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Multicenter Evaluation of the Unyvero Platform for Testing Bronchoalveolar Lavage Fluid

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ABSTRACT Bronchoalveolar lavage (BAL) culture is a standard, though time-consuming, approach for identifying microorganisms in patients with severe lower respiratory tract (LRT) infections. The sensitivity of BAL culture is relatively low, and prior antimicrobial therapy decreases the sensitivity further, leading to overuse of empirical antibiotics. The Unyvero LRT BAL Application (Curetis GmbH, Germany) is a multiplex molecular panel that detects 19 bacteria, 10 antibiotic resistance markers, and a fungus, *Pneumocystis jirovecii*, in BAL fluid in ~4.5 h. Its performance was evaluated using 1,016 prospectively collected and 392 archived specimens from 11 clinical trial sites in the United States. Overall positive and negative percent agreements with culture results for identification of bacteria that grow in routine cultures were 93.4% and 98.3%, respectively, with additional potential pathogens identified by Unyvero in 21.7% of prospectively collected specimens. For detection of *P. jirovecii*, the positive percent agreement with standard testing was 87.5%. Antibiotic resistance marker results were compared to standard antibiotic susceptibility test results to determine positive predictive values (PPVs). PPVs ranged from 80 to 100%, based on the microorganism and specific resistance marker(s). The Unyvero LRT BAL Application provides accurate detection of common agents of bacterial pneumonia and of *P. jirovecii*. The sensitivity and rapidity of this panel suggest significant clinical value for choosing appropriate antibiotics and for antibiotic stewardship.

KEYWORDS BAL, molecular diagnostics, multiplex PCR, pneumonia

Pneumonia diagnosis has traditionally been based on clinical classification (e.g., community-acquired pneumonia, ventilator-associated pneumonia), with the primary diagnostic tools being Gram staining and culture of lower respiratory tract secretions. While Gram staining can be rapidly performed on sputum and other lower respiratory tract secretions, its sensitivity for pneumonia diagnosis is low, and culture results are usually needed to allow ideal narrowing of empirical broad-spectrum antibiotic therapy

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TABLE 1 LRT BAL panel

LRT BAL panel microorganism	Associated LRT BAL panel antibiotic resistance marker(s)
Acinetobacter species	bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA58}, bla_{VIM}
Chlamydia pneumoniae	
Enterobacterales	bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM}
Citrobacter freundii	
Enterobacter cloacae complex	
Escherichia coli	
Klebsiella oxytoca	
Klebsiella pneumoniae	
Klebsiella variicola	
Morganella morganii	
Proteus species	
Serratia marcescens	
Haemophilus influenzae	
Legionella pneumophila	
Moraxella catarrhalis	
Mycoplasma pneumoniae	
Pneumocystis jirovecii	
Pseudomonas aeruginosa	bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}
Staphylococcus aureus	*mecA*
Stenotrophomonas maltophilia	
Streptococcus pneumoniae	

(1, 2). Culture is slow and imperfect; pathogens may fail to grow if patients are under antibiotic treatment, or pathogens may be reported as normal respiratory flora in polymicrobial infections where there is a focus on the predominant species isolated. Bronchoalveolar lavage (BAL) aids in identifying causative organisms in only 50 to 70% of cases, with these values varying with both the type of pneumonia and the patient population (3, 4). Infections with bacteria that do not grow in routine cultures, such as *Legionella* species or *Mycoplasma pneumoniae*, are missed if specific testing is not performed (5).

Rapid multiplex molecular panels have the possibility of improving the diagnosis and treatment of pneumonia by identifying present organisms and antimicrobial resistance genes within a short time. Rapid multiplex molecular panels have become available for a number of infectious syndromes, such as gastrointestinal infections (6), bloodstream infections (especially for testing positive blood culture bottles) (7), central nervous system infections (using cerebrospinal fluid) (8), upper respiratory tract infections, and, most recently, lower respiratory tract infections (5, 9–11).

The Unyvero LRT BAL Application (Curetis GmbH, Germany) is a U.S. Food and Drug Administration (FDA)-cleared rapid molecular multiplex *in vitro* diagnostic system for use on bronchoalveolar lavage fluid. A closed-cartridge-based approach is used for specimen lysis, DNA extraction, PCR, and array hybridization; the turnaround time is approximately 4.5 h. The panel detects the most common species observed in patients with hospital-acquired and ventilator-associated pneumonia (12, 13), in addition to *M. pneumoniae*, *Chlamydia pneumoniae*, *Legionella pneumophila*, and *Pneumocystis jirovecii*. It also detects 10 resistance markers, relevant to 3rd-generation cephalosporin or carbapenem resistance in *Enterobacterales*, *Pseudomonas aeruginosa*, and *Acinetobacter* species, penicillin resistance in *Haemophilus influenzae*, and oxacillin resistance in *Staphylococcus aureus* (Table 1). Herein, we present the results of the FDA clinical trial of the Unyvero LRT BAL Application, which included specimens collected at 11 sites in the United States, and compare it to standard of care (SoC) microbiological testing.

MATERIALS AND METHODS

Study design and sample collection. Prospective and archived specimens were collected at 11 clinical trial sites in the United States. For the prospective study arm, specimens were collected and tested

within 24 h after arrival in the laboratory at nine U.S. clinical study sites (Northwestern Memorial Hospital, Chicago, IL; University of Rochester Medical Center, Rochester, NY; Johns Hopkins Hospital, Baltimore, MD; Mayo Clinic, Rochester, MN; William Beaumont Hospital, Royal Oak, MI; University of California, Los Angeles, CA; University of Washington, Seattle, WA; Summa Health System, Akron, OH; Columbia University Medical Center/New York Presbyterian Hospital, New York, NY) between 2015 and 2016 using a predecessor of the FDA-cleared LRT BAL cartridge (clinicaltrials.gov identifier, NCT01922024). Remaining specimen aliquots were then frozen at −70°C, shipped to Curetis in Germany, and stored until they were tested with the LRT BAL Application, performed at Curetis in 2019 for this study. For the archived study arm, frozen specimens positive for at least one on-panel microorganism were collected between 2015 and 2018 at the nine prospective test sites alongside two additional sites (Medical College of Wisconsin, Milwaukee, WI; University of North Carolina, Chapel Hill, NC), stored at −70°C, and tested with the LRT BAL Application at Curetis. Study subject demographics (patient age, sex) and clinical setting (e.g., hospital ward, intensive care unit) were recorded for all prospectively collected and archived specimens. Numbers of contributed specimens per clinical site and study subject demographics are listed in Tables S1 and S2 of the supplemental material. Specimens were deidentified and assigned a study number prior to study enrollment and shipping to Curetis.

For the initial clinical trial using the predecessor cartridge, Institutional Review Board (IRB) approval was obtained from the local IRB of each site. A waiver of consent was granted by each IRB to use excess sample material and the data associated with it, which were collected for the clinical purpose of obtaining a BAL culture as part of the standard of care. Specimens were eligible for enrollment into the prospective study arm if they were collected from hospitalized patients 18 years or older with suspected or confirmed lower respiratory tract infection and if specimen testing on site had occurred within 24 h after arrival of the specimen in the laboratory. Specimens were excluded if patients had already been enrolled or patients were known to be infected with biosafety level 3 (BSL-3) microorganisms. Eligibility criteria for specimen collection for the archived study arm were similar; however, specimens from ambulatory patients and nine bronchial washings were included.

