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What Is the Role of Newer Molecular Tests in the Management of CAP?

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KEYWORDS

- Community-acquired pneumonia • Diagnosis • Influenza • Respiratory viruses
- Respiratory bacteria • PCR • Point-of-care tests

KEY POINTS

- The successful management CAP requires rapid and accurate diagnosis of the etiologic agent of CAP.
- Correct diagnosis enhances the appropriate use of antiviral agents and prevents overuse of antibiotics.
- Pathogen-directed therapy requires the use of an assay that is FDA-cleared, is highly accurate, and can be completed in a timely manner.
- New tools are becoming available and include assays that are highly accurate, especially ones that use amplified technology.

INTRODUCTION

Community-acquired pneumonia (CAP) is one of the most important infectious disease problems in the United States today and accounts for major morbidity and mortality.¹ There were approximately 4.5 million ambulatory care visits in 2007 in the United States¹ and an estimated 1.1 million hospitalizations for pneumonia, with an average length of stay of 5 days.² CAP accounts for enormous health care costs, with an estimated \$17 billion price tag annually in the United States.³

Unfortunately, in the past 20 to 25 years, with improved broad-spectrum antibiotics, the implementation of diagnostic studies has declined and most patients do not have an etiologic pathogen of CAP identified.^{1,4,5} This has been partially because of the advance of antimicrobial agents to treat CAP, resulting in a lack of the perceived need to know the etiologic pathogen, unless the patient does not respond to empiric therapy.¹ An important consideration of the diagnosis of CAP is the age and immune

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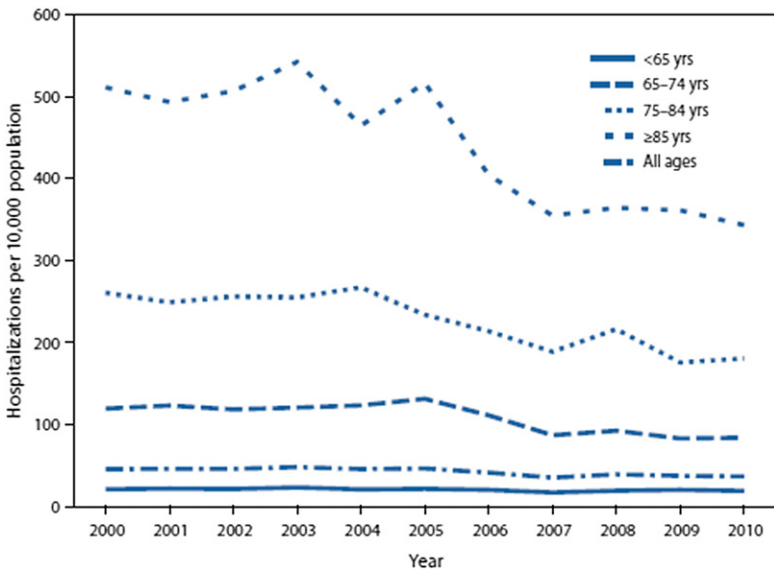
Infect Dis Clin N Am 27 (2013) 49–69
<http://dx.doi.org/10.1016/j.idc.2012.11.012>

id.theclinics.com

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state of the patient. Severe and fatal disease can occur in the elderly, the immunocompromised, the very young, and those individuals with conditions that affect cardiopulmonary function, and these syndromes can be caused by multiple types of organisms, which may be etiologically indistinguishable on presentation.⁶ The elderly represent an important factor in rapid diagnosis of CAP especially because this older generation is increasing in the United States. The Centers for Disease Control and Prevention (CDC) statistics indicate that, although pneumonia rates have been decreasing, hospitalization for the age group older than 85 years still represents the most vulnerable population (**Fig. 1**).⁷ From 2000 to 2010, the hospitalization rate for pneumonia per 100,000 population decreased by 20% for the total population. The rate decreased 30% among those aged 65 to 74 years, 31% among those aged 75 to 84 years, and 33% among those aged 85 years and older. However, throughout the period, the rate of hospitalization for 85 years and older was the highest, whereas the younger than 65 years age group was substantially lower than the rate for any other age group. Thus, accurate and rapid diagnosis of CAP is important for appropriate patient management, especially in the elderly.

The diagnosis of the etiologic agent of CAP depends on the use of rapid assays. Sensitive, specific, and rapid identification of viruses and bacteria that cause CAP



Per 10,000 population. Hospitalization for pneumonia is defined as a first-listed diagnosis on the medical record of 480-486, as coded according to the International Classification of Diseases, Ninth Revision, Clinical Modification. Rates were calculated using U.S. Census Bureau 2000-based postcensal civilian population estimates. Source: National Hospital Discharge Survey, 2000–2010. Available at <http://www.cdc.gov/nchs/nhds.htm>.

From 2000 to 2010, the hospitalization rate for pneumonia decreased by 20% for the total population. The rate decreased 30% among those aged 65–74 years, 31% among those aged 75–84 years, and 33% among those aged ≥85 years. Throughout the period, the rate of hospitalization for the <65 years age group was substantially lower than the rate for any other age group.

Fig. 1. Rate* of hospitalization for pneumonia, by age group. National hospital discharge survey, United States, 2000–2010. * - Rate per 10,000 population.

can enhance the appropriate use of antiviral agents and prevent overuse of antibiotics. Thus, being able to diagnose respiratory viruses and bacterial pathogens is an integral part of patient management today in the era of cost containment and antibiotic-sparing paradigms for CAP and other respiratory infections that are not of a bacterial origin. Several new molecular platforms exist today for identification of respiratory viruses, and some new broad-based platforms are on the horizon for the diagnosis of viruses, bacteria, and fungi.^{8,9} Although accurate diagnosis is highly desirable, perhaps more important is the rapid diagnosis of an etiologic agent of CAP, which can affect immediate patient management. Such rapid identification of a viral agent can prevent the use of unnecessary antibiotics; reduce costs; facilitate timely, effective use of antiviral drugs for viral etiologies, such as influenza; and ultimately may shorten hospital stays.

Because a variety of viruses and bacteria can be responsible for CAP, having a platform that can accurately identify multiple agents is ideal. Additionally, having a single point-of-care (POC) molecular assay that can rapidly rule in or rule out the most common agents, such as influenza or *Streptococcus pneumoniae*, is also desirable for the immediate management of CAP.

The Diagnostic Problem

The real question for optimal and rapid patient care, however, is whether the etiologic agent of CAP is bacterial or viral, and if it is bacterial, whether it is gram-positive or gram-negative. Answers in real time can direct agent-specific therapy and avoid overuse of precious broad-spectrum antibiotics. Polymerase chain reaction (PCR) and molecular tests, which have been developed for the past 20 years and have been coexisting with routine microbiology culture, have not been widely adopted, contributing little to rapid decision making for patient care, especially for bacteria, because many of them are laboratory-developed tests (LDTs), which are not Food and Drug Administration (FDA) cleared or commercially available. Urinary pathogen nonmolecular FDA-cleared diagnostic assays for *S pneumoniae* and *Legionella* are an exception, because their results are rapidly available.^{10,11}

With single-pathogen molecular tests, each test requires the suspicion of a particular pathogen, but with the development of multiple-pathogen molecular assays, particularly for viruses, the tools to advance pathogen-directed therapy may now exist.¹² This article focuses on recently developed molecular multiplex diagnostic assays and platforms, many of which are FDA-cleared, and how their use can enhance and improve the management of CAP. It provides an overview of the FDA-cleared rapid nonmolecular tests compared with the new rapid molecular tests for the diagnosis of influenza, other respiratory viruses, and bacteria, with less emphasis on those molecular tests that are not FDA-cleared, because they offer clinicians less acute medical management tools, except in research studies. Many of these FDA-cleared and noncleared assays have also been recently reviewed in great detail elsewhere.^{6,12-14}

Requirements of Molecular Assays that can Affect Patient Care

Advances in molecular diagnostic methods, which can improve management of CAP, dictate that the result be (1) rapid, (2) accurate, and (3) have the capability to detect multiple pathogens in one assay or a minimum of assays. Rapid POC assays are especially attractive and almost imperative because Infectious Diseases Society of America/American Thoracic Society guidelines recommend that it is very important that therapy for hospitalized patients be as soon as possible and that the first antibiotic dose be given in the emergency department (ED).^{15,16} These guidelines drive how rapidly a molecular test can be performed and returned to the clinician in the ED.

