Overview

Useful For
Preferred test for detection of *Pneumocystis*

Method Name
Real-Time Polymerase Chain Reaction (PCR)

NY State Available
Yes

Specimen

Specimen Type
Varies

Shipping Instructions
Specimen must arrive within 7 days of collection; specimens older than 7 days will be rejected.

Necessary Information
Specimen source is required.

Specimen Required
The high sensitivity of amplification by PCR requires the specimen to be processed in an environment in which contamination of the specimen by *Pneumocystis* species DNA is unlikely.

Submit only 1 of the following specimens:

Preferred

**Specimen Type:** Body fluid

**Sources:** Pleural

**Container/Tube:** Sterile container

**Specimen Volume:** 1 mL

**Specimen Type:** Respiratory

**Sources:** Bronchoalveolar lavage, bronchial washing, tracheal secretions, or sputum

**Container/Tube:** Sterile container

**Specimen Volume:** 1 mL

**Specimen Type:** Tissue

**Sources:** Respiratory
Test Definition: PNRP
Pneumocystis PCR

**Container/Tube:** Sterile container

**Specimen Volume:** 5-10 mm

**Collection Instructions:**

1. Submit fresh tissue.

2. Keep tissue moist with sterile water or sterile saline

**Acceptable**

**Specimen Type:** NALC/NaOH-digested respiratory specimens

**Sources:** Lavage fluid, bronchial washing, respiratory fluid, sputum, or tracheal secretion

**Container/Tube:** Sterile container

**Specimen Volume:** 2 mL

**Collection Instructions:**

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

**Specimen Minimum Volume**
0.5 mL

**Reject Due To**

| Other | Body fluid other than pleural fluid, blood, bone, nonrespiratory tissue, bone marrow, organ tissues other than lung, paraffin-embedded tissue Specimen in anaerobe vial or viral transport medium Feces Swab Tissue in formalin fluid Urine Specimen >7 days old |

**Specimen Stability Information**

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<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
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<tbody>
<tr>
<td>Varies</td>
<td>Refrigerated (preferred)</td>
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<tr>
<td></td>
<td>Frozen</td>
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**Clinical and Interpretive**

**Clinical Information**

*Pneumocystis* pneumonia is an important cause of opportunistic infection in immunocompromised patients, particularly those with HIV. The causative agent, *Pneumocystis jiroveci*, cannot be cultured in vitro and, therefore, laboratory detection has historically relied upon microscopic identification directly from patient specimens using...
Test Definition: PNRP

Pneumocystis PCR

fluorescent stains or antibodies. Unfortunately, stains often lack sensitivity and require expertise on the part of the reader in order to differentiate Pneumocystis jiroveci from staining artifacts and other fungi. This real-time PCR assay provides sensitive (21% more sensitive than direct detection using fluorescent calcofluor white stain), specific, and objective detection of Pneumocystis from bronchoalveolar lavage fluid and other specimens.

Reference Values

Not applicable

Interpretation

A positive result indicates the presence of Pneumocystis DNA.

A negative result indicates the absence of detectable Pneumocystis DNA.

Cautions

Test results should be used as an aid in diagnosis and should not be considered diagnostic in themselves. The literature indicates that Pneumocystis can cause asymptomatic colonization of healthy and immunocompromised individuals. Therefore, test results should be correlated with patient symptoms and clinical presentation.

A negative result does not rule out the presence of Pneumocystis or active disease because the organism may be present at undetectable levels.

Supportive Data

A total of 221 bronchoalveolar lavage (BAL) fluid samples were evaluated for the presence of Pneumocystis DNA by the LightCycler and compared to fluorescent microscopy using calcofluor white staining. Of the 221, 24 were positive and 190 were negative by both detection methods. The remaining 7 were positive by PCR and negative by microscopy. The 7 specimens that were positive using LightCycler PCR alone were tested using another PCR assay targeting a second Pneumocystis gene. All 7 specimens were positive using the second target suggesting that they were true positives that were undetected using the microscopic method. The sensitivity, specificity, positive and negative predictive values of this real-time PCR assay is 100%, 96%, 77%, and 100%, respectively. The analytical sensitivity of the method is 5.6 copies/mcL of positive plasmid control or approximately 28 copies/reaction. The analytical sensitivity in spiked, pooled BAL specimens was found to be 56 targets/mcL using the positive control plasmid. PCR inhibition was tested by spiking 50 extracted negative respiratory specimens (including 10 BAL fluid specimens) with 100 copies of target/mcL using a positive control plasmid. No PCR inhibition was detected. The specificity of the PCR assay was determined by evaluating DNA extracted from pure cultures of a variety of bacteria and fungi. Extracted human DNA was analyzed as well. None of the microbial or human DNA was amplified by the Pneumocystis LightCycler assay indicating that the assay is specific for Pneumocystis species.

Clinical Reference


**Performance**

**Method Description**

Bronchoalveolar lavage fluid and sputum are liquefied using N-acetyl-L-cysteine. Following liquefication, cells are pelleted by centrifugation and resuspended in S.T.A.R. buffer (Roche). Nucleic acids are extracted using the MagNA Pure LC Instrument (Roche). The extract is then transferred to individual self-contained cuvettes for amplification using the LightCycler real-time PCR platform (Roche). The LightCycler is an automated instrument that amplifies and monitors the development of target nucleic acid (amplicon) after each cycle of PCR. The detection of amplicon is based on fluorescence resonance energy transfer (FRET), which utilizes hybridization probes. The presence of the specific organism nucleic acid is confirmed by performing a melting curve analysis of the amplicon. (Arcenas RC, Uhl JR, Buckwalter SP, et al: A real-time PCR assay for detection of *Pneumocystis* from bronchoalveolar lavage fluid. Diagn Microbiol Infect Dis 2006;54:169-175)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Monday through Sunday

**Analytic Time**

1 day

**Maximum Laboratory Time**

3 days

**Specimen Retention Time**

7 days

**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**

87798

**LOINC® Information**
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