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PRACTICAL GUIDANCE FOR CLINICAL MICROBIOLOGY

Practical Guidance for Clinical Microbiology Laboratories: Viruses Causing Acute Respiratory Tract Infections

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SUMMARY Respiratory viral infections are associated with a wide range of acute syndromes and infectious disease processes in children and adults worldwide. Many viruses are implicated in these infections, and these viruses are spread largely via respiratory means between humans but also occasionally from animals to humans. This article is an American Society for Microbiology (ASM)-sponsored Practical Guidance for Clinical Microbiology (PGCM) document identifying best practices for diagnosis and characterization of viruses that cause acute respiratory infections and replaces the most recent prior version of the ASM-sponsored Cumitech 21 document, *Laboratory Diagnosis of Viral Respiratory Disease*, published in 1986. The scope of the

original document was quite broad, with an emphasis on clinical diagnosis of a wide variety of infectious agents and laboratory focus on antigen detection and viral culture. The new PGCM document is designed to be used by laboratorians in a wide variety of diagnostic and public health microbiology/virology laboratory settings worldwide. The article provides guidance to a rapidly changing field of diagnostics and outlines the epidemiology and clinical impact of acute respiratory viral infections, including preferred methods of specimen collection and current methods for diagnosis and characterization of viral pathogens causing acute respiratory tract infections. Compared to the case in 1986, molecular techniques are now the preferred diagnostic approaches for the detection of acute respiratory viruses, and they allow for automation, high-throughput workflows, and near-patient testing. These changes require quality assurance programs to prevent laboratory contamination as well as strong preanalytical screening approaches to utilize laboratory resources appropriately. Appropriate guidance from laboratorians to stakeholders will allow for appropriate specimen collection, as well as correct test ordering that will quickly identify highly transmissible emerging pathogens.

KEYWORDS clinical, guidance, laboratory, respiratory, virus

INTRODUCTION

Background

The most recent version of the American Society for Microbiology (ASM)-sponsored Cumitech 21 document, *Laboratory Diagnosis of Viral Respiratory Disease*, was published in 1986 (1). The scope of the original document was quite broad, with an emphasis on clinical diagnosis of a wide variety of infectious agents and laboratory focus on antigen detection and viral culture. The date of publication of the most recent Cumitech document was roughly 3 years after Kary Mullis' initial work on PCR technology. Since that time, the practice of clinical microbiology has significantly changed, most notably with the development of molecular approaches that have increasingly replaced traditional methods for diagnosis of respiratory viruses. Specimen collection techniques have likewise improved and have enhanced the predictive values of these new molecular methods. Development of electronic order entry systems, computerized laboratory information systems, and automated reporting has reduced turnaround times (TATs) for laboratory results dramatically even in environments where laboratory centralization has occurred. The continual emergence of new respiratory pathogens requires laboratorians to recognize laboratory testing limitations and understand when and how to refer suspicious cases to public health reference laboratories.

Purpose

This document is an ASM-sponsored Practical Guidance for Clinical Microbiology (PGCM) identifying best practices for diagnosis and characterization of viruses that cause acute respiratory infections (ARIs). The document is designed to be used by laboratorians in a wide variety of diagnostic and public health microbiology/virology laboratory settings, especially by members of the ASM worldwide. As such, this consensus document is structured to cover a wide range of practice settings, and to reflect changes in available technology, clinical practice, and viral pathogens since 1986. The document outlines the epidemiology and clinical impact of acute respiratory viral infections, including preferred methods of specimen collection and current methods for diagnosis and characterization of viral pathogens causing acute respiratory tract infections. Laboratory-developed and commercial diagnostic tools, approaches for diagnosis of emerging pathogens, and detection of antiviral resistance in influenza A virus (FLUA) and influenza A virus (FLUB) infections are also discussed. Specimen handling approaches for specimens from multiple body sites, such as nasopharyngeal swabs (NPS), nasopharyngeal aspirates (NPA), nasal swabs (NS), nasal washes (NW), oropharyngeal and throat swabs (OPS/TS), sputa, bronchoalveolar lavage (BAL) fluids, bronchoalveolar washes (BAW), and other lower respiratory tract specimens, are cov-

ered. Given the changes in turnaround time for these newer technologies and increases in clinical use, the document also addresses appropriate laboratory utilization of diagnostic respiratory viral testing. The scope of the document has shifted since the last version of the Cumitech, which included discussion on clinical overlap of viral pathogens causing acute respiratory tract infections as well as other pathogens that were shown to infect the respiratory tract, such as atypical bacterial pathogens. The current document focuses strictly on viruses that primarily cause acute respiratory infections, related syndromes, or disease processes. Viruses that can infect or shed from the respiratory tract but lead chiefly to other presentations such as rash, vesicles, parotitis, gastroenteritis, or mononucleosis-like syndromes (herpes simplex virus, varicella zoster virus, cytomegalovirus, Epstein-Barr virus, parvovirus B19, measles virus, rubella virus, mumps virus, bocavirus, and hantavirus) are not discussed in this document.

The primary focus of this document is pathogens with well-documented causal effects for acute respiratory infections, namely influenza A virus (FLUA), influenza B virus (FLUB), respiratory syncytial viruses (RSVs) A and B, respiratory enteroviruses (EVs), rhinoviruses (RVs), respiratory adenoviruses (ADVs), human metapneumovirus (hMPV), parainfluenza viruses (PIVs) 1 to 4, and coronaviruses (CoVs) (NL63, OC43, HKU-1, and 229E). The document also discusses the diagnosis and characterization of emerging respiratory viral pathogens, including CoVs (causing Middle Eastern respiratory syndrome [MERS] and severe acute respiratory syndrome [SARS]) and novel FLU strains arising from swine and avian sources.

EPIDEMIOLOGY AND CLINICAL PRESENTATION OF ACUTE RESPIRATORY VIRAL INFECTIONS

Circulation of Respiratory Viruses: a Global Problem

The increased capacity for molecular diagnostics worldwide has enhanced our understanding of global circulation patterns of respiratory viruses (2). From the clinical laboratory perspective, understanding the circulation patterns of viruses will influence the predictive value of respiratory virus testing and potentially the interpretation of respiratory virus test results based on pretest probability (3). A number of geographic regions now have well-established surveillance systems for FLU and occasionally other respiratory viruses associated with acute illness (4–7). A complicated global viral circulation pattern shows that some viruses maintain consistent seasonality, while others vary extensively. In the Northern Hemisphere, RV and respiratory EVs typically circulate in the late summer and early fall (autumn), while FLUA predictably peaks in December or January (Fig. 1). PIV types, however, have varied circulation patterns with seasonality depending on the subtype, and dominant types can change from year to year (8). Although we can begin to predict patterns of respiratory virus circulation as surveillance and detection capacities improve (9), viruses may be identified outside their normal seasonal infection patterns due to patient activities, such as travel to regions where the virus is currently circulating (10). Knowing the travel history combined with active pathogen surveillance (e.g., identifying a patient who presents during a North American summer with acute respiratory infection after travel to the Southern Hemisphere where FLU or RSV is circulating) can help direct appropriate infection prevention and control measures, as not all respiratory viruses require the same level of patient isolation (11, 12).

Acute respiratory infections. Acute respiratory infections (ARIs) are among the most common infections reported worldwide. In the 2013 global disease burden study sponsored by the World Health Organization, respiratory infections were listed as the leading cause of infectious disease and as being responsible for approximately 120 million disability-adjusted life years (DALYs) (a measure of the disease burden and its impact on quality of life) (13). Lower respiratory tract infections (LRTIs) accounted for greater than 90% of all DALYs, with approximately 35% of cases occurring in children less than 5 years old (13, 14). The impact of respiratory infections on human health is reflected in the large number of hospital and emergency room visits for both adults and children (e.g., in the United States, there are 140,000 to 710,000 FLU-related hospital-

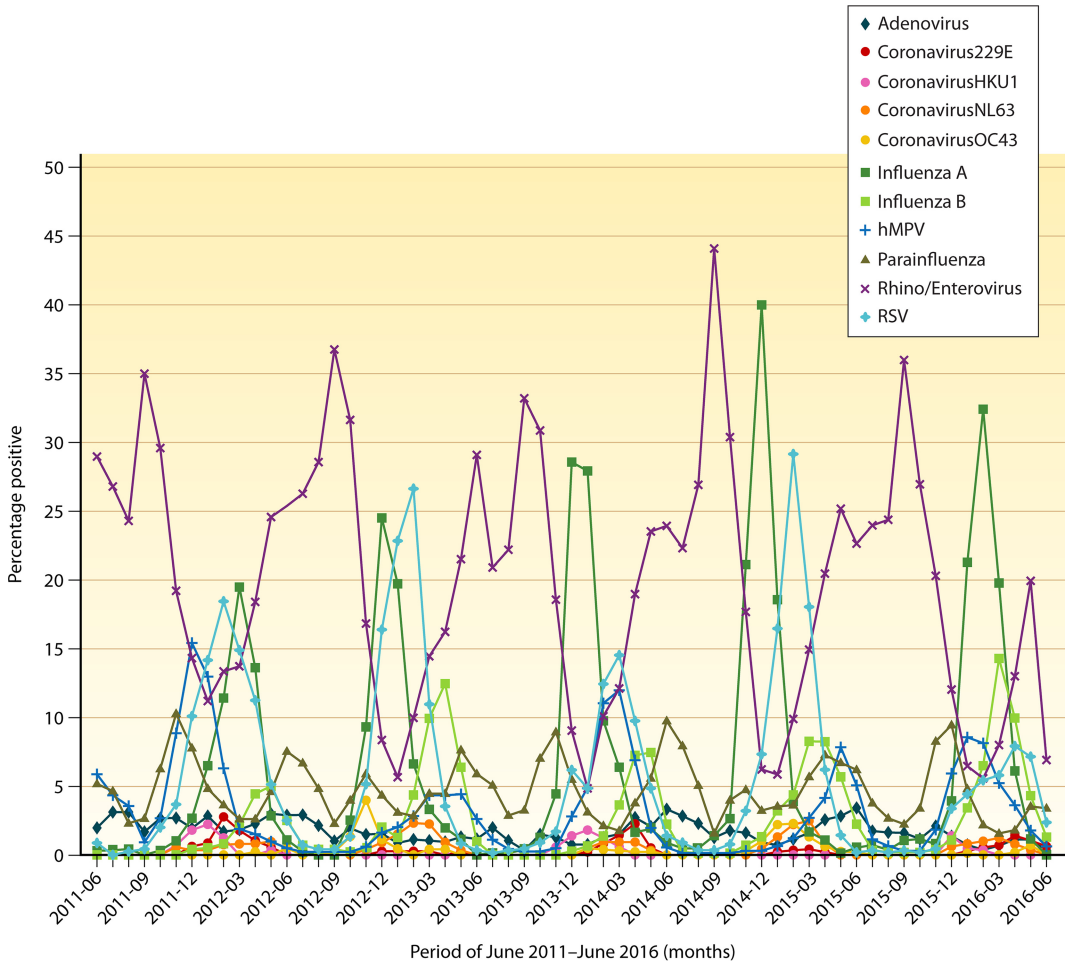

FIG 1 Circulation of common respiratory viruses in a large geographic area within the Northern Hemisphere. The data represent all acute respiratory virus testing for multiple years in a population of 4.1 million patients, using a common testing algorithm. The seasonality of viruses varies. (Generated by S. J. Drews and the ProvLab Alberta Laboratory Surveillance and Informatics Team, 2016.)

izations per year), where respiratory viral infection is the most common reason to seek medical care (15, 16).

Mechanisms of transmission. Respiratory viruses are transmitted primarily through two mechanisms: (i) inhalation of infectious droplets and (ii) contact with contaminated fomites. Aerosol transmission is the most common route of infection. Large (10 to 100 μm in diameter) aerosolized droplets can transmit viruses from the index case to a new host in close proximity ($\leq$0.9 m), while small ($<$10 μm in diameter) aerosolized droplets, produced during coughing or sneezing or through aerosol-generating procedures, can carry viral particles to new hosts several meters away ($\geq$1.8 m). Transmission via fomites from self-inoculation of the respiratory tract mucosa is the second most common route of infection (17) (Table 1). Survivability and infectivity of viruses on surfaces may vary from hours to days and depend on a number of viral and nonviral factors. Nonenveloped viruses are more likely to cause infection via direct contact, as they are more stable in the environment than enveloped viruses and are therefore more likely to survive for extended periods outside the host (18). Animal and climatological model systems suggest that respiratory virus (e.g., FLUA) transmission may also be enhanced under specific environmental conditions, such as low temperature and low humidity (19–21). It is important for laboratorians and clinicians to be aware of likely transmission routes used by respiratory viruses in order to implement adequate infection control practices, select appropriate specimen types, and safely perform laboratory manipulations (Table 1) (22).

TABLE 1 Classification and characterization of common respiratory viruses

Virus group(s)	Family (reference[s])	Nucleic acid	Nucleic acid structure[a]	Enveloped	Large-droplet transmission (reference)	Survival time as fomites (reference[s])[b]	Isolation precautions (reference[s])[c]
Adenoviruses	*Adenoviridae* (333, 334)	Linear, nonsegmented	dsDNA	No	Yes (11)	14–30 days (18)	Contact, droplet (11, 335)
Coronaviruses	*Coronaviridae* (333, 334)	Linear, nonsegmented	(+) ssRNA	Yes	Yes (11)	1 h–28 days (18, 336)	Airborne, contact, droplet, standard[d] (11, 337)
Enteroviruses, rhinoviruses	*Picornaviridae* (333, 334)	Linear, nonsegmented	(+) ssRNA	No	Yes (11)	Limited data	Droplet, standard[e] (11)
Influenza A/B viruses	*Orthomyxoviridae* (333, 334)	Linear, segmented	(−) ssRNA	Yes	Yes (11)	5 min–7 days (18, 338–341)	Droplet, standard,[f] ± airborne[g] (11, 335)
Parainfluenza viruses 1–4	*Paramyxoviridae* (333, 334)	Linear, nonsegmented	(−) ssRNA	Yes	Limited data (333)	4–10 h (18, 342)	Contact, standard (11, 335)
Human metapneumovirus	*Pneumoviridae* (333)	Linear, nonsegmented	(−) ssRNA	Yes	Limited data (333)	Limited data	Contact, standard (11)
Respiratory syncytial viruses A/B	*Pneumoviridae* (333)	Linear, nonsegmented	(−) ssRNA	Yes	Yes (343)	20 min–8 h (18, 344)	Contact, standard (11, 335)

[a]dsDNA, double-stranded DNA; ssRNA, single-stranded RNA.
[b]Survival on dry surfaces or hands. Survival times are impacted by temperature, humidity, and type of surface. Nucleic acid has been detected.
[c]Isolation precautions can be found at: https://www.cdc.gov/infectioncontrol/pdf/guidelines/isolation-guidelines.pdf. Note the caveat regarding the use of droplet precautions when undertaking procedures likely to create splashes or sprays.
[d]Isolation precautions vary by coronavirus type; the listed precautions are specific to SARS-CoV.
[e]Rhinovirus isolation precautions.
[f]Changes in hemagglutinin and neuraminidase may impact transmission of influenza viruses; for up-to-date seasonal recommendations see https://www.cdc.gov/flu/professionals/infectioncontrol/healthcaresettings.htm.
[g]Airborne transmission may be possible in some cases.

