Public Health Ontario | Santé publique Ontario

SURVEILLANCE REPORT

Influenza Genomic Surveillance in Ontario: 2024–25 Early Season

Published: January 2025

Introduction

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

Highlights

- A total of 105 specimens were included for the current season (September 1, 2024 to November 23, 2024), this represents 44.9% of specimens that tested positive at PHO.
- Of the 102 influenza A specimens sequenced from the current season, 77.5% were H1N1pdm09 and 22.5% were seasonal H3N2.
  - 67 specimens (65.7%) were H1N1pdm09 genetic subclade 6B.1A.5a.2a and 12 specimens (11.8%) 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.
  - 23 specimens (22.5%) 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.
- Of the three influenza B specimens sequenced from the current season, all 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.
- The proportion of H1N1pdm09 increased over time from 46.7% in September to 87.0% in November.
- At the molecular level, 98.7% of influenza A H1N1pdm09, all influenza A H3N2, and all influenza B Victoria specimens contained at least one amino acid substitution in an antigenic site compared to the relevant strain included in the vaccine. However, the effect of the identified antigenic site substitutions on vaccine-induced immunity is unknown.
- Of the H1N1pdm09 specimens sequenced, none had the H275Y amino acid substitution known to be associated with resistance to oseltamivir.

Background

There are two types of influenza viruses (influenza A and B) that are responsible for most cases during the influenza season. Influenza A can be further classified into subtypes (e.g., H1N1pdm09, 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]

It is estimated that PHO conducts approximately 31% of all influenza virus testing in Ontario that is reported to the Public Health Agency of Canada.[6] 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.[7]
- Individuals attending physician offices that are part of the Sentinel Practitioner Surveillance Network (see Technical Notes for additional information).[8]

To understand the diversity of the virus 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.

