

## Recommended laboratory tests for pneumococcal disease by syndrome

Clinical syndrome	Recommended tests by clinical specimen
<b>Pneumonia in children</b>	<ul style="list-style-type: none"> <li>Blood culture in cases of severe (cough, tachypnea &amp; chest indrawing), X-ray positive or failed empiric treatment</li> <li>Pleural fluid Gram stain and culture if pleural effusion is present and pleural tap can be performed safely</li> </ul>
<b>Pneumonia in adults</b>	<ul style="list-style-type: none"> <li>Sputum culture and Gram stain</li> <li>Blood culture</li> <li>Urine NOW® <i>Streptococcus pneumoniae</i> Antigen Test</li> </ul>
<b>Meningitis</b>	<ul style="list-style-type: none"> <li>Cerebrospinal Fluid (CSF) culture plus Gram stain (standard test)</li> <li>CSF NOW® <i>Streptococcus pneumoniae</i> Antigen Test or Latex agglutination or PCR if Gram stain is negative and for research or surveillance purposes, if resources allow</li> <li>Blood culture</li> </ul>

## Summary of laboratory tests used in clinical practice and their value

	Test	Sensitivity	Specificity	DV <sup>†</sup> in adults	DV <sup>†</sup> in children	Comments & Recommendations
Pneumonia	Sputum Gram stain and culture	Low	High	++++	-	Test only in high-quality pre-treatment sputum. Results should complement blood culture. Test is not applicable in young children who cannot produce sputum.
	Gram stain and culture of pleural or lung aspirate	High	High	+++	+++	Test pleural fluid only if there is evidence of effusion. Specimen collection procedure for pleural and lung aspirate is invasive, complications may arise. Limit use to only critically ill children or when there is no response to treatment.
	Gram stain and culture of other respiratory fluids*	Moderate	High	+++	+++	Endotracheal and bronchoscopic aspiration carries less risk than pleural aspiration.
	Blood culture	Low	High	++++	++++	Recommended for severe pneumonia or non-response to empiric treatment.
	Urine NOW® <i>Streptococcus pneumoniae</i> Antigen Test	Moderate <sup>§</sup>	High <sup>§</sup>	+++++	-	<sup>§</sup> Moderate sensitivity and high specificity only in adults. Not recommended for use in children. Cost may be a constraint.
	Urine Latex agglutination	Low	Moderate	++	++	Performs no better than Gram stain and culture, but test may be useful in settings with limited laboratory capacity.
Meningitis	CSF Gram stain & culture	High	High	+++++	+++++	Recommended as the standard test for meningitis.
	Blood culture	Low	High	+++	+++	Can complement CSF testing to improve diagnosis.
	CSF NOW® <i>Streptococcus pneumoniae</i> Antigen Test	High	High	+++++	+++++	Easy to use but high cost makes it impractical in resource-constrained clinical settings.
	CSF Latex agglutination	Moderate	Moderate	+++	+++	May be used as adjunct to Gram stain and culture when Gram stain is negative. Requires subjective evaluation, therefore may not perform well in places lacking highly trained personnel.
	CSF PCR	High	High	+++	+++	Test is costly and requires expertise, limiting use in many settings.
Sepsis	Blood culture	Low	High	++++	++++	Recommended as the standard test.

<sup>†</sup>DV = Diagnostic Value. Has maximum of 6" +": 1 "+" for each of the following: 1. High sensitivity; 2. High specificity; 3. Safe and easy specimen collection; 4. Easy test procedure; 5. Rapid results; 6. Low cost. \* Refers to endotracheal or bronchoscopic aspirates. Link to references & purchase information for test kits : <http://www.preventpneumo.org>

Material developed by Chizoba Wonodi & Maria Deloria Knoll (PneumoADIP) with contributions from David Murdoch (University of Otago, Christchurch, New Zealand), Thomas Cherian (WHO), Adegoke Falade (College of Medicine, University of Ibadan, Nigeria/University College Hospital, Ibadan), Asian Strategic Alliance for Pneumococcal disease prevention (ASAP) and Cynthia Whitney (CDC).

