aches. Therefore, it requires an aetiological diagnosis based on laboratory confirmation of disease. In this regard, an ideal diagnostic tool should be sensitive, specific, reliable, rapid, cheap, technically less demanding and it should also be able to detect dengue in the early stages of the disease. Detection of the

circulating serotype is very important in epidemiological surveillance of the disease.

Laboratory diagnosis of dengue infection is normally based on detection of dengue virus specific antibodies and isolation of dengue virus from patient's serum. Serological diagnosis has been proven to be of less value in the early stages of illness. Current technologies for isolation and identification of the dengue virus are based on complicated systems such as suckling mouse brain or mosquito inoculation or cell culture technique. These methods are technically demanding, expensive and time consuming.

The availability of molecular diagnostics such as the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) technique has made it possible to perform rapid detection of the viral RNA. In this study, I present an alternative design of the RT-PCR technique that can detect and simultaneously identify the sero type of the dengue virus.

I designed sets of primers and developed an RT-PCR for rapid detection and simultaneous identification of dengue virus and its serotypes in cell culture supernatant and in serum samples. It was evaluated for sensitivity, specificity and compared with other standard laboratory diagnostic methods. The test is based on 5 sets of primer pairs specific for dengue viruses within the non-structural (NS) 5 region of the dengue virus genome. A universal primer set that would bind to target sequence shared by all the four serotypes of the virus within the NS5 region, are used. The resulting PCR products are detected by gel electrophoresis and staining with ethidium bromide. The RT-PCR was developed with serotype specific primers, which were also designed within the

NS5 region of each serotype for the identification of dengue serotypes. The amplified products of different sizes were obtained with different dengue serotypes and were detected by gel electrophoresis. The RT-PCR technique is simple and rapid, capable of not only detecting the dengue virus but also identifying its serotype in clinical specimens. The RT-PCR protocol developed by me was shown to more sensitive than virus isolation in cell culture and equally sensitive in detecting dengue virus and its serotypes in serum specimens. It was also shown not to cross react with other flaviviruses.

In the preliminary phylogenetic study, I compared the nucleotide sequence homology of the Sri Lankan dengue virus isolates of the present study with dengue viruses isolated form other parts of the world. Nucleotide sequence analysis was performed by an automated nucleic acid sequencer on 14 dengue virus isolates (13 dengue type 2 and one dengue type 3).

The dengue 2 viruses were most closely related to dengue 2 virus isolated in Sri Lanka and Seychelle in 1990 and 1976 respectively. The dengue 3 virus was most closely related to dengue 3 viruses recovered in Sri Lanka in 1991, 1985 and 1981. Our results suggested that these dengue virus serotypes are evolving locally and any introduced strains are failing to become established.

## *TABLE OF CONTENTS*