

18 **KEYWORDS**

19 respiratory virus, outbreak, nucleic acid amplification, multiplex, xTAG RVP

20

ABSTRACT

21 A study was undertaken to assess the utility of the xTAG™ Respiratory Viral Panel (RVP) for
22 enhanced laboratory investigation of respiratory outbreaks. Specimens (n = 1,108) from 244
23 suspected respiratory virus outbreaks in 2006 and 2007 in Alberta, Canada were included in the
24 study. Testing by direct fluorescent antigen detection (DFA) and various in-house nucleic acid
25 amplification tests (NATs) for common respiratory viruses provided an etiological diagnosis in 177
26 outbreaks (72.5%) with 524 samples testing positive (47.3%) for a respiratory virus. Two hundred
27 samples from 51 unresolved outbreaks were further tested by RVP retrospectively. Fifty-eight
28 samples from 30 unresolved outbreaks had a respiratory virus detected by RVP (47 picornavirus, 9
29 coronavirus and 2 influenza A virus). Overall, detection of a viral etiological agent was achieved in
30 90.8% of outbreaks using a combination of DFA, NATs and RVP. Use of RVP enhances the
31 laboratory investigation of respiratory virus outbreaks and facilitates appropriate patient and outbreak
32 management.

33

INTRODUCTION

34 Identification of the etiological agent is important for the management and control of
35 respiratory outbreaks. In the case of viruses where we have options for prophylaxis and therapy
36 (such as influenza), it is particularly important that sensitive and specific outbreak diagnosis can
37 be undertaken in a timely manner (3,11). Moreover, the identification of circulating respiratory
38 agents in long term and assisted care centres (LTAC), schools or daycares provides useful
39 surveillance data for viral epidemiology in the community (7,12,19). Respiratory tract infections
40 are responsible for a significant proportion of acute morbidity and physician or emergency room
41 visits, especially where they occur in the very young (36) and the elderly (12). There is
42 considerable clinical overlap between infections caused by different respiratory viruses, and
43 accurate clinical diagnosis cannot be based on symptoms alone (10,11) .

44 Direct fluorescent antigen detection (DFA) testing and cell culture are the traditional gold
45 standard diagnostic tests for common respiratory viruses (17). Real-time nucleic acid
46 amplifications tests (NATs) are more sensitive than DFA and viral culture and can identify a
47 broader range of viruses (13,14,22). However, testing for all respiratory viral targets using
48 individual real-time NATs is expensive and laborious. Multiplexing of real-time NATs can
49 reduce the assay sensitivity and is limited by chemistry and instrumentation (13,14). Thus,
50 implementation of real-time NATs has improved the diagnostic rate in clinical laboratories but
51 the viral etiology of epidemiologically linked cases for many respiratory outbreaks remains
52 unidentified (15). Identifying the cause of these outbreaks is important for appropriate
53 management and control measures.

54 Simultaneous detection of a panel of respiratory viruses using multiplex PCR
55 amplification and detection of products by suspension microarray is one of the most promising

56 approaches for broad detection of respiratory viruses (8,23,25,27,29,32). The xTAG™
57 Respiratory Viral Panel (RVP) from Luminex Molecular Diagnostics (Toronto, Ontario, Canada)
58 allows for multiplex detection of up to 20 different respiratory viral targets using suspension
59 microarray technology. Recent studies show that the RVP assay is sensitive and specific
60 compared with antigen detection and culture (27) and in-house real-time NATs (32). In this
61 study we describe the utility of the RVP assay for detection of respiratory viruses causing
62 outbreaks in Alberta.

63

64 MATERIALS AND METHODS

65 **Clinical specimens and testing algorithm.** Respiratory outbreaks were defined by public
66 health professionals based on the presence of epidemiologically linked symptomatic cases in
67 settings such as LTAC (facilities including lodges for seniors, assisted care and group homes),
68 schools, daycares, hospitals (acute) or within a closed community (e.g. single household). The
69 Provincial Laboratory for Public Health (ProvLab) provides laboratory investigations for all
70 respiratory outbreaks in the province of Alberta (population greater than 3.3 million). The most
71 common specimen types submitted for laboratory diagnosis of respiratory virus outbreaks are
72 nasopharyngeal (NP) swabs/aspirates or throat swabs (TS) in universal transport medium (UTM,
73 Copan Diagnostic Inc. Corona, CA).

