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SURVEILLANCE REPORT

# Respiratory Syncytial Virus Genomic Surveillance in Ontario: 2024-25

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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 May 26, 2025. The 2023–24 report can be found on PHO's website.

## Highlights

- There was a total of 3,010 RSV positive specimens detected at PHO between September 1, 2024 and March 1, 2025, of which, 253 (8.4%) were sequenced.
- Of the 253 specimens sequenced, 126 (49.8%) were RSV-A and 127 (50.2%) were RSV-B.
- All RSV-A positive specimens belong to genotype GA2.3.5 (ON1-like genotype). The most common clades were A.D.5.2 (16.2% of all RSV specimens), A.D.3 (12.6%), and A.D.1 (9.5%).
- All RSV-B positive specimens belong to genotype GB5.0.5a (BA-like genotype). The most common clade was B.D.E.1 (49.4% of all RSV specimens).

## 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 clades. Although many genotypes and clades 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 clades are circulating and how this impacts the population.[4] It is important to monitor circulating genotypes as Health Canada has authorized three vaccines, ABRYSVO™, AREXVY, and mRESVIA™ for the prevention of lower respiratory tract disease caused by RSV.[5,6] Ontario has introduced a publicly funded RSV prevention program targeted to high-risk individuals and settings.[7]

PHO conducts approximately 21.8% of all RSV testing in Ontario, based on data from the Ontario Laboratory Information System. 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.[8]
- Individuals attending physician offices that are part of the Sentinel Practitioner Surveillance Network (see Technical Notes for additional information).[9]

To understand the diversity of the RSV viruses circulating in Ontario, PHO sequenced eligible specimens positive for RSV in the 2024–25 season. Specimens were eligible if they had a PCR 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, genotype and clade.

# Results

**Table 1: Number of Positive RSV Specimens, Number and Percentage Sequenced, Public Health Ontario, September 1, 2024 to March 1, 2025**

| Month | Number of Positive Specimens | Number Sequenced | Percentage Sequenced |
|---|---|---|---|
| September 2024 | 13 | 2 | 15.4% |
| October 2024 | 79 | 8 | 10.1% |
| November 2024 | 312 | 31 | 9.9% |
| December 2024 | 884 | 74 | 8.4% |
| January 2025 | 1,182 | 90 | 7.6% |
| February 2025 | 538 | 48 | 8.9% |
| March 2025* | 2 | 0 | 0.0% |
| **Total** | **3,010** | **253** | **8.4%** |

