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.

Clinical Microbiology
NEWSLETTER

CMN
Stay Current...
Stay Informed.

CMN
Vol. 40, No. 16
August 15, 2018
www.cmnewsletter.com

IN THIS ISSUE

Lower Respiratory Multiplex Panels for the Detection of Bacterial and Viral Infections

Kevin Alby,[1] Stephanie L. Mitchell,[2,3] [1]Department of Pathology and Laboratory Medicine, The Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, [2]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, [3]Department of Pathology and Laboratory Medicine, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, Pennsylvania

Abstract

Development of commercial multiplex panels for the detection and diagnosis of lower respiratory tract infections is rapidly progressing, and FDA-cleared assays are currently available. This review provides a comprehensive overview of the current or soon-to-be available commercial assays, focusing on their analytical performance, advantages, and challenges and the potential impact on patient outcomes when laboratories deploy the assays.

Introduction

To aid in the detection of various infections, the use of multiplex molecular panels for "syndromic testing," i.e., the testing of specimens for multiple pathogens common to a body site, has become commonplace in the laboratory. Available panels for detection of pathogens in blood, stool, and upper respiratory tract specimens aim to provide rapid identification of bacteria and viruses and, in some instances, to provide preliminary antimicrobial resistance testing for a limited number of antibiotics. Incorporation and widespread use of various respiratory viral panels (RVPs) on nasopharyngeal swabs revolutionized how upper respiratory tract infections are diagnosed and managed. Use of RVPs led to more rapid results, improved use of antiviral treatment, and better hospital infection control practices [1]. After RVP implementation, many laboratories received RVP requests for testing lower respiratory tract (LRT) specimens, such as bronchoalveolar lavage (BAL) fluid, to aid in the diagnosis of viral pneumonia, revealing a diagnostic gap in the syndromic testing available at the time. In response, diagnostic companies developed new molecular multiplex panels for the diagnosis of pneumonia. These panels primarily focus on improving the detection of bacterial pathogens from BAL fluid, sputum, and endotracheal aspirates. Traditional cultures are the gold standard for the detection and diagnosis of pneumonia; however, culture is not a very sensitive method, even when performed from high-quality samples [2]. Given the enhanced sensitivity of molecular methods, the use of a multiplex lower respiratory panel (LRP) could significantly improve upon current culture techniques. This review presents the current and upcoming commercial assays and discusses practical matters the laboratory should consider when deploying testing with an LRP.

Multiplex Lower Respiratory Panels (LRPs)

Laboratory-developed tests

Recent interest in the use of molecular methods for the diagnosis of LRT infections has led to the development of some multiplex laboratory-developed tests (LDTs). While the LDT methods and targets vary widely, it is worth briefly discussing these non-commercial alternatives. Development of LDTs using traditional real-time multiplex PCR assays will be somewhat limited in scope compared to commercial assays, as the number of targets one can reliably detect is approximately 4 or 5 before assay performance significantly suffers. This approach is consistent

Corresponding author: Stephanie L. Mitchell, Ph.D., D(ABMM), 4401 Penn Ave., Main Hospital, Floor B, #269, Pittsburgh, PA 15224. Tel.: 412-692-5068. E-mail: Mitchellsl5@upmc.edu.

with the detection of viral pathogens, specifically for upper respiratory tract specimens [3].

While there is limited development of LDTs for detecting bacterial pathogens of the LRT, semi-quantitative multiplex LDTs for the detection of bacterial pathogens in LRT samples are being developed to aid in the diagnosis of community-acquired pneumonia [4]. Using a combination of multiplex targets (Table 1), Gadsby et al. developed an LDT that panel can detect as many as 26 pathogens from sputum and endotracheal aspirates. Semi-quantification for a subset of bacterial targets is achieved using a standard curve generated by serial dilutions of plasmids containing each gene target, which is used to back-calculate to the bacterial density in terms of CFU per milliliter [4]. A cutoff value was applied, so that any pathogen density of $\geq 10^5$ CFU/ml was considered clinically significant. Using this cutoff, 81.1% of the samples were positive for a respiratory pathogen, while 71.5% were positive for a bacterial pathogen [4]. While there was high concordance between this bacterial PCR and standard culture (127/125; 98.4%), *Haemophilus influenzae* was missed by the PCR in two samples. Additionally, 27 (8.4%) samples grew bacteria that were not included on the PCR panel.

