



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Diagnosing Viral and Atypical Pathogens in the Setting of Community-Acquired Pneumonia



Grant W. Waterer, MD, PhD^{a,b,*}

KEYWORDS

• Pneumonia • Viral • Diagnostic • Point of care

KEY POINTS

- The concept of atypical pneumonia is outdated because clinically it is impossible to determine the pathogen.
- Nucleic acid detection is now the standard diagnostic method for all these pathogens, having replaced older serologic and antigen detection methods.
- Multiple pathogens are commonly detected in patients, particularly with *Legionella*, *Mycoplasma*, and *Chlamydia*.

INTRODUCTION

Despite many promises that molecular diagnostics would transform the management of infection, empiric therapy remains the standard of care in community-acquired pneumonia (CAP). Outside of etiologic studies, the vast majority of patients never have a pathogen diagnosed as the cause of their pneumonia. Although physicians are generally quite comfortable with empiric therapy, the need to guess and fear of missing an important pathogen inevitably leads to a broader than necessary spectrum of coverage, particularly in the setting of more severe illness.

That viruses are an important cause of pneumonia has been known since the identification of influenza in the early 1930s.¹ Despite an awareness that viruses can cause CAP, it is only recently that they have appeared as more than a footnote on the list of common pathogens. However, with modern generations of diagnostic panels, and particularly nucleic acid amplification tests, viral

pathogens are being identified increasingly as not only common causes of CAP, but possibly as being overall more common than bacteria.^{2,3} With more sensitive tests has also come confirmation that patients with CAP frequently have multiple pathogens present, particularly the combination of bacterial and viral infection.

The term “atypical pneumonia” was coined in first half of the 20th century and used to describe pneumonia owing to pathogens that were not detectable by standard Gram staining or traditional culture methods and typically associated with headache, low-grade fever, cough, and malaise. The predominant pathogens that have become associated with atypical pneumonia are *Mycoplasma pneumoniae* (first identified in human lung in 1944),⁴ *Legionella pneumophila* (first identified as a significant pneumonia pathogen in 1977 after the outbreak at a convention in Philadelphia in 1976)⁵ and *Chlamydia pneumoniae* (first identified in the respiratory tract

Disclosure Statement: The author has nothing to disclose.

^a University of Western Australia, Level 4 MRF Building, Royal Perth Hospital, Wellington Street, Perth 6000, Australia; ^b Northwestern University, 420 East Superior Street, Chicago, IL 60611, USA

* University of Western Australia, Level 4 MRF Building, Royal Perth Hospital, Wellington Street, Perth 6000, Australia.

E-mail address: grant.waterer@uwa.edu.au

Clin Chest Med 38 (2017) 21–28

<http://dx.doi.org/10.1016/j.ccm.2016.11.004>

0272-5231/17/© 2016 Elsevier Inc. All rights reserved.

in 1984).⁶ A variety of different species of these genera are now recognized as pneumonia pathogens.

This review covers the main approaches to the diagnosis of atypical and viral infections in the setting of pneumonia. The most common approach has been the use of pathogen-specific assays for use in urine, blood, or sputum. Although serologic tests based on detecting antibodies to specific pathogens were the predominant technique for decades, they all have limitations in early disease before an adaptive immune response being constituted as well as issues of cross-reactivity reducing specificity. Polymerase chain reaction (PCR)-based techniques are now the primary modality for the detection of atypical pathogens in most settings. More recently, there has been the development of multipathogen detection platforms that have become used increasingly in the setting of pneumonia.

CLINICAL DIAGNOSIS

Before moving to laboratory tests, it is worth briefly looking at the evidence of whether there are any specific clinical or radiological features in CAP that help to deduce reliably the pathogen. There are definitely clinical features that are seen more commonly in some of the atypical pathogens than with disease owing to *Streptococcus pneumoniae*. Examples include erythema multiforme with *M pneumoniae*, diarrhea with *L pneumophila*, and rhinorrhea with influenza. However, there is ample evidence that no set of clinical symptoms or signs has sufficient predictive ability to rule in or out any atypical or viral pathogens, especially *M pneumoniae*⁷ and *Legionella*.^{8–10}

A number of nonmicrobiological tests have also been proposed as being able to discriminate between “atypical” and “typical” pathogens, including the peripheral white cell count and procalcitonin. Although peripheral white cell counts do tend to be lower in viral infections compared with bacterial infections, this is not particularly discriminating at an individual patient level and certainly not accurate enough to use to determine empiric therapy.¹¹ Procalcitonin seems to be more accurate than white cell count,¹¹ but does not discriminate between atypical bacterial infection and viral infection¹² and may be misleading, particularly in critically ill patients or in patients with bacterial and viral coinfection.¹³ A definitive diagnosis based on detecting the infection pathogen(s), therefore, remains critical if we are to improve the accuracy of empiric therapy.