74 During 2006 to 2007 at the ProvLab, NP samples were first subjected to DFA using
75 monoclonal antibodies from IMAGEN™ (Lenexa, KS, USA) for influenza virus (IFV) A, IFVB,
76 respiratory syncytial virus (RSV) and parainfluenza viruses (PIV) 1-3. DFA-negative NP
77 specimens were tested by a panel of in-house real-time NATs for IFVA and B, RSV (A and B),
78 PIV (1-4), human metapneumovirus (hMPV) and respiratory adenoviruses (AdV) (13,15). All

79 other specimen types (mainly TS in the case of outbreaks) were subjected to the above
80 mentioned NATs without DFA testing (13,15). In this study, samples from outbreaks without
81 any etiological diagnosis after DFA and NAT testing were tested using the RVP assay.

82 **Extraction of specimens, NATs and RVP.** Nucleic acid extraction from respiratory
83 specimens was performed using the easyMAG® and associated reagents (bioMérieux, St.
84 Laurent, Quebec, Canada) as described previously (32,38). Real-time NATs were performed
85 according to previously published methods for IFV (A and B), RSV (A and B), PIV (1-4), hMPV
86 and AdV using the easyQ platform (bioMérieux) for real-time NASBA and 7500 SDS for other
87 real-time assays [Applied Biosystems (ABI), Foster City, CA, USA] (13,16,22,31,34,38).

88 As described above, specimens from outbreaks from 2006 to 2007 that did not have an
89 identifiable respiratory virus were tested by the RVP assay (n=200). Targets included in the RVP
90 were: IFVA, with H1 and H3 subtyping; IFVB; RSV (A and B); PIV (1-4); hMPV; AdV; human
91 coronaviruses (hCoV) 229E, OC43, NL63, HKU1 and picornaviruses including enteroviruses
92 (EV) and human rhinoviruses (HRV). RVP was performed according to the manufacturer's
93 instructions with the exception that specimens included in this study were primarily extracted for
94 in-house NATs, thus bacteriophage MS2 was not spiked into the samples as an internal control
95 prior to extraction. MS2 RNA was added into the master mix for use as an amplification and
96 detection control only. Although the RVP has been FDA-cleared for the majority of targets the
97 human coronaviruses and PIV4 components included in this study are not included in the FDA-
98 cleared version of the assay.

99 **Confirmation of RVP positive samples.** Samples which gave a positive result by RVP for
100 targets not included in the DFA/NATs screen such as hCoVs and picornaviruses were subjected
101 to in-house real-time NATs for the same targets as confirmation [(22) and unpublished] .

102 Further characterization of picornavirus positive samples was undertaken by RT-PCR of the
103 5'non-coding region (5'NCR) (as detailed below) (24). A total of 47 samples containing
104 detectable picornavirus sequences by the RVP assay were subjected to first round of
105 amplification directly from the sample extract using Primers P1-1
106 (CAAGCACTTCTGTWCCCC) and P3-1 (ACGGACACCCAAAGTAG); and semi-nested
107 amplification was performed using forward primer P1-1 and three reversed primers P2-1
108 (CAAGCACTTCTGTWCCCC), P2-2 (TTAGCCACATTCAGGAGCC) and P2-3
109 (TTAGCCGCATTCAGGGG) as described previously.

110 **Statistical Analysis.** The SPSS software v16.0 (SPSS Inc, Chicago, IL, USA) was utilized
111 for statistical analysis of data. The McNemar test was used to assess the impact of RVP on
112 outbreak resolution (compared with DFA/in-house NATs). Association of outbreaks with
113 particular seasons was assessed using Pearson Chi-squared (χ^2) analysis. In all cases, $p < 0.05$
114 was utilized to denote a statistically significant difference between parameters compared.
115 Variation in patients' age was expressed as a standard deviation (SD) and differences in results
116 between age groups was assessed by χ^2 analyses.

117

118

RESULTS

119 **Outbreak investigation analysis.** During the course of this study, 244 outbreaks related to
120 respiratory illness in LTAC, schools/daycares, hospitals and community (within a household
121 environment) were declared with 160 (65.6%) from 2006 and 84 (34.4%) from 2007 (Table 1).
122 The median number of samples per outbreak in 2006 and 2007 was 7.0 (range: 1 to 21) and 7.5
123 (range: 1 to 19), respectively. The winter season in Alberta is considered to be from October to
124 March of each year. The number of respiratory outbreaks was significantly higher in the winter

125 months compared with the rest of the year in 2006 and 2007 (χ^2 , $p = 0.0035$). Numbers used for
126 computation of these statistics are provided in Figure 1.