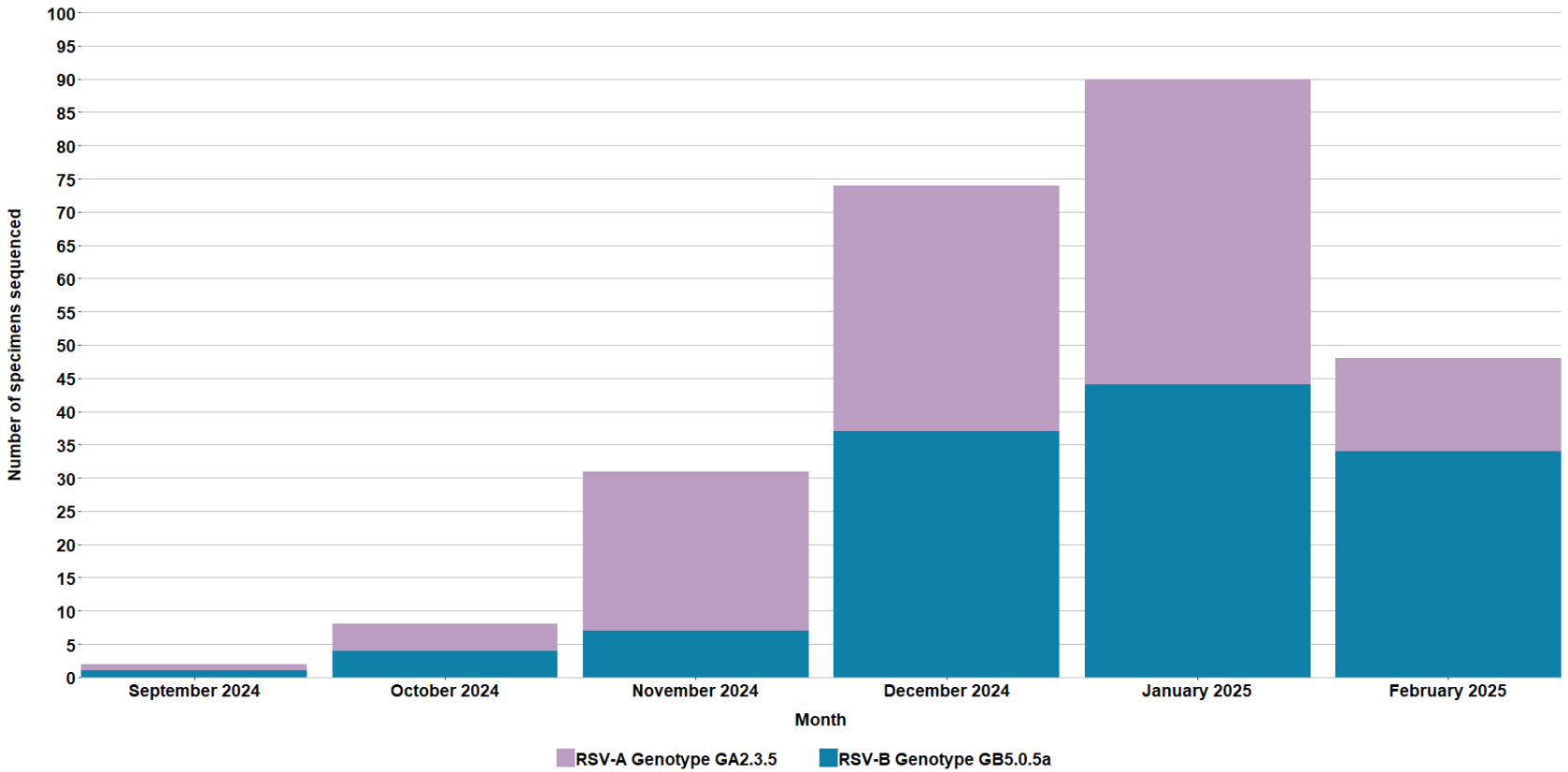
**Note:** *Includes only March 1, 2025. Of the 253 specimens sequenced, 20.9% (53/253) were outbreak-related. Results may not be representative of Ontario overall. Month was assigned based on earliest date available for a specimen. See Technical Notes for details of how specimens were selected for sequencing.

**Table 2: Number and Percentage of RSV Positive Specimens by Genetic Characterization and Month, Public Health Ontario, September 1, 2024 to March 1, 2025**

| Month | RSV-A GA2.3.5 | RSV-B GB5.0.5a | Total |
|---|---|---|---|
| September 2024 | 1 (50.0%) | 1 (50.0%) | 2 (100%) |
| October 2024 | 4 (50.0%) | 4 (50.0%) | 8 (100%) |
| November 2024 | 24 (77.4%) | 7 (22.6%) | 31 (100%) |
| December 2024 | 37 (50.0%) | 37 (50.0%) | 74 (100%) |
| January 2025 | 46 (51.1%) | 44 (48.9%) | 90 (100%) |
| February 2025 | 14 (29.2%) | 34 (70.8%) | 48 (100%) |
| **Total sequenced** | **126 (49.8%)** | **127 (50.2%)** | **253 (100%)** |

**Note:** There were no specimens successfully sequenced for March 2025. Results may not be representative of Ontario overall. Month was assigned based on the earliest date available for the specimen.

**Figure 1: Number of RSV Positive Specimens by Genetic Characterization and Month, Public Health Ontario, September 1, 2024 to March 1, 2025**




**Note:** There were no specimens successfully sequenced for March 2025. Results may not be representative of Ontario overall. Month was assigned based on the earliest date available for the specimen.

