DETECTION OF PARECHOVIRUS USING REAL-TIME QUANTITATIVE RT-PCR (REFERENCE – 2014.02.001)

Notice of Assessment

December 2014

1. GENERAL INFORMATION

1.1 Requester: CHU Sainte-Justine

1.2 Application for Review Submitted to MSSS: January 15, 2014

1.3 Application Received by INESSS: May 10, 2014

1.4 Notice Issued: October 31, 2014

> **Note:**
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> This notice is based on the scientific and commercial information submitted by the requester and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

2. TECHNOLOGY, COMPANY, AND LICENCE(S)

2.1 Name of the Technology

Real-time polymerase chain reaction (reverse transcription-quantitative polymerase chain reaction or RT-qPCR) for the detection of human parechovirus (HPeV) in blood and cerebrospinal fluid (CSF) samples.

2.2 Brief Description of the Technology, and Clinical and Technical Specifications

RT-qPCR testing detects and quantifies HPeV. The methodology was published by the authors [Renaud et al., 2011]. The purpose of this technique is to quantify viral particles by determining the number of HPeV genome copies present in the patient's blood or CSF. The RNA of the virus is first transcribed into DNA by the reverse transcriptase enzyme. It is then amplified and quantified with qPCR using an integrated standard curve constructed with quantified RNA controls. The assay involves the use of a hydrolysis probe (TaqMan). A hydrolysis probe is a probe to which a fluorescent agent and a fluorescence inhibitor are bound. When the DNA is amplified, the probe binds to the template and, as a result of Taq polymerase enzyme activity, the probe binds to the amplification product, and its inhibitor is cleaved, which allows the amplification product to be detected and quantified. The test is carried out using the ABI 7500 system. The test targets a non-coding sequence located at the 5' end of the genome (5' untranslated region: 5' UTR); this sequence is conserved among HPeV strains. The reaction mixture also contains an internal control composed of an csG4 (chlorophyll synthase G4 gene) RNA, its primers, and a specific probe that detects it. The transcription, amplification, and detection steps are thus controlled [Renaud et al., 2011].

2.3 Company or Developer

The method was developed by the requester at the University of Washington (Seattle, US) Molecular Virology Laboratory and published in 2011 [Renaud et al., 2011]. The information submitted by the requester indicates that changes have been made to the primers and the internal control used.

2.4 Licence(s): Not applicable.

2.5 Patent, If Any: Not applicable.

2.6 Approval Status (Health Canada, FDA): Not applicable; in-house protocol.

2.7 Weighted Value: 42.0

3. CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patient Group

- Patients under 18 years of age with clinical manifestations of meningitis or encephalitis with or without compatible imaging findings.
- Patients under 3 months with a clinical presentation of sepsis, with or without fulminant hepatitis or coagulopathy.
- Adults with a clinical presentation of encephalitis; the test may then be specifically ordered (otherwise, adults will be excluded).

3.2 Targeted Disease(s)

Parechoviruses belong to the family *Picornaviridae*, as do enteroviruses (EV). The *Parechovirus* genus is composed of two species, the Ljungan virus and the human parechovirus (HPeV), of which 16 types have been documented.[1]

Human parechoviruses are often the cause of pediatric infections [Esposito et al., 2014], in immunocompetent as well immunocompromised[2] individuals [Rahimi et al., 2013]. A Finnish study showed that 8 out of 9 (89%) children had antibodies against HPeV1 before the age of 2 years, and a Japanese study showed that 17 out of 20 (85%) children between the ages of 4 years and 6 years were seropositive for HPeV3 [Ito et al., 2004; Joki-Korpela and Hyypia, 1998]. These data reveal that almost all cases of HPeV infection occur in children under the age of 5, and that the majority of these infections affect very young children, as shown in the table in the Appendix.

HPeV infections may be associated with various clinical presentations: asymptomatic infection, benign disease, or more severe disease such as meningitis, encephalitis, and sepsis-like syndrome [Esposito et al., 2014]. These more serious conditions can be mistaken for bacterial meningitis, bacterial encephalitis, and sepsis [De Crom et al., 2012]. Although cases of fulminant hepatitis or disseminated intravascular coagulation syndrome have been reported (as by the requester), HPeV infections generally have a favourable outcome, and patients may be discharged soon after their diagnosis [Schuffenecker et al., 2012; Renaud et al., 2011]. Treatment for symptomatic patients is limited to supportive care, since no antiviral medication is effective against HPeV [Levorson and Jantausch, 2009]. Fever is one of the most frequently used symptoms to estimate the duration of the infection; the authors estimate the median duration to be 2 to 3 days [Felsenstein et al., 2014; Jeziorsky et al., 2014]. The majority of patients are discharged 3 to 5 days following an infection [De Crom et al., 2013; Wolthers et al., 2008]. HPeV and enteroviruses have similar clinical characteristics, frequency, and severity in young children [Fischer et al., 2014].