127 A total of 1108 specimens were submitted from the 244 outbreaks with 711 from 2006 and
128 397 from 2007. The majority of specimens received from these outbreaks were NP aspirates or
129 swabs ($n=939$, 84.7%) or TS ($n=164$, 14.8%). Other specimen types comprised of nose swab,
130 ocular swab, sputum, tracheal sample and bronchoalveolar lavage ($n=5$, 0.5%). A similar
131 distribution of specimen types was seen in 2006 (80.0%, $n=569$ NP; 19.6%, $n=139$ TS) and 2007
132 (93.2%, $n=370$ NP and 6.3%, $n=25$ TS). The distribution of sample types tested in 2006 and
133 2007 is shown in Table 1.

134 In 2006, the outbreak settings were distributed equally among LTAC and schools/daycares at
135 51.2% and 46.2%, respectively. In 2007, 78.6% of the outbreaks were in LTAC and 9.5% in
136 schools/daycares with a small number of outbreaks in hospital acute care and community
137 settings, as shown in Table 1. The median age of patients tested in 2006 was 71.5 years (range:
138 23 days to 105 years) and it was 81.1 years (range: 2 months to 101 years) in 2007.

139 **DFA and in-house NAT results.** Table 2 provides a summary of the number of outbreaks
140 and specimens tested, positive results obtained by the different methods is also shown. A total of
141 711 samples from 160 outbreaks were submitted in 2006 for diagnosis of respiratory viruses. Of
142 these 382 (53.7%) samples from 134 (83.8%) outbreaks gave a positive result for one or more
143 respiratory viral target by DFA and/or NATs. In 2007, 397 samples were tested from 84
144 outbreaks. Of these, 142 (35.8%) samples from 43 (51.2%) respiratory outbreaks gave positive
145 results for one or more respiratory viral pathogens using our DFA and/or NATs algorithm.

146 **Distribution of respiratory viruses identified by DFA/NATs.** Respiratory viral targets
147 were identified in 177 outbreaks by DFA and/or NATs in 2006 and 2007. Influenza viruses were

148 a major cause of respiratory viral outbreaks as shown in Figure 2 and IFVA and IFVB were
149 detected as the only etiological agent in 36.9% (n=90) and 4.5% (n=11) of total outbreaks
150 (n=244), respectively. IFV was identified with other non-IFV respiratory viruses in 16.4% of
151 outbreaks (n=40). Viruses other than IFVA or B detected as the etiological agents in outbreaks
152 were: 3.7% RSV (n=9), 4.9% PIV (n=12), 3.7% hMPV (n=9), and 2.5% non-IFV mixed
153 outbreaks (n=6). This resulted in 27.5% (n=67) of the total outbreaks (n=244) without an
154 etiological agent found. Total diagnosis rate for respiratory viruses by DFA and NATs in the 2
155 years combined was 72.5% (n=177) (Table 2 and Figure 2).

156 The age of patients with a positive DFA or NAT result ranged from 3 months to 106 years,
157 (median age = 70.0) in 2006. DFA or NAT positive outbreaks in 2006 (n=134) occurred in all
158 settings tested including 64 (47.8%) in LTAC, 69 (51.5%) in schools/daycares and 1 (0.7%) in
159 the community. The age of patients with a virus identified for their symptoms as part of an
160 outbreak in 2007 ranged from 18 months to 100 years (median age = 81.2) in 2007. The
161 distribution of DFA/NAT positive outbreaks (n=43) was 32 (74.4%) in LTAC, 4 (9.3%) in
162 schools/daycares and 7 (16.3%) in hospitals.

163 **Luminex xTAG™ RVP results.** RVP testing was performed on available samples from
164 outbreaks which were not resolved after testing by DFA/NATs. A total of 200 specimens
165 (2006=72, 2007=128) from 51 outbreaks (2006=22, 2007=29) that were tested previously by
166 DFA or NATs, without positive results, were available for further testing by RVP retrospectively
167 (Table 2). The total number of outbreaks used for analysis by a combination of all testing
168 methods was 228.

