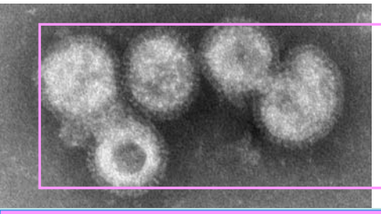

THE SEEPLEX® RV DETECTION KIT IDENTIFIES PATHOGENS IN THE MAJORITY OF RESPIRATORY OUTBREAKS FROM THE GREATER TORONTO AREA, ONTARIO, CANADA

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Abstract

Background: A key public health dilemma is that the etiology of most respiratory outbreaks is unknown when cell culture-based methods are used as the sole means of virus detection and identification. However, multiplexed molecular technologies may provide a better understanding of the etiology of respiratory virus outbreaks and may become a comprehensive public health tool. The Seeplex® RV Detection kit (Seegene, Rockville, MD 20850) is a multiplexed assay that detects 12 respiratory viruses including coronavirus OC43, coronavirus 229E/NL63, human rhinovirus A, influenza A, influenza B, respiratory syncytial (RSV) A, RSVB, parainfluenza 1, parainfluenza 2, parainfluenza 3, human metapneumovirus and adenovirus.

Objective: The purpose of this study was to compare the ability of viral isolation and the Seeplex® RV Detection kit to identify specific respiratory viruses within specimens collected from respiratory outbreaks in the Greater Toronto Area (GTA)(September 1, 2007 to February 1, 2008).

Materials and methods: All nasopharyngeal (NP) swabs from declared outbreaks in the GTA sent to the Central Public Health Laboratory (September 1, 2007- February 1, 2008) were included in this analysis. All specimens were cultured on rhesus monkey kidney and WI-38 cells (Diagnostic Hybrids, Athens, OH, USA) followed by post-culture direct fluorescence antibody (DFA) staining and/or analysis of cytopathic effect. Total nucleic acid was extracted from each specimen using the easyMag automated extraction system (bioMérieux, Montreal, QC, Canada). *gapdh* RT-PCR (ABI, Foster City, CA, USA) controlled for nucleic acid extraction. Total nucleic acid was tested by the the Seeplex® RV Detection kit protocol. Fisher's exact test was carried out using GraphPad Prism 5.01 (Graphad Software Inc., El Camino Real, CA, USA)

Results: 231 patient samples (nasopharyngeal (NP) swabs) were collected from 63 declared respiratory outbreaks in the GTA. The distribution of outbreaks with a pathogen identified by molecular means was; 30% (n=19) no identification, 52.5% (n=33) one pathogen, 14.5% (n=9) two pathogens and 3.0% (n=2) three pathogens. In contrast, culture-based protocols identified pathogens in fewer outbreaks; 63 % (n=40) no identification, 35% (n=22) 1 pathogen, and 2% (n=1) 2 pathogens (p< 0.05). Compared to virus isolation, the Seeplex assay identified a greater proportion of positive specimens for; rhinovirus: 22% (n=51/231) *vs*. 5% (n=12/231) (p<0.05), and RSV A/B: 12% (n=27/231) *vs*. 5% (n=11/231) (p< 0.05). The Seeplex assay also detected pathogens (coronaviruses and human metapneumovirus) not identified by the described isolation technique.

Conclusions: Multiplexed molecular methods allow for the identification of a respiratory viral pathogen in the majority of respiratory outbreaks in our region. Molecular methods also detected larger numbers of human rhinovirus A and RSVA/B-positive specimens than culture-based viral identification methods. Multiplexed molecular methods are a powerful tool for understanding and managing respiratory virus outbreaks.

Aim

To describe the impact of molecular tests on identification of respiratory virus pathogens in outbreak settings and provide a better understanding of the impact of molecular techniques to public health workers.