PATHOGEN-SPECIFIC APPROACHES

Legionella

Very little has changed in the diagnosis of *Legionella* infection since we reviewed this topic comprehensively 15 years ago.¹⁴ In most settings, *Legionella* is underdiagnosed and therefore underrecognized owing to routine testing not being performed.¹⁵ *Legionella* infections seem to be increasing in the United States,^{16,17} possibly owing to recent climate change, including a number of severe outbreaks with multiple fatalities,¹⁸ which has led to increased interest in its diagnosis.

Because *Legionellae* will not grow on standard culture media, the diagnosis has traditionally rested on either positive serology or a positive urinary antigen test. Both of these tests have significant limitations. In the case of serology, 20% or more of patients with culture-proven *Legionella* infection do not ever seroconvert,^{19,20} and seroconversion may take months, requiring testing out to at least 2 months if not longer.²¹ Urinary antigen testing is quite specific, but will only reliably detect *L pneumophila* serogroup 1, and usually serogroup 6, but in many areas other species (particularly *Legionella longbeachae* and *Legionella micdadei*) are more predominant. Despite these limitations, urinary antigen testing for *Legionella* is recommended in all patients with severe CAP (ie, admitted to the intensive care unit) for both diagnostic and public health reasons.²²

The mainstay of diagnosis of *Legionella* infection has been from one or more of direct antigen detection or nucleic acid detection in respiratory secretions. Direct fluorescent antigen detection was developed in the pre-PCR era but have now largely been replaced by PCR because the latter is more sensitive, less technician dependent, and easier to automate. PCR tests for *Legionella* are a mix of “home-grown” assays and commercially available products, with reported sensitivity and specificity (using all other tests as the gold standard) in the range of 91% to 99% and 94% to 99%, respectively.²³ Because PCR tests for *Legionella* are generally able to detect all species,²⁴ not surprisingly they have a greater degree of sensitivity than urinary antigen testing.^{23,25} There is, however, a reasonable argument for performing both urinary antigen testing and PCR on respiratory secretions because there is an increased diagnostic yield from this approach.²⁶ It is worth noting that both nasopharyngeal aspirates^{27–29} and throat swabs¹⁵ have substantially lower yields for the detection of *Legionella* by PCR, but may be of use in patients in whom it is not possible to get spontaneous or induced sputum samples.

Mycoplasma

Traditionally, *Mycoplasma* infections have most often been diagnosed on the basis of serology; however, as with serologic tests for many pathogens, this has significant limitations early in disease when false-negative results are common. Difficulties in making the diagnosis as well as the marked season to season variation in its prevalence probably explain the enormous variation in the estimated proportion of cases of CAP owing to *M pneumoniae*, which range from less than 1% to greater than 50%.

PCR does overcome some of the limitations of serology for the diagnosis of *Mycoplasma* infection and the nuances of assay development and relative performance characteristics has been reviewed comprehensively elsewhere.³⁰ The performance characteristics for PCR assays for *M pneumoniae* seem to be at least as good as those for *Legionella* infections and possibly better.^{31,32} As with other pathogens, the detection rate of *M pneumoniae* using PCR on nasopharyngeal aspirates is lower than in sputum samples.²⁸ Recently, there has been interest in antigen detection assays for the diagnosis of *M pneumoniae* because these offer the potential for point-of-care testing, but so far these have yet to enter the clinical mainstream.^{33,34}

Chlamydia

The nomenclature for the *Chlamydia* has changed recently with *Chlamydia* and *Chlamydophila* being combined back into a single genus.³⁵ Both *Chlamydia psittaci* and *C pneumoniae* are well-accepted as causes of CAP, although almost always being identified as much less common than either *Mycoplasma* or *Legionella* infections. A number of other *Chlamydophila*-like pathogens (such as *Parachlamydia acanthamoebae* and *Simkania negevensis*) also been suggested as potential causes of the 50% or more of cases of CAP where no pathogen is identified.^{36,37}

