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Bedside immunochromatographic test for enterovirus 71 infection in children

Kuan-Ying Arthur Huang[a,b,1], Shuan Yang[b,c,1], Kuo-Chien Tsao[b,c], Chih-Jung Chen[a,b], Yu-Chia Hsieh[a,b], Cheng-Hsun Chiu[a,b], Juo-Yu Hsieh[d], Jyh-Yuan Yang[d,**], Yhu-Chering Huang[a,b,*]

[a] Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[b] Chang Gung University College of Medicine, Taoyuan, Taiwan
[c] Department of Laboratory Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[d] Center for Research, Diagnostics and Vaccine Development, Centers for Disease Control, Taipei, Taiwan

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ABSTRACT

Background: Enterovirus 71 (EV71) causes frequent outbreaks worldwide, particularly in the Asia-Pacific area. Its quick spread is a critical challenge for public health and timely preventive measures and clinical management therefore rely on early detection. There is a need for a rapid, easy-to-use, and reliable method for detecting EV71 infections.
Objective: The study aimed to evaluate a bedside immunochromatography (ICT) kit for diagnosing acute EV71 infection in children.
Study design: Pediatric patients with herpangina or hand–foot–mouth disease were randomly and prospectively enrolled from hospitals across Taiwan. Throat or rectal swabs were collected for viral culture and reverse-transcriptase polymerase chain reaction (RTPCR). For the ICT kit, whole blood was obtained by ear piercing, finger-sticking, or venipuncture. The results of ICT, virus isolation and RTPCR in clinical samples were compared.
Results: Of the 156 patients enrolled, 91 (58%), 64 (41%) and 72 (46%) had positive results of the ICT kit, viral culture and RTPCR, respectively. Laboratory-confirmed infection with either positive EV71 culture or RTPCR was used as the diagnostic standard. The sensitivity and specificity of the ICT kit was 84% and 77%, respectively. The viral culture and RTPCR had relatively lower sensitivity but higher specificity. The patient's age did not affect the performance of the ICT, viral culture and RTPCR. However, a low sensitivity of ICT kit was noted before the second day of disease onset.
Conclusions: The ICT kit may serve as a simple, quick and reliable method for the bedside diagnosis of acute EV71 infection in children.

1. Background

Enterovirus 71 (EV71) causes epidemics annually in the Asia-Pacific area and occasional outbreaks in Europe or America, posing a major threat to child health [1]. EV71-associated diseases include herpangina, hand–foot–mouth disease (HFMD), meningitis and encephalitis with subsequent cardiopulmonary collapse or mortality. Close contact in crowded places and among family members may lead to the transmission and the development of severe illness [2]. As there are no anti-EV71 agents or vaccines available, control of infection via hygiene enhancement and patient isolation plays a key role in preventing or limiting the spread of EV71. At the same time, detection of the EV71 case is the first step in public health interventions at the start of an outbreak.

Viral culture and reverse-transcriptase polymerase chain reaction (RTPCR) are the most common methods of detecting EV71 [3,4], with 70–80% sensitivity and 80–90% specificity observed in previous studies [5]. However, these methods require well-equipped laboratories and well-trained technicians. It is unusual to provide these tests in local clinics and rural hospitals where a substantial number of EV71 patients may visit during an outbreak. Undiagnosed EV71 patients might become the primary source of spread in the communities.

* Corresponding author at: Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Memorial Hospital, No. 5, Fu-Shin Street, Kweishan 333, Taoyuan, Taiwan. Tel.: +886 3 3281200x8202; fax: +886 3 3288957.
** Corresponding author at: 161 Kun Yang Street, Nan-Kang District, Taipei 115, Taiwan. Tel.: +886 2 2653 1375; fax: +886 2 2653 0403.
E-mail addresses: jyyang@cdc.gov.tw (J.-Y. Yang), ychuang@adm.cgmh.org.tw (Y.-C. Huang).
[1] The first two authors contributed equally to this work.

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A serological test may be an alternative method for detecting EV71 infections. A patient with sero-conversion or a four fold increase in neutralisation titre against EV71 in the paired serum is considered to have acute infection [6]. Nevertheless, the collection of paired serum is rarely practised in the clinical setting and the neutralisation test takes days to obtain results. Using acute-phase serum or plasma, immunoglobulin M (IgM)-based enzyme-linked immunosorbent assay (ELISA) has been proven to be sensitive and specific, with results reported within hours. However, a laboratory is still needed to operate and interpret ELISA [7,8].

An easy-to-use, rapid and reliable diagnostic method is warranted not only in medical centres but also in local clinics during EV71 outbreaks. Based on the principle of IgM-capture ELISA, a bedside immunochromatography (ICT) kit was developed. Neither a reading machine nor well-trained personnel are needed to perform this bedside test in the first line when encountering suspected EV71 cases. The test results may serve as preliminary data for primary clinicians and public health specialists in conducting medical management and preventive measures in order to immediately arrest an outbreak.

2. Objectives

The study aimed to examine the performance of an ICT kit designed for diagnosing acute EV71 infection in paediatric patients with herpangina or HFMD.