Close contact in living environments such as long-term care facilities facilitate transmission to the elderly, who are often at higher risk for severe outcomes from respiratory virus infections, such as pneumonia, acute-care hospitalization, and death. In addition, illness may also occur in staff members. Challenges may arise because these environments are not thought of as primary health care environments and may not have infection control protocols that are as stringent as those in health care settings (23, 24).

Similarly, pediatric day care settings are another transmission setting for exposure to multiple respiratory viruses. A prospective cohort study from Washington State identified RSV, ADV, and RV as leading pathogens, with hMPV and CoV being less frequent, in children in day care settings (25), and air sampling experiments have identified RSV these settings (26). Children attending day care are at increased risk for respiratory infections (all etiologies), especially at the start of entry into day care (27), and can be a potential source of RSV infection for premature infants, who are at high risk of severe compilations and outcomes (28).

Acute respiratory viral infections. There is significant overlap in clinical symptoms associated with the different viruses causing respiratory illnesses (Table 2). The U.S. Centers for Disease Control and Prevention (CDC) has established influenza-like illness (ILI) criteria used for epidemiological surveillance to identify patients with likely influenza infection (29, 30). These clinical criteria include cough, fever (temperature greater than or equal to 100°F [37.8°C]), and/or sore throat and no identifiable cause other than influenza (31); however, the specificity of these criteria is poor, as many other patients with noninfluenza respiratory viruses can present similarly (32). In many cases, acute respiratory infection (ARI) due to these viruses is indistinguishable from illness due to bacteria on the basis of clinical presentation alone. Table 2 provides examples of diseases and disorders that are caused by respiratory viral infection; however, this table does not exclude the possibility that an unlisted virus may be the causative agent of a disease or disorder.

(i) The host. The host response to viral infections relies on elements of both the innate immunity and the adaptive immunity. Epithelial cells covering the mucosal surface of the airway constitute the first physical barrier encountered by respiratory viruses. Here, tight junctions connect the cells and provide a sealed environment, preventing viral movement outside the respiratory tract. A layer of mucus overlays the epithelial surface, and an upward directional movement of cilia effectively traps and clears virus particles from the airway epithelium (33, 34). Binding and phagocytosis of viruses result in production of several proinflammatory molecules, including interleukins (e.g., interleukin-1β [IL-1β] and IL-18), α/β defensins, collectins, type I interferons alpha/beta, and immunoglobulin A (IgA), and attract natural killer cells. Upregulation of this innate immune response limits local spread of the respiratory viruses (34) and serves as the front-line defense prior to activation of the adaptive immune system.

In infants, the immune system is still developing. The lack of complete immune memory, reduced innate and adaptive immunity, and physiological differences in airways compared to those in adults (35) increase the susceptibility to viral infections and disease severity (36). The immune response to respiratory viral infections may be augmented by protective effects of passive antibodies transmitted *in utero* (37) and other factors, including breastfeeding (38, 39). Reinfections with the same virus are not uncommon, and disease severity as well as patient outcomes is dependent on multiple factors, including viral genetic diversity and intrinsic/extrinsic patient factors (34, 40–42).

Individuals at increased risk for complications due to respiratory virus infections include children, older adults (>65 years old), patients with underlying respiratory conditions, and those with suppressed immune functions (e.g., transplant patients). In patients with underlying respiratory conditions (e.g., chronic bronchitis, chronic obstructive asthma, chronic obstructive pulmonary disease [COPD], or emphysema), a decrease (mucostasis) or increase (mucus hypersecretion) in the mucociliary escalator function may lead to decreased clearance of viral pathogens and increased risk of

TABLE 2 Diseases and disorders associated with respiratory viral pathogens[a]

Virus(es)	Respiratory diseases and disorders	Comments	Key references
ADV	Pharyngitis, common cold, laryngitis, bronchitis, bronchiolitis, pneumonia	Main cause of pharyngitis in infants and children; types 4 and 7 caused pneumonia in military recruits; multiplex NAAT-based assays which include ADV are available; latency and persistent shedding can confound interpretation of qualitative tests	(130, 236, 281, 345–349)
CoVs NL63, OC43, HKU1, 229E, SARS-CoV, and MERS-CoV	Common cold, pharyngitis, laryngitis, bronchitis, bronchiolitis, pneumonia, SARS, MERS	Multiplex NAAT-based assays are available for the detection and differentiation four genotypes of CoV (229E, OC43, NL63, and HKU1); genotypes such as SARS-CoV and MERS-CoV can be detected only by NAAT, often at reference/public health laboratories	(236, 348–356)
EV	Bronchiolitis, bronchitis, common cold, pharyngitis, pleurodynia	EV-D68 is associated with severe respiratory illness outbreaks in the USA (2014); multiplex NAAT-based assays which include EV are available; as described in the text, many panels cannot differentiate between EV and RV or detect all types of EV (e.g., EV D68); LDTs have been utilized for typing	(236, 357, 358)
hMPV	Bronchiolitis, common cold, laryngitis, bronchitis, pneumonia	hMPV infection is associated with a substantial burden of hospitalizations and outpatient visits among children throughout the first 5 years of life, especially during the first year; elderly adults also susceptible; multiplex NAAT-based assays are available for hMPV detection; two groups and four subgroups of hMPV can be detected and identified by molecular assays	(236, 281, 348, 349, 359, 360)
RVs	Bronchiolitis, bronchitis, common cold, pharyngitis, pneumonia	The leading pathogen causing common cold and the most common viral cause (8%) of pneumonia in adults in the USA; multiplex NAAT-based assays are available for RV detection; molecular assays are the only method for detection of RV genotype C; some molecular panels cannot distinguish between RV and EV	(236, 348, 349, 358, 361–363)
FLUA (including subtypes H1, H3, and H5) and FLUB	Bronchitis, bronchiolitis, common cold, influenza, laryngitis, pharyngitis, pneumonia	Common pathogen of pneumonia in adults; multiplex NAAT-based assays are available for FLUA detection; on-demand and point-of-care molecular tests are available; genotyping and subtyping can be done by molecular assays; clinical relevance of viral load determination merits further investigation	(236, 281, 286, 348, 349, 361, 364–366)
PIVs 1–4	Bronchiolitis, bronchitis, common cold, laryngitis, otitis media, pharyngitis, pneumonia	PIV 1 and PIV 3 are the most common types causing bronchitis; PIV 4 has not been confirmed to be a definite pathogen in humans; multiplex NAAT-based assays are available, including detection and differentiation of PIVs 1–4	(236, 281, 349, 354)
RSVA and -B	Bronchiolitis, bronchitis, common cold, otitis media, pneumonia	Leading cause of bronchiolitis and common pathogen of pneumonia in children; disease severity is significantly associated with viral load rather than RSV subgroup; multiplex NAAT-based assays are available for RSV, and some of them provide subgroup information on RSVA and RSVB	(236, 348, 349, 367–369)

[a]Abbreviations: ADV, adenovirus; SARS, severe acute respiratory syndrome; MERS, Middle East respiratory syndrome; CoV, coronavirus; EV, enterovirus; hMPV, human metapneumovirus; RV, human rhinovirus; FLU, influenza virus; PIV, parainfluenza virus; RSV, respiratory syncytial virus; NAAT, nucleic acid amplification test; LDT, laboratory-developed test.

infection (33). In older adults, increased susceptibility to viral infections, age-dependent vaccine effectiveness (43) and more severe disease have been attributed to waning innate and adaptive immunity. Particularly, infection with RSV has been attributed to a decrease in memory $CD8^+$ T-cell function (44, 45). Similarly, immunosuppressed patients with profound and prolonged reduction in T-cell immunity are at increased risk for severe disease from viral infection (particularly ADV, hMPV, PIV, and RSV infections) (46, 47). A few studies have suggested that genetic polymorphisms of innate immune effectors, such as Toll-like receptors (e.g., TLR-4), are associated with increased susceptibility to severe respiratory viral infection (48, 49).

(ii) Environmental factors. Environmental factors may also influence the incidence of disease caused by respiratory viral infection either alone or with other underlying factors such as asthma (50). These factors may include the number of siblings in family, environmental smoking exposure (51), air pollution, climatic conditions, or weather (52, 53).

(iii) Anatomic site of infection. As the name suggests, most acute upper respiratory tract infections (URTIs) affect sites in the upper respiratory tract, including the larynx, nasal cavities, nasopharynx, oropharynx, throat, sinuses, conjunctiva, and inner ear, and commonly manifest as rhinosinusitis or the "common cold" (54), acute sinusitis (55, 56), acute laryngitis (57–59), conjunctivitis (54, 60–65), and otitis media (64, 66, 67). (Table 2).

Viruses in lower respiratory tract infections (LRTIs) affect deeper structures below the larynx, including the trachea, bronchus, and bronchoalveolar site, and manifest as bronchiolitis (68–71), bronchitis (72–76), and acute pneumonia (77–81).

Zoonotic viruses: human-animal health interfaces. The One Health concept is an integrative and collaborative approach that works to improve the health of humans and nonhuman animals while ensuring the protection of the natural environment (82). Clinicians and laboratorians should remain aware of the potential impact of One Health human-animal interfaces to allow for the emergence of new human respiratory viral pathogens (83, 84). Recent examples include human infection with the Middle East respiratory syndrome coronavirus (MERS-CoV) with camel exposure (83), swine variants of FLUA (84), pandemic FLU (pdm09), avian FLUA (e.g., H7N9) (85), and severe acute respiratory syndrome coronavirus (SARS-CoV) associated with bats and civet cats (86, 87). Laboratorians should establish effective communication links with epidemiologists, clinicians, and animal health experts to understand the impact of zoonotic viruses on human illness (88). Identification of at-risk patients early by clinicians can reduce the potential for nosocomial transmission of zoonotic pathogens. From the laboratory perspective, this means following the epidemiology of emerging infections and communicating with clinicians and public health workers to assess risk and determine the testing required based on travel histories and animal exposures (89–91). These approaches not only will identify patients at risk and allow public health practitioners to implement strategies to reduce transmission and limit further exposure in health care facilities and the community but also will ensure that laboratories can work up specimens using appropriate biocontainment approaches to reduce the risk of laboratory transmission of pathogens (92).

Section Summary and Recommendations

Respiratory viruses are a global problem with varied temporal and geographic patterns of circulation. Laboratorians and clinicians should understand that multiple viruses can cause similar signs and symptoms when infecting the upper or lower respiratory tract. Although some viruses may be more likely to be associated with some diseases, it is difficult to use clinical presentations alone to determine the causative agent. Laboratorians should have a firm understanding of viruses that are circulating in their region, as well as emerging infections in other regions of the world, as this information may guide clinicians and laboratorians in developing appropriate algorithms to test for agents causing respiratory illness.

GUIDELINES ADDRESSING THE DIAGNOSIS AND MANAGEMENT OF SYNDROMES ASSOCIATED WITH ACUTE RESPIRATORY INFECTIONS

Laboratorians must consider how laboratory testing impacts the diagnosis and management (including infection control considerations, treatment, and prophylaxis) of patients presenting with ARIs so that they collaborate with their health care providers to develop effective utilization strategies and develop algorithms that prioritize of testing of patients for whom results can influence clinical decision making. The following section summarizes U.S. and international guidelines written in the English language for the diagnosis and management of respiratory virus infections. Although viral diagnosis does not typically affect the patient management of otherwise-healthy adult patients, these guidelines identify scenarios where respiratory virus testing has been identified to influence patient management.

Infectious Diseases Society of America

Community-acquired pneumonia. Together, the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS) published consensus guidelines for the management of community-acquired pneumonia in adults in 2007 (note that revisions of the ATS guidelines are in progress) (79). In the guidelines, they outline specific microbiological testing recommendations and discuss how to take an appropriate travel history to support the diagnosis of pneumonia. The document identifies respiratory viruses as an important cause of community-acquired pneumonia (CAP) in outpatients and inpatients and emphasizes the importance of testing for and public health reporting of emerging or novel virus strains. Improvements to diagnostic testing using molecular approaches are encouraged, and drawbacks to rapid antigen testing, including cost and false-negative and false-positive results, are discussed. The document also provides support for use of antivirals (oseltamivir, zanamivir, or peramivir) in the treatment of seasonal and pandemic FLU, and it strongly supports vaccination in the prevention of seasonal influenza disease (79).

More recently (2011), the IDSA and the Pediatric Infectious Diseases Society (PIDS) published combined guidelines for the management of CAP in infants and children older than three months (93, 94). Since viral pathogens cause the majority of CAP in preschool-aged children, antibiotic therapy is not routinely required in this population. Testing for respiratory viral infections with a rapid, highly sensitive, and specific assay is recommended, as it may reduce the use of antibiotics in patients without clinical, laboratory, or radiological findings suggestive of bacterial coinfection. Antiviral therapy should be started as early as possible in children with moderate to severe CAP when FLU is circulating and symptoms are worsening. The group suggested that treatment not be delayed for laboratory confirmation, as negative laboratory tests (especially with rapid antigen testing) may not exclude disease. The American Academy of Pediatrics, in a policy statement by the Committee on Infectious Diseases and Bronchiolitis, did not recommend ribavirin for the treatment of RSV-CAP in infants. However, palivizumab prophylaxis of RSV was recommended by the American Academy of Pediatrics (94). The palivizumab guidelines have since been updated (95) and do not emphasize laboratory testing for RSV. No recommendations were provided for the use of antivirals against PIVs, ADVs, hMPVs, or CoVs in pediatric CAP.

FLU-specific guidance. In 2009, the IDSA released guidelines on the diagnosis, institutional outbreak management, chemoprophylaxis, and treatment of FLU in adults and children (96) (an update for this document is currently in process). Specific demographic criteria were outlined for whom should be tested for FLU, and testing was recommended only if results would influence clinical management. These situations partially include the following: immunocompetent outpatients with acute febrile respiratory symptoms (within 5 days of onset) at high risk for hospitalization or death, immunocompromised outpatients with febrile respiratory symptoms (regardless of onset date), and immunocompetent and immunocompromised hospitalized patients with fever and respiratory symptoms, including CAP patients (regardless of onset date). FLU testing was also recommended for elderly and infant patients with fever of

unknown origin or sepsis (regardless of onset date), children presenting for medical care with fever and respiratory symptoms (regardless of onset date), patients who after admission develop fever and respiratory symptoms (regardless of onset date), and individuals (e.g., health care workers, residents, or visitors) with febrile respiratory symptoms (within 5 days of onset) connected to an institutional FLU outbreak.

Rhinosinusitis. The IDSA "*Clinical Practice Guideline for Acute Bacterial Rhinosinusitis in Children and Adults*" provides guidance on clinical presentations to identify patients with viral and bacterial rhinosinusitis (97). Bacterial rhinosinusitis is defined as any of the following (i) >10 days of symptoms without improvement and with onset of high fever (≥102°F [39°C], (ii) high fever with purulent nasal discharge or facial pain during the first 3 to 4 days of illness, or (iii) worsening symptoms (e.g., fever, headache, or increase in nasal discharge) after apparent resolution of an upper respiratory tract infection. This document emphasized the use of clinical approaches and not laboratory testing to distinguish between bacterial and viral rhinosinusitis due to the self- limiting nature of this illness (97).

