Respiratory Infections

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Abstract

The majority of respiratory tract infections (RTIs) are community acquired and are the single most common cause of physician office visits and among the most common causes of hospitalizations. The morbidity and mortality associated with RTIs are significant and the financial and social burden high due to lost time at work and school. The scope of clinical symptoms can significantly overlap among the respiratory pathogens, and the severity of disease can vary depending on patient age, underlying disease, and immune status, thereby leading to inaccurate presumptions about disease etiology. The rapid and accurate diagnosis of the causative agent of RTIs improves patient care, reduces morbidity and mortality, promotes effective hospital bed utilization and antibiotic stewardship, and reduces length of stay. This chapter focuses on the clinical utility, advantages, and disadvantages of viral and bacterial tests cleared by the Food and Drug Administration (FDA), and new promising technologies for the detection of bacterial agents of pneumonia currently in development or in US FDA clinical trials are briefly reviewed.

Keywords

Respiratory infections • Viral respiratory pathogens • Bacterial respiratory pathogens • Lower respiratory tract infections • Upper respiratory tract infections • Community-acquired pneumonia • Hospital-acquired pneumonia • Molecular tests

Introduction

The majority of respiratory tract infections (RTIs) are community-acquired and are the single most common cause of physician office visits and among the most common causes of hospitalizations [10, 68, 146, 182, 194]. The morbidity and mortality associated with RTIs are significant and the financial and social burden high due to lost time at work and school. Viral infections cause between 65–80 % of respiratory tract diseases with mixed viral infections present in 5–20 % of viral RTIs in adults and as high as 62 % in studies of children ≥6 years of age [66, 80, 132, 172, 182, 194]. In Europe and in the USA the incidence of pneumonia due to a bacterial pathogen is 1–10 cases per 10,000 inhabitants, depending on various factors such as age. The differentiation of viral from bacterial RTIs can be assisted considering the rate of onset of illness, patient age, symptoms, radiographic changes, biomarkers, response to treatment, and the presence of documented viral epidemics in the community. However, the scope of clinical symptoms can significantly overlap among the respiratory pathogens, and the severity of disease can vary depending on patient age, underlying disease, and immune status, thereby leading to inaccurate presumptions about disease etiology.

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Numerous studies have demonstrated that a rapid and accurate diagnosis of the causative agent of RTIs improves patient care, reduces morbidity and mortality, promotes effective hospital bed utilization and antibiotic stewardship, and reduces length of stay [9, 20, 22, 82, 92, 106, 115, 120, 121, 142, 160, 170, 191, 227, 229, 234]. Pathogen etiology may need to be considered in the clinical management of certain patients, such as immunocompromised patients or young infants, if there is a potential for the development of more severe disease [13, 65, 68, 80, 81, 86, 194]. In health care settings, the identification of a viral RTI prompts the initiation of appropriate infection control measures, thereby reducing morbidity and mortality associated with nosocomial transmission [19, 39, 40, 62, 126, 155, 217, 219].

Over the last several years great advances have been made in obtaining USA Food and Drug Administration (FDA) clearance for a variety of nucleic acid amplification tests (NAATS) that detect single or multiple viral respiratory pathogens and three bacterial pathogens (*Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Bordetella pertussis*). This chapter focuses on the clinical utility, advantages, and disadvantages of these tests. Unfortunately, no FDA-cleared assays are available for the detection of the majority of bacterial and fungal pathogens associated with community-acquired pneumonia (CAP), hospital-associated pneumonia (HAP), and ventilator-associated pneumonia (VAP). New promising technologies for the detection of bacterial agents of pneumonia currently in development or US FDA clinical trials are briefly reviewed. Although sensitive, accurate fungal diagnostics is an area of importance, the development of new diagnostic assays has been significantly delayed. Hence, for the detection of the vast majority of the nonviral pathogens, laboratory developed tests (LDTs) are the mainstay of molecular testing and will not be discussed. The importance of new rapid diagnostics for CAP, VAP, and HAP has been highlighted by a workshop conducted in November 2009 by the Infectious Diseases Society of America (IDSA) in collaboration with the FDA, the proceedings of which have been published in a special supplement of Clinical Infectious Diseases [102]. The IDSA, in an executive summary of the workshop, states that there is "a need to develop and implement modern molecular technologies to advance microbiological diagnostic testing."

Epidemiology

The common viruses that cause both lower RTIs (LRTIs) and upper RTIs (URTIs) are listed in Table 52.1. Overall, viruses are the most common cause of URTIs and the second most common etiologic cause of CAP (behind *Streptococcus pneumoniae*), ranging from 13–50 % of diagnosed cases [10, 146, 132, 172, 182, 194]. CAP in children is predominately of viral etiology, with the majority of the infections caused by respiratory syncytial virus (RSV), human rhinovirus (HRV), human metapneumovirus (HMPV), and parainfluenza viruses 1, 2, 3, 4 (PIV) [99, 194]. Influenza A (FluA) and influenza B (FluB), adenovirus (ADV), and the coronaviruses (CoVs) are additional causes of CAP in children. RTIs in non-immune compromised hospitalized children (especially < 5 years of age), are mainly due to infection with one or multiple viruses without a secondary bacterial infection. Viral CAP decreases in frequency in healthy young and

Table 52.1 Common viral and bacterial respiratory pathogens

Virus: RTI and CAP	Bacteria: RTI and CAP	Bacteria: VAP and HAP
Adenovirus (ADV)	*Bordetella pertussis* and *Bordetella parapertussis*	*Acinetobacter* spp.,
Human bocavirus (HBoV)[a]	*Chlamydophila pneumoniae*	*Corynebacterium* spp.
Coronavirus (CoV) (OC43, 229E, NL63, HKU-1) SARS, MERS	*Haemophilus influenzae*	*Enterobacteriaceae* (MDRO)
Enterovirus (EV)	*Legionella pneumophila*	*Legionella pneumophila*
Human metapneumovirus (HPMV)	*Moraxella cattharalis*	*Pseudomonas* spp.
Influenza A (seasonal H1 [A/H1], seasonal H3 [A/H3], (H1N1)pdm09 [A/2009 H1])	*Mycoplasma pneumoniae*	*Stenotrophomonas maltophilia*
Influenza B	*Neisseria meningitidis*	*Staphylococcus aureus* (MSSA and MRSA)
Parainfluenza virus 1, 2, 3, 4 (PIV)	*Staphylococcus aureus* (MSSA and MRSA)	
Human rhinovirus (HRV)	*Streptococcus pneumoniae*	
Respiratory syncytial virus (RSV)		

[a]Pathogenic status not fully determined

RTI respiratory tract infection, *CAP* community-acquired pneumonia, *VAP* ventilator-associated pneumonia, *HAP* hospital-associated pneumonia, *MSSA* methicillin-sensitive *Staphylococcus aureus*, *MRSA* methicillin-resistant *Staphylococcus aureus*, *MDRO* multidrug-resistant organism

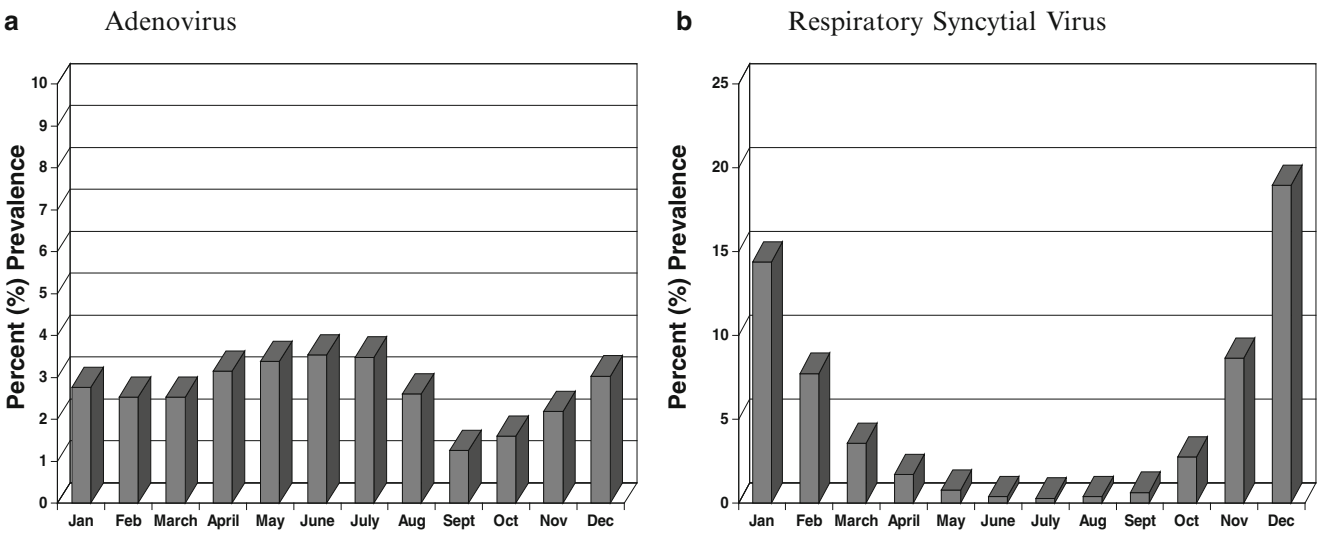

Figure 52.1 Mean prevalence of adenovirus (a) and respiratory syncytial virus (b) by month for the years 2000 through 2011. Adenovirus causes infections throughout the year, while respiratory syncytial virus infections are more seasonal

middle-aged adults to about one-third of the cases, with influenza, HRV, and CoV being the most common agents. Viral CAP rates substantially increase among the elderly, often complicated by secondary bacterial infection. The most common viral causes of CAP in the elderly are influenza and RSV; however, HMPV, PIVs, CoVs, and HRV cause both URTIs and LRTIs [66, 194].

Since 2008 several clusters of respiratory illness associated with human enterovirus 68 (HEV68) were reported in Asia, Europe, and the USA (MMWR [29]). HEV68, a unique enterovirus with similar biologic features to HRV, was associated with RTIs ranging from relatively mild illness to severe illness requiring intensive care and mechanical ventilation. Severe disease was particularly pronounced in children (MMWR [29]). Although human bocavirus (HBoV) has been implicated as a cause of RTI, in the majority of cases HBoV is detected in conjunction with other viral pathogens and the clinical significance of HBoV is still debated [105, 200]. HBoV is more commonly found in children; however, HBoV can affect persons of all ages. Underlying disease, such as cancer, is associated with severe infections requiring hospitalization.