1. Pirbright Institute. Human parechovirus [website]. Available at: http://www.picornaviridae.com/parechovirus/hpev/hpev.htm (accessed August 12, 2014).
2. Public Health England (PHE). UK Standards for Microbiology Investigations: Meningoencephalitis. London, England: PHE; 2014. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/344105/S_5i1.pdf (accessed July 8, 2014).

In general, patients are tested for HPeV if they have clinical presentations of sepsis or aseptic meningoencephalitis.[3] In these cases, rates of seropositivity for HPeV in blood and CSF samples range from 0.7% to 43% (see Appendix) [Rahimi et al., 2013; Sharp et al., 2013; Harvala et al., 2009]. HPeV infections also have a seasonal and biannual periodicity[4] [Renaud et al., 2011].

Clinical management of patients with aseptic meningitis or sepsis-like syndrome is based on supportive care and the administration of empirical antibiotic treatment until bacterial infection has been ruled out (e.g., viral identification or negative bacterial culture). Additional diagnostic tests may also be performed (magnetic resonance imaging, chest or abdominal radiograph, electroencephalogram) [Ramers et al., 2000].

3.3 Number of Patients Targeted

The requester estimates that 1,000 tests for human parechovirus using RT-qPCR will be performed each year in Quebec.

3.4 Medical Specialties and Other Professions Involved

Microbiology, infectious disease, pediatrics.

3.5 Testing Procedure

This test may be performed on whole blood and CSF samples.

The requester anticipates a turnaround of three days. The test will be carried out three times per week.

4. TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology

This is a unique test; no other test to detect human parechovirus is available in Quebec.

The National Microbiology Laboratory (NML) in Winnipeg performs the molecular detection and typing of parechovirus by RT-PCR with a turnaround time of 21 days for viral isolates/culture, CSF, stool, and other specimens.[5]

4.2 Brief Description of the Current Technological Context

RT-qPCR is the gold standard for the detection of HPeV, as it is faster, more sensitive, and simpler to use than culture [Harvala et al., 2014; de Crom et al., 2012].

Viral culture for HPeV is complex, since culture conditions and cell lines vary from one HPeV type to another. Therefore, conventional viral culture methods are not suited to HPeV detection [Selvaraju et al., 2013]. Moreover, these methods are being used less often, as their turnaround time is too long to be useful in the treatment of patients.

Although serological methods have been developed for the detection of various HPeV strains, typing reagents for HPeV3 to HPeV6 are not widely used [Nix et al., 2008; Joki-

3. Aseptic meningitis: meningitis that is not caused by pyogenic bacteria. Although it is usually caused by certain viruses, it can have infectious or non-infectious causes (Ramachandran T.S. Aseptic meningitis [website]. Medscape; 2014. Available at: http://emedicine.medscape.com/article/1169489-overview (accessed August 7, 2014)).
4. Cyclical periodicity with cases appearing every 2 years.
5. National Microbiology Laboratory (NML). Enteroviruses – Parechoviruses [website]. Available at: https://www.nml-lnm.gc.ca/guide2/pathogen_engview.php?refdiagID=301 (accessed July 8, 2014).

Korpela and Hyypia, 1998]. High rates of seropositivity for HPeV from a young age make serological results difficult to interpret [Ito et al., 2004].

4.3 Brief Description of the Advantages Cited for the New Technology

Literature searches have highlighted the following advantages regarding the rapid identification of human parechovirus with RT-qPCR:

- shorter hospital stays [Rahimi et al., 2013; Walters et al., 2011; Wolthers et al., 2008];
- promotion of appropriate antibiotic use, shorter duration of antibiotic treatment, as well as cessation of antibiotics when the diagnosis is confirmed [Rahimi et al., 2013; Shoji et al., 2013; Walters et al., 2011; Wolthers et al., 2008];
- prevention of its transmission in intensive care units [Piralla et al., 2014].

4.4 Cost of Technology and Options: Not assessed.

5. EVIDENCE

5.1 Clinical Relevance

5.1.1 Other Tests Replaced

The detection of HPeV with RT-qPCR does not replace any test listed in the 2014-2015 edition of the Index. Nor does it replace the test carried out in the National Microbiology Laboratory (NML), since NML offers parechovirus typing and also includes the detection of the Ljungan virus (non-human parechovirus). Moreover, the NML's turnaround time, added to the specimen transport time, means that results are not obtained early enough to intervene effectively.

