Public Health Ontario | Santé publique Ontario

SURVEILLANCE REPORT

Influenza Genomic Surveillance in Ontario: 2024–25 Season

Published: August 2025

Introduction

This report summarizes the results of influenza whole genome sequencing completed by Public Health Ontario (PHO) for the 2024–25 influenza season. The 2024–25 early season report and 2023–24 season report can be found on PHO's website.

Highlights

- A total of 374 specimens were included for the current season (September 1, 2024 to May 17, 2025), representing 1.8% of specimens that tested positive at PHO.
- Of the 368 influenza A specimens sequenced from the current season, 74.2% were H1N1pdm09 and 25.8% were seasonal H3N2.
  - 175 specimens (47.6%) were H1N1pdm09 genetic subclade 6B.1A.5a.2a and 98 specimens (26.6%) were genetic subclade 6B.1A.5a.2a.1. The H1N1pdm09 component of the current Northern Hemisphere influenza vaccine belongs to the genetic subclade 6B.1A.5a.2a.1.
  - 95 specimens (25.8%) were H3N2 genetic subclade 3C.2a1b.2a.2a.3a.1. The H3N2 component of the current Northern Hemisphere influenza vaccine belongs to the same genetic subclade 3C.2a1b.2a.2a.3a.1.
- All six influenza B specimens sequenced from the current season were Victoria genetic subclade V1A.3a.2. The Victoria component of the current Northern Hemisphere influenza vaccine belongs to the same genetic subclade V1A.3a.2.
- Of the H1N1pdm09 specimens sequenced, one specimen (0.4%) of subclade 6B.1A.5a.2a had the H275Y amino acid substitution in the neuraminidase (NA) gene known to be associated with resistance to oseltamivir.
- Of the 249 specimens sequenced that met quality control criteria for the polymerase acidic (PA) gene, none had the I38T amino acid substitution in the PA gene known to be associated with resistance to baloxavir.

Background

There are two types of influenza viruses (influenza A and B) that are responsible for most human cases during the influenza season. Influenza A can be further classified into subtypes (e.g., H1N1pdm09, seasonal H3N2) and influenza B can be further classified into lineages (e.g., Yamagata, Victoria). As influenza spreads through populations, changes can occur to the virus' genome.
The accumulation of these changes or mutations can result in new subdivisions beyond subtypes or lineages called clades and subclades. Although many subclades will have no differences in the ability to cause disease, some may have mutations that affect virulence, transmissibility, or allow the virus to escape natural or vaccine-induced immunity. Genomic surveillance uses whole genome sequencing 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, assess whether antivirals are working against the currently circulating viruses, and advise on vaccine strains for the upcoming seasons.[1] For the 2024–2025 influenza season, publicly funded vaccines available in Ontario are trivalent (influenza A H1N1pdm09 subclade 6B.1A.5a.2a.1, influenza A H3N2 subclade 3C.2a1b.2a.2a.3a.1, and influenza B Victoria subclade V1A.3a.2) and quadrivalent (addition of influenza B Yamagata subclade Y3) inactivated vaccines.[2-5]

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, etc.).
- Symptomatic individuals associated with an outbreak investigation.
- Hospitalized individuals, including those in intensive care.
- Symptomatic individuals, <18 years old, who receive care in an emergency department.[6]
- Individuals attending physician offices that are part of the Sentinel Practitioner Surveillance Network (see Technical Notes for additional information).[7]

PHO also subtypes influenza A specimens initially tested by other laboratories when requested and for enhanced H5N1 surveillance. As a result, PHO accumulated 52% of all influenza A positive specimens in Ontario available for sample selection.

To understand the diversity of the viruses circulating during the 2024–25 influenza season, PHO sequenced eligible specimens (Ct ≤ 27 and sufficient volume remaining) positive for influenza in the early 2024–25 season. This excludes specimens that are positive for more than one virus. Additionally, only the first positive specimen from an outbreak is selected for whole genome sequencing. Sequences are processed using bioinformatics analyses and are assigned subtypes, lineages, clades, and subclades.