Generally the CoVs (NL-63, OC43, HKU-1, 229E) cause mild, self-limiting URTIs such as the common cold. However, in the last decade, two CoVs—Severe Acute Respiratory Syndrome (SARS-CoV) and the Middle East Respiratory Syndrome (MERS-CoV) have emerged with a potential pandemic threat due to person-to-person communicability [33]. Both viruses can cause severe lower respiratory tract disease with extrapulmonary involvement and are associated with high case-fatality rates. Although SARS-CoV is currently not circulating, since 2012 MERS-CoV continues to cause outbreaks in the Middle East with secondary spread to Europe, Africa, Asia, and North America.

The prevalence of each viral pathogen can vary depending on environmental conditions (climate, season, geographic location). For example, in the New York City area, adenovirus (ADV) tends to circulate with the same prevalence year round (Fig. 52.1a), while RSV can be detected sporadically year round with the peak season in October through February (Fig. 52.1b) (unpublished data provided by author). The "classic" influenza season begins generally in late October and wanes during April. HMPV prevalence tends to rise as RSV season wanes in February and peaks in the spring, PIV-1 and PIV-4 are most prevalent in the summer through fall, while PIV-3 prevails in the spring and PIV-2 in the fall. Virus prevalence also can be patient population driven (e.g., pediatric, adult, geriatric, outpatient, or inpatient). For example, RSV and HMPV are primarily found in children <5 years of age, but can cause severe disease in all age groups [64, 65, 94, 97]. In the immunosuppressed population, other less common respiratory viral pathogens, such as herpes simplex virus (HSV) and cytomegalovirus (CMV), must be considered as a potential cause of RTIs.

The major bacterial pathogens responsible for CAP (Table 57.1) include *S. pneumoniae* (20–60 %), *Staphylococcus aureus* (methicillin-susceptible [MSSA] and methicillin-resistant [MRSA]) (3–5 %), *Haemophilus influenzae* (3–10 %), variable gram-negative rods (3–10 %), and rarely *Neisseria meningitidis* (<1 %) depending on the patient and underlying diseases such as chronic obstructive pulmonary disease (COPD) or asthma [10, 146]. *Moraxella cattharalis* is generally associated with otitis media in children and exacerbation of asthma and COPD in adults. The classic atypical pathogens that cause CAP include *C. pneumoniae*, *M. pneumoniae*, and *Legionella pneumophila*, which also can be nosocomially acquired in institutional settings [10, 17, 46, 67, 222].

The majority of RTIs (up to 70 %) caused by C. *pneumoniae* are asymptomatic or have minimal symptoms. *C. pneumoniae* accounts for 6–20 % of CAP and 5 % of cases of sinusitis, pharyngitis, tracheitis, bronchiolitis, bronchitis, and exacerbations of chronic bronchitis and asthma in both immunocompetent and immunosuppressed persons [17, 42]. *C. pneumoniae* can be present with other bacterial pathogens in up to 30 % of adult cases of CAP. *C. pneumoniae* infection can present with varied clinical courses from mild, self-limiting disease to severe forms of pneumonia, particularly in patients with cardiopulmonary disease and in the elderly [17]. All age groups are affected; however, *C. pneumoniae* infections are rarely found in young children < 5 years of age. However, by age 20, 50 % of persons have detectable antibody levels, with the elderly demonstrating a seropositivity rate between 70 – 80 % [221].

M. pneumoniae is estimated to cause 6–20 % of the cases of CAP [222]. Although most infections are asymptomatic, mild, and often self-limiting, approximately 1–5 % of infections may require hospitalization and can lead to serious extrapulmonary complications. LRTIs are more common in school age children and adolescents, with the prevalence in pediatric LRTI ranging from 10–40 %. *M. pneumoniae* infection can cause outbreaks in the community and institutions such as schools, prisons, and hospitals.

Legionella spp. causes two distinct clinical entities. Pontiac Fever is a self-limiting flu-like illness and Legionaire's disease is a severe multisystem disease involving pneumonia. Cases can be sporadic or part of outbreaks due to environmental exposure [51, 67]. *L. pneumophila* is responsible for 2–8 % of CAP and is responsible for 2–15 % of all CAP that require hospitalization. *L. pneumophila* (serogroup 1) is responsible for 90 % of the diagnosed disease, most probably because the major diagnostic tests are specific for this serogroup. Risk factors for contracting Legionellosis include smoking, immunosuppression, age ≥ 65, chronic lung disease such as emphysema, diabetes, kidney disease, cancer, or contact with environmental systems such as air conditioning cooling towers, evaporative condensers, whirlpools, and hot spring baths.

Coxiella burnetii, an obligate gram-negative intracellular bacterium has primary reservoirs in cattle, sheep, and goats. Transmission to humans occurs primarily through inhalation of aerosols from contaminated soil or animal waste. Most *C. burnetti* infections are manifested as Q Fever, a self-limited, influenza-like febrile illness (88–100 %) of abrupt onset, manifested by chills, headache, myalgia, fatigue, and sweats; ([150], CDC MMWR [30]). However, pneumonia is predominant in North America and usually mild in nature. Patients have dyspnea, pleuritic chest pain, and a dry, nonproductive cough. Rarely, *C. burnetti* infection occasionally can progress to acute respiratory distress syndrome (ARDS) ([150], CDC MMWR [30]).

B. pertussis is the cause of whooping cough [43, 119]. Pertussis-like illness can be attributed to *Bordetella parapertussis*, *Bordetella holmseii*, and rarely *Bordetella bronchioseptica* infections. Overall, *Bordetella* infections have increased dramatically over the last 10 years due to waning immunity, incomplete antibody response to vaccination with acellular vaccines, or lack of vaccination. Recent epidemics have occurred between 2010 and 2014. In 2010, more than 27,000 cases were reported, of which over 9,000 occurred in California. Nationwide by 2013 more than 28,000 cases were reported to the CDC (http://www.cdc.gov/pertussis/surv-reporting.html). The highest incidence was noted in infants < 1 year of age, but a significant amount of disease occurred among children aged 7–10 years. One large outbreak of pertussis-like illness in Ohio from 2010 to 2011 was attributed to both *B. pertussis* (68 % of the cases) and *B. holmseii* (29 % of the cases) [190]. Outbreaks of pertussis continue, highlighting the need for primary vaccination and the administration of "Tdap" booster immunizations.

HAP and VAP are more often associated with drug-resistant, multidrug-resistant, or pan-resistant bacteria, such as MRSA, extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriacae*, or carbapenemase-producing *Enterobacteriacae*, *Pseudomonas* spp., *Acinetobacter* spp., and *Stenotrophomonas maltophilia* [4]. For patients in the intensive care unit (ICU) or immunocompromised patients, other pathogens such as *Nocardia* spp, *Corynebacterium* spp., *Pneumocystis jiroveci*, *Fusarium* spp., *Aspergillus* spp, *Cryptococcus* spp., and the zygomycetes need to be considered in the differential diagnosis.

Clinical Utility

Limitations of Conventional Diagnostic Procedures

Conventional virus detection methods include rapid antigen detection tests (RADTs), direct fluorescent antibody tests (DFAs), rapid cell culture, and traditional tube culture [125]. Although these methods are acceptable diagnostic tools in certain clinical settings, they are often inferior in the breadth of pathogens identified, assay sensitivity, and result turnaround time, when compared to NAATs [18, 28, 39, 73, 74, 79, 125, 157]. The sensitivity and specificity of all the diagnostic tests, but in particular the conventional tests, are highly dependent on the viral target, age of the patient, duration of symptoms prior to sample collection, sample collection methods, and the transport and storage conditions [1, 39, 48, 49, 85, 95, 118, 127, 131, 164].

RADTs are generally the simplest tests to perform, many are waived tests under the Clinical Laboratory Improvement Amendments (CLIA), and results are generally available

within 15–30 min. Despite these benefits, RADTS have limited utility due to the narrow scope of pathogens detected (RSV, FluA, and FluB) [125], and modest to poor sensitivities [21, 39, 45, 61, 75, 79, 125, 199] which can range from 50–90 % for RSV [47] and 10–85 % for influenza viruses depending on the comparator method. Overall, the specificities of RADTs are good [21, 39, 45, 61, 75, 79, 125, 199]; however, the specificity for the detection of influenza A (H1N1) pdm09 was significantly lower than previously reported [197, 198]. RADTs generally perform better when testing pediatric samples since children shed higher titers of virus and for longer time periods than adults, especially the elderly [28, 85, 93].

DFAs detect a broader range of viruses (ADV, FluA, FluB, HMPV, PIV-1, PIV-2, PIV-3, and RSV) and can be performed in 30–60 min. The sensitivities of DFAs vary by virus, ranging from a high of approximately 60–85 % for RSV to a low of 50 % for ADV when compared to NAATs [116, 125]. DFAs are generally very specific, although specificity can be dependent on the level of technical expertise of the reader.

Rapid cell culture (Quidel/Diagnostic Hybrids, Athens, OH) can detect ADV, FluA, FluB, PIV-1, PIV-2, PIV-3, and RSV and has demonstrated sensitivities that range from a high of >80 % for some FluA strains to a low of 50 % for RSV [125, 130, 152] and excellent specificities of greater than 95 % when compared to NAATs. Rapid cell culture is generally positive within 48 h for >90 % of the seven viruses detected.

Depending on the cell lines used and antibodies available for confirmation, traditional tube culture can have a broader scope of pathogen detection when compared to RADTs, DFAs, and rapid cell culture [125]. Traditional culture will identify ADV, enterovirus (EV), HMPV, FluA, FluB, PIV-1, PIV-2, PIV-3, RSV, and HRV, plus additional viruses associated with lower RTIs in immunocompromised patients, including CMV, HSV-1, HSV-2, varicella zoster virus (VZV). Often laboratories do not specifically screen for HRV by culture, although HRV is the most common respiratory virus detected and has been shown to cause significant and serious disease in young, elderly, immunosuppressed patients, as well as patients with underlying chronic lung disease such as COPD and asthma [83]. In addition, many additional important viruses (229E-CoV, OC43-CoV, NL63-CoV, HKU-1-CoV, SARS-CoV, MERS-CoV), PIV-4, and potentially HBoV) that cause both URTIs and LRTIs are not routinely identified by traditional culture [83, 105, 200]. Finally, due to time-to-virus-detection by traditional culture (generally 3–7 days for most respiratory viruses and 3–4 weeks for slow-growing viruses such as CMV), results are usually not available within a time frame (48 h) that could affect patient management (i.e., initiate appropriate antiviral therapy and/or discontinue inappropriate antibiotic therapy). In summary, RADTs, DFAs, and rapid cell culture may provide results within a clinically relevant time frame but with limited pathogen scope and reduced sensitivity compared to NAATs. In addition, RADTs, DFAs, and rapid/traditional cell culture rarely detect more than one virus from a single sample.