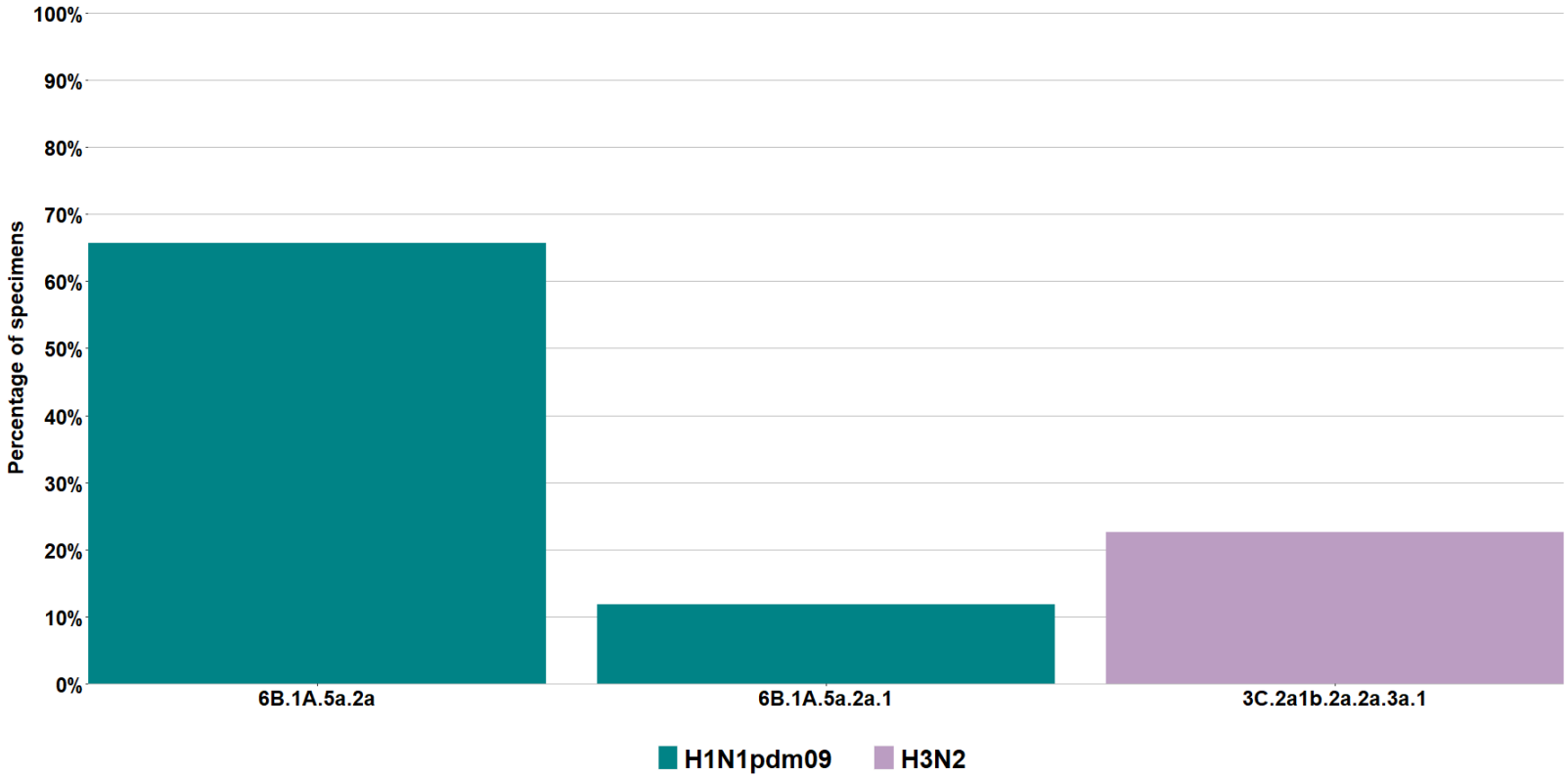
Results

Table 1a: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization, Public Health Ontario, September 1, 2024 to November 23, 2024

Genetic Characterization	2024–25 Season (September 1, 2024 – November 23, 2024)
H1N1pdm09	**79 (77.5%)**
6B.1A.5a.2a	67 (65.7%)
6B.1A.5a.2a.1	12 (11.8%)
H3N2	**23 (22.5%)**
3C.2a1b.2a.2a.3a.1	23 (22.5%)
Total sequenced	**102 (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] In total there were 230 specimens positive for influenza A at PHO during this time period, 75.7% were H1N1pdm09, 23.5% were H3N2, and 0.9% were not subtyped. Of the 230 specimens positive for influenza A, 44.3% were sequenced and included in this report.
Data sources: PHO Laboratory Information Management System

Figure 1: Percentage of Positive Influenza A Specimens, by Genetic Characterization, Public Health Ontario, September 1, 2024 to November 23, 2024



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.
Data sources: PHO Laboratory Information Management System

Table 1b: Number and Percentage of Positive Influenza B Specimens, by Genetic Characterization, Public Health Ontario, September 1, 2024 to November 23, 2024

Genetic Characterization	2024–25 Season (September 1, 2024 – November 23, 2024)
Victoria	**3 (100%)**
V1A.3a.2	3 (100%)
Total sequenced	**3 (100%)**

