Diagnostic Stewardship for Multiplex Respiratory Testing

What We Know and What Needs to Be Done

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KEYWORDS

• Syndromic panels • Respiratory infections • Pneumonia • Diagnostic stewardship • Antimicrobial resistance • Clinical utility

KEY POINTS

- Multiplex syndromic panels can rapidly diagnose infections and detect antimicrobial resistance genes allowing for more rapid therapeutic optimization.
- Randomized controlled trials evaluating respiratory and pneumonia syndromic panels in a variety of clinical settings have generated mixed results regarding the clinical utility suggested by observational studies.
- Employing diagnostic stewardship interventions to improve appropriate clinician ordering, test interpretation, as well as laboratory specimen processing, testing, and reporting can increase clinical utility.
- Stakeholders including laboratory, antimicrobial stewardship programs, clinical end users, hospital leadership, infection prevention specialists, and information and technology specialists need to be involved in active diagnostic stewardship.

INTRODUCTION

Every year, respiratory infections are a leading cause of disease and account for many medical visits. While most respiratory illnesses are caused by viral etiologies, pneumonia can be caused by various pathogens, including bacteria, viruses, and fungi. Laboratory diagnosis of respiratory infections includes a combination of routine bacterial, fungal, and mycobacteriology cultures from respiratory specimens. Viral

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cultures that attempt to grow and identify viruses are also available although now seldom used due to long turnaround times and expertise and facility requirements needed by the laboratory. Other laboratory approaches involve immunologic assays such as direct fluorescence antibody (DFA) testing , indirect fluorescence antibody (IFA) testing, and enzyme immunoassays that detect specimen antigenic proteins on the surface of pathogens, as well as serologic tests that detect antibodies produced by the human host against a specific pathogen.

More recently, the adaptation of molecular methods such as polymerase chain reaction (PCR) has revolutionized the etiologic diagnosis of infectious diseases. There are several US Food and Drug Administration (FDA)–approved multiplex PCR panels for the detection of pathogens causing upper (>12 targets consisting of bacterial, atypical, and viral targets) as well as lower respiratory infections such as pneumonia (>15 targets consisting of bacterial, viral, and fungal targets plus select antimicrobial resistance [AMR] genes). Due to their high analytical sensitivity and short turnaround time, syndromic respiratory panels can increase the likelihood of establishing a prompt etiologic diagnosis for patients presenting with respiratory infections and have the potential to enable a more appropriate antimicrobial treatment strategy. Other benefits include support for clinical decision-making in areas such as admission, isolation, and the need for additional workup.[1,2]

Approaches to optimal implementation of syndromic respiratory panels remain unclear as there are several potential drawbacks associated with their use.[3] First, detection of an organism's nucleic acid in a multiplex PCR panel does not necessarily represent the etiologic diagnosis, while prolonged shedding of viral particles after symptoms have resolved is common. Second, detection of a viral pathogen does not exclude the possibility of bacterial coinfection. Third, a sizable number of patients that undergo syndromic respiratory panel testing have multiple targets detected at once, and interpreting these results can be a challenge for clinicians. Fourth, results from an upper respiratory panel may not correlate with the etiology of a lower respiratory tract (LRT) infection. Fifth, syndromic panels are associated with a hefty cost despite the uncertain impact on relevant health outcomes. And finally, most respiratory viruses detected in syndromic panels do not have targeted antiviral therapies available, and clinicians often do not change treatment plans based on results. With such caveats in mind, reducing diagnostic error includes the concept of overdiagnosis (ie, inability to distinguish colonization from infection) and overtreatment (ie, unnecessary workup and antibiotic therapy). Therefore, diagnostic stewardship is paramount for maximum clinical utility of these panels and can be summarized as ordering the right tests for the right patient at the right time to correctly inform clinical decision-making.

Stakeholders beyond the laboratory and antimicrobial stewardship programs (ASPs) need to be involved in various stages of the diagnostic stewardship process, including information and technology specialists, hospital leadership, end users (ie, clinicians and nurses), and infection prevention specialists. Recently, the Society for Health care Epidemiology of America convened a Diagnostic Stewardship Task Force to develop a position paper on further development of the diagnostic pathway conceptual model, including the following steps: clinician testing decision and interpretation, test ordering, specimen collection and transport to the laboratory, test processing and performance, and test reporting.[4] In this article, we will follow the diagnostic pathway conceptual model as we review the available evidence related to the optimal use of syndromic respiratory panels.