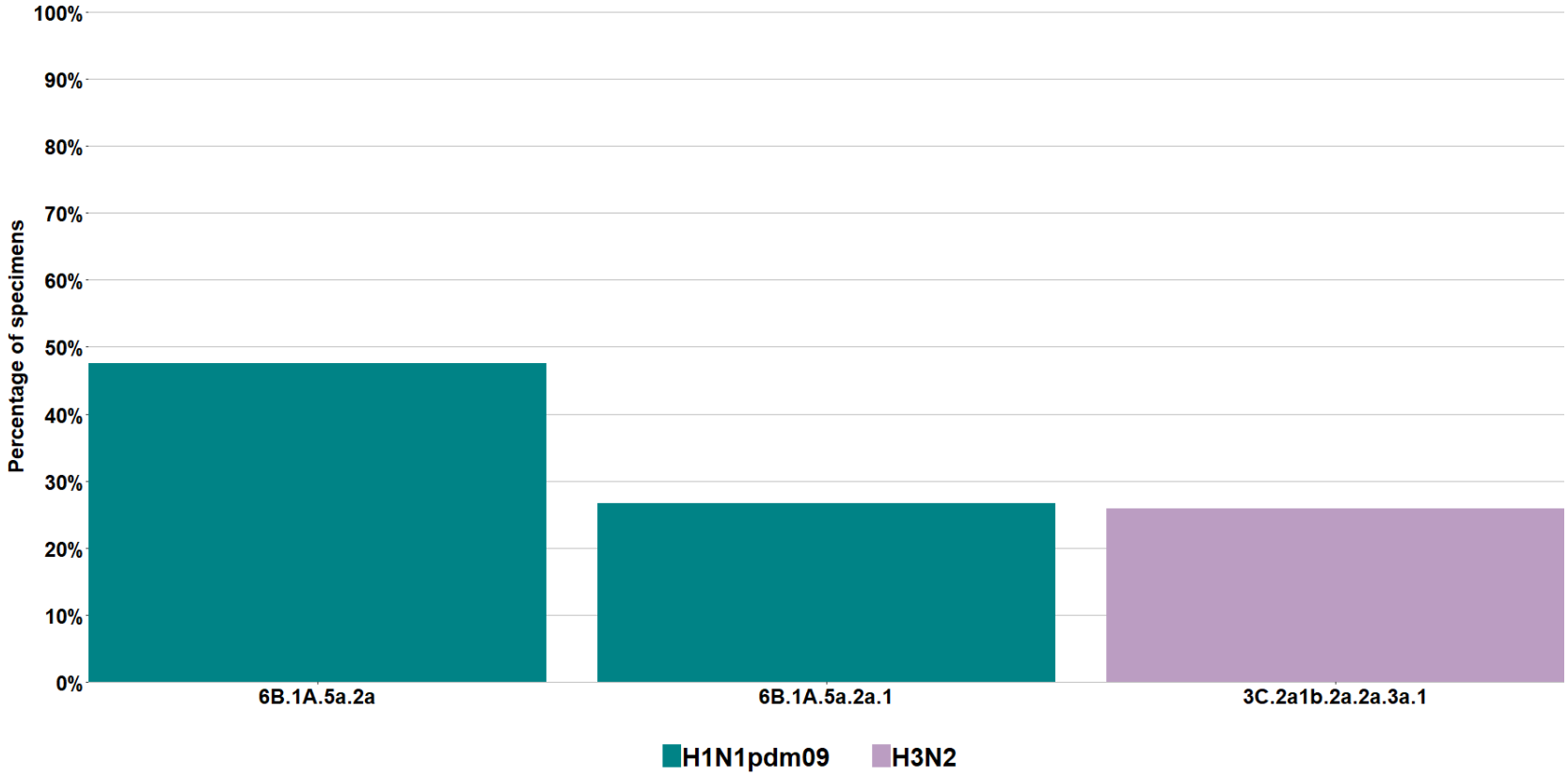
Results

Table 1a: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	2024–25 Season (September 1, 2024 – May 17, 2025)
H1N1pdm09	**273 (74.2%)**
6B.1A.5a.2a	175 (47.6%)
6B.1A.5a.2a.1	98 (26.6%)
H3N2	**95 (25.8%)**
3C.2a1b.2a.2a.3a.1	95 (25.8%)
Total sequenced	**368 (100%)**

Note: Results may not be representative of Ontario overall. Date was assigned based on the earliest date available for the specimen. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade 6B.1A.5a.2a.1, influenza A H3N2 subclade 3C.2a1b.2a.2a.3a.1, and influenza B Victoria subclade V1A.3a.2, and influenza B Yamagata subclade Y3.[4-5]

Figure 1: Percentage of Positive Influenza A Specimens, by Genetic Characterization, Public Health Ontario, September 1, 2024 to May 17, 2025



Note: The genetic subclades included in this seasons' influenza vaccine are influenza A H1N1pdm09 subclade 6B.1A.5a.2a.1, influenza A H3N2 subclade 3C.2a1b.2a.2a.3a.1, and influenza B Victoria subclade V1A.3a.2, and influenza B Yamagata subclade Y3.[4-5] Results may not be representative of Ontario overall.

Table 1b: Number and Percentage of Positive Influenza B Specimens, by Genetic Characterization, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	2024–25 Season (September 1, 2024 – May 17, 2025)
Victoria	**6 (100%)**
V1A.3a.2	6 (100%)
Total sequenced	**6 (100%)**

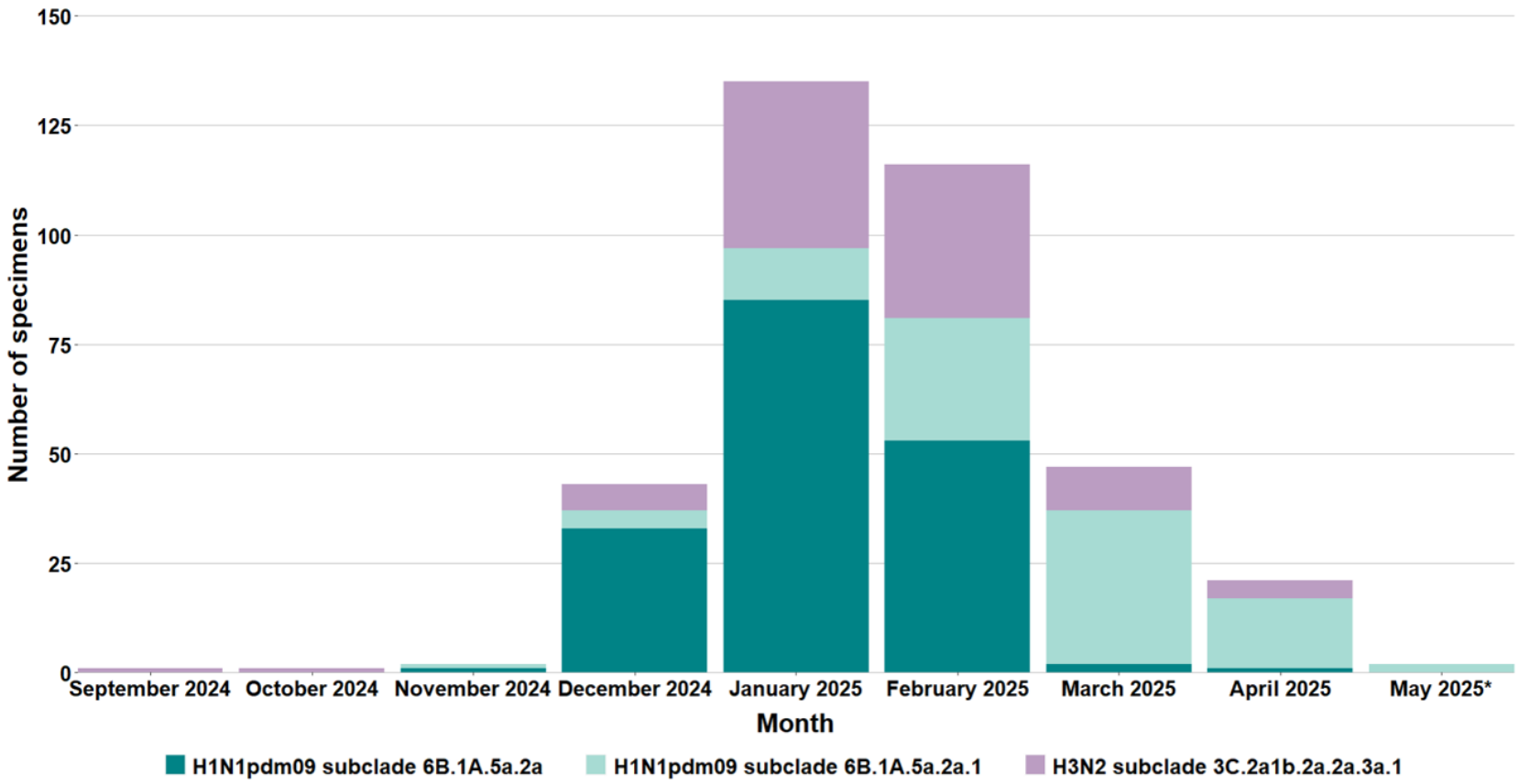
Note: Results may not be representative of Ontario overall. Date was assigned based on the earliest date available for the specimen. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade 6B.1A.5a.2a.1, influenza A H3N2 subclade 3C.2a1b.2a.2a.3a.1, and influenza B Victoria subclade V1A.3a.2, and influenza B Yamagata subclade Y3.[4-5]