Even if a molecular test is not available in time for initial therapy, a definitive diagnosis of an etiologic agent by a molecular test can promote quickly switching to pathogen-directed antibiotic or antiviral therapy, while cultures still may not be completed.

Accuracy is imperative because this is required before FDA clearance can be achieved.¹⁷ FDA-cleared assays have a great advantage over LDTs because they have undergone extensive clinical and analytical validations, their performance characteristics are well documented, and the reagents are standardized. Their use engenders confidence in the quality assurance and accuracy of results.¹⁸

The ability to detect multiple pathogens simultaneously in one assay should have a major impact on management of CAP because the menu of etiologic agents has grown with newly recognized organisms and newly emerging agents, such as severe acute respiratory syndrome, human metapneumovirus (hMPV), *Bordetella pertussis*, and new variants of influenza. Even the combination of several rapid tests can have a major impact on the type of therapy instituted (narrow agent-directed antibiotic therapy, such as for *S pneumoniae*, in the case of a positive urinary antigen test, or antiviral therapy for the influenza virus as diagnosed by a POC rapid test).

Consideration of the Diagnostic Test

Historically, in addition to the traditional Gram stain and bacterial culture of sputum or nasopharyngeal swabs for the diagnosis of CAP, there are nonmolecular and molecular assays. Before the molecular assays, there was the development of several rapid nonmolecular methods called rapid antigen direct tests (RADTs) for the detection of influenza. Additionally, the nonmolecular assay, the direct fluorescent antibody (DFA) test using monoclonal antibodies on direct patient viral transport culture sediments, has been used for rapid identification of influenza and other viruses and is still popular. The molecular tests for influenza and viruses are used the most and are FDA-cleared and have been the subject of the most advancement in diagnoses, with the exception of several newer ones for *Mycobacterium tuberculosis*,^{19,20} including the recently FDA-cleared FilmArray (Idaho Technology, Salt Lake City, UT) for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *B pertussis*.²¹ There are many non-FDA cleared assays (LDTs), but their use has been mostly limited to research studies.^{22,23} For actual use for the management of CAP, nonmolecular and molecular FDA-cleared assays are the ones most valuable for patient care.

DIAGNOSTIC TESTS FOR VIRAL INFECTIONS

Nonmolecular, Nonamplified FDA-cleared Diagnostic POC Tests for Viral Respiratory Infections

RADTs for influenza are FDA-cleared. RADTs have highly variable sensitivities (10%–75%) and specificities (50%–100%) depending on the viral target, age of the patient, sample collection, and duration of symptoms before testing. In general, RADTs perform better when testing pediatric samples, because children shed higher titers of virus and for longer time periods than adults. RADTs also perform better during periods of high prevalence (influenza seasons). They did not perform well for the detection of pandemic swine H1N1 viruses.^{14,24–26} Chartrand and colleagues¹⁴ recently provided an excellent meta-analysis describing their performance from 159 studies and reported a pooled sensitivity of 62.3% and a specificity of 98.2%. Performance was better for influenza A (64.6%) than influenza B (52.2%). There are 14 FDA-cleared RADTs available commercially, of which five are Clinical Laboratory Improvement Amendments (CLIA) waived; two of these can detect and distinguish between influenza A and B.²⁶ Development of more CLIA-waived assays with

increased accuracy and better ability to accurately differentiate influenza types and subtypes will improve use and clinical management decisions in CAP.^{26,27}

Direct fluorescent antibody

Rapid DFA testing of centrifuged sediments nasopharyngeal swabs of viral transport media can readily detect seven of the common respiratory viruses (adenovirus; influenza A and B; parainfluenza virus [PIV] 1, 2, and 3; and respiratory syncytial virus [RSV]). In addition, DFA testing can detect hMPV. The specificity of DFA testing is high, but the sensitivities can vary from a low of 50% to 80% when compared with nucleic acid amplification tests (NAATs). Because DFA testing can be performed in as little as 30 to 60 minutes, its use is ideal for POC tests if the laboratory is located near the clinic ordering the test, but a skilled microscopist is required and thus use is limited to performance in a clinical laboratory. Although the seven viruses are responsible for a large number of respiratory tract infections, other viruses are also important causes of respiratory disease. These include bocavirus, selected coronaviruses (229E, OC43, NL63, and HKU-1), PIV 4, and rhinovirus. They are only detected using PCR or NAATs.²⁵

Types of DFA The Diagnostic Hybrid method (Diagnostic Hybrids, Athens, OH) consists of staining a cell pellet from a respiratory sample, such as a nasopharyngeal swab sample, in liquid transport media after centrifugation with a monoclonal antibody mixture, which is a pool of antibodies for seven viruses (influenza A and B, RSV, PIV 1 to 3, and adenovirus). Another cell spot is stained with a monoclonal for hMPV. If the pool stains positive by fluorescent microscopy, the cell pellet is stained individually with each monoclonal to determine which virus is staining in the pool. This is a very efficient, rapid (~2–4 hours), and cost-effective method to screen respiratory samples because about 90% are negative. The disadvantage is that a fluorescent microscope and a skilled microscopist are required. Sensitivity is approximately 80% to 85% and specificity is excellent in a skilled laboratory. An advantage is that all the negative samples can be screened in one pool with a single microscopic visual screen with the pool reagent. It is difficult to use as a POC test unless the laboratory is in very close proximity to the ED or clinic.

There is a liquid DFA test available called Fastpoint (Quidel, Athens, OH). It uses three monoclonal antibodies cocktails to detect eight viruses. This method requires reading three cell spots with two filters each for each sample. This takes longer than screening one pool. A disadvantage is that the PIV are not distinguished from each other. All PIV (1–3) are labeled with phycoerythrin.

FDA-Cleared Molecular Diagnostic Tests for Viral Respiratory Infections

Non-POC FDA-cleared molecular assays

Multiplex PCR assays There are five FDA-cleared multiplex real-time reverse transcriptase (RT) PCR assays (Gen-probe; Prodesse, Madison, WI) for the qualitative detection and identification of the respiratory viruses, influenza, hMPV, PIV, and adenoviruses (**Table 1**). These assays are intended for use in only CLIA high-complexity laboratories.

1. The ProFlu+ assay targets the matrix gene for influenza A, nonstructural genes NS-1 and NS-2 for influenza B, and the polymerase gene for RSV A and RSV B.
2. The ProFAST+ assay subtypes influenza A samples as H1N1-p, H1N1-s, or H3N2. It is a qualitative multiplex real-time RT-PCR assay that targets the nucleoprotein gene of 2009 influenza A H1N1, the specific hemagglutinin genes of seasonal influenza A/H1 and seasonal influenza A/H3, and an internal control (MS2 phage).

Table 1
Food and Drug Administration cleared molecular assays for the detection of respiratory viruses

Manufacturer and Test Name	Time	Test Method	Instrument	Specimen Type	Virus Target
Gen-Probe/Prodesse ProFlu+	3–3.5 h	Real-time RT-PCR	Cepheid SmartCycler	Nasopharyngeal swab in VTM	RSV, influenza A, influenza B
Gen-Probe/Prodesse Pro hMPV	4.5–5.5 h	Real-time RT-PCR	Cepheid SmartCycler	Nasopharyngeal swab in VTM	hMPV
Gen-Probe/Prodesse ProParaFlu+ and Adenovirus+	4.5–5.5 h	Real-time RT-PCR	Cepheid SmartCycler	Nasopharyngeal swab in VTM	PIV 1–3 Adenovirus A-F
Luminex xTAG Respiratory Virus Panel	8–10 h	RT-PCR, TSPE, bead hybridization	Luminex xMap 100/200	Nasopharyngeal swab in VTM	Influenza A, influenza B, hMPV, RSV, PIV 1–3, adenovirus, rhinovirus
Nanosphere Verigene	3–3.5 h	RT-PCR, gold nanoparticle hybridization	Verigene Processor, Verigene Reader	Nasopharyngeal swab in VTM	Influenza A, influenza B, RSV
FilmArray Idaho Technology	1 h	Nested PCR, melt curve analysis	FilmArray Instrument	Nasopharyngeal swab in VTM	Adenoviruses, bocaviruses, coronaviruses, influenza A/B, A subtypes, hMPV, PIV 1–4, RSV, rhinoviruses

Abbreviations: hMPV, human metapneumovirus; PIV 1–3, parainfluenza virus types 1, 2, and 3; RSV, respiratory syncytial virus; RT-PCR, reverse-transcription polymerase chain reaction; VTM, viral transport medium.

