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COMPARISON OF TWO BROADLY MULTIPLEXED PCR SYSTEMS FOR VIRAL DETECTION IN CLINICAL RESPIRATORY TRACT SPECIMENS

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Randall T. Hayden[1], Zhengming Gu[1], Alicia Rodriguez[1], Lisa Tanioka[3], Claire Ying[3], Markus Morganstern[1], and Matthew J. Bankowski[2,3]
[1]Departments of Pathology, St. Jude Children's Research Hospital, Memphis, TN 38105; [2]John A. Burns School of Medicine and the University of Hawaii at Manoa, Honolulu, HI; and [3]Diagnostic Laboratory Services (The Queen's Medical Center), Aiea, HI

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Abstract

Background: The detection of viral respiratory tract infection has evolved greatly with the development of PCR based commercial systems capable of simultaneously detecting a wide variety of pathogens. We evaluated the relative performance of two such systems for the detection of viral agents in clinical respiratory tract specimens from immunocompromised patients.

Materials and Methods: Samples included 440 clinical respiratory tract specimens from 210 patients. Samples were deidentified and assayed in parallel using two different, broadly multiplexed PCR systems: ResPlex™ II Panel v2.0 (ResPlex), Qiagen, Hilden, Germany and; FilmArray® Respiratory Panel (FilmArray™), Idaho Technology, Inc., Salt Lake City, Utah. Method comparison was based on pairwise concordance of results, by patient and by viral target. For the purposes of analysis, viral targets were grouped into seven categories: adenovirus (ADV), coronavirus (CRV), human metapneumovirus (hMPV), influenza A (FluA), parainfluenza virus (PIV), picornavirus (PCV), and respiratory syncytial virus (RSV). No samples were positive for influenza B virus. Positive concordance by patient required at least one virus detected in common between the two systems.

Results: A total of 197 patient samples were included in the analysis, representing only the first sample collected for each patient, and excluding failed reactions on either system. The two systems showed an overall concordance, by patient, of 83.8% (p=0.0001). Eighty percent of positive results were concordant. ResPlex failed to detect 20% of FilmArray positives, and FilmArray failed to detect 4.4% of ResPlex positives. Results for the individual viruses detected showed the following distribution [Actual number positive]:

	ADV	CRV	hMPV	FluA	PIV	PCV	RSV
FilmArray™	7	30	18	14	9	84	32
ResPlex™	2	26	13	7	7	79	25

Conclusions: The two systems evaluated showed a difference in overall detection rate by patient sample. Only coronavirus and picornavirus showed significant differences in frequency of detection when results were stratified by viral group. Both broadly multiplexed PCR systems appear to provide an effective means for detecting a wide variety of respiratory viruses simultaneously.

*Revised abstract

Introduction

The detection and characterization of respiratory tract viral pathogens is of particular importance in the immunocompromised population, where agents of typically low virulence can cause severe or life-threatening illness. Recently, molecular amplification assays have become a common means of laboratory diagnosis in these cases; however, use of single-target assays can be cumbersome, time-consuming and expensive as the number of infectious agents detected increases. Widely multiplexed assays attempt to address this issue by the simultaneous detection and identification of large numbers of different pathogens; several such assays have become available, but few direct comparative studies have been published, particularly in the immunocompromised patient population. This study compared the performance of the ResPlex II Panel v2.0 (ResPlex), Qiagen, Hilden, Germany with that of the FilmArray Respiratory Panel (FilmArray), Idaho Technology, Inc., Salt Lake City, Utah).

Methods

440 nasopharyngeal specimens from 210 symptomatic pediatric oncology patients were collected for clinical diagnostic purposes from January 13th to May 4th, 2010. Samples included predominantly nasopharyngeal washes, as well as swabs and tracheal aspirates (Table 1). Following IRB review, samples remaining after clinical testing were de-identified and tested by the two multiplexed assays.

Resplex:
- Viral nucleic acid was extracted from 250µL respiratory samples using Qiagen EZ-1 Viral extraction kit and eluted in 50µL buffer.
- RT-PCR was performed using ResPlex II Panel v2.0 reagents, 10µL nucleic acid eluate.
- Detection was performed using ResPlex II bead and reaction mix on a Luminex 200 IS system, with QIAplex MDD software.

FilmArray:
- A solution of 250ul of respiratory sample was mixed with 500ul sample lysis buffer.
- About 1mL of hydration solution was added by syringe to the FilmArray pouch through hydration solution inlet port.
- 300ul of sample/lysis buffer mix was added to the pouch through sample inlet port.
- Prepared pouch was loaded and run on the FilmArray instrument.