Table 2: Number of Positive Influenza Specimens, Number and Percentage Sequenced, Public Health Ontario, September 1, 2024 to May 17, 2025

Month	Number of Positive Specimens	Number Sequenced	Percentage Sequenced
September 2024	39	1	2.6%
October 2024	95	1	1.1%
November 2024	183	2	1.1%
December 2024	2,798	43	1.5%
January 2025	6,616	135	2.0%
February 2025	6,275	118	1.9%
March 2025	3,174	49	1.5%
April 2025	1,168	23	2.0%
May 2025*	172	2	1.2%
Total	**20,520**	**374**	**1.8%**

Note: *May 2025 is a partial month. Of the 374 specimens sequenced, 5.6% (21/374) 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.

Figure 2: Number of Positive Influenza A Specimens Sequenced, by Genetic Characterization and Month, Public Health Ontario, September 1, 2024 to May 17, 2025



Note: *May 2025 is a partial month. Results may not be representative of Ontario overall. The genetic subclades included in this seasons' influenza vaccine are influenza A H1N1pdm09 subclade 6B.1A.5a.2a.1, influenza A H3N2 subclade 3C.2a1b.2a.2a.3a.1, and influenza B Victoria subclade V1A.3a.2, and influenza B Yamagata subclade Y3.[4-5] Month was assigned based on earliest date available for a specimen.

Table 3a: Number and Percentage of Positive Influenza A H1N1pdm09 Specimens with Any Antigenic Site Amino Acid Substitutions, by Genetic Characterization, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	HA Antigenic Site Ca	HA Antigenic Site Cb	HA Antigenic Site Sa	HA Antigenic Site Sb	Total
H1N1pdm09	**98.9% (270/273)**	**5.1% (14/273)**	**2.2% (6/273)**	**0.4% (1/273)**	**99.3% (271/273)**
6B.1A.5a.2a	99.4% (174/175)	8.0% (14/175)	2.3% (4/175)	0.6% (1/175)	100% (175/175)
6B.1A.5a.2a.1	98.0% (96/98)	0.0% (0/98)	2.0% (2/98)	0.0% (0/98)	98.0% (96/98)
Total sequenced	**98.9% (270/273)**	**5.1% (14/273)**	**2.2% (6/273)**	**0.4% (1/273)**	**99.3% (271/273)**

Note: The effect of the identified antigenic site substitutions on vaccine-induced immunity is unknown. This data is exploratory in nature and should be interpreted with caution. This data should not be used to directly inform clinical decisions or infer impacts on vaccine-induced immunity. See Technical Notes for details. Antigenic site amino acid substitutions were identified relative to the strain included in the 2024–25 influenza vaccine (influenza A H1N1pdm09 subclade 6B.1A.5a.2a.1). Sequenced viruses may have substitutions at more than one position within the antigenic site. Antigenic site Ca includes substitutions at positions 137, 139, 142, 168, 169, 170, 205, 223, 271 of the hemagglutinin (HA) protein. Antigenic site Cb includes substitutions at positions 69, 70, 72, 73, 75, 112 of the HA protein. Antigenic site Sa includes substitutions at positions 121, 125, 155, 157, 158, 161 of the HA protein. Antigenic site Sb includes substitutions at position 190 of the HA protein.

Table 3b: Number and Percentage of Positive Influenza A H3N2 Specimens with Any Antigenic Site Amino Acid Substitutions, by Genetic Characterization, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	HA Antigenic Site A	HA Antigenic Site B	HA Antigenic Site C	HA Antigenic Site D	HA Antigenic Site E	Total
H3N2	**100% (95/95)**	**12.6% (12/95)**	**98.9% (94/95)**	**98.9% (94/95)**	**9.5% (9/95)**	**100% (95/95)**
3C.2a1b.2a.2a.3a.1	100% (95/95)	12.6% (12/95)	98.9% (94/95)	98.9% (94/95)	9.5% (9/95)	100% (95/95)
Total sequenced	**100% (95/95)**	**12.6% (12/95)**	**98.9% (94/95)**	**98.9% (94/95)**	**9.5% (9/95)**	**100% (95/95)**