3. The Pro hMPV+ assay targets highly conserved regions of the nucleocapsid (N) gene for hMPV and a transcript derived from *Escherichia coli* bacteriophage MS2 A-protein gene (internal control).
4. The ProParaflo+ assay targets conserved regions of the hemagglutinin-neuraminidase gene for PIV 1 to 3 and a transcript derived from *E coli* bacteriophage MS2 A-protein gene (internal control).
5. ProAdeno+ assay targets the hexon gene and detects adenovirus subtypes A to F.

All five assays are approved for testing nasopharyngeal swab specimens obtained from symptomatic persons. Nucleic acids are extracted using a MagNA Pure LC Instrument (Roche Diagnostics, Indianapolis, Indiana) and the MagNA Pure Total Nucleic Acid Isolation Kit (Roche) or a NucliSENS easyMAG System and the Automated Magnetic Extraction Reagents (bioMérieux, Marcy, France). The purified nucleic acids are amplified by means of RT-PCR using target-specific oligonucleotide primers and Taqman probes complementary to highly conserved regions of the target gene. During each PCR cycle, the fluorescent intensity is monitored by the real-time instrument, the SmartCycler II (Cepheid, Sunnyvale CA).

The time to results, including extraction, is 3 to 3.5 hours for each test run. ProFlu+ sensitivities and specificities for the detection of influenza A were 100% and 92.6%, respectively; for influenza B they were 97.8% and 98.6%, respectively; and for RSV they were 89.5% and 94.9%, respectively.²⁵ In pediatric nasopharyngeal samples, results were 97% to 100% for ProFlu-1 and it detected respiratory viruses in 9% of specimens that were negative by conventional methods.²⁸ It has been compared with the FilmArray Respiratory Panel.²¹

The Pro hMPV+ assay was shown to be 94.1% sensitive and 99.3% specific compared with xTAG (Luminex Molecular Diagnostics, Austin, TX) respiratory virus panel (RVP) assay.²⁵ The Pro hMPV+ assay sensitivities were demonstrated to range from 88.9%, 96.3%, and 97.3% for PIV 1, PIV 2, and PIV 3, respectively, during clinical trials and the specificities were all higher than 99%.²⁵

xTAG RVP The xTAG RVP was the first multiplex NAAT to receive clearance by the FDA, in January 2009. The original FDA-approved version of RVP detected adenovirus, influenza A (with subtyping of seasonal influenza A/H1 and seasonal influenza A/H3), influenza B, PIV 1 to 3, hMPV, rhinovirus, RSV A and B, and was approved for use with nasopharyngeal swabs from symptomatic persons.^{29,30}

Currently, the RVP is an FDA-approved, multiplexed RT-PCR assay manufactured by Luminex (Toronto, Canada) for the detection of 12 viral targets in a single specimen: influenza A; influenza A, subtype H1; influenza A, subtype H3; influenza B; RSV A and B; PIV 1 to 3; hMPV; rhinovirus; and adenovirus. It is FDA-cleared for nasopharyngeal swabs, nasal washes, or bronchoalveolar lavage samples. It requires roughly 8 to 10 hours processing and running the assay. There is a second-generation assay called RVP FAST (Luminex) only available in Europe (not FDA-cleared in the United States) that reduces the number of steps and the time to results by 3 to 4 hours. This assay is intended for use in only CLIA high-complexity laboratories. It has been shown to be highly sensitive and specific for respiratory viruses.³¹ It has been compared with the FilmArray respiratory panel.³²

Respiratory virus plus, Verigene Respiratory Virus Nucleic Acid Test The first-generation Verigene Respiratory Virus Nucleic Acid Test (Nanosphere, Northbrook, IL) was cleared by the FDA in May 2009. This test was replaced by the automated Verigene Respiratory Virus Nucleic Acid Test_{SP}, a CLIA moderately complex test microarray-based sample-to-result that is FDA-cleared for the identification of influenza A, influenza

B (types A, Flu An H1, Flu A-2009 H1N1 Flu A-H3, and B inclusive) and RSV (A and B) from nasopharyngeal swab specimens placed in viral transport media. The Verigene System consists of two instruments (the fully automated Verigene Processor and the Verigene Reader) and single-use test cartridges, random access work flow. The entire test process only requires one user pipetting step, less than 5 minutes of technical hands-on time, and a sample-to-result turnaround time of about 2.5 hours. Test sensitivities and specificities for the detection of influenza A were 100% and 99.8%, respectively; for influenza B they were 100% and 99.1%, respectively; and for RSV they were 95.7% and 98.2%, respectively. This assay is CLIA moderate complexity, and a trained laboratory technician must perform the test. It is adequate for small- to medium-sized laboratories. Limitations of the assay include the single-test format that requires a dedicated processor for each sample for 3 to 3.5 hours.³³ The H275Y mutation for oseltamivir resistance panel is only available outside the United States.

Amplified molecular diagnostic POC tests for viral respiratory infections

The ideal influenza POC diagnostic would of necessity combine the sensitivity and specificity of RT-PCR (which are very high complexity, require experienced and highly skilled staff, and are batched, lowering clinical use) with the rapidity and simplicity of the rapid antigen test.

There are only two FDA-cleared amplified molecular assays that can be considered true POC, in that they each require approximately 1 hour to perform and thus could potentially be used for initial patient management decisions in concert with other diagnostic tests. Although FilmArray can test for 15 viruses and several bacterial pathogens, the GeneXpert System only detects influenza virus.

FilmArray technology The FilmArray system combines nucleic acid extraction, nested PCR detection, and data analysis in a single-use pouch. The automated system enables the detection of numerous viral and bacterial respiratory pathogens in a single test. The required reagents for the assay are enclosed in the single-use pouch. Water is added to hydrate the lyophilized reagents and the respiratory specimen is added. The pouch is loaded into the FilmArray instrument, and the remainder of the test is completely automated. After extraction of nucleic acid, a nested PCR reaction is performed within the pouch in an entirely closed system. The first-step PCR is a multiplexed reaction containing primers for all of the viral and bacterial targets; the amplicons from the first PCR are then diluted, and a second round of PCR reactions is performed in a multiwell array, each well containing a single primer set targeting a specific pathogen. Both amplification and melt curve analysis allow the FilmArray software to generate a result for each target.³⁴ The system is very robust, detecting a low concentration of pathogen in the presence of a high concentration of a second pathogen, with results available in 1 hour. The respiratory panel detects 15 respiratory viruses and *B pertussis*, *C pneumoniae*, and *M pneumoniae*. Originally only the viral panel was FDA-cleared, but the bacterial targets were cleared in the summer of 2012.

Several evaluations of the FilmArray platform for viral pathogens have been published. The FDA-cleared version includes the following targets: adenovirus; coronaviruses HKU1 and NL63; hMPV; influenza A virus (to type level only); influenza An H1 seasonal virus; influenza An H3 seasonal virus; influenza A virus H1-2009; influenza B virus; PIV 1 to 4; RSV; and rhinovirus/enterovirus (no differentiation).

Loeffelholz and colleagues²¹ compared the assay with Prodesse ProFlu+, ProFAST+, ProParafly+, Pro hMPV+, and ProAdeno+ real-time PCR assays on 192 nasopharyngeal-secretion specimens collected from 81 children younger than 1 year of age with upper respiratory tract symptoms. FilmArray and Prodesse assays

showed good overall agreement (181 [94.3%] of 192; kappa = 0.87; 95% confidence interval [CI], 0.79–0.94). FilmArray RP detected more PIV 1 and 3 than ProParaflu+ (18 vs 13), whereas ProAdeno+ detected more adenoviruses (11 vs 6), but these differences were not statistically significant. FilmArray and Prodesse assays showed good overall agreement (181 [94.3%] of 192; kappa = 0.87; 95% CI, 0.79–0.94). FilmArray RP detected more PIV 1 and 3 than ProParaflu+ (18 vs 13), whereas ProAdeno+ detected more adenoviruses (11 vs 6), but these differences were not statistically significant. Additionally, FilmArray RP detected 138 pathogens (confirmed as true-positives) not included in the Prodesse assays (rhinovirus/enterovirus, 118; bocavirus, 8; coronavirus, 7; PIV 4; *M pneumoniae*, 1).