Other U.S. and International Guidelines Concerning Specific Populations and Settings

SOT. In 2013, the Infectious Diseases Community of Practice of the American Society of Transplantation, the American Society of Transplantation, and the Canadian Society of Transplantation released guidelines for infectious disease testing on solid organ transplant (SOT) patients (98). The guidelines recommend testing for common respiratory viral infections, including FLU, RSV, PIV, hMPV, RV, and CoV (99) with nasopharyngeal swabs, nasal washes, or aspirates. The use of BAL fluid samples should be considered for patients with negative upper respiratory tract specimens or with clinical or radiological evidence of lower tract disease processes. Multiple approaches may be used for diagnosis (e.g., nucleic acid amplification tests [NAATs], direct fluorescent-antibody [DFA] tests, rapid antigen detection, or culture), but the guidelines emphasize that NAAT is the most sensitive approach, and use of multiplexed NAAT improves the diagnostic capacity by testing for a variety of targets, which should be seriously considered in lung transplant patients. Prophylactic interventions for FLU (vaccination and neuraminidase [NA] inhibitors [NAIs]) and RSV (palivizumab) and the use of therapeutics for influenza (neuraminidase inhibitors) and RSV (ribavirin/intravenous immunoglobulin [IVIG]) are also outlined in the document (99).

The American Society for Transplantation Infectious Diseases guidelines for the diagnosis and management of ADV in solid organ transplant patients were published in 2013 (100). The document describes posttransplantation timelines for risk of ADV infection, where the first three months following SOT represents the highest risk. The guidelines emphasize that pediatric patients had the highest incidence of ADV infection, at 6.25%, which carried an organ-specific risk level (liver > heart > kidney). In adult SOT recipients (liver, heart, kidney, and kidney-pancreas), 10.5% of those with self-limited viremia after transplant later developed ADV-associated respiratory symptoms within the first year. Although ADV subgrouping does not play a role in the clinical laboratory, it may provide a sense of molecular epidemiology. For example, respiratory tract infections were associated with subgroups B1 (serotypes 3, 7, 16, 21, and 50), B2 (serotypes 11, 14, 34, and 35), C (serotypes 1, 2, 5, 6), and E (serotype 4), while disseminated disease (involvement of two or more organs) was associated with subgroups A (serotype 31), B2 (serotypes 11, 34, and 35), C (serotypes 1, 2, and 5), and F (serotype 40). Multiple diagnostic approaches can be used for suspected ADV infection, including NAAT, culture, DFA testing, and histopathology (considered the gold standard by the guidelines group for invasive ADV infection), but due to long-term shedding in respiratory specimens (as well as urine and stool), detection of ADV is not necessarily indicative of a disease process cause by ADV. Clinical symptoms, detection of the virus in multiple sites, and histopathology may strengthen the association of ADV detection with disease; however, the American Society for Transplantation Infectious Diseases guidelines do not offer predictive algorithms to link detection of ADV in

multiple sites with disease. The lack of clear clinical cutoffs in qualitative and quantitative NAATs adds to the confusion of whether positive results represent a current active infection. Issues with false-negative ADV results with some NAAT panels are also described later in this review. The American Society for Transplantation Infectious Diseases guidelines indicate that NAAT on a blood sample may be used successfully to monitor therapy, particularly if a baseline quantitative value is determined. ADV infections can be treated with cidofovir; however ribavirin should not be routinely used to treat ADV infections even though some subtype C viruses may respond to ribavirin treatment (100).

HSC recipients. International guidelines (combined recommendations of the Center for International Blood and Marrow Transplant Research [CIBMTR], the National Marrow Donor Program [NMDP], the European Blood and Marrow Transplant Group [EBMT], the American Society of Blood and Marrow Transplantation [ASBMT], the Canadian Blood and Marrow Transplant Group [CBMTG], the IDSA, the Society for Healthcare Epidemiology of America [SHEA], the Association of Medical Microbiology and Infectious Diseases Canada [AMMI], and the [CDC]) for preventing infectious complications in hematopoietic cell transplant recipients were released in 2009 (101). Patients are at risk from respiratory virus infection (FLU, RSV, hMPV, and PIVs) at all transplant stages from preengraftment to late phase. Prolonged shedding times after viral infections were identified in hematopoietic stem cell (HSC) recipients, with the following potential shedding times for the following viruses: ADV, $\geq$2 years; FLU, $\geq$4 months; and RSV, $\geq$22 days. Preventative measures for FLU include vaccination of close contacts and antiviral prophylaxis (for close contacts and patients). No recommendations were made for the use of ribavirin as a preemptive therapy for RSV. Evidence supporting the efficacy of palivizumab prophylaxis for RSV prevention in HSC recipients $<$4 years of age was thought to be insufficient to recommend for or against use. No recommendations were made for prophylaxis of PIV or hMPV infections. Testing for RSV and FLU in HSC recipients with signs and symptoms of respiratory infection during periods of circulation was recommended; however, routine surveillance of asymptomatic patients for these respiratory viruses was not endorsed (101).

Recently, guidelines from the Fourth European Conference on Infections in Leukemia addressed the diagnosis and treatment of RSV, PIVs, hMPV, RVs, and CoVs in patients with leukemia and those undergoing HSC transplants (102). The group had several recommendations regarding diagnosis of upper and lower tract community-acquired respiratory viruses, including (i) testing to guide infection prevention and control, treatment, and decisions for deferral of chemotherapy or HSC transplant, (ii) evidence for collecting specimens from the site of involvement (e.g., pooled swabs for the upper respiratory tract and BAL fluid [or tracheal swab if BAL fluid not available] for the lower tract), (iii) evidence to support the use of first-line or routine diagnostic tests for FLU, RSV, and PIV, (iv) evidence to test for other community-associated respiratory viruses based on assessment of risk of exposure and local epidemiology, and (v) evidence to consider collection of BAL fluid or biopsy samples for broader respiratory viral pathogen testing in patients with lower tract disease. Treatment with ribavirin and IVIG was recommended for RSV infection, while ribavirin alone was recommended for patients with PIV infection (102).

In 2016, the Infectious Diseases Working Party of the German Society for Hematology and Medical Oncology released guidelines for the diagnosis and management of community-acquired respiratory viruses (103). The risk of infection with FLU, RSV, PIVs, hMPV, and ADV in cancer patients is significant, and infection is associated with high rates of pneumonia and mortality. The document highly recommends NAAT for RSV, FLU, PIV, and other circulating/prevalent viruses in symptomatic patients. NAAT is recommended over antigen detection or culture as the test of choice for identifying these viruses. For patients with lower tract infection or critical illness, expanded testing for hMPV and ADV (and potentially other rare causes of lower tract disease [e.g., RVs and CoVs]) is suggested. Moderate support for recommendations for causal treatment of FLU (oseltamivir, zanamivir, and peramivir), RSV (ribavirin and IVIG), and ADV

(cidofovir) was given. Marginal support for recommendations for causal treatments of PIVs (ribavirin) was given (103).

Patients in the ED setting. In 2016, the American Academy of Emergency Medicine approved a clinical practice paper for the vaccination, diagnosis, and treatment of FLU. For seasonal FLU in the emergency department (ED), providers should (104) (i) perform testing only if results will change clinical management, (ii) understand the limited sensitivity and false-negative rates of rapid antigen detection tests (RADTs), (iii) consider NAAT if clinical suspicion is moderate to high, and (iv) if rapid antigen detection tests are negative but clinical suspicion is high, consider empirical antiviral therapy. Additionally, FLU antivirals are recommended for patients who are (i) hospitalized, (ii) at higher risk for complications, and (iii) have progressive illness (104).

Patients requiring isolation precautions in a health care setting. The Health Care Infection Control Practices Advisory Committee (HICPAC) document "*Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings*" discusses key functions of the clinical laboratory (11). The document recommends that microbiologists help guide the limited application of rapid testing to clinical situations where this testing influences patient management decisions and that they oversee nonlaboratory workers who perform this testing. The document also recommends the application of rapid tests to support treatment decisions, bed management, and implementation of infection prevention and control measures (e.g., barrier precautions, chemoprophylaxis, and vaccination); however, the authors of this PGCM document emphasize that the test characteristics (e.g., sensitivity, specificity, and predictive values) of an assay should be taken into account when making this decision. Surveillance of FLUA and RSV was emphasized for case finding or cluster analysis, particularly when infection precautions may be implemented. Removing a patient from isolation is virus specific (see Table 1 regarding isolation precautions); however, of note, RSV antigen tests are considered inadequate to remove patients from contact precautions, as false-negative results are frequent.

Outbreak investigations. The U.S. CDC guidelines "*Unexplained Respiratory Disease Outbreaks (URDO)*" outline the steps taken to define and investigate a respiratory outbreak of unknown origin (105). Detection and characterization of the pathogen are key steps allowing for effective clinical management, infection prevention and control practices, and defining the time period of the outbreak. The document identifies a variety of testing, including NAAT, culture, serology, and antigen detection, that may be used to investigate the etiology of an outbreak (105).

Emerging pathogens. In the last few years a number of emerging viruses have been identified globally, including FLU subtypes (H5N1, H5N6, and H7N9 [106–108]) and CoV strains (MERS-CoV [109]\and SARS-CoV [110]). A number of guidelines have been published to help in the diagnosis and management of these emerging pathogens (111–113). Optimal timing of collection differs. Although the ideal specimen collection time for influenza virus is as soon as possible after symptom onset, NAAT for MERS-CoV can be performed 14 days postonset due to improved sensitivity of the assays. From the laboratory perspective, NAAT is the recommended method of detection. A wide variety of respiratory specimens may also be collected. If upper tract swabs are negative, then lower tract specimen collection should be pursued. Although the cultivation of these pathogens requires a higher level of biocontainment, the majority of activities for identification via NAAT can be done in biosafety level 2 (BSL-2) facility in a biosafety cabinet (BSC) using enhanced precautions. As new pathogens emerge (e.g., H7N4), laboratorians should confer with reference centers (e.g., the U.S. CDC) on the most appropriate testing approaches to detect and characterize these viruses.

(i) MERS-CoV. In June 2015, the most recent version of the MERS-CoV biosafety guideline was released as "*Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV)—Version 2*" (113). Activities appropriate for BSL-2 facilities using standard BSL-2 practices included molecular testing of extracted nucleic acid and final packing of specimens for transport to diagnostic laboratories for additional testing. Activities to

be undertaken in a class II BSC included aliquoting specimens, diluting specimens, performing diagnostic tests not involving propagation of potentially infected specimens, and nucleic acid extraction from potentially infectious specimens. Cell culture propagation and the characterization of propagated material should be undertaken in a BSL-3 facility using BSL-3 practices (113).

In June 2015, "Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for Middle East Respiratory Syndrome Coronavirus (MERS-CoV)—Version 2.1" was released by the CDC (114). The guidelines recommended, when possible, the collection of upper respiratory tract, lower respiratory tract, and serum specimens for the diagnosis of MERS-CoV. Potential lower respiratory tract specimens included BAL fluid, tracheal aspirate, pleural fluid, and sputum. Appropriate upper respiratory tract specimens included NPS and OPS (which could be combined in the same transport container if the test is validated for this type of combined collection) and nasopharyngeal aspirates. Upper and lower respiratory tract specimens should be collected within 7 days of symptom onset; however, NAAT can be performed 14 days postonset due to improved sensitivity of the assays (112).

(ii) Novel and emerging FLU strains. In January 2014, guidelines for possible infection with avian FLUA (H7N9) virus were released by the CDC (111), and these were later updated for novel FLU strains (116). These guidelines outline appropriate testing for emerging FLU strains such as A(H7N9) and A(H5N1), and they describe exposure risk and clinical symptoms specific for each virus. Specimens should be collected as early as possible after symptom onset (ideally within 7 days) (116). Sample collection after this point is still relevant in children, immunocompromised patients, and critically ill patients with lower tract disease, as virus can be shed for longer periods in these patient populations. As new strains emerge (e.g., H7N4), laboratorians should confer with reference centers (e.g., the U.S. CDC) on the most appropriate testing approaches to detect and characterize these viruses (117).

Acute Respiratory Viral Infection following Travel

The book *CDC Health Information for International Travel* (also known as the "Yellow Book") (118) is a reference for health professionals who care for international travelers. The "Yellow Book" identifies viral pathogens as the most common cause of respiratory infections in travelers. Etiologies can vary widely, including infection with RV, RSV, FLU, PIVs, hMPVs, ADV, or CoV (118); however, in the absence of severe illness or pneumonia, laboratory diagnosis is not always clinically necessary (118). Depending on the travel history, novel causes of respiratory illness (e.g., MERS-CoV and avian FLU strains) should be considered for symptomatic patients.

It should be noted that the positive predictive value (30 to 88%) for laboratory-confirmed influenza in returning travelers can vary widely depending on the seasonality of infection and method of detection (119). While the negative predictive value of FLU NAAT in returning travelers can be used to rule out FLU infection, earlier-generation antigen detection test methods should not be used to rule out influenza virus infection, particularly when emerging strains are suspected. Patients who should be tested for FLU infection include (i) symptomatic hospitalized patients, (ii) cases where diagnosis of FLU will affect patient management, and (iii) cases where FLU testing would affect infection prevention and control or management of close contacts (119).

Section Summary and Recommendations

Multiple guideline groups have addressed the role of laboratory diagnosis of viruses in specific patient populations. Laboratorians should be aware that many guidelines are greater than 5 years of age and may not have taken into account the changes that have occurred in the types of tests available for the diagnosis of respiratory viruses. Although some of these documents are now aging, it is clear that testing may play a more important role in the management of severely ill patients and the immunocompromised and less of a role in the management of immunocompetent and relatively healthy adults and children. Laboratory testing may assist in supporting public health

investigations (e.g., emerging pathogen investigations and outbreak investigations), epidemiological investigations, and infection control functions. Most simply, laboratory testing may be considered when it positively impacts clinical decision making and supports patient management.

SPECIMEN COLLECTION FOR LABORATORY DETECTION OF ACUTE RESPIRATORY VIRUSES

Risk Assessment for Emerging Pathogens Prior to Specimen Collection

During clinical assessment, clinicians should ask about travel history and animal exposure that could be consistent with acquisition of (or exposure to) an emerging pathogen (e.g., MERS-CoV or avian FLU). Prompt consideration of an emerging pathogen based on epidemiological risks with engagement of public health and the implementation of appropriate of infection prevention and control measures are essential to prevent nosocomial spread of these infections. In the MERS-CoV outbreak in South Korea (May to July 2015), the lack of prompt identification of risk factors in patients presenting to the ED allowed spread between patients and staff at several hospitals (120). Early identification and upfront screening procedures could have isolated the index patient and reduced the number of contacts, thus limiting the spread of infection (121). This is consistent with mathematical modeling showing that rapid identification of index cases is the most important factor in reducing spread of infection and that patient isolation and quarantine have the strongest correlation with transmission prevention (122). As soon as an emerging pathogen is suspected, the laboratory should be notified to provide advice on appropriate specimen collection and testing to ensure identification and to ensure that the specimens are handled with the appropriate biocontainment considerations for the novel pathogen.

