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Infections of the respiratory tract are among the most common health problems in children worldwide, and are associated with substantial morbidity and mortality. A wide variety of microorganisms are potential respiratory pathogens; knowledge about the likely etiologic agents of respiratory infections can help direct management and can also play an important role in disease surveillance. Beyond the identification of specific pathogens, the clinical microbiology laboratory can also provide valuable information on antimicrobial susceptibility and strain typing. Continued liaison between clinicians and laboratory staff is vital to facilitate the most cost-effective use of laboratory diagnostics.

Presently, we are still reliant on many traditional diagnostic tools that have been used for decades to determine the microbial etiology of respiratory infections. However, these tools have been increasingly supplemented by newer methods, particular molecular diagnostic techniques, which have enabled the more rapid detection of many pathogens that were previously difficult to detect. These advances have particularly led to improvements in the ability to detect respiratory viruses and other microorganisms that do not normally colonize the respiratory tract. Moreover, recent discussions about the existence of a lung microbiome have challenged traditional paradigms about the pathogenesis of respiratory infections. The concept that the healthy lung may not be a sterile organ is reshaping our interpretation of laboratory diagnostics.

This chapter focuses on the use of the clinical microbiology laboratory to determine the microbial causes of respiratory infections in children. Diagnostic aspects of some specific respiratory infections, such as tuberculosis and pertussis, are also covered in other chapters.

Respiratory Pathogens and Syndromes

Tables 22.1–22.17 show the etiologic agents associated with respiratory infections broken down by respiratory syndrome. These lists represent our current understanding and have changed little over recent decades; there have been only a relatively small number of newly discovered pathogens. The latter include human bocavirus, human metapneumovirus, and a variety of coronaviruses (SARS-CoV, CoV-NL63, CoV-HKU1 and MERS-CoV). Pathogen discovery efforts using unbiased next-generation sequencing methods have shown considerable promise but have not yet identified major new respiratory pathogens.

In general, upper respiratory infections tend to be monomicrobial and are predominantly caused by viruses, with a few notable exceptions caused by specific bacteria (e.g., acute pharyngitis caused by Streptococcus pyogenes). Lower respiratory infections are caused by a wide variety of viral and bacterial pathogens. For pneumonia at least, sequential or concurrent polymicrobial infection may be relatively common, and the exact roles of individual microorganisms and how they interact in this context are still poorly understood. The incidence of many respiratory infections follows a cyclical pattern aligned with the typical seasonal transmission of specific pathogens. Secular trends have also been noted for some vaccine-preventable infections, such as those caused by Streptococcus pneumoniae and Haemophilus influenzae type b, with decreasing burden following the successful implementation of vaccine programs.

Use of the Clinical Microbiology Laboratory

Before ordering a diagnostic test, it is important to be clear about the key clinical questions and expectations of diagnostic testing. Is knowledge about the cause of a particular respiratory infection important for patient treatment, outbreak management, epidemiological surveillance, or to reassure the clinician or caregiver of the child? It is also important to have an understanding about which specimens to collect, what tests are available, test limitations, and how to interpret results to appropriately integrate the findings into their clinical management.

The most useful specimens for diagnostic testing are those collected directly from the site of infection. Unfortunately, it is not always possible to collect these specimens, and this particularly applies to the lower respiratory tract, which is difficult to access safely in a manner that avoids contamination with colonizing organisms.

When bacteria are isolated from specific body sites, such as a throat swab, nasopharyngeal swab, or sputum, it is important to know which bacteria can be found as commensals or colonizers in the upper respiratory tract and which
ABSTRACT

A wide variety of microorganisms are potential respiratory pathogens, and the spectrum of known pathogens for each respiratory infection syndrome has not changed markers over recent years. Detection of likely etiologic agents of respiratory infections can help direct management and can also play an important role in disease surveillance. For this purpose, we are still reliant on many traditional diagnostic tools that have been used for decades in order to determine the microbial etiology of respiratory infections. However, these tools have been increasingly supplemented by newer methods, particular molecular diagnostic techniques, which have enabled the more rapid detection of many pathogens that were previously difficult to detect. These advances have particularly lead to improvements in the ability to detect respiratory viruses and also other microorganisms that do not normally colonize the respiratory tract. Recognition of the existence of the lung microbiome has challenged the traditional views of pneumonia pathogenesis and may provide the opportunity for new diagnostic tools that are focused on more than just detection of specific known pathogens. Continued liaison between clinicians and laboratory staff is vital in order to facilitate the most cost-effective use of laboratory diagnostics.

KEYWORDS

microbiology
laboratory
culture
PCR
serology
antigen detection
microbiome
diagnosis
etiology
Table 22.1 Etiologic Agents Associated With Pharyngitis

| Viral          | Bacterial                     | Fungal       |
|----------------|-------------------------------|--------------|
| Adenoviruses   | Streptococcus pyogenes        | Candida      |
| Coronaviruses  | Other β-hemolytic streptococci|              |
| Parainfluenza  | Corynebacterium ulcerans      |              |
| Respiratory syncytial virus | Corynebacterium diptheriae       |              |
| Human metapneumovirus | Arcanobacterium haemolyticum |              |
| Rhinoviruses   | Neisseria gonorrhoeae         |              |
| Influenza viruses | Mixed anaerobes             |              |
| Epstein-Barr virus | Treponema pallidum        |              |
| Enteroviruses  | Chlamydophila pneumoniae      |              |
| Herpes simplex viruses | Mycoplasma pneumoniae       |              |
| Measles        | Streptobacillus moniliformis  |              |
| Rubella        |                               |              |
| Cytomegalovirus|                               |              |
| HIV            |                               |              |

