MAYO CLINIC LABORATORIES

Test Definition: ACARP

Acanthamoeba species, Molecular Detection, PCR, Ocular

Overview

Useful For

Aids in the diagnosis of amebic keratitis in conjunction with clinical findings

Highlights

This assay is intended as an aid in the diagnosis of amebic keratitis in conjunction with clinical findings.

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

Acanthamoeba species are free-living organisms and may be found widely in the environment.

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Although verification experiments did not detect *Acanthamoeba* species DNA in contact lenses from asymptomatic adults, it is possible that the polymerase chain reaction may detect asymptomatic colonization/contamination and, therefore, testing **should not be performed on asymptomatic individuals**.

Necessary Information

1. Specimen source is required.

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

3. If submitting scrapings or swabs, specify which collection device was used (ie, scalpel or swab).

Specimen Required

The preferred specimen for this test is corneal scraping or biopsy.

Submit only 1 of the following specimens:

Specimen Type: Fresh tissue

Sources: Ocular

Container/Tube: Sterile container
Specimen Volume: 5-10 mm(3)
Collection Instructions: Submit tissue in a sterile container with 1 mL of sterile saline, minimal essential media (MEM), or viral transport media.

Preferred Paraffin-Embedded Tissue Block:
Supplies: Tissue Block Container (T553)
Specimen Type: Formalin-fixed, paraffin-embedded tissue block (FFPE)
Sources: Ocular
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 (FFPE) section
Sources: Ocular
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.**

Specimen Type: Scrapings
Sources: Eye, ocular, cornea
Container/Tube: Sterile container
Specimen Volume: 1 mL
Collection Instructions:
1. Collect corneal scrapings using a scalpel or other sharp device to remove the outer layer of cells from the eye.
2. Swish the collection device in 1 mL of sterile saline, MEM, or viral transport media.
3. Remove the scalpel blade or sharp device from the collection container before submitting to the lab.
4. Specimens containing scalpel blades will be canceled.

Specimen Type: Swabs
Sources: Eye, ocular, cornea
Container/Tube: Sterile container
Specimen Volume: 1 mL
Collection Instructions:
1. Swab must be placed into viral transport media and submitted with the specimen.
2. Specimens received without swabs will be canceled.
Additional Information: Swabs are not the preferred specimen for this test and may yield false-negative results.

Specimen Type: Contact lenses
Container/Tube: Sterile container
Specimen Volume: Entire collection
Collection Instructions:
1. Place entire contact lens in a sterile container with 1 mL sterile saline, viral transport media, or MEM.
2. Right and left lenses must be submitted individually using multiple sterile containers or in the original contact lens

case. A **separate order must be created for each lens being tested.**
3. Indicate Right or Left in the specimen source.

Specimen Type: Contact lens cases without lenses
Container/Tube: Sterile container
Specimen Volume: 1 mL solution or entire case
Additional Information:
1. Depending on the type of case submitted, it may be necessary to test right and left chambers individually. **A separate order must be created for each chamber being tested.**
2. Indicate Right or Left in the specimen source.

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

Specimen Minimum Volume
Scrapings: 0.5 mL; Other specimen types: See Specimen Required

Reject Due To

Calcium alginate-tipped swab Wood swab Transport swab containing gel Specimens containing scalpel blades Unstained slides	Reject

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information
Acanthamoeba are ubiquitous, free-living, microscopic amebae that cause rare, but severe, infections of the eye, skin, lungs, and central nervous system (CNS). They are found worldwide in water and soil and may enter the body through

inhalation, contamination of wounds, and contact lens use. As many as 24 species comprising 22 genotypes (T1-T22) have been described using 18S ribosomal RNA sequence analysis, although most human infections are due to genotype T4. Given their widespread distribution in the environment, many people will be exposed to *Acanthamoeba* during their lifetime, but very few will become sick from this exposure.

The most common form of *Acanthamoeba* infection is amebic keratitis (AK). Infection occurs primarily in contact lens wearers due to contamination of lenses, cleaning solutions, or storage cases. Amebae can also enter the cornea following trauma. AK is a painful, subacute corneal infection associated with extensive scarring and blindness if untreated. Cases generally respond to treatment, but relapse is common. Compared to corneal infection, involvement of the CNS is rare and seen primarily in severely immunocompromised individuals, such as organ transplant recipients and patients with AIDS. CNS infection may also be caused by a related ameba, *Balamuthia mandrillaris*.

Amebic keratitis is usually clinically suspected based on symptoms and confocal ophthalmologic examination. Confirmation of infection is classically identified by microscopic examination and culture of corneal tissue and contact lenses or equipment using tap water agar plate overlain with bacteria as a food source for the amebae. Unfortunately, it must be held and examined for 7 days for maximum sensitivity. A polymerase chain reaction assay provides a more rapid result with similar sensitivity to culture and is, therefore, the preferred method for confirming the clinical diagnosis of AK.