Off-label use of upper respiratory tract panels (RVPs)

Implementation of the RVP for upper respiratory tract samples has stimulated off-label use of the panel for LRT samples, requiring assay validation of LRT specimens [5]. Ruggiero and colleagues compared the performances of the BioFire (Salt Lake City, UT) FilmArray (FA) and GenMark eSensor RVPs (Carlsbad, CA) in retrospective and spiked LRT specimens. For the 52 retrospective samples, 30 were positive via viral culture, 35 via FA, and 34 via eSensor. The FA detected two coinfections, while the eSensor detected four [5]. In another study, the eSensor, Quidel (San Diego, CA) Lyra, and Panther (San Diego, CA) Fusion were compared using 105 LRT specimens, where a positive result in two of the three assays was considered a "true positive" [6]. While the Fusion and eSensor had 100% positive agreement for all targets tested (influenza A virus, influenza B virus, respiratory syncytial virus, parainfluenza virus types 1 to -3, human metapneumovirus, adenovirus, and rhinovirus), the Lyra had a variable positive agreement ranging from 50 to 100%. The negative agreement ranged between 96.1 and 100%, 79.4 and 100%, and 98.9 and 100% for the Fusion, eSensor, and Lyra respectively [6]. Other studies have included LRT samples in larger evaluations of commercially approved viral panels, noting similar performance regardless of specimen type [7-9].

BioFire

The FA Pneumonia Panel (FAPP) by BioFire Diagnostics (Salt Lake City, UT) was recently cleared by the U.S. Food and Drug Administration (FDA) for use in detecting LRT infections. The FAPP uses nested multiplex PCR to detect common bacterial and viral respiratory pathogens and also includes a selection of genetic antibiotic resistance markers, such as *mecA* and carbapenemases (Table 1). The assay can test both sputum-like (sputum and endotracheal aspirate) and BAL fluid-like (BAL fluid and bronchoalveolar wash) samples. Unique to the assay is its ability to provide semi-quantitative results, albeit this quantification is only for a subset of bacterial targets (Table 1).

The concept behind semi-quantitative results mimics the current laboratory practice that uses semi-quantitative culture. Semi-quantitation allows the interpretation of relative densities of specific bacteria to aid in the clinical interpretation of the molecular results. The semi-quantitative function is achieved by utilizing a binning algorithm based on the relative amount of quantitated target PCR amplicon compared to a known concentration of an internal standard [10]. The limit of quantification ranges from $<10^4$ to $\geq 10^7$ copies/ml, with values of $<10^{3.5}$ copies/ml considered a negative result. A negative result is similar to that of traditional quantitative BAL fluid culture, where only colonies that are $\geq 10^4$ /CFU/ml are considered clinically significant [11]. Each bin in the algorithm represents a density range of about 1 log unit, with upper and lower limits (i.e., 10^4 bin = $10^{3.5}$ to $10^{4.5}$) [12]. Additionally, resistance markers are reported only if the bacterial species that can harbor the resistance gene is present at a density above the cutoff (i.e., samples that are only *Pseudomonas aeruginosa* positive would not report *mecA* resistance).

At the time of writing, only a few studies had evaluated the performance of the FA. Initial studies showed >90% sensitivity for most targets on the panel in both sputum and BAL [13]. Some notable exceptions were 75% and 85.7% sensitivity for *Enterobacter aerogenes* in BAL fluid and sputum, respectively. Of the viral targets, adenovirus had low sensitivity in sputum (76.5%) and coronavirus sensitivity was 85.7% and 87.5% in BAL fluid and sputum, respectively [13]. Additionally, a common extended-spectrum β-lactamase recovered in clinical laboratories, CTX-M, had low sensitivities of 85.7% and 80% in both sample types [13]. Of note, some targets could not be assessed because no positive samples were included. These targets includes *Acinetobacter* species and *Moraxella catarrhalis* in BAL samples. There were no samples positive for Middle East respiratory syndrome coronavirus, *Chlamydia pneumoniae*, or the resistance markers IMP and OXA-48-like. While only one sample positive for each target was tested, the FA did not detect New Delhi metallo-beta-lactamase (NDM) in a BAL sample and missed a *Legionella pneumophila* from sputum. All the targets were >90% specific, except for the *mecA-mecC* and MREJ resistance markers in sputum [13].