UPPER RESPIRATORY PANELS

Clinician Testing Decision, Ordering, and Achieving Actionable Outcomes: What We Know

In the context of individual patient care, testing with multiplex respiratory PCR panels (RVP) is restricted to symptomatic patients as studies have demonstrated virus shedding in asymptomatic hosts. Consecutive studies performed in adults visiting a New York City tourist attraction found that at least half of the samples were positive in asymptomatic individuals.[5,6] Positive samples included human rhinovirus, non-severe acute respiratory syndrome (SARS) coronaviruses, influenza virus, respiratory syncytial virus (RSV), and parainfluenza virus. The authors did find, however, that having symptoms was predictive of testing positive among this ambulatory adult population. Additionally, in a case-control study of children, 72% of symptomatic patients but also 35% of the asymptomatic controls tested positive for respiratory viruses, particularly for rhinovirus/enterovirus.[7] These findings highlight that results from RVP must be interpreted with caution due to high detection rates among individuals without respiratory symptoms.

In 2020, the Infectious Diseases Society of America's Diagnostics Committee (IDSA-DC) published a position statement that provided a framework for ordering molecular testing for acute respiratory tract infections which considered factors such as immunosuppression, severity of illness, underlying comorbidities, pretest probability of a given pathogen, and anticipated turnaround time to results.[8] The IDSA-DC statement highlighted that testing for viral pathogens other than influenza through RVPs is not recommended for the general pediatric and adult population presenting with an acute respiratory infection but should be considered in immunocompromised hosts and in those with severe illness. Along those lines, the American Thoracic Society published a guidance statement in 2021 addressing testing of respiratory samples for non-influenza respiratory viruses in adults with suspected community-acquired pneumonia (CAP).[9] They recommended against routine use of RVPs in the general population but suggested considering hospitalized patients with severe CAP and immunocompromised hosts while acknowledging the low quality of available evidence.

In the absence of significant risk factors for unfavorable clinical outcomes, clinical benefits from RVP testing remain mixed. There is a plethora of observational studies evaluating the potential impact of RVPs on clinical outcomes, with notable limitations and inconclusive results. Only a few prospective studies with slight clinical benefits have been published (**Table 1**). An open label, single center randomized clinical trial (RCT) found that the use of an RVP in hospitalized adults with acute LRT infection only modestly reduced the duration of intravenous antibiotics when compared with the use of routine PCR testing for common pathogens.[10] Another RCT including both children and adults presenting to an emergency department with acute LRT infection found that those tested by the RVP had fewer antibiotic prescriptions and fewer complementary studies (seen in children), with improved antiviral management of participants with influenza.[11]

In contrast, an open label, multicenter RCT conducted in adult outpatients with acute respiratory infections found that implementing an RVP did not reduce antibiotic prescription rates.[12] Another RCT evaluated adults presenting to a hospital in the United Kingdom with acute respiratory illness and fever found no difference in the proportion of patients treated with antibiotics and the mean durations of antibiotic use between both groups, though the intervention group had a shorter length of stay and improved use of antiviral treatment for those with influenza.[13] Additionally, significantly fewer patients in the multiplex PCR group received more than 1 dose of antibiotics.[13]

Table 1
Overview of studies reporting the outcomes of multiplex respiratory viral panels for upper respiratory tract infections

