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SURVEILLANCE REPORT

Respiratory Syncytial Virus Genomic Surveillance in Ontario

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Introduction

This report summarizes the results of respiratory syncytial virus (RSV) whole genome sequencing (WGS) completed by Public Health Ontario (PHO) as of April 4, 2024.

Highlights

- There was a total of 2,345 RSV positive specimens detected at PHO between August 27, 2023 and March 9, 2024, of which, 238 (10.1%) were sequenced.
- Of the 238 specimens sequenced, 118 (49.6%) were RSV-A and 120 (50.4%) were RSV-B.
- All RSV-A positive specimens were lineage GA2.3.5 (ON1-like) and all RSV-B were lineage GB5.0.5a (BA-like).

Background

RSV is a major cause of lower respiratory illness, particularly among premature infants or infants under six months of age, children with underlying health conditions, and adults over 65 years of age.[1] There are two antigenic subgroups of RSV (RSV-A and RSV-B) that are based on variation within the G protein, which is a component of the viral envelope.[2] RSV undergoes changes in its genome as it spreads through populations. The accumulation of these genetic changes (i.e., mutations) can further classify RSV-A and RSV-B viruses into genotypes and lineages. Although many genotypes and lineages will have no differences in the ability to cause disease, some have mutations that may affect disease characteristics such as virulence.[3] Genomic surveillance uses WGS to monitor these changes in the genome as a virus evolves over time. This allows public health professionals to provide context to the current season, understand which genotypes and lineages are circulating and how this impacts the population.[4] It is important to monitor circulating lineages as Health Canada has recently authorized two vaccines, ABRYSVO™ and AREXVY for the prevention of lower respiratory tract disease caused by RSV.[5,6] Ontario has introduced a publicly funded RSV vaccine program targeted to high-risk individuals and settings.[7]

It is estimated that PHO conducts approximately 30% of all RSV testing in Ontario that is reported to the Public Health Agency of Canada.[8] PHO performs routine testing for seasonal respiratory viruses for select population groups, including:

- Symptomatic residents (and associated healthcare workers/staff) in congregate living settings (e.g., retirement homes, long term care homes, correctional facilities).

- Symptomatic individuals associated with an outbreak investigation.
- Hospitalized individuals, including those in intensive care.
- Symptomatic individuals, less than 18 years old, who receive care in an emergency department.[9]
- Individuals attending physician offices that are part of the Sentinel Practitioner Surveillance Network (see Technical Notes for additional information).[10]

To understand the diversity of the RSV viruses circulating in Ontario, PHO began sequencing eligible specimens positive for RSV in the 2023–24 season. Specimens were eligible if they had a cycle threshold (Ct) value ≤ 27 for RSV, sufficient volume remaining, and were positive only for RSV (no co-infection). Additionally, only the first specimen from an outbreak was eligible. Sequences were processed and analyzed using bioinformatics tools and were assigned an RSV subgroup and lineage.

Results

Table 1: Number of Positive RSV Specimens, Number and Percentage Sequenced, Public Health Ontario, August 27, 2023 to March 9, 2024

Month	Number of positive specimens	Number sequenced	Percentage sequenced
August 2023*	8	1	12.5%
September 2023	47	2	4.3%
October 2023	216	30	13.9%
November 2023	709	68	9.6%
December 2023	716	80	11.2%
January 2024	503	49	9.7%
February 2024	126	8	6.3%
March 2024*	20	0	0.0%
Total	**2,345**	**238**	**10.1%**

Note: *August 2023 and March 2024 are partial months. Of the 238 specimens sequenced, 16.4% (39/238) were outbreak-related. Results may not be representative of Ontario overall. Month was assigned based on earliest date available for a specimen. See Technical Notes for details of how specimens were selected for sequencing.
Data sources: PHO Laboratory Information Management System

Table 2: Number and Percentage of RSV Positive Specimens by Genetic Characterization, Public Health Ontario, August 27, 2023 to March 9, 2024