The standard methods for the detection of bacterial pathogens causing pneumonia are gram stain in combination with microbiological culture of lower respiratory tract specimens and blood. Often culture of respiratory specimens is not ordered on hospitalized patients and rarely performed in the outpatient setting. A gram stain result can be available within a few hours but results do not always correlate with culture [10]. Culture and antibiotic susceptibility results are usually available in 2–5 days after sample collection, and detection rates for pathogens are relatively low. A meta-analysis that evaluated 122 reports on CAP for the time period of 1966–1995 showed that a bacterial pathogen was only identified in 18 % of the samples tested [68]. A urinary antigen test for *S. pneumoniae* offers a substantial improvement over culture, with a sensitivity of 82 % and a specificity of 97 % in bacteremic adults [209, 210]. However, in non-bacteremic adults and in children, both the sensitivity and specificity are lower [210]. False-positive *S. pneumoniae* antigen tests have been reported relating to antibiotic interference. *Legionella* spp. are identified by growth on buffered charcoal yeast extract agar [158]. The sensitivity of *Legionella* culture can vary significantly from <10–80 % and DFA sensitivity from 25–70 % [158]. A urinary antigen test for *L. pneumophila* improves detection but is suboptimal since detection is limited to serogroup 1. For *L. pneumophila* serogroup 1 the sensitivity of the test varies from 70–100 %. Most laboratories do not culture for *Mycoplasma* or *Chlamydophila* [140].

Traditionally, *B. pertussis* was identified using culture on Bordet–Gengou media and/or DFA. However, the sensitivity of culture ranges from 12–60 %, the sensitivity of DFA ranges from 11–68 % [119]. Additionally, culture can take many days. Therefore, NAATS have become the gold standard for the rapid and sensitive (70–99 %) identification of pertussis [119].

Serologic antibody testing is available for some of the respiratory pathogens and can provide supplemental information. However, due to a delay in the development of detectable IgM or IgG antibodies for certain pathogens (e.g., *L. pneumophila*, *C. pneumoniae*, *B. pertussis*), usefulness for diagnosis in a clinically relevant time frame is very limited [17, 151, 158, 221]. Shortcomings of serological testing include the timing of the serum samples, difficulty in obtaining appropriately paired serum samples, and the high background of IgG antibody prevalence in some adult populations [140]. Serologic diagnosis can be misinterpreted due to prior immunization or infections, such as in the case of influenza, and require demonstration of a significant rise in antibody titers from initial to convalescent samples.

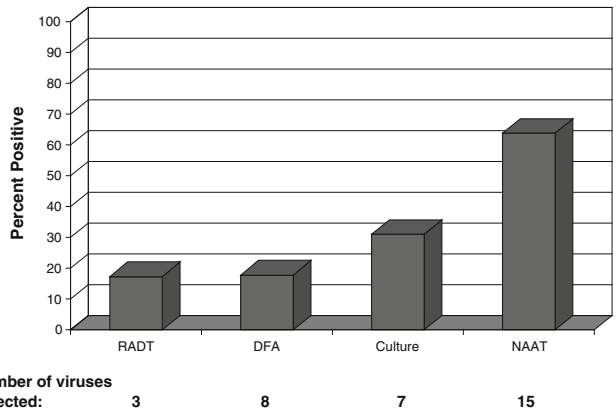

Figure 52.2 Comparison of viral test methods for identification of respiratory pathogens. Percentage of respiratory samples (n = 35,456) positive by each test methods: RADT (rapid antigen detection test) detects three viruses [influenza A (FluA), influenza B (FluB), and respiratory syncytial virus (RSV)]; DFA (direct fluorescent antibody assay) detects eight viruses [adenovirus (ADV), FluA, FluB, human metapneumovirus (HMPV), parainfluenza viruses (PIV) 1, 2, and 3, and RSV]; Culture (R-Mix rapid cell culture) detects seven viruses (ADV, FluA, FluB, PIV-1, PIV-2, PIV-3, and RSV); NAAT (nucleic acid amplification testing) detects 15 viruses (ADV, coronaviruses (CoV: OC43, NL63, HKU1, 229E), enterovirus/rhinovirus group (EV/HRV), FluA (A/H1, A/H3), FluB, HMPV, PIV (1–4), and RSV

Finally, persons with immune suppression may not develop antibodies or they may be of an insufficient level for detection, limiting the functionality of serology for monitoring vaccine response and for epidemiology studies to determine prevalence rates.

Application of Molecular Assays for the Detection of Respiratory Pathogens

Clinical Utility of NAAT for Respiratory Pathogens

Prior to the 2009 FluA(H1N1) pandemic, the infectious causes of CAP were mostly inferred based on clinical presentation which can be highly inaccurate since some bacteria, atypical pathogens, and many of the respiratory viruses cause illnesses with similar clinical symptoms [177, 194]. One study demonstrated that physicians recognized influenza in only 28 % of hospitalized children and 17 % of non-hospitalized children with laboratory-confirmed influenza when the diagnosis was based only on clinical symptoms [177]. Most diagnostic testing was limited to RADTs for FluA, FluB, and RSV as few hospitals offered comprehensive DFAs, viral culture, or laboratory-developed NAATs.

The superior ability of NAATs to rapidly and accurately detect both known and novel pathogens was best exemplified during the chaos of the 2009 FluA H1N1 pandemic [18, 39, 79, 197, 198]. Fortunately, at the start of the pandemic two NAATs were FDA-cleared for the detection of influenza viruses, the Prodesse PROFLU+ (Hologic, San Diego, CA) for the detection and differentiation of FluA and FluB ([124]; and one highly multiplexed NAAT: the Luminex xTAG RVP Respiratory Virus Panel (Luminex Molecular Diagnostics, Toronto, Canada) [113, 114, 143]. The xTAG assay enabled laboratories to detect FluA(H1N1)pdm09 and differentiate the seasonal FluA H1N1 (FluA-H1) and seasonal FluA H3N2 (FluA-H3), but also identify many other circulating viruses [79, 78]. As shown in Fig. 52.2, the number of samples positive for a respiratory virus increased dramatically with the use of the highly multiplexed xTAG RVP assay (64 %) compared to traditional test methods, including RADTS (17 %), DFA (18 %), and rapid cell culture (31 %) [79]. Interestingly, mixed viral infections containing up to four viral pathogens were identified in hospitalized patients [79]. Similarly, other studies have shown that when broad test panels are used more than one virus will be identified in 3–30 % of respiratory samples [8, 166]. Although the significance of mixed viral infections needs to be more clearly defined, the clinical impact needs to be considered as potentially severe in patients with comorbidities, immunosuppression, or other critical illnesses. During respiratory virus seasons with high influenza rates, patients with the same pathogen often are placed in hospital rooms together (cohorting) due to limited private rooms [19, 39, 40, 62, 126, 155, 165, 217, 219]. The consequences of a second viral infection in an already seriously ill hospitalized patient could be substantial, indicating that comprehensive test panels are essential in this setting.

Consequently, there has been a major shift in testing practices as numerous FDA-cleared single-and multi-analyte molecular tests for the detection of respiratory pathogens have become available (Table 52.2). Viral respiratory pathogens are particularly suited for detection using NAATs since the number of targets is relatively limited and the detection of a respiratory virus is generally considered diagnostic, although asymptomatic carriage of certain respiratory viruses has been reported in several studies [2, 14, 104, 181]. For the majority of viral and atypical bacterial respiratory pathogens, NAATs offer enhanced sensitivity over culture, RADTs and DFAs (see Tables 52.2 and 52.3 for references), and the specificity varies with the target and assay design but is generally very high. NAATs also are suited for detection of respiratory pathogens that are not routinely or easily cultured (e.g., *C. pneumoniae*, *M. pneumoniae*, HBoV, HMPV, and PIV-4), for pathogens dangerous to culture (e.g., SARS-CoV, MERS-CoV), and for pathogens where the time-to-detection by traditional testing is often too delayed to impact patient care (e.g., influenza and CMV by cell culture).

The expanded scope of pathogen detection from a previous low of three viral pathogens detected by RADTs to 17 viral and three bacterial pathogens detected by NAATs greatly enhances the clinical laboratory's diagnostic capabilities.

Table 52.2 FDA cleared tests for the detection of bacterial and viral respiratory pathogens[a,b]