169 **Analysis of RVP results for 2006.** For samples from 2006, RVP gave positive results for 20
170 samples from 12 outbreaks (LTAC, n=10; schools/daycares, n=1 and hospital, n=1) without an

171 identified cause by DFA/NAT. RVP testing was not performed on 4 unresolved outbreaks for
172 which samples were not available. Of these 20 positives detected by the RVP assay, 17 samples
173 from 9 outbreaks were positive for picornaviruses. Other respiratory viruses identified in
174 individual samples by RVP were hCoV OC43 (n=1), hCoV HKU1 (n=1) and IFVA (n=1). Thus,
175 use of the RVP assay increased the proportion of resolved outbreaks from 83.8% to 93.6% in
176 2006 (Table 2). The monthly distribution of resolved and unresolved outbreaks is shown in
177 Figure 1. The resolved outbreaks (n=12) were in all settings tested including 9 (75.0%) in LTAC,
178 1 (8.3%) in a school and 2 (16.7%) in acute care hospitals. The breakdown of positive results for
179 outbreaks by month is given in Figure 2. The age of patients with a positive RVP result ranged
180 from 2 months to 100 years (median age = 83.8) in 2006. The age of patients giving a positive
181 result by RVP was not significantly different to those with a negative result (χ^2 , p = 1.0000).

182 **Analysis of RVP results for 2007.** Of the 128 samples from 29 unresolved outbreaks in
183 2007, 38 samples (from 18 outbreaks) gave a positive result by the RVP assay. Samples from 12
184 unresolved outbreaks were unavailable for RVP testing. The targets detected in samples from
185 outbreaks without a causative agent identified by DFA/NAT were: picornavirus (n=30), hCoV
186 NL63 (n=5), hCoV OC43 (n=2) and IFVA (n=1). This resulted in an etiological diagnosis for 18
187 outbreaks which were not resolved by DFA/NAT; 17 from LTAC and 1 from the community
188 (household). The outbreaks resolved by RVP were distributed throughout the year as shown in
189 Figure 1. Thus, use of the RVP assay increased the proportion of resolved outbreaks from 51.2%
190 to 84.7% in 2007 (Table 2). The monthly distribution of resolved and unresolved outbreaks is
191 shown in Figure 1. The breakdown of positive results for outbreaks by month is given in Figure
192 2.

193 The age of patients with a positive target detected by RVP ranged from 28 to 100 years,
194 (median age = 85.7) in 2007. The age of patients giving a positive result by RVP was not
195 significantly different to those with a negative result (χ^2 , $p = 0.1753$).

196 **Analysis of results for all outbreaks 2006/2007.** Viral etiological agents detected in
197 outbreaks (n=244) by a combination of all three methodological approaches (DFA/NAT and
198 RVP) are summarized in Figure 3. Thirty samples from 51 unresolved outbreaks by DFA/NATs
199 gave positive results for picornavirus (n=22), hCoV (n=6) and IFVA (n=2) by RVP.

200 Using a combination of all three methodological approaches, 207/228 (90.8%) of outbreaks
201 were resolved (Table 2 and Figure 1). A significant difference was observed in comparison of
202 resolved outbreaks with and without RVP testing in both 2006 and 2007 ($P \leq 0.0257$, McNemar
203 analysis).

204 **Seasonality of respiratory outbreaks.** Figure 2 shows the distribution of viral targets over
205 the different months in 2006 and 2007 identified by a combination of all three methods. IFVA
206 was detected in outbreaks during the months of November to May; IFVB related outbreaks were
207 not as prevalent as IFVA during the study period and were identified from November to
208 February and also in May. Human metapneumovirus was associated with outbreaks from
209 November to March and RSV from October to March. Parainfluenza virus was detected in all
210 months tested except the summer months of July, August and September. In 2006, AdVs were
211 identified and found as the etiological agent in the months of February, October and December.
212 Coronaviruses were restricted to the winter months of January, February and March and
213 picornaviruses were distributed throughout the 2 years. Adenoviruses were not responsible for
214 any outbreak in 2007.

215 There was a significant difference in the number of resolved outbreaks by DFA/NAT in
216 winter months (October to March) compared with non-winter months (χ^2 , $p < 0.0001$),
217 presumably due to the predominance of IFV, RSV and PIV infections in winter. After the
218 additional RVP results were included, there was a trend towards enhanced resolution of
219 outbreaks in non-winter months (April to September) compared with winter months but this did
220 not reach statistical significance (χ^2 , $p = 0.0940$). It is likely that because of the enhanced
221 detection of hCoVs and picornaviruses, the RVP had more impact on identification of a viral
222 etiology for non-resolved outbreaks in the non-winter months although fewer respiratory
223 outbreaks are identified during this time.

