MAYO CLINIC LABORATORIES

Test Definition: LENT

Enterovirus, Molecular Detection, PCR, Varies

Overview

Useful For

Aiding in diagnosing enterovirus infections

This test **should not be used** to screen asymptomatic patients.

Testing Algorithm

For information see Meningitis/Encephalitis Panel Algorithm

Special Instructions

- Meningitis/Encephalitis Panel Algorithm

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

This test will detect enterovirus but will not differentiate viruses in this family or provide serotyping information.

Necessary Information

1. Specimen source is required.

2. Source information must include main anatomical site of collection.

Specimen Required

Submit a raw clinical sample (**not a culture isolate**) for enterovirus testing.

Submit only 1 of the following specimens:

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

Specimen Type: Spinal fluid
Container/Tube: Sterile vial
Specimen Volume: 0.5 mL
Collection Instructions:
1. Submit specimen from collection vial 2.
2. Do not centrifuge.

Specimen Type: Swab
Sources: Dermal, eye, rectal, genital, nasopharyngeal, oropharyngeal, throat, nasal, or urethral
Supplies:
-Culturette (BBL Culture Swab) (T092)
-E-Swab (T853)
-M4-RT (T605)
Container/Tube: Multimicrobe media (M4-RT, M4, M5, Bartels, or Jiangsu) and E-Swab or Culturette
Specimen Volume: Entire specimen
Collection Instructions:
1. Rectal swab must have no visible fecal matter
2. Place swab back into multimicrobe media.

Specimen Type: Respiratory
Sources: Bronchial washing, bronchoalveolar lavage, nasopharyngeal aspirate or washing, pleural fluid, sputum, or tracheal aspirate
Container/Tube: Sterile container
Specimen Volume: 1.5 mL
Collection Instructions: Do not centrifuge.

Forms

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

Specimen Minimum Volume

Body and respiratory fluids: 0.5 mL; Spinal fluid: 0.3 mL; Swab: See Specimen Required

Reject Due To

Calcium alginate-tipped swab	Reject
Wood swab	Reject
Transport swab containing gel	Reject
Heat-inactivated specimen	Reject
Dry-flocked ESwab	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Refrigerated (preferred)	7 days	
	Frozen	7 days	

Clinical & 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 polymerase chain reaction (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 feces. 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 is a qualitative assay. 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 polymerase chain reaction (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/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 (non-spiked) specimens of each specimen type. Of the spiked specimens, 97% to 100% were positive, and 100% of the non-spiked specimens were negative. A total of 489 spiked and non-spiked 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:
The lower limit of detection 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

1. Khetsuriani N, Lamonte-Fowlkes A, Oberst S, et al. Enterovirus surveillance-United States, 1970-2005. MMWR Surveill Summ, 2006;55(8):1-20
2. Abedi GR, Watson JT, Nix WA, Oberste MS, Gerber S. Enterovirus and Parechovirus surveillance - United States, 2014-2016. MMWR Morb Mortal Wkly Rep. 2018;67(18):515–518
3. Foray S, Pailloud F, Thouvenot D, Aymard M, Lina B. Evaluation of combining upper respiratory tract swab samples with cerebrospinal fluid examination for the diagnosis of enteroviral meningitis in children. J Med Virol. 1999;57(2):193-197
4. Furione M, Zavattoni M, Gatti M, Percivalle E, Fioroni N, Gerna G. Rapid detection of enteroviral RNA in cerebrospinal fluid (CSF) from patients with aseptic meningitis by reverse transcription-nested polymerase chain reaction. New Microbiol. 1998;21(4):343-351

Performance

Method Description

For this real-time reverse-transcription laboratory-developed polymerase chain reaction (PCR) assay, viral nucleic acid is extracted from specimens, followed by amplification and detection on the Roche LightCycler 480 instrument. This PCR assay has been optimized to detect a target sequence in the polyprotein region. Primers amplify a 193 base-pair product.

Enterovirus genomic RNA is first transcribed to complementary DNA (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 light of a different wavelength that can be measured with a signal 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 C, which allows the probes to bind to the amplified product, the temperature in the thermal chamber is then slowly raised to 80 degrees C and the fluorescence 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. (Kies KD, Thomas AS, Binnicker MJ, Bashynski KL, Patel R. Decrease in Enteroviral Meningitis: An Unexpected Benefit of Coronavirus Disease 2019 [COVID-19] Mitigation. Clin Infect Dis. 2021;73(9):e2807-e2809; Bernard PS, Reiser A, Pritham GH. Mutation detection by fluorescent hybridization probe melting curves. In: Meuer S, Wittwer C, Nakagawara K, eds. Rapid Cycle Real-Time PCR Methods and Applications. Springer; 2012:11-20)

PDF Report

No

Day(s) Performed

Monday through Sunday

Report Available

2 to 3 days

Specimen Retention Time

7 days

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

87498

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
LENT	Enterovirus PCR	93856-3

Result ID	Test Result Name	Result LOINC® Value
SRC68	Specimen Source	31208-2
80066	Enterovirus PCR	93856-3