Manufacturer/test[c]	Amplification and detection platform(s)[d]	Extraction Platforms	Targets[e]	Specimen types approved[f]	Method(s)[g]	References
Alere i NAT Flu A/B	Alere i Instrument	Included	FluA, FluB	NS	Isothermal amplification, Fluorescence detection	[11, 12, 35, 91, 98, 162]
Argene/bioMerieux Argene R-Gene Adenovirus Assay	Cepheid SmartCycler	bioMerieux NucliSENS easyMAG	ADV	NPS	Real-Time PCR, Fluorescence detection	[148]
BioFire/bioMerieux Film Array Respiratory Virus Panel	BioFire Film Array	Included	ADV, CoV (OC43, NL63, 229E, HKU-1), HMPV, FluA (H1, H3, 2009-H1N1), HRV/EV, PIV 1,2,3,4, *M. pneumoniae*, *C. pneumoniae*, *B. pertussis*	NPS	Real-Time RT-PCR/PCR, Fluorescence detection	[7, 23, 26, 57, 87, 90, 133, 174, 176, 179, 180, 183, 188, 193, 220, 230]
CDC Influenza Division[g] CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panels 1. Influenza A/B typing Kit 2. Influenza A subtyping Kit 3. Influenza A /H5 (Asian lineage) Kit 4. Influenza B lineage genotyping assay	ABI 7500 Fast Dx	Qiagen QIAamp, Qiagen QIAcube, Roche Magna Pure compact, Roche Magna Pure LC, bioMerieux NucliSENS easyMAG	1. FluA, FluB 2. A/H1, A/H3, A/2009 H1 3. A/H5N1 (Asian lineage) 4. B/Victoria, B/Yamagata lineages	Varies by test including: NPS, NS, NA, NW, NPS/TS, BAL, TA, BW, VC	Real-Time RT-PCR, Fluorescence detection	NA
Cepheid 1. XpertFlu Assay 2. Xpert Flu/RSV XC Assay	Cepheid GeneXpert	Included	1. FluA (A/2009 H1), FluB 2. FluA, FluB, RSV	1. NPS, NA, NW NPS, NW, NA (in VTM)	Real-Time RT-PCR, Fluorescence detection	[35, 56, 59, 107, 129, 163, 178, 195, 196, 198, 199]
Focus Diagnostics 1. Simplexa Influenza A H1N1 2. Simplexa FluA/B and RSV 3. Simplexa Flu A/B Direct	3 M Integrated Cycler	1 and 2: Qiagen QIAamp Viral RNA 3: Included	1. FluA (A/2009 H1) 2. FluA, FluB, RSV 3. FluA, FluB, RSV	1. NPS, NA, NPA 2. NPS, NA, NPA 3. NPS	Real-Time RT-PCR, Fluorescence detection	[3, 111, 117, 204, 205, 212, 229]
GenMark eSensor Respiratory Viral Panel	Thermocycler GenMark eSensor XT-8	bioMerieux NucliSENS easyMAG	FluA, (A/H1, A/H3, A/2009 H1), FluB, RSV (A, B), ADV (B/E, C), PIV (1–3), HMPV, HRV	NPS	RT-PCR/PCR, Electrochemical detection of bound signal probes	[175, 179, 193]
Hologic/Gen-Probe/ Prodesse 1. ProFlu+ 2. ProFAST+ 3. ProAdeno+ 4. ProParaFlu+ 5. ProhMPV+	Cepheid Smartcycler II	bioMerieux NucliSENS easyMAG, Roche Magna Pure LC, Roche Magna Pure Total NA	1. FluA, FluB, RSV 2. A/H1, A/H3, A/2009 H1 3. ADV 4. PIV 1,2,3 5. HMPV	All NPS	Real-Time RT-PCR/PCR, Fluorescence detection	[26, 71, 124, 130, 133, 174, 202, 205, 220, 221]
IntelligentMDx IMDx FluA/B and RSV	Abbott m2000*rt*	Abbott m2000*sp*	FluA (A/H1, A/H3, A/2009 H1), FluB, RSV	NPS	Real-Time RT-PCR, Fluorescence detection	NA
Iquum/Roche Liat Influenza A and B	Iquum/Roche Liat Analyzer	Included	FluA, FluB	NPS	Real-Time RT-PCR, Fluorescence detection	NA

(continued)

Table 52.2 (continued)

Manufacturer/test[c]	Amplification and detection platform(s)[d]	Extraction Platforms	Targets[e]	Specimen types approved[f]	Method(s)[g]	References
Luminex 1. xTag Respiratory Virus Panel 2. xTag RVP *Fast*	Thermocyclers Luminex Lx100/200	bioMerieux NucliSENS easyMAG, bioMerieux NucliSENS miniMAG, Roche MagnaPure	1. ADV, hMPV, HRV, FluA (A/H1, A/H3), FluB, PIV 1,2,3, RSV (A, B) 2. AdV, HMPV, HRV, FluA (A/H1, A/H3), FluB, RSV (A, B)	1. NPS 2. NPS	RT-PCR/PCR Primer extension xTAG Bead Array Fluorescent detection	[5, 7, 8, 34, 47, 60, 72, 74, 78, 79, 88, 101, 108, 113, 114, 143, 144, 149, 151, 153, 157, 166–168, 179, 183, 185, 202, 203, 208, 213, 227]
Meridian Bioscience 1. *illumi*gene Pertussis 2. *illumi*gene Mycoplasma	Meridian Illumipro-10	1. Heat 2. Qiagen QIAmp DSP DNA miniKit	1. *B. pertussis* 2. *M. pneumoniae*	1. NPS 2. NPS, TS	Isothermal amplification with turbimetric detection	[184]
Nanosphere Verigene Respiratory Virus Nucleic Acid Test Plus	Nanosphere Verigene Processor SP Verigene Reader	Included	FluA (A/H1, A/H3, A/2009 H1), FluB, RSV (A, B)	NPS	RT-PCR Gold nanoparticle detection	[3, 20, 26, 41, 101, 160, 221]
Qiagen Artus Influenza A/B Rotor-gene RT-PCR Kit	Qiagen Roto-Gene Q MDx	Qiagen QIAsymphony RGQ	FluA, FluB	NPS	Real-Time RT-PCR, Fluorescent detection	[73]
Quidel 1. Lyra Influenza A+B Assay 2. Lyra RSV+HMPV Assay 3. Lyra Parainfluenza Virus Assay 4. Lyra Adenovirus Assay 5. Amplivue Bordetella Assay	1. and 2. Cepheid SmartCycler II, ABI 7500 Fast Dx, Life Technologies QuantStudio 3. and 4. ABI 7500 Fast Dx 5. Thermocycler and Amplivue Cassette	1–4: bioMerieux NucliSENS easyMAG Heat treatment	1. FluA, FluB 2. HMPV, RSV 3. PIV-1,2,3 4. ADV 5. *B. pertussis*	1. and 2. NPS, NS, NA, NW 3 and 4. NPS, NS 5. NPS	1–4. Real-Time RT-PCR/PCR, Fluorescent detection 5. Helicase dependent amplification, Lateral flow	NA
US Army JBAIDS 1. Influenza A/H5[g] 2. Influenza A&B Detection Kit[g] 3. Influenza A Subtyping Kit[g]	Idaho Technologies JBAIDS	Included	1. H5N1 (Asian lineage) 2. FluA and FluB 3. A/H1, A/H3, A/2009 H1	1. NPS, TS 2. NPS, NPW 3. NPS, NPW	Real-Time RT-PCR, Fluorescent detection	NA

[a]Adapted from FDA website

[b]Test methods and availability of products may change by publication date

[c]BioFire/bioMerieux, Salt Lake City, Utah; CDC: Centers for Disease Control and Prevention, Atlanta, GA; Cepheid, Sunnyvale, CA; Focus Diagnostics, Cypress, CA; Gen-Probe, San Diego, CA; Roche Molecular Diagnostics/Iquum, Marlborough, MA; Luminex, Austin TX; Nanosphere, Northbrook, IL; Qiagen, Valencia, CA; Quidel, San Diego, CA, JBAIDS: US Army: Joint Biological Agent Identification and Diagnostic System

[d]ABI: Applied Biosystems

[e]Abbreviations: RT: reverse transcriptase; PCR: polymerase chain reaction; NA: none available; ADV: adenovirus; CoV: coronavirus; FluA: all influenza A types; A/H1: seasonal H1N1; A/H3: seasonal H3N2; A/2009 H1: influenza A (H1N1)pdm09; H5N1: avian influenza A H5N1: FluB: influenza B; HMPV: human metapneumovirus: EV: enterovirus; HRV: human rhinovirus; PIV: parainfluenza virus; RSV; respiratory syncytial virus; *M. pneumoniae*: *Mycoplasma pneumoniae*; *C. pneumoniae*: *Chlamydophila pneumoniae*: *B. pertussis*: *Bordetella pertussis*

[f]These specimen types are specified in product package information and cleared by the Food and Drug Administration (FDA). Abbreviations: NPS; nasopharyngeal swab; NPW: nasopharyngeal wash; NPA: nasopharyngeal aspirate; NS: nasal swab; TS: throat swab; NA: nasal aspirate; NW: nasal wash; NPS/TS: dual specimen consisting of nasopharyngeal swab and throat swab; BAL: bronchial alveolar lavage; BA: bronchial aspirate; BW: bronchial wash; EA: endotracheal aspirate; EW: endotracheal wash; TA: tracheal aspirate; VC: viral culture

[g]Available only to qualified Department of Defense (DoD) Laboratories, US Public Health Laboratories, and National Respiratory and Enteric Virus Surveillance System (NREVSS) collaborating laboratories

Table 52.3 Research use only, investigational use only, or CE marked tests for the detection of respiratory pathogens[a]

Manufacturer/test[b]	Amplification and detection platform(s)	Extraction platforms	Targets[c]	Specimen types	Method(s)[d]	References
Abbott Ibis PLEX-ID/Flu assay PLEX-ID Respiratory Virus Assay	Abbott Ibis T5000 platform	Thermo King-Fisher	Pan-influenza (PB1) Five pan-FluA (NP, M1, PA, PB2, NS1) ADV (A-F), CoV HMPV, FluA, FluB, PIV (1–3), RSV	Respiratory samples (not specified) NPA	Broad range RT-PCR, Electrospray Ionization Mass Spectrometry, (RT-PCR/ ESI-MS)	[36, 37, 44, 55, 69, 88, 100, 159, 207, 214, 215]
Autogenomics 1. Infiniti RVP Plus 2. Infiniti Flu A-sH1N1	Thermocycler Autogenomics Infiniti Analyzer	Not specified	1. ADV (A, B, C, E) FluA (A/2009-H1N1), FluB, RSV, PIV (1–4), HRV (A, B), EV (A, B, C, D), CoV (HKU1, OC43, NL63, 229E), HMPV (A, B) 2. FluA, A/2009 H1	Respiratory samples (not specified)	RT-PCR BioFilmChip Microarray	NA
bioMerieux 1. NucliSENS Influenza A+B 2. NucliSENS RSV A+B 3. NucliSENS HMPV 4. NucliSENS *Mycoplasma* 5. NucliSENS *Chlamydophila*	bioMerieux NucliSENS easyQ	bioMerieux NucliSENS easyMAG	1. FluA and FluB 2. RSV (A, B) 3. HMPV 4. *M. pneumoniae*, *C. pneumoniae*	NPA, NPS, NS, NA, BAL	NASBA, Fluorescence detection	[15, 32, 50, 76, 134–138, 147, 154, 218]
Curetis Pneumonia Panel Bacterial	Curetis Unyvero System	Included	*A. baumanii*, *E. coli* *Enterobacter* spp., *M. morganii* *K. oxytoca*, *K. pneumoniae* *H. influenzae*, *Proteus* spp. *M. catarrhalis*, *S. aureus* *P. aeruginosa*, *S. marcescens* *S. maltophilia*, *S. pneumoniae* *C. pneumoniae*, *L. pneumophila* *P. jiroveci*, Plus 18 antibiotic resistance markers	BAL, TA, BW, BB, PB	Multiplex End Point PCR	[103, 201]
Hologic/Gen-Probe ProPneumo-1	Smartcycler II Qiagen Roto-Gene Applied Biosystems GeneAmp PCR 7500	bioMerieux NucliSENS easyMag	*C. pneumoniae* *M. pneumoniae*	NPS, NPW, BAL, sputum	Real-Time RT-PCR, Fluorescence detection	[96]
Icubate 1.Respiratory Panel V (viral) 2.Flu Typing 3. Respiratory Panel B (Bacterial)	Icubate Processor Incubator	Included	1. FluA (A/2009 H1), FluB, PIV (1–4), RSV (A,B), HMPV (A,B), HRV, ADV (3/7, 4), Cox A, B, Echo, HRV, Corona (OC43, NL63, 229E, HKU-1), HBoV 2. FluA (A/H1, A/H3, A/2009 H1, A/H5 (avian), N1 (shared), N2 (seasonal), FluB *H. influenzae* (non-typeable) *H. influenzae* (a, b, c, d) *H. influenzae* (e, f), *S. aureus* *N. meningitidis*, *S. pneumoniae* *C. pneumoniae*, *L. pneumophila* *M. pneumoniae*	Respiratory samples (not specified)	ARM-PCR technology (Amplicon Rescued Multiplex PCR), End point detection	NA

