Journal of Virological Methods 179 (2012) 104–107

Contents lists available at SciVerse ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Detection of enterovirus RNA in cerebrospinal fluid: Comparison of two molecular assays

S.C.M. de Crom[a], C.C. Obihara[a,b], A.M. van Loon[c], A.A. Argilagos-Alvarez[d], M.F. Peeters[d,1], A.M. van Furth[b], J.W.A. Rossen[c,d,*]

[a] Department of Pediatrics, St. Elisabeth Hospital, Post-office Box 90151, 5000 LC, Tilburg, The Netherlands
[b] Department of Pediatric Infectious Diseases Immunology and Rheumatology, VU Medical Centre, Post-office Box 7057, 1007 MB, Amsterdam, The Netherlands
[c] Department of Virology, Eijkman-Winkler Center, University Medical Center Utrecht, Post-office Box 85500, 3508 CX, Utrecht, The Netherlands
[d] Laboratory of Medical Microbiology and Immunology, St. Elisabeth Hospital, Post-office Box 90151, 5000 LC, Tilburg, The Netherlands

Article history:
Received 26 May 2011
Received in revised form
22 September 2011
Accepted 10 October 2011
Available online 15 October 2011

Keywords:
Enterovirus
Parechovirus
Meningitis
GeneXpert enterovirus assay
Real-time PCR

A B S T R A C T

Enterovirus (EV) and human parechovirus (HPeV) are a major cause of infection in childhood. A rapid diagnostic test may improve the management of patients with EV and HPeV infection.

The aim of this study is to evaluate the performance of the GeneXpert enterovirus assay (GXEA) for detection of EV RNA compared to a user-developed reverse-transcriptase (RT) quantitative real-time PCR (qPCR) in routine clinical practice. Also a RT-qPCR assay for detection of HPeV RNA in different clinical samples was developed and evaluated. Cerebrospinal fluid (CSF) from 232 patients suspected for meningitis was collected and tested for EV and HPeV using RT-qPCR assays. In parallel an aliquot of the samples was tested using the GXEA and viral culture.

EV RNA was detected in 22 (19.0%) and 28 (24.1%) of 116 samples using the GXEA and RT-qPCR assay, respectively. EV was isolated from 10 of 116 (8.6%) samples by viral culture. GXEA had a sensitivity, specificity, positive predictive value and negative predictive value of 82.1%, 100%, 100% and 96.2%, respectively.

In this study, molecular assays were superior to viral culture for detecting EV RNA in CSF. GXEA showed a high specificity but a lower sensitivity for the detection of EV RNA compared to the RT-qPCR assay.

1. Introduction

Human non-polio enterovirus (EV) is a major cause of infection in children, with 10 to 30 million infections annually, especially in neonates and young infants (Hyppiä et al., 1992; Khetsuriani et al., 2006b; Nijhuis et al., 2002; Pichichero et al., 1998; Rotbart et al., 1998). Recently, human parechovirus (HPeV) types 1–18 have been described and show epidemiological and clinical characteristics similar to EV (Khetsuriani et al., 2006b). The clinical spectrum of EV and HPeV infections varies from nonspecific febrile illness to severe systemic illnesses involving the central nervous system (CNS) (Khetsuriani et al., 2006b; Modlin, 2009, 3rd edition; Rittichier et al., 2005; Wolthers et al., 2008).

EV infections are the most common cause of aseptic meningitis and account for 80–90% of all cases of CNS infections for which a possible causative agent is identified (Rotbart et al., 1994). EV infections are associated with significant morbidity and mortality in neonates, particularly ante- or perinatal infections. EV infections have also been associated with severe neurodevelopmental sequelae (Chang et al., 2007; Huang et al., 1999; Rittichier et al., 2005).

Viral culture used to be the "gold standard" for the diagnosis of EV infection in different clinical specimens such as feces, throat swabs and cerebrospinal fluid (CSF). However, viral culture takes 4–8 days and the diagnosis is often too late to influence clinical decision making (Sawyer, 2002). Sensitivity of viral culture is relatively low (53–75%) and some EV serotypes grow poorly (Byington et al., 1999; Ramers et al., 2000; Rotbart et al., 1997; Sawyer, 1999, 2002; Stellrecht et al., 2000). A rapid diagnostic test may improve the management of patients with EV infections. Rapid diagnostic tests include nucleic acid amplification technology, such as reverse-transcriptase (RT) quantitative real-time PCR (qPCR) and nucleic acid sequence-based amplification (NASBA) (Fox et al., 2002; Kämmerer et al., 1994; Manayani et al., 2002; Nicholson et al., 1994; Read and Kurtz, 1999; Rotbart et al., 1994;

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; EV, enterovirus; GXEA, GeneXpert enterovirus assay; HPeV, human parechovirus; NASBA, nucleic acid sequence-based amplification; RT-qPCR, reverse-transcriptase (RT) quantitative real-time PCR; tMK cells, tertiary Cynomolgus monkey kidney cells.

* Corresponding author at: Laboratory of Medical Microbiology and Immunology, St. Elisabeth Hospital, Hilvarenbeekseweg 60, 5022 LC, Tilburg, The Netherlands. Tel.: +31 13 5391313; fax: +31 13 5359433.
E-mail address: j.rossen@elisabeth.nl (J.W.A. Rossen).

[1] Deceased.

0166-0934/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jviromet.2011.10.007

Table 1
Primer and probes used for real-time PCR detection.