Genetic characterization	Number sequenced (percentage)
RSV-A	**118 (49.6%)**
GA2.3.5	118 (49.6%)
RSV-B	**120 (50.4%)**
GB5.0.5a	120 (50.4%)
Total sequenced	**238 (100%)**

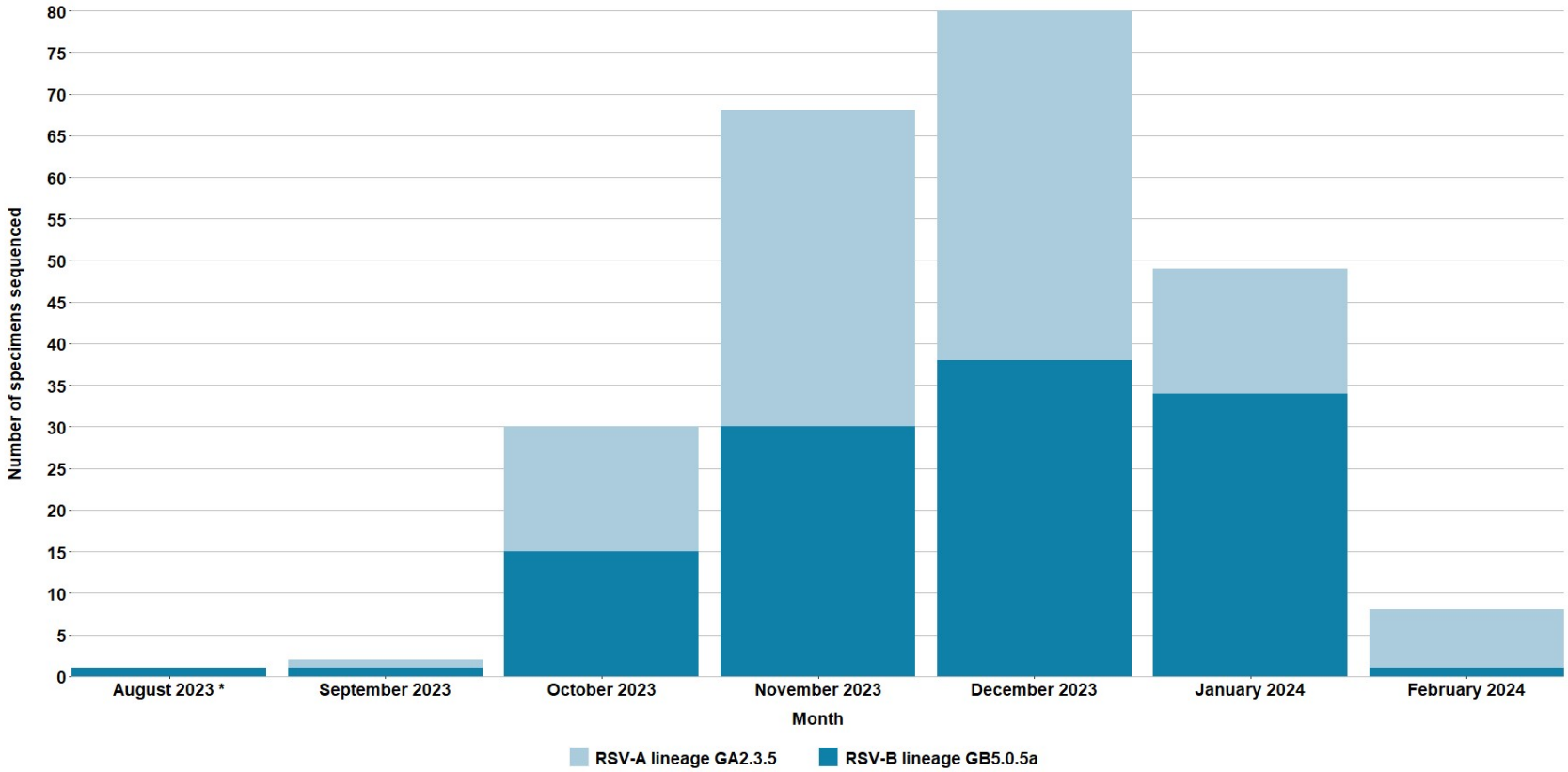
Note: Results may not be representative of Ontario overall. Date was assigned based on the earliest date available for the specimen.
Data sources: PHO Laboratory Information Management System