Unyvero LRT BAL Application testing. The LRT BAL panel detects 19 bacteria and one fungus (Table 1). LRT BAL testing was performed according to the manufacturer's instructions using 180 μl of specimen. After lysis in the Unyvero Lysator, samples were processed on LRT BAL cartridges. Results were generated by the Unyvero software and electronically exported to a database.

SoC reference testing. Gram staining, routine aerobic culturing, and various pathogen identification and antimicrobial susceptibility testing methods (matrix-assisted laser desorption ionization–time of flight [MALDI-TOF] mass spectrometry, Vitek 2, Phoenix, MicroScan, Sensititre, disk diffusion, broth dilution, or agar dilution) were performed by following routine standard procedures at each study site. SoC testing was initiated prior to prospective Unyvero testing; however, SoC testing was not complete before Unyvero results became available. Culture results, including the presence of oropharyngeal flora as well as any off-panel organisms (listed in Table S3 of the supplemental material), were reported semiquantitatively (as "rare," "few," "moderate," or "numerous") or quantitatively according to local practices; for quantitative cultures, a reporting threshold of 10^3 CFU/ml or higher for mini-bronchoalveolar lavage specimens and 10^4 CFU/ml or higher for bronchoalveolar lavage specimens was applied, according to recommendations by the Infectious Diseases Society of America and the American Society for Microbiology (14). Antimicrobial susceptibility results were interpreted using CLSI breakpoints (15). *C. pneumoniae*, *M. pneumoniae*, *L. pneumophila*, and *P. jirovecii* SoC testing (e.g., PCR, direct or indirect fluorescence antibody tests [DFA or IFA, respectively], culture [applied for *L. pneumophila* only]) was performed only if clinically ordered and was therefore limited to a subset of subjects. Reference testing data were collected and submitted via an electronic case report form system by an individual blind to the Unyvero results. Isolates recovered in culture were deidentified and saved using the Cryobank system (Mast Diagnostica), frozen at −70°C, and then shipped to Curetis.

Isolate whole-genome sequencing. Isolates (11 *Acinetobacter* species, 1 *Citrobacter freundii*, 19 *Enterobacter cloacae* complex, 18 *Escherichia coli*, 10 *Haemophilus influenzae*, 27 *Klebsiella* species, 4 *Moraxella catarrhalis*, 6 *Proteus* species, 57 *Pseudomonas aeruginosa*, 14 *Serratia marcescens*, 57 *Staphylococcus aureus*, 25 *Stenotrophomonas maltophilia*, and 3 *Streptococcus pneumoniae* isolates) from SoC cultures were regrown and analyzed by whole-genome sequencing to confirm their species and assess for the presence or absence of LRT BAL panel antibiotic resistance genes. Isolates were available for most prospective and archived specimens. In four cases, species within the *Klebsiella pneumoniae* complex, including *K. variicola*, were reported as *K. pneumoniae* by the study sites. Isolates that failed to regrow in culture at Curetis were evaluated by PCR followed by bidirectional sequencing from frozen stocks for the target genes listed under "Specimen PCR/sequencing" below.

The preparation of isolates for whole-genome sequencing was performed at IHMA Europe Sàrl, Switzerland, with DNA extraction performed using the DNeasy UltraClean kit (Qiagen). Following Illumina Nextera XT library preparation, DNA extracts underwent whole-genome sequencing by Microsynth, Switzerland, based on an extract quality of >0.2 μg DNA, with a concentration of >10 ng/μl. Sequencing was conducted using v2 Illumina NextSeq high-output kits (2 × 150), resulting in a minimum depth of 100× for 5-Mb bacterial genomes. Adaptor-trimmed, demultiplexed, and quality-checked raw reads were *de novo* assembled and analyzed for taxonomic identification and the presence of resistance markers by Ares Genetics (Austria) using ARESdb (16). *De novo* assemblies delivered to Curetis were screened using sequences of LRT BAL panel resistance markers, 23S rRNA genes, or other LRT BAL target genes to assess for resistance marker presence, confirm the identification of whole-genome sequence results by GenBank BLAST of corresponding assembled sequence reads, and ensure that provided assemblies were free of contamination from other species.

TABLE 2 Comparison of results of SoC and Unyvero testing in the prospective study arm

Result type	No. of cases (n = 1,016)	%
All concordant results	774	76.2
Unyvero and SoC negative	635	62.5
Unyvero and SoC positive	139	13.7
All discordant results	242	23.8
Unyvero detection of additional microorganisms	214	21.1
Unyvero positive, SoC negative	151	14.9
Unyvero and SoC positive (partially concordant)	63	6.2
SoC detection of additional microorganisms	22	2.2
Unyvero negative, SoC positive	18	1.8
Unyvero and SoC positive (partially concordant)	4	0.4
Unyvero and SoC detection of different microorganisms	6	0.6
Partially concordant results	2	0.2
Fully discordant results	4	0.4

Specimen PCR/sequencing. Discrepant results were analyzed by PCR, followed by bidirectional Sanger sequencing using LRT BAL assay primer pairs as well as other company-proprietary primer pairs targeting genetic loci other than those of the corresponding LRT BAL assays, as follows. For most organisms, the 23S rRNA gene was assessed; other gene targets assessed included *dhaK* for *C. freundii*, *rpoB* for *Morganella morganii* and *Enterobacter*, *Klebsiella*, and *Proteus* species, *copB* for *M. catarrhalis*, P1 adhesin for *M. pneumoniae*, *psaA* and *lyt* for *S. pneumoniae*, and the 26S mitochondrial rRNA gene for *P. jirovecii*. DNA was extracted from 180 μl of specimen using QIAamp Blood Mini Kits (Qiagen). PCRs were set up in a volume of 30 μl using 3 μl of extracted DNA and amplified (35 cycles) using an Eppendorf EP Gradient thermocycler. Amplified DNA was subjected to gel electrophoresis (Agilent Bioanalyzer) to confirm expected sizes; if the size was confirmed and amplicons had a molarity of 15 nM or higher, amplified DNA was bidirectionally sequenced (Microsynth).

Statistical analysis. LRT BAL Application microorganism detection results were compared to SoC culture results to assess true-positive (TP), false-negative (FN), false-positive (FP), and true-negative (TN) rates and to calculate positive percent agreement [PPA; TP/(TP + FN)] and negative percent agreement [NPA; TN/(TN + FP)], with two-sided 95% confidence intervals (95% CI), determined according to the Wilson score method.

LRT BAL resistance marker results were compared for genotypic agreement with samples positive for a pathogen carrying a specific resistance marker (as confirmed by isolate sequencing) and with samples positive for a pathogen not carrying this marker, with 95% CIs. LRT BAL resistance marker results were also compared to results of phenotypic antimicrobial susceptibility testing (AST) by determining the positive predictive value (PPV, rate of agreement of the predicted phenotype with the phenotype as determined by AST), with 95% CIs.

