CONCISE COMMUNICATION

Respiratory Outbreak Investigations: How Many Specimens Should Be Tested?

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To determine the optimal number of specimens for virus detection in a respiratory outbreak, laboratory results from 2 Canadian public health laboratories were reviewed. The evidence suggests that 3 specimens are sufficient for detection of a virus in >95% of outbreaks, thereby reducing laboratory costs.

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INTRODUCTION

Laboratory testing identifies the etiologic agent of respiratory outbreaks, and results are essential to inform public health outbreak mitigation and response. The main goal of testing is to detect the presence of influenza, which prompts prophylaxis and treatment. Guidelines have often recommended testing 6 specimens for respiratory outbreak investigation[1]; however, this number is dependent upon test sensitivity and can be reduced for nucleic acid amplification tests (NAATs).[2] An earlier study looked at the role of point-of-care tests for influenza detection in outbreaks in contrast to NAATs but did not identify an ideal number of specimens for either technology.[3] In this study, we aimed to determine the optimal number of outbreak specimens to initially test using NAAT to facilitate a high probability of detecting influenza and other viral respiratory pathogens.

METHODS

In Alberta and Nova Scotia, unique investigation numbers are assigned to reported clusters of respiratory illness detected in any setting. Specimens were ranked by accession timing in laboratory information systems and linked to outbreaks through shared investigation numbers.

Results for respiratory specimens tested as part of reported outbreak investigations between January 1, 2011, and December 31, 2014, were included. Specimens were tested following established algorithms in the provincial public health laboratories in Nova Scotia and Alberta, which test the majority of and all outbreak specimens for their jurisdictions, respectively. During influenza season in Nova Scotia, all specimens were initially tested using an RT-PCR assay targeting influenza A, influenza B, and respiratory syncytial virus (RSV).[4] Negative specimens were reflexively tested using the Seeplex RV15 OneStep ACE Detection kit (Seegene, Inc., Seoul, Korea), a multiplexed RT-PCR that detects influenza A, influenza B, RSV A and B, adenovirus, human metapneumovirus, coronavirus 229E/NL63 and OC43, parainfluenza viruses 1–4, rhinovirus A/B/C, enterovirus, and bocaviruses 1–4. Outside influenza season, the Seeplex RV15 assay was used as a standalone test. In Alberta, respiratory specimens were first tested using an RT-PCR assay targeting influenza A and B.[5] Negative specimens were subsequently tested by xTAG respiratory viral panel classic assay (Luminex Molecular Diagnositcs, Texas, USA),[6] which detects the same viruses as the Seeplex RV15, except that enterovirus and rhinovirus are recognized as a combined entero-rhinovirus, HKU1 is included as a coronavirus target, and bocavirus is not detected.

RESULTS

In Nova Scotia, a total of 1,058 specimens related to 308 respiratory outbreaks were tested, with a median of 3 specimens per outbreak (range: 1–17 specimens; see Online Supplementary Material). A virus was detected in 276 outbreaks (89.6%). Similarly, in Alberta, 2,705 specimens related to 646 respiratory outbreaks were tested, with a median of 4 specimens per outbreak (range: 1–69 specimens; see Online Supplementary Material). A virus was detected in 578 outbreaks (89.5%). Overall, the proportional distribution of viruses detected in outbreaks was similar in the 2 provinces (Table 1). Influenza A was detected in the largest number of outbreaks at 33.7% (n = 93) in Nova Scotia and 51.0% (n = 295) in Alberta. The second most common finding was enterovirus/rhinovirus outbreak, with similar proportions in Nova Scotia (24.3%) and Alberta (21.5%). In Nova Scotia and Alberta, the proportions of outbreaks with ≥2 viruses detected were also markedly similar at 20.6% (n = 57) and 20.4% (n = 118), respectively.

A virus was detected among the first 3 specimens in 97.5% of outbreaks in Nova Scotia and 96.7% in Alberta (Table 2). In Nova Scotia, influenza A and B were detected among the first 3 specimens in 98.9% and 100% of influenza outbreaks, respectively. In Alberta, influenza A and B were detected among the first 3 specimens in 95.9% and 93% of influenza outbreaks, respectively. Overall, in 450 of 467 influenza A and B outbreaks (96.3%) in Alberta and Nova Scotia influenza was detected within the first 3 specimens. In Nova Scotia and Alberta, respectively, 270 and 1,022 tests were performed on the fourth or later specimen.