Virus	Target	Forward primer (s) (5′–3′)	Reverse primer (s) (5′–3′)	Probe(s)[a]	Reference
Enterovirus	5′ UTR	TCC TCC GGC CCC TGA	AAT TGT CAC CAT AAG CAG CCA	6FAM-CGG AAC CGA CTA CTT TGG GTG ACC GT	Vuorinen et al. (2003)
			GAT TGT CAC CAT AAG CAG CCA	6FAM-CGG AAC CGA CTA CTT TGG GTG TCC GT	
HPeV	5′ UTR	TGC AAA CAC TAG TTG TAW GGC CC	TCA GAT CCA TAG TGY CAC TTG TTA CCT	6FAM-CGA AGG ATG CCC AGA AGG TAC CG	This study
			TCA GAT CCA CAG TGT CTC TTG TTA CCT		

[a] FAM, 6-carboxyfluorescein.

Zoll et al., 1992). The advantages of RT-qPCR are the small amounts of samples required and its rapidity (7–24 h), high sensitivity and specificity (almost 100%) (Ahmed et al., 1997; Byington et al., 1999; Khetsuriani et al., 2006a; Rittichier et al., 2005; Sawyer, 2002). Nowadays traditional viral culture has therefore been replaced by nucleic acid amplification tests as the gold standard for detection of EV in CSF.

The GeneXpert enterovirus assay (GXEA) is designed as an integrated system combining specimen processing, EV amplification and detection in a disposable cartridge which takes 2.5 h to detect EV in CSF (Kost et al., 2007; Marlowe et al., 2008; Seme et al., 2008).

The aim of this study was to evaluate the performance of the GXEA for the detection of EV RNA compared to a user-developed RT-qPCR in routine clinical practice. In addition, a RT-qPCR assay for detection of HPeV RNA in different clinical samples was developed and evaluated.

2. Materials and methods

2.1. Clinical specimens

CSF of 232 patients suspected with meningitis was collected from May 2007 till December 2009 and tested for the presence of EV and HPeV RNA, using RT-qPCR assays based on the 5′ UTR region of the EV and HPeV genome, respectively. In parallel, an aliquot of the samples was tested using the GXEA. Of 116 samples enough material was still available to perform a viral culture on tertiary Cynomolgus monkey kidney (tMK) cells. Samples were stored at 4 °C before being used within 16 h of receipt.

2.2. Viral RNA isolation

An aliquot of the samples (200 μl) was used to extract viral RNA using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Basel, Switzerland) as described previously (van de Pol et al., 2006). Each sample was eluted in 50 μl buffer. All samples had been spiked before extraction with an internal control virus (phocine distemper virus) to monitor for efficient extraction and amplification, as described previously (van Doornum et al., 2003).

2.3. RT-qPCR

The isolated viral RNA was reverse transcribed using MultiScribe RT and random hexamers (both from Applied Biosystems, Foster City, CA, l). Detection of EV was performed using a RT-qPCR assay as previously described (Nijhuis et al., 2002). In addition, an in-house RT-qPCR assay was developed for the detection of HPeV using Primer Express (Applied Biosystems). Conserved target regions were identified using BLAST (www.ncbi.nlm.nih.gov/blast). Sequences of the primers and probes used are summarized in Table 1. Potential cross-reactivity of the HPeV-specific assay with other related picornaviruses was excluded by using the RT-qPCR assay on samples known to contain RNA of Coxsackievirus A (A2, A9, A13, A14, A16, A24), Coxsackievirus B (B1, B3, B4, B5), Echovirus (1–7, 9, 12, 14, 15, 17, 19–21, 24–27, 29, 31, 32), Enterovirus 71, Poliovirus 1, Poliovirus 3 and/or Rhinoviruses. Real-time PCR procedures were performed as described previously (van de Pol et al., 2006).

2.4. GeneXpert

The GXEA was performed according to the manufacturer's instructions (Cepheid, Sunnyvale, CA, USA) within 16 h of receipt of the samples as previously described (Kost et al., 2007; Marlowe et al., 2008). In short, 140 μl of CSF was added to the GeneXpert cartridge and then processed automatically for the different steps of sample preparation and amplification. Results were available within 2.5 h.

2.5. Viral culture

Viral culture was performed on confluent layers of tMK cells. After inoculation of 0.25 ml of clinical specimen and absorption to the cells for 1 h, 1 ml of culture medium was added and cells, maintained at 37 °C on roller drums, were examined daily during 14 days for a cytopathic effect. Typing of the virus isolates was carried out by neutralization or complement fixation with intersecting antiserum pools by standard procedures.

3. Results

A RT-qPCR specific for the detection of HPeV was developed (Table 1). As both EV and HPeV belong to the picornavirus family, the specificity of the RT-qPCR was tested for cross-reactivity with samples known to contain picornavirus RNA. No detection of other picornaviruses was observed (data not shown). Results of the user-developed Rt-qPCR assay were taken as the gold standard.

A total of 232 patients were included in this study. The mean age was 28.8 years (range 0–84.9 years). There were more males than females (ratio 1.3:1).

EV was isolated from 10 of 116 (8.6%) samples with viral culture. EV RNA was detected in 22 (19.0%) and 28 (24.1%) of these samples using the GXEA and RT-qPCR assay, respectively (Table 2). Two viral culture positive samples were negative for both molecular assays.

All 232 samples were tested with the GXEA and RT-qPCR assays. EV RNA was detected in 32 (13.8%) and 40 (17.2%) of these samples, respectively (Table 3).

Fifteen (6.5%) of the 232 samples tested with the GXEA gave an invalid result. No invalid results were reported using the RT-qPCR assay. In 8 patients (20%) EV RNA was detected with the RT-qPCR assay. They would have been missed if only a GXEA was performed. In 4 of these 8 patients EV RNA was also detected by RT-qPCR assay in feces and throat swabs of the same subject, making the possibility of a false positive result, generated by this assay, unlikely. Unfortunately, no additional material of the other 4 patients was available.