**Table 3: Number and Percentage of RSV Positive Specimens by Genetic Characterization, Public Health Ontario, September 1, 2024 to March 1, 2025**

| Genetic Characterization | Number Sequenced (Percentage) |
|---|---|
| **RSV-A genotype GA2.3.5** | **126 (49.8%)** |
| A.D.1 | 24 (9.5%) |
| A.D.1.4 | 9 (3.6%) |
| A.D.1.5 | 12 (4.7%) |
| A.D.3 | 32 (12.6%) |
| A.D.3.1 | 5 (2.0%) |
| A.D.3.2 | 1 (0.4%) |
| A.D.5.1 | 2 (0.8%) |
| A.D.5.2 | 41 (16.2%) |
| **RSV-B genotype GB5.0.5a** | **127 (50.2%)** |
| B.D.4.1.1 | 2 (0.8%) |
| B.D.E.1 | 125 (49.4%) |
| **Total sequenced** | **253 (100%)** |

**Note:** Results may not be representative of Ontario overall. Date was assigned based on the earliest date available for the specimen.

**Table 4: Number and Percentage of RSV Positive Specimens by Genetic Characterization and Age Group, Public Health Ontario, September 1, 2024 to March 1, 2025**

| Genetic Characterization | Less than 1 Year | 1–4 Years | 5–19 Years | 20–64 Years | 65 Years and Over | Total |
|---|---|---|---|---|---|---|
| **RSV-A genotype GA2.3.5** | **21 (65.6%)** | **23 (76.7%)** | **3 (100%)** | **12 (60.0%)** | **67 (39.9%)** | **126 (49.8%)** |
| A.D.1 | 5 (15.6%) | 4 (13.3%) | 0 (0.0%) | 6 (30.0%) | 9 (5.4%) | 24 (9.5%) |
| A.D.1.4 | 1 (3.1%) | 0 (0.0%) | 1 (33.3%) | 2 (10.0%) | 5 (3.0%) | 9 (3.6%) |
| A.D.1.5 | 0 (0.0%) | 4 (13.3%) | 0 (0.0%) | 1 (5.0%) | 7 (4.2%) | 12 (4.7%) |
| A.D.3 | 4 (12.5%) | 5 (16.7%) | 0 (0.0%) | 2 (10.0%) | 21 (12.5%) | 32 (12.6%) |
| A.D.3.1 | 0 (0.0%) | 1 (3.3%) | 0 (0.0%) | 1 (5.0%) | 3 (1.8%) | 5 (2.0%) |
| A.D.3.2 | 1 (3.1%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 1 (0.4%) |
| A.D.5.1 | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 2 (1.2%) | 2 (0.8%) |
| A.D.5.2 | 10 (31.2%) | 9 (30.0%) | 2 (66.7%) | 0 (0.0%) | 20 (11.9%) | 41 (16.2%) |
| **RSV-B genotype GB5.0.5a** | **11 (34.4%)** | **7 (23.3%)** | **0 (0.0%)** | **8 (40.0%)** | **101 (60.1%)** | **127 (50.2%)** |
| B.D.4.1.1 | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 2 (1.2%) | 2 (0.8%) |
| B.D.E.1 | 11 (34.4%) | 7 (23.3%) | 0 (0.0%) | 8 (40.0%) | 99 (58.9%) | 125 (49.4%) |
| **Total sequenced** | **32 (100%)** | **30 (100%)** | **3 (100%)** | **20 (100%)** | **168 (100%)** | **253 (100%)** |

**Note:** Results may not be representative of Ontario overall. Age was assigned based on the birth date provided on the test requisition.