Population	Location	Study Design	Outcomes Impacted	Active ASP Present	Reference
Adults	Inpatients (China)	Open-label, prospective, single-center RCT	Modestly reduced the duration of intravenous antibiotics	Yes	Shengchen et al,[10] 2019
Children and adults	Emergency Department (Argentina)	Open-label, prospective, single-center RCT	Fewer antibiotic prescriptions, fewer complementary studies (in children), and improved antiviral management for influenza	Yes	Echavarría et al,[11] 2018
Adults	Outpatients (Sweden)	Open-label, prospective, multi-center RCT	No reduction in antibiotics	Not specified	Brittain-Long et al,[12] 2011
Adults	Emergency Department or Acute Medical Unit (UK)	Open-label, prospective, open-label, single-center RCT	No difference in the proportion of patients treated with antibiotics and the mean durations of antibiotic use, shorter length of stay, improved use of antiviral treatment for those with influenza	Not specified	Brendish et al,[13] 2017
Adults	Emergency Department (France)	Open-label, prospective, single-center RCT	No difference in the duration of antibiotic therapy between groups	Yes	Velly et al,[37] 2023
Adults	Inpatients (US)	Open-label, single-center RCT	No difference in antibiotic use (even with procalcitonin results), no difference in antibiotic use in hospitalized	Not specified	Branche et al,[36] 2015

Children	Inpatients (US)	Retrospective	Duration of antibiotic use shorter (in group with TAT <7 h)	Not specified	Rogers et al,[2] 2015
Adults	Emergency Department (US)	Retrospective	Reduced antibiotics in positive test admitted without radiographic findings (in group with TAT <7 h)	Yes	Weiss et al,[20] 2019
Children and Adults	Inpatient and outpatients (Turkey)	Retrospective	Higher rate of antibiotic discontinuation and lower antibiotic prescription	Yes	Keske et al,[15] 2018
Adults	Inpatients (Canada)	Retrospective	Reduction of antibiotic treatment duration with ASP	Yes	Lowe et al,[16] 2017

Abbreviations: ASP, antimicrobial stewardship program; hrs, hours; RCT, randomized controlled trial; TAT, turnaround time; UK, United Kingdom; US, United States.

In general, interventions to optimize diagnostic testing at the testing decision level include educational activities and clinician decision support tools. A recent survey of hospital epidemiology and infectious disease experts showed that nearly half of respondents did not believe that RVP improved clinical outcomes, despite other perceived benefits related to diagnosis and patient care.[14] Also, 58% of surveyed sites had implemented diagnostic stewardship to enhance the usefulness of RVPs, with education being the most common intervention (54%) but was perceived as having limited impact. Other interventions included order sets to guide test ordering, restrictions on test ordering based on clinician or patient characteristics, or structured communication of results. Along with heterogeneous prospective data assessing clinical outcomes of RVPs, the limited data on the impact of potential interventions in optimizing RVP utility have also shown mixed results.

Some retrospective interventional studies in hospitalized patients found a significantly higher rate of antibiotic discontinuation as well as an overall decrease in the total number of antibiotic prescriptions in both children and adults following implementation of the RVP plus education by the ASP.[15] An acceptance rate as high as 77% for ASP recommendations and a reduction of antibiotic treatment duration in patients with viral respiratory infections were demonstrated when there was an active targeted ASP audit and feedback.[16] However, in contrast, retrospective studies assessing the impact of real-time ASP pharmacist intervention on antibiotic de-escalation, change, or discontinuation showed that clinicians only accepted 19% to 47% of ASP recommendations.[17,18] These findings suggest that resource-intensive interventions such as direct audit-and-feedback in patients with positive RVP by trained antimicrobial stewardship providers warrant further study before widespread implementation.

Individuals presenting with an influenza-like viral illness may benefit from testing with focused molecular testing, such as influenza A/B and RSV. This includes potential candidates for antivirals during high influenza virus activity (ie, age$\geq$65 years, history of chronic pulmonary disease, immunocompromised hosts) and patients with risk factors for complications from RSV infection (ie, history of stem cell transplant, hematologic malignancy on chemotherapy, infants<6 months). Options include primary testing for specific, single viruses (when prevalence is high) with reflex syndromic testing if initial testing is negative. Nonetheless, the American Academy of Pediatrics does not recommend RSV testing for children presenting with bronchiolitis.[19] Other variables that need to be considered include patient age, acuity of infection, vaccination status, and virus seasonality and epidemiology. Some individuals may also require testing for public health, work, or school-related reasons. Additionally, besides influenza and RSV, supportive care is typically recommended for other viral etiologies which may not warrant the need for a multiplex PCR panel, unless the test results may aid in the avoidance of unnecessary antibiotic therapy. Even in the context of SARS coronavirus 2 (SARS-CoV-2), focused molecular respiratory tests can be performed to assist in clinical decisions related to clinical interventions.