Table 22.2 Etiologic Agents Associated With Croup

| Viral          | Bacterial                      |
|----------------|-------------------------------|
| Parainfluenza viruses | Mycoplasma pneumoniae        |
| Influenza viruses     |                               |
| Respiratory syncytial virus |                           |
| Human metapneumovirus |                               |
| Coronavirus         |                               |
| Human bocavirus      |                               |
| Adenoviruses        |                               |
| Measles             |                               |
| Rhinoviruses        |                               |
| Enteroviruses       |                               |
| Herpes simplex viruses |                           |

Table 22.3 Etiologic Agents Associated With Sinusitis

| Viral             | Bacterial             | Fungal                |
|-------------------|-----------------------|-----------------------|
| Rhinoviruses      | Haemophilus influenzae| Aspergillus species   |
| Parainfluenza viruses | Streptococcus pneumonia | Alternaria species       |
| Adenoviruses      | Anaerobes             | Penicillium species   |
|                   | Moraxella catarrhalis | Zygomyces             |
|                   | Staphylococcus aureus |                       |
|                   | Streptococcus pyogenes|                       |
|                   | Mycoplasma pneumoniae |                       |
|                   |                       |                       |

Table 22.4 Etiologic Agents Associated With Acute Bronchitis

| Viral          | Bacterial                      |
|----------------|-------------------------------|
| Adenoviruses   | Mycoplasma pneumoniae         |
| Influenza viruses |                               |
| Parainfluenza viruses |                           |
| Respiratory syncytial virus |                       |
| Rhinoviruses   |                               |
| Coronaviruses  |                               |
| Human metapneumovirus | Herpes simplex viruses       |
| Enteroviruses  |                               |
| Measles        |                               |
| Mumps          |                               |
| Human bocavirus|                               |

Table 22.5 Etiologic Agents Associated With Bronchiolitis

| Viral          | Bacterial                      |
|----------------|-------------------------------|
| Respiratory syncytial virus | Mycoplasma pneumoniae       |
| Parainfluenza viruses     |                               |
| Adenoviruses             |                               |
| Influenza viruses        |                               |
| Human metapneumovirus    |                               |
| Rhinoviruses             |                               |
| Enteroviruses            |                               |
| Mumps                    |                               |
| Herpes simplex viruses   |                               |

Table 22.6 Etiologic Agents Associated With Pneumonia

| Viral                         | Bacterial                          | Fungal                       |
|-------------------------------|------------------------------------|------------------------------|
| Respiratory syncytial virus   | Streptococcus pneumoniae           | Pneumocystis jiroveci       |
| Parainfluenza viruses         | Haemophilus influenzae             | Aspergillus species         |
| Influenza viruses             | Staphylococcus aureus              | Zygomyces                    |
| Coronaviruses                 | Mycoplasma pneumoniae              | Coccidioides immitis        |
| Adenoviruses                  |                                   | Cryptococcus neoformans     |
| Human metapneumovirus         | Enterobacteriaceae                 | Histoplasma capsulatum      |
| Rhinoviruses                  | Pseudomonas aeruginosa             |                             |
| Epstein-Barr virus            | Acinetobacter species              |                             |
| Enteroviruses                 | Mixed anaerobes                    |                             |
| Human bocavirus               | Streptococcus agalactiae           |                             |
| Herpes simplex viruses        | Chlamydia psittaci                 |                             |
| Varicella zoster virus        | Chlamydia pneumoniae               |                             |
| Measles                       | Chlamydia trachomatis              |                             |
| Rubella                       | Burkholderia pseudomallei          |                             |
| Cytomegalovirus               | Streptococcus pneumoniae           |                             |
| HIV                           | Neisseria meningitidis             |                             |
|                               | Coxiella burnetii                  |                             |
|                               | Mycobacterium species              |                             |

when found would indicate definitive infection. Table 22.15 outlines microorganisms that are regarded as part of the normal respiratory flora. Importantly, given the right conditions, some bacteria that can harmlessly colonize the respiratory tract may also be respiratory pathogens. As will be discussed further in this chapter, several microbiological diagnostic tests employed in the diagnosis of childhood respiratory disease have limited ability to differentiate between colonization and disease and are therefore of limited value when considered in isolation.
Table 22.7  Etiologic Agents Associated With the Common Cold

| Pathogen | Typical Gram Stain Appearance | Likely to Be Significant |
|----------|-------------------------------|-------------------------|
| Staphylococcus aureus | Gram-positive cocci in chains | | |
| Haemophilus influenzae | Gram-positive lancet-shaped diplococci | Predominant pathogen in Gram stain with abundant neutrophils |
| Streptococcus pneumoniae | Gram-positive cocci in chains | | |
| Haemophilus parainfluenzae | Small pleomorphic gram negative coccobacilli | | |
| Mycoplasma pneumoniae | Gram-positive diphtheroid-shaped bacilli | | |
| Enterobacter aerogenes | Pleomorphic diphtheroid gram-positive bacilli | | |
| α-Hemolytic streptococci | Special stain (Loeffler’s methylene blue stain) demonstrates typical club-shaped ends | | |

Table 22.10  Etiologic Agents Associated With Lung Abscess

| Pathogen | Typical Gram Stain Appearance | Likely to Be Significant |
|----------|-------------------------------|-------------------------|
| Staphylococcus aureus | Gram-positive cocci in chains | | |
| Haemophilus influenzae | Gram-positive lancet-shaped diplococci | Predominant pathogen in Gram stain with abundant neutrophils |
| Streptococcus pneumoniae | Gram-positive diphtheroid-shaped bacilli | | |
| Other gram-negative bacilli | Pleomorphic diphtheroid gram-positive bacilli | | |
| Entamoeba histolytica | Special stain (Loeffler’s methylene blue stain) demonstrates typical club-shaped ends | | |
| Anaerobes | | | |
| Mycoplasma pneumoniae | Absence of organisms as they lack a cell wall and cannot be visualized on Gram stain | | |