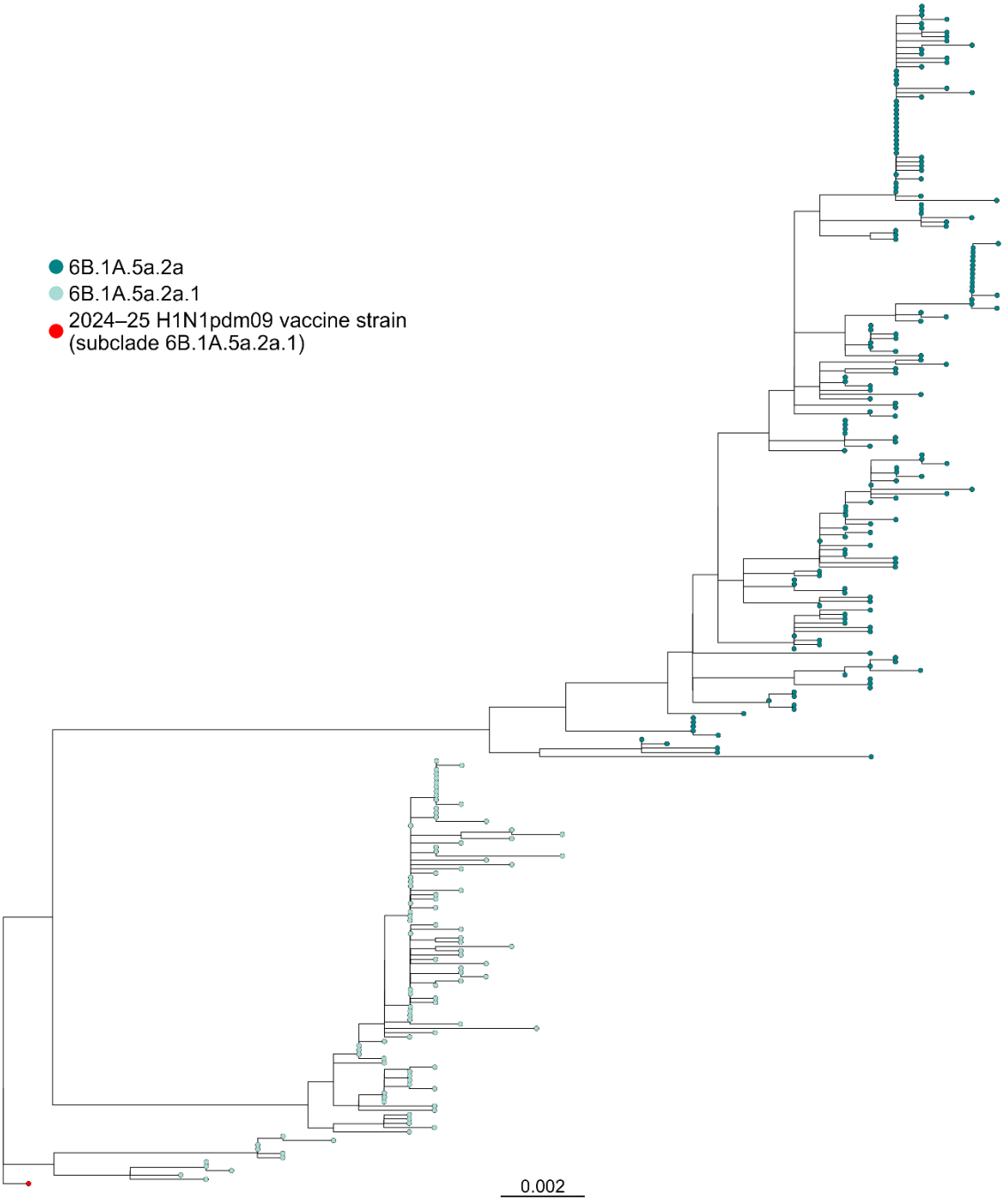
Note: The effect of the identified antigenic site substitutions on vaccine-induced immunity is unknown. This data is exploratory in nature and should be interpreted with caution. This data should not be used to directly inform clinical decisions. See Technical Notes for details. Antigenic site amino acid substitutions were identified relative to the strain included in the 2024–25 influenza vaccine (influenza A H3N2 subclade 3C.2a1b.2a.2a.3a.1). Sequenced viruses may have substitutions at more than one position within the antigenic site. Antigenic site A includes substitutions at positions 122, 124, 135, 260, 261, 265 of the HA protein. Antigenic site B includes substitutions at positions 158, 159, 160, 189 of the HA protein. Antigenic site C includes substitutions at positions 50, 273, 276, 278, 280 of the HA protein. Antigenic site D includes substitutions at positions 96, 121, 173, 182, 203, 207, 212, 214, 242 of the HA protein. Antigenic site E includes substitutions at positions 63, 78, 83, 91, 94 of the HA protein.

Table 3c: Number and Percentage of Positive Influenza B Victoria Specimens with Any Antigenic Site Amino Acid Substitutions, by Genetic Characterization, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	120-loop	150-loop	160-loop	190-helix	Total
Victoria	**100% (6/6)**	**0.0% (0/6)**	**0.0% (0/6)**	**100% (6/6)**	**100% (6/6)**
V1A.3a.2	100% (6/6)	0.0% (0/6)	0.0% (0/6)	100% (6/6)	100% (6/6)
Total sequenced	**100% (6/6)**	**0.0% (0/6)**	**0.0% (0/6)**	**100% (6/6)**	**100% (6/6)**