5.1.2 Diagnostic or Prognostic Value

The availability of a highly sensitive and rapid test (turnaround time of less than 3 hours), improves the clinical management of patients by reducing their hospital stay and the duration of antibiotic treatment [Bennett et al., 2011].

For example, a retrospective study of 15 neonates and young infants hospitalized for fever and sepsis-like syndrome (maculopapular and palmoplantar rash) showed that antibiotic treatment during hospitalization can be discontinued after real-time PCR detection of HPeV3 in serum and CSF samples [Shoji et al., 2013].

Patients who test positive do not have to undergo further diagnostic tests to determine the cause of their symptoms. Depending on how the patient's condition improves, he or she can be discharged more rapidly.

No treatment is currently available for HPeV infections [Levorson and Jantausch, 2009]. A positive result means a patient's antibiotic treatment can be discontinued, and a negative result does not compromise patient treatment.

Tests for HPeV should not produce additional adverse effects. In the situations where the tests would be used, blood or CSF samples need to be taken to test for enteroviruses and for basic bacterial cultures in any case.

Several authors (including the requester) have decided in favour of introducing tests to detect human parechovirus:

- HPeV testing should be performed with RT-qPCR in children under 3 months who have fever and other symptoms of sepsis during the summer and fall [Renaud et al., 2011];
- Tests for HPeV should be performed on CSF samples from children under the age of one who are hospitalized for sepsis or meningoencephalitis syndrome [Pineiro et al., 2010];
- Tests for parechovirus infections should be performed on neonates and infants who have a fever without an identifiable bacterial cause [Eyssette-Guerreau et al., 2013];
- HPeV testing with RT-qPCR should be performed on children under the age of 6 months who have symptoms of sepsis during the summer and fall [Sharp et al., 2013].

5.2 Clinical Validity

COMPONENT	PRESENCE	ABSENCE	NOT APPLICABLE
Sensitivity	X		
Specificity	X		
Positive predictive value (PPV)	X		
Negative predictive value (NPV)	X		
Likelihood ratio (LR)		X	
ROC curve		X	
Accuracy		X	

Sensitivity and Specificity

A Dutch prospective study was conducted from 2008 to 2011 on 285 patients aged 0 years to 16 years with systemic symptoms of meningitis, diarrhea, respiratory tract infections, and other. RT-qPCR was used to identify 44 cases of HPeV infection, 16 of which were cases of meningitis. The results of this study demonstrate the diagnostic performance of RT-qPCR and viral culture on different specimens. RT-qPCR was performed on 281 stool, 296 nasopharyngeal, 189 blood, 250 urine, and 141 CSF samples. Viral culture was used to test 159 stool, 146 nasopharyngeal, and 80 CSF samples. Patients were considered to have tested positive if at least one type of sample was positive using one of the two techniques. This creates a bias in the calculations of the first table for the specificity and positive predictive value, which are 100% in every case with positive tests.

Table 1: Comparison of the diagnostic performance of rt-qpcr and viral culture for the detection of HPeV in different samples

	STOOL		NASOPHARYNGEAL SWABS		CSF		BLOOD	URINE
	RT-qPCR	Viral culture	RT-qPCR	Viral culture	RT-qPCR	Viral culture	RT-qPCR	RT-qPCR
	%							
SENSITIVITY	95.1	18.5	63.6	0	84.2	0	79.3	56.8
SPECIFICITY	100	100	100	100	100	100	100	100
PPV	100	100	100	-	100	-	100	100
NPV	99.0	84.3	93.1	86.3	97.4	81.6	95.7	92.0

Adapted from Table 3a, De Crom et al., 2013.

The sensitivity of RT-qPCR is higher than that of viral culture regardless of the type of specimen being tested. The sensitivity of RT-qPCR on blood and CSF samples is 84.2% and 79.3%, respectively, whether the sampling is composed of patients with symptoms of meningitis, patients with systemic symptoms, or patients with other types of symptoms. Tables 2 and 3 present results for the same parameters, based on whether the diagnosis is that of a systemic HPeV infection with or without meningitis.