(continued)

Table 52.3 (continued)

Manufacturer/test[b]	Amplification and detection platform(s)	Extraction platforms	Targets[c]	Specimen types	Method(s)[d]	References
Luminex FluA/B, RSV Assay	Aries	included	FluA/B and RSV	NPS	Multi-code Real-Time RT-PCR, Fluorescence detection	NA
Pathofinder 1. Respifinder 15 2. Respifinder 19 3. Respifinder Smart 22	Thermocycler ABI310, ABI3100, ABI3130, ABI3730, ABI3500 Beckman CEQ Roche Lightcycler 480 Corbett RotorGgene 3000/6000 Qiagen RotorGene Q	Not specified	1. FluA (H5N1), FluB, PIV (1–4), RSV (A,B), hMPV, HRV, ADV, CoV (OC43, NL63, 229E) 2. 15 plus: *B. pertussis*, *M. pneumoniae*, *C. pneumoniae*, *L. pneumophila* 3. FluA (H1N1-2009), FluB, PIV (1–4), RSV (A,B), hMPV, EV/HRV, ADV, CoV (OC43, NL63, 229E, HKU-1), HBoV *B. pertussis*, *C. pneumoniae*, *L. pneumophila*, *M. pneumoniae*	NPW, NPA, NPW, BAL, Sputum	RT-PCR/PCR, Capillary electrophoresis, Multiplex PCR, Melt curve analysis	[24, 47, 141, 185, 186]
Qiagen Resplex II	GeneAmp PCR system 9700 LiquiChip 200 Workstation	QIAamp viral RNA QIAamp MiniElute QIASymphony QIAxtractor	FluA, FluB, PIV (1–4), RSV (A,B), HMPV, HRV, Cox/Echo Pan-ADV (B,E), CoV (OC43, NL63, 229E, HKU-1), HBoV	Respiratory Samples (not specified)	RT-PCR/PCR, Bead Array, Hybridization detection	[8, 69, 74, 90, 128, 129, 141, 145, 224, 227]
Seegene 1. Seeplex Respiratory Assay Group of 12: 2. Seeplex Respiratory Assay Group of 15: 3. Seeplex Typing Influenza A Virus	Variety including: Qiagen Rotor-gene Applied Biosystems GeneAmp PCR system 9700	Variety including: NucliSENS easyMAG Qiagen BioRobot MDx iNtRON Biotechnology Viral Gene-spin Kit	1. Set A: ADV, CoV (229E/NL63), PIV (1–3) 1. Set B: FluA/B, RSV (A,B), HRV A, CoV (OC43) 2. Set A: ADV (A/B/C/D/E), PIV (1–3), CoV (229E/NL63) 2. Set B: CoV (OC43), HRV (A/B/C), FluA, RSV (A, B) 2. Set C: HBoV (1/2/3/4), FluB, HMPV, EV, PIV 4 3. FluA (generic), A/H1, A/H3, A/2009-H1	Respiratory Samples (not specified)	Real-Time RT-PCR, Fluorescence detection	[16, 58, 74, 109, 110, 122, 192, 211, 231, 232]

[a]Test methods and availability of products may change by publication date

[b]Abbott, Chicago, IL; Autogenomics, Vista, CA; bioMérieux, Marcy, France, Curetis, Stutgart, GR; Luminex, Austin, TX; Hologic/Gen-Probe, San Diego, CA; GenMark Diagnostics, Carlsbad, CA; Icubate, Huntsville, AL; Qiagen, Valencia, CA; Pathofinder, Maastricht, NL; Seegene, Seoul, Korea, Thermo King-Fisher (Waltham, MA)

[c]Abbreviations: ADV: adenovirus; HBoV: human bocavirus; CoV: coronavirus; FluA: all influenza A types; H1: seasonal H1N1; H3: seasonal H3N2; 2009-H1: Influenza A(H1N1)pmd09; FluB: influenza B; HMPV: human metapneumovirus: EV: enterovirus; HRV: human rhinovirus; PIV: parainfluenza virus; RSV; respiratory syncytial virus; *M. pneumoniae*: *Mycoplasma pneumoniae*; *C. pneumoniae*: *Chlamydophila pneumoniae*: *L. pneumophila*: *Legionella pneumophila*, *M. morganii*: *Morganella morganii*, *N. meningitidis*: *Neisseria meningitidis*; *Strep. pneumoniae*: *Streptococcus pneumoniae*; *S. aureus*; *Staphylococcus aureus*

[d]Specimen types listed were identified either on manufacturer websites or from publications. Respiratory Samples (not specified): no specific information available. Abbreviations: NPS; nasopharyngeal swab; NPW: nasopharyngeal wash; NPA: nasopharyngeal aspirate; NS: nasal swab; NA: nasal aspirate; BAL: bronchial alveolar lavage; BB: bronchial brush; BW: bronchial wash; PB: protected brush; TA: tracheal aspirate

[e]RT: reverse transcriptase; PCR: polymerase chain reaction

The data derived from studies utilizing comprehensive viral and bacterial NAATs has and will continue to provide invaluable insights into the clinical manifestations of viral and bacterial infections, significance of mixed viral infections, and surprisingly the realization that viruses can colonize a host without overt disease.

Impact on Antimicrobial Selection and Stewardship

Aside from the use of RADTs during influenza season, RTI diagnostic testing including viral DFAs and culture, bacterial culture, NAATs, serologic testing for the atypical pathogens, and urinary antigen testing for *S. pneumoniae*

and *L. pneumophila* are still underutilized in the outpatient setting [10, 189]. Therefore, treatment of patients with CAP is generally empiric and based on guidelines by the American Thoracic Society and IDSA [146] rather than on a confirmed laboratory diagnosis. Antibiotic selection must cover both the most prevalent bacterial pathogens and the atypical pathogens.

Despite the high prevalence of viral infections, approximately 22.6 million (55 %) out of 41 million antibiotic prescriptions were prescribed for viral lower and upper RTIs, despite the fact that a bacterial etiology was highly unlikely [82]. Conversely the identification of the viral pathogen can lead to the administration of an appropriate antiviral. For example, during the early weeks of the 2009 influenza A H1N1 pandemic, four different influenza viruses were circulating with varying antiviral susceptibility patterns. According to surveillance data provided by the Center for Disease Control and Prevention (CDC, Atlanta, GA), FluA-H1 strains demonstrated >99 % resistance to the first line therapeutic oseltamivir, a neuraminidase inhibitor, and susceptibility to adamantine, FluA-H3 was oseltamivir-susceptible and >99 % adamantine-resistant, FluA(H1N1)pdm09 was oseltamivir-susceptible and >99 % adamantine-resistant; FluB was oseltamivir-susceptible and adamantine-resistant. Therefore, the identification of what specific strain of influenza virus was causing the infection of specific patients was essential to ensure proper drug selection, especially in high-risk or critically ill patients. Additionally, the more appropriate use of influenza antivirals can be achieved when an accurate, rapid diagnosis is made. Recent studies demonstrated that rapid testing permitted the timely administration of oseltamivir [229] and allowed for a more rapid discontinuation of treatment in persons without documented influenza [191]. Finally, new viral therapeutic agents for respiratory viruses other than influenza are in development and/or in clinical trials [171, 233]. Proper administration of these new agents will depend on the laboratory providing accurate tests that detect a broad range of viral pathogens [189].

For HAP and VAP, initial empiric therapy choices may be standardized and initiated based on patient clinical status, underlying disease, and/or risk for infection with a multidrug-resistant pathogen. Initial therapy often is inadequate, thereby extending the course of the disease and increasing morbidity, mortality, and hospital length of stay. The mortality rate in ICU pneumonia cases ranges from 20–30 % when initial therapy was adequate, to 50–80 % when initial therapy was inappropriate and changed after culture results were obtained [112]. Furthermore, inadequate and/or unnecessary broad spectrum antibiotic therapy can enhance the spread of drug-resistant pathogens within institutional settings and increase the risk of hospital-acquired infections such as *Clostridium difficile*, MRSA, and vancomycin-resistant enterococci.

Comprehensive NAATs provide key information not only for antiviral therapy selection but can also aid in restricting antibiotic use to those circumstances where antibiotic therapy is appropriate and in promoting switches to targeted specific therapies, thus reducing the use of broad spectrum antibiotics when not indicated [170, 142, 189]. This practice is in keeping with the goals of antibiotic stewardship, especially considering the steady and critical rise of antibiotic resistance and limited or no options available for the treatment of multidrug or pan-resistant bacterial infections.

Prevention of Nosocomial Infections

The burden of nosocomial infections can be significant, incurring additional costs for supplemental diagnostic tests, extended hospitalization, and increased morbidity and mortality [19, 40, 62, 126, 155, 217, 219]. Therefore, rapid diagnostic tests are needed to identify infected patients upon admission, thereby preventing nosocomial transmission by facilitating isolation and appropriate cohorting decisions [165, 206]. Studies have documented significant nosocomial transmission of ADV, influenza, RSV, HMPV, PIV, and HRV in hospital units, chronic care facilities, and pediatric units [19, 40, 62, 126, 155, 217, 219, 232]. During the height of RSV season, when prevalence can be >50 %, high numbers of hospital admissions often require the cohorting of RSV-positive children due to a lack of private rooms. However, limiting diagnostic testing to RSV alone in a cohorting scenario could put other seriously ill children at risk for acquisition of a second viral infection with other pathogens such as HMPV or HRV [80]. The rapid identification of health care facility-acquired *Legionella* infection is essential so that the environment source can be identified and eradicated, thus preventing further transmission. In addition, PCR techniques are used to proactively routinely screen potential environmental sources.

