

## **Detection of Dengue Co Infections Using a Novel Single Tube Multiplex Reverse Transcription Polymerase Chain Reaction**

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Co-infection in individuals by more than one Dengue Virus (DENV) serotype has been reported in regions where multiple serotypes co-circulate. Co-infections can be detected using Polymerase Chain Reaction (PCR). Semi-nested multiplex PCR with Lanciotti's primers is a widely used PCR method for serotyping DENV and it has also been used for detecting co-infections. Despite of being widely used, Lanciotti's method may be sub-optimal in detecting co-infections as overlapping primer targets will create a bias in the amplification of the serotype with a low viral load. This could lead to underreporting of co-infections. Nine new non-overlapping primers were designed to independently amplify each serotype with minimal competition between primers to their target. In mixed infections, novel PCR assay exhibited higher sensitivity in detecting the minor serotype compared to Lanciotti's method. The new method can also detect all four serotypes in viral RNA isolated from viral cultures and patient samples in a single tube multiplex PCR. This enables rapid and cost-effective serotyping with improved sensitivity in detection of co-infections in clinical samples.

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