Table 2: Comparison of the diagnostic performance of RT-qPCR and viral culture for HPeV in cases of systemic infection without meningitis

	STOOL		NASOPHARYNGEAL SWABS		BLOOD	URINE
	RT-qPCR	Viral culture	RT-qPCR	Viral culture	RT-qPCR	RT-qPCR
	%					
SENSITIVITY	100	22.2	73.3	0	100	76.9
SPECIFICITY	9.1	88.9	58.3	100	85.7	90.9
PPV	74.4	80.0	81.5	-	95.7	95.2
NPV	-	36.4	46.7	26.3	100	62.5

Adapted from Table 3b, De Crom et al., 2013.

Testing for parechovirus on blood samples is ideal for the diagnosis of systemic HPeV infection (sensitivity: 100%; specificity: 85%). The specificity obtained with blood samples is 85%, since the cases of meningitis also tested positive from blood tests. Tests carried out on stool samples are very sensitive (100%), but they are not specific for systemic infections (9.1%).

Table 3: Comparison of the diagnostic performance of RT-qPCR and viral culture for HPeV in the case of meningitis

	STOOL		NASOPHARYNGEAL SWABS		CSF		BLOOD	URINE
	RT-qPCR	Viral culture	RT-qPCR	Viral culture	RT-qPCR	Viral culture	RT-qPCR	RT-qPCR
	%							
SENSITIVITY	100	0	81.3	0	100	0	100	73.3
SPECIFICITY	0	100	0	100	100	100	0	50.0
PPV	84.2	-	81.3	-	100	-	66.7	92.3
NPV	-	18.2	0	16.7	100	14.3	-	20.0

Adapted from Table 3c, De Crom et al., 2013.

For the diagnosis of meningitis, RT-qPCR shows a sensitivity of 100% with stool, CSF, and blood samples. HPeV-positive stool and blood samples are not specific to a meningitis infection. Although the specificity is 100% in CSF samples, this type of sample was taken only when indicated by the clinical presentation. Cases of meningitis are defined as symptomatic with the presence of HPeV in the CSF. Therefore, biases may be noted in the rates of sensitivity, specificity, PPV, and NPV for CSF samples.

The first study in which RT-qPCR was used shows a sensitivity of 100% among 36 clinical specimens comprising CSF and stool samples, rectal and nasopharyngeal swabs, and lung and spleen tissues containing HPeV1 to HPeV4 and HPeV6 (confirmed with sequencing) [Nix et al., 2008].

During a clinical validation study of the RT-qPCR method for the detection of HPeV, 33 patients were tested using RT-qPCR on blood and CSF samples. Three patients had a positive reaction in the two sample types, and thirty had a negative reaction in both sample types [Noordhoek et al., 2008]. However, the study did not include a method to confirm the positive results (for example, sequencing).

Nix et al. developed a nested PCR[6] method, which is known for its specificity, and tested it for the detection of parechovirus on 107 clinical specimens confirmed positive by RT-qPCR. They obtained a sensitivity of 94% (CSF: 7/8, stool: 86/92, rectal swabs: 3/3, nasopharyngeal swabs: 2/2, spleen: 2/2) [Nix et al., 2010].

In a US retrospective study, RT-qPCR showed a specificity of 100% with a bank of 867 clinical specimens of CSF, which supports direct use of the test on clinical specimens [Renaud et al., 2011].

The RT-qPCR method did not yield any positive results for HPeV when 129 clinical specimens of CSF containing Coxsackievirus (A9, B4, B5), echovirus (6, 7, 9, 11, 13, 15, 18, 30), and enterovirus 71 were tested [Nix et al., 2008].

6. "Nested" PCR is a two-step PCR procedure involving 2 pairs of primers specific to the same DNA sequence. The first step of 10 cycles is carried out with the first pair of primers, and an excess from the second pair of primers is then added before extending the reaction to approximately 25 cycles. This technique increases the level of specificity for homologous sequences (Université Pierre et Marie Curie, Faculté de médecine Pitié-Salpêtrière (FMPMC-PS). Biologie génique – Objectifs au cours de formation de base IFTAB [website]. Available at: http://www.chups.jussieu.fr/polys/biochimie/BGbioch/POLY.Chp.8.11.html (accessed August 8, 2014)).

5.3 Analytical (or Technical) Validity

COMPONENT	PRESENCE	ABSENCE	NOT APPLICABLE
Repeatability		X	
Reproducibility	X		
Analytical sensitivity	X		
Analytical specificity	X		
Matrix effect		X	
Concordance	X		
Correlation between test and comparator		X	

Sensitivity

RT-qPCR showed a sensitivity of 100% (30/30) after 30 cycles with specimens containing 30 copies, and a sensitivity of 80% (24/30) with specimens containing 10 copies of HPeV1 RNA. At 3 copies, 11/30 (36.7%) were positive [Nix et al., 2008]. The authors report that RT-qPCR is 100 to 1,000 times more sensitive than viral culture [Nix et al., 2008].