224 **Confirmation of RVP positive samples.** Samples that gave a positive result by the RVP
225 assay for IFVA and hCoVs (OC43, NL63 and HKU1) were confirmed by in-house NATs (13
226 ,22,31 and unpublished). Confirmation of a picornavirus positive result by amplification and
227 sequencing of the 5'NCR of HRV was successful in 44/ 47 positive samples using primers
228 reported previously (24). No amplified product was obtained for 3 picornavirus positive samples
229 identified by RVP, likely due to a low viral load in the sample. Interestingly, many of the
230 rhinoviruses associated with outbreaks belonged to the divergent group reported to be associated
231 with childhood infection (24). Further detailed analysis of these HRVs will be undertaken in
232 future studies.

233

234

DISCUSSION

235 Respiratory virus outbreaks are associated with a considerable impact on health care
236 resources (4,20,35). There is a limitation in using DFA and culture for detection of respiratory
237 viruses because of low sensitivity and the range of viral pathogens which can be identified. Probe

238 based NATs, in a real-time detection format, are sensitive and specific but can be expensive and
239 laborious when a broad range of respiratory viruses need to be identified. The use of a panel of
240 singleplex or small multiplex real-time assays for detection of the main causes of respiratory
241 virus outbreaks would not be practical in many diagnostic laboratories, nor would such an
242 approach be cost effective where a full range of possible viral causes needs to be investigated.

243 The use of technology involving multiplex amplification of targets with detection of
244 products using suspension microarrays is now well established and has been applied to the
245 diagnosis of individual cases of respiratory virus infection with great success (8,23,25,27,29,32).
246 In this study, we investigated the utility of the xTAGTM RVP assay for detection of respiratory
247 viruses associated with outbreaks for which a viral cause could not be found using DFA/in-house
248 NATs for a range of common respiratory viruses. The RVP assay provides the additional
249 identification of coronaviruses (229E, OC43, NL63 and HKU1), picornaviruses and the sub-
250 typing of IFVA hemagglutinin, which are not included in our routine diagnostic algorithm using
251 DFA and in-house NATs.

252 Despite the use of a range of NATs, we were only able to find a viral cause for a respiratory
253 outbreak in 50% of outbreaks in 2007 using our then routine algorithm of DFA/NATs
254 combination. As expected, RVP had the most impact on identification of viruses not included in
255 our testing algorithm (especially rhinoviruses) but it also identified two IFVA positive samples
256 which had been missed by DFA/NAT in 2006/2007.

257 Although it has been reported that HRVs cause a significant proportion of acute infections in
258 children and may be associated with lower respiratory infections and severe presentations (5,30),
259 the impact of such infections in the elderly has not been reported widely. In Alberta in 2007,
260 outbreaks found to be associated with HRV infection after RVP testing, caused considerable

261 concern and resulted in severe symptoms in elderly individuals as part of outbreaks at LTAC.
262 We found that rhinoviruses were an important cause of outbreaks outside of the winter months
263 and especially impacted LTAC. The presentation of HRV infection is clearly not always mild
264 and the cause of “off season” outbreaks could not have been predicted easily based on clinical
265 presentation. Interestingly, many of the HRVs found associated with outbreaks in 2006 and 2007
266 were closely related to the novel group of viruses identified recently (24,30).

267 Although we did not find many outbreaks caused by hCoVs in Alberta in 2006-2007 such
268 infections can be quite variable from year to year. In one study, hCoV OC43 was shown to be
269 associated with outbreaks in elderly care facilities with “IFV-like” clinical presentation (6).
270 Despite early data indicating that hMPV may not be associated with significant disease in adults,
271 we (34) and others (7,9,21,26,37), have shown that hMPV is also associated with respiratory
272 outbreaks, with the elderly being particularly vulnerable to severe presentations. Thus the broad
273 spectrum detection of viruses which cannot be identified easily by DFA/culture (for example,
274 hCoVs and hMPV), using assays such as RVP, will be very important in enhancing our
275 understanding of respiratory virus morbidity and mortality.