Epidemiologic Surveillance and Outbreak Investigation

The identification of a wide range of viral pathogens is essential for epidemiologic surveillance and establishes both seasonal and population patterns which can serve as excellent indicators for predicting immunization scheduling (e.g., influenza), or for administering preventive measures (e.g., RSV immune globulin). Previously, such surveillance was done by state Departments of Health or on a national level by the CDC. Comprehensive NAATs now enable local laboratories to monitor in real time viral prevalence and provide to their clinicians and health care facilities regular updates on circulating viruses. Testing decisions should use local data and additional resources and epidemiology information provided on the CDC web site (http://www.cdc.gov/flu/).

A study by S. Wong et al. demonstrated that the use of a multiplex NAAT (17 viruses) identified a virus in 59 % of the outbreaks and 29 % of the outbreak specimens that were negative using DFAs and a limited number of individual NAATs (ten viruses) [226]. Overall, the detection rate increased from 72–91 % for outbreaks and from 47–56 % for outbreak specimens. Comprehensive testing can identify reemerging or new emerging viral pathogens, as was the case during the influenza A H1N1 2009 pandemic.

Additionally, NAATs have been a key component in understanding both the epidemiology and features of large outbreaks of pertussis-like illness [190]. For example the large percentage of cases (29 %) of *B. holmseii* identified in an outbreak in Ohio in 2010–2011 was in contrast to some previous reports where the frequency of detection was very low [190]. The USA has seen peaks of pertussis activity every 3–5 years, with increasing cases since 1980. Accurate detection of pertussis is essential to better control the spread of disease from a public health standpoint through antimicrobial prophylaxis of asymptomatic household contacts, children less than 1 year of age, pregnant women in their third trimester, persons with preexisting conditions that are at risk for the development of severe respiratory failure, and all contacts in high-risk settings (http://www.cdc.gov/pertussis/outbreaks/pep.html).

Available Assays

Sample Types, Transport, and Storage

The recovery of respiratory pathogens is highly dependent on using the appropriate sample type and collection methods [139]. Laboratories using commercial FDA-cleared assays should refer to the manufacturer's package information to determine what sample types have been validated for use with the specific test (Table 52.2). The use of alternate samples types is permitted after the laboratory has performed their own validation studies that establish acceptable performance characteristics when testing the alternate sample type.

Applicable upper respiratory tract specimens for viral, *C. pneumoniae*, and *M. pneumoniae* testing include nasopharyngeal (NP) washes, NP aspirates, NP swabs, and midturbinate swabs placed in viral transport media [1, 48, 49, 95, 118, 131, 164, 223]. Oropharyngeal swab specimens are less sensitive (54 %) than either NP swabs (73 %) or NP wash specimens (85 %) due to the substantially lower levels of virus present in the oropharynx than the nasopharynx [131]. However, the combined use of nasal-oropharyngeal swabs can enhance the recovery of both avian influenza and SARS-CoV [49]. NP flocked synthetic swabs should be used in lieu of traditional synthetic NP swabs since flocked NP swabs yield a greater recovery of viral pathogens, with sensitivity comparable to NP wash specimens [1, 48, 95]. The rates of positivity for *B. pertussis* by real-time PCR were shown to be comparable when specimens were collected with either NP rayon swabs on aluminum shafts in Amies gel with charcoal or NP flocked swabs in universal transport media [6].

Lower respiratory tract (LRT) samples appropriate for viral pathogens, *C. pneumoniae*, *M. pneumoniae*, *L. pneumophila*, and bacterial pathogens include induced sputum, bronchial alveolar lavages, bronchial washings, protected brushes, and Combicath specimens. Studies that examined the detection of FluA(H1N1)pdm09 found that in seriously ill patients requiring intensive care, upper respiratory tract samples can be negative while LRT samples are positive [120, 123, 156, 230].

Additional factors that influence pathogen recovery include the time of sample collection after the onset of clinical symptoms and the age of the patient (children tend to shed higher titers of virus and for longer periods of time than adults). Therefore, for optimal detection, samples should be collected within 3 days for adults and within 5 days for children after the onset of symptoms [39, 85, 95, 120, 123]. Samples should be transported to the laboratory as soon as possible, preferentially on wet ice or refrigerated (2–8 °C) if testing will be performed within 48 h. If testing is delayed, the samples should be stored at −80 °C. Multiple freeze-thaws should be avoided as this process can decrease pathogen titers.

Nucleic Acid Extraction

Target lysis in a stabilizing matrix to prevent target degradation by deoxyribonucleases (DNases) and/or ribonucleases (RNases), followed by isolation and purification of the nucleic acids (NAs) are essential and critical steps of every NAAT. This is particularly important for respiratory samples that can be highly viscous and contain inhibitory substances and enzymes that destroy the target NAs. Some sample types may require a pretreatment with proteinase K or a similar enzyme. Highly efficient commercial NA extraction systems ensure sufficient NA recovery and the removal of inhibitory substances that could result in inefficient or no amplification of the target NAs. Presently, the majority of NA extraction systems use a chaotropic agent to lyse viral particles or bacterial cells, silica particles or a membrane to capture the released NAs, and a series of wash steps to remove inhibitory substances. NAs are generally eluted in either RNase/DNase free water or a stabilizing buffer such as EDTA-Tris. Although NA extraction can be performed manually using for example spin columns, the majority of laboratories currently

use automated platforms that can extract as few as one sample at a time or more than 96 samples in microwell plate formats. Many NAATs have been FDA-cleared with specific extraction platforms or methods and laboratories should be aware that the substitution of another extraction platform or method constitutes a major change in the assay protocol. From a regulatory perspective, if the extraction procedure is modified or changed then the entire test is now considered an LDT. Laboratories must be compliant with all applicable state and federal CLIA standards, and may choose to comply with the College of American Pathologists regulations to meet state or federal regulatory requirements.

Amplification and Detection of Nucleic Acids

Many amplification and detection methods are used in the current FDA-cleared assays (Table 52.2) and additional assays that are not FDA cleared but may be Conformité Européenne (CE) marked for use as an in vitro diagnostic device (IVD) in Europe (Table 52.3). Amplification methods include traditional reverse transcription polymerase chain reaction (RT-PCR), PCR, real-time RT-PCR and PCR, RT-PCR and primer extension, nested PCR, amplicon rescued multiplex PCR (ARM-PCR), and isothermal amplification such as nucleic acid sequence based amplification (NASBA), helicase-dependent amplification (HAD), nicking enzyme amplification reaction (NEAR), and loop mediated amplification (LAMP). Primers sets can be broad range (e.g., family), short range (e.g., genus), or pathogen-specific (e.g., genus and species). Targets may be single or multiple copy and include genomic RNA, or DNA, or messenger RNA. Detection technologies utilize fluorogenic intercalating dyes, fluorogenic probes (Taqman, fluorescence resonance energy transfer [FRET] hydridization, molecular beacons, scorpions, locked nucleic acid [LNA]), arrays (liquid bead, gold nanoparticles, or solid chip), electrochemical-based methods, melt curve analysis, lateral flow, or simple turbidity.

Tests vary considerably with the number of targets detected, ranging from one pathogen target plus an internal control (IC) to 20 targets plus an IC. They differ in which targets are detected (viral and/or bacterial) and if the tests are able to detect and differentiate various types within a virus family (e.g., PIV-1, -2, -3, and -4) (Tables 52.2 and 52.3). Platforms can be all inclusive with NA extraction, amplification, and detection performed in one cartridge (e.g., GeneXpert, Cepheid, Sunnyvale, CA; cobas Liat System, Roche Molecular Systems/Iquum, Marlborough, MA), in one pouch (Film Array, BioFire/bioMérieux, Salt Lake City, UT), or one chamber of a multi-test wheel cartridge (Simplexa Direct, Focus Diagnostics, Cypress, CA). Testing can be performed using modular systems that incorporate NA extraction with amplification but a separate unit for detection (e.g., Verigene, Nanosphere, Northbrook, IL), modular systems that separate isolation, from amplification combined with detection (Simplexa, Focus Diagnostics,; ProFlu+, Gen-Probe, San Diego, CA; Lyra, Quidel, San Diego, CA) and systems where isolation, amplification, and detection are all performed separately (xTag RVP, Luminex, Austin, TX; eSensor XT-8 System, GenMark Diagnostics, Carlsbad, CA; Infiniti RVP Plus, Autogenomics, Vista, CA; Resplex II, Qiagen, Valencia, CA).

Only a limited number of tests are FDA-cleared for the detection of bacterial pathogens. One highly multiplexed test, the FilmArray Respiratory Panel (BioFire/bioMérieux) tests for 17 viral pathogens and three bacterial pathogens, *C. pneumoniae*, *M. pneumoniae*, and *B. pertussis*. One additional assay is FDA-cleared for the detection of *M. pneumoniae*, the *illumi*gene Mycoplasma Assay (Meridian Biosciences, Inc, Cincinnati, OH). Two addition assays are FDA-cleared for the detection of *B. pertussis*, the *illumi*gene Pertussis assay (Meridian) and the Amplivue *Bordetella* assay (Quidel, San Diego, CA).

Time-to-results for the FDA-cleared assays ranges from 15 min to approximately 12 h depending on the platform, with the most rapid results (< 1.5 h) for the all-inclusive cartridge/pouch based tests. Technical hands-on time varies from < 2 min to approximately 3 h. Currently, most NAATs are rated as CLIA moderate to high complexity. In January 2015, the first waived NAAT under CLIA was cleared by the FDA, the Alere i Influenza A&B test (Alere Scarborough, Scarborough, ME). To perform the test, a sample receiver and test base are inserted into the Alere i instrument (Alere). After a 3 min heating step, the sample is eluted from the nasal collection swab directly into the sample receiver buffer. The transfer cartridge is used to transfer the sample to the test cartridge, where NEAR-amplification and detection occur in approximately 10 min [11, 12, 35, 162].