Table 22.11  Etiologic Agents Associated With Cystic Fibrosis

| Pathogen | Typical Gram Stain Appearance | Likely to Be Significant |
|----------|-------------------------------|-------------------------|
| Staphylococcus aureus | Gram-positive cocci in chains | | |
| Haemophilus influenzae | Gram-positive diphtheroid-shaped bacilli | | |
| Mycoplasma pneumoniae | Pleomorphic diphtheroid gram-positive bacilli | | |
| Pseudomonas aeruginosa | Special stain (Loeffler’s methylene blue stain) demonstrates typical club-shaped ends | | |
| Burkholderia cepacia | | | |
| Stenotrophomonas maltophilia | | | |
| Mycobacterium species | | | |

Table 22.8  Etiologic Agents Associated With Epiglottitis

| Pathogen | Typical Gram Stain Appearance | Likely to Be Significant |
|----------|-------------------------------|-------------------------|
| Staphylococcus aureus | Gram-positive lancet-shaped diplococci | Predominant pathogen in Gram stain with abundant neutrophils |
| Haemophilus influenzae | Gram-positive diphtheroid-shaped bacilli | | |
| Streptococcus pneumoniae | Pleomorphic diphtheroid gram-positive bacilli | | |
| Other gram-negative bacilli | Special stain (Loeffler’s methylene blue stain) demonstrates typical club-shaped ends | | |

Table 22.9  Etiologic Agents Associated With Pleural Effusion and Empyema

| Pathogen | Typical Gram Stain Appearance | Likely to Be Significant |
|----------|-------------------------------|-------------------------|
| Staphylococcus aureus | Gram-positive lancet-shaped diplococci | Predominant pathogen in Gram stain with abundant neutrophils |
| Haemophilus influenzae | Gram-positive diphtheroid-shaped bacilli | | |
| Streptococcus pneumoniae | Pleomorphic diphtheroid gram-positive bacilli | | |
| Mycoplasma pneumoniae | Special stain (Loeffler’s methylene blue stain) demonstrates typical club-shaped ends | | |
| Arcanobacterium haemolyticum | | | |
| Corynebacterium diphtheriae | | | |
| α-Hemolytic streptococci | | | |

Table 22.12  Respiratory Specimens and Diagnostic Testing

| Specimen Type | Microbiological Investigations | Comment |
|---------------|-------------------------------|---------|
| Sputum/induced sputum | Microscopy; culture; susceptibilities; DFA; PCR | Provided it is a good-quality specimen, it can be a highly informative specimen; can be difficult to obtain in children |
| Nasopharyngeal aspirate/swab | Microscopy; culture; susceptibilities; DFA; PCR | Most useful in viral infections; requires a skilled operator to obtain specimen; in some ways, it is easier to obtain than a throat swab, because the nares are always accessible |
| Nasal swab | Microscopy; culture; susceptibilities; DFA; PCR | Limited usefulness as it only recovers organisms present in the nasal cavity and not beyond |
| Throat swab | Microscopy; culture; susceptibilities; DFA; PCR | Probably the most representative specimen for disease of the upper respiratory tract; many bacterial pathogens are also common colonizers at various stages of childhood; can be difficult to obtain without child and parent cooperation; may represent organisms present in the nose as well as the oropharynx |
| Endotracheal aspirate | Microscopy; culture; susceptibilities; DFA; PCR | Invasive specimen, but is likely to represent pathogens from the lower respiratory tract; can be contaminated by organisms present in the oropharynx that can make result interpretation difficult |
| Bronchoalveolar lavage fluid | Microscopy; culture; susceptibilities; DFA; PCR | Invasive specimen but is likely to represent pathogens from the lower respiratory tract; can be contaminated by organisms present in the oropharynx, which can make result interpretation difficult |
| Transthoracic needle aspiration Lung tissue | Microscopy; culture; susceptibilities; DFA; PCR | Highly invasive specimen; risk of complications; microbiologically of high value provided the correct area has been biopsied |
| Pleural fluid | Microscopy; culture; susceptibilities; DFA; PCR | Highly invasive specimen; risk of complications; microbiologically of high value provided the correct area has been biopsied |
| Blood cultures Serum/whole blood | Microscopy; culture; susceptibilities; DFA; PCR | Invasive specimen but is the specimen of choice in a child with empyema |
| Urine | Antigen detection tests; microscopy; culture | Antigen detection tests are of limited value in children; pathogen is rarely cultured from urine |

Table 22.13  Gram Stain Appearance of Bacterial Respiratory Pathogens

| Pathogen | Typical Gram Stain Appearance | Likely to Be Significant |
|----------|-------------------------------|-------------------------|
| Streptococcus pneumoniae | Gram-positive lancet-shaped diplococci | | |
| Staphylococcus aureus | Gram-positive cocci in chains | | |
| Haemophilus influenzae | Small pleomorphic gram negative coccobacilli | | |
| Streptococcus pyogenes | Gram-positive cocci in chains | | |
| Arcanobacterium haemolyticum | Gram-positive diphtheroid-shaped bacilli | | |
| Corynebacterium diphtheriae | Pleomorphic diphtheroid gram-positive bacilli | | |
| Mycoplasma pneumoniae | Absence of organisms as they lack a cell wall and cannot be visualized on Gram stain | | |

DFA, Direct fluorescent antibody; PCR, polymerase chain reaction.
**Table 22.14** Screening of Respiratory Specimen Quality

| Specimen                     | Acceptable for Culture |
|------------------------------|------------------------|
| Sputum                       | <10 SEC/average 10× field |
| Endotracheal aspirate        | <10 SEC/average 10× field and bacteria seen in at least 1 of 20 oil immersion fields |
| Bronchoalveolar lavage fluid | <1% of cells present are SEC |

This table has been modified from Jorgensen JH, Pfaller MA, Carroll KC, et al. *Manual of Clinical Microbiology*, 11th ed. Washington, DC: American Society of Microbiology, 2015. SEC, Squamous epithelial cells.

