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SHORT REPORT
Optimal number of samples to test for institutional respiratory infection outbreaks in Ontario

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SUMMARY

The objective of this study was to determine the optimal number of respiratory samples per outbreak to be tested for institutional respiratory outbreaks in Ontario. We reviewed respiratory samples tested for respiratory viruses by multiplex PCR as part of outbreak investigations. We documented outbreaks that were positive for any respiratory viruses and for influenza alone. At least one virus was detected in 1454 (85·2%) outbreaks. The ability to detect influenza or any respiratory virus increased as the number of samples tested increased. When analysed by chronological order of when samples were received at the laboratory, percent positivity of outbreaks testing positive for any respiratory virus including influenza increased with the number of samples tested up to the ninth sample, with minimal benefit beyond the fourth sample tested. Testing up to four respiratory samples per outbreak was sufficient to detect viral organisms and resulted in significant savings for outbreak investigations.

Key words: Influenza, laboratory tests, outbreaks, respiratory infections.

Laboratory testing is crucial in the identification of causative organisms for respiratory outbreaks, particularly for institutional outbreaks where vulnerable residents live. The relatively recent utilization of more sensitive molecular-based *vs.* culture-based testing has improved the ability to detect a wider range of viruses in a timely fashion. This can better inform outbreak management policies such as visitor restrictions, antiviral use, and other measures. Outbreak management practices when influenza is identified as a causative organism in institutional outbreaks are well documented [1–3]. Despite this and the prominence of laboratory results in the formulation of outbreak case definitions, there is a dearth of published evidence providing guidance on the optimal number of samples to submit to identify outbreak-associated viruses. Some jurisdictions' guidelines recommend submitting six samples for respiratory outbreak investigations [4–8]. It has been assumed that this number originates from analogous studies for enteric outbreaks [9]. Providing guidelines on the number of samples to be submitted during an outbreak is important as outbreak management needs and testing costs must be balanced.

This study aimed to determine the optimal number of respiratory samples per outbreak to be tested by multiplex polymerase chain reaction (PCR) during institutional respiratory outbreaks in Ontario. The optimal number of respiratory samples refers to the

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ideal number of samples submitted per outbreak in order to maximize detection of viral organisms while efficiently using laboratory resources.

We reviewed respiratory samples tested as part of routine outbreak investigations at Public Health Ontario Laboratories (PHOL) from 1 September, 2009 to 31 August, 2011. PHOL provides testing of most of the respiratory samples submitted as part of community, hospital, long-term care facility (LTCF) or other outbreak-related settings. PHOL provides service to the entire province of Ontario (population 13·4 million) through 11 laboratories located throughout the province including a central laboratory in Toronto. In Ontario, PHOL relies on the medical officer of health or designate to determine if an outbreak meets the provincial case definition [4]. PHOL does not receive sufficient information to verify that declared outbreaks have met the provincial case definition.

Multiplex PCR (Seeplex® RV, Seeplex® RV15 ACE; Seegene, USA) or Luminex xTAG™ respiratory viral panel (Luminex Molecular Diagnostics, Canada) were used to test for adenovirus, coronavirus, enterovirus/rhinovirus, influenza A and B, metapneumovirus, parainfluenza and respiratory syncytial virus (RSV). An in-house assay specific for influenza A and B detection and influenza subtyping was also performed. Only multiplex results were analysed for this study.

Descriptive analyses were performed to first characterize outbreaks in terms of organisms identified by number of samples submitted per outbreak and then by chronological order of samples submitted in an outbreak. The chronological order of samples within an outbreak was defined based on the date samples were received at PHOL. A random order was generated using StataSE v. 10 (StataCorp, USA) when samples were received on the same day. We documented outbreaks that were positive for any respiratory viruses (including influenza) and because outbreak management differs when influenza is identified we also documented outbreaks that were positive for influenza alone.

A total of 5760 samples were received from 1707 respiratory outbreaks during the study period. A range of 1–12 samples were submitted per outbreak with a mean and median of 3·4 and three samples, respectively. Across all outbreaks, 89% of samples were either the first sample or received on the same day as the first sample. The mean and median of submission time lag from the first received sample was 0·6 and 0 days, respectively, with a range of 0–36 days.

