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Comparative evaluation of Diatherix TEM-PCR™ and BioFire FilmArray® in the detection of viral and bacterial respiratory pathogens

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Background

The speed and efficiency of etiologic determination of influenza-like illness (ILI) has been greatly improved with multiplex diagnostic assays. We conducted a comparative evaluation of two multiplex PCR-based platforms with comparable coverage of viral and bacterial respiratory pathogens.

Methods

- Acute Respiratory Infection Consortium (ARIC) Natural History Study (NHS) is an observational, longitudinal study of influenza-like illness (ILI). Since 2009, we enrolled otherwise healthy military personnel and beneficiaries into ARIC at five military treatment facilities across the continental United States.
- Eligibility: patients presenting for care <72h after the onset of ILI, defined as fever (temperature of 100.4° F or greater at the time of evaluation, or by self-report) and sore throat or one of the following respiratory symptoms: cough, sputum production, shortness of breath, or chest pain. Patients with underlying medical conditions were excluded from participation.
- Nasal/oral specimens were collected by study coordinators using nylon flocked swabs (Copan Diagnostics, Corona, CA). at the time of enrollment and tested by two assays: [1] target-enriched multiplex PCR (TEM-PCR™; Diatherix Laboratories, Inc.; Huntsville, AL) and [2] BioFire FilmArray® Respiratory Panel (FilmArray®; BioFire Diagnostics, Salt Lake City, UT). Viral and bacterial pathogens covered by the two assays are listed in Table 1. Specimens (n=396) were selected based on availability, and tests were performed in separate laboratories.

Table 1. Respiratory pathogens covered by TEM-PCR™ and BioFire FilmArray®

Viral pathogens	Diatherix TEM-PCR™	BioFire FilmArray®
Human Rhinovirus and Enterovirus	✓ HRV, Coxackievirus/Echovirus	✓ HRV/Enterovirus
Influenza A (pH1N1, H1N1 and H3N2)	✓	✓
Influenza B	✓	✓
Coronavirus (HKU1, NL63, 229E, and OC43)	✓	✓
Respiratory Syncytial virus (A and B)	✓	✓
Parainfluenza (PIV1 -4)	✓	✓
Human Metapneumovirus	✓	✓
Adenovirus	✓	✓
Bocavirus	✓	
Bacterial pathogens		
Streptococcus pneumoniae	✓	
Haemophilus influenzae	✓	
Moraxella catarrhalis	✓	
Staphylococcus aureus (MSSA/MRSA)	✓	
Streptococcus pyogenes	✓	
Klebsiella pneumoniae	✓	
Mycoplasma pneumoniae	✓	✓
Acinetobacter baumannii	✓	
Pseudomonas aeruginosa	✓	
Neisseria meningitidis	✓	
Bordetella pertussis	✓	✓
Chlamydophila pneumoniae	✓	✓

- Specimens were also tested by single-plex PCR for influenza at the Naval Health Research Center (San Diego, CA) using the CDC human influenza virus real-time RT-PCR diagnostic panel.
- Kappa (κ) coefficients and 95% confidence intervals (CI) were computed. In addition, the sensitivity and specificity of each platform for detecting influenza virus was computed using single-plex PCR tests as a gold standard. Statistical analyses were performed using SAS software (Version 9.3; SAS Institute, Cary, NC).

Results

- Of the 396 specimens tested by both platforms, TEM-PCR™ and FilmArray® detected at least one viral pathogen among 256 (64.7%) and 262 (66.2%) specimens, respectively (Figure 1). The frequency of viral co-detection (i.e. two or more viral pathogens in a specimen) was 7.3% for TEM-PCR™ and 5.8% for BioFire FilmArray®.