**Table 22.15** Normal Respiratory Flora

| Species                        | Table 22.12 |
|--------------------------------|-------------|
| *Streptococcus pneumoniae*     | *Staphylococcus aureus* |
| *Moraxella catarrhalis*        | *Neisseria meningitidis* |
| *Kingella*                     | *Haemophilus influenzae* |

**Table 22.16** Molecular Assays Commonly in Use for the Diagnosis of Respiratory Diseases

| Molecular Assay       | Principle                                                   | Main Use                                                      | Comment                                      |
|-----------------------|-------------------------------------------------------------|---------------------------------------------------------------|----------------------------------------------|
| Singleplex PCR        | Single DNA or RNA target that is amplified                  | Can be designed for the detection of any known DNA or RNA sequence | Generally higher sensitivity than multiplex PCR as the targets are not competing |
| Multiplex PCR         | Simultaneous amplification of several DNA or RNA targets    | Respiratory pathogens; immunocompromised protocols; detection of various pathogens in blood cultures | Wide coverage of pathogens in a single test informs clinical management in a timely manner |
| 16S rRNA sequencing   | Amplification of 16S ribosomal RNA followed by sequencing of the product | Used to detect bacterial species in a clinical specimen that has failed to detect pathogens in culture | Covers a wide range of pathogens listed in accessible sequence databases |
| Next-generation sequencing | Sequencing of a whole bacterial or viral genome or simultaneous sequencing of multiple bacterial or viral genes | Resistance testing and outbreak investigations | Can offer multiple gene sequences simultaneously or whole genome sequencing as well as de novo sequencing; currently, high cost prohibits routine use |

**Table 22.17** Molecular Terms Commonly Used in Diagnostics

| Molecular Term     | Explanation                                                                                     |
|--------------------|-------------------------------------------------------------------------------------------------|
| PCR                | An in vitro chemical reaction that leads to the synthesis of large quantities of a target nucleic acid sequence. |
| Reverse transcriptase PCR | RNA targets are converted into cDNA that is then amplified. This is needed for the amplification of RNA viruses (most common respiratory viruses). |
| RT PCR             | The target amplification and the detection step occur simultaneously in the same tube. These assays require special thermal cyclers. |
| SNPs               | Useful markers of genetic differences between strains, e.g., in outbreak investigations. |
| Target amplification techniques | Copies of a specific target nucleic acid are synthesized, and the products of amplification are detected by specifically designed oligonucleotide primers that bind to the complementary sequence on opposite strands of the double-stranded targets. |
| Signal amplification techniques | The target itself is not amplified; instead, the concentration of labeled molecules attached to the target nucleic acid is increased and measured. |

**CLINICAL SPECIMENS FOR RESPIRATORY PATHOGEN DIAGNOSIS**

Detection of respiratory pathogens is dependent on the type and quality of specimen collected, the timing of collection after the onset of clinical symptoms, the age of the patient, and transportation and storage of the sample before being tested in the laboratory. Ensuring high-quality collection of the right specimens is essential for making an accurate and interpretable laboratory diagnosis.

A range of specimens can be used for identifying the microbial etiology of respiratory infections in children and are shown in Table 22.12. Not all specimens are easily obtainable, and the diagnostic utility varies with each specimen type. The inability to obtain good-quality specimens from the lower respiratory tract is a fundamental problem with pneumonia diagnostics, and obtaining representative and uncontaminated specimens from the lungs is a challenge. Specimens collected by sputum induction or bronchoscopy may be contaminated by normal respiratory flora. Thoracic needle aspiration is the best technique to obtain specimens from the site of infection in pneumonia, but it is performed in few centers despite a good safety profile.16

Specimens should be collected as early as possible in the acute stage of an infection, preferably prior to administration of antimicrobial or antiviral drugs. During this period, higher pathogen concentrations are likely to be present; however,
the duration of pathogen shedding depends on the microorganism involved and the severity of the infection and other factors. With uncomplicated influenza virus infections, virus shedding is usually 3 to 5 days following symptom onset; however, this may be extended in severe respiratory disease to 5 to 10 days. Children may also shed for up to 10 days and many weeks in immunocompromised individuals.

**Throat and Nasopharyngeal Specimens**

The majority of respiratory tract specimens received in the diagnostic laboratory from children are aspirates or swabs obtained from the upper respiratory tract. Nasopharyngeal aspirates are generally superior to swabs for the detection of respiratory viruses, since large numbers of respiratory epithelial cells are aspirated during the collection process. However, aspirates are more difficult to obtain, especially outside the hospital setting, as they require a specific suction device. A range of commercial swabs are available, which include rayon tipped swabs and polyurethane sponges with wooden, plastic, or wire shafts. The availability of flocked nylon swabs, designed for the collection of respiratory samples, allows for the improved collection and release of respiratory epithelial cells and secretions from both children and adults. Their use for obtaining nasopharyngeal specimens has been shown to have a similar performance to nasopharyngeal aspirates for the detection of common respiratory viruses in children, and the technique is relatively noninvasive.

Nasal or oropharyngeal samples are generally not recommended for routine diagnostic use. The combining of nasal and throat swabs has been trialed in children in hospital and community settings and shown to have a reduced sensitivity. In general, viral loads are higher in the nasopharynx than in the oropharynx, but with some respiratory virus infections, avian influenza H5N1, for example, titers may be highest in the lower respiratory tract. There may also be a higher yield from throat swabs compared to other samples for the detection of *Mycoplasma pneumoniae.*

**Induced Sputum**

Culture of sputum specimens is commonly used as part of the evaluation of pneumonia in adults. Despite difficulties with interpretation of results, carefully collected and processed sputum specimens have been shown to be useful in some contexts. Nonetheless, there is still ongoing controversy about the value of routinely examining sputum. Furthermore, sputum microscopy and culture are not routinely performed in children due to difficulties in obtaining specimens in this age group who are typically unable to expectorate.

