**Supplementary material**

Article title:

**Respiratory virus co-infections with SARS-CoV-2 continue to be rare one year into the pandemic in Alberta, Canada (June 2020 – May 2021)**

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**Supplementary Methods.**

*Specimen storage and processing*

Once collected, respiratory specimens were transported from collection sites to the laboratory at room temperature. Respiratory specimens were first tested for SARS-CoV-2 with aliquots removed for RPP testing and long-term storage, both stored at -20oC. Specimens were then tested using the RPP assay the following day after thawing to room temperature (undergoing one freeze-thaw cycle).

Specimens undergoing RPP testing first underwent an extraction procedure (NucliSENS easyMag, bioMerieux, Marcy-l’Etoile, France). Any bronchoalveolar lavage or endotracheal aspirate specimens were extracted using the MagMAX Total Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Waltham, USA). Extracts were then tested using the RPP assay. All extraction and testing was conducted as per manufacturer’s instructions.

*Data analysis*

Duplicate testing (i.e. repeat SARS-CoV-2 and RPP) testing on the same patient conducted within six days of the first positive SARS-CoV-2 test was reviewed. Any positive RPP results were considered to be a co-infection if they were found within the incubation period of the specific virus (as outlined by Chow *et al*).1 If discordant testing results from multiple samples from the same patient were found, all positive RPP targets were included. Duplicate positive RPP targets were counted only once.

Given rhinovirus has been reported as the most common co-infection with SARS-CoV-2 in the literature thus far, we also wanted to evaluate how often rhinovirus is seen as a co-infecting virus with other viruses detected on the RPP. To do this, we extracted respiratory virus testing data from January 1, 2016 – December 31, 2019 to evaluate the proportion of each RPP target that was found positive along with ERV (as this is how rhinovirus is detected on the RPP assay). Data was extracted from the same centralized provincial laboratory information system (Millennium, Cerner, North Kansas City, USA) as the SARS-CoV-2 and RPP data from June 1, 2020 – May 31, 2021.

Data were summarized using basic epidemiologic analyses. Confidence intervals (CIs; 95%) were expressed using Wilson’s method. Continuous variables were compared using Mann-Whitney tests while categorical variables were compared using Chi-square or Fisher’s exact tests, as appropriate. Analysis was done using GraphPad Prism (Version 9.2.0; GraphPad Software LLC). A p-value<0.05 was considered significant. Adult patients were ≥18 years of age at the time of testing while pediatric patients were ≤17 years of age. Patient type (community, emergency room, inpatient – non-intensive care unit [ICU], ICU, and nursing home) was recorded at the time of testing.

**Supplementary Results**

*Description of duplicate testing*

A total of 146 duplicate samples (representing 92 patients) were removed. Of these, 123/146 (84.2%) were negative for any RPP target. The remainder 23 samples were positive for ERV (n=21), HMPV (n=1), and PIV-4 (n=1). Discordant testing results among these 23 samples, occurred for HMPV and PIV-4.

**Table S1.** Proportion of co-infections based on patient setting.

|  |  |  |  |
| --- | --- | --- | --- |
| Patient setting | Total samples of this patient type tested for SARS-CoV-2 and RPP | Number of specimens found to have a co-infection | Proportion of patients with co-infections (%)a |
| Community | 2431 | 55 | 2.3 |
| Emergency Room | 1197 | 40 | 3.3 |
| Inpatient (non-ICU) | 875 | 30 | 3.4 |
| ICU | 155 | 7 | 4.5 |
| Nursing Home | 100 | 2 | 1.3 |

Abbreviations: ICU – intensive care unit; RPP – respiratory pathogen panel

aComparison of all groups (Pearson’s chi-square; p=0.08).

**Table S2.** Historical proportion of respiratory viral panel testing results (2016-2019) in the entire province of Alberta with enterovirus/rhinovirus co-infection.a

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Respiratory Viral Target | 2016 | 2017b | 2018 | 2019 |
|  | N (%) | N (%) | N (%) | N (%) |
| Influenza A | 6/5771  (0.1) | 129/5391  (2.4) | 214/4826  (4.4) | 107/3084  (3.5) |
| Influenza B | 2/1717  (0.1) | 37/1593  (2.3) | 43/1589  (2.7) | 35/1047  (3.3) |
| hCoV 229E | 20/147  (13.6) | 18/257  (7.0) | 4/67  (6.0) | 18/305  (5.9) |
| hCoV OC43 | 29/269  (10.8) | 14/409  (3.4) | 33/593  (5.6) | 62/700  (8.9) |
| hCoV NL63 | 27/205  (13.2) | 23/248  (9.3) | 65/483  (13.5) | 23/267  (8.6) |
| hCoV HKU1 | 2/41  (4.9) | 15/180  (8.3) | 8/240  (3.3) | 11/150  (7.3) |
| PIV 1 | 39/396  (9.8) | 99/611  (16.2) | 14/178  (7.9) | 60/445  (13.5) |
| PIV 2 | 11/116  (9.5) | 34/172  (19.8) | 39/267  (14.6) | 15/161  (9.3) |
| PIV 3 | 57/560  (10.2) | 83/777  (10.7) | 117/680  (17.2) | 98/1028  (9.5) |
| PIV 4 | 48/303  (15.8) | 52/342  (15.2) | 63/347  (18.2) | 29/228  (12.7) |
| RSV A | 159/1648  (9.4) | 66/981  (6.7) | 60/680  (8.8) | 146/1302 (11.2) |
| RSV B | 115/1175  (9.8) | 99/1391  (7.1) | 211/2192  (9.6) | 91/1252  (7.3) |
| HMPV | 65/1271  (5.1) | 183/1281  (14.3) | 140/1772  (7.9) | 80/1104  (7.2) |
| Adenovirus | 85/340  (25) | 148/487  (30.4) | 194/636  (30.5) | 197/640 (30.8) |

Abbreviations: ERV – enterovirus/rhinovirus; hCoV – human coronavirus (non-SARS-CoV); HMPV – human metapneumovirus; PIV – parainfluenza virus; RSV – respiratory syncytial virus.

aDenominators in each cell indicate total number of specimens testing positive for the given virus in that year, with the numerator indicating the number of tests where ERV was found as a co-infecting virus.

bIn 2017, the laboratory changed mid-way from using the Respiratory Viral Panel (Luminex Corporation) to using the NxTAG Respiratory Pathogen Panel (Luminex Corporation).

**Supplementary Material References**

**1.** Chow EJ, Mermel LA. Hospital-Acquired Respiratory Viral Infections: Incidence, Morbidity, and Mortality in Pediatric and Adult Patients. *Open forum infectious diseases* 2017;4:ofx006-ofx006.