**Table 5: Number and Percentage of RSV Positive Specimens by Genetic Characterization and Setting, Public Health Ontario, September 1, 2024 to March 1, 2025**

| Genetic Characterization | Intensive Care Unit | Hospital/Emergency Department | Congregate Living | Ambulatory or No Setting Reported | Total |
|---|---|---|---|---|---|
| **RSV-A genotype GA2.3.5** | **0 (0.0%)** | **55 (67.9%)** | **56 (44.4%)** | **15 (34.9%)** | **126 (49.8%)** |
| A.D.1 | 0 (0.0%) | 10 (12.3%) | 10 (7.9%) | 4 (9.3%) | 24 (9.5%) |
| A.D.1.4 | 0 (0.0%) | 4 (4.9%) | 5 (4.0%) | 0 (0.0%) | 9 (3.6%) |
| A.D.1.5 | 0 (0.0%) | 5 (6.2%) | 6 (4.8%) | 1 (2.3%) | 12 (4.7%) |
| A.D.3 | 0 (0.0%) | 12 (14.8%) | 16 (12.7%) | 4 (9.3%) | 32 (12.6%) |
| A.D.3.1 | 0 (0.0%) | 1 (1.2%) | 4 (3.2%) | 0 (0.0%) | 5 (2.0%) |
| A.D.3.2 | 0 (0.0%) | 1 (1.2%) | 0 (0.0%) | 0 (0.0%) | 1 (0.4%) |
| A.D.5.1 | 0 (0.0%) | 0 (0.0%) | 1 (0.8%) | 1 (2.3%) | 2 (0.8%) |
| A.D.5.2 | 0 (0.0%) | 22 (27.2%) | 14 (11.1%) | 5 (11.6%) | 41 (16.2%) |
| **RSV-B genotype GB5.0.5a** | **3 (100%)** | **26 (32.1%)** | **70 (55.6%)** | **28 (65.1%)** | **127 (50.2%)** |
| B.D.4.1.1 | 0 (0.0%) | 0 (0.0%) | 2 (1.6%) | 0 (0.0%) | 2 (0.8%) |
| B.D.E.1 | 3 (100%) | 26 (32.1%) | 68 (54.0%) | 28 (65.1%) | 125 (49.4%) |
| **Total sequenced** | **3 (100%)** | **81 (100%)** | **126 (100%)** | **43 (100%)** | **253 (100%)** |

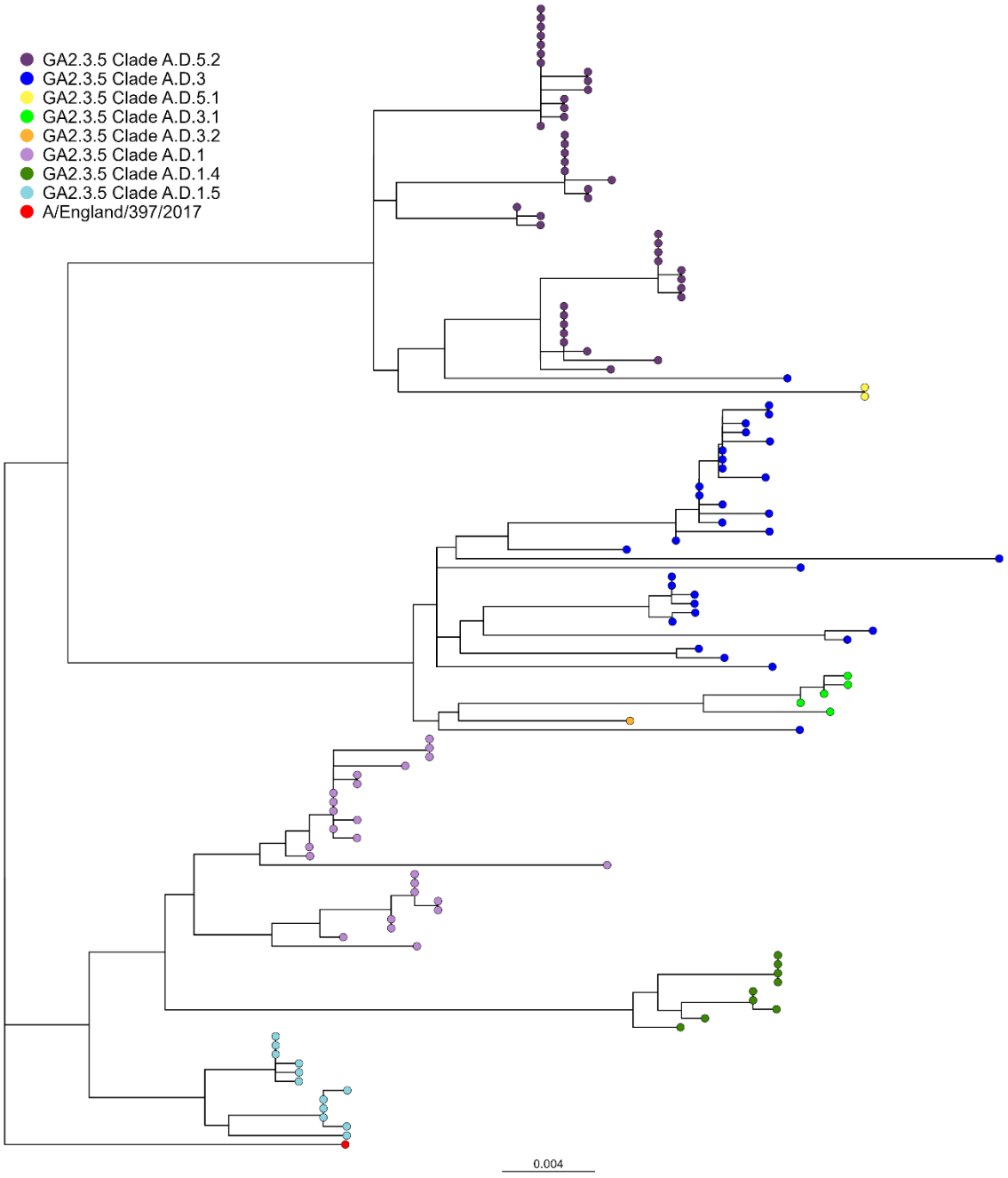
**Note:** Results may not be representative of Ontario overall. Setting represents the health care facility at which an individual received care. Congregate living includes long-term care homes, retirement homes, correctional facilities, and undefined institutions (excluding hospitals). Only one specimen per outbreak was eligible for sequencing. Approximately 11% of specimens are missing information on setting and are grouped into 'Ambulatory or no setting reported' category.