Table 3: Number and Percentage of RSV Positive Specimens by Genetic Characterization and Month, Public Health Ontario, August 27, 2023 to March 9, 2024

Month	RSV-A GA2.3.5	RSV-B GB5.0.5a	Total
August 2023*	0 (0.0%)	1 (100%)	1 (100%)
September 2023	1 (50.0%)	1 (50.0%)	2 (100%)
October 2023	15 (50.0%)	15 (50.0%)	30 (100%)
November 2023	38 (55.9%)	30 (44.1%)	68 (100%)
December 2023	42 (52.5%)	38 (47.5%)	80 (100%)
January 2024	15 (30.6%)	34 (69.4%)	49 (100%)
February 2024	7 (87.5%)	1 (12.5%)	8 (100%)
Total sequenced	**118 (49.6%)**	**120 (50.4%)**	**238 (100%)**

Note: *August 2023 is a partial month and had one specimen sequenced. There were no samples successfully sequenced for March 2024. Results may not be representative of Ontario overall. Month was assigned based on the earliest date available for the specimen.
Data sources: PHO Laboratory Information Management System

Figure 1: Number of RSV Positive Specimens by Genetic Characterization and Month, Public Health Ontario, August 27, 2023 to March 9, 2024



Note: *August 2023 is a partial month and had one specimen sequenced. There were no samples successfully sequenced for March 2024. Results may not be representative of Ontario overall. Month was assigned based on the earliest date available for the specimen.
Data sources: PHO Laboratory Information Management System

Table 4: Number and Percentage of RSV Positive Specimens by Genetic Characterization and Age Group, Public Health Ontario, August 27, 2023 to March 9, 2024

Genetic characterization	Ages: Less than 1	Ages: 1-4	Ages: 5-19	Ages: 20-64	Ages: 65 and older	Total
RSV-A	**26 (63.4%)**	**17 (54.8%)**	**3 (42.9%)**	**15 (62.5%)**	**57 (42.2%)**	**118 (49.6%)**
GA2.3.5	26 (63.4%)	17 (54.8%)	3 (42.9%)	15 (62.5%)	57 (42.2%)	118 (49.6%)
RSV-B	**15 (36.6%)**	**14 (45.2%)**	**4 (57.1%)**	**9 (37.5%)**	**78 (57.8%)**	**120 (50.4%)**
GB5.0.5a	15 (36.6%)	14 (45.2%)	4 (57.1%)	9 (37.5%)	78 (57.8%)	120 (50.4%)
Total sequenced	**41 (100%)**	**31 (100%)**	**7 (100%)**	**24 (100%)**	**135 (100%)**	**238 (100%)**

Note: Results may not be representative of Ontario overall. Age was assigned based on the birth date provided on the test requisition.
Data sources: PHO Laboratory Information Management System

Table 5: Number and Percentage of RSV Positive Specimens by Genetic Characterization and Setting, Public Health Ontario, August 27, 2023 to March 9, 2024

Genetic characterization	Intensive care unit	Hospital/emergency department	Congregate living	Ambulatory or no setting reported	Total
RSV-A	**3 (75.0%)**	**46 (59.0%)**	**45 (44.6%)**	**24 (43.6%)**	**118 (49.6%)**
GA2.3.5	3 (75.0%)	46 (59.0%)	45 (44.6%)	24 (43.6%)	118 (49.6%)
RSV-B	**1 (25.0%)**	**32 (41.0%)**	**56 (55.4%)**	**31 (56.4%)**	**120 (50.4%)**
GB5.0.5a	1 (25.0%)	32 (41.0%)	56 (55.4%)	31 (56.4%)	120 (50.4%)
Total sequenced	**4 (100%)**	**78 (100%)**	**101 (100%)**	**55 (100%)**	**238 (100%)**