276 In this study, we investigated the utility of RVP to provide enhanced etiological diagnosis of
277 respiratory outbreaks as compared to DFA/NAT testing. Our previous studies confirmed the
278 excellent sensitivity and specificity of RVP for detection of the main causes of respiratory virus
279 outbreaks in Alberta (IFVA, IFVB, hMPV, RSV, PIV1-3) (32). We were also able to show that,
280 in the majority of cases, we were able to obtain subtyping information for seasonal IFVA
281 simultaneously with a positive result by RVP. This provides critical information to guide
282 prophylaxis and therapy as IFVA H3 viruses resistant to adamantanes and IFVA H1 viruses
283 resistant to neuraminidase inhibitors are increasingly identified (1,2,33). Based on this data, the

284 Luminex xTAGTM RVP assay has been implemented in our laboratory for investigation of acute
285 respiratory infection outbreaks that have no etiological diagnosis by DFA. The RVP has thus
286 replaced in-house NATs in our routine testing algorithm providing an efficient and cost-effective
287 means of sensitive, broad respiratory virus testing.

288 A recent analysis of the use and clinical impact of RVP confirmed that this test is very cost
289 effective compared with DFA and or culture when the prevalence of infection is > 11% (28).
290 Using a combination of DFA and RVP had an intermediate cost and provides the best
291 combination of rapid turn-around and sensitivity for many laboratories. Although clearly this
292 approach provides expanded detection of respiratory viruses, there are still scope for increasing
293 the number of viruses detected. In a recent study, the RVP test demonstrated superior sensitivity
294 for the detection of all influenza strains, including the novel swine-origin H1N1 (18). Further
295 evaluation and appropriate enhancement to the assay will be needed to ensure that novel viruses
296 (including potential pandemic influenza viruses) are detected by RVP and other multiplexed
297 NATs. Inclusion of viruses such as human bocavirus, respiratory polyomaviruses and bacteria
298 causing overlapping symptoms will enhance our ability to identify causes for respiratory
299 infection and disease.

300

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307 outbreaks are identified and specimens forwarded to ProvLab.

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310 **Table 1: Location and specimen type for respiratory virus outbreaks during 2006 and 2007**
311 **in Alberta, Canada**

312 NP: nasopharyngeal aspirate or swab; TS: throat swab; Others: nose swab, ocular swab, sputum,
313 tracheal aspirate and bronchoalveolar lavage.

314

315 **Table 2: Summary of outbreak investigations by DFA, NATs and RVP assays**

316 The number and percentage of outbreaks which gave a positive result by DFA/NATs and the
317 added value of RVP testing for DFA/NAT negative samples is shown. The percentages are
318 indicated in parentheses.

319 ^a Only samples and outbreaks available for analysis by RVP are included in these columns.

320 ^b Samples from 4 outbreaks in 2006 and 12 in 2007 were not available for RVP testing as
321 indicated in the Results section.

322

323 **Figure 1: Monthly distribution of outbreaks analyzed by a combination of testing methods.**

324 **DFA** = Direct fluorescent antigen

325 **NAT** = nucleic acid amplifications test

326 **RVP** = Luminex xTAGTM Respiratory Viral Panel

327 The numbers of resolved outbreaks reported by DFA and NATs, additional positive results by
328 RVP, unresolved outbreaks not tested by RVP in 2006 and 2007 are included. October to March
329 was considered as the winter months in Alberta, Canada as denoted by the double arrow bars.

330

331 **Figure 2: Monthly distribution of viruses identified by a combination of methods in**
332 **resolved outbreaks**

333 Influenza A (IFVA), Influenza B (IFVB), respiratory syncytial virus (RSV), parainfluenza
334 viruses (PIV), human metapneumovirus (hMPV), respiratory adenoviruses (AdV), human
335 coronaviruses (hCoVs – OC43, NL63, HKU1)

336

337 **Figure 3: Summary of viruses identified in resolved outbreaks**

338 The pie chart show the distribution of viral targets identified using a combination of DFA and
339 NATs. The bar graph represents outbreaks tested by RVP and the respiratory viruses identified.