Appropriate Specimen Collection Is Critical for Virus Detection in the Laboratory

When to collect a specimen. Clinicians should collect specimens from symptomatic individuals with acute respiratory illness within 5 days of symptom onset (preferably within 48 h). Specimen collection later than 5 days after onset is recommended only when symptoms persist or worsen, in young children, or in the immunocompromised (96, 123).

Virus-specific shedding estimates can further direct best collection guidelines for respiratory specimens; however, it should be noted that estimates are typically performed on select patient populations, and differences may be due to differences in study designs, differences in specimen types, and differences in detection technologies between studies (124–126). NAAT is the most sensitive method of detection, and sampling as soon as possible after the onset of symptoms is considered ideal for healthy individuals; most viral targets can be effectively identified in the first 2 days after symptom onset, and multiple studies indicate that viral loads in respiratory specimens will generally decrease over time. Furthermore, a delay in specimen collection following onset of respiratory symptoms will negatively impact the sensitivity of laboratory tests to detect a pathogen.

In RV infection, NAAT identified peak shedding within 2 days of symptom onset, with decreasing viral loads up to 7 days after onset (127). When virus culture and NAAT were both used to test specimens, 57% of human hMPV isolates were detected within the first 2 days of symptom onset, while only 19% were detected greater than 4 days after onset (128). Only 27% of hMPV NAAT-positive specimens collected after day four were positive by culture (128). In children tested for RSV by DFA testing, viral shedding (measured in upper respiratory tract specimens [e.g., nasal, throat, and NPS specimens]) peaked 2 days after onset of illness, and the median shedding duration was 4.5 days. Similar shedding patterns were identified for FLU infection. In community patients with acute respiratory illness, FLUA viral loads measured by NAAT peaked at day one following symptom onset and were detected until day eight, while in patients who had one symptom (but did not meet the case definition for acute respiratory illness), loads peaked on day one with detection until day six (129). In contrast, FLUB viral loads were

found to be highest on the day of symptom onset and to persist until day six to eight (129).

It should be noted that there are no standard "case definitions" on how long positive respiratory virus results detected by NAAT should be considered part of the same infection event. Some preliminary studies propose a 30-day period for ADV infection in children and for RV infection in infants as a definition of a single case (130, 131); however, the temporal definition of a new viral infection should be assessed in the clinical context, as the presence of comorbidities can significantly alter viral shedding times. The duration of shedding can be influenced by multiple factors. Although prior infection may not completely prevent reinfection, it may alter the duration of shedding. Older individuals (suggested to have prior exposure) and children with prior RSV infection generally shed for shorter periods of time (125, 132). The strain of virus or subtype or coinfection with different viruses (133, 134) can also influence shedding patterns. RSVA was detected 5.8 days longer than RSVB (135). Similarly, when children with acute expiratory wheezing were found to be coinfected with EV and RV, shedding of RV persisted for 2 to 3 weeks, whereas EV shedding persisted for 5 to 6 weeks (136).

Other factors may increase shedding time and still allow for productive specimen sampling and detection of viral pathogens. Some studies suggest that viral shedding may also be extended in patients with more severe disease (125, 137). Shedding can also be prolonged in immunosuppressed patients. Although the number of patients with detectable virus (FLU, PIV, or RSV) was highest in the first 2 weeks following symptom onset, long-term virus detection (>30 days) with NAAT on upper and lower respiratory tract specimens has been described for FLU, PIV, and RSV in patients with hematological disorders (138). Testing these patients for "test of cure" is not recommended or appropriate for viral upper respiratory tract infections, as viral shedding often does not represent active infection (139).

Biosafety considerations and PPE required for collection. Respiratory viruses such as FLU can be efficiently transmitted through the air (140, 141); however, the direct risk to health care workers who are collecting upper and lower respiratory tract specimens by different aerosol-generating procedural methods (e.g., bronchoscopy, sputum induction, endotracheal intubation, positive pressure ventilation, nebulizer treatment, airway suction, tracheostomy, chest physiotherapy, and high-frequency oscillatory ventilation) is currently unknown (142). Analysis of historical data is confounded by growing evidence that infection prevention and control practices for respiratory viruses may not be uniformly followed (143). A recent analysis of practices in multiple U.S. states found low practice adherence, with many health care workers unsure of when appropriate personal protective equipment (PPE) should be worn (143). Droplet precautions for patients with confirmed or suspected infection with FLU should be practiced to prevent transmission during collections. The need for N95 masks can be controversial, and local infection prevention and control procedures should be followed to minimize aerosolization and risk of health care worker infection (144–146). Even if more "effective" respirators are used when clinicians are in contact with patients, their benefit may be negated if generally poor infection prevention and control practices are utilized (145). The laboratorian with expertise in respiratory virus transmission and viral characteristics can be a valuable member of local teams when creating respiratory protection program protocols.

Sampling from upper respiratory tract sites: which specimen to use? For an upper respiratory tract infection, a variety of specimens can be used to diagnose respiratory infections (NPA, NPS, NW, NS, OPS/TS, and sputum) (Table 3), and the U.S. CDC offers collection guidance for each; however, laboratories should use manufacturers' recommended specimen types in U.S. Food and Drug Administration (FDA)-cleared or validated/verified laboratory tests (147, 148). Selection of specimen type is dependent on a variety of factors: patient age, patient willingness to undergo a specific procedure, clinical presentation, the nature of the potential pathogen, and the appropriateness of the specimen type for verified laboratory diagnostic approaches. Although a combination of different specimen types can improve the sensitivity of NAATs (149–155), this

TABLE 3 Sensitivity of respiratory viral detection from different specimen types[a]

Specimen type	Sensitivity of detection[b] of:						
	FLUA/B[c]	RSV	RV/EV	ADV	hMPV	PIVs	CoVs[c]
NPS	++	++	++	++	++	+++	++
NPA	+++	+++	+++	+++	+++	+++	+++
OPS	++(+)[d]	++	+	++	+	+	+
TS	++	++	+	++	+	++	++
Sputum[f]	+++	+++	+++	+++	++	+(+)	++(+)[e]
BAL fluid	+++	+++	++	++	++	+(+)	++
Lung biopsy specimen	++	++	+	+	+	o	+++

[a]For specimen collection, it is important that appropriate infection control practices are followed, as collection can be aerosol generating. FDA clearance and laboratory-based validation/verification of the specimen source for assay need to be considered. Appropriate collection methods should consider downstream testing to ensure that specimens are handled, stored, and shipped properly prior to testing. Preanalytical specimen storage information provided by the laboratory should indicate storage temperature, retention time, and stability of the specimens (123, 178, 179, 370). Combinations of different specimen types can significantly increase the yield for viral detection. Results for nasal specimens are not included in this table because the literature describing their efficacy in detection is variable (372–374).

[b]+++, specimen type has high detection rates for the indicated virus; ++, specimen type is acceptable for viral detection, but sensitivity may be reduced due to the sampling or testing method used for detection; +, specimen type has reduced sensitivity for indicated virus; ++(+), minor reduction; +(+), moderate reduction; o, limited utility.

[c]For emerging avian influenza virus strains or for CoVs such as SARS-CoV or MERS-CoV, lower respiratory samples are additionally recommended for enhanced detection.

[d]NPS were more sensitive for detection of FLUB, while OPS were more sensitive for FLUA strains (153).

[e]Sputum sensitivity varies between CoV strains (180).

[f]Sensitivity of sputum results can vary widely depending on the quality of the specimen received. Sputa received for viral testing are not screened for specimen adequacy as for those received for bacterial workup (371).

must be balanced in such a way to maintain high detection rates yet still maintain a cost-effective approach. For emerging pathogens (e.g., novel FLUA H5/H7/H9 or emerging CoV), a collection of multiple different types (OPS, NS, NPS, BAL fluid, etc.) may be necessary to identify specimens that most reliably result in detection of the pathogen. Depending on the pathogen (e.g., emerging CoV or novel FLUA), other, atypical specimens such as blood or stool for direct virus detection may also be suggested for collection (156–158).

Traditionally, NPA were used as the gold standard for detection of respiratory viruses (159). Previous publications suggest that NPS is equivalent to NPA for the detection of multiple viruses in children (160). Although NPS/NPA are generally more sensitive than throat swabs for detection of most viruses (152, 154, 161, 162), NS are easier to obtain, are less painful (163–165), and can be self-collected with yields equivalent to those collected by a clinician (166). Reduced diagnostic sensitivity using NS samples is often considered an acceptable trade-off for increased compliance, particularly when the prevalence of disease is high (159, 167). In addition, there are increasing data suggesting that the combination of both an NS and an OPS in adults and children has a yield equivalent to that of NPS/NPA (10, 151, 155). Use of a flocked swab with a liquid viral transport medium may additionally improve viral detection (161, 168, 169). Easier midturbinate collection with flocked swabs may provide an alternative to proper nasopharyngeal specimens, albeit with potentially a lowered sensitivity (170, 171). Finally, when using commercially available rapid antigen detection tests (RADTs), laboratories should use the kit-recommended swab unless the performance of the test with a different specimen type has been verified (172).

Approaches to specimen collection from the lower respiratory tract. Lower respiratory tract specimens such as sputum, bronchoalveolar lavage/wash, and lung tissue may be considered in cases where the patient may be infected with an emerging pathogen (173, 174) or is under intensive/critical care for pneumonia (175), in cases involving autopsy (176), or where molecular detection requires pathological evidence of invasive disease (e.g., ADV infection in lung specimens of lung transplant patients) (177). In severe illness due to influenza and emerging pathogens, upper respiratory

tract sampling may yield false-negative results (112). Accurate diagnosis in these cases often will require a variety of specimens from the upper and lower respiratory tracts. Selection of lower respiratory tract specimens should be dependent on the disease course (e.g., anatomic location of the diseases process, stability of the patient/risk in sampling, and ability to access the anatomic site) (176, 178–180). Given these issues, specimen collection and therefore determination of the lower respiratory tract infection may not be possible.

Lower respiratory tract specimen types vary in their ability to be used to detect specific viral etiologies (Table 3). Sputum may be considered an appropriate specimen for sampling the lower respiratory tract in some patients (178–180). However, data are limited. Specimen viscosity and higher rates of PCR inhibition make sputum a more difficult specimen type to use in the laboratory (174), and most FDA-cleared assays for respiratory viruses are not validated by the manufacturer for sputum or other lower respiratory tract samples (e.g., BAL fluid). Bronchial washes and lavage fluids can be useful specimen types, provided that they are collected appropriately in sterile containers, as the viral load for lower respiratory tract infections can be higher in these specimen types. Lung tissue collected during bronchoscopy, open surgical procedure, or autopsy should be placed in a sterile container with a small amount of sterile saline to keep it moist (176). Specimens should not be put into formalin, as it reduces the sensitivity of NAAT, and formalin-preserved samples are not commonly verified sample types for most laboratory test systems. Procedural variability for specimen collection (e.g., volumes collected and dilution factors) makes comparison of the performances of these off-label specimens difficult.

Transport medium and transport considerations. Viral transport medium or universal transport medium facilitates viral culture, direct fluorescent-antibody (DFA) testing (181), rapid antigen detection tests (RADTs) (182), and molecular testing (181, 183, 184). Stability guidelines outlined in the package insert (storage at room temperature, refrigeration, or freezing) should be used as per the manufacturer's instructions. Other transport devices may be considered (e.g., dry swabs [185, 186] or alcohol-based transport medium [185]) but are not widely used. Transport should be in accordance with regulators' guidelines in each jurisdiction.

Section Summary and Recommendations

Always ensure that clinicians are aware of processes for safe specimen collection from patients who are suspected of being infected with routine and emerging respiratory viruses. For the detection of routine seasonal respiratory viruses, samples should be collected as early as possible from patients following onset of illness. Shedding studies of multiple viruses indicate that viral titers drop daily following the onset of illness. Thus, sampling from patients at later time points is expected to negatively impact the sensitivity of diagnostic assays. Sample collection from the upper respiratory tract may be easiest, but upper tract sampling may not detect viruses causing lower tract disease. Following specimen collection, ensure that appropriate transport and storage conditions are used for specimens.

LABORATORY DETECTION OF ACUTE RESPIRATORY VIRUSES

The Role of Cell Culture is Limited

Cell culture was long considered the gold standard for virus isolation and identification prior to the availability of molecular assays (187, 188). Modification of cell lines (including primary lines, immortalized lines, mixed cell lines, and transfected lines) has improved the ability to detect respiratory viral pathogens (189). For laboratories offering cell culture analyses, detailed procedures can be found in the Clinical and Laboratory Standards Institute document "*M41: Viral Culture*." (190).

There are a variety of drawbacks to using cell culture compared to molecular methods, and many virology laboratories have opted to discontinue viral culture in the laboratory for these reasons. It is well established that cell culture has a lower sensitivity than molecular techniques (191, 192), the turnaround time and hands-on time required

to perform cell culture compared to molecular testing are increased, and the technical expertise for performing cell culture is often not available. Traditional tube cultures are slow and can take up to 10 days for detection of respiratory infections (36). A study in pediatric patients indicated that positive viral culture results would not impact the management of healthy children hospitalized for illness attributed to community-acquired respiratory viral infection due to the delay in time to culture positivity (193). Shell vial assays can decrease the time to detection; however, 1 to 2 days is still required for growth and identification of the virus. Care must be taken when selecting cell lines for viral growth, as not all cell lines will allow for propagation of all viruses, and cell lines may be viral strain specific (194). Yields from cell culture are often decreased following freezing, due to reduced numbers of viable virus particles; therefore, samples that are frozen prior to culturing may be falsely negative (139, 195). As a safety note, culture approaches may inadvertently propagate emerging pathogens and compromise laboratory biosafety (195); however, maintaining cell culture capabilities in public health laboratories remains important for identification of unknown or emerging pathogens, particularly when specific molecular amplification processes are not available, and can provide an understanding of the virus viability within a clinical specimen (196).

Direct Fluorescent-Antibody and Immunofluorescent-Antibody Assays for Respiratory Viruses

Direct fluorescent-antibody (DFA) and immunofluorescent-antibody (IFA) assays have been used to detect a variety of respiratory viruses from primary specimens (chromatographic immunoassays for the detection of respiratory viruses are discussed in the following section). Commercial and standardized clinical reagents are available for select respiratory pathogens (e.g., FLUA/B, PIVs 1 to 3, ADV, hMPV, and RSV) (197). Like that of traditional cell culture techniques, the quality of DFA/IFA assays is impacted by specimen quality and collection method (171). Unlike the case for traditional cell culture, DFA and IFA assays do not require viable viruses and the turnaround times are short ($<$4 h) and on a single-specimen basis can be shorter than those for older laboratory-developed molecular approaches (which have, e.g., separate extraction steps, greater numbers of manual steps, and manual interpretation and data entry in laboratory information systems) or batched-based testing; however, DFA/IFA technologies are labor-intensive, require a skilled technologist to read and interpret results, require a fluorescence microscope, and are subject to reader error. Furthermore, the hands-on time required per test is not structured for high-throughput result reporting. Compared to molecular detection methods, DFA and IFA assays have significantly reduced sensitivity and specificity (197). Some argue that the lower sensitivity can identify "clinically relevant infections" in some patient populations (e.g., hospitalized pediatric patients) (198) in contrast to detection of free nucleic acid as in molecular detection. Additionally, microscopic examination of samples for DFA testing can directly determine specimen quality (199) by allowing for observation of the number of epithelial cells present in the sample.