For comprehensive diagnosis of CAP and to rapidly identify pathogens associated with HAP and VAP, additional tests are needed that target the main bacterial pathogens [63]. The Research Use Only (RUO) Unyvero System (Curetis, Stutgart, GR) has an assay developed as an aid for the diagnosis of bacterial pneumonia. The specimen is preprocessed in Unyvero L4 Lysator, and then added to the assay-specific cartridge which is then inserted into the Unyvero A50 analyser. All steps are controlled by the Unyvero C8 Cockpit. The assay detects the major bacterial pathogens (*Acinetobacter baumanii*, *Enterobacter* spp. *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Moraxella. catarrhalis*, *Morganella morganii*, *Proteus* spp., *S. aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *S. maltophilia*, *S. pneumoniae*), the atypical pathogens

(C. *pneumoniae*, *L. pneumophila*), and one fungal pathogen (*Pneumocystis jiroveci*) known to cause CAP, HAP, and VAP. In addition, the assay will detect 18 genes associated with the major categories of antibiotic resistance. Time-to-results is approximately 4–5 h. The assay serves as a preliminary screen to be followed by culture and traditional antimicrobial susceptibility testing. Preliminary studies demonstrated that the assay detected more pathogens than routine culture. The Unyvero system detected numerous resistance markers and allowed for a change in empiric antibiotic therapy within 5–6 h for 67 % of the patients tested [103, 201].

The RUO Abbott Plex-ID system (Abbott, Abbott Park, IL) is comprised of broad range PCR or RT-PCR and Electrospray Ionization Mass Spectrometry (PCR/ESI-MS) for pathogen detection [36, 37, 44, 55]. Following NA extraction, PCR and/or RT-PCR is performed in a microwell plate using multiple primer pairs, with one primer set per well. Following amplification, the automated platform performs post-PCR desalting, purification, and high-resolution mass spectrometry using ESI-MS. The raw spectra are analyzed and calibrated with an internal mass standard. Spectral analysis determines the nucleotide base composition of the single-stranded oligonucleotides complementary to the initial target. To evaluate the relative concentration of the target or targets present, a semiquantitative value can be obtained by comparing the peak heights with the internal PCR calibration internal mass standard present in every well. The initial Plex-ID (Ibis T5000) system has been replaced by the Abbott IRIDICA RUO system (Abbott) that is comprised of PLEX-ID SP (nucleic acid extraction platform), PLEX-ID FH (liquid handler for assay setup), and a new version of the PLEX-ID PCR/ESI-MS platform. Time-to-results is <8 h. PCR- or RT-PCR/ESI-MS has been used to detect and identify a variety of respiratory viruses, influenza subtypes, bacteria, and fungi associated with RTIs ([88, 159, 207, 214, 215]; Huttner et al. 2014).

NAAT Performance

Overall, NAATs for respiratory viral and the atypical bacterial pathogens are highly sensitive (85 – 100 %) and very specific (>95 %) when samples are collected shortly after the onset of clinical symptoms (see references in Tables 52.2 and 52.3). Assays can detect mixed infections [79] and a broad range of viral types within a family [34]. Performance results from clinical trials for FDA clearance and from investigator-initiated studies need to be reviewed carefully when evaluating an assay because many factors can affect the overall results, such as patient population and age, testing conditions, specimen collection factors, storage time from sample collection to testing, and comparator method used in the analysis. Similarly, results obtained in different studies can vary significantly for assays and targets, depending on the study design (see references in Tables 52.2 and 52.3). Numerous studies have demonstrated that not all targets within a particular assay are detected with the same sensitivity. NAATs also have certain limitations. Due to the complexity of primer and probe designs and interactions, highly multiplexed NAATs can sometimes be less sensitive than individual NAATs [70, 166, 167]. Reduced analytical sensitivity, however, does not always correlate with reduced clinical sensitivity since viral quantity in clinical samples, depending on the virus and timing of sample collection, are often much higher than the limit of detection of the assays. NAATs often are less sensitive for viruses with multiple serotypes, such as ADV [37, 74, 113, 133]. Decreased performance for the detection of ADV can be clinically significant, particularly when testing samples from immunosuppressed patients (e.g., transplant patients) for whom ADV infection has a high mortality rate if not treated. For high-risk patients, laboratories may elect to supplement testing with either a more sensitive, broadly reactive ADV-specific assay or with cell culture [25, 62, 148, 173]. Many of the assays cannot distinguish between HRV and EV due to the fact that the assays target the 5′ UTR which is genetically similar for the two viruses [113]. The performance of the assays, in particular for RNA viruses, also may be affected by sequence mutations that occur over time. These mutations can result in primer/probe mismatches that decrease assay sensitivity, result in a total lack of target amplification and detection, or result in cross reactivity [153]. Any modifications to the assays to accommodate such genetic changes would have to be cleared by the FDA. In highly multiplexed assays, revalidation can require extensive assessment of all target interactions, thus making changes extremely difficult.

The FDA-cleared assays for the detection of *B. pertussis* target the multicopy IS481 gene. This gene is found not only in *B. pertussis* (50–238 copies/cell) but also in *B. holmseii* (8-10 copies.cell) and in 1–5 % of the strains of *B. bronchioseptica* [190]. Therefore, the assays are not specific for *B. pertussis* and alternative targets must be tested to differentiate the three species and also to detect *B. parapertussis* [89, 190, 216, 225]. Additionally, pseudo-outbreaks of pertussis have been described, indicating that great caution must be used not only in performing the NAAT but also in the collection of test samples, particularly in areas where vaccine is administered. Finally, due to the short time of localization of the atypical bacterial pathogens to the URT, the detection of these pathogens may require a combination of various sample types and various test methods, including NAATs, serology, and antigen testing, to provide the most sensitive results.

Interpretation of Test Results

The advantages of NAATs compared with traditional testing methods include improved sensitivity, not needing a viable organism for detection, broader scope of pathogens identified, and the ability to detect mixed infections. These same advantages can also raise issues with the interpretation of results. Until recently the detection of any respiratory virus had been considered significant. Several studies have shown that respiratory viruses can be detected in asymptomatic patients [2, 14, 104, 181]. One study found that NAATs identified a virus in 83 % of specimens from symptomatic children, but also detected a virus in 42 % of specimens from children without symptoms [2]. Another study demonstrated similar colonization with CoV (7.6 %) in symptomatic hospitalized children versus (7.1 %) in an asymptomatic outpatient control population. The overall prevalence of CoV or the types of CoV was not significantly higher among hospitalized children than controls. Respiratory viruses in NP swabs were identified from both asymptomatic and symptomatic solid organ transplant recipients early after transplantation [14]. The data suggest that due to the high prevalence of positive results in children and immunocompromised patients without symptoms, results should be interpreted cautiously, and in the context of clinical presentation, radiographic findings, and other laboratory tests. Additionally, clinicians must be aware that NAATs will detect nucleic acids lingering from a previous infection and that samples submitted for assessment of therapeutic response may remain positive for days to weeks after treatment due to the presence of nonviable organisms and not due to treatment failure. In these situations culture or quantitative NAATs (see Future Directions below) may provide the best option. With the increased use of highly multiplexed respiratory virus assays, a variety of mixed viral infections are detected and the clinical importance of each virus is not always evident. Nonviable virus from a previous infection may be detected, making it impossible to determine which or if all viruses contribute to the current illness. Test reports need to explain what viruses are included in the NAAT panel, sensitivity and specificity of the test, and that a negative result does not preclude infection with a specific pathogen due to many factors that can lead to decreased assay sensitivity.

Aside from the detection of the atypical pathogens and *Bordetella* spp. the detection of other bacterial pathogens, such as those included in the Unyvero test, directly from clinical samples raises several interesting questions that need to be addressed: (1) How do we differentiate between colonization and infection so as not to promote overuse of antibiotics? Are quantitative assays necessary or can assay cut off values be established at clinically relevant thresholds? (2) How many pathogens need to be included in a screening test? The detection of the broad scope of potential gram-positive and gram-negative bacterial pathogens is limited by multiplexing capabilities. Should these assays detect at a minimum those pathogens with the highest clinical impact or those difficult to treat? (3) How can we detect a broad range of resistance mechanisms, some of which do not have genetic markers (such as porin down-regulation in *Pseudomonas* spp.)? (4) Can we trust a positive result to direct antibiotic therapy? (5) Can we trust a negative result when there is the possibility of the presence of unknown resistance mechanisms? (6) Do we need to link a resistance marker to a specific bacterial target? For example, how do we interpret a result positive for a specific resistance gene when we do not detect the organism in which it would reside? (7) Will this type of testing provide actionable results, i.e., will this change clinical practice? The answers to these questions will only be resolved during clinical trials that compare NAAT results to classic microbiology testing, clinical practice, and patient outcomes. However, the future of testing may be a combination of multiplexed NAAT viral and bacterial screens.

Laboratory Issues

Laboratories must consider multiple factors in selecting the most appropriate NAAT or in developing testing algorithms (Table 52.4) [60]. Considerations include patient population(s), FDA status of the NAAT and the implications for regulatory issues, platform type, test complexity and technical expertise required, turnaround time, testing volumes, batch vs single unit testing, and the number and type of pathogens detected. The higher costs of NAATs can easily be offset by replacing the less sensitive test methods or by testing on site in lieu of referral to a reference laboratory [60, 144]. Additionally costs can be offset by other demonstrated benefits, such as improvements in patient care and financial outcomes, including 30 % reduction in antibiotic use, up to 20 % reduction in unnecessary diagnostic tests and procedures, and 50 % reduction in hospital days [9, 22, 82, 92, 115, 120, 123, 142, 170, 191, 227, 229]. To achieve these benefits NAATs should be performed within 24 h of sample collection so that results are available within a clinically relevant time frame. With the advent of single unit cartridge/pouch based tests that require minimal hands-on time and very minimal molecular technical expertise, all size laboratories can perform NAATs on all shifts. Considering the impact of global travel, comprehensive and highly specific NAATS should be performed year round and not limited to specific seasons. Although the positive predictive value of these tests remains high during times of low viral prevalence, laboratories should consider confirmatory testing when a virus is detected during an unusual time period. Some pathogens are more prevalent in specific groups