RESULTS

Overall concordance in the prospective study arm. The prospective study arm included 1,016 specimens. When we compared Unyvero to SoC results, a specimen was regarded as concordant if reported results for panel organisms were fully identical. Specimens were regarded as partially concordant if one or more organisms were concordantly reported by both methods while additional organisms were reported by one method only. Specimens were regarded as discordant if both methods reported entirely different results. The overall concordance of Unyvero to SoC results in the prospective study arm was 76.2% (774/1,016) (Table 2). Unyvero and SoC testing reported positive results for at least one panel microorganism for 35.7% (363/1,016) and 22.9% (233/1,016) of the prospective specimens, respectively. Unyvero and SoC testing reported positive results for three or more on-panel microorganisms for 9.6% and 3.4% of positive prospective specimens, respectively (Tables 2 and 3). For 21.7% (220/1,016) of specimens, Unyvero identified potential pathogens that were not reported by SoC testing, including 34 specimens (3.3%) for which Unyvero reported three or more panel analytes. In only 2.8% (28/1,016) of specimens, SoC testing reported additional on-panel organisms not reported by Unyvero. The negative predictive value for Unyvero testing was 97.2% (635/653, specimens reported negative by both Unyvero and SoC testing/

TABLE 3 Positivity rates and numbers of microorganisms detected by SoC and Unyvero testing in the prospective study arm

Result	No. (%) of cases (n = 1,016)	
	Unyvero	SoC
Negative	653 (64.3)	786 (77.4)
Positive	363 (35.7)	230 (22.6)
With the following no. of organisms detected:		
1	250 (68.9)	191 (83.0)
2	78 (21.5)	31 (13.5)
3	19 (5.2)	5 (2.2)
4	7 (1.9)	3 (1.3)
5	7 (1.9)	
6	2 (0.6)	

specimens reported negative by Unyvero). For a full comparison listing all prospective specimens, please refer to Table S4 in the supplemental material.

Detection of bacteria that grow in routine cultures. Results from LRT BAL compared with SoC results for on-panel microorganisms that would be expected to be detectable in routine culture in the prospective and archived study cohorts are shown in Table 4. PPAs for on-panel analytes with SoC results of 90% or higher were observed with the following exceptions. For the *E. cloacae* complex, *K. pneumoniae*, and *K. variicola*, PPAs were 77.8% (28/36), 89.1% (49/55), and 50.0% (2/4), respectively. For the prospective study arm, the overall PPA was 90.1% (247/274). For both study arms combined, the overall PPA was 93.4% (669/716).

SoC testing reported two on-panel analytes that were not detected by Unyvero, for which whole-genome sequencing of isolated organisms confirmed off-panel analytes (SoC, *K. pneumoniae*; whole-genome sequencing, *Raoultella ornithinolytica*; SoC, *S. pneumoniae*; whole-genome sequencing, *Streptococcus cristatus*). PCR followed by bidirectional sequencing on two FN specimens identified related species in specimen DNA extracts, which might suggest possible misidentification by SoC testing (SoC, *H. influenzae*; PCR/sequencing, *Haemophilus parainfluenzae*; SoC, *K. pneumoniae*; PCR/sequencing, *K. oxytoca* [Unyvero reported also *K. oxytoca*]); further analysis was not possible due to isolate nonavailability. Remaining false-negative (FN) cases were analyzed by PCR/sequencing from specimen DNA extracts. Analyte presence was confirmed for 31/47 (66.0%) FN cases, as follows: 0 of 1 *Acinetobacter* species case, 6 of 8 *E. cloacae* complex cases, 3 of 4 *E. coli* cases, 0 of 1 *H. influenzae* case, 1 of 2 *K. oxytoca* cases, 2 of 6 *K. pneumoniae* cases, 2 of 2 *K. variicola* cases, 4 of 5 *P. aeruginosa* cases, 2 of 2 *S. marcescens* cases, 5 of 10 *S. aureus* cases, 5 of 5 *S. maltophilia* cases, and 1 of 1 *S. pneumoniae* case.

Individual microorganism NPAs for prospective study specimens ranged from 95.2% to 99.8%, with an overall NPA of 98.4% (15,705/15,967). A high rate of additional (FP) detections was observed for the prospective specimens (262 FP/1,016 specimens) (Table 4). For archived specimens, 123 FP/392 specimens with a slightly lower overall NPA (97.9%; 5,707/5,830) were observed (which may be explained because archived specimens were selected for being positive for at least one on-panel microorganism). For both study arms combined, the overall NPA was 98.3% (21,412/21,797).

Organism presence was confirmed by PCR/sequencing in the original specimen for 84.9% of false-positive Unyvero detections (327/385), thereby indicating that such results represent true detections that had not been reported by SoC testing. (Table 5). Many of these additional confirmed detections are clinically relevant pathogens, such as *Acinetobacter* species, *S. aureus*, or *P. aeruginosa*. For one specimen, the *C. freundii* assay was positive, whereas PCR/sequencing and culture detected *Citrobacter youngae*.

TABLE 4 Unyvero LRT BAL panel performance compared to that of SoC testing for bacterial species as isolated in routine culture