The specificity of positive serology for *C pneumoniae* has also been questioned, because studies using PCR-based diagnosis typically find much lower rates of infection than earlier serology-based studies and a large variety of assays with different performance characteristics have been used.³⁸ As with *Mycoplasma*, in early disease *Chlamydophila* serology is often negative making PCR a superior diagnostic test.³⁹

Unlike *Legionella* and *Mycoplasma*, *Chlamydophila* cannot be detected by 16S-based PCR assays. Because culture of *Chlamydophila* is difficult and has a low yield, it is rarely done⁴⁰; therefore, PCR assays that have been developed are

generally compared with serologic tests, with their known limitations as discussed. The true sensitivity of PCR for *Chlamydophila* species is, therefore, unknown. However the reported specificity of most assays is well over 95%⁴¹ and, therefore, a positive result in the right clinical context should be acted on.

Influenza

All major etiologic studies of CAP have identified influenza as a significant cause of CAP, particularly in hospitalized patients. Since the recent H1N1 09 influenza pandemic, there is evidence that the use of empiric antiinfluenza therapy in the setting of CAP has increased significantly, with an unclear impact on outcome.⁴² A fast and reliable diagnostic test for influenza is, therefore, attractive not only to prescribe antivirals appropriately (for treatment and prophylaxis), but also to aid in the allocation of respiratory isolation beds, which are often in limited supply, especially in influenza season. For this reason, the diagnostic tools available for influenza have significantly outpaced those for the other causes of atypical pneumonia.

In the United States, there are more than a dozen approved rapid influenza tests primarily based on the detection of influenza antigens in respiratory samples. Most available assays have been compared with a gold standard of real-time reverse transcription PCR in the same sample. The sensitivity of these assays varies between 10% and 75% depending on age, quality of the sample, and duration of symptoms. Complicating the assessment of the usefulness of these assays is that the performance seems to vary between influenza strains and, unfortunately, during the H1N1 09 pandemic they were less than optimal.^{43–47} A recent metaanalysis of 159 published studies of rapid influenza tests found the pooled sensitivity, sensitivity, specificity, and positive and negative predictive values to be 62%, 98%, 34%, and 38%, respectively.⁴⁷

Not surprisingly, given these data, there is little evidence that rapid influenza tests are currently used by clinicians to alter patient management.⁴⁸ However, this is a rapidly changing field and more recent publications suggest that there are incremental improvements with a range of sensitivity from 68% to 79% and specificity of 99% to 100%.^{49–51} This is clearly an area where we can expect to see significant advances over the next few years.

In the absence of rapid diagnostic tests, existing commercial PCR assays for influenza have well-documented good performance characteristics for influenza A and B, and these data are

well-reviewed elsewhere.⁵² What is interesting from etiologic studies is the high degree of copathogen involvement with influenza, particularly the codetection of bacterial infection with *S pneumoniae*.^{2,3} Whether this is genuine coinfection or sequential infection is a current controversy and major area of research interest. Unlike bacterial pathogens, the constant genomic shifts in influenza A do affect the performance of assays and they need to be revalidated constantly as new strains appear.⁵³

A variety of point-of-care platforms have been developed for detecting influenza, of which the GeneXpert system (Cepheid, Sunnyvale, CA) is perhaps so far the best studied.⁵⁴ GeneXpert is an “all-in-one” platform requiring minimal technical expertise, and is a potential point-of-care platform for diagnosing influenza. A sputum sample is placed in a cartridge that plugs into the platform without the need for further processing or expert microbiological assistance. With a turnaround time of less than 2 hours, results can be available fast enough to impact on empiric therapy. This system has been evaluated extensively for the diagnosis of tuberculosis, including multidrug-resistant tuberculosis, where it has been proven to have excellent sensitivity and specificity.⁵⁵ The influenza A and B GeneXpert assay has been evaluated in comparison to a number of commercially available rapid antigen tests and PCR tests and found to have excellent sensitivity (97%–100%) and specificity (99%–100%).^{56–60} The potential clinical usefulness has been studied in the emergency department setting, again with good performance and efficiency.^{61,62} Point-of-care testing for influenza is a highly competitive area with potential new products regularly entering the market offering greater speed, lower cost, and/or greater accuracy (for example^{63–66}).