A separate study found similar performance rates for the bacterial targets compared to the standard of care (culture and PCR methods), where the overall percent positive agreement (PPA) was 94.7% (BAL fluid) and 95.8% (sputum) and the negative percent agreement was 98.6% (BAL fluid) and 96.5% (sputum) [14]. Viral target evaluation was limited, and antibiotic markers were not assessed in the study. Interestingly, targets for *P. aeruginosa* and *Staphylococcus aureus* had a lower than average PPA of 75% (BAL fluid) and 88.9% (sputum), respectively, which were not the same problematic targets observed in the studies by Kerr and Faron [13,14]. Additionally, while false-positive results were observed for the FA, the majority (18/34; 52.9%) were from a patient who had

Table 1. Lower respiratory panel targets

	Target[b]		
LRP[a]	**Bacteria**	**Viruses**	**Antimicrobial resistance markers**
Biofire	*Acinetobacter calcoaceticus-baumannii* complex *Serratia marcescens* *Proteus* spp. *Klebsiella pneumoniae* group *Enterobacter aerogenes* *Enterobacter cloacae* *Escherichia coli* *Haemophilus influenzae* *Moraxella catarrhalis* *Pseudomonas aeruginosa* *Staphylococcus aureus* *Streptococcus pneumoniae* *Klebsiella oxytoca* *Streptococcus pyogenes* *Streptococcus agalactiae* *Legionella pneumophila* *Mycoplasma pneumoniae* *Chlamydia pneumoniae*	Influenza A virus Influenza B virus Respiratory syncytial virus Rhinovirus/enterovirus Human metapneumovirus Parainfluenza virus Adenovirus Coronavirus Middle East respiratory syndrome coronavirus[c]	*mecA/C* and MREJ KPC NDM Oxa48-like CTX-M VIM IMP
Unyvero	*Acinetobacter* spp. *Chlamydophila pneumoniae* *Citrobacter freundii* *Enterobacter cloacae* complex *Escherichia coli* *Haemophilus influenzae* *Klebsiella oxytoca* *Klebsiella pnuemoniae* *Klebsiella variicola* *Legionella pneumophila* *Moraxella catarrhalis* *Morganella morganii* *Mycoplasma pneumoniae* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens* *Stapylococcus aureus* *Stenotrophomonas maltophilia* *Streptococcus pneumoniae*	None	KPC NDM Oxa-23 Oxa-24/40 Oxa-48 Oxa-58 VIM CTX-M *mecA* TEM
Gadsby et al. LDT	*Acinetobacter baumannii* *Klebsiella pneumoniae* *Escherichia coli* *Haemophilus influenzae* *Moraxella catarrhalis* *Pseudomonas aeruginosa* *Staphylococcus aureus* *Streptococcus pneumoniae* *Streptococcus agalactiae* *Legionella pneumophila* *Legionella* spp. *Mycoplasma pneumoniae* *Chlamydia pneumoniae* *Chlamydophila psittaci*	Influenza A virus Influenza B virus Respiratory syncytial virus Rhinovirus Human metapneumovirus Parainfluenza virus 1–3 Adenovirus Coronavirus (229E, HKU1, NL63, OC43)	None

[a] Upper respiratory tract panels used off label with LRT specimens are excluded.
[b] Bacterial, viral and resistance marker targets that available on each panel are listed. Boldface targets are semi-quantifiable results.
[c] Target is not available in the United States.

received antibiotic therapy for >12 hours before sample collection, which likely explains the negative culture results [14].

Curetis

The Curetis Unyvero (Curetis, GmbH) system was cleared earlier this year (2018) with an LRT panel for testing endotracheal aspirates. The Unyvero system consists of a lysator instrument for prepping the sample, an analyzer for testing the cartridge, and a cockpit system for controlling the process and interfacing with the laboratory information system (LIS). The cleared LRT panel contains 19 bacterial targets and 10 markers of resistance (Table 1). Importantly, the system does not provide any type of quantitative information, simply reporting the presence or absence of the target. To date, the most extensive study was a multicenter clinical trial study that tested 603 prospective tracheal aspirate samples [15]. In this study, 312 samples were positive for 1 or more pathogens. The overall weighted sensitivity for organism identification compared to culture was 92.5%, with a range of positive agreement of 25 to100%. Excluding organisms with fewer than 10 identifications in the sample set (7 of 16), the range of positive agreement was 87.5 to100%. The weighted specificity was 97.4%, with a range of negative agreement from 92.5 to 99.7%. Importantly, of the 239 positive LRT detections, 213 (89%) were confirmed via targeted PCR followed by bidirectional sequencing. For determination of resistance markers, the LRT had an overall weighted sensitivity of 93%, with a range of positive agreement ranging from 85.7 to 100%. The overall weighted specificity was 98.8%, with a negative percent agreement range from 86.9 to100%. Contrived samples were utilized to augment low-frequency targets and had a range of positive agreement of 86 to 100%.