Species	Study arm[a]	No. positive by Unyvero and SoC/ no. positive by SoC	PPA (%) (95% CI)	No. negative by Unyvero and SoC/ no. negative by SoC	NPA (%) (95% CI)
Acinetobacter species	Prospective	10/11	90.9 (62.3–98.4)	993/1,004	98.9 (98.0–99.4)
	Archived	18/18	100.0 (82.4–100.0)	371/374	99.2 (97.7–99.7)
	Total	**28/29**	**96.6 (82.8–99.4)**		
C. freundii	Prospective	1/1	100.0 (20.7–100.0)	1,011/1,014	99.7 (99.1–99.9)
	Archived	5/5	100.0 (56.6–100.0)	382/387	98.7 (97.0–99.4)
	Total	**6/6**	**100.0 (61.0–100.0)**		
E. cloacae complex	Prospective	13/17	76.5 (52.7–90.4)	991/998	99.3 (98.6–99.7)
	Archived	15/19	78.9 (56.7–91.5)	373/373	100.0 (99.0–100.0)
	Total	**28/36**	**77.8 (61.9–88.3)**		
E. coli	Prospective	17/18	94.4 (74.2–99.0)	968/998	97.0 (95.7–97.9)
	Archived	46/49	93.9 (83.5–97.9)	326/343	95.0 (92.2–96.9)
	Total	**63/67**	**94.0 (85.6–97.7)**		
H. influenzae	Prospective	8/9	88.9 (56.5–98.0)	958/1,006	95.2 (93.7–96.4)
	Archived	50/50	100.0 (92.9–100.0)	321/342	93.9 (90.8–95.9)
	Total	**58/59**	**98.3 (91.0–99.7)**		
K. oxytoca	Prospective	6/7	85.7 (48.7–97.4)	1,001/1,009	99.2 (98.4–99.6)
	Archived	16/17	94.1 (73.0–99.0)	369/375	98.4 (96.6–99.3)
	Total	**22/24**	**91.7 (74.2–97.7)**		
K. pneumoniae	Prospective	20/24	83.3 (64.1–93.3)	982/992	99.0 (98.2–99.5)
	Archived	29/31	93.5 (79.3–98.2)	351/361	97.2 (95.0–98.5)
	Total	**49/55**	**89.1 (78.2–94.9)**		
K. variicola	Prospective	0/2	0.0 (0.0–65.8)	1,012/1,014	99.8 (99.3–99.9)
	Archived	2/2	100.0 (34.2–100.0)	386/390	99.0 (97.4–99.6)
	Total	**2/4**	**50.0 (15.0–85.0)**		
M. catarrhalis	Prospective	2/2	100.0 (34.2–100.0)	997/1,010	98.7 (97.8–99.2)
	Archived	21/21	100.0 (84.5–100.0)	362/371	97.6 (95.5–98.7)
	Total	**23/23**	**100.0 (85.7–100.0)**		
M. morganii	Prospective	0/0	NA	1,009/1,012	99.7 (99.1–99.9)
	Archived	1/1	100.0 (20.7–100.0)	391/391	100.0 (99.0–100.0)
	Total	**1/1**	**100.0 (20.7–100.0)**		
Proteus species	Prospective	4/4	100.0 (51.0–100.0)	1,006/1,012	99.4 (98.7–99.7)
	Archived	15/15	100.0 (79.6–100.0)	370/377	98.1 (96.2–99.1)
	Total	**19/19**	**100.0 (83.2–100.0)**		
P. aeruginosa	Prospective	69/72	95.8 (88.5–98.6)	900/943	95.4 (93.9–96.6)
	Archived	54/56	96.4 (87.9–99.0)	334/336	99.4 (97.9–99.8)
	Total	**123/128**	**96.1 (91.2–98.3)**		
S. marcescens	Prospective	12/12	100.0 (75.8–100.0)	998/1,003	99.5 (98.8–99.8)
	Archived	23/25	92.0 (75.0–97.8)	364/367	99.2 (97.6–99.7)
	Total	**35/37**	**94.6 (82.3–98.5)**		
S. aureus	Prospective	63/71	88.7 (79.3–94.2)	904/945	95.7 (94.2–96.8)
	Archived	56/58	96.6 (88.3–99.1)	313/334	93.7 (90.6–95.9)
	Total	**119/129**	**92.2 (86.3–95.7)**		
S. maltophilia	Prospective	19/21	90.5 (71.1–97.4)	972/994	97.8 (96.7–98.5)
	Archived	37/40	92.5 (80.1–97.4)	342/352	97.2 (94.9–98.4)
	Total	**56/61**	**91.8 (82.2–96.4)**		
S. pneumoniae	Prospective	3/3	100.0 (43.9–100.0)	1,003/1,013	99.0 (98.2–99.5)
	Archived	34/35	97.1 (85.5–99.5)	352/357	98.6 (96.8–99.4)
	Total	**37/38**	**97.4 (86.5–99.5)**		
Total	**Prospective**	**247/274**	**90.1 (86.0–93.1)**	**15,705/15,967**	**98.4 (98.2–98.5)**
	Archived	**422/442**	**95.5 (93.1–97.1)**	**5,707/5,830**	**97.9 (97.5–98.2)**
	Total	**669/716**	**93.4 (91.4–95.0)**	**21,412/21,797**	**98.3 (98.1–98.4)**

[a]Prospective study arm, $n = 1,016$; archived study arm, $n = 392$.

In 9/69 (13.0%) FP *H. influenzae* cases, PCR/sequencing detected other *Haemophilus* species or *Aggregatibacter* species (formerly considered *Haemophilus* species).

***C. pneumoniae*, *L. pneumophila*, and *M. pneumoniae*.** Standard of care tests for *C. pneumoniae*, *L. pneumophila*, and *M. pneumoniae* were performed on selected specimens only. *L. pneumophila* was routinely tested by culturing at two study sites

TABLE 5 PCR/sequencing of positive Unyvero specimens negative by SoC testing

Species	Study arm	No. of false-positive specimens	No. confirmed by PCR followed by sequencing/no. of false-positive specimens	No. with possible cross-reactivity/total (%)
Acinetobacter species	Prospective	11	11/11	
	Archived	3	3/3	
	Total	**14**	**14/14 (100.0)**	
C. freundii	Prospective	3	3/3	
	Archived	5	4/5	1/5
	Total	**8**	**7/8 (87.5)**	**1/8 (12.5)**[a]
E. cloacae complex	Prospective	7	7/7	
	Archived	0		
	Total	**7**	**7/7 (100.0)**	
E. coli	Prospective	30	18/30	
	Archived	17	15/17	
	Total	**47**	**33/47 (70.2)**	
H. influenzae	Prospective	48	42/48	5/48
	Archived	21	17/21	4/21
	Total	**69**	**59/69 (85.5)**	**9/69 (13.0)**[b]
K. oxytoca	Prospective	8	7/8	
	Archived	6	6/6	
	Total	**14**	**13/14 (92.9)**	
K. pneumoniae	Prospective	10	5/10	
	Archived	10	7/10	
	Total	**20**	**12/20 (60.0)**	
K. variicola	Prospective	2	2/2	
	Archived	4	2/4	
	Total	**6**	**4/6 (66.7)**	
M. catarrhalis	Prospective	13	13/13	
	Archived	9	8/9	
	Total	**22**	**21/22 (95.5)**	
M. morganii	Prospective	3	3/3	
	Archived	0		
	Total	**3**	**3/3 (100.0)**	
Proteus species	Prospective	6	6/6	
	Archived	7	6/7	
	Total	**13**	**12/13 (92.3)**	
P. aeruginosa	Prospective	43	41/43	
	Archived	2	2/2	
	Total	**45**	**43/45 (95.6)**	
S. marcescens	Prospective	5	4/5	
	Archived	3	1/3	
	Total	**8**	**5/8 (62.5)**	
S. aureus	Prospective	41	31/41	
	Archived	21	18/21	
	Total	**62**	**49/62 (79.0)**	
S. maltophilia	Prospective	22	21/22	
	Archived	10	10/10	
	Total	**32**	**31/32 (96.9)**	
S. pneumoniae	Prospective	10	10/10	
	Archived	5	4/5	
	Total	**15**	**14/15 (93.3)**	

[a]In one of eight false-positive *C. freundii* cases, PCR/sequencing identified *Citrobacter youngae*.
[b]In 9 of 69 false-positive *H. influenzae* cases, PCR/sequencing identified *Haemophilus haemolyticus* (5 cases), *Aggregatibacter aphrophilus* (3 cases), or *Haemophilus parainfluenzae* (1 case).