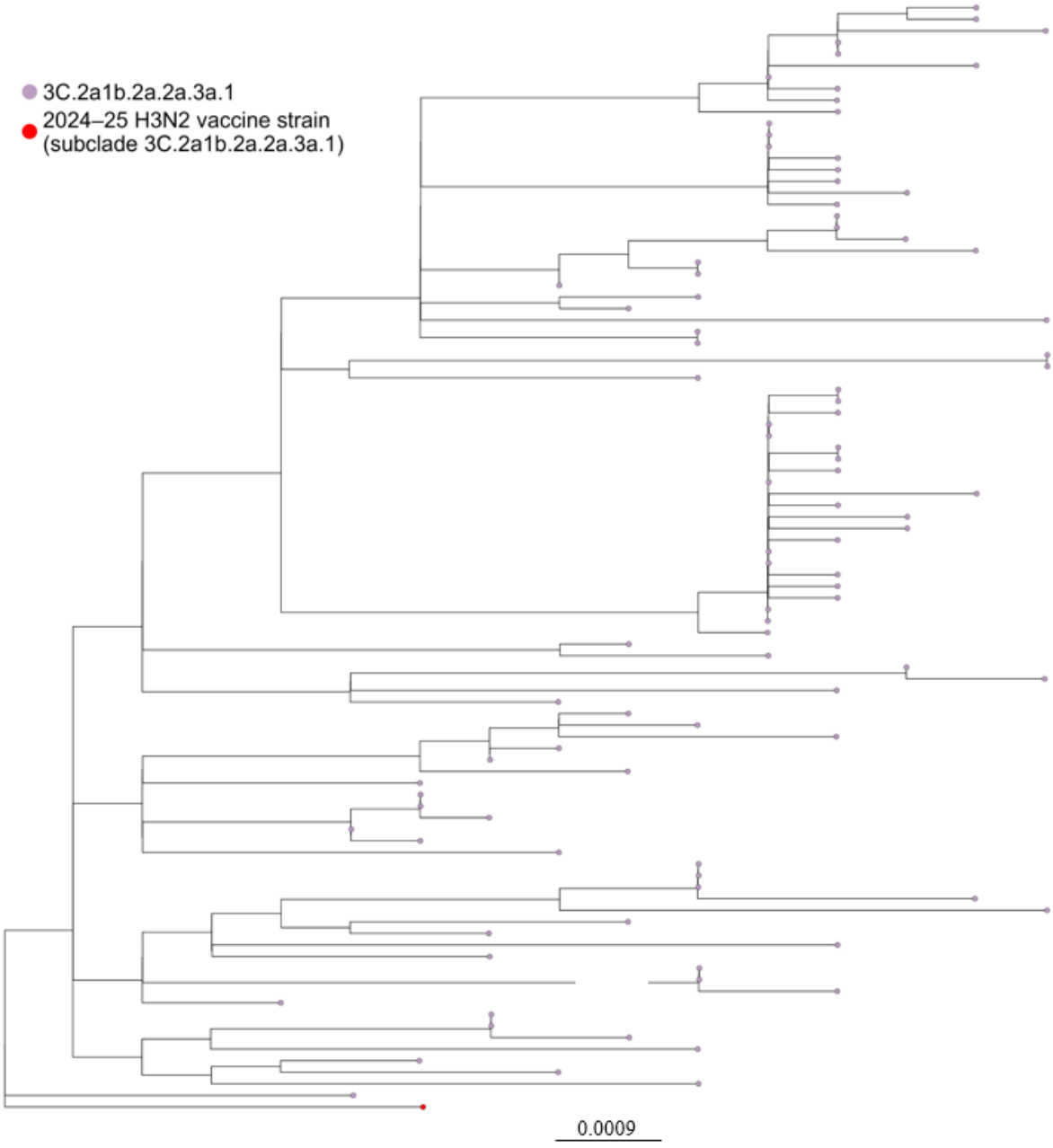
Note: The effect of the identified antigenic site substitutions on vaccine-induced immunity is unknown. This data is exploratory in nature and should be interpreted with caution. This data should not be used to directly inform clinical decisions. See Technical Notes for details. Antigenic site amino acid substitutions were identified relative to the strain included in the 2024–25 influenza vaccine (influenza B Victoria subclade V1A.3a.2). Sequenced viruses may have substitutions at more than one position within the antigenic site. Antigenic site 120-loop includes substitutions at positions 128 and 129 of the HA protein. Antigenic site 190-helix includes substitutions at positions 194 and 196 of the HA protein.

Figure 3a: Phylogenetic Tree of Positive Influenza A H1N1pdm09 Specimens, Public Health Ontario, September 1, 2024 to May 17, 2025



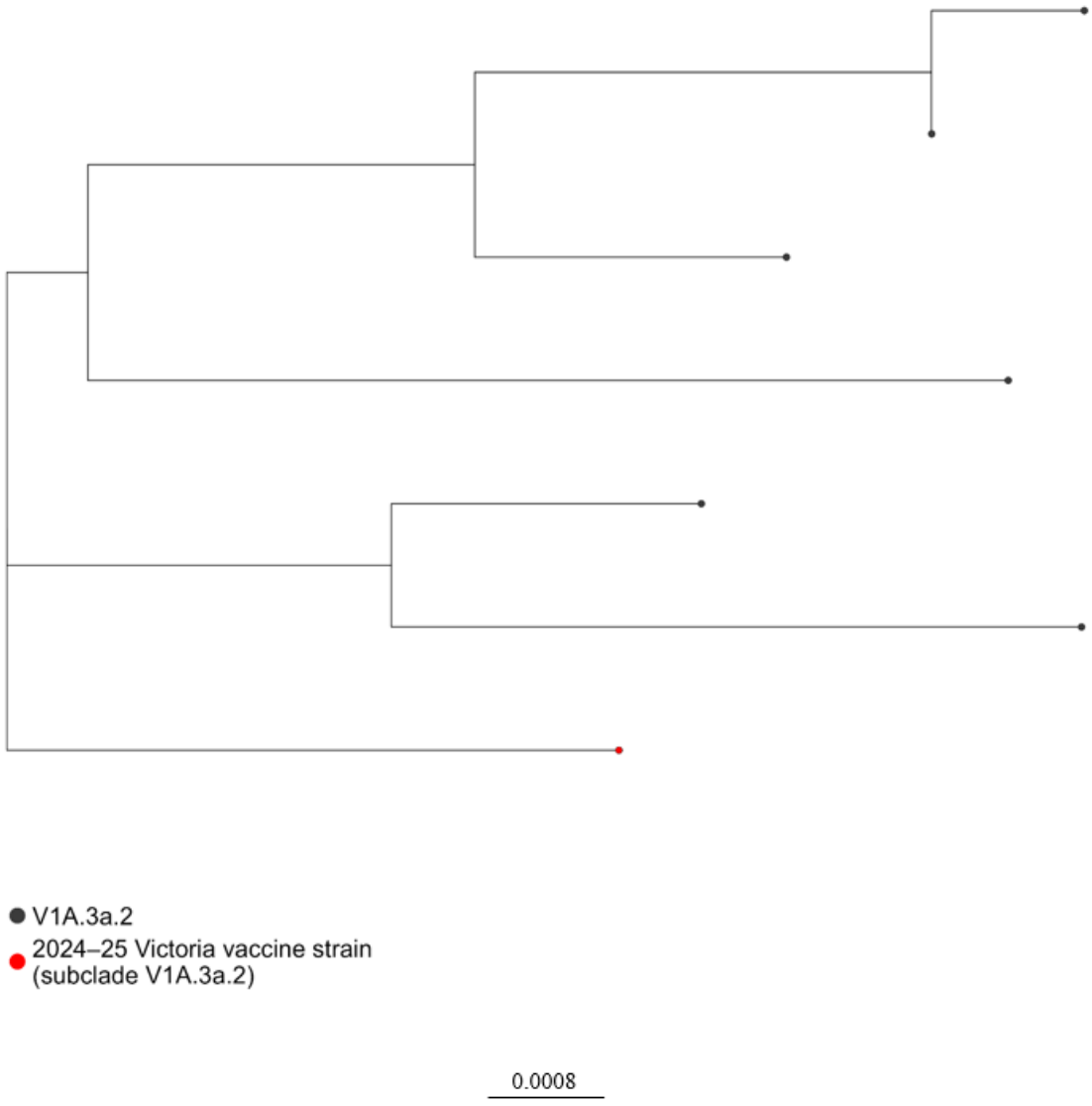
Note: Each circle represents a separate specimen. Results may not be representative of Ontario overall. The maximum likelihood phylogenetic tree was generated based on the HA region of the influenza genome using the IQ-TREE GTR model with 100 bootstrap replicates. Identical sequences are retained in the tree. The tree is rooted with the vaccine reference strain A/Victoria/4897/2022_H1N1_pdm09-like-virus (EPI_ISL_16714268).

