Tuesday-59

Comparison of a Multiplex PCR Lower Respiratory Tract Assay with Bacterial Culture

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Abstract

(UPDATED)

Background: Lower respiratory tract infections (LRTI) present with a wide variety of symptoms, severity, and causative agents. Therefore, a wide range of diagnostic methods are needed to detect and differentiate bacterial, viral and fungal causative agents of LRTI, including quantitative bacterial culture, direct staining and molecular methods. Quantitative culture of bronchoalveolar lavage fluids (BALF) is labor intensive, and the delay involved in culture, definitive identification and susceptibility testing often results in extended use of broad spectrum antibiotics. The Investigational Use Only Multiplex PCR Unyvero LRT Assay (Curetis; Holzgerlingen, Germany) allows for the rapid detection and identification of 20 potential etiologic agents within 5 hours of collection. In addition to offering multiple targets known to cause LRTI, the assay includes detection of 17 target gene sequences that confer antimicrobial resistance.

Methods: Remnant BALFs ordered for quantitative bacterial culture were included (n=127). Specimens from CF patients and patients ≤16 yo were excluded. Quantitative bacterial culture was performed as part of routine care by the UNC Hospitals' Microbiology Laboratory. Multiple aliquots of BALFs were frozen at -70°C within 24 h of collection, and an aliquot was thawed immediately before testing on the Unyvero instrument.

Results: Among the 127 samples tested, culture results included no growth (n=13), growth not further identified with <10,000 CFU/mL (n=6), and mixed organisms (n=18). Of the no growth group, Unyvero produced 10 negative results and in three samples it detected a target that was not isolated from culture. Of these (n=3), one produced a Unyvero result of *S. aureus.* This sample came from the right upper lobe of a patient that grew *S. aureus* in their right middle lobe. All six of the <10,000 CFU/mL samples were negative by Unyvero. All of the mixed growth culture samples had two or more bacterial targets detected by PCR, some with corresponding resistance markers.

Oropharyngeal flora (OPF) grew in a fifth of the samples (n=27), of which 23 also grew additional potential pathogens. Two with OPF present on culture were negative by Unyvero and two others had targets identified. Of the 23 with additional potential pathogens, Unyvero successfully identified all isolated pathogens in 16 BALF. In 7 cases, Unyvero identified all isolated pathogens included on the panel but in addition called one or more bacterial targets that were not included in the culture report.

Twenty BALFs grew isolates not included on the LRT panel. When these were the only isolates grown (n=9), the Unyvero was negative for all but one sample. When other pathogens were also isolated, Unyvero identified the organisms (n=6) that are included on the panel, and organisms that were not reported by culture (n=2). The remaining three samples, Unyvero did not detect any cultured pathogen.
There were three additional BALFs for which Unyvero did not detect any cultured pathogen.

Conclusions: The performance of the Unyvero LRT assay as compared to culture proved to be largely concordant. The LRT panel is unique in that it provides sample to answer results in less than 5 h and detects a variety of pathogens and antibiotic resistance markers. In the cases of mixed cultures for which identification results were available, Unyvero was able to identify multiple pathogens present that were not reported by culture. Limiting our ability to analyze concordance with culture was the "mixed organisms" report used by the Microbiology Laboratory. Additionally, susceptibility testing was not performed on all isolates cultured, which made it difficult to interpret resistance gene detection by Unyvero. From an antimicrobial stewardship prospective, having resistance results within 5h of specimen collection could be beneficial. While the analytical performance of the Unyvero LRT assay is comparable to traditional methods, the clinical utility of a qualitative molecular assay as compared to quantitative culture deserves further investigation.

Table 1: Organism and resistance targets on IUO Unyvero LRT Panel

Type	Target
Gram-positive	*Staphylococcus aureus*
	Streptococcus pneumoniae
Enterobacteriaceae	*Citrobacter freundii*
	Escherichia coli
	Enterobacter cloacae complex
	Proteus spp.
	Klebsiella pneumoniae
	Klebsiella oxytoca
	Klebsiella variicola
	Serratia marcescens
	Morganella morganii
Non-fermenters	*Moraxella catarrhalis*
	Pseudomonas aeruginosa
	Acinetobacter baumannii complex
	Stenotrophomonas maltophilia
	Legionella pneumophila
Others/Fungi	*Pneumocystis jirovecii*
	Haemophilus influenzae
	Mycoplasma pneumoniae
	Chlamydia pneumoniae

Gene	Resistant Against
*erm*B	macrolide/lincosamide
mecA	oxacillin
*mec*C (LGA251)	
tem	penicillin
shv	
ctx-M	3rd generation cephalosporin
kpc	carbapenem
imp	
ndm	
oxa-23	
oxa-24/40	
oxa-48	
oxa-58	
vim	
*sul*1	sulfonamide
*gyr*A83	fluoroquinolone
*gyr*A87	

Results

Figure 1: Study population, September 2017-April 2018 (n=73)

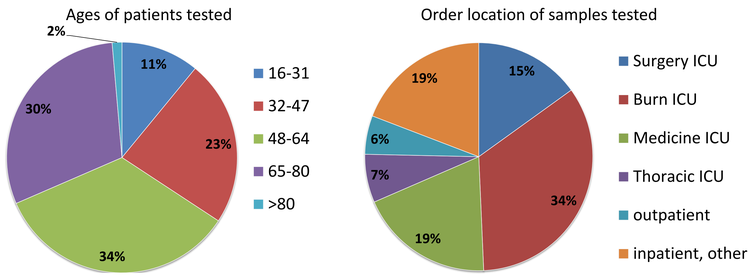


Figure 2: Unyvero sample results, by target (n=127)

