

COMPARISON OF THE FILMARRAY® RESPIRATORY PANEL AND PRODESSE® ASSAYS FOR THE DETECTION OF RESPIRATORY PATHOGENS

M. Loeffelholz, D. Pong, R. Pyles, Y. Xiong, A. Miller, K. Buffon, T. Chonmaitree
University of Texas Medical Branch, Galveston, TX 77555



CVS 2011
Poster M75

Updated Abstract

Aim: Compare diagnostic performance and overall respiratory pathogen detection rate of pre-market FilmArray (FA) (Isho Technology) and In vitro Diagnostic Use Prodesse® (PRO) (Gen-Probe) assays.

Methods: Nasopharyngeal aspirates (NPAs) were collected within 7 days of upper respiratory infection onset as part of a prospective study (2008-2010) of healthy children (birth-1 yr). NPAs were stored frozen, and aliquots tested by FA and PRO. ProFAST™, ProParafu™, ProHMPV™, and ProAdeno™ assays. FA detects 21 pathogens, while the PRO assays detect 10 viruses. Laboratory-developed PCR assays were performed to resolve discordant results and confirm pathogens detected only by FA.

Results: A total of 192 NPAs were tested by FA and PRO assays. Overall, 163/192 (84.9%) of NPAs were positive for a respiratory pathogen by FA and 45/192 (23.4%) by PRO. FA and PRO detected a total of 204 and 48 pathogens, respectively. Among viruses common to both assays, FA and PRO showed good agreement (181/192 (94.3%); Kappa = 0.87, 95% CI 0.79-0.94). FA detected more parainfluenza viruses 1 and 3, and Prodesse detected more adenoviruses (Table 1).

Virus	FA+/PRO+	FA-/PRO+	FA+/PRO-	FA-/PRO-
Influenza A H1-2009	1	0	0	0
Influenza B	1	0	0	0
RSV	10	0	1 (1)*	0
Parainfluenza 1	3	0	2 (0)	0
Parainfluenza 2	0	0	0	0
Parainfluenza 3	10	0	3 (2)	0
hMPV	12	0	0	0
Adenovirus	6	5 (5)	0	0
Total	43	5	0	0

*Number in () = number of discordant results confirmed by laboratory-developed PCR or detection of virus in an adjacent episode

Additionally, FA detected 155 pathogens not included in the PRO assays [rhinovirus (RV) enterovirus (EV), 129; bocavirus, 12; coronavirus, 9; parainfluenza virus 4, 4; *Mycoplasma pneumoniae*, 1]. Ninety-nine (76.7%) of the RV/EV positive specimens were confirmed either by in-house PCRs or by a positive adjacent episode.

Conclusions: The larger panel in the pre-market FA assay allowed for the detection of additional respiratory pathogens. In this young patient population with upper respiratory infection, RV/EV accounted for the majority of the additional pathogens detected by FA. PRO was superior for detection of adenoviruses.

Introduction

Nucleic acid amplification tests (NAATs) such as PCR are well established as the most sensitive method for the detection of respiratory viruses. As of March 28, 2011 there are five manufacturers of Food and Drug Administration (FDA)-cleared PCR assays for respiratory viruses: Centers for Disease Control and Prevention (CDC); Focus Diagnostics; Gen-Probe (Prodesse); Luminex; and Nanosphere (source: amp.org). Additional FDA-cleared NAATs for respiratory pathogens are likely to be available in the future.

The aim of this study was to compare the pre-market FilmArray Respiratory Panel (RP) (Isho Technology) with the FDA-cleared Gen-Probe (Prodesse) PCR assays (ProFlu, ProFAST, ProParafu, ProHMPV, ProAdeno). We evaluated clinical accuracy, overall pathogen yield, labor, throughput, and reagent costs.

Materials and Methods

Study Design

- Subjects**
- Prospective study (2008-2010) of healthy children (birth to 1 yr)
- Specimens**
- Nasopharyngeal aspirates (NPAs) collected within 7 days of upper respiratory infection
 - 192 NPAs collected from 81 children (mean, 2.4 / child; range, 1-8 / child)
 - 159 of 192 (83%) NPAs collected from children ≤6 mo. of age
 - NPAs stored frozen at -70° C prior to testing

FilmArray RP

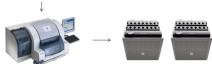
Batch of 1 patient specimen



- Multiplex PCR detects 21 targets: adenovirus, bocavirus, coronavirus (HKU1, NL63, OC43, 229E), enterovirus, inf A H1, inf A H1-2009, inf A H3, inf B, metapneumovirus, parainfluenza 1-4, RSV, rhinovirus, *Bordetella pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* (pre-market version), FDA clearance pending
- Pouch contains all reagents for nucleic acid extraction, first-round multiplexed PCR, and single, nested, real time PCRs

Gen-Probe Prodesse Assays

Batch of 22 patient specimens



Perform sequentially:

- ProAdeno+
- ProFlu+ (inf A, inf B, RSV)
- ProFAST+ (H1, H1-2009, H3)
- ProParafu+ (Parainfluenza 1-3)
- ProHMPV+

Laboratory-Developed PCRs

- qPCR tests developed for adenovirus, parainfluenza 1 / 3, influenza A and B, HMPV, RSV, coronavirus NL63, OC43 and 229E, bocavirus, rhinovirus, enteroviruses
- qPCR for human glyceraldehyde 3-phosphate dehydrogenase served as an internal control

Table 1. Sample Input Volume

Assay	PCR sample volume
FilmArray	~100 µl to first stage PCR
Prodesse	4.5 µl

Table 2. Correlation of FilmArray and Prodesse—Viruses Common to Both Panels

Virus	FA+/PRO+	FA-/PRO+	FA+/PRO-	FA-/PRO-
Influenza A H1-2009	1	0	0	0
Influenza B	1	0	0	0
RSV	10	0	1 (1)*	0
Parainfluenza 1	3	0	2 (0)	0
Parainfluenza 2	0	0	0	0
Parainfluenza 3	10	0	3 (2)	0
hMPV	12	0	0	0
Adenovirus	6	5 (5)	0	0
Total	43	5	6	0

*Number in () = number of discordant results confirmed by laboratory-developed PCR or by detection of virus in an adjacent episode

Agreement = 181/192 (94.3%). Kappa 0.87 (95% CI 0.79-0.94)

Table 3. Detection of Pathogens Unique to FilmArray RP

Pathogen	Number detected
Bocavirus	12
Corona 229E	4
Corona HKU1	1
Corona NL63	1
Corona OC43	3
<i>M. pneumoniae</i>	1
Parainfluenza 4	4
RV/EV	129

Results

Table 4. Confirmation of Pathogens Unique to FilmArray RP

Pathogen	No.	Confirmed [No. (%)]		Total
		Positive by In-house PCR	Positive adjacent episode ^a	
RV/EV	129	90 (69.8)	9 (7.0)	99 (76.7)
Corona OC43	3	2 (66.7)	0	2 (66.7)
HKU1	1	NA ^b	-	0
NL63	1	0	-	0
229E	4	4 (100)	-	4 (100)
Parainfluenza 4	4	NA	2 (50)	2 (50)
Bocavirus	12	8 (66.7)	1 (8.3)	9 (75)
<i>M. pneumoniae</i>	1	NA	-	0

^aAdjacent symptomatic episode within 2-40 days
^bNA = Not available

Table 5. Co-Infections Detected by FilmArray and Prodesse

Co-infection	Assay	Viruses	No.	
Two viruses	Prodesse	hMPV, Para3	2	
		RSV, Para3	1	
		FilmArray	RV/EV, Para1	2
			RV/EV, Para3	4
			RV/EV, Para4	2
			RV/EV, Adeno	5
RV/EV, hMPV	2			
RV/EV, Boca	6			
RV/EV, Corona	6			
RV/EV, RSV	2			
Three viruses	FilmArray	Boca, Corona	1	
		RSV, Corona	1	
		Para3, hMPV	2	
		Para3, Adeno	1	
		Boca, RV/EV, hMPV	1	
Boca, RV/EV, Para1	1			
Boca, RV/EV, RSV	1			
Boca, RSV, Para3	1			

Table 6. Unresolved / Failed Samples

Assay	No. (%)
FilmArray	2 (1.0)
ProFlu+	11 (5.7)
ProParafu+	0
ProHMPV+	2 (1.0)
ProAdeno+	6 (3.1)

Conclusions

- For viruses common to the FilmArray and Prodesse assays, the systems have good overall agreement
- Prodesse is more sensitive for detection of adenoviruses
- FilmArray is more sensitive for detection of parainfluenza viruses
- The larger panel in the FilmArray RP allowed for the detection of additional respiratory pathogens
- In this cohort of young children, rhino/enteroviruses are the most common causes of URI
- While FilmArray hands-on time per patient sample is less, Prodesse allows larger number of samples to be analyzed in the same amount of time

Table 7. Throughput, Labor and Consumable Costs

Assay	Specimens per 8 hr shift ^a	Hands-on time (min per shift/min per sample) ^b	Reagent, consumable cost per patient ^c
FilmArray	8	32/4	\$129
Prodesse ^d	22	194/8.8	\$184.87

^aBased on 1 FilmArray instrument, 1 easyMAG nucleic acid extractor, 2 Smartcyclers

^bBased on list prices for PCR reagents

^cProdesse assays: ProFlu+, ProHMPV+, ProParafu+, ProAdeno+

Note: ProFAST+ labor and reagent cost not included, as only 1 sample in this study was positive for influenza A

Acknowledgments

Supported by NIH grants R01 DC005841 and UL1 RR029876, and by a grant from Isho Technology