Collins reported results from a single-center study examining the performance of the LRT with 127 BAL samples [16]. The positive agreement with culture ranged from 50 to 100% and negative agreement from 93 to 100%.

Advantages and Challenges to Implementation

Advantages

One main advantage of having FDA-approved panels for LRT samples is that it eliminates some of the barriers to performing LRT molecular diagnostics outside larger hospitals and laboratories. By removing the development, validation, and regulatory requirements of LDTs or off-label use of upper respiratory tract panels, FDA approval of LRT panels allow better diagnosis and treatment in the local community hospital setting.

The goal of diagnostic testing is to provide information that has a direct impact on patient care. The molecular revolution in microbiology has had a significant impact by allowing quicker identification of potential pathogens, which results in downstream benefits, such as improved antibiotic utilization. One small retrospective study was conducted to assess the potential impact of the FAPP on antimicrobial modification and prescribing versus standard of care culture and PCR methods. The study found that for 47.9% of the patients, use of the assay could result in more appropriate antibiotic de-escalation and a significant reduction in time to de-escalation [14]. Use of the FAPP to guide antibiotic therapy was estimated to have a minimal risk for inappropriate modifications [17]. Of note, the study also found that for 46.9% of the patients, there was no potential change in therapy if the FAPP was used. A single-center study performed at Beaumont Hospital in Michigan reported on the theoretical impact of the LRT panel on the management of patients with suspected pneumonia [18]. This study utilized information from the panel that was submitted to the FDA and so included some targets not ultimately approved. An infectious disease physician evaluated patient charts as to the appropriateness of antibiotic therapy concerning their final cultured organism at the time the LRT result would have been available. Only 15% of patients had appropriate antibiotics at the time an LRT result would have been available, with 63% being overtreated and 22% undertreated. Seventy-six percent of the undertreated cohort were modifiable based on an LRT result.

When assessing the performance of the LDT for pneumonia, Gadsby and colleagues also retrospectively assessed the potential impact of the LDT on antimicrobial prescribing [4]. Upon review, 84.8% of the study subjects had antimicrobials <72 hours before the LRT sample was obtained. Indeed, the LDT was able to detect bacteria in 77.6% of the pre-treated patients, while culture was positive in only 32.1% [4]. As with the FAPP, the biggest impact of the LDT was in the potential for more appropriate de-escalation of antimicrobials (77.2% of the patients). There was a smaller impact on the potential for escalation (5.9%), and 16.9% had no potential change. While additional, more comprehensive studies are needed, these preliminary studies strongly suggest a benefit to using the LRP, either as a commercial assay or as an LDT, to improve antibiotic stewardship and improved patient outcomes.

Challenges

While there are some clear advantages to implementing an LRP, there are challenges that should be addressed by the laboratory before adopting the test. Similar to other multiplex panels, an LRP includes a large number of targets, which raises the concern of variable sensitivities of each target, some of which may be unacceptable. Variable detection appears to be present for some viral and antibiotic resistance targets on the FAPP [13]. While this may not discourage the laboratory from implementing these panels, it is important to be aware of sensitivity issues when determining the utility of the panel for certain patient populations, interpretation of negative results, and obtaining a reliable negative predictive value. Furthermore, the breadth of these panels results in the inclusion of targets that are clinically distinct and that often apply to specific patient populations. The inclusion of both hospital-acquired and community-acquired pathogens presents a challenge in deciding when and on which patients the test should be performed.