When compared with laboratory-developed real-time PCR assays for the detection of various respiratory viruses and certain bacterial pathogens on a total of 215 frozen archived pediatric respiratory specimens previously characterized as either negative or positive for one or more pathogens by real-time PCR examined using the FilmArray RP system, the overall agreement between the FilmArray RP and corresponding real-time PCR assays for shared analytes was 98.6% (kappa = 0.92; 95% CI, 0.89–0.94).³⁵

Rand and colleagues³² compared FilmArray RP and the xTAG RVP multiplex respiratory virus PCR methods for the detection of respiratory viruses in a set of 200 patient specimens frozen at -70°C after standard viral culture and antigen detection methods had been performed. Both systems detected between 40% and 50% more viruses than traditional methods, which were mostly rhinoviruses and hMPV. The FilmArray RP detected significantly more total viruses either alone or as part of mixed infections than the xTAG RVP, and an additional 21.6% more RSVs. The xTAG RVP required 5 to 6 hours with 2.5 to 3 hours of hands-on time, whereas the FilmArray RP took about an hour with 3 to 5 minutes of hands-on time.

Fifteen viruses can be detected and differentiated including H1N1/2009/pdm influenza. Sensitivity ranged from 88.9% for adenovirus to 100% for many of the viruses with nearly perfect specificity. The ability to detect multiple pathogens in 1 hour with a simple set-up and fully automated system offers a significant benefit. It is user friendly, with about 3 to 5 minutes of labor involved, and could eventually allow POC testing in the ED or an outpatient clinic by minimally trained health care workers especially if the assay becomes CLIA waived. The time of the assay is 1 hour test time, which is a great advance.

The test is limited to a single patient test per run. Thus, its low throughput could be a significant drawback for laboratories receiving large numbers of specimens, because only a single sample can be processed at a time with one instrument.

GeneXpert system The Cepheid GeneXpert System platform for influenza is an on-demand molecular diagnostics assay that is a fully integrated system. The rapid, sophisticated genetic testing for organisms and genetic-based diseases automates otherwise complex manual laboratory procedures. The easy-to-use system integrates several complicated and time-intensive steps, including sample preparation, DNA amplification, and detection, which enable the analysis of influenza samples in a single proprietary test cartridge. It is a closed, self-contained, cartridge-based, random access assay for performing nucleic acid extraction, PCR amplification, and real-time detection of PCR products with no intermediate sample-handling steps. It is an automated platform that represents a paradigm shift in the automation of molecular analysis, producing accurate results in a timely manner with minimal risk of contamination.

The assay was first released under the FDA Emergency Use Authorization Rule during the 2009 pandemic for detection of influenza A, including 2009 H1N1 pdm.

The GeneXpert Emergency Use Authorization assay demonstrated 91% sensitivity for influenza A compared with the CDC rRT-PCR assay.³⁶

Compared with the Luminex xTAG Respiratory Virus rRT-PCR Panel, the GeneXpert was 91.2% sensitive for detection of influenza A; additionally, it demonstrated a 92.1% sensitivity for the detection of 2009 H1N1 compared with the Focus Diagnostics Influenza A/H1N1 (2009) rRT-PCR assay.³⁷

A newer version of the Xpert Flu (second generation) was developed subsequently with an identification for 2009 H1N1 pdm. This assay cartridge demonstrated 93% overall sensitivity for detection of influenza A and B in one study and 100% and 80.8% sensitivity for 88 influenza A and B, respectively, in another when compared with laboratory-developed rRT-PCR assays.³⁸ Tested against a laboratory-developed rRT-PCR assay Xpert Flu showed a sensitivity of 78.8% for influenza A and 76.5% for influenza B.³⁹ In contrast, the second-generation Xpert Flu test showed excellent sensitivities (98.1% and 93.8% sensitivity for influenza A and B, respectively) compared with commercially available and FDA-cleared Gen-Probe ProFlu+ rRT-PCR.⁴⁰

DiMaio and colleagues⁴¹ compared the X-pert Flu rapid nucleic acid testing assay with other rapid POC antigen tests for the diagnosis of influenza A. Influenza A sensitivity was 97.3% for Xpert Flu, 95.9% for DFA testing, 62.2% for BinaxNOW, and 71.6% for BD Directigen. Influenza B sensitivity was 100% for Xpert Flu and DFA testing, 54.5% for BinaxNOW, and 48.5% for BD Directigen. Specificity for influenza A was 100% for Xpert Flu, BinaxNOW, and BD Directigen, and 99.2% for DFA testing. All methods demonstrated 100% specificity for influenza B (**Table 2**).

Test performance uses three easy steps: (1) transfer 300 μ l of prepared sample into the large cartridge opening, (2) dispense elution reagent into the small opening, and (3) insert cartridge and start assay. The advantage is that it is FDA-cleared and can meet the requirements for an accurate POC test for influenza for a POC assay for a clinic or ED or out-patient setting. The POC assay could eventually allow POC testing in clinics by minimally trained health care workers, if the assay becomes CLIA waived. Pandemic 2009 influenza A virus and H3N2 (including H2N2v), seasonal H1N1, and influenza B can be identified. One, or two, four 16, samples, can be run simultaneously; or 64 samples can be run if larger random access equipment is used. The test time is approximately 1 hour. The disadvantage is that only influenza A and B are identified and differentiated. No other viruses are identified.

The GeneXpert System combines on-board sample preparation with real-time PCR amplification and detection functions for fully integrated and automated nucleic acid

Table 2
Gen Xpert Fu amplified assay compared with rapid influenza tests

	Influenza A % Sensitivity	Influenza A % Specificity	Influenza B % Sensitivity	Influenza B % Specificity
Xpert Flu	97.3	100	100	100
Direct Fluorescent Antibody	95.9	99.2	100	100
Binax NOW	62.2	100	54.5	100
BD Directogen	71.6	100	48.5	100

Sensitivity and specificity were calculated using rRT-PCR as the comparator standard.

Data from DiMaio MA, Sahoo MK, Waggomer J, et al. Comparison of Xpert Flu rapid nucleic acid testing with rapid antigen testing for the diagnosis of influenza A and B. *J Virol Methods* 2012; 86(1–2):137–40.

analysis. The system is designed to purify, concentrate, detect, and identify targeted nucleic acid sequences thereby delivering answers directly from unprocessed samples. Modular in design, the GeneXpert System has a variety of configurations from a single test format to a random access 64-test sample robot to meet the broad range of testing demands of any clinical environment. More studies are required to determine its use in various testing algorithms, and the cost-effectiveness of allowing a more rapid, accurate diagnosis and treatment of influenza-infected patients at the POC setting.

Non-FDA-cleared Molecular Diagnostic Tests for Viral Respiratory Infections

This field is rapidly emerging and mostly involves assays that are multiplexed. These assays were recently reviewed in detail by Caliendo.⁴² They may soon achieve FDA clearance and should be evaluated for their advantages on the management of CAP.

Multiplex PCR-electrospray ionization mass spectrometry platform for viruses

A novel molecular approach to the identification of nearly all human respiratory viral pathogens directly from clinical patient specimens, PCR-electrospray ionization mass spectrometry (Ibis Biosciences, Carlsbad, CA; Abbott Molecular, DesPlains, IL) has the potential to revolutionize diagnostic virology, but is not yet FDA-cleared.⁴³ The platform involves the nucleic acid extraction, rt-PCR, or PCR using sets of primers targeting highly conserved genes that flank variable genomic regions, and then followed by injection of amplicons into an electrospray ionization time-of-flight mass spectrometer, where the mass and number of each nucleic is measured. An excellent review of the literature that discusses the advantages and limitations of the relatively new platform has been published.¹² Thus far, several publications point to its usability for identification of a broad range of viral respiratory pathogens.⁴⁴⁻⁴⁶ Several formats are available, in addition to the one for common respiratory viruses, including specifically for rapid influenza typing of all H and N types.⁴⁷⁻⁵¹ This platform was instrumental in identifying and characterizing the H1N1 2009 influenza strain.⁵² FDA clearance may be coming soon for influenza typing.