Rapid Antigen Detection Tests for the Detection of Respiratory Viruses

Clinical Laboratory Improvement Amendments (CLIA)-waived tests are intended for use in "professional" settings (e.g., physicians' offices, mobile clinics, and pharmacies) and/or by untrained operators with no laboratory expertise (200). A summary of rapid antigen detection test (RADT) technologies that may be used as near-patient or point-of-care (POC) tests is given in Table 4. Technologies for these guidelines are discussed in general here; specific products are not discussed, and company names are not mentioned.

Earlier RADT assays detected antigens of FLUA, FLUB, and RSV. Use was often restricted to specific specimen types (e.g., NPS or NS), the sensitivities of these assays in pediatric and adult populations varied but were considered to be poor, and the assays could not be used to rule out infection (201, 202). Performance characteristics of these assays were typically determined during normal respiratory virus seasons, with

TABLE 4 Respiratory viral testing approaches with CLIA waivers[a]

Patient population(s)	Virus(es)	Viral targets	Technologies	Sensitivity (%) vs real-time PCR assays	Specificity (%) vs real-time PCR assays	Specimen type(s)	General TAT	References
Clinically ill, no age restrictions	FLUA, FLUB	Antigens	Immunochromatographic (first generation)	FLUA, 15–56; FLUB, 24–56; combined, 23–69	FLUA, 99–100; FLUB, 99–100; combined, 96–97	NPS, NS, NW/ nasal aspirate	Minutes	207, 375–378
Clinically ill, neonatal and pediatric, age varies	RSV	Antigens	Immunochromatographic (first generation)	58–80	91–100	NPS, NPA, NW/ nasopharyngeal wash	Minutes	212, 375, 379–381
Clinically ill, no age restriction	FLUA, FLUB	Antigen	Assisted/automated reading of immunoassay (second generation)	FLUA, 67–81; FLUB, 33–92	FLUA, 98–100; FLUB, 90–100	NPS, NS, NW/ nasal aspirate	Minutes	211, 288, 376, 381, 382
Clinically ill, pediatric patients, age varies	RSV	Antigen	Assisted/automated reading of immunoassay (second generation)	78–82	97–99	NPS, NW/nasal aspirate	Minutes	214, 383
Clinically ill, no age restriction	FLUA, FLUB	Nucleic acid	Isothermal amplification and molecular beacon probe detection	FLUA, 73– 94; FLUB, 75–97	FLUA, 63–100; FLUB, 54–100	Direct NS or NPS	≤1 h	286–288, 384
Clinically ill	FLUA, FLUB	Nucleic acid	Automated sample preparation, amplification, detection, and result interpretation, real-time multiplex RT-PCR	FLUA, 98–99; FLUB, 99–100	FLU, 99–100; FLUB, 99–100	NPS	≤1 h	289, 385
Clinically ill	FLUA, FLUB, RSV	Nucleic acid	Automated sample preparation, amplification, detection, and result interpretation, real-time multiplex RT-PCR	FLUA, 95; FLUB, 100; RSV, 99	FLUA, 98; FLUB, 99; RSV, 99	NPS	≤1 h	386, 387
Clinically ill adult and pediatric patients	FLUA H3 subtype and 2009 H1 subtype, FLUB, ADV, CoV, hMPV, PIV 1–3, RSV, RV, EV	Nucleic acid	Single-instrument configuration, real-time multiplex NAAT	96.8% positive agreement vs other FDA-cleared molecular comparative method	99.5% negative agreement vs other FDA-cleared molecular comparative method	NPS	Approx 1 h	388, 389

[a]Abbreviations: FLU, influenza virus; RSV, respiratory syncytial virus; ADV, adenovirus; CoV, coronavirus; hMPV, human metapneumovirus; PIV, parainfluenza virus; RV, rhinovirus; EV, enterovirus; NAAT, nucleic acid amplification test; RT-PCR, reverse transcription-PCR; NPS, nasopharyngeal swab; NPA, nasopharyngeal aspirate; NW, nasal wash.

acceptable specificity for RSV and FLU (203, 204); however, the performance characteristics are significantly reduced when assays are used out of season (205–207). Many believe that the clinical utility of employing FLU and RSV POC assays, given the high numbers of both false-positive and false-negative results, is questionable (205–207), and the future long-term availability of rapid antigen detection kits is in doubt. On 23 February 2017, the U.S. Food and Drug Administration (FDA) reclassified rapid antigen influenza virus test kits from class I to class II medical devices (208). This was meant to address growing concern about the variable performance of these assays as well as poor sensitivity compared to other methods such as NAATs and culture. Existing kits could be purchased until 12 January 2018 and used until the kit expiry date. Following that point in time, manufacturers were expected to monitor kit reliability and provide updates to users. Additionally, some assays are unable to differentiate between FLUA and FLUB, which may impact epidemiological investigations (209), and they have particularly poor sensitivity to detect avian influenza virus and other emerging subtypes (210). However, RADT may still have a place in management of outbreaks or in locations with limited access to molecular diagnostics (209), but consideration of the assay performance and the seasonality should be taken into account when using these assays.

A second generation of viral antigen POC tests improved the sensitivity for FLUA/B and RSV detection compared to that of earlier technologies (211, 212); however, the performance characteristics were still reduced compared to those of routine molecular testing (Table 4). Similar to the case for earlier generations, respiratory viral infection could not be ruled out with the newer POC tests, and sensitivities and specificities varied depending on the FLU target and the comparator molecular method used (Table 4). For RSV, sensitivity and specificity were reduced compared to those with molecular methods (Table 4). The sensitivity of these tests is highest during the RSV season when the positive predictive value is high (213–215). If clinicians feel there is a need for RSV antigen-based POC testing for pediatric patients (e.g., when there is no nearby laboratory access or in resource-poor environments), laboratorians need to inform clinicians of the newer test technologies, provide information on the current prevalence of these pathogens, and assist in generating algorithms that reduce the risks of these technologies.

Molecular Detection Approaches as the New Reference Standard

Extraction considerations. The first step in NAAT requires extraction, purification, and preservation of target organism nucleic acids. Extraction technologies should be able to cleanly isolate both high-quality viral RNA and DNA and, depending on the assay, to additionally sample human nucleic acids to allow the detection of human genes (e.g., that for glyceraldehyde 3-phosphate dehydrogenase [GAPDH] or β_2-microglobulin [β2M]) as control targets. The ubiquitous presence of RNase enzymes in most human samples makes isolation of RNA nucleic acid targets (e.g., FLU, RSV, and CoV) (Table 1) more difficult than isolation of DNA (e.g., adenovirus) (Table 1) and often requires additional steps for processing. Multiple extraction methods may be employed for respiratory virus detection. Heat-mediated lysis is an approach where target organisms are lysed or homogenized to release target nucleic acids (216). This approach is used in some commercial NAATs. Manual extraction using phase separation, capture via magnetic beads, or immobilized silica spin or vacuum wash columns may also be used. Automated extraction systems may be employed and generally use magnetic silica or other particles designed to capture RNA, DNA, or both. In fully automated instrument systems, all steps from extraction through to amplification are incorporated into a single cartridge or pouch.

Commercially available respiratory virus NAAT kits for detection of respiratory viral targets generally have a specific extraction method that is qualified for sample processing as part of the FDA clearance. Often, the FDA-cleared NAATs will have claims for specific specimen types (NPS, NS, etc.) but may or may not specify the type of transport medium. If the laboratory chooses to use specimen types besides those that are FDA

cleared, the laboratory should perform a verification study to document recovery of the target nucleic acids and acceptable performance of the NAAT (217). A validation plan should consider a variety of factors, including the frequency of specimen type being tested and the risk that specimen types may not be compatible with the assay. Similarly, if the testing laboratory chooses to use a different extraction protocol, verification for comparable performance is required. The requirement for verification of additional specimen types is outlined in the College of American Pathologists' *Microbiology Checklist, Molecular Microbiology, MIC.64810* (sections titled "Test performance—manufacturer's instructions" and "Laboratory-developed or modified FDA-cleared/approved tests") (217). Many in-depth documents and reviews discussing the requirements of molecular assay validation have previously been published (218, 219); therefore, a detailed discussion will not be included in this article.

Assay control considerations. All NAATs, whether laboratory-developed tests (LDTs) or FDA-cleared assays, should include a set of controls, including external positive and negative controls for respiratory viral targets that are tested by all steps in the assay. An internal amplification control should be added to all specimens except in assays where inhibition rates of the NAAT have been shown to be below acceptable limits (often defined by the laboratorian) (220, 221). These controls ensure that target nucleic acid is recovered and any potential NAAT inhibitors are removed during the sample processing stage. While commercial NAAT kits are designed to flag invalid results (when internal controls fail), LDTs require manual checks and result review to detect invalid specimens. The number of external controls and their frequency of use should be established by the laboratory based on regulatory requirements and its individualized quality control plan (IQCP), with a focus on risk assessment. Rules for review and result-based actions items should be addressed in the laboratory IQCP (222, 223).

Contamination. Molecular target amplification assays are susceptible to false-positive results caused by contamination, and false-positive results may occur at any step in sample collection and processing. Preanalytical contamination may occur when specimen integrity is breached during the collection process or when integrity is breached during early handling processes in the laboratory (221). Even when using a biosafety cabinet, steps should be taken to limit the production of aerosols and to process specimens in a manner that prevents cross contamination (224). Given that respiratory viruses can be identified in health care environments, it is possible that inappropriately handled swabs or other specimens could be contaminated with these viruses (225). It is also possible that a laboratory worker infected with a respiratory virus may act as a contaminating vector in the laboratory. The greatest risk of contamination is from amplicons created (and possibly aerosolized) during previous molecular runs. Most commercial assays using either real-time reporters or array-based detection are designed to minimize risks of amplicon contamination unless the laboratorian fails to correctly handle the reaction vessels (221).

Assays that incorporate manual postamplification processing present the highest risk of contamination to the laboratory. Multiple amplicon sterilization processes have been established to decrease the chance of amplicon carryover in molecular assays. These include the use of UV light to create thymidine dimers (cross-linking contaminating DNA), altered amplification chemistry using modified nucleotides, addition of uracil DNA glycosylase (UNG), and the use of hydroxylamine to prevent cytosine and guanine base pairings in subsequent reactions; however, numerous chemical approaches may be used (226–228).

Good laboratory practices can also be used to control contamination or carryover of amplicons (Table 5). These are particularly relevant when multiple processes such as reagent preparation, nucleic acid extraction, amplification, and postamplification processing are utilized. Open systems (where extraction, amplification, and/or detection stages are exposed to the environment) and closed systems (where extraction, amplification, and detection are completed within a single compartment not exposed to the environment) have different contamination control requirements (Table 4). Staff training protocols and laboratory standard operating procedures (SOPs) should emphasize

TABLE 5 Good laboratory practices for molecular assays

Laboratory practice to decrease contamination events	Recommendation for type of molecular system[a]	
	Open	Closed
Unidirectional flow (clean to dirty)	Recommended	Not required
Physical separation of pre- and postwork areas	Recommended	Not required
Regular decontamination of work areas	Recommended	Recommended
Use of aerosol-resistant pipette tips	Recommended	Recommended
Change of PPE between processing steps	Recommended	Not required
Restricting worker movements postamplification	Recommended	Not required
Centrifuging reagents	Recommended	Recommended
Ensuring that only one specimen is uncapped at a time	Recommended	Recommended
Process to monitor contamination events	Recommended	Recommended
Dedicated equipment for pre- and postamplification areas	Recommended	Not required
Monitoring environment for contamination (e.g., by environmental swipe tests)	Recommended	Recommended

[a]Based on the type of molecular system, laboratory practices to decrease contamination are either recommended or not required (217, 221–223, 228).

the organization of workflow process (such as unidirectional flow, separate areas for pre- and postamplification processing, regular decontamination of work areas with bleach, strict adherence to use of aerosol resistant pipette tips, mandating changing of gloves and lab coats between processing steps, and restricting work on new samples after handling postamplification reaction mixtures [228]) and technical practices (such as aliquoting of reagents, centrifuging of reagents, and care in capping and uncapping tubes, which may also prevent cross contamination). Physical separation of workspaces dedicated to different assay steps (e.g., pre-PCR and post-PCR) can also decrease the risk of contamination (221) and is recommended for open systems, but it is not necessary for closed systems.

Additionally, laboratorians should develop processes to monitor contamination events. Sentinel systems, such as running negative or no-template controls in each amplification assay, can be used for detecting large-scale contamination (221), while low-level contamination events may be identified by laboratorians as an excessive or unusual amount of low-level positive specimens (e.g., positive results near the cutoff). Care should be taken when interpreting results for higher numbers of low-level positive results outside the normal respiratory virus season, as many low-level positive results may represent contamination. Care should be taken when interpreting specimens that are positive for multiple targets, and laboratorians should have a sense of the coinfection rates within their settings. Coinfection rates may vary widely between adult and pediatric patient populations and may account for over 10% of all specimens in some pediatric populations (229–231). Environmental swipe tests should be considered to monitor workspaces for contamination from current or recently circulating viruses as well as control materials, and they can be used to detect widespread amplicon contamination events (232); however, sporadic contamination events may be missed due to sampling bias. Some FDA-cleared assays have specific recommendations for environmental monitoring and outline routine decontamination measures. For other tests, it is up to the laboratory to define intervals as part of their quality assurance program or IQCP (217, 221–223).

Positive predictive value and false-positive tests. In general, molecular tests for respiratory viruses have high sensitivity and excellent negative predictive values, which can reliably rule out infection when assay results are negative. Most molecular assays for respiratory viruses also have excellent positive predictive values, in the range of 90% or higher. Because molecular amplification assays for these pathogens are generally more sensitive than culture-based methods (233), it is often difficult to determine if a molecular result is a false positive when the reference culture method is negative. In some instances, a second molecular assay using a different gene target may be used to resolve discrepant results; however, it should have analytical sensitivity equal to (or better than) that of the first assay (220). Additionally, when the respiratory viral pathogen is present at a level close to the assay's limit of detection, discrepant results

due to Gaussian distribution effects can be observed (234). Finally, sampling error can affect the results of comparative studies if two separate swabs or collection protocols are utilized.

Labor and cost of molecular assays. The use of molecular approaches has traditionally been accompanied by higher supply costs than for antigen- or culture-based methods (235); however, modern molecular technologies provide improved performance characteristics compared to culture and/or DFA/IFA (197). Automation and integrated molecular test platforms can provide labor savings to the laboratory to offset increased reagent and platform costs (236) and may also decrease downstream costs for the health care system by providing more rapid and accurate results. Incorporation of molecular assays has resulted in variable patient management outcomes depending on studies, with some studies showing positive effects and other studies showing no effect, as identified in a recent review by one of the authors of this article (237). Negative effects on patient management have not been identified. Positive effects on patient management include decreased patient isolation times (238), length of stay (LOS) (239), administration of antibiotics and oseltamivir (240), and duration of antibiotic therapy (241).