contributing to 143/238 (60.1%) reported test results for *L. pneumophila*. Standard of care testing was reported for *C. pneumoniae* in a single (negative) case. Standard of care testing was reported for *M. pneumoniae* in 34 cases, with 28 negative tests from the prospective specimen collection and 6 positive archived specimens. Standard of care testing was reported for *L. pneumophila* in a large number of cases, with 237 negative and one positive test from the prospective specimen collection and 19 positive archived specimens. For prospective and archived specimens combined, PPAs were 83.3% (5/6) for *M. pneumoniae* and 85.0% (17/20) for *L. pneumophila* (Table 6). For the

TABLE 6 *C. pneumoniae*, *L. pneumophila*, *M. pneumoniae*, and *P. jirovecii* detected or not detected by SoC testing and by Unyvero

Species	Study arm	No. (%) tested by SoC testing	No. (%) not tested by SoC testing	No. positive by Unyvero and SoC testing/no. positive by SoC testing	PPA (%) (95% CI)	No. negative by Unyvero and SoC testing/no. negative by SoC testing	NPA (%) (95% CI)
C. pneumoniae	Prospective	1 (0.1)	1,015 (99.9)	0/0		1/1	100.0 (20.7–100.0)
	Archived	0					
	Total			**0/0**			
L. pneumophila	Prospective	238 (23.4)	778 (76.6)	0/1	0.0 (0.0–79.3)	237/237	100.0 (98.4–100.0)
	Archived	19		17/19	89.5 (68.6–97.1)		
	Total			**17/20**	**85.0 (64.0–94.8)**		
M. pneumoniae	Prospective	28 (2.8)	988 (97.2)	0/0		28/28	100.0 (87.9–100.0)
	Archived	6		5/6	83.3 (43.7–97.0)		
	Total			**5/6**	**83.3 (43.7–97.0)**		
P. jirovecii	Prospective	105 (10.3)	911 (89.7)	5/5	100.0 (56.6–100.0)	99/100[a]	99.0 (94.6–99.8)
	Archived	19		16/19	84.2 (62.4–94.5)		
	Total			**21/24**	**87.5 (69.0–95.7)**		

[a]For one additionally detected *P. jirovecii* result, analyte presence was confirmed by PCR/sequencing.

prospective study cohort, the NPA was 100.0% for *M. pneumoniae* (28/28) and *L. pneumophila* (237/237).

For most prospective and archived specimens, no SoC test for *C. pneumoniae*, *L. pneumophila*, or *M. pneumoniae* was performed, and therefore, such specimens were not used to calculate PPA or NPA. Among these, Unyvero detected *L. pneumophila* in one (from the prospective study arm, confirmed by PCR/sequencing) and *M. pneumoniae* in nine (six from the prospective study arm, three of which were confirmed by PCR/sequencing, and three from the archived study arm, two of which were confirmed by PCR/sequencing). False negative (FN) cases were analyzed by PCR/sequencing from specimen DNA extracts. Analyte presence was confirmed for 2 of 3 FN *L. pneumophila* cases and one FN *M. pneumoniae* case.

P. jirovecii. Standard of care testing for *P. jirovecii* was performed on selected specimens only. There were 100 negative and five positive SoC *P. jirovecii* tests in the prospective study, as well as 19 *P. jirovecii* positive archived specimens. For prospective and archived specimens combined, the PPA was 87.5% (21/24) (Table 6), with the five SoC-positive *P. jirovecii* detections by DFA, IFA, or PCR from the prospective cohort (100.0%, 5/5) and 84.2% (16/19) from the archived cohort being detected by Unyvero. For the prospective study cohort, the NPA was 99.0% (99/100; 95% CI, 94.6 to 99.8%); one specimen tested negative by a DFA SoC test but was positive by Unyvero and confirmatory PCR/sequencing.

Among the 911 and 373 specimens in the prospective and archived arms with no SoC *P. jirovecii* testing, Unyvero detected *P. jirovecii* in 16 in the prospective (14 confirmed by PCR/sequencing) and 13 in the archived (10 confirmed by PCR/sequencing) arms. FN cases were analyzed by PCR/sequencing from specimen DNA extracts, and analyte presence was confirmed for all three FN *P. jirovecii* cases.

Antibiotic resistance markers. When *Enterobacterales*, *P. aeruginosa*, *Acinetobacter* species, *H. influenzae*, or *S. aureus* is detected, Unyvero reports the presence or absence of select antibiotic resistance markers, indicating a possible resistance phenotype (3rd-generation cephalosporin resistance for *Enterobacterales*, *Acinetobacter* species, and *P. aeruginosa* [based on the detection of $bla_{\text{CTX-M}}$], carbapenem resistance for *Enterobacterales*, *Acinetobacter* species, and *P. aeruginosa* [based on the detection of bla_{KPC}, bla_{NDM}, bla_{VIM}, or $bla_{\text{OXA-48}}$], carbapenem resistance for *Acinetobacter* species [based on the detection of $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, or $bla_{\text{OXA-58}}$], penicillin resistance for *H. influenzae* [based on the detection of bla_{TEM}], and oxacillin resistance for *S. aureus* [based on the detection of *mecA*]).

Table 7 summarizes the positive detections for panel antibiotic resistance markers observed for each bacterial species or group for prospective and archived specimens. Positive gene detections by Unyvero were analyzed by PCR/sequencing for confirmation of these markers in corresponding specimens, with 95.7%, 100%, 95.0%, and

TABLE 7 Antibiotic resistance gene detection in the prospective and archived arms

Type of resistance	Resistance gene	Study arm[a]	No. positive/ no. reported (%)[b]	No. confirmed by PCR followed by sequencing/ no. positive (%)
Resistance to 3rd-generation cephalosporins in *Enterobacterales*, *P. aeruginosa*, or *Acinetobacter* species	bla_{CTX-M}	Prospective	9/208 (4.3)	8/9
		Archived	14/212 (6.6)	14/14
		Total	**23/420 (5.5)**	**22/23 (95.7)**
Resistance to carbapenems in *Enterobacterales*, *P. aeruginosa*, or *Acinetobacter* species[c]	bla_{KPC}	Prospective	4/208 (1.9)	4/4
		Archived	2/212 (0.9)	2/2
		Total	**6/420 (1.4)**	**6/6 (100.0)**
	bla_{NDM}	Prospective	1/208 (0.5)	1/1
		Archived	0/212 (0.0)	
		Total	**1/420 (0.2)**	**1/1 (100.0)**
	bla_{VIM}	Prospective	1/208 (0.5)	1/1
		Archived	0/212 (0.0)	
		Total	**1/420 (0.2)**	**1/1 (100.0)**
	bla_{OXA-48}	Prospective	1/112 (0.9)	1/1
		Archived	0/166 (0.0)	
		Total	**1/278 (0.4)**	**1/1 (100.0)**
Resistance to carbapenems in *Acinetobacter* species conferred by bla_{OXA} panel markers	bla_{OXA-23}	Prospective	3/21 (14.3)	3/3
		Archived	4/21 (19.0)	4/4
		Total	**7/42 (16.7)**	**7/7 (100.0)**
	bla_{OXA-24}	Prospective	4/21 (19.0)	4/4
		Archived	3/21 (14.3)	3/3
		Total	**7/42 (16.7)**	**7/7 (100.0)**
	bla_{OXA58}	Prospective	0/21 (0.0)	
		Archived	1/21 (4.8)	1/1
		Total	**1/42 (2.4)**	**1/1 (100.0)**
Resistance to penicillin in *H. influenzae*	bla_{TEM}	Prospective	16/56 (28.6)	15/16
		Archived	24/71 (33.8)	23/24
		Total	**40/127 (31.5)**	**38/40 (95.0)**
Resistance to oxacillin (methicillin) in *S. aureus*	*mecA*	Prospective	47/104 (45.2)	32/47
		Archived	44/77 (54.1)	34/44
		Total	**91/181 (50.3)**	**66/91 (72.5)**