The age range of individuals associated with outbreaks was 1–104 years, with a mean and median of 80·7 and 85 years, respectively. Of all institutional respiratory outbreaks reported, 1426 (83·5%) were in LTCFs and retirement homes, 64 (3·8%) in hospitals, 54 (3·2%) in schools and daycare centres, and 163 (9·5%) had no setting information reported. Viruses detected among the 1707 outbreaks included enterovirus/rhinovirus in 608 (35·6%), influenza A in 447 (26·2%), parainfluenza in 195 (11·4%), RSV in 179 (10·5%), coronavirus in 161 (9·4%), metapneumovirus in 155 (9·1%), influenza B in 11 (0·6%) and adenovirus in four (0·2%). At least one virus was detected in 1454 (85·2%) outbreaks and more than one virus was identified in 260 (15·2%) outbreaks. Overall, the ability to detect influenza or any respiratory virus increased with an increased number of samples tested – up to seven samples and up to five samples, respectively (Table 1). When analysed by chronological order of when samples were received at the laboratory, percent positivity of outbreaks for any respiratory virus including influenza increased with the number of samples tested up to the ninth sample tested. Three hundred and forty-two (20%) outbreaks were positive for influenza with the first sample tested, 457 (26·3%) up to the fourth sample tested and 458 (26·8%) by the ninth sample tested. Similarly, 1130 (66·2%) outbreaks were confirmed for any respiratory virus with the first sample tested, 1445 (84·7%) up to the fourth sample tested and 1454 (85·2%) by the seventh sample tested. Testing after the ninth sample provided no benefits to the identification of new outbreaks positive for any respiratory virus (Fig. 1).

Seven-hundred and fifty (13%) samples from 422 outbreaks were tested as the fifth or subsequent sample and a respiratory virus was detected for the first time in 74 (17·5%) of these outbreaks, representing 4·3% of all outbreaks tested. The most common virus detected in these 74 outbreaks included enterovirus/rhinovirus in 29 (39%) outbreaks, coronavirus in 15 (20%), parainfluenza in 10 (15%) and influenza A in nine (12%) outbreaks. Five (6·7%) of these outbreaks had more than one virus identified. In nine of the 74 outbreaks all prior samples tested negative and in one of the nine outbreaks influenza was the virus later identified.

In this study we provide evidence about the optimal number of respiratory samples to test during

Table 1. *Number and percentage of outbreaks positive for influenza or any respiratory virus by number of samples submitted per outbreak, Public Health Ontario Laboratories, 1 September 2009 to 31 August 2011**

No. of samples submitted per outbreak	Total no. of outbreaks	No. of outbreaks positive for any virus	Percentage of outbreaks positive for any virus	No. of outbreaks positive for influenza	Percentage of outbreaks positive for influenza
1	226	124	54·9	40	17·7
2	361	286	79·2	89	24·7
3	418	378	90·4	108	25·8
4	280	263	93·9	76	27·1
5	197	190	96·4	59	29·9
6	174	164	94·3	60	34·5
7	26	24	92·3	12	46·2
8	7	7	100·0	3	42·9
9	11	11	100·0	7	63·6
10	6	6	100·0	3	50·0
12	1	1	100·0	1	100·0
Total	1707	1454	85·2	458	26·8

* Outbreaks are counted positive for a particular virus only once regardless of how many samples from the outbreak were positive for that target.