340 Data shown here includes the outbreaks in the years of 2006 and 2007 (n=244).

341 IFV related mixed outbreaks were those with detectable IFVA and/or IFVB, together with PIV,
342 RSV, hMPV and AdV.

343 Non-IFV mixed outbreaks included PIV, hMPV, RSV and AdV.

344

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Table 1: Location and specimen type for respiratory virus outbreaks during 2006 and 2007 in Alberta, Canada

Year	Outbreak location					Specimen type			
	Total outbreaks	Long term care (%)	Schools/daycare (%)	Hospitals (%)	Community (%)	Total tested	NP (%)	TS (%)	Others (%)
2006	160	82 (51.2%)	74 (46.2%)	2 (1.3%)	2 (1.3%)	711	569 (80.0%)	139 (19.6%)	3 (0.4%)
2007	84	66 (78.6%)	8 (9.5%)	4 (4.8%)	6 (7.1%)	397	370 (93.2%)	25 (6.3%)	2 (0.5%)
Total	244	148 (60.6%)	82 (33.6%)	6 (2.6%)	8 (3.3%)	1108	939 (84.7%)	164 (14.8%)	5 (0.5%)

NP: nasopharyngeal aspirate or swab; TS: throat swab; Others: nose swab, ocular swab, sputum, tracheal aspirate and bronchoalveolar lavage.

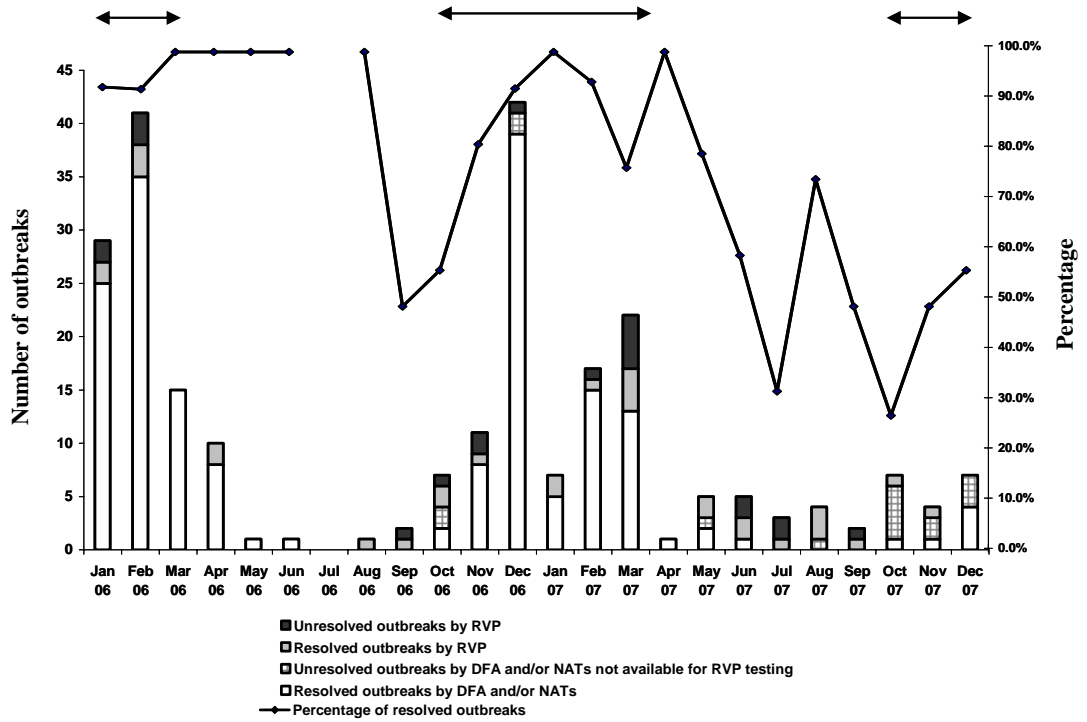
Table 2: Summary of outbreak investigations by DFA, NATs and RVP assays

Year	Outbreaks positive by DFA/NATs (%)	Outbreaks negative by DFA/NATs and positive by RVP (%) ^a	Outbreaks positive by DFA/NATs/RVP (%) ^a	Samples from outbreaks positive by DFA/NATs (%)	Samples from outbreaks negative by DFA/NATs and positive by RVP (%) ^a	Samples from outbreaks positive by DFA/NATs/RVP (%) ^a
2006	134/160 (83.8)	12/22 (54.5)	146/156 ^b (93.6)	382/711 (53.7)	20/72 (27.8)	402/677 ^b (59.4)
2007	43/84 (51.2)	18/29 (62.1)	61/72 ^b (84.7)	142/397 (35.8)	38/128 (29.7)	180/355 ^b (50.7)
Total	177/244 (72.5)	30/51 (58.8)	207/228 ^b (90.8)	524/1108 (47.3)	58/200 (29.0)	582/1032 ^b (56.4)

^a Only samples and outbreaks available for analysis by RVP are included in these columns.