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## Laboratory approaches for the detection of pneumococcal disease



This pamphlet is a concise overview of laboratory tests for *S. pneumoniae* (pneumococcus) disease. It provides comparative information on the sensitivity, specificity, cost and practical utility of different pneumococcal diagnostic tests. We hope this tool will support clinical decision making and pneumococcal disease surveillance and epidemiologic efforts.

### **Streptococcus pneumoniae is a global killer**

Diseases caused by *S. pneumoniae* are important contributions to mortality and morbidity the world over. Pneumococcus is the primary bacterial cause of pneumonia in children and older adults. Other pneumococcal syndromes include meningitis, sepsis, otitis media, cellulitis and other soft tissue infections.

### **Diagnosing pneumococcal disease is challenging**

Diagnosis often requires a combination of clinical, radiological and microbiological assessments. *S. pneumoniae* is a fastidious organism; detecting the bacterium is especially difficult because conventional tests such as culture have low sensitivity, particularly for pneumonia, where only a minority of cases are bacteremic. Furthermore, tests performed on specimens from non-sterile sites, such as sputum, poorly distinguishes colonization from infection.

### **No single Pneumococcal test offers optimal detection**

The most common laboratory diagnostic approaches for pneumococci are: 1) Gram staining; 2) bacterial culture; 3) antigen detection; 4) genetic material detection and 5) serologic assays for antibodies. Each laboratory assay/test has advantages and disadvantages that depend on the resources, lab capacity and expertise available. The costs and benefits of conducting these tests must be weighed against the likelihood that a test result will alter empirical treatment. We hope that this pamphlet will offer some guidance to individualize testing decisions.

## Common tests validated for clinical, surveillance and research purposes

### Gram stain

Gram stain is one of oldest microbiological tests used to differentiate bacteria. *S. pneumoniae* are Gram positive diplococci and appear as bluish, lancet-shaped pairs or chains under microscopy.

**Clinical specimens:** sputum, cerebrospinal fluid (CSF), pleural fluid & endotracheal, bronchoscopic or lung aspirates

### Advantages

- Rapid results
- Relatively inexpensive
- Good specificity in experienced hands
- Requires minimal equipment

### Disadvantages

- Morphology may be altered by antimicrobial therapy
- Organisms may appear as Gram negative (pink) if there is excessive decolourization of Gram stain
- Requires experienced microscopists

### Culture

Bacterial culture is considered the gold standard because of its high specificity and because supplemental testing (serotyping and antibiotic resistance) can be done on cultured isolates to characterize strains better. However, due to its low sensitivity, culture should be combined with other tests to optimize pneumococcal detection.

Bacterial culture of *S. pneumoniae* requires 24 – 48 hrs incubation in 5% CO<sub>2</sub> on 5% sheep blood agar. Colonies of *S. pneumoniae* are identified by gross appearance or microscopy. Normally, identification is based on optochin susceptibility. Due to rare optochin resistance, confirmation by bile solubility is necessary. Commercial slide agglutination, coagulation and DNA hybridization tests can also be used for confirmation.

**Clinical specimens:** sputum, CSF, blood, pleural fluid, endotracheal, bronchoscopic or lung aspirates

### Advantages

- Highly specific
- Relatively inexpensive
- Can conduct antibiotic resistance testing, serotyping or other tests to characterize strains
- Blood culture from suspected meningitis cases can significantly increase detection over CSF culture alone

### Disadvantages

- Requires 24-48 hrs incubation
- Sputum cultures prone to be false positive due to contamination by upper respiratory pneumococci
- Young children are unlikely to produce sputum samples
- Culture can have low sensitivity due to
  - recent antibiotic use
  - most pneumonia cases are not bacteremic
  - *Streptococcus pneumoniae* is fastidious; delay in processing, contamination and extreme ambient temperatures kill the organism
  - inadequate blood specimen volume
  - use of human blood in agar

### Antigen detection – Latex-particle agglutination

Antibody coated latex particles are used to detect the presence of pneumococcal polysaccharide in clinical specimens. A positive test has visible signs of agglutination in reagents/ specimen mixture.