DISCUSSION

The use of sensitive NAATs compared to traditional virus culture methods has reduced the number of specimens

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TABLE 1. Viruses Detected in Outbreaks with ≥1 Detected Virus, Nova Scotia and Alberta, January 1, 2011, Through December 31, 2014

	Nova Scotia		Alberta	
	No. and Proportion of Outbreaks (n = 276)[a]		No. and Proportion of Outbreaks (n = 578)[a]	
Virus Detected	n	%	n	%
Influenza A	93	33.7	295	51.0
Influenza B	22	8.0	57	9.9
RSV A/B	46	16.7	70	12.1
Rhinovirus	65	23.6	ND	ND
Parainfluenza viruses 1–4	40	14.5	50	8.7
Coronavirus 229E/NL63, OC43, HKU1[b]	38	13.8	62	10.7
Human metapneumovirus	31	11.2	60	10.4
Adenovirus	1	0.4	5	0.9
Enterovirus	3	1.1	ND	ND
Bocavirus	0	0.0	ND	ND
Enterovirus-Rhinovirus[c]	67[c]	24.3[c]	124	21.5

NOTE. ND, specific test not done on specific testing platform.
[a]Sum of outbreaks by virus is greater than total number of outbreaks as some outbreaks were positive for >1 virus.
[b]Coronavirus HKU1 is not targeted by Nova Scotia platform, only for Alberta.
[c]Data combined in Nova Scotia for comparison against Alberta. Enterovirus and rhinovirus are identified individually in Nova Scotia, note 1 outbreak with both viruses detected.

TABLE 2. Virus Detection by Specimen Number, Outbreaks with ≥1 Detected Virus, Nova Scotia, and Alberta, January 1, 2011, Through December 31, 2014

A) Nova Scotia

	Influenza A		Influenza B		Any Virus[a]	
n^{th} Specimen	n	Cumulative %	N	Cumulative %	n	Cumulative %
1	74	79.6	15	68.2	226	81.9
2	15	95.7	2	77.3	37	95.3
3	3	98.9	5	100.0	6	97.5
4	0	98.9	0	100.0	3	98.6
5	1	100.0	0	100.0	3	99.6
6	0	100.0	0	100.0	1	100.0
≥7	0	100.0	0	100.0	0	100.0
Total	93		22		276	

B) Alberta

	Influenza A		Influenza B		Any Virus[a]	
n^{th} Specimen	n	Cumulative %	N	Cumulative %	n	Cumulative %
1	229	77.6	44	77.2	453	78.4
2	39	90.8	6	87.7	84	92.9
3	15	95.9	3	93.0	22	96.7
4	4	97.3	1	94.7	9	98.3
5	3	98.3	0	94.7	6	99.3
6	1	99.3	1	96.5	2	99.7
≥7	4	99.7	2	100.0	2	100.0
Total	295		57		578	

[a]Includes influenza.

required to confirm the presence of a virus in an outbreak investigation, and multiplex NAATs have allowed the simultaneous detection of multiple viral agents. Restricting initial testing to 3 specimens could significantly reduce laboratory costs while maintaining sensitivity for detecting an outbreak agent. Despite testing specimens from different provinces and using

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different testing platforms and algorithms, >95% of all outbreaks for which an agent was detected had detected an agent within the first 3 specimens. For influenza, an agent was detected among the first 3 specimens in 96% of outbreaks. Although another study deemed 4 specimens optimal, the findings of marginal increases in virus detection when testing >3 specimens by NAAT mirror the findings here.[2]

During the time period studied, testing 3 specimens per outbreak would have reduced the number of specimens tested by 25.5% in Nova Scotia and 37.8% in Alberta. The associated resources could have been redirected to test additional specimens from outbreaks with few submissions. It is possible that in some outbreaks specimens were not collected because the time lag from symptom onset was considered too long. While specimen collection ideally occurs within 48 hours of symptom onset, this guideline can likely be extended, as both culturable virus and viral RNA can be detected for longer periods of time.[1,7,8]

These findings have several limitations. Like others,[1] this study ranked specimens by accession time in the laboratory information system. The fact that 2 laboratories within 2 distinct provinces had such strikingly similar findings supports the validity of the results and the unlikelihood that these are chance findings. Second, clinical information was not available, limiting our ability to compare characteristics by viruses detected. Third, classification of outbreak specimens was dependent on proper identification and recording. Similar to other data,[2] we found mixed pathogens in a considerable proportion of outbreaks. However, 3 specimens may not be suitable when mixed pathogens are identified. These situations may require further testing to inform infection control and public health practices, where simultaneous outbreaks or pseudo-outbreaks may be present. Also, further research is required to understand the clinical and epidemiological relevance of respiratory virus coinfection.

Optimal specimen number ultimately depends on sensitivities and specificities of assays in place as well as testing algorithms. However, as an initial step, the routine testing of 3 specimens in an outbreak allows for sensitive detection of influenza and an understanding of the other viruses that cause respiratory outbreaks, all while containing costs. In addition, testing high numbers of specimens can potentially lead to false-positive identification of an outbreak etiologic agent, further supporting the guidance to limit testing numbers.[9,10]

In summary, these findings demonstrate that 3 specimens is the ideal number when using NAAT to initially detect viral respiratory pathogens in an outbreak investigation. The focus of this study was to determine the minimum number of specimens required to identify a viral pathogen, and these results do not rule out the need for further testing in some circumstances, such as persistence of an outbreak over a long time period; no agent detected; detection of multiple pathogens and/or coinfection; concern related to illness severity; or, for influenza, suspicion of antiviral resistance. Further research is also required to understand the epidemiology of noninfluenza respiratory viruses and to understand how the reporting of these viruses influences medical interventions and/or public health measures.

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SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/ice.2015.171.

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