To overcome specimen collection problems, methods such as hypertonic saline nebulization have been used to induce sputum production. Induced sputum is now widely used to investigate lower respiratory infections in immunocompromised adults, especially for diagnosing *Pneumocystis jirovecii* infection, and has also been used to diagnose pneumonia in children from settings with a high prevalence of tuberculosis. However, few studies have collected induced sputum routinely from children with pneumonia. Recent studies of children hospitalized with community-acquired pneumonia from Finland, Kenya, and New Caledonia showed that collection of induced sputum was well tolerated, with a diagnostic yield from culture ranging from 12% to 65% using different interpretative criteria.

The most rigorous evaluation of induced sputum for the diagnosis of pneumonia in children was performed as part of the Pneumonia Etiology Research for Child Health (PERCH) study. In this large study, there was no clear evidence that isolation of specific potential pathogens by culture or detection by polymerase chain reaction (PCR) was associated with pneumonia case status. In addition, for PCR, there was no evidence that induced sputum provided additional evidence over and above testing a nasopharyngeal specimen. In contrast, a recent longitudinal study from South Africa found that testing of induced sputum in addition to nasopharyngeal swabs provided incremental yield for detection of *Bordetella pertussis* and several respiratory viruses.

**Bronchoscopy Specimens**

Although obtaining a lower respiratory sample via bronchoscopy is more invasive than sputum collection and is only available in certain facilities, there are potential advantages in being confident that the sample actually comes from the lower respiratory tract and in the avoidance of upper airway contamination. However, despite best efforts to avoid contamination with normal upper airways flora (including with use of protected specimen brushes), this is often difficult to achieve and must be considered when interpreting routine bacterial culture findings. In practice, the use of bronchoscopy to obtain specimens in the context of childhood respiratory infections is largely restricted to immunocompromised individuals and those with problematic cystic fibrosis or with persistent focally abnormal chest radiographic changes. Bronchoscopy can also have an important role in the diagnosis and management of pediatric pulmonary tuberculosis.

**Endotracheal Aspirates**

Despite widespread use, the value of endotracheal aspirates to diagnose the cause of ventilator-associated pneumonia is debatable. Even though quantitative culture methods have been recommended, tracheal aspirate microscopy and culture do not appear to distinguish between infection and colonization. There is also evidence that specimens should be rejected from further processing if no organisms are seen on Gram stain.

**Transthoracic Lung Aspiration**

Needle aspiration of an area of suspected pneumonia is theoretically most likely to obtain the ideal clinical specimen for determining microbial etiology of pneumonia. Experience with large numbers of procedures at some locations has demonstrated the good safety profile of this technique. In The Gambia, which has the greatest experience with transthoracic lung aspiration in children, a review of over 500 lung aspirates over 25 years reported complications in six patients (all transient) and no deaths from the procedure. Diagnostic yield with both culture and nucleic acid detection methods is appreciable, with about a two-fold increase in yield with nucleic acid detection over culture alone. However, the interpretation of results from highly sensitive molecular diagnostic techniques needs to consider new concepts of the lung microbiome that question whether the lungs are normally sterile. Transthoracic needle aspiration is not indicated.
for all children with pneumonia and is only appropriate for peripheral lesions confirmed on chest radiography.

**Lung Tissue**

The use of lung tissue to determine the microbial etiology of pneumonia is largely restricted to postmortem studies.\(^4\) These are rarely performed on children but may provide valuable information on the causes of fatal cases of pneumonia and can confirm antemortem microbiological diagnoses.

**Blood Specimens**

Blood can be collected for culture, serological testing and, occasionally, nucleic acid tests. The yield from blood cultures is enhanced by obtaining adequate specimen volume, collecting the specimen prior to antimicrobial therapy, and avoidance of skin contamination through good phlebotomy technique.\(^3\),\(^4\) Although there is good evidence that yield increases with increasing blood volume, the optimal collection volume in children is unclear. One current guideline recommends the collection of 3% to 4% of total patient blood volume in patients weighing less than 12.7 kg and 1.8% to 2.7% in patients weighing greater than 12.8 kg.\(^5\) Anaerobic blood culture is usually unnecessary in children.

**Urine**

The main reason to collect urine specimens as part of the workup of respiratory infections in children is to test for specific antigens. For this purpose, the timing of specimen collection in relation to antimicrobial therapy is less important than for urine culture for suspected urinary tract infection. Collection of acute phase urine specimens can be challenging in young children, and a variety of techniques have been deployed to enhance collection in a clinically relevant time frame.

Testing of urine for antimicrobial activity by simple biosay methods has been a valuable tool for detecting prior antimicrobial administration in epidemiological studies, although the timely collection of urine samples in young children can be challenging.\(^4\)

**MICROBIOLOGICAL TOOLS**

**Microscopy**

As part of the investigation of respiratory infections, specimens obtained from the lower respiratory tract, pleural space, or abscesses that are sent for bacterial culture are usually examined first by Gram stain microscopy. Microscopy provides information on specimen quality and can provide early clues about the cause of infection. For example, the presence of large numbers of polymorphonuclear leukocytes indicates an inflammatory response, while the presence of bacteria with characteristic morphology may provide an early indication of the culture result and give guidance about treatment. When performed by experienced microscopists, some findings can be very specific. For example, the detection of Gram-positive cocci in clusters in a pleural fluid sample is highly suggestive of *Staphylococcus aureus*. The presence of a predominance of small gram-negative pleomorphic coccobacilli in a good-quality sputum sample is suggestive of infection with *H. influenzae*. Table 22.1 lists the typical Gram stain picture of three commonly found pathogens.