Data Analysis:
- To reduce bias from multiple samples collected from one patient, only results from the first sample collected during the study period were included for analysis.
- Results from individual samples in which the first run failed were excluded from analysis.
- Results from consecutive runs in which the first run failed were included in the analysis.
- Positive results by FilmArray were compared to positive results by Resplex at three different cut offs for Resplex results, 100, 150, and 200. Only results using a cutoff of 200 are displayed here.
- Besides overall comparative analysis, positive rates were compared with results stratified into five age categories.

Results

- A total of 440 samples were collected from 210 patients (Table 1).
  - 440 samples from 210 patients were tested by FilmArray
  - 417 samples from 199 patients were tested by Resplex
  - Mean patient age in years was 8, the youngest being 0.16 (2 months) and the oldest 26.

Table 1. Sample attributes

Characteristic	Frequency	Percent
Specimen type		
BAL	2	1.02
NPS	1	0.51
NPW	188	95.43
TA	6	3.05
Age group (years)		
< 2	51	25.89
3–5	43	21.83
6–13	57	28.93
14–18	28	14.21
> 19	18	9.14

Table 2. Targets detected

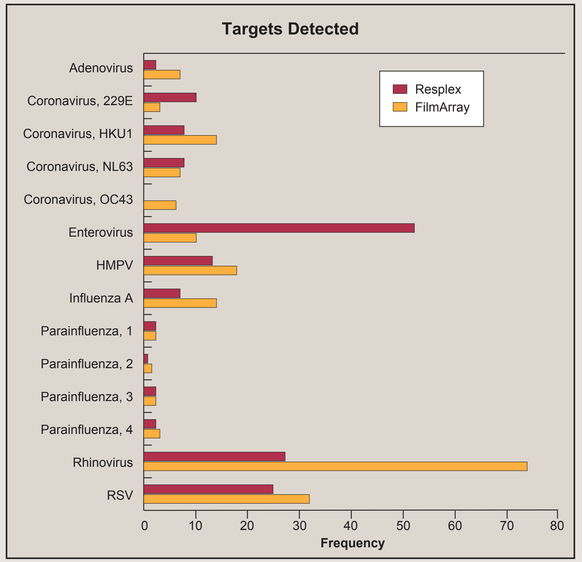
	Frequency (%), n = 197		
Results	**FilmArray**	**Resplex**	***p* value**
Samples testing positive	135 (68.5)	113 (57.4)	0.0001
Respiratory pathogen			
Adenovirus	7 (3.6)	2 (1.0)	0.0625*
Boca virus	0	0	-
Coronavirus, 229E	3 (1.5)	10 (5.1)	0.0156*
Coronavirus, HKU1	14 (7.1)	8 (4.1)	0.0703*
Coronavirus, NL63	7 (3.6)	8 (4.1)	1*
Coronavirus, OC43	6 (3.1)	0	-
HMPV	18 (9.1)	13 (6.6)	0.0625*
Influenza A	14 (7.1)	7 (3.6)	0.0156*
Influenza B	0	0	-
Parainfluenza, 1	2 (1.0)	2 (1.0)	-
Parainfluenza, 2	2 (1.0)	1 (0.51)	1*
Parainfluenza, 3	2 (1.0)	2 (1.0)	1*
Parainfluenza, 4	3 (1.5)	2 (1.0)	1*
Enterovirus	10 (5.1)	52 (26.4)	<0.0001
Rhinovirus	74 (37.6)	27 (13.7)	<0.0001
RSV	32 (16.2)	25 (12.7)	0.1185

*exact *p* value

Table 3. Positive results by age group

	Positive		Negative	
Age group	**FilmArray**	**Resplex**	**FilmArray**	**Resplex**
< 2	37 (72.6)	33 (64.7)	14 (27.4)	18 (35.3)
3–5*	36 (83.7)	29 (67.4)	7 (16.3)	14 (32.6)
6–13*	36 (63.2)	29 (50.9)	21 (36.8)	28 (49.1)
14–18	15 (53.6)	12 (42.9)	13 (46.4)	16 (57.1)
> 19	11 (61.1)	10 (55.6)	7 (38.9)	8 (44.4)

*exact *p* value: Age group 3–5: 0.0391; age group 6–13: 0.0156.



* Significant differences were observed for these target groups. Qiagen cut off = 200

Conclusions

- Broadly multiplexed PCR methods provide a rapid and sensitive means for simultaneous detection of respiratory tract pathogens in nasopharyngeal wash specimens, collected from pediatric oncology patients
- The two systems examined showed a high degree of overall concordance
- Specific differences among the systems were seen in targeted viruses, sensitivity, hands-on and total run times
- Concordance rates varied depending on positive cutoff criteria used in the ResPlex system
- The FilmArray system demonstrated significantly reduced total run time and hands-on time, compared to ResPlex

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Contact Information
Email: Randall.Hayden@stjude.org

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