Poster No. 1403

Evaluation of Diatherix Laboratories TEM-PCR™: a novel multiplex diagnostic panel for detection of bacterial and viral respiratory pathogens

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Background

- A broad diversity of viral and bacterial pathogens cause influenza-like illness (ILI). Determination of etiology is important for selection of appropriate treatment, design of prevention strategies (e.g. vaccines), and determining the relative contribution of specific pathogens to the overall burden of disease.
- Molecular-based diagnostic methods (i.e. PCR) are highly sensitive and specific. However, reliance upon single PCR-based assays for diagnosis is costly and inefficient.
- Multiplex diagnostic methods increase the efficiency and decrease the turnaround time for etiologic determination. The development of novel multiplex platforms requires evaluation of their performance using clinical isolates.

Methods

- A target-enriched multiplex PCR (TEM-PCR™) panel for 14 bacterial and 12 viral respiratory pathogens has been developed by Diatherix Laboratories, Inc. (Huntsville, AL, USA; Table 1). TEM-PCR™ is protected by US 7,851,148 and is the property of Diatherix Laboratories.

Table 1. Viral and Bacterial Pathogens Included on Diatherix Laboratories TEM-PCR™ Respiratory Infection Panel

Adenovirus types 3, 4, 7, 21	*Acinetobacter baumannii*	*Pseudomonas aeruginosa*
Coxsackievirus/Echovirus	*Bordetella pertussis*	*Staphylococcus aureus*
Human bocavirus	*Chlamydophila pneumoniae*	*Streptococcus pneumoniae*
Human coronavirus (4 types)	*Haemophilus influenzae*	*Streptococcus pyogenes* (Group A)
Human metapneumovirus	*Klebsiella pneumoniae*	
Human rhinovirus	*Legionella pneumophila*	
Influenza A - Human influenza	MRSA	
Influenza A - H1N1-09	*Moraxella catarrhalis*	
Influenza B	*Mycoplasma pneumoniae*	
Parainfluenza virus 1, 2, 3, 4	*Neisseria meningitidis*	
Respiratory syncytial virus A and B		

- After nucleic acid extraction, low concentration of nested gene-specific primers are (Fo-forward out; Fi-forward in; Ri-reverse in; Ro-reverse out) used for target enrichment during the initial PCR cycles. Later in the procedure, a pair of universal SuperPrimers is used to amplify all targets. The Reverse SuperPrimer is labeled with biotin for subsequent detection of amplicons. PCR products are hybridized to complimentary detection probe covalently coupled to a solid surface substrate and detection is facilitated by addition of Streptavidin-labeled Phycoerythrin (Figures 1 and 2).

Figure 1. Schematic of target enrichment procedure in Diatherix Laboratories TEM-PCR multiplex diagnostic panel

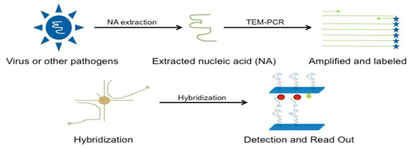

Figure 2. Workflow diagram of sample processing using Diatherix's TEM-PCR multiplex diagnostic panel

- The source of the clinical isolates for this evaluation was an observational study of febrile ILI among otherwise healthy 0-65y subjects presenting for care at five US military hospitals. Nasopharyngeal specimens were collected from participants and tested by single reaction PCR for influenza, human rhinovirus (HRV), and adenovirus.
- A subset of 403 specimens (77 influenza PCR-positive, 54 HRV PCR-positive, 10 adenovirus PCR-positive (including 2 co-detection of adenovirus and HRV), and 264 influenza- , HRV- and adenovirus PCR-negative) was selected for testing by TEM-PCR. The median (range) age of patients was 24.5y (16 d-63.7 y). Thirty-one percent (n=125) of specimens were from subjects <18y.
- Samples were processed for TEM-PCR at Diatherix Laboratories, Huntsville, AL. Single reaction PCR was performed at the Naval Health Research Center.

Results

- Of 403 specimens, 387 (96%) were evaluated by TEM-PCR™ with 259 (67%) positive for at least one virus.
- Viral detection rates were: HRV, n=61 (16%); Influenza A, n=51 (13%); Coxsackie/Echovirus, n=45 (12%); Coronavirus, n=42 (11%); RSV, n=40 (10%); Parainfluenza, n=22 (5.7%); Human Metapneumovirus, n=18 (5%); Influenza B, n=17 (4%); Adenovirus, n=4 (1%); Bocavirus, n=1 (0.3%; Table 2). *Streptococcus pneumoniae* was the most frequently detected bacterial pathogen (Table 3).