Microscopy is also an important tool to assess the quality of lower respiratory samples, which itself has a large impact on the interpretation of culture results.\(^4\) Specimens from the lower respiratory tract can be contaminated by upper respiratory secretions during collection. Also, a poorly collected “lower respiratory” specimen may be predominantly composed of upper respiratory secretions. Either situation can lead to incorrect interpretations of culture results. To overcome this issue, it is standard practice for diagnostic laboratories to assess the quality of lower respiratory samples before they are cultured. This typically involves assessing the number of squamous epithelial cells (SECs) and polymorphonuclear cells (PMNs) in a Gram-stained smear of the specimen.\(^10\),\(^31\) The presence of low numbers of SECs and high numbers of PMNs per low-power field are regarded as being indicative of a high-quality specimen.\(^5\) Conversely, specimens with relatively low numbers of PMNs and high numbers of SECs are likely to represent oropharyngeal contamination and are rejected for routine culture. Detection of a potential pathogen in such a specimen that is contaminated with oropharyngeal flora may represent nothing more than the patient’s oropharyngeal microbiota.

**Table 22.1** summarizes some commonly used criteria for assessment of lower respiratory specimens. Other rejection criteria have been described that also include the presence of PMNs,\(^5\) and it is the responsibility of the laboratory to have a standard operating procedure that specifies rejection criteria. Although there is a paucity of data from children, quantity of SECs alone was demonstrated to be a useful quality measure for induced sputum from young children with pneumonia.\(^5\) Notable exceptions to sputum rejection criteria are specimens for detection of *Legionella* spp.\(^5\) and *Mycobacterium tuberculosis*; any specimen submitted for investigation of legionellosis or tuberculosis should be processed by the laboratory regardless of the specimen quality.

**Culture**

Traditional bacterial culture techniques continue to be a fundamental diagnostic tool in diagnostic laboratories. In contrast, viral culture is now infrequently performed as a routine test, as it is time-consuming, requires a specialist laboratory area and has been largely superseded by molecular diagnostic techniques.

Although most important bacterial pathogens grow on standard laboratory media, such as sheep blood agar, special media environmental conditions are required to optimize the growth of some bacteria. For example, chocolate agar is the usual medium used to isolate *H. influenzae*, an atmosphere of 5% CO\(_2\) is required to isolate *S. pneumoniae*, and special media are required for culturing *Legionella* species and *B. pertussis*. As a rule of thumb, it takes most bacterial pathogens 24 to 48 hours to grow in culture, and a further 24 to 48 hours are required to perform antimicrobial susceptibility testing.

The recent availability of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized the workflow in diagnostic laboratories.\(^5\)–\(^7\) MALDI-TOF MS allows the rapid identification of cultured microorganisms at a relatively low cost. The identification is based on the generation of mass spectra from whole cell extracts that are then compared to a library of well-characterized protein profiles. Although this method still
Among the most common antigen detection assays for respiratory infections are the RADTs for respiratory viruses, particularly influenza viruses and respiratory syncytial virus. The usefulness of RADTs is limited by variable and often suboptimal sensitivity, typically 50% to 98% for RSV and 10% to 85% for influenza viruses, although specificities are generally high (80% to 100%). The clinical usefulness of these tests is affected by disease prevalence, being poor when there are few cases in the community (positive predictive value is low and false-positive cases are more likely). During peak virus circulation, although the positive predictive value approaches 100%, the negative predictive value is lower and false-negative results are more likely. Due to concerns about poor sensitivity, most authorities recommend that RADTs for influenza are only used with caution outside the influenza season and only when a result will influence patient management; they emphasize that negative RADT results do not exclude influenza in patients with typical signs and symptoms.

Other commonly used antigen detection assays for respiratory infections are those that detect *S. pneumoniae* and *Legionella pneumophila* serogroup 1 antigens in urine. These assays, typically in immunochromatographic test format, provide results within a short time frame, but are almost exclusively used on adults with suspected pneumonia. The specificity of currently used pneumococcal urinary antigen tests in children is poor, with frequent false positives due to nasopharyngeal carriage of *S. pneumoniae*. This has limited the clinical utility of this test in children, but there may be some value as a diagnostic adjunct in cases with radiologically confirmed pneumonia. There is considerable interest in the development of serotype-specific pneumococcal urinary antigen tests. Early assessments in children indicate that these next-generation assays may have some diagnostic value, at least in epidemiological studies, but assay cutoffs need to differ from adults to distinguish between carriage and disease. Pneumococcal antigen detection assays have also been successfully applied to pleural fluid samples in children with pleural effusion or empyema. A positive test result has high specificity in this context.

RADTs are also available for the diagnosis of *S. pyogenes* in throat swab specimens. These tests have high specificity for detection of *S. pyogenes*, but have relatively low sensitivity (70% to 90%), which is even lower in those with less severe disease. As a consequence of suboptimal sensitivity, it is commonly recommended to perform bacterial culture on any samples that test negative by RADTs.

**Serology**

Serological testing for respiratory pathogens was commonly performed in the past, relying either on the detection of immunoglobulin M (IgM) in the acute phase of the disease or the demonstration of seroconversion. More recently, the use of serological testing has largely been replaced by molecular-based assays that provide a rapid diagnosis with greater sensitivity and specificity.

Serological assays still have a limited place in the diagnosis of childhood respiratory disease. Detection of IgM antibodies is still a routine diagnostic tool for *M. pneumoniae* infection. However, older children may not mount an IgM response because of reinfection rather than primary infection, and IgM antibodies may persist for months after the acute...
infection. For detection of *B. pertussis* infection, IgG antibody responses to pertussis toxin may be an indicator of infection, although these assays cannot differentiate between an immune response induced by infection and that due to vaccination. Serological diagnosis of pertussis has largely been replaced by molecular-based assays. The serological diagnosis of *Chlamydia pneumoniae* infection is complicated by the lack of species-specific tests and the resultant potential of cross-reactions in the assay. A single positive IgM response in any disease investigation may represent possible cross-reactivity or nonspecific interference in the assay and needs to be interpreted with caution and in the context of the clinical presentation.

Although detection of antistreptolysin O (ASO) and deoxyribonuclease (DNase) antibodies can be used when investigating the potential complications of *S. pyogenes* infections, such as glomerulonephritis and rheumatic fever, they are not useful for the diagnosis of acute *S. pyogenes* pharyngitis.

