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## Incorrect identification of recent Asian strains of Coxsackievirus A16 as human enterovirus 71: Improved primers for the specific detection of human enterovirus 71 by RT PCR

David Perera, Yuwana Podin, Winnie Akin, Cheng-Siang Tan and Mary Jane Cardoso\*

Address: Institute of Health & Community Medicine, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, 94300 Malaysia

Email: David Perera - davidperera@yahoo.com; Yuwana Podin - ypodin@yahoo.com; Winnie Akin - winnie\_akin@yahoo.com; Cheng-Siang Tan - kennytcs@hotmail.com; Mary Jane Cardoso\* - janecardosa@yahoo.co.uk

\* Corresponding author

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### Abstract

**Background:** Human enterovirus 71 has emerged as an important pathogen in the Asia Pacific region and it is important to be able to make a rapid and specific diagnosis for outbreak control. Recent Asian strains of Coxsackievirus A16 have changes in the VP1 gene which causes mispriming of widely used primers for human enterovirus 71 specific identification.

**Methods:** Local strains of Coxsackievirus A16 were sequenced in the VP4 and VP1 genes and using sequence alignment tools, an improved set of primers were designed for specific identification of human enterovirus 71. These primers were evaluated against virus isolates as well as primary clinical specimens.

**Results:** A total of 218 virus strains were tested. All 39 human enterovirus 71 isolates were positive and none of the 38 Coxsackievirus A16, 127 other enteroviruses and 14 prototype flaviviruses and adenoviruses were positive when tested with the new primers. When aliquots of primary specimens known to have yielded human enterovirus 71 were retrospectively tested, we found that within 2 months of collection of the specimens, greater than 90% were positive but that the success rate diminished rapidly to 18% after 2 years storage.

**Conclusions:** Our new primers will be useful in rapid diagnosis of human enterovirus 71 infection, and can also be used as a screening tool in surveillance programmes for early warning of human enterovirus 71 transmission.

### Background

Hand, foot and mouth disease (HFMD) is an unremarkable illness that commonly occurs in young children. This condition is caused by human species A enteroviruses [1] most commonly Coxsackievirus A16 (CVA16) but has more recently been associated with human enterovirus 71 (HEV71) which can also cause neurological complica-

tions [2]. In the most common manifestation which gives the syndrome its name, children typically present with vesicular exanthema on the soles of their feet, the palms of their hands and in their mouths, causing discomfort and feeding difficulties. CVA16 and HEV71 are also associated with herpangina, but this is often also caused by other species A enteroviruses such as Coxsackievirus A8