[a]Prospective study arm, $n = 1,016$; archived study arm, $n = 392$.
[b]Unyvero reports results for antibiotic resistance markers only if one or more corresponding host organism(s) is simultaneously detected (otherwise, marker results are masked).
[c]The carbapenem markers bla_{KPC}, bla_{NDM}, and bla_{VIM} are associated with *Enterobacterales*, *P. aeruginosa*, or *Acinetobacter* species (420 reported results for the prospective and archived study arms), while bla_{oxa-48} is associated with *Enterobacterales* only (278 reported results for the prospective and archived study arms).

72.5% confirmed positive for bla_{CTX-M}, carbapenemase genes, bla_{TEM}, and *mecA*, respectively. Neither the Unyvero assay nor PCR/sequencing establish marker linkage to host genomes, so detected antibiotic resistance markers and bacteria may be codetected but unrelated to one another, instead originating from other on- or off-panel bacteria.

Two hundred nineteen isolates (11 *Acinetobacter* species, 1 *C. freundii*, 19 *E. cloacae* complex, 18 *E. coli*, 10 *H. influenzae*, 6 *K. oxytoca*, 17 *K. pneumoniae*, 3 *K. variicola*, 6 *Proteus* species, 57 *P. aeruginosa*, 14 *S. marcescens*, and 57 *S. aureus*) isolated from SoC cultures underwent whole-genome sequencing to assess the presence or absence of panel resistance markers in their genomes. All whole-genome sequence results in which an antibiotic resistance marker on the Unyvero panel was found had corroborating phenotypic AST results (bla_{CTX-M}, 3 cases; bla_{KPC}, 1 case; bla_{NDM}, 1 case; bla_{OXA-48}, 1 case; bla_{OXA-23}, 3 cases; bla_{OXA-24}, 3 cases; bla_{TEM}, 4 cases; *mecA*, 26 cases). For *mecA* in *S. aureus* and bla_{TEM} in *H. influenzae*, the absence of the marker in the genome correlated with a susceptible phenotype for all cases; for other resistance markers, the absence of the marker in the genome cannot be correlated with a susceptible phenotype due to the possibility of other resistance mechanisms in corresponding host organisms.

TABLE 8 Genotypic agreement and phenotypic agreement of Unyvero resistance marker results with isolates for corresponding specimens, with 95% confidence intervals

Type of resistance	Genotypic agreement[a]			Phenotypic agreement (PPV)[b]
Resistance to 3rd-generation cephalosporins in *Enterobacterales*, *P. aeruginosa*, or *Acinetobacter* species	Unyvero results	No. of isolates harboring bla_{CTX-M}/total, % (95% CI)	No. of isolates not harboring bla_{CTX-M}/total, % (95% CI)	No. of bla_{CTX-M} genes detected and resistant phenotype of isolate(s)/total, % (95% CI)
	Host(s) and bla_{CTX-M}	3/3, 100.0 (43.9–100.0)	4	12/12, 100.0 (75.8–100.0)
	Host(s) without bla_{CTX-M}	0	117/124, 94.4 (88.8–97.2)	
	Host(s) not detected	0	3	
Resistance to carbapenems in *Enterobacterales*, *P. aeruginosa*, or *Acinetobacter* species	Unyvero results	No. of isolates harboring resistance marker(s)/total, % (95% CI)	No. of isolates not harboring resistance marker(s)/total, % (95% CI)	No. of resistance markers detected and resistant phenotype of isolate(s)/total, % (95% CI)
	Host(s) and marker(s)	2/2,[c] 100.0 (34.2–100.0)	3	2/2, 100.0 (34.2–100.0)
	Host(s) without marker(s)	0	119/125, 95.2 (89.9–97.8)	
	Host(s) not detected	0	3	
Resistance to carbapenems conferred by the bla_{OXA} panel markers in *Acinetobacter* species	Unyvero results	No. of isolates harboring resistance marker(s)/total, % (95% CI)	No. of isolates not harboring resistance marker(s)/total, % (95% CI)	No. of resistance markers detected and resistant phenotype of isolate/total, % (95% CI)
	Acinetobacter species and bla_{OXA}	6/6,[d] 100.0 (61.0–100.0)	0	8/9, 88.9 (56.5–98.0)
	Acinetobacter species without bla_{OXA}	0	5/5, 100.0 (56.6–100.0)	
	Acinetobacter species not detected	0	0	
Resistance to penicillin in *H. influenzae*	Unyvero results	No. of *H. influenzae* isolates harboring bla_{TEM}/total, % (95% CI)	No. of *H. influenzae* isolates not harboring bla_{TEM}/total, % (95% CI)	No. of bla_{TEM} genes detected and resistant phenotype of the isolate/total, % (95% CI)
	H. influenzae and bla_{TEM}	4/4, 100.0 (51.0–100.0)	0	17/19, 89.5 (68.6–97.1)
	H. influenzae without bla_{TEM}	0	6/6, 100.0 (61.0–100.0)	
	H. influenzae not detected	0	0	
Resistance to oxacillin (methicillin) in *S. aureus*	Unyvero results	No. of *S. aureus* isolates harboring *mecA*/total, % (95% CI)	No. of *S. aureus* isolates not harboring *mecA*/total, % (95% CI)	No. of *mecA* genes detected and resistant phenotype of the isolate/total, % (95% CI)
	S. aureus and *mecA*	21/26, 80.8 (62.1 to 91.5)	4	47/59, 79.7 (67.7–88.0)
	S. aureus without *mecA*	2	24/30, 80.0 (62.7–90.5)	
	S. aureus not detected	3	2	

[a]Genotypic agreement was determined for the subset of positive specimens for which isolates from corresponding host organisms were available for whole-genome sequencing.

[b]Phenotypic agreement of positive resistance markers (positive predictive value) was determined for the subset of SoC-positive specimens with available antimicrobial susceptibility test results for the corresponding host organisms. For a comparison of all samples with resistance markers reported by Unyvero to SoC results, please refer to Table S5 in the supplemental material.

[c]Two isolates distributed to the following host/marker combinations: *E. cloacae* complex/bla_{KPC} and *K. pneumoniae*/bla_{NDM} bla_{OXA-48}.

[d]Six *Acinetobacter* isolates harbored the following bla_{OXA} genes: bla_{OXA-23} (3 cases) and bla_{OXA-24} (3 cases).

