# Test Definition: ENTP

Enterovirus, Molecular Detection, PCR, Plasma

## Overview

### Useful For

Aiding in diagnosing enterovirus infections using plasma specimens

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

### Method Name

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

### NY State Available

Yes

## Specimen

### Specimen Type

Plasma EDTA

### Ordering Guidance

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

### Specimen Required

Submit a raw clinical sample (not a culture isolate) for enterovirus polymerase chain reaction .

**Collection Container/Tube:** Lavender top (EDTA)
**Submission Container/Tube:** Screw-capped, sterile container
**Specimen Volume:** 1 mL
**Collection Instructions:** Centrifuge blood collection tube and aliquot plasma into plastic vial.

### Forms

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

### Specimen Minimum Volume

0.3 mL

### Reject Due To

| | |
|---|---|
| Gross hemolysis | Reject |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Plasma EDTA | 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, 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. 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.

### Supportive Data

Accuracy/Diagnostic Sensitivity and Specificity:
Mayo Clinic Laboratories compared the generic 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 (cerebrospinal 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.

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 did not crossreact with a specificity panel containing other RNA-containing viruses (rhinovirus; reovirus; influenza virus, types A and B; respiratory syncytial virus; and parainfluenza virus) and DNA-containing viruses (herpes simplex, 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. Harvala H, Broberg E, Benschop K, et al: Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. J Clin Virol. 2018 Apr;101:11-17
2. Khetsuriani N, Lamonte-Fowlkes A, Oberst S, et al: Enterovirus surveillance-United States, 1970-2005. MMWR Surveill Summ, 2006 Sep 15;55(8):1-20
3. Foray S, Pailloud F, Thouvenot D, Floret 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 Feb;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 Oct;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 using the MagNA Pure automated instrument (Roche Applied Science), 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 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.(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
Same day/1 to 5 days

### Specimen Retention Time
1 week

### 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.

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## 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 |
|---|---|---|
| ENTP | Enterovirus PCR, P | 97151-5 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 56068 | Enterovirus PCR, P | 97151-5 |