Other Viruses

A large number of other viruses are well-known to cause pneumonia, with the most common being adenovirus, respiratory syncytial virus, metapneumovirus, parainfluenza, and coronaviruses. In the absence of specific treatments for any of these viruses, discussion of specific diagnostic tests is relatively superfluous; however, many of the multipathogen approaches are discussed herein and include 1 or more of these viruses in their “panels.”

MULTIPATHOGEN APPROACHES

With an ever-expanding list of pneumonia-causing pathogens, it is both time consuming and expensive to test for each organism individually. The

ability to detect multiple pathogens in a single test is, therefore, highly appealing and has been the subject of significant research, development, and validation in the setting of respiratory tract infection. Starting with “home-grown” multiplex PCR assays, a variety of new platforms have been developed to speed up pathogen identification, and in some cases combining this with antibiotic sensitivity testing. Because the focus of multipathogen detection tools is to find the cause of the pneumonia, they all combine assays for “typical” pathogens such as *S pneumoniae* with the “atypical” pathogens.

Multipathogen detection systems can in general these can be categorized into those specifically designed to speed up pathogen recognition from positive blood cultures (eg, including systems such as The Verigene GramPositive Blood Culture Nucleic Acid Test; Nanosphere, Northbrook, IL), Prove-it Sepsis StripArray technology (Mobidiag, Espoo, Finland), and FilmArray (BioFire Diagnostics, Salt Lake City, UT), and those designed for clinical samples such as sputum, blood, or urine (eg, GeneXpert, SeptiFast [Roche Diagnostics, Mannheim, Germany], SepsisTest [Molzym, Bremen, Germany], Curetis Unyvero [Curetis AG, Holzgerlingen, Germany], and VYOO [SIRS Lab, Jena, Germany]). The systems designed to speed up blood culture results are not particularly relevant to a discussion of atypical pathogens. Systems designed to work on clinical samples are, however, of interest, especially those with point-of-care applications. Unfortunately data in the specific setting of CAP are relatively limited at present, so only those with relevant published studies on viral and atypical pathogens are discussed briefly herein.

GeneXpert

The GeneXpert system has already been discussed, but it is worth noting that the range of pathogen assays is steadily increasing and now includes respiratory syncytial virus and methicillin-resistant *Staphylococcus aureus*, which are clearly relevant to pneumonia.

FilmArray

FilmArray is another novel “all-in-one” multiplex PCR platform with minimal technical expertise required and a turnaround time of approximately 1 hour. Manual handling is very limited, as with GeneXpert, and a variety of panels are available. The commercially available respiratory panel detects 17 viral and 3 bacterial pathogens. The performance of the respiratory panel has been compared with “in-house” PCR tests with

favorable results^{67,68} and the system seems to be robust enough to be useful in routine clinical practice.^{69,70}

Curetis Unyvero

The Curetis Unyvero P50 pneumonia cartridge can detect 17 bacterial and fungal pathogens and 22 antibiotic resistance markers from respiratory samples in a single run in approximately 4 hours.⁷¹ The panel includes *L pneumophila* and *M pneumoniae*, but specific performance data on these pathogens from clinical studies has not been reported. A preliminary study in critically ill patients found the performance of the Curetis Unyvero to be questionable, but noted the system was still under development.⁷²

Mass Spectrometry

Mass spectrometry has been available for decades, but improvements in size, speed, and cost have brought this technology to a point where it can be used for both broadrange and target-specific identification of pathogens. PCR-electrospray ionization mass spectrometry holds particular promise given that it can identify minute quantities and mixtures of nucleic acids from microbial isolates or directly from clinical specimens. The performance of PCR-electrospray ionization mass spectrometry for detecting influenza in clinical samples seems at least as good as conventional PCR assays.⁷³ A single study from Taiwan indicates that PCR-electrospray ionization mass spectrometry has promise for the detection of multiple viruses in the setting of respiratory tract infection but this was done retrospectively rather than in real time.⁷⁴

A different use of mass spectrometry, matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) is also a protein/peptide diagnostic tool that has been shown to have usefulness in identifying microorganisms at a species level. MALDI-TOF-MS has been assessed predominantly as a means of rapidly identifying the identity of both bacteria and their bacterial products from positive blood cultures, up to 24 hours faster than conventional methods. A comparison of the diagnostic accuracy of MALDI-TOF-MS with liquid chromatography MS for influenza A, metapneumovirus, and respiratory syncytial virus suggested the latter may be superior.⁷⁵ A potential and significant limitation of current MALDI-TOF-MS is that when a large mixture of bacteria are present, as occurs more commonly in hospital-acquired pneumonia and ventilator-acquired pneumonia, the sensitivity and specificity become suboptimal.⁷⁶