Understanding Applications of Molecular Detection Approaches

Limited role of viral loads in predicting patient outcomes. A growing body of evidence shows a correlation of respiratory viral load and patient outcome. In one study of immunocompetent adult patients, age and hospitalization time were associated with earlier reverse transcriptase PCR (RT-PCR) cycle threshold (C_T) values for FLUA/B of $\leq$20 than later C_T values (242). Association of viral load and outcome can also vary by genotype, as RV-A viral loads were higher in patients with severe disease than in patients without severe disease, while no difference in viral load was observed for patient groups infected with RV-C (243). Furthermore, increased fatalities in adult CAP patients were associated with sustained viremia and high viral loads of ADVs in sputum and tracheal aspirates (244).

However, current laboratory practices generally report qualitative results for a respiratory virus NAAT, rather than determining a true viral load. The currently available laboratory-developed viral load assays have multiple problems, including the lack of an international standard, lack of standardized technology, and lack of consensus on specimen types (245). Additionally, the timing of specimen collection can influence viral load results. In fact, viral load samples taken on day 3 postonset may have a stronger association with clinical outcome than samples taken on day 0, 1, or 2 (246). Given the viral load data described in this section, the viral shedding data (described above), and the impact of age, immune status, and/or coinfection with other respiratory viruses (134), additional studies are needed to determine when viral loads are appropriate in different patient populations and how to appropriately interpret the results. Due to sampling errors, time of collection, patient age, etc., viral loads may not be comparable from one patient to another. In the future, possible roles for these viral load assays may include monitoring an individual patient over time to assess for viral clearance or response to antiviral therapy.

Molecular panel testing for respiratory viruses. (i) Defining multiplex assays. Multiplexing of molecular assays was traditionally restricted by the number of targets that could be efficiently amplified within a single reaction vessel (247–249). The earliest approaches were often batch-based assays that relied on a single nucleic acid extraction followed by one or more molecular assays. Often, panels of multiple individual targets or small multiplexes with 1 to 3 targets could overcome some of the inefficacies of massively multiplexed reactions (250); however, development of new technologies with improved multiplexing capabilities has allowed detection of multiple virus targets from a single sample (251–253).

(ii) Recommendations for patient populations in which multiplexed respiratory viral panel testing may be appropriate. Testing requirements may vary depending on the patient setting and resources, as the costs of the multiplex assays are high. The

most appropriate patients to test may vary depending on the health care setting, as some studies show questionable utility in testing adult outpatients for viruses other than FLU (254), Instead, for FLU patients who meet ILI criteria and are at high risk for complications, a highly accurate rapid test may have the greatest utility. Others have shown that multiplexed viral panels can directly influence antibiotic utilization practices (241).

Hematology and oncology patients may be appropriate patient populations for testing. The Infectious Diseases Working Party of the German Society for Haematology and Medical Oncology identified community-acquired respiratory virus infection as a significant cause of morbidity and mortality in oncology patients (103). Infectious viral etiologies were widely varied and included both single and mixed infections. For example, RSV infection has a high likelihood of progressing to a lower respiratory tract infection (30%) and a high chance of mortality (27%) in oncology patients. Therefore, testing for FLU, RSV, PIV, and other prevalent community-circulating viruses in all oncology patients presenting with symptoms (103) is suggested.

Transplant patients may also be an appropriate patient population for multiplex testing. Given the poor predictive value of the U.S. CDC's ILI criteria not only in adult transplant patients but in general, some authors have suggested an increased role for the use of multiplex respiratory NAAT assays in adult transplant patients with suspected respiratory virus infection (32). In lung transplant patients, identification of mixed viral infections using a multiplex panel could be used as a predictor of poor outcome (e.g., biopsy-proven rejection or sustained decline in forced expiratory volume [FEV1]) (255). In lung transplant patients, the detection of one or more viruses using a respiratory virus panel in a BAL fluid sample during the first year after transplant has also been associated with significantly faster development of bronchiolitis obliterans syndrome (BOS) (256).

Intensive care unit (ICU) patients may be another appropriate patient population for respiratory viral multiplex panel testing. In a recent review, respiratory viruses such as FLU, RSV, and RVs were suggested to cause immunosuppression in ICU patients (257). Given the clinical severity of illness in patients in the ICU, they are good candidates for respiratory virus panel testing. Appropriate identification of the severity of patient illness as well as the patient location (including the ICU) within the health care facility can often be challenging for the laboratory. Therefore, identification of critically ill patients with suspected pneumonia has previously been used as a selection criterion in the absence of accurate hospital location data (258).

Pediatric patients with an underlying illness may also be an appropriate patient population for respiratory viral panel testing. Panel testing may allow for identification of pathogens associated with specific risks in pediatric patients. This may include increased risks for asthma and wheezing in critically ill patients (259) or a lack of FEV1 improvement in pediatric cystic fibrosis patients (260).

(iii) To multiplex or not to multiplex? A variety of commercial and FDA-cleared *in vitro* diagnostic tools are currently available. Incorporation of these highly multiplexed assays into the laboratory significantly decreases turnaround time compared to that when performing all assays individually (252). Additionally, ease of use is improved with many assays giving "sample-to-answer" detection of respiratory viruses. Multiplex assays often have excellent performance characteristics, allowing clinicians to be confident with test results and make informed clinical decisions with concrete patient and health system benefits. Compared to complex algorithms involving multiple ordering of tests for small numbers of viral targets (e.g., FLU, FLU/RSV, EV, and RV alone), multiplex panels used as a routine test ordering choice can remove some of the confusion or indecision described by clinicians when ordering tests for smaller numbers of viral targets individually (261, 262). However, given that these panels are expensive, demonstration that the results impact patient care help justify the increased cost to the laboratory. A variety of studies have looked at indirect benefits of multiplexed panel testing; however, the identified outcomes are not consistent between studies. In patients 3 months to 21 years old, panel use has been associated with decreased length

of stay (LOS) in emergency departments and inpatient wards (241). Identification of a viral etiology has also shown improvements in hospital isolation resource use, which can be removed as appropriate and targeted only to patients who require isolation. Compared to other methods, multiplex panels can decrease the amount of antibiotic and antiviral use, and they may be used to appropriately triage patients in acute care settings (239, 263, 264). A significant decrease in the duration of antibiotic use and the number of chest radiographs was observed in an adult tertiary care center when rapid multiplexed panels were used compared to traditional antigen detection and older molecular methods (239). Adult outpatient outcomes were assessed at a Connecticut VA Center that used an on-demand respiratory panel. Outpatients were divided into those with FLU detected, those with a non-FLU virus detected, and those with no pathogen detected. Antibacterial prescription rates did not vary between groups; however, there was a statistically significant difference between antiviral prescription rates: the FLU-positive group was more likely to be treated with an antiviral agent (80/105 [81%] treated) than were patients in the non-influenza virus pathogen group (6/109 [5.5%]) and the no-pathogen-detected group (2/81 [2.5%]) ($P < 0.001$) (254). Respiratory panel use allows for more comprehensive characterization of viruses for general epidemiology/surveillance (15, 265) and outbreak investigation. Other, less tangible but important, benefits to respiratory viral panel use may also include improved patient and physician satisfaction with an improved test turnaround time.

Multiplexed respiratory virus panels may have significant costs for implementation, and some may have significant costs to operate. Health care administrators need to be made aware of the indirect and direct benefits of panels and how cost savings may be generated through improved workflow practices and lower labor costs (266, 267). Laboratorians and clinicians may need to reassess how clinical utility studies are undertaken and consider group efforts to undertake well-controlled and standardized studies (264).

(a) Multiplexing and the utility of identifying mixed infections. Multiplexing of molecular assays can facilitate identification of mixed viral infections (268–270). Coinfections are defined as the detection of more than one virus in a patient specimen. The rate of coinfection will depend on the particular virus, the methodology used for detection, the patient population demographics, and the geographic location of the study (271). However, understanding the impact of coinfections on patient outcomes is challenging, particularly when molecular tools are used for diagnostics. Nonviable virus from a remote infection or virus not associated with the current infection may be detected by molecular methods. Important considerations include (i) whether identification of mixed infections leads to a better understanding of patient prognosis, (ii) whether identification of mixed infections leads to changes in patient management, (iii) whether identification of mixed infections leads to changes in infection prevention and control practices, and (iv) whether the increased identification of viruses not routinely identified in nonmultiplex panels allow for placing patients in cohorts based on etiology during isolation.

In some cases, coinfections may make up a significant proportion of total viral cases within a population. In one recent study, coinfections with bacteria and viruses were identified in 40% of viral respiratory tract infections requiring hospitalization (272). For example, in Japan, a recent study found that 43.8% of patients who were diagnosed with a CoV infection were also infected with an additional virus (273). In another study, coinfections of two or more viruses were identified in approximately 18% of infants with an acute respiratory illness; RV was the most common coinfecting virus, but other viruses, such ADV, hMPV, and PIVs, were also codetected (270). Thus, the impact of mixed infection on patient outcomes is still under debate. Some studies show no difference in patient outcomes when coinfections are compared to single virus-infections, even in highly immunocompromised patient populations (268). Additionally, studies in immunocompetent children with lower respiratory tract infection found that RSV coinfection with any other respiratory virus was not associated with more severe disease than RSV infection alone (274). Conversely, other studies show that coinfection

with RSV and a second virus in infants with lower respiratory tract infections is associated with increased length of stay (LOS) (275). In another study, an increased risk of life-threatening disease (e.g., intensive care unit [ICU] admission, need for mechanical ventilation, or death) was identified in patients with ADV-RSV coinfections compared to RSV single-virus infections. In a secondary outcome analysis, FLU-RSV coinfections had an increase in LOS compared to RSV single-virus infections (274), while ADV coinfections were more likely to be associated with the need to treat with supplemental oxygen than were ADV single-virus infections (276). Furthermore, in cases of community-acquired pneumonia, viral-bacterial infection has been associated with a more complicated course (e.g., hospital death or mechanical ventilation for >7 days) than infections with bacteria alone, viruses alone, or no identified etiology (277).

(b) Commercially available molecular test panels may not fulfill all testing needs. A major drawback of multiplexed panels is the inability to differentiate closely related viruses or to detect all targets with equivalent sensitivity, and some targets on commercial multiplex panels continue to be detected more efficiently by singleplex assays (278) (also see comments on emerging pathogens below). In one study, detection of RSVA and FLUA had decreased sensitivity in panel tests compared to that with singleplex NAAT (279). Likewise, detection of ADV in multiplex panels often has decreased sensitivity compared to that with in-house NAAT assays (280), particularly for ADV group E (279). Of note, only respiratory species of ADV (B, C, and E) will be detected in multiplex panels, while nonrespiratory ADV species (A, D, and F) will be missed. In commercial panels, the proprietary nature of primers and probes does not allow investigation for detection of emerging viral pathogens, which may be missed by commercial assays (281).

Another limitation in some available assays is the inability to distinguish EVs from RVs. This can lead to secondary laboratory differentiation algorithms to characterize infection (282), and this is compounded by the limited ability to detect emerging EV strains (278). For example, enterovirus D68 may require altered patient management compared to seasonal EV strains, as it is associated with extrapulmonary syndromes such as acute flaccid paralysis (282). Additionally, detection of nonrespiratory ADV in the respiratory tract can precede systemic infection in immunocompromised children (283). Unfortunately, there is currently no practical gold standard to determine whether ADV detection in the respiratory tract is causal or incidental (284).

(iv) Near-patient or POC tests. As highlighted above, CLIA-waived tests are intended for use in "professional" settings (e.g., physicians' offices, mobile clinics, and pharmacies) and/or by untrained operators with no laboratory expertise (200). A summary of NAAT assays that can be used as point-of-care (POC) or near-point-of-care tests is in Table 4. Technologies for these guidelines are discussed in general here; specific products are not discussed, and company names are not mentioned.

The availability of newly developed CLIA-waived NAAT assays which detect FLUA/B or both FLUA/B and RSV is increasing. Multiple assays are now emerging in the marketplace and may have similar test characteristics (285); users should consult up-to-date resources for a list of waived products. Users should note that in general, reverse transcriptase PCR technologies may have higher sensitivities than isothermal assays (286–289).

Benefits of near-patient NAAT assays include ease of use and reduced process steps compared to those with older molecular assays, software that allows for easier result interpretation, and closed systems to reduce contamination (286–289). Drawbacks of near-patient NAAT assays include the potential to cause unforeseen strain on the laboratory (e.g., for confirmatory testing and quality assurance program support), the impact on resource utilization outside central laboratories, and the limited scope of specimen types that can be used (290–292).

A recent review of POC testing, including NAAT, identifies several barriers to understanding the benefits of point-of-care testing for respiratory viruses (237). Implementation of rapid nucleic acid testing could be associated with decreases in number of hospital admissions, length of stay, emergency department length of stay, duration

of antimicrobial use, droplet contact days, total isolation days, and receipt of antibiotics (238–241).

Appropriate Test Utilization in the Era of Molecular Testing

Respiratory virus testing algorithms vary between health care institutions. Resources, types of laboratory facilities, and different patient populations (to name a few) may all play a role in the testing algorithm chosen. Choosing Wisely is a campaign started in 2012 that focuses on initiating discussions with both the patient and physicians about unnecessary procedures, treatments, and tests (293). This section focuses on Choosing Wisely and discusses (i) which testing options might be suitable to perform depending on needs, (ii) what laboratories can do when resources are limited, (iii) how the importance of preanalytics plays into the testing decision being made, and (iv) what additional considerations need to be discussed up front before any test or piece of equipment is adopted by the laboratory or health care environment. The following sections describe key steps in ensuring that health care workers choose respiratory tests appropriately.

Stakeholder engagement. To provide high-quality, cost-effective laboratory services, it is imperative to understand the clinical needs of the end users when considering solutions for detection of respiratory viruses (294). Depending on the health care system, the laboratory may be asked to offer testing within the main laboratory or to play a role in determining the best test for near-patient testing. Because diagnostic needs vary, it is important to identify the right stakeholders at the beginning in order to determine appropriate process development and assay deployment.

Stakeholder discussion should include the needs of primary care providers, characteristics of the patient population, clinical practice settings, required test turnaround time, availability and expertise of nonlaboratory staff to perform POC testing, the volume of testing, and potential outcomes of a new assay/process. Physician groups utilizing testing are broad and may include the emergency department, inpatient/ICU, infection prevention and control groups, and pediatric and adult outpatient services such as urgent care or family practice. The laboratory, along with infectious diseases physicians, should engage these providers to completely understand the provider/patient need.

In order to choose wisely for respiratory virus testing, one must have a fundamental understanding of the needs of the organization. Early engagement with the provider and operational stakeholders (departmental administrators or managers overseeing specimen collection and/or testing) is paramount to successful test implementation. It is crucial for an institution to consider and understand the potential clinical and financial impact of a diagnostic test. Some decisions may be made based on outcome data in the literature or data that are internally generated (263, 295–304). Outcomes can include (but are not limited to) cost, TAT, infection prevention and control decisions, antibiotic administration, antiviral administration, inpatient LOS, rates of admission to the hospital, referrals, and ancillary testing (chest radiography or other laboratory testing) (299, 302). A positive or defined outcome not only demonstrates the utility of a specific test but can also be presented to administrators to support the proposal. Many institutions today are implementing test algorithm changes in part due to evidence-based medicine and outcome data.