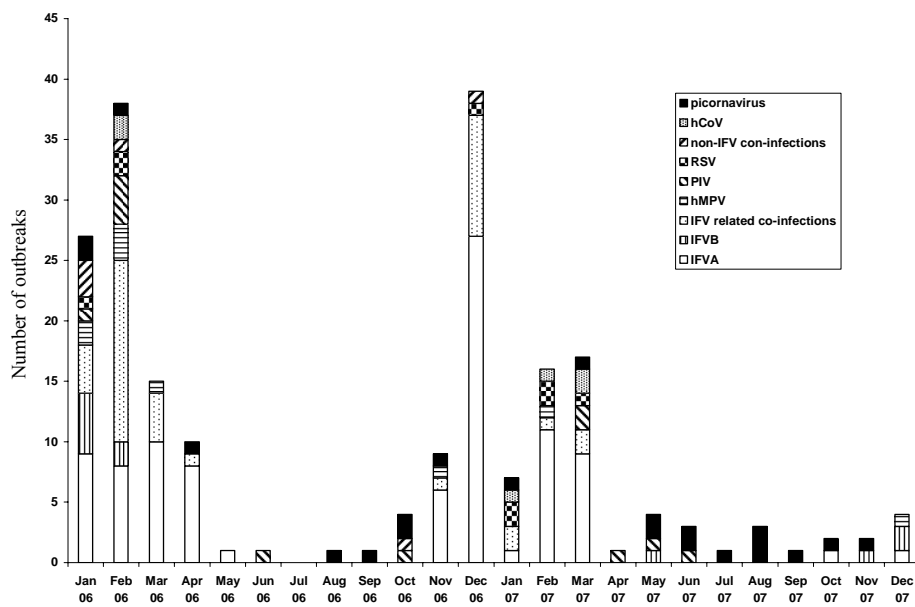
^b Samples from 4 outbreaks in 2006 and 12 in 2007 were not available for RVP testing as indicated in Results.

Figure 1: Monthly distribution of outbreaks analyzed by a combination of testing methods



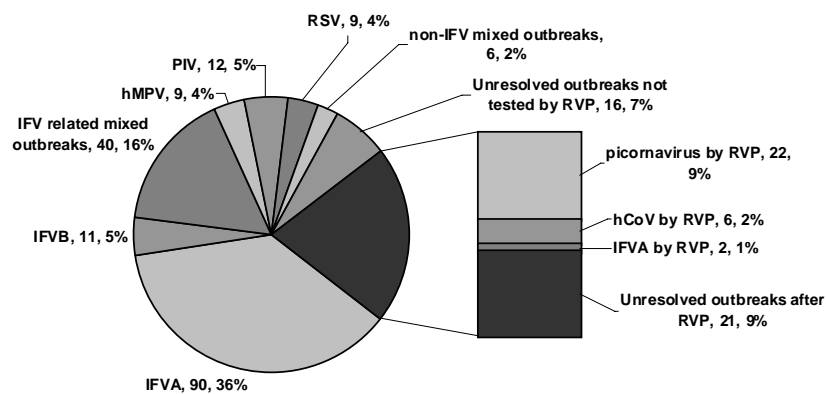
NP: nasopharyngeal aspirate or swab; TS: throat swab; Others: nose swab, ocular swab, sputum, tracheal aspirate and bronchoalveolar lavage.

Figure 2: Monthly distribution of viruses identified by a combination of methods in resolved outbreaks



Influenza A (IFVA), Influenza B (IFVB), respiratory syncytial virus (RSV), parainfluenza viruses (PIV), human metapneumovirus (hMPV), respiratory adenoviruses (AdV), human coronaviruses (hCoVs – OC43, NL63, HKU1)

Figure 3: Summary of viruses identified in resolved outbreaks



The pie chart show the distribution of viral targets identified using a combination of DFA and NATs. The bar graph represents outbreaks tested by RVP and the respiratory viruses identified. Data shown here includes the outbreaks in the years of 2006 and 2007 (n=244).

IFV related mixed outbreaks were those with detectable IFVA and/or IFVB, together with PIV, RSV, hMPV and AdV.

Non-IFV mixed outbreaks included PIV, hMPV, RSV and AdV.