Overview

Useful For
Aids in diagnosing enterovirus infections

Method Name
Real-Time Polymerase Chain Reaction (PCR)/RNA Probe Hybridization

NY State Available
Yes

Specimen

Specimen Type
Varies

Necessary Information
1. Specimen source is required.
2. Source information should include main anatomical site of collection.

Specimen Required
Submit a raw clinical sample (not a culture isolate) for enterovirus PCR. This test will detect enterovirus, but will not differentiate viruses in this family or provide serotyping information.

Submit only 1 of the following specimens:

Specimen Type: Body fluid
Sources: Pericardial, peritoneal, or pleural
Container/Tube: Sterile container
Specimen Volume: 0.5 mL
Collection Instructions: Do not centrifuge.

Specimen Type: Respiratory
Sources: Bronchial washing, bronchoalveolar lavage, nasopharyngeal aspirate, swab or washing, throat or nasal swab, sputum, or tracheal aspirate
Container/Tube: Sterile container
Specimen Volume: 1.5 mL

Specimen Type: Spinal fluid
Container/Tube: Sterile vial
Specimen Volume: 0.5 mL

Collection Instructions: Do not centrifuge.

Specimen Type: Swab

Supplies:
- Culturette (BBL Culture Swab) (T092)
- M4-RT (T605)

Sources: Dermal, eye, rectal, genital, nasopharyngeal, throat, nasal or urethral

Container/Tube: Multimicrobe media (M4-RT) (T605) or similar viral transport media (M4 or M5) and Eswab

Specimen Volume: Entire specimen

Collection Instructions:
1. Rectal swab must have no visible stool.
2. Place swab back into multimicrobe media (M4-RT, M4, or M5).

Forms
If not ordering electronically, complete, print, and send a Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume
Body Fluid, Spinal Fluid: 0.3 mL
Respiratory Specimen: 1 mL

Reject Due To

<table>
<thead>
<tr>
<th>Swabs</th>
<th>Calcium alginate-tipped swab, wood swab, or transport swab containing gel</th>
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Specimen Stability Information

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<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
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<tbody>
<tr>
<td>Varies</td>
<td>Refrigerated (preferred)</td>
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</tr>
<tr>
<td></td>
<td>Frozen</td>
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Clinical and Interpretive

Clinical Information
Enteroviruses are positive-sense RNA viruses in the Picornaviridae family. These viruses were initially classified by serotype as polioviruses (3 types), echoviruses (31 types, including types 22 and 23, which are now classified as parechoviruses), coxsackievirus A (23 types), and coxsackievirus B (6 types). However, genomic studies have
demonstrated that there is significant overlap in the biological characteristics of different serotypes and more recently isolated enteroviruses are now named with consecutive numbers (eg, EV68, EV69).

The normal site of enterovirus replication is the gastrointestinal tract where the infection is typically subclinical. However, in a proportion of cases, the virus spreads to other organs, causing systemic manifestations, including mild respiratory disease (eg, the common cold); conjunctivitis; hand, foot, and mouth disease; aseptic meningitis; myocarditis; and acute flaccid paralysis. Collectively, enteroviruses are the most common cause of upper respiratory tract disease in children. In addition, the enteroviruses are the most common cause of central nervous system (CNS) disease; they account for almost all viruses recovered in culture from spinal fluid. Differentiation of enteroviruses from other viruses and bacteria that cause CNS disease is important for the appropriate medical management of these patients.

Traditional cell culture methods require 6 days, on average, for enterovirus detection. In comparison, real-time PCR allows same-day detection. Detection of enterovirus nucleic acid by PCR is also the most sensitive diagnostic method for the diagnosis of CNS infection caused by these viruses.

**Reference Values**

**Negative**

**Interpretation**

A positive result indicates the presence of enterovirus RNA in the specimen.

**Cautions**

A negative result does not rule out the possibility of enterovirus infection.

This assay may detect virus from a variety of specimen types in asymptomatic individuals, including stool. This assay should only be used for patients with a clinical history and symptoms consistent with enterovirus infection, and must be interpreted in the context of the clinical picture. This test should not be used to screen asymptomatic patients.

This is a qualitative assay and results are reported as either negative or positive for targeted enterovirus RNA.