A PubMed search for the terms “respiratory,” “virus,” “testing,” “utilization,” and “compliance” found no articles related to utilization and compliance for respiratory virus testing; however, we have identified a need for monitoring usage after implementing algorithms to ensure compliance and appropriate utilization of tests by the ordering health care workers.

Choosing the right test. As evidenced by the diversity of institutional provider groups discussed above, a single solution might not work for all patient populations or specialties of care. In choosing wisely, regardless of the test or the ability to be reimbursed, the emphasis should be on what the provider will do with the result and how implementation will impact the clinical outcome, the quality of care given

to the patient (e.g., reduction in unnecessary antibiotic use or duration) or the institution (e.g., reduced length of stay [LOS] in the hospital). Because many laboratories are being asked to do more with less, it is incumbent on not just the laboratory personnel, but all health care professionals, to spend money wisely and show the impact of testing that is implemented. Quality of care is also improved when physicians understand how to best use a result from a laboratory test. In many electronic medical records (EMR), decision trees can be adopted to aid in appropriate test selection, and tests can be restricted by patient location (e.g., inpatient versus outpatient) to promote effective ordering habits. As fee-for-service models are replaced with integrated care delivery systems, test reimbursement becomes less of a driver for best practices for respiratory virus testing. For example, laboratorians should consider the importance of providing influenza A virus subtype data when using/considering molecular assays, as some FDA-cleared tests do not provide the subtype. In some settings, clinicians may not voice concerns about lacking subtype information. An argument against subtyping is that subtyping matters only when circulating subtypes have different patterns of resistance to antiflu drugs. In other settings, clinicians may use subtyping data to place patients in cohorts in health care settings with low bed-to-patient ratios.

As described above, many providers have historically relied on RADTs, culture, or direct fluorescent-antibody (DFA) testing for the detection of respiratory viruses. RADTs have still maintained their popularity because of their rapidity even though they are suboptimal in regard to sensitivity (209, 305). Over the last decade, the use of NAATs with relatively faster sample-to-answer times has replaced that of more traditional methods (306). Sample-to-answer methods with TATs of ~1 h may be acceptable for hospitalized patients, or perhaps patients in the ED, but TATs exceed those required in outpatient setting. More recently, FDA-cleared and CLIA-waived NAATs with sensitivities and specificities comparable to those of FDA-cleared laboratory-based molecular tests have become available (307).

Complex multiplex PCR assays are often restricted to hospital settings and reserved for the most ill patients with associated comorbidities. Diagnosis of respiratory illness in this setting is deemed important to the physician even though treatment might not be available for a specific pathogen. The infection prevention and control needs of a health care institution may warrant the implementation of multiplexed testing to appropriately place patients with similar infections in cohorts when bed space is restricted. These multiplex assays can be further divided into random-access and batched testing platforms (306). Both routine and unplanned-for laboratory needs may require the laboratorian to consider utilizing both batched testing and random-access test systems. Random-access platforms are suggested for daily use in laboratories with low to medium specimen volumes, with the benefit of a rapid turnaround time and simplified workflow. As test volumes increase, the laboratorian may reconsider test algorithms and utilize a batched testing platform (308). Some algorithms may improve cost-effectiveness by offering a less-expensive upfront singleplex assay for FLU or duplexed or triplexed assays, including FLU and RSV, and using multiplex panels only if the sample is negative for FLU; however, algorithms will vary by institution, time of year, and prevalence of influenza. Furthermore, algorithms should be chosen based on stakeholder engagement and the individual testing needs of the patient population.

So, how is this made operational? We have provided a risk assessment flow chart in Fig. 2. We realize that a single approach will not be applicable to all laboratories. Therefore, laboratorians should work with their clinical partners and manufacturers to establish risk-based algorithms which can be used to determine the appropriateness of testing. Test ordering systems, clinical information, and patient location, as well as demographic identifiers, can be used to streamline the placing of specimens into appropriate test algorithms (e.g., no testing, testing for limited targets, or broad panel testing). Laboratorians should offer clinicians the opportunity to discuss cases that do

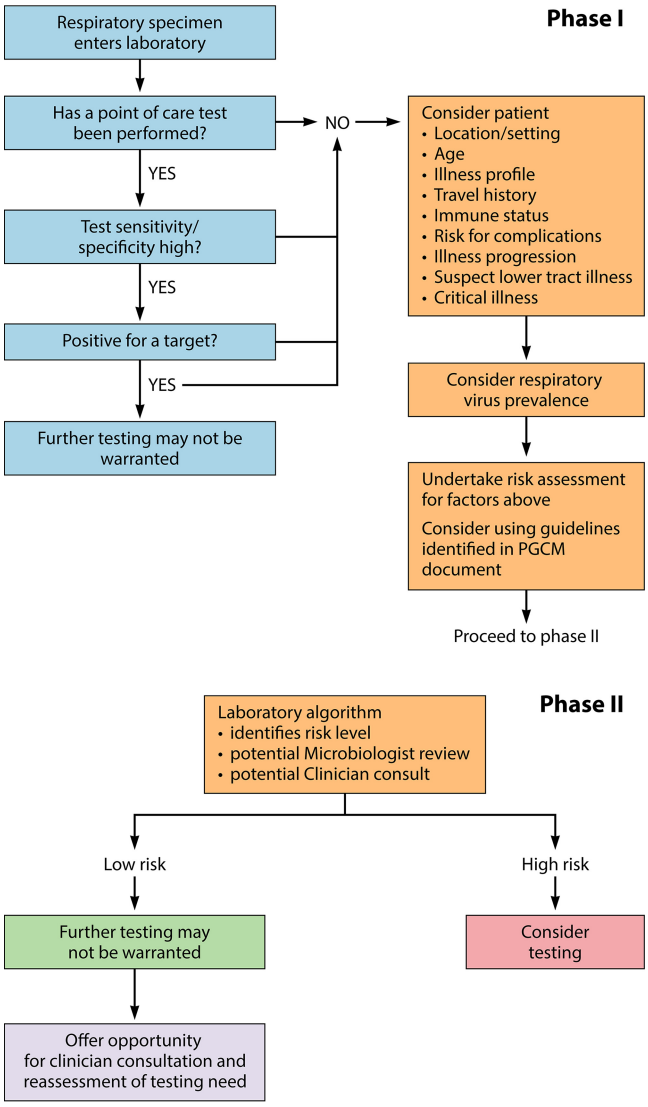

FIG 2 A risk assessment approach to determine populations most effectively served by acute respiratory virus testing. The decision-making model can be used to identify the level of test complexity for patient populations.

not fit into general risk groups (e.g., low versus high), where patients may benefit from specific laboratory tests.

Recent Issues Surrounding LDTs for the Diagnosis of Acute Respiratory Viral Infections

LDTs may find a role in the clinical laboratory under the following scenarios: where commercial assays are not available, when performance issues emerge with commercial respiratory virus assays, or when a new assay is required immediately (e.g., in the event of an emerging respiratory pathogen) (309). LDTs are defined as assays developed and performed by high-complexity laboratories (e.g., "home brew" or "in-house" assays) that are "intended for clinical use" (310). Draft guidance documents surrounding LDT use were released in 2014 by the FDA, which provide guidance for clinical laboratories, industry, and drug administration staff (310). As of 2016 to 2017, the FDA proposed a "risk-based, phased-in approach, in combination with continued exercise of enforcement discretion for certain regulatory requirement and certain types of LDTs"; however, it is up to the individual laboratory to calculate the risk associated with the use of LDTs (311). These issues are not specific to the United States (312). This proposed framework

would place each LDT into a specific risk class (305), and laboratories in other countries may benefit from comparing how they and their U.S. colleagues perform risk assessments (313).

Section Summary and Recommendations

Older methods such as rapid antigen detection techniques, DFA tests, and viral culture have essentially been replaced by more rapid and sensitive NAAT assays, which have improved the characteristics of laboratory tests for the diagnosis of acute respiratory viral infections. However, the highly sensitive nature of these tests as well as the possibility for molecular contamination means that laboratorians need to develop processes and practices to prevent molecular contamination. Laboratorians should understand the risks and benefits of using LDTs and potential regulations restricting their use. Rapid POC NAATs are allowing for the rapid detection of multiple respiratory pathogens compared to routine laboratory NAATs. Multiplexed NAATs, including POC tests, now allow for rapid detection with faster turnaround times (TATs) and sensitivity and specificity equivalent to those of laboratory-based NAATs. Apart from patient management for FLU, the patient and system benefits of multiplexed NAATs require further study, and current study outcomes may be confounded by multiple factors. Laboratorians should consider strong utilization approaches when initiating supporting NAAT POC test and multiplexed NAAT implementation. Laboratory utilization discussions should take into account the clinical utility of testing in specific patient populations. Finally, although NAATs are the primary method of detection, laboratorians should coordinate testing in a reference laboratory that undertakes viral culture techniques to allow for phenotypic influenza virus characterization and/or antiviral susceptibility testing as part of ongoing public health surveillance.

ANTIVIRAL AND PROPHYLACTIC AGENTS: IMPACT ON THE CLINICAL LABORATORY

RSV Prophylaxis and Antiviral Agents

The use of palivizumab (314) has been described above. Laboratory diagnosis of RSV has no direct impact on the decision-making on when to initiate palivizumab prophylaxis, but general laboratory testing trends may help in the determination of when the RSV season starts and ends in some locations (95).

Although the use of multiple agents to treat respiratory viral infections has been described, the number of antiviral agents with FDA approval is limited. For treatment of RSV infection, the only approved agent is ribavirin (in aerosolized form). The use of aerosolized ribavirin can pose health hazards to health care workers and is not easy to deliver to patients, making it a less-than-ideal treatment choice. The 2012 *Report of the Committee on Infectious Diseases* (*Red Book*) focuses on pediatric infections and indicates that primary treatment for RSV is supportive. The *Red Book* does not recommend the routine use of ribavirin but does indicate that use may be considered in "selected patients with documented, potentially life-threatening RSV infections" (315). Research-focused approaches regarding RSV mutations is not described further here; however, potential mutations driving resistance against palivizumab and issues with ribavirin are described in a recent review (215). A comprehensive review of the effectiveness of antivirals for these viruses is beyond the scope of this guidance document, but there are emerging data supporting the use of oral ribavirin in treatment of URTI and LRTI in stem cell transplant patients (102, 103, 316–318). As new antiviral agents for RSV (and other viruses) become approved, laboratorians may need to develop processes for systematic antiviral resistance testing and surveillance.

Treatment and Prevention of Influenza

FLU is the only respiratory virus discussed in these guidelines that currently has a vaccine available for prevention (315). Clinical laboratories should work with their public health laboratories to ensure that appropriate FLU characterization by culture and molecular methods occurs. Culture may still be required for phenotypic strain

typing as well as antiviral susceptibility testing as part of studies or national surveillance systems. These data may also help support decision-making regarding FLU vaccine effectiveness (41).

Currently licensed antivirals for influenza include the adamantanes, which block the activity of the M2 protein (active only against FLUA), and neuraminidase (NA) inhibitors (NAIs), which block the activity of the NAs of influenza A and B viruses. At the time of this publication, NAIs are the only drugs that are effective for the prevention or treatment for influenza. Adamantanes, which do not have activity against FLUBs, are no longer effective against seasonal FLUA (319). Two NA inhibitors, oral oseltamivir and inhaled zanamivir, are licensed in many countries. In addition, intravenous peramivir is licensed in Japan, China, South Korea, Canada, and the United States. A fourth drug of this class, long-acting inhaled laninamivir, is licensed in Japan. Similar to the case for M2 blocker-resistant viruses, viruses resistant to an NA inhibitor(s) may gain an evolutionary advantage and spread beyond countries employing NA inhibitor therapy. In 2007 to 2009, oseltamivir-resistant A(H1N1) seasonal prepandemic viruses rapidly emerged and spread globally (320, 321). In contrast, influenza A H1N1 (pdm09) virus strains are almost universally susceptibility to oseltamivir and zanamivir (322). Continuous antiviral susceptibility testing of seasonal FLU viruses is imperative to identify and track the emergence and spread of viruses resistant to NA inhibitors and M2 blockers.

Relevance of FLU Antiviral Resistance Testing

Guidelines from the Community Network of Reference Laboratories for Human Influenza in Europe suggest that testing for antiviral resistance is typically indicated in the following instances: (i) in patients lacking virological improvement (persistent virus shedding after 5 days of treatment using ab NAAT that "delivers semi-quantitative information" [e.g., a real-time PCR with a C_T value]), (ii) in patients treated with antivirals with severe FLU who do not clinically improve (time frame not given), (iii) in fatal cases where an understanding of resistance may influence prophylaxis of contacts, (iv) in cases of FLU developed during or after antiviral prophylaxis, and (v) in contacts of antiviral-treated FLU patients who developed respiratory symptoms or in contacts of FLU patients for whom the presence of resistant virus had been confirmed (323). One group that may benefit from antiviral testing is patients who shed virus for long periods of time and who do not improve after treatment (e.g., highly immunocompromised patients) (324, 325).

As molecular markers of resistance are not well established and vary depending on virus type/subtype and NA inhibitor, determination of antiviral resistance should be carried out in a reference laboratory with experience in these techniques (326). Documents created by the WHO's Global Influenza Surveillance and Response System (GISRS) and the WHO Influenza Antiviral Working Group (WHO-AVWG) can assist in the interpretation of these results (327, 328). Other documents may be available from other committees which provide guidance on the use of influenza antivirals (329).

Section Summary and Recommendations

Laboratorians should identify a reference laboratory for the characterization of influenza and antiviral susceptibility testing. Antiviral testing is not a routine test, and the time required to undertake such testing limits the clinical relevance of this testing in most patient populations. Antiviral testing may be required for epidemiological studies as well as cases of failure in prophylaxis. One patient population that may benefit from this testing is patients who are highly immunocompromised who do not clinically improve following antiviral treatment and who may shed virus for an extended period of time.

CODING AND REIMBURSEMENT

This section was introduced into the guidance document following presentation of these guidelines in the draft from at an ASM general meeting. Current procedural

terminology (CPT) is a set of guidelines, codes, and descriptions used to elucidate and standardize services by health care professionals, including testing in the clinical laboratory. The CPT codes for microbiology and virology are established through the Pathology Coding Caucus (PCC) of the American Medical Association (AMA). CPT codes in microbiology and virology have a 5-digit identifier with a description of the target and procedure (e.g., 87,633, CPT code in the category "infectious agent detection by nucleic acid [DNA or RNA]"). New codes are published yearly. Inclusion of a code in the CPT manual does not imply endorsement of the test, nor does it cover insurance or reimbursement policies.

In general, when a new test that needs a code is available, a proposal for coding is presented to the PCC. Among the criteria used by the PCC to review the request are test methodology definition, the volume of test utilization, the medical necessity, and scientific publications detailing performance and outcomes studies for the new test. After each caucus meeting, a document entitled "CPT Editorial Summary of Panel Actions" is prepared, which summarizes the actions that were taken by the panel on each of the code applications.

