01663

Evaluation of the Respiratory Pathogen ID/AMR Panel Workflow Compared to Standard-of-Care for the Diagnosis of Lower Respiratory Tract Infections

04. Diagnostic microbiology

4h. Clinical metagenomics

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Background Immunocompromised patients are at risk of lower respiratory tract infections. Current standard-of-care (SOC) methods require a battery of laboratory tests, which may take weeks to result. The Respiratory Pathogen ID/AMR Panel (RPIP) next-generation sequencing (NGS) test (IDbyDNA/Illumina) offers rapid detection and quantification of >280 respiratory pathogens and >2,100 AMR markers with a single assay. We assessed accuracy of RPIP and automated Explify data analysis (IDbyDNA) compared to SOC testing from bronchoalveolar lavage (BAL) specimens.

Methods BAL (n=51) from hospitalized patients (03/2019-12/2020) were evaluated by SOC testing (i.e. direct staining, culture, antigen testing, targeted and multiplex PCR assays for a broad range of bacterial, viral, fungal and mycobacterial pathogens). Remnant samples (stored at -80°C) were spiked with internal controls (MS2/T7, Microbiologics), nucleic acid extracted (MagMAX™ Pathogen RNA/DNA Kit, Applied Biosystems), sequencing libraries prepared and target-enriched (RPIP reagents, Illumina), and sequenced on a NovaSeq (Illumina) to a depth of 3M reads/sample. Sequencing data was analyzed using the Explify Analysis Software on BaseSpace. Explify results were interpreted using reporting guidelines adapted from SOC criteria utilized by the Johns Hopkins Hospital Microbiology Laboratory.

Results SOC detected a putative pathogen in 16 (31%) patients and RPIP detected 18 putative pathogens in 15 (29%) patients. Overall agreement of RPIP and SOC was 73.6% (53.3% positive agreement, 81.6% negative agreement, Table 1) prior to discordant analysis. Most SOC-positive/RPIP-negative results were bacteria with low colony counts (<300 CFU/ml) or off-panel targets (CoNS, P. alcaligenes). RPIP detected 10 additional pathogens (8 viruses, 2 bacteria, Table 1). Ureaplasma parvum was detected by RPIP from a lung transplant patient with altered mental status and elevated ammonia levels consistent with donor-derived Ureaplasma syndrome. Discordant analysis is underway.

Conclusions Similar to respiratory culture, reporting guidelines are required for appropriate interpretation of NGS results. RPIP should be considered as an adjunct to SOC and would have resulted in an etiologic diagnosis in 6 additional patients

(43% etiologic diagnosis with SOC + RPIP). Targeted NGS workflows can detect pathogens that are missed by SOC in the setting of high concentrations of normal respiratory microbiota and pathogens that are not detectable by standard algorithms.

Summary of respiratory pathogens detected by RPIP

	SOC Bacterial Positive	**SOC Viral Positive**	**SOC Fungal Positive**	**SOC No Pathogen**
SOC-positive, RPIP-positive	2 *Enterococcus faecalis*[a] 2 *Pseudomonas aeruginosa* 1 *Moraxella catarrhalis* 1 MRSA (*mecA* detected) 1 MSSA (*mecA* not detected)[b]	1 SARS-CoV-2[c]		26 Normal respiratory microbiota 5 No growth
SOC-positive, RPIP-negative	2 MRSA (300 CFU/ml) 1 *Pseudomonas alcaligenes** (100 CFU/ml) 1 Coagulase-Negative *Staphylococcus* species* (>10,000 CFU/ml) 2 *Haemophilus influenzae* (100 CFU/ml, 200 CFU/ml) 1 *Enterococcus faecalis* (2000 CFU/ml)		1 *Penicillium* species	
SOC-negative, RPIP-positive	*1 Burkholderia cepacia* complex (BCC)[d] *1 Ureaplasma parvum*	3 Epstein Barr Virus 1 Human metapneumovirus		

[a] SOC and RPIP positive for *E. faecalis*. RPIP also detected EBV & HHV6 in one sample.

[b] SOC and RPIP positive for MSSA. RPIP also detected HSV1

[c] SOC and RPIP positive for SARS-CoV-2. RPIP also detected HHV6

[d] SOC > 10,000 CFU/mL normal respiratory flora; RPIP BCC 3.2 X10^6 copies/mL among 4.9 X 10^8 copies/mL normal respiratory flora

* not targeted by RPIP

Keyword 1
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Keyword 2
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Conflicts of interest

Do you have any conflicts of interest to declare?

I have the following potential conflict(s) of interest to report
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