Specimen Collection, Transport, and Processing

All commercially available RVPs are validated for nasopharyngeal swabs (NPSs) (**Table 2**). NPSs are to be immediately placed into acceptable transport media, which is typically viral transport media or universal transport media but can also be in saline, M4 media, M4RT media, or into the Liquid Amies (ESwab). Specimens should be tested as soon as possible but can be refrigerated for 72 hours to 7 days and frozen at −70C for years with relatively low impact on stability. However, studies have also shown that reduced antibiotic usage and duration were seen only when test results

Table 2
Laboratory targets for diagnostic stewardship

	Upper Respiratory Viral Panel	Pneumonia Panel
Define acceptable specimens	• Nasopharyngeal swabs (manufacturer-validated) • Lower respiratory specimens (eg, BAL, sputum) if in-house validated	• BAL (including mini-BAL) • Sputum (induced, expectorated sputum, or endotracheal aspirates)
Additional concordant laboratory testing	• Not applicable	1. Gram-stains to determine sputum specimen quality. 2. Respiratory culture
Test performance concerns	• Reported low sensitivity for non–SARS-CoV-2 coronavirus, human metapneumovirus, adenovirus. Reflex testing in immunocompromised patients could be warranted. • Reported low sensitivity for atypical bacteria: *C. pneumoniae, M. pneumoniae, L. pneumophila* and *Bordetella pertussis.* Reflex testing in sputum or oropharyngeal swabs in appropriate patient population could be warranted.	• PPA and NPA varies as gold standard is culture. Molecular methods are more sensitive making difficult to distinguish non-viable cells vs colonizer vs pathogen • Low concordance rate in lower semiquantitative bins ($<10^5$ DNA copies/mL)
Test reporting	• Qualitative test: 'Detected' vs 'Not detected'	• Qualitative test: 'Detected' vs 'Not detected' • Semiquantitative bins for positive bacterial targets: 10^4, 10^5, 10^6, or $\geq 10^7$ copies/mL
Support for interpretation	• Institutional guidelines	• Provide a comment regarding semiquantitative units (in copies/mL) not being equivalent to CFU/mL • Provide a comment regarding low amounts of bacteria potentially being indicative of colonization or normal respiratory flora • Provide interpretation linking the organism and the antimicrobial susceptibility gene and therapeutic comments devised with ASP and ID teams • Institutional guidelines

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Table 2
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	Upper Respiratory Viral Panel	Pneumonia Panel
Nudges and alerts	• Best Practice Alerts devised with ASP and IC/IP	• Best Practice Alerts devised with ASP and IC/IP
Selective reporting	• Not applicable	• Semiquantitative bins • Antimicrobial resistance genes
Framing results	• Inclusion of procalcitonin levels	• Inclusion of Gram-stain results, WBC count, and procalcitonin levels
Repeat testing	• Potential hard stop with a 10-d block • Approval with new or worsening symptoms • Testing on a BAL specimen after a negative result from NPS	

Abbreviations: ASP, antimicrobial stewardship program; BAL, bronchoalveolar lavage; CFU/mL, cell-forming units per milliliter; IC/IP, infection control and infection prevention; NPA, negative percent agreement; NPS, nasopharyngeal swabs; PPA, positive percent agreement; WBC, white blood cell.

were posted less than 7 hours after time of specimen collection suggesting that utility is greatest when testing is in-house, as opposed to being performed in reference laboratories where turnaround times can be a few days.[2,20] Some laboratories have validated the off-label use of lower respiratory specimen types such as sputum, bronchoalveolar lavage fluid samples (BAL), and bronchial washings on upper respiratory panels. Pre-processing steps such as digestion with a solution containing dithiothreitol was shown to help with specimens that originally yielded an invalid result.

