# Test Definition: FLARP

Free-Living Amebae, Molecular Detection, PCR, Varies

## Overview

### Useful For

Aids in the diagnosis of primary amebic meningoencephalitis and granulomatous amebic encephalitis in spinal fluid and tissue in conjunction with clinical and radiologic findings

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

### Testing Algorithm

For more information see Meningitis/Encephalitis Panel Algorithm.

### Special Instructions

- Meningitis/Encephalitis Panel Algorithm

### Highlights

This assay is intended as an aid in the diagnosis of primary amebic meningoencephalitis and granulomatous amebic encephalitis in conjunction with clinical and radiologic findings.

This test has similar sensitivity and specificity to culture but provides a more rapid result.

### Method Name

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

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

### Necessary Information

**Specimen source is required.**

### Specimen Required

**Submit only 1 of the following specimens:**

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

**Collection Instructions:** Send vial number 2.

**Specimen Type:** Tissue: Fresh
**Sources:** Brain, skin, lung
**Container/Tube:** Sterile container
**Specimen Volume:** 5 to 10 mm
**Collection Instructions:** Submit tissue in a sterile container with 1 mL of sterile saline or minimal essential media (MEM).

**Preferred: Paraffin-embedded tissue block:**
**Supplies:** Tissue Block Container (T553)
**Specimen Type:** Formalin-fixed, paraffin-embedded tissue block (FFPE)
**Sources:** Brain, skin, lung
**Container/Tube:** Tissue block
**Collection Instructions:** Submit a FFPE tissue block to be cut and returned.

**Acceptable: Paraffin-embedded tissue block:**
**Specimen Type:** Formalin-fixed, paraffin-embedded tissue block (FFPE)
**Sources:** Brain, skin, lung
**Container/Tube:** Sterile container for each individual cut section (scroll).
**Collection Instructions:** Perform microtomy and prepare five separate 10-micron sections. **Each section (scroll) must be placed in a separate sterile container for submission.**

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

### Specimen Minimum Volume
Spinal Fluid: 0.3 mL; Tissue: 5 mm biopsy

### Reject Due To
All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

### Specimen Stability Information

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

## Clinical & Interpretive

### Clinical Information
Several free-living amebae can infect the central nervous system (CNS) and cause devastating, usually fatal, disease. The route of entry and clinical course of infection varies with the type of ameba involved. *Naegleria fowleri* typically causes rapidly progressive primary amebic meningoencephalitis (PAM) in previously healthy children or adults. Infection is

acquired during contact with contaminated water, including swimming and diving in warm stagnant freshwater lakes and by nasal irrigation with nonsterile water. During contact, the amebae enter the nasal sinuses and travel along the olfactory nerve through the cribriform plate of the skull and into the CNS. PAM is almost uniformly fatal within several days of exposure. Because of the rarity of the infection and difficulty in initial detection, about 75% of diagnoses are made after the death of the patient. In contrast, *Acanthamoeba* species and *Balamuthia mandrillaris* usually cause a subacute CNS illness, usually in adults who are immunocompromised, called granulomatous amebic encephalitis (GAE). The presentation of GAE can mimic a brain abscess, aseptic or chronic meningitis, or CNS malignancy. The amebae usually disseminate to the CNS from the lungs or a primary skin lesion.

These amebae are usually identified by microscopic examination of cerebrospinal fluid or brain tissue and agar culture. Culture is more sensitive than microscopy alone but takes up to 7 days to produce a positive result. Also, *B mandrillaris* will not grow in routine culture. Real-time polymerase chain reaction assays offer a rapid and sensitive alternative to microscopy and culture.

### Reference Values

Negative

### Interpretation

A positive result indicates the presence of free-living amoeba DNA and is consistent with active or recent infection. While positive results are highly specific indicators of disease, they should be correlated with symptoms and clinical findings of primary amebic meningoencephalitis and granulomatous amebic encephalitis.

### Cautions

Primary amebic meningoencephalitis due to *Naegleria fowleri* is a rapidly fatal disease, and rapid detection may improve the likelihood of survival. The fastest way to make a diagnosis is through examination of spinal fluid for characteristic trophozoites. This should be performed in addition to polymerase chain reaction testing.

While this assay is designed to detect symptomatic infection with free-living amoeba species, the widespread distribution of these microscopic amebae in the environment may contaminate inanimate objects. Thus, testing should be reserved for patients with a clinical history and symptoms consistent with granulomatous amebic encephalitis and primary amebic meningoencephalitis.

Inadequate specimen collection or improper storage may invalidate test results.

Free-living amoeba DNA may be detectable for an unknown period of time after adequate treatment.

### Supportive Data

The following assay verification data supports the use of this assay for clinical testing.

Accuracy/Diagnostic Sensitivity and Specificity:

Species Inclusivity:
Thirty-seven strains of free-living amoeba were tested. All 20 *Acanthamoeba* species and all strains of *Naegleria fowleri* and *Balamuthia mandrillaris* were detected, while other *Naegleria* and *Balamuthia* species were not detected with this assay.

Results from this free-living ameba polymerase chain reaction (PCR) assay were compared to culture results on 6 cerebrospinal fluid (CSF) specimens. All were negative by both culture and PCR (100% concordance).

Twenty-eight formalin-fixed paraffin-embedded (FFPE) tissue blocks (up to 34 years old) were tested with the free-living ameba PCR assay and results were compared to histopathologic diagnosis. Nineteen of the tissues were positive by histopathology diagnosis. Of these 19, 15 were positive by PCR (83% sensitivity). Slides were not available for review of the histopathology-positive, PCR-negative cases, and thus the presence of organisms within the blocks tested by PCR could not be confirmed. Specificity was 100%.