Reference Values

Negative

Interpretation

A positive result indicates the presence of *Acanthamoeba* species DNA and is consistent with active or recent infection. While positive results are highly specific indicators of disease, they should be correlated with symptoms, clinical findings, radiologic features, or confocal ophthalmologic examination.

Cautions

While this assay is designed to detect symptomatic infection with *Acanthamoeba* species, the widespread distribution of these free-living microscopic amebae in the environment may contaminate inanimate objects such as contact lenses and cases. Thus, it should be used for patients with a clinical history and ocular symptoms consistent with amebic keratitis.

Inadequate specimen collection or improper storage may invalidate test results.

Acanthamoeba species DNA may be detectable for an unknown period after adequate treatment.

Supportive Data

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

Species Inclusivity:
The *Acanthamoeba* polymerase chain reaction (PCR) assay detected all 20 strains of *Acanthamoeba* that were included in the validation study, including genotypes that cause human disease.

Accuracy/Diagnostic Sensitivity and Specificity-Fresh Specimens:
Results from this PCR assay detecting the 18S ribosomal RNA gene of *Acanthamoeba* species were compared to culture

results on 112 contact/ocular specimens. Of the 12 specimens that were positive by culture, 11 were detected by PCR (sensitivity 92%). PCR also detected an additional 2 positive specimens, which were both from the same patient with a clinical diagnosis of amebic keratitis (AK), thus indicating that they were true positive results. Ninety-eight specimens were negative by both culture and PCR.

Accuracy/Diagnostic Sensitivity and Specificity-Formalin-Fixed Paraffin-Embedded Specimens:
Twenty-four formalin-fixed paraffin embedded (FFPE) archived tissue blocks (up to 34 years old) were tested by the *Acanthamoeba* species PCR assay and results were compared to histopathologic (light microscopic) diagnosis. Fourteen of the tissues had a morphologic diagnosis of Acanthamoeba keratitis; of these, 11 were positive by PCR (sensitivity 79%). The three falsely negative specimens had scant amounts of tissue in the blocks and may no longer have contained diagnostic tissue. Ten specimens were negative by both histopathology and PCR (specificity 100%).

Supplemental Accuracy Data:
Spiking studies were performed using ocular material in transport media (contact lens fluid, minimal essential media), fresh tissue, and FFPE tissue spiked with *Acanthamoeba* genomic DNA at an approximate concentration of 50 targets/mcL. All samples were then extracted and tested in a blinded fashion. At 50 targets/mcL, 100% of the ocular material, the fresh, and the FFPE tissue were positive by PCR.

Analytical Sensitivity/Limit of Detection:
-The limit of detection (LOD) determined with serial dilution of cultured *Acanthamoeba* cysts (counted using a hemocytometer) is 1 cyst per processed sample.
-The LOD established using genomic DNA spiked into contact lens solution/minimal essential transport media is 1.26 target copies/mcL.
-The LOD established using genomic DNA spiked into fresh tissue matrix is 6.5 target copies/mcL.
-The LOD established using genomic DNA spiked into FFPE tissue matrix is 5.7 target copies/mcL.

Analytical Specificity:
No PCR signal was obtained from the extracts of 47 bacterial, viral, parasitic, and fungal isolates from similar organisms or from organisms commonly found in the specimens tested.

Precision:
Qualitative inter- and intra-assay precision was 100%. All crossing point values were within 2 cycles of the mean.

Reference Range:
The reference range is "Negative" for this assay. PCR and culture performed on 291 contact lenses from asymptomatic individuals failed to detect *Acanthamoeba* DNA or growth.

However, PCR may detect *Acanthamoeba* species colonization due to the widespread distribution of this free-living ameba in the environment, and PCR testing should only be used for patients with a clinical history and ocular symptoms consistent with AK.

Reportable Range:
This is a qualitative assay, and the results are reported as negative or positive for targeted *Acanthamoeba* species

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 March 27, 2025. Accessed May 6, 2025. Available at www.cdc.gov/acanthamoeba/about/index.html?CDC_AAref_Val

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. The LC 480 II instrument is an automated instrument that amplifies and monitors the development of target nucleic acid (amplicon) after each cycle of polymerase chain reaction (PCR).

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

The PCR mix contains a forward and reverse primer specific for *Acanthamoeba* species template amplification and 1 TaqMan probe (CY5). The CY5 probe contains 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 *Acanthamoeba* 18S rRNA, it is subsequently degraded by a DNA polymerase with 5'-3' exonuclease activity, resulting in release of the fluorophore and production of detectable fluorescent signal.(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;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;152[6]:799-807)

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

87798

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
ACARP	Acanthamoeba species Detection, PCR	41429-2

Result ID	Test Result Name	Result LOINC® Value
SRCAS	Specimen Source	31208-2
38058	Acanthamoeba species PCR	41429-2