Test Performance

While the overall sensitivity of RVPs is high, there are some viral targets that have slightly lower accuracy such as the non–SARS-CoV-2 coronavirus, human metapneumovirus, and adenovirus.[21,22] In immunocompromised patients, the decreased sensitivity and negative predictive value for adenovirus may miss early intervention before progression to systemic infection. Retesting negative specimens on a single-plex PCR testing showed an additional 5% increase in adenovirus cases, typically those with low viral load (Ct values >30, <106 copies/ml) and adenovirus genotypes A, D, and F.[23] The sensitivity for influenza using the BioFire FilmArray respiratory panel was greater than 73% but the updated RP.2 panel improved detection to greater than 94%.[24,25]

The other targets with variable performance are the atypical bacteria (*Chlamydia pneumoniae, Mycoplasma pneumoniae,Legionella pneumophila*) and *Bordetella pertussis*. *M pneumoniae* clinical sensitivities range from 64% to 98% depending on the platform used. The specimen type can also significantly affect clinical sensitivity.[26] Sputum was recommended for PCR detection of *M pneumoniae* by the British Thoracic Society Guidelines in 2009 after it was shown to have the highest positivity rate in a study comparing sputum, NPS, and throat swab.[27] The sensitivity for sputum versus NPS for *M pneumoniae* was 95.2% and 38.1%, respectively, whereas the specificity was 100% and 93.9%, respectively. For *C pneumoniae,* the sensitivity for sputum is greater than 95% compared to greater than 30% for NPS.[28] The sensitivity is generally greater than 80% when LRT specimens, not NPS, are tested.[29]

For detection of *B pertussis,* RVPs may use the pertussis toxin promoter target which is detected typically in highly concentrated samples (Ct value <27.0). The

insertion sequence IS*481* can be used instead but IS*481* is also present in *B holmesii* and in some *B bronchiseptica*.[30] Compared to a single-plex PCR, the BioFire FilmArray RVP panel detected approximately 30% less cases.[31] In contrast, the QIAstat-DX RP assay which utilizes the IS481 insertion sequence showed 100% sensitivity in a study where the GenMark ePlex RPP assay only detected 66.7% of the specimens.[32] Hence, it is important for the clinical teams and microbiology laboratories to understand their panel targets' limitations. If clinical suspicion for atypical bacterial and/or *B pertussis* is high (eg, in pediatric populations, immunocompromised populations), a single target PCR against these targets or using a LRT specimen type may be considered for additional testing.

Laboratories may perform an off-label validation using lower respiratory specimen types on an RVP given its additional diagnostic value. Various upper RVPs can reliably detect all the targets in the BAL matrix with high precision.[33] The limit of detection (LoD) between BAL and NPS were also very comparable for viral targets; some targets even reported lower LoD in BALs.[33,34] Specificities of 100% were achieved for the targets, although false negative results with low bacterial load (CT > 30) for the atypical bacteria may also occur. However, the viscosity of the specimen could affect the sensitivity of the assay. A negative result may not necessarily rule out an infection.

Test Reporting

All the RVPs are manufactured and validated for qualitative testing. Reporting of the test results typically is either "detected" or "not detected" for each pathogen. The specimen type needs to be clearly indicated given that different clinical implications and performance characteristics may occur. Some suggest that inclusion of procalcitonin (PCT) levels into the report could help assist the clinicians in optimizing antimicrobial therapy. In a retrospective interventional study, the impact of an automated ASP electronic health record (EHR) best practice alert in inpatients with low PCT levels and a virus detected found a reduction in antibiotic use and discharge prescribing rates.[35] Meanwhile, several RCTs demonstrated that no difference in the duration of antibiotic therapy was seen when PCT levels were considered, though the standard of care group had high utilization of PCT at baseline for one of the studies.[36,37]

LOWER RESPIRATORY PANELS

Clinician Testing Decision, Ordering, and Achieving Actionable Outcomes: What We Know

A single-center prospective feasibility study evaluated the diagnostic impact of the BioFire FilmArray pneumonia panel *plus* in adults admitted with suspected CAP and found a significant reduction in the time to potentially actionable results as well as an increased microbiological yield compared with standard diagnostic microbiology methods.[38] The use of the BioFire FilmArray pneumonia panel significantly increased the detection of potential viral and bacterial pathogens in adult inpatients with CAP in another single-center prospective study.[39]

To date, there is only 1 published multicenter, randomized controlled trial evaluating the potential clinical impact of multiplex lower respiratory pneumonia PCR panels in hospitalized patients with clinical suspicion for pneumonia and risk factors for infection with gram-negative bacilli[40] (see **Table 1**). Of note, the PCR group also received active ASP recommendations approximately 5 hours after sample collection, resulting in a statistically significant reduction of 45% in inappropriate antibiotic therapy. Notably, there were no significant differences in overall antibiotic duration, the proportion of patients reaching clinical stability, length of hospital stays, rate of ICU admission, and

proportion of patients being discharged from hospital. Additionally, the pneumonia PCR used in this trial showed a poor positive predictive value at 39%, a known shortcoming of these tests.