When traditionally culturing endotracheal aspirates and sputum samples, laboratories include acceptability specimen criteria to determine if the specimen is appropriate for culture. For sputum, calculation of a white blood cell/squamous epithelial cell ratio is performed via Gram stain to ensure the sputum is not saliva and represents an LRT specimen [19]. Endotracheal aspirates undergo a similar quality check via Gram stain, where the presence or absence of bacteria in the context of white blood cell quantity is

used [20,21]. While there are internal controls included on these panels, they do not serve as a specimen quality assessment, and no targets for white blood cells or squamous cells are included. The ommission of quality assessment may result in testing specimens of inferior quality or inappropriate samples. The laboratory should strongly consider the continued use of Gram stains to assess specimen quality prior to running the molecular panels.

Another challenge is the interpretation of positive results in the absence of an assessment of normal flora. An advantage to traditional culture is its unbiased nature, with both commensals and pathogens recovered. Interpretation of pathogen significance from an LRT specimen is often guided by the presence (or absence) of normal flora and its amount compared to the pathogen in question. This is especially true for those organisms that are both colonizers and opportunistic pathogens, such as *Streptococcus pneumoniae*, *M. catarrhalis*, and *H. influenzae*. Determination of these organisms as true pathogens is often challenging, particularly for pediatric patients, where colonization rates are higher than for adults. Placing the density of these potential pathogens in the context of the density of normal flora is helpful for clinicians when attempting to identify the cause of pneumonia. While the semi-quantitative bacterial results aim to address this concern, assays still lack the ability to definitively interpret organisms that are colonizers. This issue is also a potential problem for patients who have chronic tracheostomies, where over time, the tracheostomy tube becomes colonized, commonly with Gram-negative bacteria. This condition is currently a challenge for proper interpretation of traditional endotracheal aspirate cultures and could be even more confounding when using the LRP.

While most commercial assays include some resistance markers, specimens still need to be cultured in order to perfrom antimicrobial susceptibility testing. Limited studies suggest the potential impact of LRP panels in de-escalating antibiotics, but studies document only a small to modest impact on antimicrobial escalation, with a significant percentage of patients having no modifications to therapy when the LRP is used. Given that these samples still require traditional culture, laboratory workflow and budget will be impacted. Concerns about how to incorporate LRP into the workflow will likely affect the ability of the assay to impact patient care. Table 2 provides critical questions that should be addressed before the LRP is adopted.

Conclusions

Given the challenges and limitations, the LRP is not likely to completely replace traditional culture and will, therefore, be a supplemental method. This limitation adds cost to the workup and diagnosis of LRT infections and will likely have a significant impact on laboratory budget and workflow. However, the ability to significantly impact antibiotic use and modify to provide more appropriate therapy early in the disease course. If this occurs, it could dramatically improve patient outcomes. Formation of diagnostic stewardship teams that include clinical microbiology, antibiotic stewardship pharmacists, infection control practitioners, and infectious disease physicians will be beneficial to establish institutional practices guidelines for LRP implementation, thereby ensuring the prudent and appropriate use of LRPs. Larger, multicenter outcome studies are needed to fully assess the impact of LRPs on patient care and hospital costs to determine if the advantages of LRPs justify the cost.

References

[1] Rogers BB, Shankar P, Jerris RC, Kotzbauer D, Anderson EJ, Watson JR, et al. Impact of a rapid respiratory panel test on patient outcomes. Arch Pathol Lab Med 2015;139:636-41.

[2] Marimon JM, Morales M, Cilla G, Vicente D, Perez-Trallero E. Detection of bacteria and viruses in the pleural effusion of children and adults with community-acquired pneumonia. Future Microbiol 2015;10:909-15.

[3] Anderson TP, Werno AM, Barratt K, Mahagamasekera P, Murdoch DR, Jennings LC. Comparison of four multiplex PCR assays for the detection of viral pathogens in respiratory specimens. J Virol Methods 2013;191:118-21.

[4] Gadsby NJ, Russell CD, McHugh MP, Mark H, Conway Morris A, Laurenson IF, et al. Comprehensive molecular testing for respiratory pathogens in community-acquired pneumonia. Clin Infect Dis 2016;62:817-23.

[5] Ruggiero P, McMillen T, Tang YW, Babady NE. Evaluation of the BioFire FilmArray respiratory panel and the GenMark eSensor respiratory viral panel on lower respiratory tract specimens. J Clin Microbiol 2014;52:288-90.