Table 2. Viral etiology by TEM-PCR™ among children and adults presenting with influenza-like illness

Virus detected and subtypes	No.	%
Human Rhinovirus	61	(15.8)
Influenza A	51	(13.2)
H3N2	35	
pH1N1	16	
Coxsackievirus/Echovirus	45	(11.6)
Coronavirus	42	(10.9)
CoV-229E	11	
CoV-HKU1	10	
CoV-NL63	6	
CoV-OC43	15	
Respiratory Syncytial Virus	40	(10.3)
RSV-A	28	
RSV-B	12	
Parainfluenza Virus	22	(5.7)
PIV-1*	6	
PIV-2	4	
PIV-3*	10	
PIV-4	2	
Human metapneumovirus	18	(4.7)
Influenza B	17	(4.4)
Adenovirus	4	(1.0)
ADV3	3	
ADV4	1	
Bocavirus	1	(0.3)

* Due to sequence homology, PIV-1 and PIV-3 are known to cross-react. PIV-1 through -4 are reported as Parainfluenza Virus 'Detected'.

Table 3. Bacterial etiology by TEM-PCR™ among children and adults presenting with influenza-like illness

Bacteria detected	No.	%
Streptococcus pneumoniae	150	(37.4)
Staphylococcus aureus	115	(28.7)
Haemophilus influenzae	106	(26.4)
Moraxella catarrhalis	93	(23.2)
Streptococcus pyogenes	23	(5.7)
Klebsiella pneumoniae	20	(5.0)
Acinetobacter baumannii	9	(2.2)
Pseudomonas aeruginosa	8	(2.0)
Mycoplasma pneumoniae	6	(1.5)
Neisseria meningitidis	4	(1.0)
Other Detection	94	(23.4)

- Twelve percent of specimens had more than one viral pathogen detected in the specimen (Figure 3). Among cases with Influenza A infection, 23.3% had co-detection of at least 2 bacterial pathogens targeted by the platform (Figure 4).

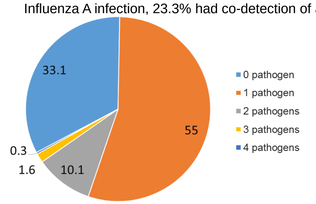

Figure 3. Proportion of nasopharyngeal specimens by number of viral pathogens detected (n=387). Three and 4 pathogens were detected in 6 (1.3%) and 1 (0.6%) samples, correspondingly.

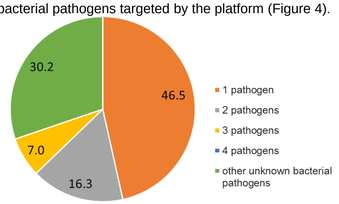

Figure 4. Proportion of nasopharyngeal specimens by number of bacterial pathogens detected among Influenza A-positive cases (n=43). Thirty percent of cases (green) had detection of bacterial pathogen not targeted by the platform.

- Compared to single reaction PCR, the sensitivity/specificity of TEM-PCR (Table 4) was as follows: influenza A (94%, 99%); influenza B (65%, 99%); HRV (68%, 90%); adenovirus (40%, 100%).

Table 4. Sensitivity and specificity of TEM-PCR as compared to single PCR for influenza, rhinovirus and adenovirus

Singleplex PCR results	Diatherix results No	Yes	Total	Sensitivity and Specificity estimates	Numbers.	Estimates	95% CI
Influenza A							
No	333	1	334	Spe.	333/334	0.99	(0.98, 1.00)
Yes	3	50	53	Sen.	50/53	0.94	(0.84, 0.99)
Total	336	51	387				
Influenza B							
No	362	2	364	Spe.	362/364	0.99	(0.98, 1.00)
Yes	8	15	23	Sen.	15/23	0.65	(0.43, 0.84)
Total	370	17	387				
HRV							
No	189	3	192	Spe.	189/192	0.98	(0.96, 1.00)
Yes	17	36	53	Sen.	36/53	0.68	(0.54, 0.80)
Total	206	39	245#	# Singleplex HRV test was not available in a proportion of samples			
Adenovirus							
No	377	0	377	Spe.	377/377	1.00	(0.99, 1.00*)
Yes	6	4	10	Sen.	4/10	0.40	(0.12, 0.74)
Total	383	4	387	*one-sided, 97.5%			

Conclusions

- The sensitivity and specificity of the Diatherix panel for detecting Influenza A, the most clinically relevant of the viral pathogens, was 94% and 99%, correspondingly.
- Decreased Diatherix panel sensitivity for detection of Influenza B and Adenovirus can be explained by low sample volume submitted for nucleic acid extraction used for TEM-PCR™. Of the 403 tested specimens, 180 (44.7%) had suboptimal sample volumes.
- It is unknown whether bacterial co-detection represents colonization or co-infection. However, carriage of multiple bacterial pathogens together with viruses may result in respiratory disease, especially in children with an immature immune system.
- Multiplex molecular assays can be used for accurate detection of ILI.

Acknowledgements and Disclaimer

- The work was supported by the Infectious Disease Clinical Research Program (IDCRP), a Department of Defense (DoD) program executed through the Uniformed Services University of the Health Sciences (USU). This project has been funded in whole, or in part, with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), under Inter-Agency Agreement [Y1-AI-5072].
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