**Supportive Data**

**Accuracy/Diagnostic Sensitivity and Specificity:**

Mayo Clinic Laboratories compared the detection of enteroviruses from spinal fluid by conventional tube cell culture (MCR-5) and by LightCycler PCR. Of 715 specimens tested, enteroviruses were detected in 65 (9%) by conventional cell culture and 82 (11%) by LightCycler PCR. Twenty-two of 82 (27%) were exclusively positive by PCR; whereas, only 5 of 65 (8%) were exclusively positive by conventional cell cultures.

**Supplemental Data (Spiking Studies):**

To supplement the data above, 30 or more negative specimens of each specimen type (spinal fluid [CSF]/sterile body fluid, dermal/ocular/rectal swabs, plasma, and upper and lower respiratory specimens) were spiked with enterovirus culture control at approximately 10 to 50 targets/mcL (the approximate limit of detection). The spiked specimens were run in a blinded manner along with negative (nonspiked) specimens of each specimen type. Of the spiked specimens, 97% to 100% were positive, and 100% of the nonspiked specimens were negative. A total of 489 spiked and nonspiked specimens were tested.

**Assay Inclusivity:**

The assay detected all 64 members of an enterovirus panel, consisting of coxsackieviruses, polio viruses, echoviruses, and other enteroviruses. Importantly, the detection of parechovirus serotypes was found to be variable.
Analytical Specificity/Limit of Detection (LoD):

The lower LoD of this assay is approximately 10 to 50 RNA target copies/mcL. This was confirmed in all specimen types accepted for this assay.

Specificity:

The assay cannot reliably distinguish between enterovirus and rhinovirus. However, no cross-reactivity was observed when a specificity panel was tested, including influenza A/B, respiratory syncytial virus, parainfluenza virus, herpes simplex virus, Epstein-Barr virus, varicella zoster virus, and cytomegalovirus.

Reportable Range:

This is a qualitative assay and results are reported as either negative or positive for targeted enterovirus RNA.

Clinical Reference


Performance

Method Description

For this real-time reverse-transcription laboratory-developed PCR assay, viral nucleic acid is extracted by the MagNA Pure automated instrument (Roche Applied Science) from specimens, followed by amplification and detection on the Roche LightCycler 2.0 instrument. This PCR assay has been optimized to detect a target sequence in the polyprotein region. Primers amplify a 193-bp product.

Enterovirus genomic RNA is first transcribed to cDNA by reverse transcriptase, followed by amplification of the cDNA product. The LightCycler instrument can rapidly (30-40 minutes) detect amplicon development through stringent air-controlled temperature cycling in capillary cuvettes. The detection of amplified products is based on the fluorescence resonance energy transfer (FRET) principle. For FRET product detection, a hybridization probe with a donor fluorophore, fluorescein, on the 3'-end is excited by an external light source and emits light that is absorbed by a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5'-end. The acceptor fluorophore then emits a light of a different wavelength that can be measured with a signal that is proportional to the amount of specific PCR product. FRET (with subsequent production of a detectable fluorescent signal) only occurs when the probes have specifically annealed to the target sequence of the amplicon.

Melting-curve analysis is performed following PCR amplification and is the detection phase of the assay, since it offers greater sensitivity than the amplification phase and maintains high specificity.

The melting phase of the assay occurs as follows:

Starting at 45 degrees Celsius, which allows the probes to bind to the amplified product, the temperature in the
thermal chamber is then slowly raised to 80 degrees Celsius and the fluorescence is measured at frequent intervals to determine the point where half of the fluorescence is lost as the probes are denatured (ie, "melt") off of the target. This is called the melting temperature (Tm) of that virus. Analysis of the PCR amplification and probe melting curves is accomplished through the use of LightCycler software. (Cockerill FR III, Uhl JR; Applications and challenges of real-time PCR for the clinical microbiology laboratory. In Rapid Cycle Real-Time PCR Methods and Applications. Edited by U Reischl, et al. Germany, Springer, 2002, pp 3-30)

PDF Report
No

Day(s) and Time(s) Test Performed
Monday through Sunday; Varies

Analytic Time
Same day/1 day

Maximum Laboratory Time
3 days

Specimen Retention Time
1 week

Performing Laboratory Location
Rochester

Fees and Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
87498

LOINC® Information

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