**Molecular Methods**

The development and implementation of molecular methods is the single biggest recent advance in the diagnostics of respiratory infections. While nucleic acid detection tests (NATs), such as PCR, have been used to detect respiratory pathogens for over two decades, the widespread adoption of these tests by diagnostic laboratories has occurred only recently, largely due to the increased availability of commercial assays. Table 22.16 discusses some of the more commonly used molecular assays, and Table 22.17 gives explanation of commonly used terms in molecular diagnostics.

NATs have several advantages over other diagnostic tools, including rapid turnaround time, the ability to detect low levels of all known pathogens, the lack of dependence on the viability of the target microorganism, little influence of antimicrobial therapy on diagnostic sensitivity, and the ability to be automated. NATs may also provide additional information, such as antimicrobial susceptibility data and strain typing.

For the diagnosis of respiratory infections, the most widely used NATs are those that detect respiratory viruses and non-colonizing bacteria (e.g., *M. pneumoniae*, *Legionella* species, *B. pertussis*). For these microorganisms, detection in a respiratory sample from a child with a compatible clinical syndrome is regarded as sufficient evidence to assign causation. In contrast, NATs for other bacteria that may also be found in normal respiratory flora, including some of the most important pneumonia pathogens (e.g., *S. pneumoniae*, *H. influenzae*, and *S. aureus*), have struggled for a role outside research laboratories. As with culture-based methods, the problem with detection of these targets by NAT is the inability to distinguish colonization and carriage from disease.

NATs have particularly revolutionized the diagnosis of viral respiratory tract infections and are now the testing method of choice. Respiratory viruses are now commonly detected by large multiplex panels that typically include influenza A and B viruses, respiratory syncytial virus, para-influenza viruses, human metapneumovirus, human rhinoviruses, enteroviruses, parechovirus, adenoviruses, human bocavirus, and several coronaviruses (OC43, 229E, NL63, HKU1). There are now many commercial multiplex assays available in a variety of formats, and the landscape is continually changing. In the right clinical context, the detection of a respiratory virus in a respiratory sample is generally regarded as being sufficient to assign causation. However, this assumption is not always reliable as there is uncertainty about the pathogenic role of some viruses, leading some to question the wisdom of using large multiplex NAT panels as first-line tests for respiratory pathogens, given potential problems with interpretation of positive results. Furthermore, respiratory viruses are often detected in a similar proportion of both subjects with and without pneumonia in childhood pneumonia etiology studies although this observation typically does not apply to influenza A and B viruses, respiratory syncytial virus, and human metapneumovirus, which are disproportionately associated with case status.

NATs have also been used to detect *S. pyogenes* in throat swab samples, although these methods have not been used widely. This situation is likely to change with the recent increased availability of commercial methods and the motivation to improve turnaround times to better guide antimicrobial therapy.

Detection of microbial load by quantitative molecular methods has been explored in the effort to help distinguish infection from contamination or colonization. Microorganisms detected in greater quantities may be more likely to be clinically significant. Quantitative multiplex PCR has been used to determine the etiology of community-acquired pneumonia in adults using cutoffs developed for interpretation of culture results from lower respiratory tract specimens. Greater confidence in the diagnostic cutoffs will be needed before this approach can be introduced into routine diagnostic use. Quantitative approaches using NATs have also been applied to nasopharyngeal specimens. Among human immunodeficiency virus (HIV)-infected adults in South Africa, quantitative PCR testing of nasopharyngeal samples distinguished between pneumococcal pneumonia and asymptomatic pneumococcal colonization with reasonable diagnostic accuracy. Nasopharyngeal pneumococcal load also distinguished colonization from microbiologically confirmed pneumococcal pneumonia in a large pediatric study, although the diagnostic accuracy was inadequate for clinical use.

NATs have also been applied, with limited success, to non-respiratory specimens for determining the microbial etiology of respiratory infections in children. There has been particular interest in the testing of blood for *S. pneumoniae* by PCR. Among Italian children, blood PCR detected invasive pneumococcal disease with high specificity. However, in other populations positive results have been reported in control participants who do not have suspected pneumococcal disease, with false-positive results being relatively common in children from developing countries where there is a high prevalence of pneumococcal carriage.

The potential application of whole genome sequencing in the diagnostic laboratory is still being realized. This method is already being increasingly used for strain characterization of bacterial isolates as part of epidemiological investigations. However, its precise role in determining the etiology of respiratory infections is uncertain.

**Antimicrobial Susceptibility Testing**

Most antimicrobial susceptibility testing methods are performed on pure live bacterial cultures using a variety of
standard methods. Several guidelines have been established for interpretation of findings; the most commonly used guidelines are produced by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). These guidelines are comparable, and it is essential that each diagnostic laboratory chooses an approved guideline for interpretation of their antimicrobial susceptibility test results. Increasingly, molecular methods with rapid turnaround times are being used to detect specific antimicrobial resistance mechanisms. This trend is likely to continue given the constant demands of rapid identification of resistant pathogens.

Antiviral susceptibility testing against respiratory pathogens is rarely indicated and has mainly focused on influenza viruses.

**DIAGNOSTIC APPROACH BY SYNDROME**

**Common Cold**

Manifestations of the common cold are so typical that diagnostic testing is usually unnecessary. If there is a reason to determine the specific virus involved, testing a nasopharyngeal specimen for respiratory viruses by NAT is the current test of choice.

**Pharyngitis**

The main reasons to diagnose the cause of acute pharyngitis are to detect cases caused by *S. pyogenes* and to identify the occasional case due to less common causes, such as *Arcanobacterium haemolyticum* and *Corynebacterium diphtheriae*. Throat swab culture is still the mainstay although antigen detection assays are available. In future, molecular point of care tests are likely to become available to clinicians in primary care.

