MAYO CLINIC LABORATORIES

Test Definition: FLARP

Free-living Amebae Detection, PCR

Overview

Useful For

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

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

Highlights

This assay is intended as an aid in the diagnosis of primary amebic meningoencephalitis and granulomatous amebic encephalitis in conjunction with clinical 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: Cerebrospinal fluid

Container/Tube: Sterile container

Specimen Volume: 0.5 mL

Collection Instructions: Send vial #2.

Specimen Type: Tissue: Fresh

Sources: Brain, skin, lung

Container/Tube: Sterile container

Specimen Volume: 5-10 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.

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Minimum Volume

CSF: 0.3 mL; Tissue: 5 mm biopsy

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 may enter the nasal sinuses like *N fowleri* or can 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 offers 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 amoeba 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 were tested with the free-living amoeba 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. The internal control presence indicated no inhibition in these specimens. The sensitivity of PCR for clinical FFPE specimens is 83% and specificity is 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. Trabelsi H, Dendana A, Sellami A, et al: Pathogenic free-living amoebae: epidemiology and clinical review. Pathol Biol (Paris). 2012;60:399-405

2. Thompson PP, Kowalski RP, Shanks RM, Gordon YJ: Validation of real-time PCR for laboratory diagnosis of *Acanthamoeba* keratitis. J Clin Microbiol. 2008;46:3232-3236

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 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;44:3589-3595; 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)

PDF Report
No

Specimen Retention Time
7 days

Performing Laboratory Location
Rochester

Fees & Codes

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 US Food and Drug Administration.

CPT Code Information
87798 x 3

87798 (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	Reporting Name	LOINC®
SSFLA	Specimen Source	31208-2
38061	Acanthamoeba species PCR	41429-2
38063	Balamuthia mandrillaris PCR	41432-6
38062	Naegleria fowleri PCR	87758-9