Isolates with and without a genomic presence of resistance markers were compared to Unyvero results for genomic agreements for corresponding specimens (Table 8, genotypic agreements). In addition, positive predictive values (PPVs) compared to AST results for Unyvero detections are shown in Table 8 (phenotypic agreements).

One *E. coli* isolate and two *K. pneumoniae* isolates subjected to whole-genome sequencing harbored $bla_{\text{CTX-M}}$, all three of which were detected by Unyvero. For four discordant specimens, Unyvero detected $bla_{\text{CTX-M}}$, the presence of which was not confirmed in available corresponding isolates. In three of these, Unyvero reported additional bacterial species that were SoC negative and therefore not assessed with whole-genome sequencing. For the fourth discordant specimen, from which $bla_{\text{CTX-M}}$-negative *E. coli* was isolated, $bla_{\text{CTX-M}}$ was found in *Providencia stuartii* (which is not on the Unyvero panel). Determining phenotypic PPVs is challenging for specimens with more than one organism present and not reported by SoC testing. Therefore, determining whether a Unyvero resistance marker result was concordant or discordant with the isolate phenotype was performed only if AST data were available for all detected bacteria. For this limited subset, a phenotypic PPV of 100.0% (12/12) was observed.

bla_{KPC} was found in a single isolate of *E. cloacae* complex and detected by Unyvero; both bla_{NDM} and $bla_{\text{OXA-48}}$ were found in a single isolate of *K. pneumoniae* and detected by Unyvero. There were three discordant specimens, with Unyvero detecting bla_{KPC} (two cases) or bla_{VIM} (one case) together with additional host bacteria that were negative by SoC testing and with isolates of *Acinetobacter* species and *P. aeruginosa* testing negative for these resistance genes. Both bla_{KPC}-positive specimens tested positive for *K. pneumoniae* by Unyvero. For one of these specimens, SoC testing found *K. pneumoniae* below the reporting threshold for lavage specimens; the presence of bla_{KPC} in this isolate was confirmed by whole-genome sequencing. For the second specimen, *K. pneumoniae* was reported by both Unyvero and SoC testing (together with a carbapenem-resistant phenotype), but an isolate was not available to Curetis. Like $bla_{\text{CTX-M}}$, carbapenem resistance markers were often observed in specimens with multiple species detected or specimens for which Unyvero reported additional bacteria not detected by culture. A phenotypic PPV (100.0% [2/2]) was determined only for the subset of specimens (both bla_{KPC} positive) with available AST results for all applicable bacteria.

For the 11 specimens positive for *Acinetobacter* isolates, the presence or absence of $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, or $bla_{\text{OXA-58}}$ was concordantly determined by Unyvero and isolate sequencing, with three isolates each harboring $bla_{\text{OXA-23}}$ or $bla_{\text{OXA-24}}$. A phenotypic PPV of 88.9% (8/9) was observed. For the eight concordant specimens, Unyvero reported $bla_{\text{OXA-23}}$ (3 cases) and $bla_{\text{OXA-24}}$ (5 cases); for one specimen, Unyvero reported $bla_{\text{OXA-24}}$, and a carbapenem-susceptible phenotype was found with no isolate available for whole-genome sequencing.

All specimens positive for *H. influenzae* for which isolates were available with and without a confirmed genomic presence of bla_{TEM} (4 and 6 isolates, respectively) were correctly detected by Unyvero. A phenotypic PPV of 89.5% (17/19) was observed. Two specimens that were reported by Unyvero as having *H. influenzae*, and bla_{TEM} had discordant AST results (penicillin susceptible), but isolates were not available for further testing.

Specimens positive for *S. aureus* with a confirmed genomic presence of *mecA* in the corresponding isolate were detected by Unyvero with an agreement of 80.8% (21/26). Lack of agreement resulted from not detecting *mecA* (two cases) or *S. aureus* (three cases). Specimens positive for *S. aureus* without a genomic presence of *mecA* in the corresponding isolate were detected by Unyvero with an agreement of 80.0% (24/30). For four specimens, *mecA* was reported, although sequencing showed the absence of *mecA* in the corresponding isolates and AST indicated susceptibility to oxacillin. A phenotypic PPV of 79.7% (47/59) was observed.

DISCUSSION

Diagnosis of the etiology of pneumonia in clinical practice is challenging. With an extensive microbial differential diagnosis and the ever-increasing challenge of

antimicrobial resistance, more precise diagnostics for pneumonia, especially severe pneumonia, are likely to be beneficial so that patients with pneumonia receive timely, effective, and not overly broad-spectrum therapy. BAL fluid is considered an excellent specimen for the assessment of lower respiratory tract infections; however, culture yield can be low, especially in the context of antecedent antibiotic therapy (17). Here, we evaluated the Unyvero LRT BAL multiplex PCR panel approach for detecting 19 bacteria and one fungus, alongside 10 resistance genes. There was an overall high negative predictive value of 97.2% on a per-sample basis for microorganism detection, potentially allowing for de-escalation of antibiotics. The overall PPA and NPA with culture for the detection and identification of microorganisms that grow in routine cultures were 93.4% and 98.3%, respectively.

Similar agreements have been published recently by Collins et al. (10), who compared the performance of the Unyvero LRT panel (same targets as Unyvero LRT BAL, except for *P. jirovecii*) to routine bacterial culture methods on 175 BAL specimens and reported a sensitivity of 96.5% and a specificity of 99.6% among the microbial targets. For antibiotic resistance marker analytes of the LRT BAL panel, a PPV of 100% was reported. In another recent publication, Pickens et al. (18) reported a sensitivity of 85.7% and a specificity of 98.4% for 620 respiratory specimens (395 bronchoscopic or nonbronchoscopic BAL specimens, 225 aspirates) using the Unyvero LRT panel.

The Unyvero HPN/P55 panel (commercialized outside the United States for use with lavage, aspirate, or sputum samples) includes additional analytes, and therefore performance data may be not comparable to those of the FDA-cleared Unyvero pneumonia panels. Peiffer-Smadja et al. (19) evaluated 95 bronchoalveolar samples from ventilated patients with hospital-acquired pneumonia using the HPN panel and reported an overall sensitivity and specificity of 80% and 99%, respectively. Gadsby et al. (20) evaluated 74 bronchoalveolar lavage fluid specimens from patients admitted to a Scottish intensive care unit using the Unyvero P55 panel and reported an overall sensitivity for on-panel targets of 63.5%. Ozongwu et al. (21) studied 85 respiratory specimens using the Unyvero P55 assay, with an overall sensitivity and specificity for on-panel targets of 88.8% and 94.9%, respectively. Studies have also been published on another precursor version (P50) with a different target gene panel that is no longer commercialized (22, 23).