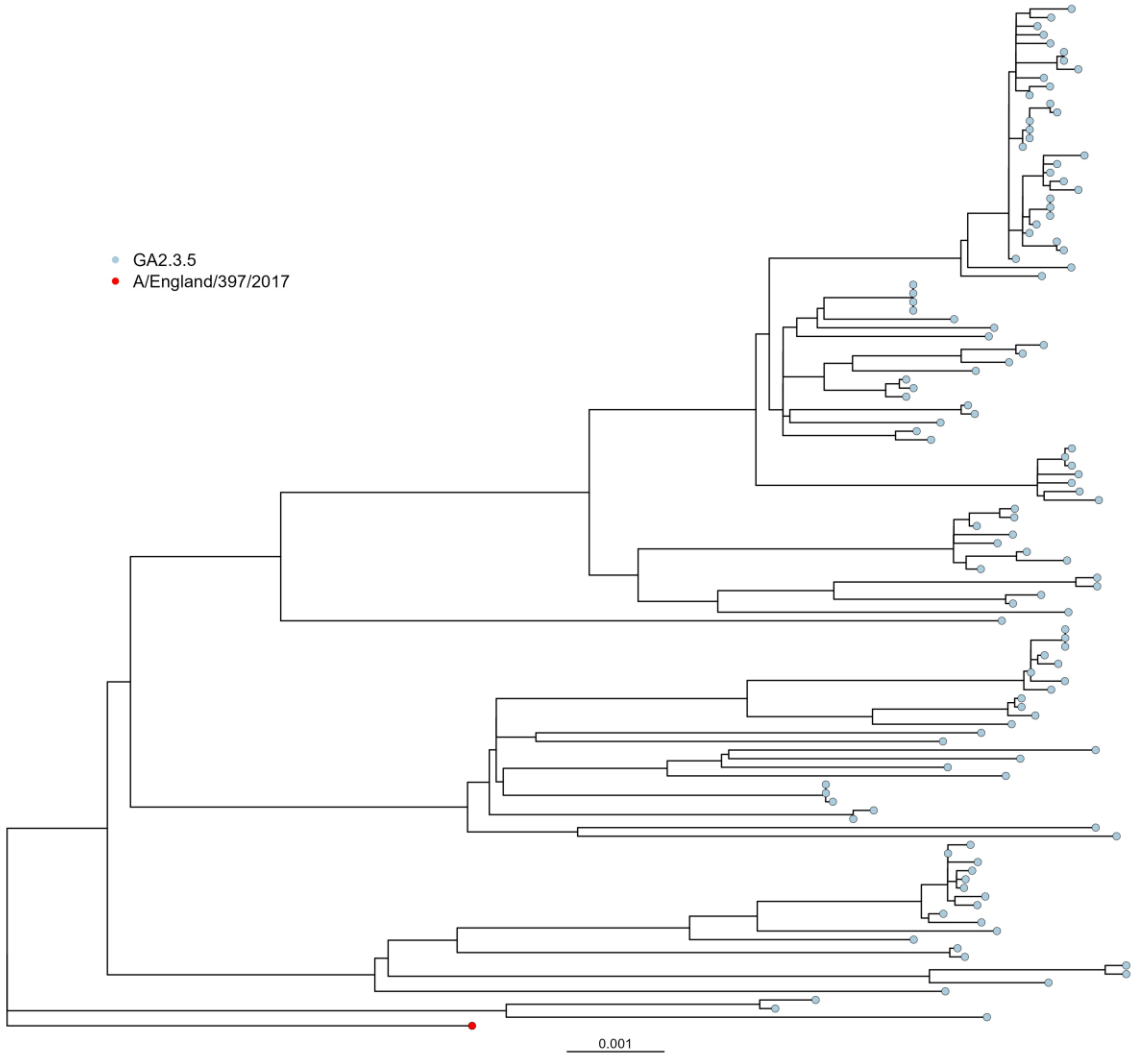
Note: Results may not be representative of Ontario overall. Setting represents the health care facility at which an individual received care. Congregate living includes long-term care homes, retirement homes, correctional facilities, and undefined institutions (excluding hospitals). Only one specimen per outbreak was eligible for sequencing. Approximately 13% of specimens are missing information on setting and are grouped into 'Ambulatory or no setting reported' category.
Data sources: PHO Laboratory Information Management System