**Table 6: Number and Percentage of RSV Positive Specimens by Genetic Characterization and Region, Public Health Ontario, September 1, 2024 to March 1, 2025**

| Genetic Characterization | Northern | Eastern | Central East | Toronto | South West | Central West | Total |
|---|---|---|---|---|---|---|---|
| **RSV-A genotype GA2.3.5** | **10 (66.7%)** | **2 (6.7%)** | **57 (69.5%)** | **19 (44.2%)** | **13 (44.8%)** | **25 (46.3%)** | **126 (49.8%)** |
| A.D.1 | 7 (46.7%) | 1 (3.3%) | 4 (4.9%) | 3 (7.0%) | 1 (3.4%) | 8 (14.8%) | 24 (9.5%) |
| A.D.1.4 | 0 (0.0%) | 0 (0.0%) | 2 (2.4%) | 2 (4.7%) | 1 (3.4%) | 4 (7.4%) | 9 (3.6%) |
| A.D.1.5 | 0 (0.0%) | 0 (0.0%) | 2 (2.4%) | 3 (7.0%) | 4 (13.8%) | 3 (5.6%) | 12 (4.7%) |
| A.D.3 | 1 (6.7%) | 1 (3.3%) | 16 (19.5%) | 4 (9.3%) | 5 (17.2%) | 5 (9.3%) | 32 (12.6%) |
| A.D.3.1 | 0 (0.0%) | 0 (0.0%) | 2 (2.4%) | 3 (7.0%) | 0 (0.0%) | 0 (0.0%) | 5 (2.0%) |
| A.D.3.2 | 0 (0.0%) | 0 (0.0%) | 1 (1.2%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 1 (0.4%) |
| A.D.5.1 | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 1 (3.4%) | 1 (1.9%) | 2 (0.8%) |
| A.D.5.2 | 2 (13.3%) | 0 (0.0%) | 30 (36.6%) | 4 (9.3%) | 1 (3.4%) | 4 (7.4%) | 41 (16.2%) |
| **RSV-B genotype GB5.0.5a** | **5 (33.3%)** | **28 (93.3%)** | **25 (30.5%)** | **24 (55.8%)** | **16 (55.2%)** | **29 (53.7%)** | **127 (50.2%)** |
| B.D.4.1.1 | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 2 (3.7%) | 2 (0.8%) |
| B.D.E.1 | 5 (33.3%) | 28 (93.3%) | 25 (30.5%) | 24 (55.8%) | 16 (55.2%) | 27 (50.0%) | 125 (49.4%) |
| **Total sequenced** | **15 (100%)** | **30 (100%)** | **82 (100%)** | **43 (100%)** | **29 (100%)** | **54 (100%)** | **253 (100%)** |