Table 52.4 Factors to consider in selecting an appropriate NAAT

Topic	Parameter	Factors to consider
Patient population	Age	Neonates, children, elderly
	Immune status	BMT, SOC, oncology, HIV
	Underlying disease	COPD, asthma, CHF, CF
	Health care inpatient setting	Risk of mixed infections, infection control
	Health care outpatient setting	Risk to family members
Regulatory issues	FDA status/CE marked	IVD, RUO, IUO, ASRs
	Regulatory requirements	CLIA, CAP, State, Federal
Laboratory issues	Test complexity	Technical expertise required, training
	Turnaround time	STAT (ED) versus routine, number of times tested per day
	Volume	Single unit cartridge versus larger batch testing
	Instrumentation/space	Complexity, cost, number of units
Assay performance	Appropriate sample types	Applicable for patient population(s)
	Sensitivity	Clinically relevant level (>90 %)
	Specificity	No cross reactivity (>95 %)
	Reactivity	Detect all subtypes (100 %)
	Consistent results	Reproducible, rare failures
Cost	Implementation	Assay validation or verification, training
	Instrumentation	Number of units
	Cost per test	Assessed by clinical benefit
	Quality	Quality control, proficiency testing

ASRs analyte specific reagents, *BMT* bone marrow transplant, *CAP* College of American Pathologists, CE cConformite Européenne, *CF* cystic fibrosis, *CHF* congestive heart failure, *CLIA* Clinical laboratory Improvement Act, *COPD* chronic obstructive pulmonary disease, asthma, *ED* emergency department, *HIV* human immunodeficiency virus, *IUO* investigational use only, *IVD* in vitro diagnostic, *NAAT* nucleic acid amplification test *RUO* research use only, *SOC* solid organ transplant,

(e.g., RSV and HMPV in children and the elderly), but limiting testing to specific age groups will miss clinically relevant disease in other patient populations [64, 83]. Infections with these pathogens can also sometimes have atypical presentations, such as pericarditis due to HMPV in an otherwise healthy adult [97]. Therefore, age may be useful in triaging initial testing but should not govern the final scope of what viruses are included in diagnostic testing. In addition, if step wise testing is considered based on risk factors such as age or immune status, coinfections that could lead to serious nosocomial transmission in health care settings should not be missed. Unexpected local, national, and international events, such as the H1N1-2009 pandemic can change our testing algorithms and laboratories must be prepared to adapt quickly to such events.

Quality Control

Kit positive and negative controls, ICs, and external controls should be used to verify the performance of the reagents and to ensure no inhibitory substances remain after extraction that could lead to poor amplification, reduced assay sensitivity, and false-negative results [77]. Controls should be tested in accordance with regulatory requirements as outlined by CLIA, CAP, and state or other federal regulatory agencies, and in accordance with the manufacturer's instructions. Ideally all controls should go through the same process as the patient sample. ICs are best added at the NA extraction step to ensure efficient recovery of NAs. ICs added after extraction will just confirm the amplification efficiency and an external extraction control must be used unless the extraction method has demonstrated negligible inhibition (generally <1 %) for the sample types tested. During amplification and detection, the IC is essential to demonstrate a lack of amplification failure or decreased efficiency. External positive and negative controls (not provided in the kit) must be used to verify each new lot and/or shipment of reagents. Each analyte of the multiplex must be verified individually in either a single reaction or as a component of a pooled control. Daily positive (individual or pooled) and negative controls must be run thereafter if batch testing is performed. Rotating controls after lot/shipment validation is acceptable. Tests using a single unit cartridge/pouch that contain a procedural control (IC or process control) do not require external controls to be run with each individual cartridge/pouch once the performance of the procedural control has been verified. External positive controls for each analyte and negative controls are only required for verification of each new lot/shipment or at a minimum once per month.

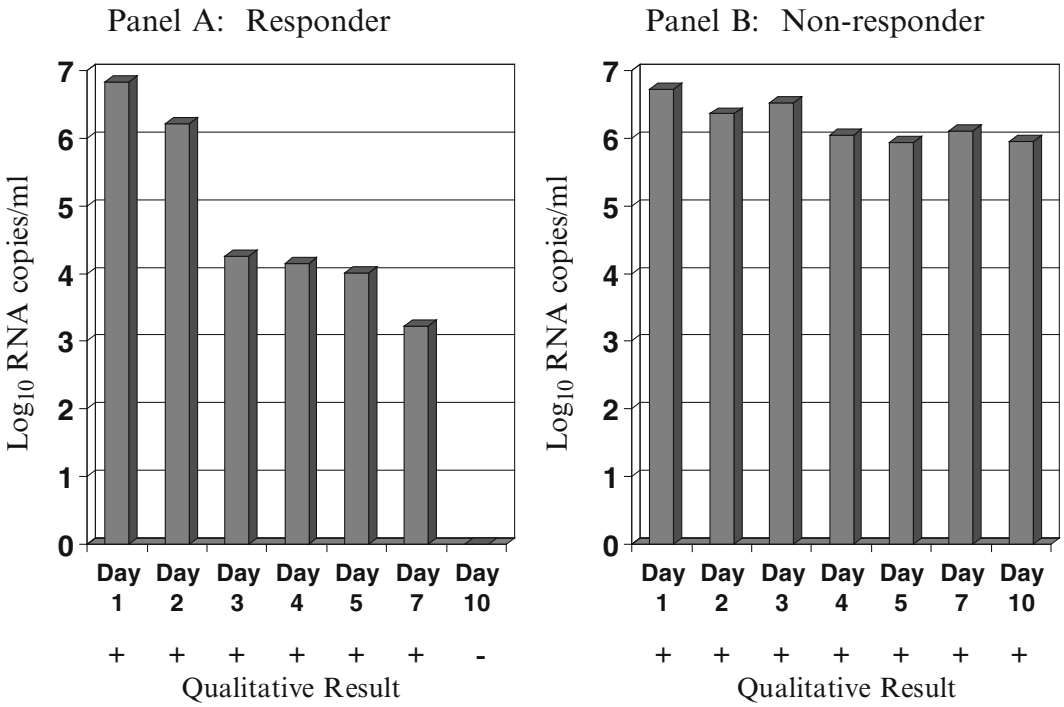

Figure 52.3 Quantitative versus qualitative testing for assessing antiviral response. Panel A: Influenza A viral load results over time in a patient with oseltamivir-susceptible influenza A virus. Panel B: Influenza A viral load results over time in a patient with oseltamivir-susceptible influenza A virus. +, positive for Influenza A detection; -, negative for influenza A detection

Laboratories must ensure proper procedures to prevent both sample and amplicon cross contamination that could cause false-positive results. Laboratories need to interpret negative results and IC values in the context of the presence or absence of potential nucleic acid degradation and amplification inhibition. Laboratories are responsible to continually assess the performance of their assays to ensure that over time, the performance has not declined due to factors such as genetic shifts in the target analytes. Finally, ongoing assessment of technical competency and participation in proficiency testing programs are essential to ensure high-quality performance and results.

Future Directions

Quantitative Viral Assays

Both virus type and the amount of virus present (viral load) can significantly impact the clinical characteristics and clinical course of RTIs. Quantitative detection of viral respiratory pathogens can help to assess the dynamics of viral proliferation, better understand viral pathogenesis, and permits a means to evaluate the significance of coinfections. Since NAATs detect both viable and nonviable virus, monitoring patients for treatment response with qualitative testing provides little information as tests may remain positive for days even with successful therapy. Quantitative tests that demonstrate a decline in viral load during therapy more accurately assess patient response (Fig. 52.3a) and the failure to see a decline (Fig. 52.3b) would potentially indicate earlier a need to consider alternative therapies. This information is especially important for critically ill patients and immunosuppressed patients. Finally, viral load assays provide important information in the assessment of new antiviral agents in FDA clinical trials. Future assay development should consider quantification of the viral targets.

Influenza Resistance Testing

Antiviral resistance to neuraminidase inhibitors can be assessed both phenotypically and genotypically [84, 161, 187]. Neuraminidase inhibition assays detect decreases in susceptibility by determining the 50 % inhibitory concentration (IC_{50}). However, these assays require growth of the virus and the presence of quasi-species can lead to unreliable results. Alternatively, genotypic assays are easier and identify known resistance mutations. However, newly identified mutations require phenotypic confirmation. Although no molecular assays are FDA-cleared for influenza resistance testing, several methods are used including traditional Sanger sequencing, pyrosequencing, PCR genotyping assays, and next-generation sequencing (NGS) [84, 161].

Pyrosequencing is currently the method of choice since the method is fast, has a high throughput, is sensitive (can detect a mutation if present in 10 % of the population), can assess multiple known mutations (e.g., H275Y mutation found in resistant H1N1-2009 strains) as well as unknown mutations and polymorphisms [52–54]. NGS has the added advantage of generating longer sequence lengths and has identified new genetic mutations associated with neuraminidase resistance. Laboratories should consider offering resistance testing in seasons where circulating strains may have varying resistance patterns (for example, H1N1 in 2007–2008 that demonstrated variable oseltamivir susceptibility), especially for patients at high risk to develop severe disease (e.g., pregnant or immunosuppressed) [38]. In addition, screening for resistance is indicated in seriously ill patients who continue to shed virus and are not clinically improving after prolonged antiviral therapy.

Next-Generation Sequencing

Over the last decade the development of NGS has transformed a labor-intensive slow process into a real-time method with applicability to respiratory diagnostics. The complexity of NGS is beyond the scope of this chapter but the use of NGS will continue to evolve in the clinical laboratory, particularly in light of easy-to-use bench top sequencers such as the Ion Torrent Personal Genome Machine (PGM) (Life Technologies, Carlsbad, CA) and the MiSeq (Illumina, San Diego, CA). Already NGS is used to detect and identify respiratory pathogens, resistance and pathogenicity markers, genetically characterize viruses, explore the respiratory microbiome, and understand the epidemiology of respiratory pathogens [169]. With new emerging technologies and simple methods to perform the required bioinformatics, this testing will increasingly become part of routine diagnostics.

Summary

The IDSA Diagnostics Task Force report: "Better Tests: Better Care: Improved Diagnostics for Infectious Diseases" highlighted the importance of diagnostic testing in the management of infectious diseases [27]. Likewise, the 2013 CDC [31] report "Antibiotic Resistance Threats in the US" states that both the development of new drugs and new diagnostics are essential to combat the threat of multiple-drug-resistant pathogens [30]. The incorporation of NAATs into routine practice for the diagnosis of infectious diseases will continue to grow, bringing new advanced technologies that allow the rapid and accurate detection of viral, bacterial, and fungal respiratory pathogens.

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