Pricing/fee setting for a CPT code is the purview of the Centers for Medicare and Medicaid Services (CMS). Annually, the CMS holds the Clinical Laboratory Fee Schedule (CLFS) meeting at its headquarters in Baltimore, MD. Stakeholders present the code(s) (as established by the PCC) and a proposed reimbursement amount (based on an existing rate or as a recommend new rate based on a comprehensive cost analysis). The CMS Advisory Panel on Clinical Laboratory Diagnostic Tests functions to establish payment rates based on crosswalking or gapfilling and establishes factors used for determining coverage and payment processes (330).

Per the CMS (331), crosswalking occurs when a new test (or substantially revised test) is determined to be similar to multiple existing test codes, portions of an existing test code, or an existing test code. Gapfilling occurs when there is no existing comparable test available (331).

As of 2017, reimbursement compliance is a system in place to ensure that the testing being performed is medically relevant for the clinical situation. Here, appropriate testing for specific clinical conditions and clinical outcomes is critical. The issue of medical relevance has been raised in virology recently in regard to multiplex respiratory virus and gastrointestinal panels. In brief, CPT code 87,633, defined as respiratory virus (e.g., ADV, FLU, CoV, hMPV, PIV, or RV), includes multiple NAAT reactions, and multiplex NAAT panels with target numbers (including types or subtypes) ranging from 12 to 25 targets. The medical necessity and reimbursement for these multiplex assays have been challenged, and Medicare and Medicare administrative contractor (MACs) alerted providers that a "broad-net" or "one-size-fits-all" panel contributes to test overutilization and increased health care costs without specific benefit to a given patient. They assert that testing should be limited to organisms with the greatest likelihood of occurrence in a given patient population and, if results are negative, to provide reflexive testing to more "exotic" organisms.

A consortium of clinical organizations whose members represent testing laboratories has submitted comments directly to MACs, recommending a thorough review of this issue. At the time of this writing, only a partial resolution has occurred (as per verbal communication by one of the authors).

Payment rates continue to be under scrutiny and have been discussed during implementation of the Protecting Access to Medicare Act (PAMA). This statute calls for a market-based fee schedule based on a weighted median of individual private payor test reimbursements reported by "applicable laboratories," which by specific requirements excluded hospital laboratories. Applicable laboratories included 45% of all commercial laboratories and 5% of physician office laboratories. As such, the data for reimbursement are heavily weighted by discounted pricing by large commercial entities to major payors (MACs). Beginning in January 2018, the intention was for price reductions to be implemented at 10% in each of the next 3 years, followed by a 15% reduction for the following 3 years, until the established

weighted median price is hit. These new fees were to be applied to all who are paid using the CLFS. Of note, concerned organizations and individuals have contacted CMS about the detrimental effect of the act and the predicted closure of many laboratories and the impact on patient care. The status of these new fees was in question as of January 2018 (332).

Section Summary and Recommendations

Laboratorians should be aware of reimbursements for existing and new diagnostics for respiratory in their locations.

CONCLUSIONS

This is the most recent update of ASM practice guidelines for clinical microbiology, addressing changes to acute respiratory viral infection diagnostics since the previous document, which was published in 1986. Since that time, laboratory practices as well as clinical practices have changed extensively. The guidelines were developed for the laboratory diagnosis of viruses causing acute respiratory illness, with technologies ranging from low- to high-complexity testing. Respiratory virus testing may be considered if a diagnosis has impact on patient management, especially when FLU treatment decisions are based on test results or in immunocompromised patients. In general, testing immunocompetent patients will not impact patient management. However, testing may be undertaken for surveillance in sentinel labs, to guide infection control decisions/practices, or when highly pathogenic emerging pathogens are suspected.

The landscape of respiratory virus testing has significantly changed in the last 30 years. The decreased use of older technologies such as viral culture and direct antigen detection represents a significant programmatic change in the diagnosis of respiratory viruses. Many front-line clinical laboratories have completely phased out viral culture, and testing such as strain typing and antiviral resistance testing is generally limited to reference laboratories. Molecular techniques are now the preferred diagnostic approaches for the detection of acute respiratory viruses and are more amenable to automation and high-throughput workflows. Good molecular laboratory practices and quality assurance programs are keys to preventing laboratory contamination. The decreasing complexity of platforms used for molecular testing has expanded the geographic capacity of these assays, which can now be placed closer to patients as POC tests, while newer technologies have made multitarget panels widely available. For novel and emerging respiratory viruses, laboratory-developed tests will still be required to compensate for testing gaps that often need to be filled quickly. With all the advances in technology, however, effective communication between clinicians and the laboratory is still essential to quickly identify highly transmissible emerging pathogens and reduce health care worker exposure. Laboratorians should work closely within their teams as well as with other clinicians and public health practitioners to ensure that health systems are prepared for the inevitable emergence of new respiratory viral pathogens.

Implementation of clinically relevant testing algorithms can ensure optimized patient care and improve laboratory resource management. Particularly, strong preanalytical screening approaches can facilitate appropriate specimen collection and direct providers to correctly order diagnostic tests as needed. Laboratorians should ensure that they continue to work with their public health reference laboratory colleagues to align processes to enable continued virus characterization and antiviral resistance testing.

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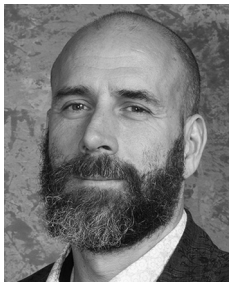
Christine C. Ginocchio, Ph.D., MT(ASCP), is Vice President, Medical Affairs, bioMérieux, Vice President, Scientific and Medical Affairs, BioFire Diagnostics, and Professor of Medicine, Northwell School of Medicine, New York. She has 40 years of experience in all phases of laboratory management and clinical diagnostics. She has been the principal investigator for more than 60 diagnostic industry and pharmaceutical clinical trials, including 23 studies of *in vitro* diagnostic devices for U.S. FDA clearance. Her areas of extramural funded research included HIV, cytomegalovirus, respiratory viruses, human papillomavirus, antibiotic resistance, and molecular diagnostics for infectious diseases. Her awards include the President's Award and Irving Abrahams Award for outstanding basic science research, the PASCV 2012 award in diagnostic virology, and an ASM 2013 BD award for research in clinical microbiology. She is co-editor-in-chief for the *Journal of Clinical Virology*, a section editor for the 10th, 11th, and 12th editions of the *Manual of Clinical Microbiology*, and on the editorial board for *Clinical Microbiology Reviews*.



Todd F. Hatchette, M.D., F.R.C.P.C., completed his M.D. and internal medicine residency at Memorial University of Newfoundland. After completing residency training in infectious diseases and medical microbiology at Dalhousie University, Dr. Hatchette completed postdoctoral research training in virology at St. Jude Children's Research Hospital in Memphis, TN. Dr. Hatchette is the Chief of Service for the Division of Microbiology, Department of Pathology and Laboratory Medicine, in the Nova Scotia Health Authority's Central Zone and a Professor in the Department of Pathology with cross-appointments in the Departments of Immunology and Microbiology and Medicine, where he is a consultant in infectious diseases. Dr. Hatchette has expertise in the clinical and laboratory diagnosis of viral infections and serves as an advisor on a number of local, provincial, and national committees. He is the President of the Association of Medical Microbiology and Infectious Disease, Canada.

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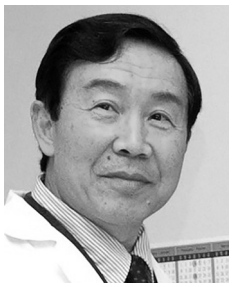
Robert C. Jerris, Ph.D., D(ABMM), is Medical Director, Microbiology, at Children's Healthcare of Atlanta and Adjunct Professor at Emory University School of Medicine. He completed his Ph.D. in experimental pathology at Emory University in 1981 and a postdoctoral fellowship at the Centers for Disease Control and Prevention. He has concentrated his research career in rapid methods (molecular and matrix-assisted laser desorption ionization–time of flight [MALDI-TOF]) for microbial identification and antimicrobial resistance. Dr. Jerris is the founding father of the South Eastern Association for Clinical Microbiology, has served for over 30 years in numerous positions with the American Society for Microbiology, and currently is the Chair of the ASM Committee on Professional Affairs.



Yan Li joined the National Microbiology Laboratory (NML) of the Public Health Agency of Canada in September 1998. He is the Chief of the Influenza and Respiratory Viruses Section of NML. He received his Ph.D. from the University of Ottawa in 1992. As the head of the WHO National Influenza Center in Canada, Dr. Li takes a leadership role in planning, developing, and maintaining a comprehensive national surveillance programs for influenza virus and other respiratory pathogens. He provides ongoing scientific advice and technical expertise to stakeholders in influenza surveillance and diagnostics. He has 187 peer-reviewed publications.

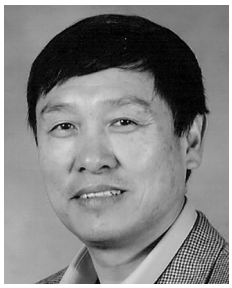
Mike Loeffelholz earned a Ph.D. in microbiology from Ohio University in 1987 and completed a postdoctoral fellowship in medical and public health microbiology at the University of Rochester in 1990. Dr. Loeffelholz is currently a Professor in the Department of Pathology and Director of the Clinical Microbiology Laboratory and the American Society for Microbiology CPEP-accredited Medical Microbiology Fellowship program at the University of Texas Medical Branch at Galveston. Dr. Loeffelholz has over 80 peer-reviewed publications and book chapters. He is editor-in-chief of the *Clinical Virology Manual*, 5th edition, and an editor of the *Journal of Clinical Microbiology*. Dr. Loeffelholz has served on a number of committees at the national level, including the ASM Committee on Professional Affairs, the CDC Board of Scientific Counselors/Office of Infectious Diseases, and the Association of Public Health Laboratories board of directors. He is a diplomate of the American Board of Medical Microbiology (ABMM).

Yvette S. McCarter, Ph.D., D(ABMM), is a Professor of Pathology and Laboratory Medicine at the University of Florida College of Medicine-Jacksonville and Director of the Clinical Microbiology Laboratory at UF Health Jacksonville. Dr. McCarter received her Ph.D. in pathology from the Medical College of Virginia and completed a postdoctoral fellowship in medical and public health microbiology at Hartford Hospital under the mentorship of Dr. Raymond C. Bartlett and Dr. Ann Robinson. She is a diplomate of the American Board of Medical Microbiology. Dr. McCarter recently completed a term as Chair of the American Board of Medical Microbiology. She is the microbiology associate editor for *Lab Medicine* and currently serves on the American Society for Clinical Pathology LabQ editorial board and Workshops and On-Demand Webcasts Committee. Her research interests include utilization controls in the clinical microbiology laboratory, cost-effective laboratory medicine, and evaluation of new diagnostic tests.

Melissa B. Miller, Ph.D., D(ABMM), F(AAM), is a Professor of Pathology and Laboratory Medicine at the University of North Carolina at Chapel Hill School of Medicine. She has been the Director of the Clinical Mycobacteriology, Mycology, and Molecular Microbiology Laboratories and Associate Director of the Clinical Microbiology-Immunology Laboratory at UNC Medical Center since 2004. Dr. Miller received her Ph.D. in molecular biology from Princeton University and completed the Medical and Public Health Microbiology Fellowship at UNC. She has a long-standing interest is respiratory viral diagnosis, including assessing new technologies, determining appropriate testing algorithms, and assessing impact on patient outcomes. As a past member and chair of the ASM Committee on Laboratory Practices and current chair of the ASM Professional Practice Committee, Dr. Miller has a keen interest in establishing best practices and advocating for standardization and dissemination of these practices.

Susan Novak-Weekley, Ph.D., S(M)A.S.C.P., D(ABMM), is the Vice President of Medical Affairs at Qvella. Dr. Novak-Weekley received her B.S. in microbiology at Colorado State University and her Ph.D. in microbiology at the University of Arizona. She completed a postdoctoral fellowship in clinical microbiology at the University of California, Los Angeles, Medical Center and Wadsworth VA Medical Center in Los Angeles, CA. Dr. Novak-Weekley is currently a member of the American Society for Microbiology (ASM) *Journal of Clinical Microbiology* editorial board. Dr. Novak-Weekley is currently the Councilor for the Southern California American Society for Microbiology (SCASM), in addition to serving as annual meeting and fundraising cochair. Additionally, she is a member of the Committee on Microbial Sciences (COMS) for ASM and also serves on the Nominating Committee for ASM and the New Technologist Mentoring Committee (CMMC).

Audrey N. Schuetz, M.D., M.P.H., D(ABMM), is an Associate Professor of Laboratory Medicine and Pathology at the Mayo Clinic School of Medicine and Science in Rochester, MN. She received her M.D. and completed a pathology residency and medical microbiology fellowship at Emory University School of Medicine. She is board certified in clinical pathology, anatomic pathology, and medical microbiology through the American Board of Pathology and is board certified by the American Board of Medical Microbiology. Dr. Schuetz is Director of Initial Processing and Media Laboratories and Co-Director of Bacteriology in the Division of Clinical Microbiology at Mayo. She is a member of the Expert Panel of Microbiology of the Clinical and Laboratory Standards Institute. Her interests include infectious diseases pathology and evaluation of rapid diagnostic techniques to improve antimicrobial stewardship.



Yi-Wei Tang is currently the Chief of the Clinical Microbiology Service at the Memorial Sloan-Kettering Cancer Center and a Professor of Pathology and Laboratory Medicine at the Weill Medical College of Cornell University in New York, NY, USA. He obtained his medical training from Fudan University Shanghai School of Medicine and Ph.D. in microbiology and immunology from Vanderbilt University. He has been engaged in medical and molecular microbiology translational research, aimed at developing and evaluating new and advanced microbiological diagnostic testing procedures. Dr. Tang is a Fellow of the American Academy for Microbiology and of the Infectious Disease Society of America. Dr. Tang has been recognized for his extraordinary expertise in molecular microbiology, diagnosis, and monitoring and has over 200 peer-reviewed articles and book chapters in this field.

Ray Widen is the Scientific Director, Esoteric Testing and Research, Tampa General Hospital. He has more than 34 years of clinical microbiology experience, 28 years of flow cytometry research experience, and over 24 years of molecular diagnostics assay development and validation expertise. Dr. Widen has an extensive background in applications in leukemia/lymphoma diagnostics as well as infectious disease diagnostics for viral, bacterial, and fungal targets.

Steven J. Drews, Ph.D., F.C.C.M., D(ABMM), is a clinical virologist at the Provincial Laboratory for Public Health (ProvLab), Alberta, Canada. He completed his Ph.D. in experimental medicine (infectious diseases) in 2003 at the University of British Columbia. He then completed his clinical microbiology fellowship at the University of Toronto. Since then, he has focused on both the clinical microbiology and research aspects of respiratory infections and has been involved in respiratory virus surveillance and preparedness planning at a national level in Canada. Dr. Drews currently heads the province-wide influenza and acute respiratory viral diagnostics program at ProvLab, Alberta. Dr. Drews has held faculty positions at both the University of Toronto and the University of Calgary. He is currently an Associate Professor in Laboratory Medicine and Pathology at the University of Alberta, Edmonton, Canada, and the outgoing President of the Canadian College of Microbiologists.