ResPlex technology

ResPlex Technology (QIAplex Technology; Qiagen, Gaithersburg, MD) uses PCR of extracted nucleic acid followed by a liquid-phase bead-based array technology.⁵³ There is now a second-generation of the assay called ResPlex II and it detects more than 15 viruses; it was compared with conventional virology and the PCR-electrospray ionization mass spectrometry assay in which both assays performed well, detecting more viruses than conventional virology.^{46,54} It is a highly complex assay with multiple steps including postamplification handling of samples, which can increase the potential for contamination.⁴²

Infiniti system

This system (AutoGenomics) uses multiplex RT-PCR primers for more than 20 viruses with automated microarray hybridization, requiring 3.5 to 4 hours for first result for 24 specimens.⁴² One evaluation comparing it to single real-time virus-specific demonstrated an overall concordance of 94.1%.⁵⁵

Jaguar system

This system (Handy Lab, acquired with Becton Dickinson Diagnostics, Becton Dickinson, Sparks, MD) uses automated nucleic acid extraction with real-time PCR for multiple respiratory pathogens, requiring 3.5 hours for 24 samples.⁴² For the detection of influenza A/B and RSV, it demonstrated sensitivities of 100%, 90%, and 100%, respectively.⁵⁶

MOLECULAR DIAGNOSTIC TESTS FOR BACTERIAL INFECTIONS

Although most of the advances in molecular diagnosis of CAP, such as PCR, have been in the area of viral infections, and there has been less development in assays for bacterial infections that are FDA-cleared and commercially available, there have been some outstanding ones, mostly for tuberculosis.¹⁹ However, in the area of non-FDA-cleared research studies, there have been many assays developed, especially for pneumococcus, and some are in use in laboratories, such as LDTs. However, the LDTs are not widely available and are less useful to practicing clinicians until they become commercially available.^{6,57}

FDA-Cleared Molecular Amplification Assays for Bacteria

The commercialization and FDA clearance of molecular amplified assays for bacteria is rapidly taking place and more of these assays will soon be available for the practicing clinician for the management of CAP.

Mycobacterium tuberculosis

One of the most outstanding success stories in molecular amplification for an important worldwide respiratory pathogen had been for mycobacteria. In view of the lack of poor sensitivity (45%–80%) of acid-fast smear results and expensive and complex culture methods, which can take 1 to 4 weeks for identification, the development of FDA-cleared molecular tests had played an important role in improved patient outcomes.²⁵

The CDC recommends that NAATs be used for at least one respiratory sample from patients suspected of having *M tuberculosis*.¹⁹ There are now several FDA-cleared assays to choose from: (1) Amplicor *Mycobacterium tuberculosis* Test (Amplicor; Roche Diagnostics)⁵⁸; (2) Amplified *Mycobacterium tuberculosis* Direct Test (Gen-Probe, San Diego, CA)⁵⁹; and (3) the Xpert MTB/RIF (Cepheid, Sunnyvale, CA), which detects *M tuberculosis* and rifampin resistance directly from clinical specimens in approximately 2 hours, allows nucleic acid extraction within the cartridge, has low complexity, and demonstrates high sensitivity compared with standard methodologies (Table 3).^{20,60,61}

Mycoplasma pneumoniae, Chlamydia pneumoniae, and Bordetella pertussis

M pneumoniae, *C pneumoniae*, and *B pertussis* have been recently FDA-cleared (FilmArray).²¹ Only one published article mentions the detection of one *M pneumoniae* organism in its evaluation of viral pathogens.²¹ It is expected that the FDA clearance for these organisms will greatly enhance the understanding of the role they each play in CAP in the near future (Table 4).

Staphylococcus aureus

Although *S aureus* was usually considered to be mostly a hospital-acquired cause of pneumonia, it is now recognized as a more common cause of CAP, especially methicillin-resistant *S aureus* (MRSA).⁶² The FDA has cleared several PCR tests for the detection of MRSA in nasal swab specimens; however, there are no cleared assays to detect MRSA or methicillin-sensitive *S aureus* as agents of CAP.⁶² Several POC assays that are cleared for screening using nasal/nares swabs have sensitivities from 95% to 100% sensitivity and provide results in less than 2 hours.^{63,64} It has also been evaluated in skin and soft tissue and blood cultures.⁶⁵

Gram-positive bacteria Verigene platform

The FDA cleared the Verigene platform (Verigene GP Blood Culture Nucleic Acid Test) in June 2012 for the identification of gram-positive bacteria from positive blood culture

Table 3

FDA-cleared assays for the detection of tuberculosis bacterial agents in community-acquired pneumonia and lower respiratory infections

Manufacturer and Test Name	Test Method	Instrument	Specimen Type	Gene Target	Turnaround Time
Gen-Probe Amplified <i>Mycobacterium tuberculosis</i> Direct Test	TMA, HPA	Gen-Probe Leader 50	AFB smear-positive and smear-negative respiratory specimens	<i>M tuberculosis</i> complex rRNA	2.5–3.5 h
Roche Amplicor <i>Mycobacterium tuberculosis</i> Direct Test	PCR, colorimetric detection	Thermocycler EIA reader	AFB smear-positive respiratory specimens	<i>M tuberculosis</i> complex DNA	6.5 h
Cepheid Xpert <i>Mycobacterium tuberculosis</i>	PCR	Cepheid SmartCycler	Respiratory specimens	<i>M tuberculosis</i> , rifampin resistance	<2 h

Abbreviations: AFB, acid-fast bacilli; EIA, enzyme immunoassay; HPA, hybridization protection assay; TMA, transcription mediated amplification.

Table 4

FDA-cleared assays for the detection of bacterial agents in community acquired pneumonia and lower respiratory infections

	Assay	Manufacturer	Date Cleared	Turnaround Time
<i>Chlamydia pneumoniae</i>	FilmArray	Idaho Technology	2012	1 h
<i>Mycoplasma pneumoniae</i>	FilmArray	Idaho Technology	2012	1 h
<i>Bordetella pertusis</i>	FilmArray	Idaho Technology	2012	1 h

bottles. The targets identified include staphylococci (including MRSA), streptococci, *Enterococcus* (including vancomycin-resistant *Enterococcus*), and *Listeria* spp.

Non-FDA-Cleared Molecular Assays for Bacteria

The advance in the development of NAATs, such as PCR, in the last two decades has offered a huge understanding of the diagnostic capability of such assays for the management of CAP. The development of research assays has paved the way for commercialization and FDA clearance rapidly taking place today.

Notably, these non-FDA cleared assays include the ones for atypical agents, such as *C pneumoniae*, *M pneumoniae*, and *Legionella pneumophila*.^{66–71}

Chlamydia pneumoniae

Multiple in-house nucleic acid amplification (PCR) methodologies have been published, but the literature has been confounded by lack of standardization and validation.^{72,73} The CDC workshop identified a few assays that were considered to be “validated” enough to be used for research studies; others have been developed and used.^{66,74–82}

The advantages of these assays are their sensitivity, decreased possibility of contamination, and ability to quantify DNA. A PCR assay has recently been used to identify an outbreak of CAP among navy SEALs.⁸³ More research using FDA-cleared assays is necessary to further understand the role that *C pneumoniae* has in CAP. An extensive study of real-time PCR in a CAP study tested 355 samples and compared them with nested PCR, and touchdown enzyme time-released PCR⁷⁹ demonstrated increased sensitivity compared with traditional PCR methods.⁸²

There has previously never been a commercially available NAAT assay developed, until recently (FilmArray). A LCx research-use-only PCR developed by Abbott Laboratories was used in a multicenter study for comparison with PCR results using in-house PCRs from five different laboratories, which performed very well but it was never taken to a trial.⁸⁴ Becton Dickinson performed a clinical trial for a strand displacement amplification assay (SDA), but it was not cleared by the FDA.

Mycoplasma pneumoniae

Many assays have been developed for the detection of *Mycoplasma* and have been reported to work very well, especially in outbreak situations.^{67,71,85} Becton Dickinson performed a clinical trial for SDA, but it was not cleared by the FDA. There has previously never been an FDA-cleared commercially available NAAT assay developed, until recently (FilmArray).

Legionella pneumophila

Several PCR assays have been published and are reviewed by Murdoch.⁶⁸ Becton Dickinson performed a clinical trial for SDA, which was cleared by the FDA, but never commercialized.