Table 6: Number and Percentage of RSV Positive Specimens by Genetic Characterization and Region, Public Health Ontario, August 27, 2023 to March 9, 2024

Genetic characterization	Northern	Eastern	Central East	Toronto	South West	Central West	Total
RSV-A	**20 (60.6%)**	**13 (59.1%)**	**40 (42.1%)**	**16 (51.6%)**	**7 (70.0%)**	**21 (45.7%)**	**117 (49.4%)**
GA2.3.5	20 (60.6%)	13 (59.1%)	40 (42.1%)	16 (51.6%)	7 (70.0%)	21 (45.7%)	117 (49.4%)
RSV-B	**13 (39.4%)**	**9 (40.9%)**	**55 (57.9%)**	**15 (48.4%)**	**3 (30.0%)**	**25 (54.3%)**	**120 (50.6%)**
GB5.0.5a	13 (39.4%)	9 (40.9%)	55 (57.9%)	15 (48.4%)	3 (30.0%)	25 (54.3%)	120 (50.6%)
Total sequenced	**33 (100%)**	**22 (100%)**	**95 (100%)**	**31 (100%)**	**10 (100%)**	**46 (100%)**	**237 (100%)**

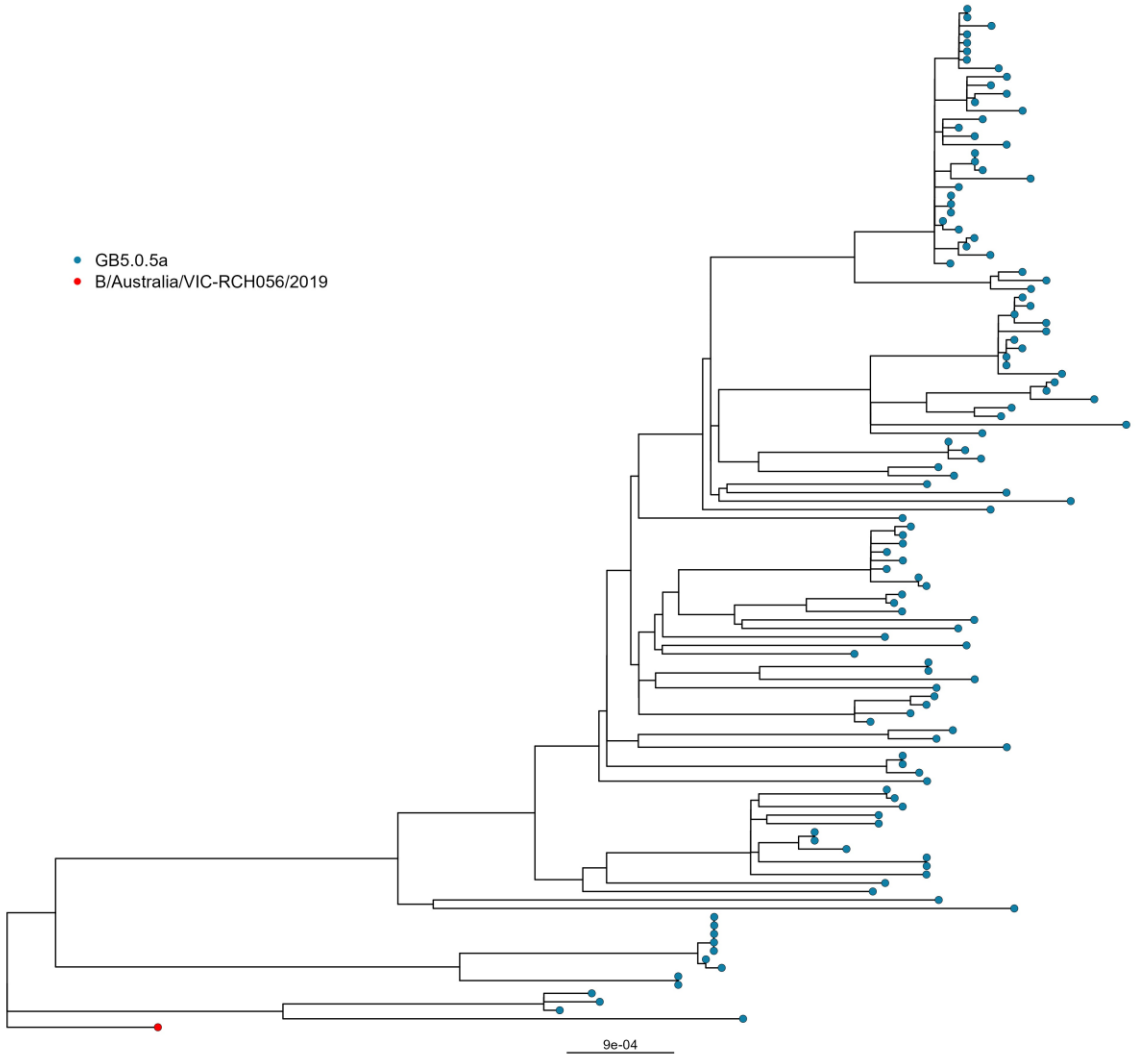
Note: One specimen was excluded as the location was unknown. Results may not be representative of Ontario overall. Region was assigned using patient address when available. If missing, region was assigned using submitter address. For additional information on which public health units are included in each region, see Technical Notes.
Data sources: PHO Laboratory Information Management System