Figure 3b: Phylogenetic Tree of Positive Influenza A H3N2 Specimens, Public Health Ontario, September 1, 2024 to May 17, 2025



Note: Each circle represents a separate specimen. Results may not be representative of Ontario overall. The maximum likelihood phylogenetic tree was generated HA region of the influenza genome using the IQ-TREE GTR model with 100 bootstrap replicates. Identical sequences are retained in the tree. The tree is rooted with the vaccine reference strain A/Thailand/8/2022_H3N2_like_virus (EPI_ISL_16014504).

Figure 3c: Phylogenetic Tree of Positive Influenza B Victoria Specimens, Public Health Ontario, September 1, 2024 to May 17, 2025



Note: Each circle represents a separate specimen. Results may not be representative of Ontario overall. The maximum likelihood phylogenetic tree was generated HA region of the influenza genome using the IQ-TREE GTR model with 100 bootstrap replicates. Identical sequences are retained in the tree. The tree is rooted with the vaccine reference strain B/Austria/1359417/2021_Victoria-like-virus (EPI_ISL_1519459).

Table 4: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Age Group, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	0–4 Years	5–19 Years	20–64 Years	65 Years and Over	Total
H1N1pdm09	**49 (79.0%)**	**20 (54.1%)**	**63 (68.5%)**	**141 (79.7%)**	**273 (74.2%)**
6B.1A.5a.2a	38 (61.3%)	17 (45.9%)	48 (52.2%)	72 (40.7%)	175 (47.6%)
6B.1A.5a.2a.1	11 (17.7%)	3 (8.1%)	15 (16.3%)	69 (39.0%)	98 (26.6%)
H3N2	**13 (21.0%)**	**17 (45.9%)**	**29 (31.5%)**	**36 (20.3%)**	**95 (25.8%)**
3C.2a1b.2a.2a.3a.1	13 (21.0%)	17 (45.9%)	29 (31.5%)	36 (20.3%)	95 (25.8%)
Total sequenced	**62 (100%)**	**37 (100%)**	**92 (100%)**	**177 (100%)**	**368 (100%)**

Note: Results may not be representative of Ontario overall. Age was assigned based on the birth date provided; excludes specimens with missing birth dates. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade 6B.1A.5a.2a.1, influenza A H3N2 subclade 3C.2a1b.2a.2a.3a.1, and influenza B Victoria subclade V1A.3a.2, and influenza B Yamagata subclade Y3.[4-5]

Table 5: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Setting, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	Intensive Care Unit	Hospital/ Emergency Department	Congregate Living	Ambulatory or No Setting Reported	Total
H1N1pdm09	**3 (60.0%)**	**133 (67.9%)**	**42 (85.7%)**	**95 (80.5%)**	**273 (74.2%)**
6B.1A.5a.2a	2 (40.0%)	98 (50.0%)	20 (40.8%)	55 (46.6%)	175 (47.6%)
6B.1A.5a.2a.1	1 (20.0%)	35 (17.9%)	22 (44.9%)	40 (33.9%)	98 (26.6%)
H3N2	**2 (40.0%)**	**63 (32.1%)**	**7 (14.3%)**	**23 (19.5%)**	**95 (25.8%)**
3C.2a1b.2a.2a.3a.1	2 (40.0%)	63 (32.1%)	7 (14.3%)	23 (19.5%)	95 (25.8%)
Total sequenced	**5 (100%)**	**196 (100%)**	**49 (100%)**	**118 (100%)**	**368 (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 selected for sequencing. Approximately 27% of influenza A specimens are missing information on setting and are grouped into 'Ambulatory or no setting reported' category. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade 6B.1A.5a.2a.1, influenza A H3N2 subclade 3C.2a1b.2a.2a.3a.1, and influenza B Victoria subclade V1A.3a.2, and influenza B Yamagata subclade Y3.[4-5]

Table 6: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Region, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	Northern	Eastern	Central East	Toronto	South West	Central West	Total
H1N1pdm09	**13 (92.9%)**	**19 (95.0%)**	**72 (69.9%)**	**41 (68.3%)**	**53 (82.8%)**	**75 (70.1%)**	**273 (74.2%)**
6B.1A.5a.2a	6 (42.9%)	10 (50.0%)	51 (49.5%)	27 (45.0%)	28 (43.8%)	53 (49.5%)	175 (47.6%)
6B.1A.5a.2a.1	7 (50.0%)	9 (45.0%)	21 (20.4%)	14 (23.3%)	25 (39.1%)	22 (20.6%)	98 (26.6%)
H3N2	**1 (7.1%)**	**1 (5.0%)**	**31 (30.1%)**	**19 (31.7%)**	**11 (17.2%)**	**32 (29.9%)**	**95 (25.8%)**
3C.2a1b.2a.2a.3a.1	1 (7.1%)	1 (5.0%)	31 (30.1%)	19 (31.7%)	11 (17.2%)	32 (29.9%)	95 (25.8%)
Total sequenced	**14 (100%)**	**20 (100%)**	**103 (100%)**	**60 (100%)**	**64 (100%)**	**107 (100%)**	**368 (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. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade 6B.1A.5a.2a.1, influenza A H3N2 subclade 3C.2a1b.2a.2a.3a.1, and influenza B Victoria subclade V1A.3a.2, and influenza B Yamagata subclade Y3.[4-5]

Table 7: Number and Percentage of Positive H1N1pdm09 Specimens with Amino Acid Substitution H275Y Associated with Oseltamivir Resistance, by Genetic Characterization, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	Amino Acid Substitution H275Y
H1N1pdm09	**0.4% (1/273)**
6B.1A.5a.2a	0.6% (1/175)
6B.1A.5a.2a.1	0.0% (0/98)
Total sequenced	**0.4% (1/273)**

Note: H275Y substitution has been associated with oseltamivir resistance in influenza A H1N1pdm09 viruses.[8] This data is exploratory in nature and should be interpreted with caution. Antiviral resistance is determined by investigation at specific sites previously identified to confer resistance and does not account for all potential mechanisms of resistance. This data should not be used to directly inform clinical decisions. See Technical Notes for details.