The sensitivity of the PCR primers was tested on strains of parechovirus (reference strains and clinical isolates), and RT-qPCR successfully amplified all the strains (32 strains of HPeV1, 4 of HPeV2, 2 of HPeV3, 4 of HPeV4, 9 of HPeV5, 1 of HPeV6, and 4 of Ljungan virus) [Nix et al., 2008].

Table 4: Various detection limits reported in the literature

STUDY	METHOD	TYPE OF HPEV VIRUS	DETECTION LIMIT	
			$CCID_{50}$/mL*	Number of copies/mL
Felsenstein et al., 2014	5 µL of nucleic acid solution	HPeV3 Other types HPeV3 Other types	32 1.6×10^3 to 3.2×10^5 1.6 7.9 to 1.6×10^3	
Selvaraju et al., 2013		HPeV3 With MS2† IC Without MS2 IC Other types Without MS2 IC	 $1 \times 10^{0.6}$ $1 \times 10^{1.6}$ $10^{0.001}$ and $10^{0.1}$	
Schuffenecker et al., 2012	Parechovirus r-gene by bioMérieux	HPeV1	13.9	
Bennett et al., 2011	Platinum RT-PCR EXPRESS qPCR	HPeV1		1.5×10^{3}§ 2.3×10^{3}§
Renaud et al., 2011		HPeV1		5×10^2
Van der Sanden et al., 2008		HPeV1 HPeV2	4×10^{4}‡ 1.6×10^{5}‡	2.7×10^{4}§ 1.1×10^{5}§

* CCID stands for *cell culture infectious dose*. $CCID_{50}$/mL is a measurement of the concentration of infectious agents contained in a solution. For example, a solution of 1 $CCID_{50}$/mL indicates that 1 mL of this solution has a 50% chance (or 5 out of 10 tubes) of infecting a cell culture; a solution of 14 $CCID_{50}$/mL is 14 times more concentrated than its $CCID_{50}$/mL. Therefore, the greater the $CCID_{50}$/mL, the greater the concentration of infectious agents in the solution.

† Bacteriophage MS2, which has a single-stranded, positive-sense RNA genome.

‡The values were converted based on the articles; $10^{6.6} \times 10^{-2} = 4 \times 10^4$ and $10^{6.2} \times 10^{-1} = 1.6 \times 10^5$

§The number of copies per reaction was converted into concentration.

The variability among the detection limits established in the various studies is a result of $CCID_{50}$ values, which depend on the ability of HPeV to grow in the cell culture used. The limits established in this case are generally lower than those expected with viruses that have a more clearly standardized growth.

Specificity

The specificity of the PCR primers did not yield any positive results when it was tested with the following six viral strains: Coxsackievirus (A16, A24), echovirus 30, enterovirus 68, encephalomyocarditis virus, and Theiler murine encephalomyelitis virus [Nix et al., 2008].

There was no cross-reactivity of the HPeV RT-qPCR with 103 rhinovirus specimens obtained from viral culture and confirmed with RT-qPCR. However, a mild cross-reactivity of the RT-qPCR was observed with enterovirus-positive specimens (142) validated under the same conditions. The specimens of enterovirus included Coxsackievirus A, Coxsackievirus B3 to B5, echovirus 6, 9, 11, 30, and enterovirus 71 [Renaud et al., 2011]. High virus concentration in a viral culture may reduce the specificity of the test.

There was no cross-reactivity of HPeV with 84 viral culture specimens positive for EV [Van der Sanden et al., 2008].

No cross-reactivity was reported between HPeV RT-qPCR and the following pathogens:

- Influenza [A, A/H1N1, B] virus, parainfluenza [1 to 4] virus, respiratory syncytial virus, human metapneumovirus, coronavirus [229E, OC43, NL63, and HKU1], adenovirus, *Pneumocystis jirovecii*, *Mycoplasma pneumoniae*, norovirus, rotavirus, measles, herpesvirus (types 1 and 2), varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, and mumps virus [Bennett et al., 2011];
- Coxsackievirus (A2, A9, A13, A14, A16, A24, B1, B3, B4, B5), echovirus (1 to 7, 9, 12, 14, 15, 17, 19 to 21, 24 to 27, 29, 31, 32), enterovirus 71, poliovirus (1 and 3) and rhinovirus [De Crom et al., 2012];
- Herpesvirus (types 1 and 2), varicella-zoster virus, adenovirus, respiratory syncytial virus (A and B), parainfluenza (1 to 3), influenza (A and B), human metapneumovirus, rhinovirus, coronavirus (OC43, 229E, NL63), norovirus, rotavirus, sapovirus, and astrovirus [Nielsen et al., 2013];
- Coxsackievirus A9 and B6, echovirus 1, rhinovirus, norovirus, influenza, parainfluenza, human metapneumovirus, respiratory syncytial virus, adenovirus, BK virus, cytomegalovirus, Epstein-Barr virus, herpesvirus, human herpesvirus, and varicella-zoster virus [Felsenstein et al., 2014];
- Coxsackievirus (A9, B1, B2, B3, B4, B5), echovirus (3, 4, 5, 6, 7, 9, 11), rhinovirus (8, 13, 26, 27, 39), herpesvirus 1 and 2, human herpesvirus 6, cytomegalovirus, and Epstein-Barr virus [Selvaraju et al., 2013];
- There is no cross-reactivity of the Parechovirus r-gene kit, referred to by Schuffenecker, with 26 strains of non-HPeV *Picornaviridae*. Among these are Coxsackievirus, echovirus, enterovirus, poliovirus, and rhinovirus [Schuffenecker *et al.*, 2012].

Concordance

A concordance of 96% was established on sequencing of 58 samples with a positive RT-qPCR test; 53 of these were successfully sequenced. Sequencing could not be performed on 3 samples and failed for the last 2 samples, despite repeated attempts [Selvarangan et al., 2011].

Bennett et al. tested 120 CSF samples using both the multiplex RT-qPCR method and an in-house nested PCR method. They obtained a concordance of 100% (7 HPeV positive and 113 negative) [Bennett et al., 2011].

Seventy clinical samples (33 positive and 37 negative), for which results were established with RT-PCR, were tested using HPeV3-specific RT-qPCR and a pan-HPeV assay. The resulting concordances were 98.6% and 100%, respectively [Selvaraju et al., 2013].

Reproducibility

Bennett et al. assessed the intra-assay and inter-assay reproducibility of their in-house RT-qPCR method with the Platinum one-step kit and the Express qPCR one-step kit [Bennett et al., 2011]. The assays were carried out 20 times in both cases. The resulting reproducibility was high, since the variation was low (Table 5).

Table 5: Reproducibility of RT-qPCR with the Platinum RT-PCR and Express qPCR reagent kits

TYPE OF REPRODUCIBILITY	METHOD	DETECTION				COEFFICIENT OF VARIATION
		Mean threshold (cycle)	Standard deviation	Max*	Min*	
Inter-assay	Platinum RT-PCR	27.02	0.48	28.45	25.6	0.0178
	Express qPCR	26.86	0.32	27.31	26.23	0.0119
Intra-essay	Platinum RT-PCR	27.34	0.28	28.81	26.51	0.0102
	Express qPCR	27.54	0.35	28.03	27.02	0.0127

Adapted from Table 2, Bennett et al., 2011.

*The maximum and minimum represent the mean threshold value ± 3 standard deviations.

The reproducibility of an in-house RT-qPCR method was analyzed using HPeV3 diluted to $1 \times 10^{1.6}$ $CCID_{50}$/mL. Reactions were run in triplicate on 3 different days. The author obtained excellent reproducibility, finding an intra-assay variation of 0.5 cycles of amplification (coefficient of variation of 1.3%) and inter-assay variation of 1 cycle of amplification (coefficient of variation of 2.8%) [Selvaraju et al., 2013].

5.4 Recommendations from Other Organizations

A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases, published by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM), refers to the use of nucleic acid amplification tests (NAAT) for the diagnosis of parechovirus in CSF samples in meningitis and encephalitis [Baron et al., 2013]. It also refers to stool tests using NAAT and viral culture in cases of gastroenteritis.

In the United Kingdom, parechovirus screening is regulated by the UK Standards for Microbiology Investigations, issued by Public Health England, which today encompasses the Health Protection Agency. The UK Standards for Microbiology state that parechovirus testing is performed on CSF samples using NAAT when the clinical presentation is suggestive of meningoencephalitis in children under the age of 3 and in immunocompromised patients

only.[7] Monitoring procedures have also been implemented in the United Kingdom since July 1, 2014, for infants younger than 90 days who have parechovirus meningitis.[8]

6. ANTICIPATED OUTCOMES OF INTRODUCING THE TEST

6.1 Impact on Material and Human Resources

Not assessed.

6.2 Economic Consequences of Introducing Test Into Quebec's Health Care and Social Services System

Not assessed.