Supplemental Data:
Spiking studies were performed using free-living amoeba negative CSF, fresh tissue, and FFPE tissue spiked with *Acanthamoeba, N fowleri*, and *B mandrillaris* genomic DNA. The sensitivity of the PCR assay was 97% to 100% and the specificity with spiked specimens was 100%. The samples were extracted and tested in a blinded fashion.

Analytical Sensitivity/Limit of Detection:

The limit of detection (LOD) established using genomic DNA spiked into CSF is:
*A polyphaga* ATCC 30173 - 2.5 target copies/mcL
*B mandrillaris,* ATCC PRA 290 - 3.05 target copies/mcL
*N fowleri,* ATCC 30896 - 1.16 target copies/mcL

The LOD established using genomic DNA spiked into fresh tissue matrix is:
*A polyphaga,* ATCC 30173 - 6.5 target copies/mcL
*B mandrillaris,* ATCC PRA 290 - 1.84 target copies/mcL
*N fowleri,* ATCC 30896 - 4.01 target copies/mcL

The LOD established using genomic DNA spiked into formalin-fixed paraffin-embedded tissue matrix is:
*A polyphaga,* ATCC 30173 - 5.7 target copies/mcL
*B mandrillaris,* ATCC PRA 290 - 21.94 target copies/mcL
*N fowleri,* ATCC 30896 - 7.82 target copies/mcL

Analytical Specificity:
No PCR signal was obtained from the extracts of 33 bacterial, viral, parasitic, and fungal isolates from similar organisms or from organisms commonly found in the specimens tested.

Precision:
Interassay precision was 100% and intra-assay precision was 100%.

Reference Range:
The reference range is "negative" for this assay. This assay is intended for detection of DNA from *Acanthamoeba* species, *N fowleri*, and *B mandrillaris* in CSF, and fresh and FFPE tissue (skin, lung, and brain) to aid in the diagnosis of primary amebic meningoencephalitis (PAM) and granulomatous amebic encephalitis (GAE) in conjunction with clinical findings.

Reportable Range:
This is a qualitative assay, and the results are reported as negative or positive for targeted free-living amoeba (*Acanthamoeba* species, *N fowleri*, and *B mandrillaris*) detected.

### Clinical Reference

1. Cope JR, Ali KM, Visvesvara GS: Pathogenic and opportunistic free-living amebae. In: Carroll KC, Pfaller MA, Landry ML, et al, eds. Manual of Clinical Microbiology. 12th Ed. ASM Press; 2019:chap142
2. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED): Parasites - *Acanthamoeba* - Granulomatous Amebic Encephalitis (GAE); Keratitis. CDC; Updated December 29, 2021. Accessed March 28, 2023. Available at www.cdc.gov/parasites/acanthamoeba/health_professionals/acanthamoeba_keratitis_hcp.html
3 Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED): *Naegleria fowleri* - Primary Amebic Meningoencephalitis (PAM) - Amebic Encephalitis. Updated July 7, 2022. Accessed February 19, 2023. Available at www.cdc.gov/parasites/naegleria/health_professionals.html

## Performance

### Method Description

The assay is performed on the Roche LightCycler (LC) 480 II instrument following DNA extraction on the Roche MagNA Pure or the Siemens Tissue Preparation System (TPS). The LC 480 II instrument is an automated instrument that amplifies and monitors the development of target nucleic acid (amplicon) after each polymerase chain reaction (PCR) cycle.

The DNA target for this PCR assay is a gene encoding the nuclear small subunit ribosomal 18S ribosomal RNA (rRNA).

The PCR is run as 2 separate assays: primers and probes for Acanthamoeba species and an internal control will comprise 1 reaction, while primes and probes for *Balamuthia mandrillaris* and *Naegleria fowleri* are contained in the second reaction. The forward and reverse primers are for template amplification and the TaqMan probes (FAM and CY5) are for detection. The FAM and CY5 probes contain a fluorophore (5'-end) and a quencher (3'-end) in close proximity; the quencher inhibits the fluorescence signal from the fluorophore while the probe is intact. After the probe anneals to the targeted ameba 18S rDNA, it is subsequently degraded by a DNA polymerase with 5'-3' exonuclease activity, resulting in release of the fluorophore and production of signal. Presence of the specific organism nucleic acid is confirmed by the presence of an amplification curve, which is used to determine if a specimen is positive or negative.(Qvarnstrom Y, Visvesvara GS, Sriram R, da Silva AJ: Multiplex real-time PCR assay for simultaneous detection of Acanthamoeba spp., Balamuthia mandrillaris, and Naegleria fowleri. J Clin Microbiol. 2006 Oct;44[10]:3589-3595; Connelly L, Anijeet D, Alexander CL: A descriptive case of persistent Acanthamoeba keratitis: raising awareness of this complex ocular disease. Access Microbiol. 2019 Nov 28;2[3]:acmi000084; Norgan AP, Sloan LM, Pritt BS: Detection of Naegleria fowleri, Acanthamoeba spp, and Balamuthia mandrillaris in formalin-fixed, paraffin-embedded tissues by real-time multiplex polymerase chain reaction. Am J Clin Pathol. 2019 Nov 4;152[6]:799-807)

### PDF Report

No

### Day(s) Performed
Monday through Saturday

### 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
87798 x 3
87999 (if appropriate for government payers)

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| FLARP | Free-living Amebae Detection, PCR | 96910-5 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| SSFLA | Specimen Source | 31208-2 |
| 38061 | Acanthamoeba species PCR | 41429-2 |
| 38063 | Balamuthia mandrillaris PCR | 41432-6 |
| 38062 | Naegleria fowleri PCR | 87758-9 |