Figure 2a: Phylogenetic Tree of RSV-A Lineage GA2.3.5 Specimens, Public Health Ontario, August 27, 2023 to March 9, 2024



Note: Each circle represents a separate specimen and colour indicates the G-gene lineage (noted in light blue). Results may not be representative of Ontario overall. The maximum likelihood phylogenetic tree was generated based on complete RSV genomes using the IQ-TREE GTR model with 100 bootstrap replicates. Identical sequences are retained in the tree. The tree is rooted with the reference strain A/England/397/2017 (GISAID ID EPI_ISL_412866; noted in red).
Data sources: PHO Laboratory Information Management System

Figure 2b: Phylogenetic Tree of RSV-B Lineage GB5.0.5a Specimens, Public Health Ontario, August 27, 2023 to March 9, 2024



Note: Each circle represents a separate specimen and colour indicates the G-gene lineage (noted in dark blue). Results may not be representative of Ontario overall. The maximum likelihood phylogenetic tree was generated based on complete RSV genomes using the IQ-TREE GTR model with 100 bootstrap replicates. Identical sequences are retained in the tree. The tree is rooted with the reference strain B/Australia/VIC-RCH056/2019 (GISAID ID EPI_ISL_1653999; noted in red).
Data sources: PHO Laboratory Information Management System

Technical Notes

Data Sources

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- Data were extracted from the PHO Laboratory Information Management System on April 2, 2024 at approximately 4:30 p.m.
- Bioinformatics processing of data by the Biocomputing Centre were completed on April 12, 2024 at approximately 2:00pm.

Public Health Ontario's RSV Whole Genome Sequencing Strategy

- Public Health Ontario used random sampling to select 250 eligible RSV PCR positive specimens for whole genome sequencing. Of the 250 selected, 238 (95.2%) were successfully sequenced.
- Specimens were eligible if they had Ct value ≤ 27 for RSV, sufficient volume remaining, and were PCR positive only for RSV (no co-infection). Additionally, only the first specimen from an outbreak was eligible for WGS and specimens that were submitted on behalf of the Sentinel Practitioner Surveillance Network (SPSN) were excluded.
- Only upper respiratory specimens (e.g. nasopharyngeal or throat swabs) were included.
- Genetic characterization of specimens was completed using WGS and analyzed by a bioinformatics pipeline using ivar (1.4.2), bwa-mem (0.7.17), bcftools (1.10.2), and vcftools (0.1.16).[11-14] Lineage was assigned with Nextclade (2.14.0) analysis.[15] Phylogenetic tree was created using IQ-TREE (2.2.3).[16]