**Croup**

The diagnosis of croup is usually based on the characteristic clinical picture (fever, hoarseness, barking cough, inspiratory stridor, and varying degrees of respiratory distress) and epidemiology. Identification of specific microbial causes can be accomplished by testing a nasopharyngeal specimen for respiratory viruses by NAT.

**Sinusitis**

Diagnostic testing is not usually performed on cases of acute sinusitis as the microbial etiology is well described. However, sinus puncture should be performed to obtain specimens for bacterial culture in patients with severe sinusitis, in those who have not responded to empiric antibiotics, and in patients with severe immunosuppression.

**Epiglottitis**

*H. influenzae* type b is isolated in cultures of blood and/or epiglottis in most children with epiglottitis. Direct visualization of the epiglottis should be performed in a setting where immediate securing of the airway is possible.

**Bronchiolitis**

A specific diagnosis of the causative agent of bronchiolitis can be made by testing a nasopharyngeal specimen for respiratory viruses by NAT.

**Pneumonia**

Determining the microbial etiology of pneumonia in children remains challenging, largely due to difficulties obtaining a sample from lungs. Current guidelines for the management of community-acquired pneumonia in children generally recommend that diagnostic tests should mainly be used on patients with severe disease, with a focus on blood cultures and detection of respiratory viruses. The development of improved urinary antigen tests and quantitative molecular assays holds hope for the future.

**Pleural Effusion and Empyema**

Gram stain and culture of fluid aspirated from the pleural cavity is indicated in patients for whom a diagnosis of infection is considered. The sample can also be tested for pneumococcal antigen by a RADT and nucleic acid detection methods. Testing of pleural fluid increased the yield of *S. pneumoniae* detection by 31% in South African children with empyema.

**Lung Abscess**

Needle aspiration provides the best opportunity to identify the microbial cause of an abscess. Abscess fluid may also be recovered by bronchoscopy if it has ruptured. Blood cultures should also be performed in children with suspected lung abscess.

**Infections Associated With Cystic Fibrosis**

There is often a close working relationship between clinicians caring for patients with cystic fibrosis and laboratory scientists. Special attention is given by the laboratory to lower respiratory specimens from patients with cystic fibrosis with a particular focus on classic pathogens associated with this disease, such as *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, and *S. aureus*. The use of synergy testing to assess antimicrobial combinations is often used in cystic fibrosis patients with multiresistant organisms, although the value of this practice has been questioned.

**Microbiome**

Recognition of the possible existence of the lung microbiome has been a major recent revelation in respiratory medicine. Until recently, the lungs in health were regarded as sterile, but the use of modern culture-independent techniques has consistently found evidence of bacteria in the lower airways. Most of these studies have been performed on bronchoscopic specimens, which may be susceptible to contamination, but there is certainly mounting evidence supporting the non-sterility of the lung.

The existence of the lung microbiome has challenged our traditional paradigm of pneumonia pathogenesis, as the traditional view is that pneumonia is caused by a single invasive pathogen in a normally sterile site. There is increasing recognition that bacteria and viruses frequently interact in the causative pathway to pneumonia, and the common finding of polymicrobial infection adds further complexity to our understanding of how pneumonia develops. The traditional bacterial versus viral pneumonia concept may be too simplistic. Consequently, we are likely to need...
more sophisticated approaches to pneumonia diagnosis and interpretation of laboratory results than simply using assays that target single specific putative pathogens.

We have a lot to learn about the lung microbiome and are only just beginning to understand changes in the lung ecosystem during acute infections. Analysis of the lung microbiome may provide insights into pneumonia etiology and reveal novel markers for pneumonia prognosis and treatment guidance. The following are some examples of recent findings about the respiratory microbiome that may have clinical implications, and they give an indication of the applications that may be available in the future.

Using 16S ribosomal RNA sequencing, a recent study showed that certain taxa in the respiratory microbiota were associated with the clinical course of pediatric pneumonia. In children aged 6 months to 5 years, high relative abundance in sputum of Actinomyces, Veillonella, Rothia, and Lactobacillales was associated with decreased odds of length of stay ≥ 4 days, and high relative abundance of Haemophilus and Pasteurellaceae was associated with increased odds of intensive care unit admission. In children aged 5 to 18 years, high relative abundance in sputum of Porphyromonadaceae, Bacteriodales, Lactobacillales, and Prevotella was associated with increased odds of length of stay ≥ 4 days.

In another recent study, the composition of the nasopharyngeal bacterial community of children was related to the prior history of acute sinusitis. History of acute sinusitis was associated with significant depletion in relative abundance of taxa including Faecalibacterium prausnitzii and Akkermansia spp. and enrichment of Moraxella nonliquefaciens. Children who experienced more frequent upper respiratory infections had significantly diminished nasopharyngeal microbiota diversity.

Other recent data indicate that interactions between RSV and nasopharyngeal microbiota might modulate the host immune response, potentially affecting clinical disease severity. The nasopharyngeal microbiome at the time of upper respiratory viral infections during infancy may contribute to the ensuing risk for development of asthma, and that the microbiome of children with cystic fibrosis is susceptible to environmental influences, suggesting that interventions to preserve the community structure found in young patients and slow disease progression might be possible.

We can expect to see an exponential increase in publications on the role of the respiratory microbiome in health and disease over the next few years. The extent to which these findings can be readily translated into clinical applications is uncertain.

**Future Prospects**

The trend towards increased use of molecular diagnostic tools will probably continue with increased availability of point of care testing. It is also likely that measurement of bacterial and viral pathogen load will be part of those developments, both for distinguishing between colonization and disease and for monitoring response to treatment. Any future developments in diagnostics for respiratory infections must incorporate new knowledge about the lung microbiome. For lower respiratory infections, there is likely to be a move away from the detection of specific known pathogens to measurement of markers of change in the lung microbial ecology during disease. The development of new and better urinary antigen tests would be welcome, as these can be readily adapted to point of care testing.

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Access the reference list online at [ExpertConsult.com](http://ExpertConsult.com).
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