3. Study design

3.1. Patients and samples

A total of 150 paediatric patients with severe EV infections reported to the Centers for Diseases Control of Taiwan (CDC-Taiwan) in 2004, 2006 and 2011. Among them, 105 patients with virology data, available blood samples, and complete records were enrolled in this study. In 2012, 72 paediatric patients who were diagnosed with herpangina or HFMD, either as out patients or in patients treated in Chang Gung Memorial Hospital, were approached and 51 more were found eligible and enrolled in this study.

Severe EV infections were defined by the presence of herpangina or HFMD plus the occurrence of one or more complications such as poliomyelitis-like syndrome, aseptic meningitis, encephalitis, pulmonary oedema/haemorrhage or death. Herpangina was defined as febrile illness and the presence of oral ulcers predominantly affecting the posterior oral cavity, including the soft palate, anterior tonsillar pillars or uvula. Patients with HFMD presented with multiple oral ulcers and vesicular rashes on the hands, feet, knees or buttocks. Throat and/or rectal swabs were collected and whole blood was obtained by ear piercing, finger-sticking, or venipuncture.

The study complied with both the Good Clinical Practice guidelines and the Declaration of Helsinki. The hospital's ethics review committee approved the study protocol and all enrolled patients and/or their parents or other surrogates provided written informed consent.

3.2. Viral culture with immunofluorescent assay

Throat and/or rectal swabs were collected for viral culture [3]. The specimens were inoculated into human MK2, Hep2, MRC-5 and RD cell lines. Cells with cytopathic effect were harvested and an immunofluorescent assay (IFA) was performed to identify EV, respiratory syncytial virus, herpes simplex virus, influenza virus, para-influenza virus and cytomegalovirus. Specimens positive for EV were subsequently examined by IFA using type-specific monoclonal antibodies against viruses, including Coxsackievirus A2, A4, A5, A6, A9, A10, A16, A24 and B1-6; Echovirus 4, 6, 9, 11 and 30; Poliovirus 1–3; and EV71.

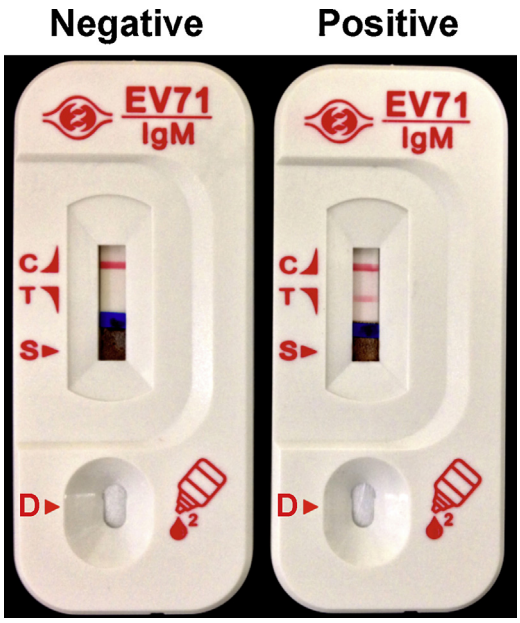

Fig. 1. Detection of EV71 infection by the ICT kit. The test was performed with whole blood sample. The presence of both colored test line (T) and control line (C) indicated an EV71-positive result. If only one colored band appeared in the control area (C) of the device, the result was negative. S, sample loading zone; D, diluent loading zone.

3.3. Reverse-transcriptase polymerase chain reaction

Viral RNA was extracted from throat and/or rectal swab using a QIAamp Mini Viral RNA Extraction Kit (Qiagen, Germany). One-step RTPCR was performed to amplify the VP1 region of EV71 [9]. The reaction mixture contained the extracted RNA template, forward primer 5′-ACYATGAAAYTGTGCAAGG-3′, reverse primer 5′-CCRGTAGGKGTRCACGCRAC-3′ and reagents provided by the QIAGEN OneStep RT-PCR kit (Qiagen, Germany). Negative controls (sterile water) and positive controls (EV71 RNA template) were included. The PCR products were checked by 2% agarose gel electrophoresis and the size of EV71-specific PCR products was approximately 484 bp.

3.4. ICT kit

The Formosa One Sure EV71 IgM Rapid Test kit (Formosa Biomedical Technology Corp., Taiwan) is an ICT test device intended for the qualitative detection of IgM antibodies to EV71 in human whole blood, serum or plasma. The test was based on the capture of IgM using immobilised anti-human IgM μ-chain antibodies and the subsequent detection of the captured EV71 VP1 antigens using mouse anti-VP1 antibodies conjugated to latex.

Briefly, 15 μl of whole blood or 7 μl of serum/plasma was dropped to the sample loading zone (S). When the wet front passed the blue indicator line of the device, two drops of the diluent were added to the diluent loading zone (D). Both the drop tube and diluent were provided by the manufacturer. The result was read after 15 min (Fig. 1). The test kit was kept at room temperature and had an expiry date of after 1 year.