Next-Generation Sequencing

Next-generation sequencing, also known as high-throughput sequencing, is a generic term used to describe a group of different modern sequencing technologies including Illumina (Solexa, San Diego, CA) sequencing, Roche 454 sequencing, Ion torrent: Proton/PGM Sequencing (ThermoFisher Scientific, Waltham, MA), and SOLiD sequencing (ThermoFisher Scientific, Waltham, MA). These recent technologies allow sequencing of DNA and RNA much more quickly and cheaply than the previously used Sanger sequencing (ThermoFisher Scientific, Waltham, MA). To date, there are few data on the applicability of next-generation sequencing to immediate clinical care, but it has been particularly useful in diagnosing new and/or novel pathogens for which there are no available assays.

SUMMARY

As technology has improved, we have moved from relying on serologic tests to diagnose atypical and viral pathogens to direct detection of these pathogens in clinical specimens. Starting from a base of homegrown PCR assays, an increasing array of commercial assays have appeared, first as single pathogen assays, and increasingly as multiplexed tests. Increasing focus on point-of-care testing, or at least rapid enough turnaround time to influence initial clinical management, has driven development of a host of new platforms and technologies that are likely to change the way we manage pneumonia over the coming decade.

REFERENCES

1. Shope RE. The etiology of swine influenza. *Science* 1931;73(1886):214-5.
2. Jain S, Self WH, Wunderink RG, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. *N Engl J Med* 2015;373(5):415-27.
3. Holter JC, Muller F, Bjorang O, et al. Etiology of community-acquired pneumonia and diagnostic yields of microbiological methods: a 3-year prospective study in Norway. *BMC Infect Dis* 2015; 15:64.
4. Eaton M, Meiklejohn G, va Herick W. Studies on the etiology of primary atypical pneumonia: a filterable agent transmissible to cotton rats, hamsters and chick embryos. *J Exp Med* 1944;79(6):649-68.
5. McDade JE, Shepard CC, Fraser DW, et al. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. *N Engl J Med* 1977;297(22):1197-203.