Table 8a: Number and Percentage of Positive Influenza A Specimens with Amino Acid Substitution I38T Associated with Baloxavir Resistance, by Genetic Characterization, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	Amino Acid Substitution I38T
H1N1pdm09	**0.0% (0/151)**
6B.1A.5a.2a	0.0% (0/103)
6B.1A.5a.2a.1	0.0% (0/48)
H3N2	**0.0% (0/92)**
3C.2a1b.2a.2a.3a.1	0.0% (0/92)
Total sequenced	**0.0% (0/243)**

Note: Includes specimens with ≥90% coverage and 30x depth for the polymerase acidic gene. As such, the 'Total sequenced' does not align with other tables in the report. I38T substitution has been associated with baloxavir resistance in influenza A viruses.[9] This data is exploratory in nature and should be interpreted with caution. Antiviral resistance is determined by investigation at specific sites previously identified to confer resistance and does not account for all potential mechanisms of resistance. This data should not be used to directly inform clinical decisions. See Technical Notes for details.

Table 8b: Number and Percentage of Positive Influenza B Specimens with Amino Acid Substitution I38T Associated with Baloxavir Resistance, by Genetic Characterization, Public Health Ontario, September 1, 2024 to May 17, 2025

Genetic Characterization	Amino Acid Substitution I38T
Victoria	**0.0% (0/6)**
V1A.3a.2	0.0% (0/6)
Total sequenced	**0.0% (0/6)**

Note: Includes specimens with ≥90% coverage and 30x depth for the polymerase acidic gene. I38T substitution has been associated with baloxavir resistance in influenza B viruses.[9] This data is exploratory in nature and should be interpreted with caution. Antiviral resistance is determined by investigation at specific sites previously identified to confer resistance and does not account for all potential mechanisms of resistance. This data should not be used to directly inform clinical decisions. See Technical Notes for details.

Technical Notes

Data Sources

Public Health Ontario (PHO)

- Data were extracted from the PHO Laboratory Information Management System on May 22, 2025 at approximately 9:00 a.m.
- Bioinformatics processing of data by the Biocomputing Centre were completed on July 8, 2025 at approximately 2:00 pm.

Public Health Ontario's Influenza Whole Genome Sequencing Strategy

- Specimens were eligible for whole genome sequencing with the following criteria:
  - Real-time PCR Ct ≤ 27.
  - Upper respiratory specimens (e.g. nasopharyngeal or throat swabs).
  - Specimens were positive for only one influenza A subtype or influenza B and not any other viruses.
  - If outbreak related, only the first specimen from an outbreak was eligible.
  - Specimens were not tested as part of the Sentinel Practitioner Surveillance Network (SPSN).
- A random sample of eligible specimens were selected to obtain approximately 400 specimens with sufficient volume for sequencing from November 24, 2024 to May 17, 2025. Of the 400 specimens selected, 370 were successfully sequenced (≥90% coverage at 30x depth, and mean depth of ≥30 for both hemagglutinin and neuraminidase segments). This represents 1.8% of influenza positive specimens at PHO. The early season (September 1 to November 23, 2024) was subsampled to include a random sample of 1.8% of specimens as well. In total, 374 specimens were included in this report.
- Genetic characterization of specimens was completed using whole genome sequencing and analyzed by a bioinformatics pipeline using Fastp (0.23.2), CFIA-NCFAD/nf-flu (3.3.6), bwa (0.7.17), bedtools (2.31.0), bcftools (1.10), and emboss (6.6.0).[10-15] Clade was assigned with Nextclade (3.15.0) analysis.[16] Phylogenetic tree was created using IQ-TREE (2.2.3).[17]

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, which detects influenza A, influenza B, respiratory syncytial virus, and SARS-CoV-2. FLUVID is performed 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 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)[7] are tested by MRVP and are exempt from laboratory testing restrictions.

Testing Methods

- Testing for influenza at PHO is performed using:
  - A laboratory-developed multiplex respiratory virus PCR panel assay (MRVP). The assay includes 11 targets including influenza A, influenza A H1N1pdm09, influenza A seasonal H3N2, and influenza B.
  - FLUVID assay includes influenza A and B, as well as respiratory syncytial virus (RSV A/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.
  - A separate influenza A subtype real-time PCR assay. This assay is mainly used for influenza A positive specimens referred to PHO for subtype testing.

Antigenic Characterization

- Antigenic characterization of influenza viruses involves an investigation of key proteins present on the outer surface of the influenza virus that can stimulate an immune response in the infected host. The main antigenic sites are contained within proteins which are involved in the entry and release of viral particles in host cells (the hemagglutinin (HA) and neuraminidase (NA) proteins). Antibodies that bind to specific regions of these proteins can initiate recognition of the virus by the infected host cells.[17]
- Within a respiratory season, antigenic characterization (typing/matching) of circulating influenza viruses can be assessed by in vitro laboratory experiments that measure the strength of antibody responses and by sequence-based analysis of the viral genome. The similarity in genetic sequence can be used to determine the degree of relatedness between currently circulating influenza strains and those included in the recommended annual influenza vaccine.
- The data presented provides a summary of the mutations identified in the main antigenic sites relative to the influenza viruses in circulation at the time of this report. This data is exploratory in nature and should be interpreted with caution. The potential outcomes of the identified mutations on vaccine-induced immunity or antiviral response is unknown. This data should not be used to directly inform clinical decisions.