Table 2. Laboratory questions to be addressed prior to LRP adoption

When to test	Who to test	What to test	Question related to reporting results and ensure proper interpretation
• Is the LRP run only on day shift versus all shifts? • Is there staffing available to run 24/7? • Appropriately trained staff? • What is the proper length of time before repeat testing can be performed?	• All patients • All inpatients • A pre-defined subset of high-risk patients • By approval only	• All LRT samples or a subset? • Will the laboratory perform specimen quality assessment via Gram stain? • Do the specimen assessment criteria need to be modified for LRP use? • Is testing performed only on first-time specimens or repeat specimens?	• Enter into the LIS only? • Phone the primary physician? • Phone diagnostic or antibiotic stewardship? • Are results to be treated as a critical value? • Should results be incorporated into final culture results? • Should testing for biomarkers (i.e., C-reactive protein, procalcitonin) be performed in conjunction with the LRP?

[6] Sam SS, Caliendo AM, Ingersoll J, Abdul-Ali D, Hill CE, Kraft CS. Evaluation of performance characteristics of Panther Fusion assays for the detection of respiratory viruses from nasopharyngeal and lower respiratory tract specimens. J Clin Microbiol JCM.00787-18; Accepted manuscript posted online 23 May 2018, doi:10.1128/JCM.00787-18.

[7] Hammond SP, Gagne LS, Stock SR, Marty FM, Gelman RS, Marasco WA, et al. Respiratory virus detection in immunocompromised patients with FilmArray respiratory panel compared to conventional methods. J Clin Microbiol 2012;50:3216-21.

[8] Rand KH, Rampersaud H, Houck HJ. Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. J Clin Microbiol 2011;49:2449-53.

[9] Costa E, Rodríguez-Domínguez M, Clari MA, Gimenez E, Galan J, Navarro D. Comparison of the performance of 2 commercial multiplex PCR platforms for detection of respiratory viruses in upper and lower tract respiratory specimens. Diagn Microbiol Infect Dis 2015;82:40-3.

[10] Buccambuso M. Precision of the FilmArray Pneumonia Panel: considerations for interpreting relative abundance of bacterial nucleic acids in lower respiratory specimens. Basel: European Society of Clinical Microbiology and Infectious Diseases; 2018.

[11] Gilligan PH, Alby K, York MK. Guidelines for performance of respiratory tract cultures. Washington, DC: ASM Press; 2016.

[12] Judd D. Verification of BioFire FilmArray Pneumonia Panel plus detections by alternate methods. Basel: European Society of Clinical Microbiology and Infectious Diseases; 2018.

[13] Kerr S. Clinical evaluation of the BioFire FilmArray Pneumoniae Panel plus. Paper presented at: European Society of Clinical Microbiology and Infectious Diseases Meeting; 2018; Basel, Switzerland.

[14] Faron ML. Clinical evaluation of a semi-quantitative multiplex molecular assay for the identification of bacteria, viruses and fungi in lower respiratory specimens. Paper presented at: European Society of Clinical Microbiology and Infectious Diseases Meeting; 2018; Basel, Switzerland.

[15] Qi C. Multicenter clinical trial of the Unyvero lower respiratory tract infection application. Paper presented at: European Society of Clinical Microbiology and Infectious Diseases Meeting; 2018; Basel, Switzerland. Paper presented at: European Society of Clinical Microbiology and Infectious Diseases Meeting; 2018; Basel, Switzerland.

[16] Collins ME. Comparison of a multiplex PCR lower respiratory tract assay with bacterial culture. Clinical Virology Symposium; 2018; Palm Beach, Florida.

[17] Huang AM. Potential clinical impact of a semi-quantitative multiplex molecular assay for the identification of bacteria, viruses, and fungi in lower respiratory specimens. Paper presented at: European Society of Clinical Microbiology and Infectious Diseases Meeting; 2018; Basel, Switzerland.

[18] Agarwal M. Potential impact of rapid diagnostics in management of suspected pneumonia. Paper presented at: European Society of Clinical Microbiology and Infectious Diseases Meeting; 2018; Basel, Switzerland.

[19] Linscott AJ. Specimen collection, transport, and acceptability. Washington, DC: ASM Press; 2016.

[20] Zaidi AK, Reller LB. Rejection criteria for endotracheal aspirates from pediatric patients. J Clin Microbiol 1996;34:352-4.

[21] Morris AJ, Tanner DC, Reller LB. Rejection criteria for endotracheal aspirates from adults. J Clin Microbiol 1993;31:1027-9.