6. Grayston JT, Kuo CC, Wang SP, et al. A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. *N Engl J Med* 1986;315(3):161–8.
7. Wang K, Gill P, Perera R, et al. Clinical symptoms and signs for the diagnosis of *Mycoplasma pneumoniae* in children and adolescents with community-acquired pneumonia. *Cochrane Database Syst Rev* 2012;(10):CD009175.
8. Wingfield T, Rowell S, Peel A, et al. Legionella pneumonia cases over a five-year period: a descriptive, retrospective study of outcomes in a UK district hospital. *Clin Med (Lond)* 2013;13(2):152–9.
9. Viasus D, Di Yacovo S, Garcia-Vidal C, et al. Community-acquired Legionella pneumophila pneumonia: a single-center experience with 214 hospitalized sporadic cases over 15 years. *Medicine* 2013;92(1):51–60.
10. Sopena N, Sabria-Leal M, Pedro-Botet ML, et al. Comparative study of the clinical presentation of Legionella pneumonia and other community-acquired pneumonias. *Chest* 1998;113(5):1195–200.
11. Jereb M, Kotar T. Usefulness of procalcitonin to differentiate typical from atypical community-acquired pneumonia. *Wien Klin Wochenschr* 2006;118(5–6):170–4.
12. Kruger S, Ewig S, Marre R, et al. Procalcitonin predicts patients at low risk of death from community-acquired pneumonia across all CRB-65 classes. *Eur Respir J* 2008;31(2):349–55.
13. Wu MH, Lin CC, Huang SL, et al. Can procalcitonin tests aid in identifying bacterial infections associated with influenza pneumonia? A systematic review and meta-analysis. *Influenza Other Respir Viruses* 2013;7(3):349–55.
14. Waterer GW, Baselski VS, Wunderink RG. Legionella and community-acquired pneumonia: a review of current diagnostic tests from a clinician's viewpoint. *Am J Med* 2001;110(1):41–8.
15. Maze MJ, Slow S, Cumins AM, et al. Enhanced detection of Legionnaires' disease by PCR testing of induced sputum and throat swabs. *Eur Respir J* 2014;43(2):644–6.
16. Centers for Disease Control and Prevention (CDC). Legionellosis — United States, 2000–2009. *MMWR Morb Mortal Wkly Rep* 2011;60(32):1083–6.
17. Adams D, Fullerton K, Jajosky R, et al. Summary of notifiable infectious diseases and conditions - United States, 2013. *MMWR Morb Mortal Wkly Rep* 2015;62(53):1–122.
18. Garrison LE, Kunz JM, Cooley LA, et al. Vital signs: deficiencies in environmental control identified in outbreaks of legionnaires' disease - North America, 2000–2014. *MMWR Morb Mortal Wkly Rep* 2016;65(22):576–84.
19. Edelstein PH, Meyer RD, Finegold SM. Laboratory diagnosis of Legionnaires' disease. *Am Rev Respir Dis* 1980;121(2):317–27.
20. Zuravleff JJ, Yu VL, Shonnard JW, et al. Diagnosis of Legionnaires' disease. An update of laboratory methods with new emphasis on isolation by culture. *JAMA* 1983;250(15):1981–5.
21. Monforte R, Estruch R, Vidal J, et al. Delayed seroconversion in Legionnaire's disease. *Lancet* 1988;2(8609):513.
22. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2007;44(Suppl 2):S27–72.
23. Avni T, Bieber A, Green H, et al. Diagnostic accuracy of PCR alone and compared to urinary antigen testing for detection of Legionella spp.: a systematic review. *J Clin Microbiol* 2016;54(2):401–11.
24. Benitez AJ, Winchell JM. Rapid detection and typing of pathogenic nonpneumophila Legionella spp. isolates using a multiplex real-time PCR assay. *Diagn Microbiol Infect Dis* 2016;84(4):298–303.
25. Botelho-Nevers E, Grattard F, Viallon A, et al. Prospective evaluation of RT-PCR on sputum versus culture, urinary antigens and serology for Legionnaire's disease diagnosis. *J Infect* 2016;73(2):123–8.
26. Gadsby NJ, Russell CD, McHugh MP, et al. Comprehensive molecular testing for respiratory pathogens in community-acquired pneumonia. *Clin Infect Dis* 2016;62(7):817–23.
27. Blaschke AJ, Allison MA, Meyers L, et al. Non-invasive sample collection for respiratory virus testing by multiplex PCR. *J Clin Virol* 2011;52(3):210–4.
28. Herrera M, Aguilar YA, Rueda ZV, et al. Comparison of serological methods with PCR-based methods for the diagnosis of community-acquired pneumonia caused by atypical bacteria. *J Negat Results Biomed* 2016;15:3.
29. Cho MC, Kim H, An D, et al. Comparison of sputum and nasopharyngeal swab specimens for molecular diagnosis of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila*. *Ann Lab Med* 2012;32(2):133–8.
30. Parrott GL, Kinjo T, Fujita J. A compendium for *Mycoplasma pneumoniae*. *Front Microbiol* 2016;7:513.
31. Loens K, Beck T, Ursi D, et al. Evaluation of different nucleic acid amplification techniques for the detection of *M. pneumoniae*, *C. pneumoniae* and *Legionella* spp. in respiratory specimens from patients with community-acquired pneumonia. *J Microbiol Methods* 2008;73(3):257–62.
32. Chou RC, Zheng X. A comparison of molecular assays for *Mycoplasma pneumoniae* in pediatric patients. *Diagn Microbiol Infect Dis* 2016;85(1):6–8.
33. Li W, Liu Y, Zhao Y, et al. Rapid diagnosis of *Mycoplasma pneumoniae* in children with pneumonia by an immuno-chromatographic antigen assay. *Sci Rep* 2015;5:15539.