Antiviral Resistance

- Antiviral resistance was based on screening of genomic data for molecular markers of resistance as opposed to susceptibility testing.
- H275Y on the neuraminidase gene is considered a clinically relevant amino acid substitution associated with oseltamivir resistance in influenza A H1N1pdm09 viruses.[8] The effect of other substitutions (including those in H3N2 viruses) within this gene on oseltamivir resistance are not well described.
- I38T on the polymerase acidic (PA) gene is considered a clinically relevant amino acid substitution associated with baloxavir resistance in influenza A and influenza B viruses.[9] The effect of other substitutions within this gene on baloxavir resistance are not well described.

Data Caveats

This report is based on specimens tested at PHO and may not be representative of Ontario, as other hospitals and private laboratories also provide respiratory pathogen testing services. In addition, specimen selection for genetic characterization may not fully represent all patient settings across Ontario.

- Specimens available for this report represented 52% of all influenza A positive specimens in Ontario during this time period.
- PHO primarily tests individuals over 65 years of age and that reside in congregate living settings. For this reason, results may overrepresent older individuals and those residing in congregate living settings.
- During this respiratory season, specimens from hospitalized patients initially tested by other laboratories were sent to PHO for subtype testing due to enhanced H5N1 surveillance. For this reason, results may overrepresent hospitalized individuals.
- Additional biases may be introduced due to eligibility criteria for diagnostic testing, catchment area of PHO testing, the volume of specimen available, whole genome sequencing specimen selection criteria, and whether a specimen can be successfully sequenced. As a result, the results may not represent Ontario overall.
- 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.
- Geographic Regions:
    - Northern region includes Algoma Public Health, Northeastern Health Unit, Northwestern Health Unit, North Bay Parry Sound District Health Unit, Public Health Sudbury & Districts, and Thunder Bay District Health Unit;
    - Eastern includes Eastern Ontario Health Unit, Ottawa Public Health, Renfrew County and District Health Unit, and South East Health Unit;
    - Central East includes Durham Region Health Department, Haliburton Kawartha Northumberland Peterborough Health Unit, Peel Public Health, Simcoe Muskoka District Health Unit, and York Region Public Health;
    - Toronto includes Toronto Public Health;
    - South West includes Chatham-Kent Public Health, Grey Bruce Health Unit, Huron Perth Public Health, Lambton Public Health, Middlesex-London Health Unit, Southwestern Public Health, and Windsor-Essex County Health Unit;
    - Central West includes City of Hamilton Public Health Services, Grand Erie Health Unit, Halton Region Public Health, Niagara Region Public Health, Region of Waterloo Public Health and Emergency Services, and Wellington-Dufferin-Guelph Public Health.
- Age was assigned based on the birth date provided and the specimen collection or login date.
- Patient setting is missing for approximately 27% of influenza specimens. Therefore, results by patient setting should be interpreted with caution.

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Appendix A: Number of Positive Influenza A and B Specimens Tested at Public Health Ontario by Subtype

Table A1: Number and Percentage of Positive Influenza A and B Specimens, Number and Percentage Sequenced, by Subtype, Public Health Ontario, September 1, 2024 to May 17, 2025

Subtype	Number of Positive Specimens (Percentage)	Number Sequenced (Percentage)
Influenza A	**19,975 (97.3%)**	**368 (98.4%)**
H1N1pdm09	13,952 (68.0%)	273 (73.0%)
Seasonal H3N2	5,182 (25.3%)	95 (25.4%)
Unknown subtype	887 (4.3%)	0 (0.0%)
Influenza B	**551 (2.7%)**	**6 (1.6%)**
Total Positives	**20,520 (100%)***	**374 (100%)**

Notes: *Includes specimens that tested positive for both influenza A and influenza B (n=6); and specimens that tested positive for both H1N1pdm09 and seasonal H3N2 (n=41). 'Unknown subtype' refers to specimens that were unable to be assigned to a subtype or not subtyped. 'Number of Positive Specimens' includes positive specimens initially tested at PHO or positive specimens initially tested by other laboratories and sent to PHO for subtyping. 'Number Sequenced' includes positive specimens randomly selected for whole genome sequencing and included in this report.

Appendix B: Jurisdictional Comparison

Table B1: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Jurisdiction, August 26, 2024 to May 18, 2025

Genetic Characterization	Ontario (September 1 – May 17	Canada (September 1 – May 17)	United States of America (September 29 – May 17)	United Kingdom (August 26 – May 18)
H1N1pdm09	**273 (74.2%)**	**824 (67.9%)**	**1,722 (44.0%)**	**1,141 (76.6%)**
6B.1A.5a.2a	175 (47.6%)	325 (26.8%)	637 (16.3%)	1,042 (69.9%)
6B.1A.5a.2a.1	98 (26.6%)	499 (41.1%)	1,085 (27.7%)	99 (6.6%)
H3N2	**95 (25.8%)**	**390 (32.1%)**	**2,189 (56.0%)**	**349 (23.4%)**
3C.2a1b.2a.2a.3a	0 (0.0%)	0 (0.0%)	6 (0.2%)	2 (0.1%)
3C.2a1b.2a.2a.3a.1	95 (25.8%)	390 (32.1%)	2,183 (55.8%)	347 (23.3%)
Total sequenced	**368 (100%)**	**1,214 (100%)**	**3,911 (100%)**	**1,490 (100%)**

Notes: Prevalence may not be directly comparable across jurisdictions due to varying time periods and sampling strategies.
Data Sources: Public Health Ontario, Public Health Agency of Canada [4], Centres for Disease Control and Prevention[19], UK Health Security Agency [20]

Table B2: Number and Percentage of Positive Influenza B Specimens, by Genetic Characterization and Jurisdiction, August 26, 2024 to May 18, 2025

Genetic Characterization	Ontario (September 1 – May 17)	Canada (September 1 – May 17)	United States of America (September 29 – May 17)	United Kingdom (August 26 – May 18)
Victoria	**6 (100%)**	**142 (100%)**	**656 (100%)**	**850 (100%)**
V1A.3a.2	6 (100%)	142 (100%)	656 (100%)	850 (100%)
Total sequenced	**6 (100%)**	**142 (100%)**	**656 (100%)**	**850 (100%)**

Notes: Prevalence may not be directly comparable across jurisdictions due to varying time periods and sampling strategies.
Data Sources: Public Health Ontario, Public Health Agency of Canada [4], Centres for Disease Control and Prevention [19], UK Health Security Agency [20]

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