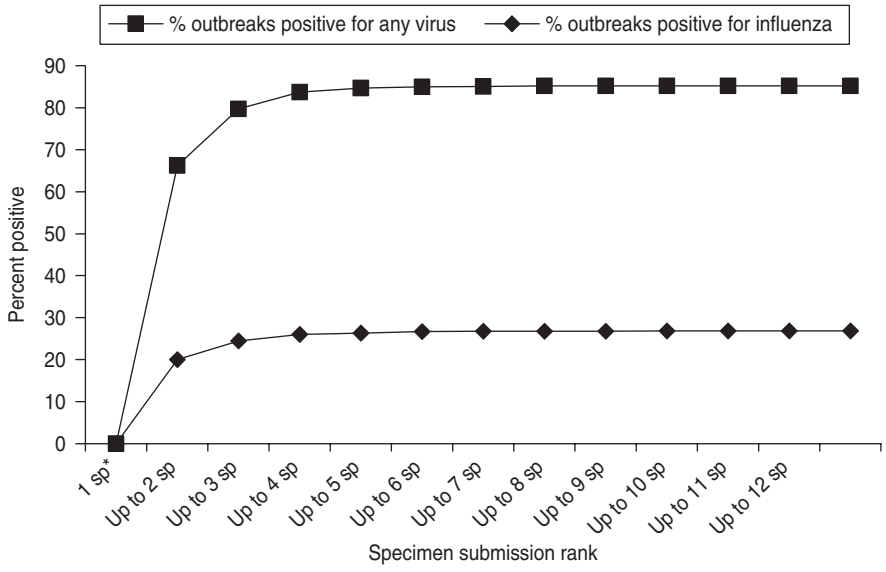

Fig. 1. Percent positivity of outbreaks for influenza or any respiratory virus by chronological order of specimens tested at Public Health Ontario Laboratories, 1 September 2009 to 31 August 2011. * sp stands for specimen.

institutional respiratory outbreaks. Overall, there was an increased number of outbreaks for which an organism was identified as number of samples tested per outbreak increased; however, there was an insufficient number of outbreaks with more than seven samples submitted for valid comparison. Submitting only one sample per outbreak limited our ability to detect a virus; therefore submitting more than one sample should be encouraged for all respiratory outbreak investigations. Testing up to four samples per outbreak identified influenza in 26·3% of outbreaks and any respiratory virus in 84·7% of outbreaks. In comparison, testing up to nine samples per outbreak identified influenza in 26·8% of outbreaks, and testing up to seven samples identified any respiratory virus in 85·2% of outbreaks. These provided a marginal increase (0·5%) in virus detection compared to testing up to four samples. Moreover, there was no

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benefit in virus detection when testing more than seven samples for non-influenza viruses or more than nine samples for influenza. Guidelines for respiratory outbreak investigations in other jurisdictions suggest testing up to six samples, and once a positive result is confirmed in at least two cases, no further testing is recommended [7]. Similarly, results from gastroenteritis outbreaks indicated that testing up to three samples confirmed 91% of norovirus outbreaks and testing more than seven samples added nothing to the ability to detect norovirus [10]. Reviewing all outbreaks to determine the effect of limiting testing to four samples at PHOL, we detected a range of viruses in additional samples tested, including enterovirus/rhinovirus in 29 (1·7%) outbreaks, coronavirus in 15 (0·9%), parainfluenza in 10 (0·6%), and influenza in nine (0·5%) outbreaks. Overall, only nine (0·5%) positive outbreaks would have had no virus identified had testing been limited to four samples per outbreak; and in only one of the nine outbreaks was influenza the virus identified. Additional sample submission may be indicated in the event of prolonged outbreaks or when clinical severity increases, which can improve detection. Over the study period, limiting testing to four samples per outbreak equated to 750 (13%) fewer samples. This would have resulted in significant savings for outbreak investigation and laboratory resources.

Our study does have some limitations. The observational design of the study limits our ability to determine causation between detected viruses and outbreaks. Second, we had limited control on sample submission practices which impacts the ability to identify an organism. Third, we did not have access to clinical data or patient symptom onset date. The lack of clinical data limited our ability to associate identified viruses to outbreak severity. Absence of symptom onset date prevented us from assessing the well established effect of delays in sample collection on virus detection during outbreak testing. Last, ranking order was randomly assigned for samples received at the same time. To account for that we repeated analyses assigning different ranking order for samples received at the same day and found that only 12 (0·8%) outbreaks had the first virus detected on the fifth or subsequent samples. Despite these limitations, this study provides good evidence that testing one sample may not be sufficient to detect respiratory viral organisms. Testing up to four samples per outbreak was sufficient to detect viral organisms in respiratory outbreaks and results in significant savings for local public health outbreak investigation and laboratory resources. Further studies are required to outline the impact of sample collection practices on outbreak management outcomes.

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DECLARATION OF INTEREST

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