Public Health Ontario's Respiratory Testing Algorithm

- PHO's laboratory respiratory testing algorithm is based on patient setting.
- PHO laboratory performs multiplex respiratory virus PCR (MRVP) on symptomatic children (< 18 years) seen in the emergency department, symptomatic hospitalized patients (ward and Intensive or Critical Care Unit), symptomatic residents in institutional settings (non-outbreak), and specimens from the first four symptomatic individuals (including healthcare workers/staff) in an outbreak that requests respiratory virus testing.
- PHO laboratory performs FLUVID PCR on symptomatic residents and healthcare workers/staff in the institutional settings in an outbreak beyond the first four that have been tested for SARS-CoV-2 and MRVP.
- Individuals attending physician offices that are part of the Sentinel Practitioner Surveillance Network (SPSN).[10] SPSN patients are exempt from laboratory testing restrictions.

Testing Methods

- Testing for RSV at PHO is performed using:
  - A laboratory-developed multiplex respiratory virus PCR panel assay (MRVP). The assay detects 11 viral targets including RSV.

- A FLUVID PCR assay which detects respiratory syncytial virus (RSV-A/B), influenza A and B, and SARS-CoV-2 (COVID-19). This assay may be used as an initial test prior to MRVP to provide earlier results during influenza and RSV seasons. FLUVID detects RSV but does not differentiate between RSV-A and RSV-B.

Data Caveats

- PHO conducts approximately 30% of RSV testing in Ontario. Further, only 10.1% of positive specimens were sequenced during the current season. Biases may be introduced due to eligibility criteria for diagnostic testing, catchment area of PHO testing, the volume of specimen available, WGS specimen selection criteria, and whether a specimen can be successfully sequenced. As a result, the results may not represent Ontario overall.
- Numbers and proportions may not align with the Ontario Respiratory Virus Tool as only specimens eligible (Ct value ≤ 27 for RSV, sufficient volume remaining, and first specimen from an outbreak) were included.
- The report includes specimens tested from the start of the RSV season to when a stable decrease in the percent positivity was observed. Thus, the time period covered may not represent the entire season. Counts based on specimens do not represent unique individuals, as some individuals may have more than one specimen tested.
- Region was assigned based on patient address when available and submitter address when missing. As such, individuals with missing patient address on the requisition may be misclassified.
    - Northern region included Northwestern Health Unit, Thunder Bay District Health Unit, Porcupine Health Unit, Algoma Public Health, Public Health Sudbury & Districts, Timiskaming Health Unit, and North Bay Parry Sound District Health Unit.
    - Eastern region included Renfrew County and District Health Unit, Ottawa Public Health, Eastern Ontario Health Unit, Leeds, Grenville & Lanark District Health Unit, Kingston, Frontenac and Lennox & Addington Public Health (KFLA), and Hastings Prince Edward Public Health.
    - Central East region included Haliburton, Kawartha, Pine Ridge District Health Unit (HKPR), Peterborough Public Health, Durham Region Health Department, Simcoe Muskoka District Health Unit, York Region Public Health, and Peel Public Health.
    - Toronto region included Toronto Public Health.
    - South West region included Grey Bruce Health Unit, Huron Perth Public Health, Southwestern Public Health, Middlesex-London Health Unit, Lambton Public Health, Chatham-Kent Public Health, and Windsor-Essex County Health Unit.
    - Central West region included Niagara Region Public Health, Halton Region Public Health, Hamilton Public Health Services, Brant County Health Unit, Wellington-Dufferin-Guelph Public Health, Region of Waterloo Public Health and Emergency Services, and City of Haldimand-Norfolk Health Unit.
- Age was assigned based on the birth date provided and the specimen collection or login date.
- Patient setting is missing for almost 13% of specimens. Therefore, results by patient setting should be interpreted with caution.

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