Streptococcus pneumoniae

Many research assays have been developed for the PCR amplification of the pneumococcus, but thus far none are FDA-cleared.^{22,86,87} One of the main problems for using these assays on sputa, nasopharyngeal swabs, and oropharyngeal swabs is how to interpret the result, because a very sensitive assay can also detect pneumococcal carriage and serious infection. The evolution of the noncleared PCR assays and the challenges in the interpretation of these assays are discussed in an excellent review by Blaschke.²³ Early assays targeted the pneumolysin gene, but showed rather poor sensitivity and specificity because of cross-reactivity with viridians streptococci.⁸⁸ A comparison of several genes as targets for PCR indicated that the autolysis (lyt) gene was the most specific target.⁸⁹ Sheppard and colleagues⁹⁰ demonstrated the specificity of this target in clinical samples. Progress is being made with regard to distinguishing carriage or colonization from pneumonia by detecting the colonization density using real-time PCR.⁹¹

Multiplex PCR-electrospray ionization mass spectrometry platform for bacteria

A novel approach to the identification of nearly all human bacterial pathogens directly from clinical patient specimens has the potential for revolutionize diagnostic bacteriology, but is not yet FDA-cleared. An excellent review of the literature that discusses the advantages and limitations of the relatively new platform, and that covers thousands of bacteria and does not require the designation of a specific agent for the test target, has been recently published.¹²

OTHER TESTS THAT ARE NOT DIAGNOSTIC

A newly recognized biomarker assay that is neither diagnostic nor molecular deserves mentioning in consideration of CAP, and this is the procalcitonin test.⁹² The blood level of procalcitonin is elevated in patients who have a bacterial infection, who have sepsis with a bacterial agent, or who are in shock.⁹² Although a thorough discussion is beyond the scope of this article, it needs to be mentioned as a new tool in the consideration of other rapid diagnostic tests in making decisions regarding the diagnosis of possible bacterial cause versus a viral cause of CAP. There is only one FDA-cleared assay at this time (Biomerieux), and it is a 20-minute assay, making it a valuable adjunct in clinical management of CAP when other rapid diagnostic tests are available. The potential use of this assay has been reviewed recently and clinicians will want to evaluate its use in future study of CAP.^{6,92,93}

Recommendations for Use of Molecular Assays Based on Present Evidence

For optimal patient care and management of CAP, clinicians require an accurate test that is available in real time, so that decisions about pathogen-directed antimicrobial therapy or antiviral therapy can be instituted rapidly. Antivirals should be prescribed when the etiologic agent is influenza.^{94,95} Presently, pathogen-directed therapy requires the use of an assay that is FDA-cleared, is highly accurate, and can be completed in a timely manner. The last 20 years have greatly advanced the molecular and nonmolecular diagnostic assays for routine care of patients with CAP, and the clinician now has assays that can meet these requirements. Some of these include:

- Rapid influenza tests that are highly accurate, especially ones that use amplified technology
- Rapid molecular tests for *M tuberculosis*, especially ones that can be completed in a few hours

- Accurate and rapid tests for most other common respiratory viruses that can cause CAP and lower respiratory infections
- Rapid assays that have the ability to accurately detect atypical pathogens, such as *M pneumoniae*, *C pneumoniae*, *Legionella* spp, and *B pertussis*

Future Molecular Studies Needed to Assist Clinicians in the Medical Management of CAP

More research is required before accurate and rapid assays are available, especially for the FDA clinical trials clearing them for routine use, and comparisons involving the newer POC molecular assays, which now have the potential to influence immediate management decisions for CAP. The Infectious Diseases Society of America has stated that “Better, rapid molecular diagnostic tests are an unmet need for respiratory tract infections.”⁵⁷ FDA-cleared assays are especially needed for the rapid diagnosis of *S pneumoniae*. More research is required to ascertain the ability of rapid molecular identification of genetic markers to predict antibiotic and antiviral resistance, which may be the next generation of new assays. More research is needed to define the role of highly multiplexed assays that can identify hundreds of organisms to know how they can be implemented, especially in light of cost-containment issues in medicine. Lastly, clinicians have to consider the role of antimicrobial stewardship in improving outcomes of patients with CAP (eg, changing, de-escalation, and duration of antimicrobials) and whether molecular diagnostic tools play a role in changing therapy when the etiologic agent is identified.⁹⁶

SUMMARY

Although clinicians have many new and sometimes rapid molecular diagnostic tools that are the result of the explosion of new assays, and need to be using the ones that are available, future research will advance the field such that etiologic agent identification for agents of CAP will be the routine⁹⁷ rather than the exception.

REFERENCES

1. Bartlett J. Diagnostic tests for agents of community-acquired pneumonia. *Clin Infect Dis* 2011;52(Suppl 4):S296–304.
2. Hall MJ, DeFrances CJ, Williams SN, et al. National hospital discharge survey: 2007 summary. *Natl Health Stat Report* 2010;(29):1–20 Hyattsville (MD).
3. File TM, Marrie TJ. Burden of community-acquired pneumonia in North American adults. *Postgrad Med* 2010;122:130–41.
4. Bartlett J. Diagnostic test for etiologic agent of community-acquired pneumonia. *Infect Dis Clin North Am* 2004;18:809–27.
5. Bartlett JG. Decline in microbial studies for patients with pulmonary infections. *Clin Infect Dis* 2004;39:170–2.
6. File T Jr. New diagnostic tests for pneumonia: what is their role in clinical practice? *Clin Chest Med* 2011;32:417–30.
7. Centers for Disease Control and Prevention. Rate of hospitalization for pneumonia, by age group. National Hospital Discharge Survey, United States, 2000–2012. *MMWR Morb Mortal Wkly Rep* 2012;61:657.
8. Ecker DJ, Sampath R, Blyn LB, et al. Rapid identification and strain-typing of respiratory pathogens for epidemic surveillance. *Proc Natl Acad Sci U S A* 2005;102:8012–7.

9. Ecker DJ, Sampath R, Willett P, et al. The microbial Rosetta Stone database: a compilation of global and emerging infectious microorganisms and bioterrorist threat agents. *BMC Microbiol* 2005;5:19.
10. Selickman P, Paxos M, File TM Jr, et al. Performance measure of urinary antigen in patients with *Streptococcus pneumoniae* bacteremia. *Diagn Microbiol Infect Dis* 2010;67:129–33.
11. Shimada T, Noguchi Y, Jackson JL, et al. Systematic review and meta-analysis: urinary antigen tests for legionellosis. *Chest* 2009;136:1576–85.
12. Wolk DM, Kaleta EJ, Wysocki VH. PCR-electrospray ionization mass spectrometry. *J Mol Diagn* 2012;14:295–304.
13. Bhat N, O'Brien KL, Karron RA, et al. Use and evaluation of molecular diagnostics for pneumonia etiology studies. *Clin Infect Dis* 2012;54(Suppl 2):S153–8.
14. Chartrand C, Leeflang MM, Minion J, et al. Accuracy of rapid influenza diagnostic tests: a meta-analysis. *Ann Intern Med* 2012;156(7):500–11.
15. Lindenauer PK, Remus DW, Roman S, et al. Public reporting and pay for performance in hospital quality improvement. *N Engl J Med* 2007;356:486–96.
16. Houck PM, Bratzler DW, Nsa W, et al. Timing of antibiotic administration and outcomes for Medicare patients hospitalized with community-acquired pneumonia. *Arch Intern Med* 2004;164:637–44.
17. Russek-Cohen E, Feldblyum T, Whitaker KB, et al. FDA perspectives on diagnostic device clinical studies for respiratory infections. *Clin Infect Dis* 2011;52(Suppl 4):S305–11.
18. Ginocchio CC. Quality assurance in clinical virology. In: Specter S, Hodinka R, Young SA, et al, editors. *Clinical virology manual*. 4th edition. Washington, DC: ASM Press; 2009. p. 3–17.
19. Centers for Disease Control and Prevention. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. *MMWR Morb Mortal Wkly Rep* 2009;58:7–10.
20. Van Rie A, Page-Shipp L, Sanne I, et al. Xpert M tuberculosis RIF for point-of-care diagnosis of TB in high-HIV-burden, resource limited countries: hype or hope? *Rev Molecular Diagnosis* 2010;10:937–46.
21. Loeffelholz M, Pong D, Pyles R, et al. Comparison of the filmarray respiratory panel and Prodesse real-time PCR assays for detection of respiratory pathogens. *J Clin Microbiol* 2011;49:4083–8.
22. Yang S, Shin L, Khalil A, et al. Quantitative PCR assay using sputum samples for rapid diagnosis of pneumococcal pneumonia in adult emergency department patients. *J Clin Microbiol* 2005;43:3221–6.
23. Blaschke AJ. Interpreting assays for the detection of *Streptococcus pneumoniae*. *Clin Infect Dis* 2011;52(Suppl 4):S331–7.
24. Babin SM, Hsieh YH, Rothman RE, et al. Meta-analysis of point-of-care laboratory tests in the diagnosis of novel 2009 swine-lineage pandemic influenza A(H1N1). *Diagn Microbiol Infect Dis* 2011;69:410–8.
25. Ginocchio CC. Strengths and weaknesses of FDA-approved/cleared diagnostic devices for the molecular detection of respiratory pathogens. *Clin Infect Dis* 2011;52(Suppl):S312–25.
26. Kumar S, Henrickson KJ. Update on influenza diagnostics: lessons from the novel H1N1 influenza A pandemic. *Clin Microbiol Rev* 2012;25:361.
27. Lee CK, Cho HC, Woo MK, et al. Evaluation of Sofia fluorescent immunoassay analyzer for influenza A/B virus. *J Clin Virol* 2012;55(3):239–43.
28. LeGoff J, Kara R, Moulin F, et al. Evaluation of the one-step multiplex real-time reverse transcriptase-PCR ProFlu-1 assay for the detection of influenza A and