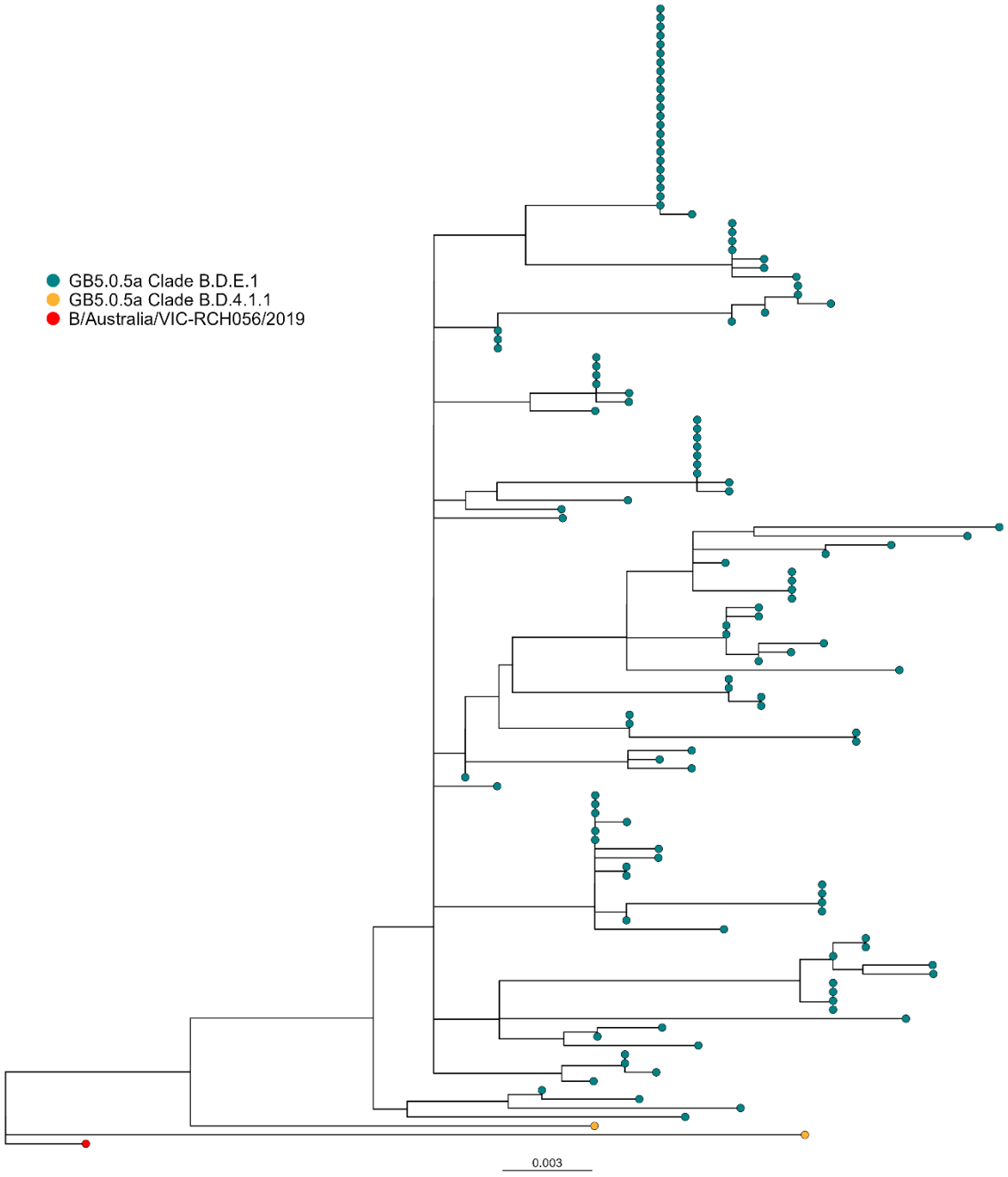
**Note:** Results may not be representative of Ontario overall. Region was assigned using patient address when available. If missing, region was assigned using submitter address. For additional information on which public health units are included in each region, see Technical Notes.