Additional data on diagnostic stewardship interventions are limited. A single-center, retrospective, preintervention and postintervention study that evaluated the impact of BioFire FilmArray pneumonia panel implementation in adult ICU patients with pneumonia was recently published.[41] That institution's pneumonia treatment guidelines recommended the use of multiplex pneumonia panel in all patients with HAP and VAP, as well as some patients with CAP (if severe, receiving broad-spectrum agents, or not improving), and the results were subject of prospective audit and feedback by their ASP. The authors found a decreasing trend (while not statistically significant) in the time to discontinuation of methicillin-resistant *Staphylococcus aureus* and antipseudomonal therapy after the implementation of the panel. It is possible that the small sample size, confounding introduced by the early months of the COVID-19 pandemic, could have impacted the results.

Specimen Collection, Transport, and Processing

Acceptable specimen types for pneumonia panels are BAL (including mini-BAL) and sputum (induced, expectorated sputum, or endotracheal aspirates) from symptomatic individuals (see **Table 2**). Specimens should be tested as soon as possible but can be stored for 1 day in 2 to 8°C. Manufacturers do not recommend specimens to be preprocessed, centrifuged, treated with any mucolytic or decontaminating agents, or placed into transport media before testing.

In general, invasive specimen types such as BAL have higher diagnostic yield than noninvasive specimens like sputum, although patients with COVID-19 have been hospitalized for pneumonia with low bacterial loads recovered in their sputum.[42] During the COVID-19 pandemic, coinfections and pneumonia were commonly seen in severely ill patients. Invasive sampling techniques were contraindicated among COVID-19 patients due to the risk of aerosol generation. That said, given the disease severity and clinical need of the patient, sputum specimens do have diagnostic advantages over BAL despite their lower yield.

Upon every lower respiratory panel request, Gram-stains are performed to determine sputum specimen quality. Typically, an acceptable sputum specimen has greater than 25 polymorphonuclear neutrophils (PMN) and less than 10 epithelial cells per high-power field (hpf) and a poor-quality specimen has greater than 25 squamous cells/hpf, and less than 25 PMN/hpf. A concomitant culture is required for organism isolation and further antimicrobial susceptibility testing.

Test Performance

Overall, the positive percent agreement (PPA) and negative percent agreement with bacterial cultures can range from 16% to 100% and 92% to 100%, respectively, with differences seen in sample types tested (eg, BAL or sputum). Instances where the panel is positive but cultures are negative may not always be interpreted as a 'false positive' since reflex testing of these same specimens using another molecular assay confirmed the original LRT panel results. At the same semi-quantification level, the concordance rate can be as low as 43% for culture-positive specimens but samples with targets detected at $\geq 10^5$ DNA copies/mL grew significantly in culture.[43] Positive predictive values of AMR genes to phenotypic antibiotic susceptibility test results range from 80% to 100%, depending on the microorganism and specific resistance marker(s).[44] The panel has excellent negative predictive value for on-panel targets. Negative results have the potential to assist in de-escalation of broad-spectrum

therapy. Like RVP panels, the adenovirus target suffers from lower sensitivity. Within 6 months of the expiration date, there is a 10 to 100X loss in sensitivity for adenovirus genotype C. If there is high clinical suspicion, communication with the laboratory is necessary to ensure that the kits are not within 6 months of expiration, or another confirmatory test is recommended.