Methods

- All nasopharyngeal (NP) swabs from declared outbreaks in the GTA sent to the Central Public Health Laboratory (September 1, 2007- February 1, 2008) were included in this analysis.
- Specimens were collected using the flocculated Starswab® Multitrans Collection and Transport system.
- All specimens were cultured on rhesus monkey kidney and WI-38 cells (Diagnostic Hybrids, Athens, OH, USA) followed by post-culture direct fluorescence antibody (DFA) staining and/or analysis of cytopathic effect.
- Total nucleic acid was extracted from each specimen using the easyMag automated extraction system (bioMérieux, Montreal, QC, Canada) as per manufacturer's protocols.
- To control for extraction all specimens were tested for human target *gapdh* by using the *gapdh* RT-PCR kit (ABI, Foster City, CA, USA) as per the manufacturer's instructions.
- Total nucleic acid was tested by the the Seeplex® RV Detection kit protocol (Seegene, Inc., Rockville, MD) for the following pathogens; influenza A, influenza B, respiratory syncytial virus A (RSVA), RSVB, parainfluenza (PIV) 1, PIV2, PIV3, human rhinovirus A, human metapneumovirus, adenovirus, coronavirus OC43, and coronavirus 229E/NL63. Multiplexed molecular testing was carried out as per the manufacturer's instructions.
- Specimens for multiplexed molecular testing were batched and tested once a week on a Thursday
- Turnaround times from data of specimen submission to completion date and report date were stored in an Excel file (Microsoft® Excel 2003, Microsoft, Seattle, WA).
- Basic statistical analysis was carried out using GraphPad Prism 5.01 (Graphad Software Inc., El Camino Real, CA, USA)

Results

- Between September 1, 2007 and February 1, 2008, 231 patient specimens (NP swabs) were collected from 63 declared respiratory outbreaks in the GTA.
- Of the outbreaks identified; 58 (92%) were from long-term care facilities, 3 (5%) were from acute care facilities, 2 (3%) were from an unknown setting.
- The median turnaround times from specimen collected to specimen reported was determined for the following methods; culture 13 days (standard deviation (SD) 4 days), rapid antigen detection 1 day (SD 2 days), multiplex assay: 6 days (SD 3 days).
- Turnaround times were determined to be significant between all methods (Kruskal-Wallis test, P<0.05 and Dunn's Multiple Comparison Test, p<0.05)

Results

Table I. Distribution of outbreaks positive for pathogens by culture and multiplexed molecular testing.

Viral pathogen	# culture positive outbreaks	# molecular positive outbreaks
Influenza A	3	3
Influenza B	2	2
RSV	5	4
RSV **PIV1**	1	2
RSV **Human rhinovirus**	0	1
RSV **Human coronavirus OC43**	ND	1
RSV **Human coronavirus 229/NL63**	ND	1
PIV1	4	3
PIV1 **Human rhinovirus**	0	1
PIV2	1	1
PIV3	1	1
Human rhinovirus	6	15
Human rhinovirus **Human metapneumovirus**	ND	1
Human metapneumovirus	ND	1
Human metapneumovirus **Coronavirus 229E/NL63** **Human rhinovirus**	ND	1
Coronavirus 229E/NL63 **Human rhinovirus**	ND	1
Coronavirus 229E/NL63 **Coronavirus OC43**	ND	1
Coronavirus OC43	ND	3
Coronavirus OC43 **Human rhinovirus**	ND	1

ND - Not done, RSV - Respiratory syncytial virus, PIV - parainfluenza virus

Results II

Table II. The distribution of outbreaks associated with respiratory viral pathogens.

	Culture n (%)	Molecular n (%)
No pathogens identified	40 (63)	19 (30)
Single pathogen	22 (35)	33 (52.5)
Dual pathogens	1 (2)	9 (14.5)
Three pathogens	0	2 (3)

Conclusion

- Multiplexed molecular methods allow for the identification of a respiratory viral pathogen in the majority of respiratory outbreaks in our region.
- Molecular methods also detected larger numbers of human rhinovirus A and RSVA/B-positive specimens than culture-based viral identification methods.
- Multiplexed molecular methods are a powerful tool for understanding and managing respiratory virus outbreaks.

References

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