- influenza B viruses and respiratory syncytial viruses in children. *J Clin Microbiol* 2008;46:789–91.
29. Mahony J, Chong S, Erante F, et al. Development of a respiratory virus panel test for detection of twenty human respiratory viruses by use of a multiplex PCR and a fluid micro-bead-based assay. *J Clin Microbiol* 2007;45:2965–70.
 30. Merante F, Yaghoubian S, Janeczko R. Principles of the xTAG respiratory viral panel (RVP assay). *J Clin Virol* 2007;40:S31–5.
 31. Pabbaraju K, Tokaryk KL, Wong S, et al. Comparison of the Luminex xTAG respiratory panel with in-house nucleic acid amplification tests for diagnosis of respiratory virus infections. *J Clin Microbiol* 2008;49:3056–62.
 32. Rand KH, Rampersaud H, Houck H. Comparison of two multiplex methods for detection of respiratory viruses: filmarray RP and xTAG RVP. *J Clin Microbiol* 2011;49:2449.
 33. Jannetto PJ, Buchan BW, Vaughan KA, et al. Real-time detection of influenza a, influenza B, and respiratory syncytial virus a and B in respiratory specimens by use of nanoparticle probes. *J Clin Microbiol* 2011;48:3997–4002.
 34. Poritz M, Blaschke A, Byington C, et al. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. *PLoS One* 2011;6:e26047.
 35. Pierce V, Elkan M, Leet M, et al. Comparison of the Idaho Technology FilmArray system to real-time PCR for detection of respiratory pathogens in children. *J Clin Microbiol* 2012;50:364–71.
 36. Miller S, Moayeri M, Wright C, et al. Comparison of GeneXpert FluA PCR to direct fluorescent antibody and respiratory viral panel PCR assays for detection of 2009 novel H1N1 influenza virus. *J Clin Microbiol* 2010;48:4684–5.
 37. Sambol AR, Iwen PC, Pieretti M, et al. Validation of the Cepheid Xpert Flu A real time RT-PCR detection panel for emergency use authorization. *J Clin Virol* 2010; 48:234–8.
 38. Popowitch EB, Rogers E, Miller MB. Retrospective and prospective verification of the cepheid Xpert influenza virus assay. *J Clin Microbiol* 2011;49:3368–9.
 39. Li M, Brenwald N, Bonigal S, et al. Rapid diagnosis of influenza: an evaluation of two commercially available RT-PCR assays. *J Infect* 2012;65:60–3.
 40. Novak-Weekley SM, Marlowe EM, Poulter M, et al. Evaluation of the Cepheid Xpert Flu Assay for rapid identification and differentiation of influenza A, influenza A 2009 H1N1, and influenza B viruses. *J Clin Microbiol* 2012;50:1704–10.
 41. DiMaio MA, Sahoo MK, Waggomer J, et al. Comparison of Xpert Flu rapid nucleic acid testing with rapid antigen testing for the diagnosis of influenza A and B. *J Virol Methods* 2012;86(1–2):137–40. <http://dx.doi.org/10.1016/j.jviromet.2012.07.023>.
 42. Caliendo AM. Multiplex PCR and emerging technologies for the detection of respiratory pathogens. *Clin Infect Dis* 2011;52(Suppl 4):S326–30.
 43. Ecker DJ, Sampath R, Massire C, et al. Ibis T5000: a universal biosensor approach for microbiology. *Nat Rev Microbiol* 2008;6:553–8.
 44. Chen KF, Rothman RE, Ramachandron P, et al. Rapid identification of viruses from nasal pharyngeal aspirates in acute viral respiratory infections by RT-PCR and electrospray ionization mass spectrometry. *J Virol Methods* 2011;173:60–6.
 45. Chen KF, Blyn L, Rothman RE, et al. Reverse transcriptase polymerase chain reaction and electrospray ionization mass spectrometry for identifying acute viral upper respiratory tract infections. *Diagn Microbiol Infect Dis* 2011;69:179–86.
 46. Forman MS, Advani S, Newman C, et al. Diagnostic performance of two highly multiplexed respiratory virus assays in a pediatric cohort. *J Clin Virol* 2012; 55(2):168–72.

47. Sampath R, Russell KL, Massire C, et al. Global surveillance of emerging influenza viruses genotypes by mass spectrometry. *PLoS One* 2007;2:2489.
48. Deyde VM, Sampath R, Garten RJ, et al. Genomic signature-based identification of influenza A viruses using RT=PCR/electrospray ionization mass spectrometry (ESI-MS) technology. *PLoS One* 2010;5:e13293.
49. Deyde VM, Sampath R, Gubareva LV. RT-PCR/electrospray ionization mass spectrometry approach in detection and characterization of influenza viruses. *Expert Rev Mol Diagn* 2011;11:41–52.
50. Jeng K, Massire C, Zembower TR, et al. Monitoring seasonal influenza A evolution: Rapid 2009 pandemic H1N1 surveillance with a reverse transcription-polymerase chain reaction/electrospray ionization mass spectrometry assay. *J Clin Virol* 2012. <http://dx.doi.org/10.1016/j.jcv.2012.05.002>.
51. Tang YW, Lowery K, Valsamakis A, et al. Evaluation of a PLEX-ID flu assay for simultaneous detection and identification of influenza viruses A and B. *J Clin Microbiol* 2013;51. [Epub ahead of print].
52. Metzgar D, Baynes D, Myers CA, et al. Initial identification and characterization of an emerging zoonotic influenza virus prior to pandemic spread. *J Clin Microbiol* 2010;48:4228–34.
53. Benson R, Tondella ML, Bhatnagar J, et al. Development and evaluation of a novel multiplex PCR technology for molecular differential detection of bacterial respiratory disease pathogens. *J Clin Microbiol* 2008;46:2074–7.
54. Balada-Llasat JM, LaRue H, Kelly C, et al. Evaluation of commercial ResPlex II v2.0, Multicode-PLx and xTAG respiratory viral panels for the diagnosis of respiratory viral infections in adults. *J Clin Virol* 2011;50:42–5.
55. Raymond F, Carbonneau J, Boucher N, et al. Comparison of automated microarray detection with real-time PCR assays for detection of respiratory viruses in specimens obtained from children. *J Clin Microbiol* 2009;47:743–50.
56. Beck ET, Jurgens LA, Ehl SC, et al. Development of a rapid automated influenza A, influenza B, and respiratory syncytial virus A/B multiplex real-time RT-PCR assay and its use during the 2009 H1N1 swine-origin influenza virus epidemic in Milwaukee, Wisconsin. *J Mol Diagn* 2010;12:74–81.
57. Infectious Diseases Society of America. An unmet medical need: rapid molecular diagnostics tests for respiratory tract infections. *Clin Infect Dis* 2011;52(Suppl 4):S384–95.
58. D'Amato RF, Wallman AA, Hochstein LH, et al. Rapid diagnosis of pulmonary tuberculosis by using the Roche AMPLICOR *Mycobacterium tuberculosis* PCR test. *J Clin Microbiol* 1995;33:1832–4.
59. Lemaitre N, Armand S, Vachee A, et al. comparison of the real-time PCR method and the gen-probe amplified *Mycobacterium tuberculosis* direct test for detection of *Mycobacterium tuberculosis* in pulmonary and nonpulmonary specimens. *J Clin Microbiol* 2004;42:4307–9.
60. Dorman SE, Chihota VN, Ewis JJ, et al. Performance characteristics of the Cepheid Xpert MTB/RIF test in a tuberculosis prevalence survey. *PLoS One* 2012;7(8):e43307.
61. Scott LE, McCarthy K, Gous N, et al. Comparison of Xpert MTB/RIF with other nucleic acid technologies for diagnosing pulmonary tuberculosis in a high HIV prevalence setting: a prospective study. *PLoS Med* 2011;8(7):e1001061.
62. Peterson LR. Molecular laboratory tests for the diagnosis of respiratory tract infection due to *Staphylococcus aureus*. *Clin Infect Dis* 2011;52(Suppl 4):S361–6.
63. Paule SM, Hacek DM, Kufner B, et al. Performance of the BDGeneOhm methicillin-resistant *Staphylococcus aureus* test before and during high-volume clinical use. *J Clin Microbiol* 2007;45:2993–8.