The Curetis Unyvero pneumonia panel includes *Pneumocystis jirovecii* (PJP) as a target. The PPA with standard DFA and IFA testing was 87%.[44] Studies have suggested that colonizers are more likely to have less than 10^4 copies/ml, but the panel is a qualitative test. However, the LoD of PJP on the panel is 10^5 copies/ml, so some may consider that positive detection may be associated with *P jirovecii* pneumonia.[45,46] In cases like this, reflex testing to quantitative *P jirovecii* PCR may be recommended.[47]

Test Reporting

Reporting results from pneumonia panels depends on the commercial pneumonia panel used. If the laboratory is running the Curetis Unyvero test, all targets are reported as 'detected' and 'not detected.' On the BioFire FilmArray pneumonia panel, the viral, atypical bacteria, and AMR genes are also reported as 'detected' and 'not detected.' Negative bacterial targets are reported as 'not detected' but positive bacterial targets are reported as 'detected' and in semiquantitative 'bins' of 10^4, 10^5, 10^6, or $\geq 10^7$ copies/ml.[48] However, clinical teams should not consider copies/mL as equivalent to cell-forming units (CFU)/mL, the standard quantification approach for such routine bacterial cultures as urine culture. Notating a comment in the report explaining that semiquantitative results (in copies/mL) are not equivalent to CFU/mL and may not correlate with the quantity of bacteria reported by respiratory culture may help clinical teams interpret results. Some have suggested implementing cutoffs, suppressing results from certain 'bins,' that correlate to an amount that is more commonly seen in routine culture although this would require laboratories to pursue their own validation to develop a threshold cutoff.[49]

The reports could also contain a reminder that pneumonia panels may detect low amounts of bacteria which could be indicative of colonization or normal respiratory flora, especially at institutions where there is not an active ASP. To prevent confusion in such cases, incorporating other clinical laboratory information into the same report may help clinical teams interpret the results. For example, a report could have Gram-stain result, rapid molecular result, final identification, and phenotypic susceptibility. Other strong relationships between the pneumonia panel results and true pneumonia are host inflammatory responses such as temperature (eg, fever), white blood cell count, percent polymorphonuclear lymphocytes, and PCT levels.[36,50] A benefit of combining all the results into one view is that a follow-up targeted stewardship alert can be placed to aid in the interpretation of results.

An important aspect of the pneumonia panels is the ability to detect AMR genes. Incomplete understanding of the molecular terminology can lead to ineffective treatment or missed opportunities for antimicrobial optimization. Laboratories should avoid reporting AMR results simply as 'detected' and 'not detected' without linking the organism and the AMR gene (if possible) and providing interpretation guidance.[51] It is also a College of American Pathologists requirement (checklist item MIC.21855) to link AMR determinants and phenotypic susceptibility results to a specific organism in the final patient report. For example, when *mecA* and *S aureus* are detected, proper reporting would say 'methicillin-resistant *S aureus*' or 'extended-spectrum β-lactamase (ESBL)–producing *Escherichia coli*' for an *E coli* with CTX-M gene detected. Inclusion of therapeutic comments are helpful and can be broad such as

referring the team to consult the infectious diseases team, pharmacy, or specific treatment guidelines. Alternatively, the comments can contain more specific therapeutic information such as 'in the presence of a ESBL producer (CTX-M detected), a carbapenem is the drug of choice,' or 'ceftriaxone is recommended for initial therapy pending susceptibility results' (when CTX-M is not detected). Interestingly, some institutions may choose not to report the absence of CTX-M or *blaKPC* to prevent the assumptions that the organism would be susceptible to expanded-spectrum cephalosporins or carbapenems. Some laboratories only report AMR genes that are detected and suppress all 'not detected' results. Depending on your institutional and local governmental public health surveillance programs, certain drug-resistant isolates trigger isolation protocols. Laboratories should work with their antimicrobial stewardship, infection prevention, and infectious diseases teams to develop an appropriate reporting structure.

In rare cases, discrepant phenotypic and genotypic AST results may arise. Major errors defined as when an AMR gene is detected in an isolate that was susceptible to phenotypic testing or very major errors defined as when an AMR gene is not detected in an isolate found to be resistant by phenotypic testing can occur. Sometimes the AMR gene may not be detected in the organism causing the disease. Laboratories should have a process in place to resolve discordant phenotypic and genotypic susceptibility results. There are publications and resources provided by the Clinical and Laboratory Standards Institute that offer guidance to troubleshoot discrepant results.[52]

Repeat Testing

In a Centers for Medicare & Medicaid Services guidance document, repeat testing using syndromic panels with the same pathogens within 14 days for the same clinical indication is typically not covered for payment.[53] Both upper and lower respiratory panels are considered equivalent, but it must also be noted there are also less than 5 target panels that include viruses such as influenza, RSV, and SARS-CoV-2 included in the policy. Hence, it is crucial that there is judicial usage of repeat testing.