**Clinical specimens:** urine or sputum from adults, CSF

### Advantages

- Moderate sensitivity and specificity in CSF especially in settings lacking highly trained personnel
- Requires minimal equipment
- Rapid and portable
- Sensitivity less affected by prior antibiotic use than culture
- Antigen detection occurs in spite of autolysis
- Test kits combining *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis* antigens also available

### Disadvantages

- Low sensitivity and moderate specificity in urine
- Performs no better than gram stain and culture in some cases
- Requires subjective evaluation of test result, therefore test may not perform well in labs lacking highly trained personnel

### Antigen detection – The Binax NOW® *Streptococcus pneumoniae* Antigen Test

The NOW® *Streptococcus pneumoniae* Antigen Test (Binax) is a relatively new test. Although still expensive, it is a promising addition to other antigen detection methods such as latex agglutination.

This is a rapid immunochromatographic assay for detection of *S. pneumoniae* antigen in the urine of adults with pneumonia and in the cerebrospinal fluid (CSF) of patients of all ages with meningitis. Its use on other specimen types is under investigation. The test targets the C polysaccharide antigen common to all 91 pneumococcal serotypes.

**Clinical specimens:** urine of adults or CSF

### Advantages

- High sensitivity and specificity in CSF
- Moderate sensitivity and high specificity in urine of adults.
- Can be used at point of care
- Requires minimal equipments and expertise
- Rapid and portable; 15 minutes for test results
- Kit is stable between 15-25°C
- Sensitivity less affected by prior antibiotic use than culture

### Disadvantages

- Test on urine of children has low specificity because a positive test can result from nasopharyngeal colonization, even in the absence of disease
- Does not identify organisms other than *Streptococcus pneumoniae*
- Relatively expensive compared to culture and PCR



## Methods currently for surveillance and research purposes only

### Genetic material detection: Polymerase Chain Reaction (PCR)

PCR is a major and relatively new advance for pneumococcal diagnosis. Although it is currently expensive and its use in resource-poor settings is limited to research and surveillance, lower costs in the future may allow PCR testing to become more widely used in clinical settings in developing countries.

Standard PCR techniques amplify gene fragments in clinical specimens using enzymes and DNA primers through repeated thermal cycles.

**Clinical specimens:** CSF, blood, pleural fluid, endotracheal, bronchoscopic or lung aspirates

### Advantages

- Good sensitivity in CSF
- Can detect DNA from specimens lacking viable pneumococci

### Disadvantages

- Presence of inhibitors affect assay sensitivity
- Relatively expensive because of equipment and materials cost
- Requires a high level of expertise
- Potential for contamination is high

### Serological methods

Several serological assays have been developed to detect host antibody or the immune complex response to pneumococcal infection.

### Experimental tests not yet validated

- NOW® *SP* Antigen Test on blood culture bottles
- PCR test on sera
- Real-time PCR for quantitative assays
- Multiplexed PCR
- Analysis of breath samples for volatile organic compounds

### Other procedures less often used

- Counter Immunoelectrophoresis
- Enzyme-linked immunosorbent assays

## How to get the most out of your tests

The diagnostic value obtained from a test depends partly on the quality of the specimen tested. To ensure the highest quality of clinical specimen the following should be observed:

- Process all specimens without delay or store appropriately
- **CSF for Latex:** test specimens as soon as possible, or store between 2-8°C for up to 48 hrs, or at -20°C
- **CSF & other sterile body fluids for culture:** process as soon as possible, never store in a refrigerator or freezer.
- **Blood for culture:** collect enough volume, 10-30mls from adults and 2-5 mls from children. Inoculate a second bottle to increase yield
- Bring blood culture bottles to room temperature before inoculation. Place inoculated blood culture bottles in an incubator as soon as possible and never in a refrigerator or freezer.
- **Sputum for Gram stain or culture:** process only specimens with <10 squamous epithelial cells and >25 polymorphonuclear cells per low-power field