64. Wolk DM, Picton E, Johnson D, et al. Multicenter evaluation of the Cepheid Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) test as a rapid screening method for detection of MRSA in nares. *J Clin Microbiol* 2009;47:758–64.
65. Wolk DM, Struelens MJ, Pancholi P, et al. Rapid detection of *Staphylococcus aureus* and methicillin *S. aureus* in wound specimens and blood cultures: multi-center preclinical evaluation of the Cepheid Xpert MRSA/SA skin and soft tissue and blood culture assays. *J Clin Microbiol* 2009;47:823–6.
66. Kumar S, Hammerschlag MR. Acute respiratory infection due to *Chlamydia pneumoniae*: current status of diagnostic methods. *Clin Infect Dis* 2007;44:568–76.
67. Loens K, Goossens H, Ieven M. Acute respiratory infections due to *Mycoplasma pneumoniae*: current status of diagnostic methods. *Eur J Clin Microbiol Infect Dis* 2010;29:1055–69.
68. Murdoch DR. Diagnosis of *Legionella* infection. *Clin Infect Dis* 2003;36:64–9.
69. Cloud JL, Carroll KC, Pixton P, et al. *Legionella* species in respiratory specimens using PCR with sequencing confirmation. *J Clin Microbiol* 2000;33:1709–12.
70. Diederens BM, Van Der Eerden MM, Viaspolder F, et al. Detection of respiratory viruses and *Legionella* spp. by real-time polymerase chain reaction in patients with community acquired pneumonia. *Scand J Infect Dis* 2009;41:45–50.
71. Liu FC, Chen PY, Huang FL, et al. Rapid diagnosis of *Mycoplasma pneumoniae* infection in children by polymerase chain reaction. *J Microbiol Immunol Infect* 2007;40:507–12.
72. Apfalter P, Blasi F, Boman J, et al. Multicenter comparison trial of DNA extraction methods and PCR assays for detection of *Chlamydia pneumoniae* in endarterectomy specimens. *J Clin Microbiol* 2001;39:519–24.
73. Dowell SF, Peeling RW, Boman J, et al. Standardizing chlamydia pneumoniae assays: recommendations from the Centers of Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). *Clin Infect Dis* 2001;33:492–503.
74. Gaydos CA, Quinn TC, Eiden JJ. Identification of *Chlamydia pneumoniae* by DNA amplification of the 16S rRNA gene. *J Clin Microbiol* 1992;30:796–800.
75. Gaydos CA, Roblin PM, Hammerschlag MR, et al. Diagnostic utility of PCR-enzyme immunoassay, culture, and serology for detection of *Chlamydia pneumoniae* in symptomatic and asymptomatic patients. *J Clin Microbiol* 1994;32:903–5.
76. Gaydos CA, Fowler CL, Gill VJ, et al. Detection of *Chlamydia pneumoniae* by polymerase chain reaction-enzyme immunoassay in an immunocompromised population. *Clin Infect Dis* 1993;17:718–23.
77. Gaydos CA, Eiden JJ, Oldach D, et al. Diagnosis of *Chlamydia pneumoniae* infection in patients with community acquired pneumonia by polymerase chain reaction enzyme immunoassay. *Clin Infect Dis* 1994;19:157–60.
78. Boman J, Allard A, Person K, et al. Rapid diagnosis of respiratory *Chlamydia pneumoniae* infection by nested touchdown polymerase chain reaction compared with culture and antigen detection by EIA. *J Infect Dis* 1997;175:1523–6.
79. Madico G, Quinn T, Boman J, et al. Touchdown enzyme time release-PCR for detection and identification of *C. trachomatis*, *C. pneumoniae*, and *C. psittaci* using the 16S and 16S-23S spacer rRNA genes. *J Clin Microbiol* 2000;38:1085–93.
80. Tondella ML, Talkington DF, Holloway BP, et al. Development and evaluation of real-time PCR-based fluorescence assays for the detection of *Chlamydia pneumoniae*. *J Clin Microbiol* 2002;40:575–83.
81. Apfalter P, Barousch W, Nehr M, et al. Comparison of a new quantitative ompA-based real-time PCR TaqMan assay for detection of *Chlamydia pneumoniae*

- DNA in respiratory specimens with four conventional PCR assays. *J Clin Microbiol* 2003;41:592–600.
82. Hardick J, Maldeis N, Theodore M, et al. Real-Time PCR for *Chlamydia pneumoniae* utilizing the Roche Lightcycler and a 16S rRNA gene target. *J Mol Diagn* 2004;6:132–6.
 83. Coon RG, Balansay MS, Faix DJ, et al. *Chlamydophilia pneumoniae* infection among basis underwater demolition/SEALS (BUD/S) candidates, Coronado, California, July 2008. *Mil Med* 2011;176:320–3.
 84. Chernesky MS, Schachter J, Summersgill J, et al. Comparison of an industry derived LCx *Chlamydia pneumoniae* PCR research kit to in-house assays performed in five laboratories. *J Clin Microbiol* 2002;40:2357–62.
 85. Morozumi M, Ito A, Murayama SY, et al. Assessment of real-time PCR for diagnosis of *Mycoplasma pneumoniae* in pediatric patients. *Can J Microbiol* 2006;52:125–9.
 86. Avni T, Mansur N, Leibovici L, et al. PCR using blood for diagnosis of invasive pneumococcal disease: systematic review and meta-analysis. *J Clin Microbiol* 2010;48:489–96.
 87. Smith MD, Sheppard CL, Hogan A, et al. Diagnosis of *Streptococcus pneumoniae* infections in adults with bacteremia and community-acquired pneumonia: clinical comparison of pneumococcal PCR and urinary antigen detection. *J Clin Microbiol* 2009;47:1046–9.
 88. Murdock DR, Anderson TP, Beynon KA, et al. Evaluation of a PCR assay for detection of *Streptococcus pneumoniae* in respiratory and nonrespiratory samples from adults with community-acquired pneumonia. *J Clin Microbiol* 2003;41:63–6.
 89. Carvalho Mda MG, Tondella ML, Mc Caustland K, et al. Evaluation and improvement of real-time PCR assays targeting *lytA*, *ply*, and *psaA* genes for detection for pneumococcal DNA. *J Clin Microbiol* 2007;45:2460–6.
 90. Sheppard CL, Harrison TG, Morris R, et al. Autolysin-targeted LightCycler assay including internal process control for detection of *Streptococcus pneumoniae* DNA in clinical samples. *J Med Microbiol* 2004;53:189–95.
 91. Albrich WC, Madhi SA, Adrian PV, et al. Use of a rapid test of pneumococcal colonization density to diagnose pneumococcal pneumonia. *Clin Infect Dis* 2012;54:601–9.
 92. Gilbert DN. Procalcitonin as a biomarker in respiratory tract infection. *Clin Infect Dis* 2011;52(Suppl 4):S346–50.
 93. Fowler CL. Procalcitonin for triage of patients with respiratory tract symptoms: a case study in the trial design process for approval of a new diagnostic test for lower respiratory tract infections. *Clin Infect Dis* 2011;52(Suppl 4):S351–6.
 94. Jain S, Benoit SR, Skarbinski J, et al. Influenza-associated pneumonia among hospitalized patients with 2009 pandemic influenza A (H1N1)—United States, 2009. *Clin Infect Dis* 2012;54:1221–9.
 95. Harper SA, Bradley JS, Englund JA, et al. Seasonal influenza in adults and children: diagnosis, treatment chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2009;48:1003–32.
 96. Avdic E, Cushinotto LA, Hughes AH, et al. Impact of an antimicrobial stewardship intervention on shortening the duration of therapy for community-acquired pneumonia. *Clin Infect Dis* 2012;54:1581–7.
 97. Johansson N, Kalin M, Tiveijung-Lindell A, et al. Etiology of community-acquired pneumonia: increased microbiological yields with new diagnostic methods. *Clin Infect Dis* 2010;50:202–9.