Note: No tested samples were positive for *C. pneumoniae, K. oxytoca, M. morganii, M. pneumoniae*

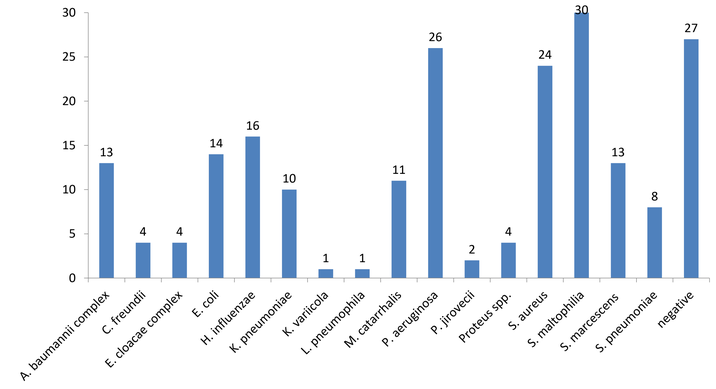


Table 2: Positive and negative agreement of Unyvero compared to culture

Note: Table does not include 38 samples (44 targets) that we were unable to analyze in comparison to culture

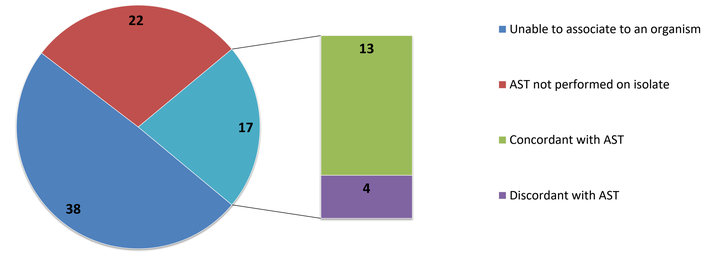
Organism	TP	FN	FP	TN	PPA/sensitivity	NPA/specificity
S. aureus	20	2	3	64	91%	96%
S. pneumoniae	5	1	3	80	83%	96%
C. freundii	3	0	0	86	100%	100%
E. coli	2	0	3	84	100%	97%
E. cloacae complex	2	2	0	85	50%	100%
Proteus spp.	0	0	3	86	n/a	97%
K. pneumoniae	8	0	1	80	100%	99%
K. oxytoca	0	0	0	89	n/a	100%
K. variicola	0	0	0	89	n/a	100%
S. marcescens	7	0	0	82	100%	100%
M. morganii	0	0	0	89	n/a	100%
M. catarrhalis	5	0	2	82	100%	98%
P. aeruginosa	23	0	1	65	100%	98%
A. baumannii complex	10	0	0	79	100%	100%
S. maltophilia	17	0	2	70	100%	97%
L. pneumophila	1	0	0	88	100%	100%
P. jirovecii	1	0	0	88	100%	100%
H. influenzae	9	0	6	74	100%	93%
M. pneumoniae	0	0	0	89	n/a	100%
C. pneumoniae	0	0	0	89	n/a	100%
Total	113	5	24	1638	96%	99%

Additional organisms identified by culture: *Achromobacter* sp., *Burkholderia cepacia* complex (3), *Burkholderia gladioli*i, *Candida* sp. (4), *Corynebacteium* sp. (2), *E. faecalis, E. faecium*, *Klebsiella aerogenes*, *N. meningitidis*(2), *Nocardia* sp. (2), *Raoultella ornithinolytica*, *S. mitis*

Results

Figure 3: Resistance genes detected

3a: All resistance genes detected by Unyvero (n=77), relative to the ability to associate the gene to an isolate



3b: Resistance genes that could be associated with an organism, compared to phenotypic susceptibility testing results, n=39

Organism	Unyvero resistance gene detected	Phenotypic AST (ND=not done)
S. aureus	*mecA*	oxacillin resistant (n=7)
		ND (n=2)
E. coli	*gyrA*	ND (n=10)
	tem	ampicillin resistant (n=1)
		ND (n=3)
	sul1	ND (n=2)
K. pneumoniae	*tem*	ampicillin resistant (n=1)
K. variicola	*tem*	ampicillin resistant (n=1)
P. aeruginosa	*gyrA*	ciprofloxacin susceptible (n=4)
		ciprofloxacin intermediate (n=1)
		levofloxacin resistant (n=2)
		ND (n=2)
A. baumannii complex	*tem*	ND (n=1)
H. influenzae	*tem*	ND (n=2)

Conclusions

- Our study used the IUO Unyvero LRT panel which includes additional targets not included in the FDA cleared panel.
- Unyvero identified 181 organism targets and 77 resistance genes.
- Concordance for organism targets was 98.4% and positive agreement rate for resistance genes was 76.5% when compared to bacterial culture and phenotypic susceptibility testing.
- In cases of mixed growth, the Unyvero was able to identify separate targets (n=42) from 36 samples that were not further differentiated on routine culture.
- The limit of detection for the instrument is 10,000 CFU/mL, which is comparable to the clinical threshold used for culture reporting.
- Results obtained by this panel within 5h of collection provides clinicians with an early organism differential, including resistance, allowing earlier treatment decisions and support for antimicrobial stewardship efforts.

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