34. Miyashita N, Kawai Y, Kato T, et al. Rapid diagnostic method for the identification of *Mycoplasma pneumoniae* respiratory tract infection. *J Infect Chemother* 2016;22(5):327–30.
35. Pannekoek Y, Qin QL, Zhang YZ, et al. Genus delimitation of Chlamydiales by analysis of the percentage of conserved proteins (POCP) justifies the reunifying of the genera *Chlamydia* and *Chlamydophila* into one single genus *Chlamydia*. *Pathog Dis* 2016;74 [pii:ftw071].
36. Friedman MG, Dvoskin B, Kahane S. Infections with the chlamydia-like microorganism *Simkania negevensis*, a possible emerging pathogen. *Microbes Infect* 2003;5(11):1013–21.
37. Greub G. Parachlamydia acanthamoebae, an emerging agent of pneumonia. *Clin Microbiol Infect* 2009;15(1):18–28.
38. Villegas E, Sorlozano A, Gutierrez J. Serological diagnosis of *Chlamydia pneumoniae* infection: limitations and perspectives. *J Med Microbiol* 2010; 59(Pt 11):1267–74.
39. Benitez AJ, Thurman KA, Diaz MH, et al. Comparison of real-time PCR and a microimmunofluorescence serological assay for detection of *chlamydophila pneumoniae* infection in an outbreak investigation. *J Clin Microbiol* 2012;50(1):151–3.
40. She RC, Thurber A, Hymas WC, et al. Limited utility of culture for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* for diagnosis of respiratory tract infections. *J Clin Microbiol* 2010;48(9): 3380–2.
41. Rodriguez-Dominguez M, Sanbonmatsu S, Salinas J, et al. Microbiological diagnosis of infections due to *Chlamydia* spp. and related species. *Enferm Infecc Microbiol Clin* 2014;32(6):380–5 [in Spanish].
42. Aziz M, Vasoo S, Aziz Z, et al. Oseltamivir overuse at a Chicago hospital during the 2009 influenza pandemic and the poor predictive value of influenza-like illness criteria. *Scand J Infect Dis* 2012;44(4):306–11.
43. Gao F, Loring C, Laviolette M, et al. Detection of 2009 pandemic influenza A(H1N1) virus Infection in different age groups by using rapid influenza diagnostic tests. *Influenza Other Respir Viruses* 2012;6(3):e30–4.
44. Nutter S, Cheung M, Adler-Shohet FC, et al. Evaluation of indirect fluorescent antibody assays compared to rapid influenza diagnostic tests for the detection of pandemic influenza A (H1N1) pdm09. *PLoS One* 2012;7(3):e33097.
45. Ciblak MA, Kanturvardar M, Asar S, et al. Sensitivity of rapid influenza antigen tests in the diagnosis of pandemic (H1N1)2009 compared with the standard rRT-PCR technique during the 2009 pandemic in Turkey. *Scand J Infect Dis* 2010;42(11–12): 902–5.
46. Ganzenmueller T, Kluba J, Hilfrich B, et al. Comparison of the performance of direct fluorescent antibody staining, a point-of-care rapid antigen test and virus isolation with that of RT-PCR for the detection of novel 2009 influenza A (H1N1) virus in respiratory specimens. *J Med Microbiol* 2010;59(Pt 6):713–7.
47. 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.
48. Nicholson KG, Abrams KR, Batham S, et al. Randomised controlled trial and health economic evaluation of the impact of diagnostic testing for influenza, respiratory syncytial virus and *Streptococcus pneumoniae* infection on the management of acute admissions in the elderly and high-risk 18- to 64-year-olds. *Health Technol Assess* 2014; 18(36):1–274, vii–viii.
49. Peci A, Winter AL, King EC, et al. Performance of rapid influenza diagnostic testing in outbreak settings. *J Clin Microbiol* 2014;52(12):4309–17.
50. Busson L, Hallin M, Thomas I, et al. Evaluation of 3 rapid influenza diagnostic tests during the 2012–2013 epidemic: influences of subtype and viral load. *Diagn Microbiol Infect Dis* 2014;80(4): 287–91.
51. Ryu SW, Lee JH, Kim J, et al. Comparison of two new generation influenza rapid diagnostic tests with instrument-based digital readout systems for influenza virus detection. *Br J Biomed Sci* 2016;73:1–6.
52. Vemula SV, Zhao J, Liu J, et al. Current approaches for diagnosis of influenza virus infections in humans. *Viruses* 2016;8(4):96.
53. Huzly D, Korn K, Bierbaum S, et al. Influenza A virus drift variants reduced the detection sensitivity of a commercial multiplex nucleic acid amplification assay in the season 2014/15. *Arch Virol* 2016; 161(9):2417–23.
54. Salez N, Nougairede A, Ninove L, et al. Xpert Flu for point-of-care diagnosis of human influenza in industrialized countries. *Expert Rev Mol Diagn* 2014; 14(4):411–8.
55. Steingart KR, Schiller I, Horne DJ, et al. Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev* 2014;(1):CD009593.
56. 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(12):4684–5.
57. Wahrenbrock MG, Matushek S, Boonlayangoor S, et al. Comparison of cepheid xpert Flu/RSV XC and BioFire FilmArray for detection of influenza A, influenza B, and respiratory syncytial virus. *J Clin Microbiol* 2016;54(7):1902–3.
58. Popowitch EB, Miller MB. Performance characteristics of xpert Flu/RSV XC assay. *J Clin Microbiol* 2015;53(8):2720–1.

