

## Overview

### Useful For

Aids in diagnosing enterovirus infections

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

### Testing Algorithm

See [Meningitis/Encephalitis Panel Algorithm](#) in Special Instructions.

### 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

### Advisory Information

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 should include main anatomical site of collection.**

### Specimen Required

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

**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:** Do not centrifuge.

**Specimen Type:** Swab

**Supplies:**

-Culturette (BBL Culture Swab) (T092)

-M4-RT (T605)

**Sources:** Dermal, eye, rectal, genital, urethral

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

**Specimen Volume:** Entire specimen

**Collection Instructions:**

1. Rectal swab must have no visible fecal matter
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

### Reject Due To

Swab	Calcium alginate-tipped swab, wood swab, or transport swab containing gel
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### Specimen Stability Information

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

## 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

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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 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 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,

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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**

1. Khetsuriani N: Enterovirus surveillance-United States, 1970-2005. MMWR Morbidity Mortality Weekly Report Sept 15 2006;55(SS08):1-20
2. Abedi GR, Watson JT, Nix WA, Oberste MS et al: Enterovirus and Parechovirus Surveillance - United States, 2014-2016. MMWR Morb Mortal Wkly Rep 2018;67:515-518
3. Foray S, Pailloud F, Thouvenot D, et al: Evaluation of combining upper respiratory tract swab samples with cerebrospinal fluid examination for the diagnosis of enteroviral meningitis in children. J Med Virology 1999;57(2):193-197
4. Furione M, Zavattoni M, Gatti M, et al: 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 PCR assay, viral nucleic acid is extracted 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 (T<sub>m</sub>) 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**

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



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Result ID	Test Result Name	Result LOINC Value
SRC68	Specimen Source	39111-0
80066	Enterovirus PCR	93856-3