A study comparing repeat testing on NPS on both a full (>12 targets) RVP versus a smaller multiplex (<4 targets) showed that 75% of repeat tests were consistently negative, with 12% remaining positive with the same organisms upon repeat.[54] Similar findings have been reported in both adult and pediatric settings. In the immunocompromised population, especially those with concurrent symptoms like pulmonary infiltrates, fever, and hypoxia, a BAL is often performed and may include a repeat RVP. A study by Azadeh and colleagues compared the effectiveness of RVP testing on BAL samples versus NPS and found that 83% had a corresponding match in a subsequent BAL testing.[55] However, in 20% of the patients, pathogens were identified in the BAL that were not detected from the NPS. These findings indicate that once a pathogen is identified by testing NPS on RVP, subsequent testing of BAL will seldom provide new actionable clinical information. Another study performed in a bone marrow transplant pediatric population also showed that out of 140 specimens and 67 instances of repeat testing, new clinical information was only obtained in 30% of the cases and in most cases, repeat testing from an initial negative result did not change clinical management.[56] A median of 11 days elapsed between the initial and second result suggesting that a 10-day hard stop block may be a reasonable approach.[56,57] In a study with greater than 1400 specimens, savings of $140,000 per year would accrue if all repeated respiratory testing were eliminated.[54] Repeat testing may hinder clinical gain especially in cases with discordant results due to collection inadequacy. Differing results from initial runs were associated with new/

worsening symptoms and in some cases, testing on a differing specimen type such as a BAL specimen after a negative result from NPS may at times offer valuable information.

FUTURE DIRECTIONS AND SUMMARY

Many new molecular developments for multiplex testing of respiratory infections are on the horizon. At the time of writing this article, bioMérieux's SPOTFIRE respiratory panel, which consists of 15 targets, became FDA-cleared and Clinical Laboratory Improvement Amendments–waived, pioneering the introduction of molecular syndromic testing as a point of care test. For high complexity clinical laboratories, the Respiratory Pathogen ID/AMR enrichment kit (Illumina, Inc., San Diego, CA, USA), a next-generation sequencing assay developed to detect greater than 280 respiratory pathogens and AMR sequences from respiratory specimens, is also available as a laboratory-developed test. To maximize clinical utility to balance the hefty laboratory costs, respiratory panels should only ultimately be ordered if the result will affect patient management, and results should be interpreted in the clinical context. However, a strong diagnostic stewardship action plan requires robust data from research studies, preferably prospective clinical trials. Proposed areas of diagnostic stewardship research include the role of clinical decision tools (clinical decision supporting software), the impact of pairing results with clinical biomarkers, ways to enhance adherence to ASP recommendations and management guidance through EHR interventions (educational alerts, limiting test ordering according to institutional guidelines), and important health outcomes and cost-effectiveness analyses in different key populations. Diagnostic stewardship is crucial to improving patient care, but there must be a call to action for all stakeholders involved to participate in research and active implementation.

CLINICS CARE POINTS

- Multiplex syndromic upper and lower respiratory tract panels are now widely available in clinical microbiology laboratories and healthcare institutions in high resource areas.
- Observational studies have shown a potential for syndromic respiratory panels to increase the likelihood of establishing an etiological diagnosis and enabling prompt optimization of antimicrobial therapy.
- These panels have significant limitations, and evidence from prospective clinical studies have shown mixed results when evaluating actionable clinical outcomes such as reduction in inappropriate antimicrobial use, duration of therapy, and length of hospital stay.
- The approach to optimal implementation of syndromic respiratory panels remains uncertain, and further diagnostic stewardship research is needed to determine how to best use these panels to improve clinical outcomes.
- Currently, syndromic respiratory panels should only be considered in symptomatic individuals, particularly those with severe illness or immunocompromised.

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