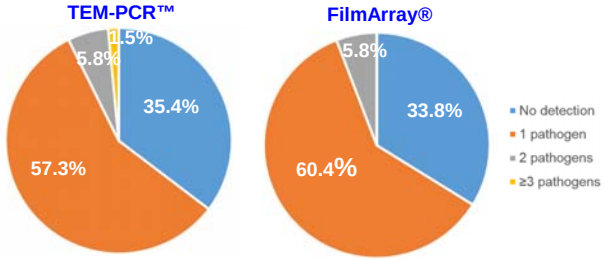

Figure 1. Number of viral pathogens detected among 396 ARIC NHS patients using TEM-PCR™ and FilmArray® Panels

- Viral pathogens: TEM-PCR™ had a higher frequency of detection of influenza A/B, coronavirus, human metapneumovirus, RSV, and parainfluenza virus, while FilmArray® more frequently detected adenovirus and enterovirus. There was substantial pathogen-specific agreement between the panels, with the exception of Adenovirus and influenza B (Table 2).

Table 2. Viral pathogen-specific agreement between TEM-PCR™ and FilmArray®

		FilmArray®		Agreement	
	TEM-PCR™	Negative	Positive	*K*	95%CI
Adenovirus	Negative	387	7	0.19	(-0.14, 0.52)
	Positive	1	1		
Influenza A	Negative	358	2	0.76	(0.63, 0.88)
	Positive	12	24		
A(H1N1)pdm09	Negative	374	2	0.80	(0.66, 0.94)
	Positive	5	15		
A(H3N2)	Negative	380	0	0.71	(0.51, 0.91)
	Positive	7	9		
Influenza B	Negative	384	2	0.58	(0.3, 0.86)
	Positive	5	5		
Enterovirus (including HRV)	Negative	289	21	0.74	(0.66, 0.82)
	Positive	15	71		
Coronavirus	Negative	329	2	0.91	(0.86, 0.97)
	Positive	7	58		
Human metapneumovirus (hMPV)	Negative	362	3	0.86	(0.76, 0.95)
	Positive	5	26		
RSV	Negative	344	5	0.85	(0.77, 0.93)
	Positive	7	40		
Parainfluenzavirus	Negative	377	0	0.97	(0.92, 1)
	Positive	1	18		

- Bacterial pathogens: TEM-PCR™ had a higher frequency of detection of *M. pneumoniae*. The prevalence of *C. pneumoniae* and *B. pertussis* was too low to allow a comparative evaluation.

Table 3. Bacterial pathogen-specific agreement between TEM-PCR and FilmArray®

		FilmArray®		Agreement	
	TEM-PCR™	Negative	Positive	*K*	95%CI
M. pneumoniae	Negative	389	0	0.72	(0.42, 1)
	Positive	3	4		

- Both assays were sensitive and specific in detecting influenza A and B, when using CDC human influenza virus real-time RT-PCR diagnostic panel as a gold standard (Table 4).

Table 4. Sensitivity and specificity of detecting influenza virus (A/B) using TEM-PCR™ and FilmArray®

	FilmArray®				TEM-PCR™			
	Single-plex PCR				Single-plex PCR			
	Negative	Positive	Sen.(%)	Spe.(%)	Negative	Positive	Sen.(%)	Spe.(%)
Influenza A								
Negative	368	2	90.48	98.13	359	1	95.24	95.73
Positive	7	19			16	20		
A(H1N1)pdm09#								
Negative	377	0	100.0	98.18	374	0	100.0	97.4
Positive	7	10			10	10		
A(H3N2)#								
Negative	385	0	100.0	100.0	379	0	100.0	98.44
Positive	0	9			6	9		

Two specimens were tested positive for influenza A by NHRC, but no subtype was identified and were excluded from the analysis. Sen: sensitivity; Spe: specificity

Conclusions

These results reveal a high degree of concordance between Diatherix Laboratories TEM-PCR™ and BioFire FilmArray® in the detection of viral respiratory pathogens. Low correlation between the panels with respect to adenovirus may be due to differences in assay inclusivity for detection of the specific targets.

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