**Figure 2a: Phylogenetic Tree of RSV-A Genotype GA2.3.5 Specimens, Public Health Ontario, September 1, 2024 to March 1, 2025**




**Note:** Each circle represents a separate specimen and colour indicates the G-gene clade. 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).

**Figure 2b: Phylogenetic Tree of RSV-B Genotype GB5.0.5a Specimens, Public Health Ontario, September 1, 2024 to March 1, 2025**




**Note:** Each circle represents a separate specimen and colour indicates the G-gene clade. 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).

# Technical Notes

## Data Sources

**Public Health Ontario**

- Data were extracted from the PHO Laboratory Information Management System on March 26, 2025 at approximately 12:00pm.
- Bioinformatics processing of data by the Biocomputing Centre were completed on June 4, 2025 at approximately 12:00pm.

## Public Health Ontario's RSV Whole Genome Sequencing Strategy

- Public Health Ontario used random sampling to select 320 eligible RSV PCR positive specimens for whole genome sequencing. Of the 320 randomly selected, 272 had sufficient volume for WGS, 268 were subtyped to 136 (50.7%) RSV-A or 132 (49.3%) RSV-B. Of the specimens that were successfully sequenced (including supplemental Sanger sequencing, where applicable), 126 (49.8%) were RSV-A and 127 (50.2%) were RSV-B.
- Specimens were eligible for WGS 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).[10-13] Clade was assigned with Nextclade (3.15.0) analysis.[14] Phylogenetic tree was created using IQ-TREE (2.2.3).[15]

## 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, inpatient, and Intensive or Critical Care Unit), symptomatic residents in institutional settings (non-outbreak), and specimens from the first four symptomatic patients/residents in an outbreak setting that request respiratory virus testing.
- PHO laboratory performs FLUVID PCR on symptomatic healthcare workers/staff in the institutional setting in an outbreak setting requesting COVID-19 and respiratory virus testing or residents after the first four that have been tested for COVID-19 and MRVP. Additionally, FLUVID PCR is performed on symptomatic adult individuals seen in the Emergency Department who are at risk for severe illness or outcome and for whom care or treatment decisions may be impacted by test results.
- Individuals attending physician offices that are part of the Sentinel Practitioner Surveillance Network (SPSN).[9] 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 21.8% of RSV testing in Ontario. Further, only 8.4% 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.
- The proportion of specimens that were RSV-A is slightly underestimated compared to RSV-B as fewer RSV-A specimens were successfully sequenced, 126/136 (92.7%) for RSV-A and 127/132 (96.2%) for RSV-B.
- 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, Algoma Public Health, Public Health Sudbury & Districts, Northeastern 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, and South East Health Unit.
    - Central East region included Haliburton Kawartha Northumberland Peterborough Health Unit, 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, City of Hamilton Public Health Services, Wellington-Dufferin-Guelph Public Health, Region of Waterloo Public Health and Emergency Services, and Grand Erie Health Unit.
- Age was assigned based on the birth date provided and the specimen collection or login date.
- Patient setting is missing for approximately 11% of specimens. Therefore, results by patient setting should be interpreted with caution.

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## Citation

Ontario Agency for Health Protection and Promotion (Public Health Ontario). Respiratory syncytial virus genomic surveillance in Ontario. Toronto, ON: King's Printer for Ontario; 2025.

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