6.3 Main Organizational, Ethical, and Other (Social, Legal, Political) Issues

Not assessed.

7. IN BRIEF

7.1 Clinical Relevance

A positive result for human parechovirus in CSF samples rules out bacterial meningitis or encephalitis, and in blood samples rules out bacterial sepsis (according to the symptoms). Obtaining these results promptly allows empirical antibiotic treatment to be discontinued, which in turn reduces the cost of treatment and exposure to antibiotics. It also allows patients whose clinical condition is not considered critical to be discharged and reduces the number of diagnostic procedures.

7.2 Clinical Validity

RT-qPCR showed a clinical sensitivity of 94% to 100% and a specificity of 100% for the detection of HPeV in cases of systemic infection and meningitis.

The detection of HPeV with RT-qPCR in blood samples, in cases of systemic infection without meningitis, yields a positive predictive value (PPV) greater than 95%, and detection in CSF, in cases of meningitis, yields a PPV of 100%.

The detection of HPeV by RT-qPCR in blood and CSF samples shows a negative predictive value greater than 95%.

7.3 Analytical Validity

RT-qPCR can be completed in three hours instead of several days—as is the case for viral culture—and its efficiency (sensitivity and specificity) is greater than that of viral culture. Results show high reproducibility and very high concordance.

Detection limits of 9 to 500 viral copies were established. Assays also showed detection limits ranging from $10^{0.001}$ to 3.2×10^5 $CCID_{50}$/mL. However, as cell growth for different HPeV types differs from one cell culture to another, the data are difficult to interpret. The detection limit of RT-qPCR was shown to increase by a factor of 10 when an internal control

7. Public Health England (PHE). UK Standards for Microbiology Investigations: Meningoencephalitis. London, England: PHE; 2014. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/344105/S_5i1.pdf (accessed July 8, 2014).
8. Public Health England (PHE). Enterovirus and Parechovirus meningitis in infants <90 days of age. Beginning 01 July 2014. Available at: http://www.his.org.uk/files/5414/0181/4935/Study_Flyer_Template_TO_MICROBIOLOGISTS_FINAL_s_k_29_5_14.pdf (accessed August 12, 2014).

(MS2) was used. RT-qPCR is considered to be 100 to 1,000 times more sensitive than viral culture for the detection of HPeV.

Moreover, 8 out of 9 studies showed no cross-reactivity with the numerous viruses tested. The ninth study showed a specificity of 97% in positive viral cultures (high-titre virus).

Reproducibility was established by intra-assay coefficients of variation of 1.0% to 1.3% and inter-assay coefficients of variation of 1.2% to 2.8%.

Concordance, ranging from 96% to 100%, was established among RT-qPCR, an HPeV3-specific RT-qPCR, a nested PCR, and the sequencing of positive results obtained.

7.4 Recommendations from Other Organizations

The Infectious Diseases Society of America (IDSA), the American Society for Microbiology (ASM), and Public Health England recommend the use of NAAT methods for the diagnosis of meningitis and parechovirus encephalitis. Moreover, Public Health England limits its use to infants under the age of 3 and immunocompromised patients.

8. INESSS NOTICE IN BRIEF

Detection of Parechovirus Using Real-Time Quantitative RT-PCR

Status of the Diagnostic Technology

- ☒ Established
- ☐ Innovative
- ☐ Experimental (for research purposes only)
- ☐ Replacement for technology:_____, which becomes obsolete

INESSS Recommendation

- ☒ Include test in the Index, conditional upon the development of an algorithm related to enterovirus testing. It is necessary to clearly establish whether the test will be performed sequentially or in parallel with NAAT for enterovirus (code 40061). In both cases, it must be determined whether the weighted values should be changed, as the extraction process is the same for both tests.
- ☐ Do not include test in the Index
- ☐ Reassess test

Additional Recommendation

- ☐ Draw connection with listing of drugs, if companion test
- ☐ Produce an optimal use manual
- ☐ Identify indicators, when monitoring is required

NOTE

The market availability of kits combining tests for both types of viruses on panels should also be considered.