59. Salez N, Nougairède A, Ninove L, et al. Prospective and retrospective evaluation of the Cepheid Xpert(R) Flu/RSV XC assay for rapid detection of influenza A, influenza B, and respiratory syncytial virus. *Diagn Microbiol Infect Dis* 2015;81(4):256–8.
60. DiMaio MA, Sahoo MK, Waggoner 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;186(1–2):137–40.
61. Dugas AF, Valsamakis A, Gaydos CA, et al. Evaluation of the Xpert Flu rapid PCR assay in high-risk emergency department patients. *J Clin Microbiol* 2014;52(12):4353–5.
62. Soto M, Sampietro-Colom L, Vilella A, et al. Economic impact of a new rapid PCR assay for detecting influenza virus in an emergency department and hospitalized patients. *PLoS One* 2016;11(1):e0146620.
63. Jokela P, Vuorinen T, Waris M, et al. Performance of the Alere i influenza A&B assay and mariPOC test for the rapid detection of influenza A and B viruses. *J Clin Virol* 2015;70:72–6.
64. Wang CH, Chang CP, Lee GB. Integrated microfluidic device using a single universal aptamer to detect multiple types of influenza viruses. *Biosens Bioelectron* 2016;86:247–54.
65. Liu J, Zhao J, Petrochenko P, et al. Sensitive detection of influenza viruses with Europium nanoparticles on an epoxy silica sol-gel functionalized polycarbonate-polydimethylsiloxane hybrid microchip. *Biosens Bioelectron* 2016;86:150–5.
66. Hirama T, Minezaki S, Yamaguchi T, et al. HIRA-TAN: a real-time PCR-based system for the rapid identification of causative agents in pneumonia. *Respir Med* 2014;108(2):395–404.
67. Pierce VM, 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(2):364–71.
68. Andersson ME, Olofsson S, Lindh M. Comparison of the FilmArray assay and in-house real-time PCR for detection of respiratory infection. *Scand J Infect Dis* 2014;46(12):897–901.
69. Rappo U, Schuetz AN, Jenkins SG, et al. Impact of early detection of respiratory viruses by multiplex PCR assay on clinical outcomes in adult patients. *J Clin Microbiol* 2016;54(8):2096–103.
70. Gilbert D, Gelfer G, Wang L, et al. The potential of molecular diagnostics and serum procalcitonin levels to change the antibiotic management of community-acquired pneumonia. *Diagn Microbiol Infect Dis* 2016;86(1):102–7.
71. Jamal W, Al Roomi E, AbdulAziz LR, et al. Evaluation of Curetis Unyvero, a multiplex PCR-based testing system, for rapid detection of bacteria and antibiotic resistance and impact of the assay on management of severe nosocomial pneumonia. *J Clin Microbiol* 2014;52(7):2487–92.
72. Kunze N, Moerer O, Steinmetz N, et al. Point-of-care multiplex PCR promises short turnaround times for microbial testing in hospital-acquired pneumonia—an observational pilot study in critical ill patients. *Ann Clin Microbiol Antimicrob* 2015;14:33.
73. Mengelle C, Mansuy JM, Da Silva I, et al. Evaluation of a polymerase chain reaction-electrospray ionization time-of-flight mass spectrometry for the detection and subtyping of influenza viruses in respiratory specimens. *J Clin Virol* 2013;57(3):222–6.
74. Shih HI, Wang HC, Su IJ, et al. Viral respiratory tract infections in adult patients attending outpatient and emergency departments, Taiwan, 2012-2013: a PCR/electrospray ionization mass spectrometry study. *Medicine* 2015;94(38):e1545.
75. Majchrzykiewicz-Koehorst JA, Heikens E, Trip H, et al. Rapid and generic identification of influenza A and other respiratory viruses with mass spectrometry. *J Virol Methods* 2015;213:75–83.
76. La Scola B, Raoult D. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. *PLoS One* 2009;4(11):e8041.