Detection of typically cultivatable microorganisms by molecular approaches but not culture may occur due to the presence of nonviable organisms, including those treated with antibiotics. Conversely, culture-based tests may be biased toward the fastest-growing or most predominant organisms and may report respiratory or oropharyngeal flora only, missing pathogens hidden within the overgrowth. Culture-based tests are also more likely to be impacted by specimen transportation or storage than are molecular tests. Unyvero detected organisms not reported by SoC testing in 21.7% of specimens in the prospective study arm, with an increased rate of polymicrobial detections compared to that of SoC testing. Cross-reactivity to closely related species was observed by molecular methods (PCR/sequencing) for a few samples (*H. influenzae*, 13.0% [9/69]), likely caused by the close genetic similarity of the gene target (23S rRNA) to those of other *Haemophilus* or *Aggregatibacter* species. The majority of detected additional organisms were confirmed by molecular methods (PCR/sequencing). Culture and molecular assays combined may therefore provide a better gold standard than culture alone (18). Increased detection rates compared to that of SoC culture have been observed for other syndromic molecular panels (24, 25).

For respiratory specimens, differentiation of colonizing or contaminating organisms from pathogens, by culture and/or molecular techniques, can be challenging. This is especially so in intubated patients, whose endotracheal tubes provide a pathway for microorganisms to enter the lower respiratory tract and a site for colonization related to biofilm formation. Although quantitative reporting may be helpful, there is scant

evidence supporting how to incorporate such quantities into patient management, and bronchoalveolar lavage and other specimens are not necessarily homogeneous, adding challenges to quantification. Besides the Unyvero test, the BioFire pneumonia panel (BioFire, Salt Lake City, UT) is the only other FDA-cleared lower respiratory tract panel (24, 26–30). Its configuration is different from that of the Unyvero panel, including, for example, several viruses, but not *P. jirovecii*. This panel reports results of detected bacteria that can be isolated in routine bacterial cultures semiquantitatively using four different bin categories corresponding to 10^4, 10^5, 10^6, or $\geq 10^7$ copies/ml (24), whereas the Unyvero LRT BAL Application does not. Correlations to quantitative culture results reported as CFU/ml can be challenging; whether results should be reported quantitatively or qualitatively for ideal clinical utility is as-yet undefined (9, 24, 29). In general, such molecular panels may have the most promising impact on clinical utility when they are integrated into the standard testing practices (Gram stain, culture, AST). We recognize that, as with SoC culture, it may sometimes be difficult to discriminate pathogens from colonizers. We also consider the possibility of off-panel organisms or resistance markers, not covered by such panels, being missed. However, these panels still provide valuable information on a comprehensive range of common pathogens and resistance markers days before SoC results become available and often identify potential pathogens missed in SoC culture.

In this study, several detections of *L. pneumophila*, *M. pneumoniae*, and *P. jirovecii* occurred outside clinically ordered testing. Although we are unable to ascertain the clinical significance of these findings, it is possible that such diagnoses are missed in clinical practice. Peiffer-Smadja et al. (19) recently reported two unexpected cases of severe legionellosis detected in ventilator-associated pneumonia (VAP) patients using the Unyvero HPN panel (both of which were confirmed by culture). Nucleic acid amplification testing is a recommended approach for *P. jirovecii* (14); the Unyvero platform is the only FDA-cleared panel to offer this testing. The PPA and NPA for detection of *P. jirovecii* were 100.0% (5/5) and 99.0% (99/100), respectively, for the prospective study arm for the subset of samples routinely tested by SoC methods (IFA, DFA, or PCR). Interestingly, Unyvero LRT BAL detected *P. jirovecii* in another 16 samples in the prospective study arm, with 14 confirmed by an additional molecular test. Eight of 16 samples were reported negative for all other panel organisms by both SoC and Unyvero testing; two were reported negative by SoC testing but positive by Unyvero. Although this organism is a known colonizer at concentrations lower than 10^4 copies/ml, concentrations of 10^5 copies/ml or higher (the analytical limit of detection for the *P. jirovecii* assay on the Unyvero panel) may be associated with *P. jirovecii* pneumonia (31–34). As such, the additional Unyvero findings may be indicative of *P. jirovecii* pneumonia (PCP) that would otherwise remain undiscovered if only routinely ordered SoC tests are applied, especially in cases of non-HIV patients. As the potential for PCP is often not even considered in such patients and may be rare, routine testing for *P. jirovecii*, provided by the Unyvero panel, may be beneficial, in particular for patients whose etiology is difficult to determine.

Antibiotic resistance marker PPVs were 100% based on the detection of $bla_{\text{CTX-M}}$, bla_{KPC}, bla_{NDM}, bla_{VIM}, or $bla_{\text{OXA-48}}$, 88.9% based on the detection of bla_{OXA} markers in *Acinetobacter* species, 89.5% based on the detection of bla_{TEM}, and 79.7% based on the detection of *mecA*. A limitation of a PCR-based approach is that it does not specifically link the detected antibiotic resistance gene to the detected microorganism. This limitation affected the detection of methicillin-resistant *S. aureus* in cases in which *mecA* was detected in specimens alongside *S. aureus*, that were methicillin susceptible, likely due to the presence of *mecA* in coagulase-negative staphylococci from respiratory flora. A low-grade *mecA* background originating from respiratory flora may also be the reason why it was sometimes difficult to confirm a particular *mecA* result reported by Unyvero using molecular assays.

For the Gram-negative resistance genes, detection of a resistance gene does not necessarily link it with its host bacterium. Nevertheless, for Gram-negative bacilli, there

were strong genotypic and phenotypic correlations of Unyvero results to corresponding isolates. Reporting of resistance genes may provide a clue to the presence of an underlying resistant organism, which may have implications for infection prevention and control (e.g., if bla_{KPC}, bla_{NDM}, bla_{VIM}, or bla_{OXA-48} is detected), even if the species with which the gene is associated is unknown.

Ultimately, it is clinical correlation to signs and symptoms of pneumonia that lead to the diagnosis. The Unyvero LRT BAL assay does not distinguish a colonizer from a pathogen, but it gives the clinicians more data to guide them in their treatment choices. This is especially true for a patient who is not responding to broad-spectrum antibiotics and from whom a BAL specimen is then collected; given the necessity of performing an invasive procedure to collect this specimen, maximizing the data obtained may be helpful to increase the ability of a clinician to determine appropriate antibiotics. The detection of *Acinetobacter* species in combination with bla_{OXA}-type resistance markers, *S. maltophilia*, or *P. jirovecii* in a patient for whom those were not suspected or finding *K. pneumoniae* with a bla_{KPC} gene hidden among the "oropharyngeal flora" may change the management of a patient and potentially improve outcomes. Molecular detection of bla_{KPC} has been associated with positive outcomes, including reduced times to optimal antibiotic therapy, shorter lengths of intensive care unit stays, and reduced mortality (35). A recent paper looking at the potential for the Unyvero assay to guide therapy found that it could potentially have changed the management of 87.6% of patients, including possibly facilitating antibiotic de-escalation (66%) or escalation (10%) (18). Prospective, randomized, controlled trials that measure the clinical impact of this platform when used with appropriate antibiotic stewardship (7, 36) can further assess such clinical utility.

Early diagnosis and proper choice of antimicrobials are crucial for successful management of pneumonia. The Unyvero LRT BAL Application provides accurate detection of 19 bacteria alongside *P. jirovecii* and 10 antibiotic resistance genes from bronchoalveolar lavage fluid, allowing enhanced diagnosis of lower respiratory tract infections.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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