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APPENDIX A

Epidemiology of Cases of HPeV Infection With Symptoms of Meningitis, Encephalitis, and Sepsis

STUDY (COUNTRY)	SAMPLES	NUMBER OF PATIENTS (AGE)	NUMBER OF POSITIVE CASES (%)	AGE OF POSITIVE CASES	DURATION OF FEVER (DAYS)	DURATION OF STAY (DAYS)
Abed and Boivin, 2006 (Canada)*	Various‡	28 (-)	28 (-)	27/28 < 4 years	-	Av. 3.7
De Crom et al., 2012 (Netherlands)	CSF	232 (0-85 years)	8 (3.4)	-	-	-
De Crom et al., 2013 (Netherlands)	Various‡	285 (< 16 years)	44 (15.0)	median 68 days	-	Med. 3
Escuret et al., 2013 (France)	CSF	380 (0-95 years)	9 (2.4)	< 2 years	-	Av. 4
		137 (< 2 years)	9 (6.6)			
		82 (< 3 months)	8 (9.7)			
Felsenstein et al., 2014 (United States)	CSF	440 (children)	12 (2.7)	< 5 years	Med. 2	-
		334 (< 5 years)	12 (3.6)			
Fischer et al., 2014 (Denmark)	Various‡	6,817 (children)	202 (3.0)	< 3 months	-	-
Ghanem-Zoubi et al., 2013 (Israel) †	CSF	367 (0-5 years)	13 (3.5)	< 3 months	-	Med. 4
Han et al., 2013 (South Korea) †	CSF	183 (1-15 years)	12 (6.5)	< 1 year	-	Av. 5.7
		105 (< 1 year)	12 (11.4)			
Harvala et al., 2009 (United Kingdom) †	CSF	1,575 (all ages)	14 (0.7)	< 3 months	-	-
		480 (< 3 months)	14 (2.9)			
Harvala et al., 2014 (United Kingdom)	Blood	128 (< 3 years)	7 (5.5)	-	-	-
Jeziorsky et al., 2014 (France)	CSF	120 (< 1 year)	9 (7.5)	-	Med. 3	Med. 3.5
Mirand et al., 2012 (France)	CSF	98 (0-77 years)	4 (4.1)	< 4 months	-	Med. 3.5
		67 (0-16 years)	4 (5.9)			
Nielsen et al., 2013 (Denmark)	Various‡	2,187 (all ages)	66 (3.0)	95% < 4 months	-	-
Noordhoek et al., 2008 (Netherlands)	Various‡	186 (all ages)	18 (9.7)	16/18 < 1 year	-	-
		106 (< 1 year)	16 (15.2)			
Pineiro et al., 2010 (Spain)	CSF	397 (< 12 months)	9 (2.3)	-	-	Av. 6.8
		265 (<2 months)	8 (3)			

STUDY (COUNTRY)	SAMPLES	NUMBER OF PATIENTS (AGE)	NUMBER OF POSITIVE CASES (%)	AGE OF POSITIVE CASES	DURATION OF FEVER (DAYS)	DURATION OF STAY (DAYS)
Piralla et al., 2014 (Italy)	CSF, blood	60 (< 1 months)	3 (5.0)	-	-	-
Rahimi et al., 2013 (Iran)	CSF	148 (< 8 years)	64 (43.0)	-	-	-
Renaud et al., 2011 (United States)	CSF, blood	499 (1-88 years)	17 (3.4)	< 3 months	3.5[§]	Av. 3
Schuffenecker et al., 2012 (France)	CSF	1,765 (< 5 years)	33 (2.9)	< 5 months		Av. 2.9
Selvarangan et al., 2011 (United States)	CSF	780 (children)	58 (7.0)	Av. 46 days	Av. 2.3	Av. 3.6
Sharp et al., 2013 (United States)	CSF	388 (1-18 years)	66 (17.0)	< 5 months	Av. 2.7	Av. 3.9
Shoji et al., 2013 (Japan)	CSF, blood	15 (-)	-	< 3 months	Med. 3	Med. 8
Walters et al., 2011 (United States)	CSF	421 (< 18 years)	10 (2.4)	< 2 months	-	-
		317 (< 3 years)	10 (3.2)			
Wolthers et al., 2008 (Netherlands)	CSF	716 (< 5 years)	33 (4.6)	Median 1.2 months	Med. 3	Med. 5
		662 (< 2 years)	32 (4.8)			

Abbreviations: CSF = cerebrospinal fluid; Med. = median; Av. = average.

*The test is conducted using PCR.

† These studies were carried out using nested PCR. This technique consists in amplifying DNA samples using two pairs of primers for a single locus. The first pair performs a regular amplification of the locus. The second pair of primers (nested primers) binds within the previously amplified sequence to produce a shorter PCR product. This type of PCR has improved specificity, since the amplification product depends on the specificity of two pairs of primers.

‡ Studies of the various samples did not focus solely on cases of meningitis, encephalitis, and sepsis.

§ The author does not indicate whether this is an average or median value.