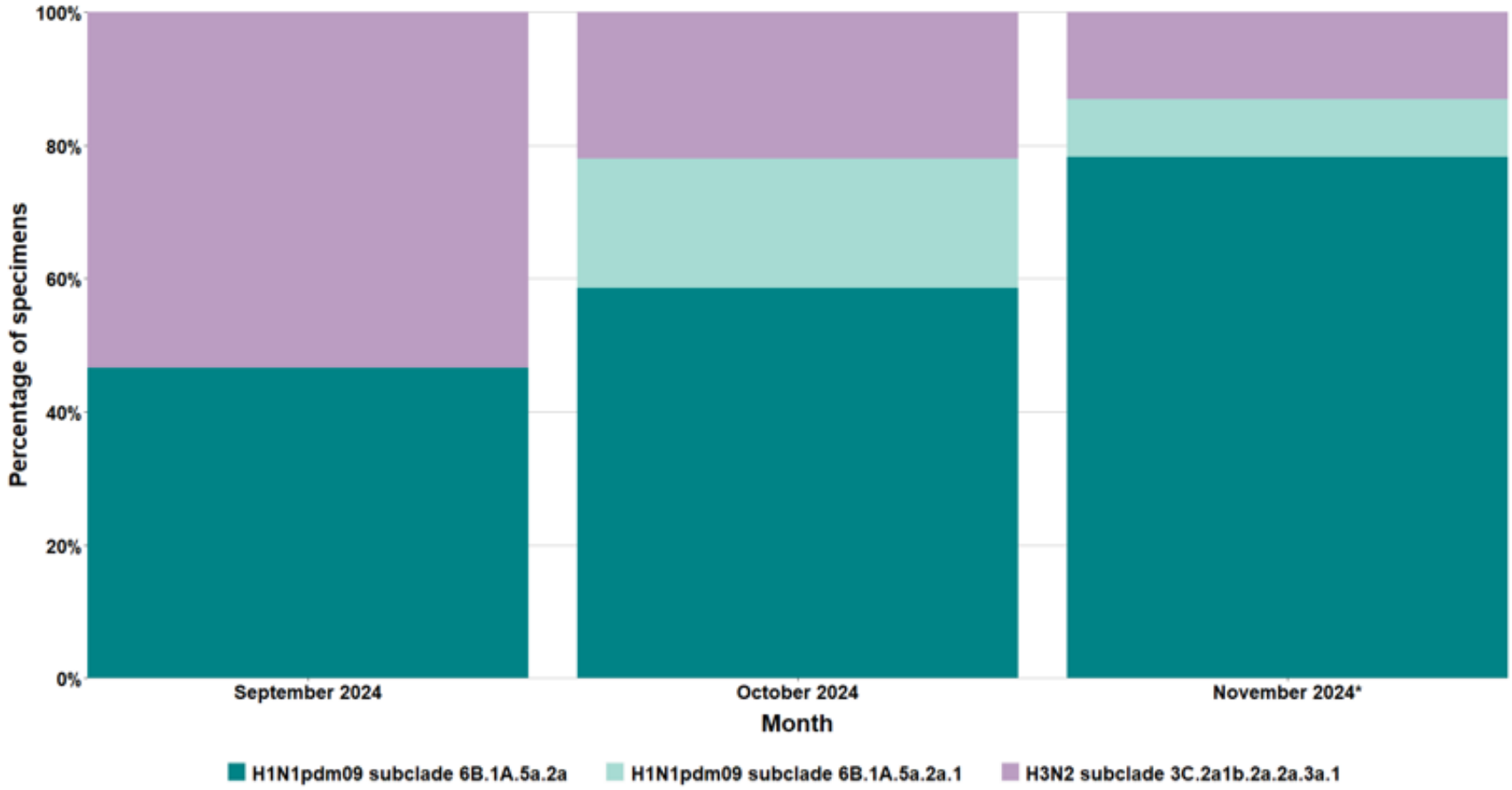
Note: Results may not be representative of Ontario overall. Date was assigned based on the earliest date available for the specimen. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade 6B.1A.5a.2a.1, influenza A H3N2 subclade 3C.2a1b.2a.2a.3a.1, and influenza B Victoria subclade V1A.3a.2, and influenza B Yamagata subclade Y3.[4-5] In total there were 4 specimens positive for influenza B at PHO during this time period. Of the four specimens positive for influenza B, three were sequenced and included in this report.
Data sources: PHO Laboratory Information Management System

Table 2: Number of Positive Influenza Specimens, Number and Percentage Sequenced, Public Health Ontario, September 1, 2024 to November 23, 2024

Month	Number of Positive Specimens	Number Sequenced	Percentage Sequenced
September 2024	39	15	38.5%
October 2024	95	42	44.2%
November 2024*	100	48	48.0%
Total	**234**	**105**	**44.9%**

Note: *November is a partial month. Of the 105 specimens sequenced, 2.9% (3/105) 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

Figure 2: Percentage of Positive Influenza A Specimens Sequenced, by Genetic Characterization and Month, Public Health Ontario, September 1, 2024 to November 23, 2024



Note: *November 2024 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.
Data sources: PHO Laboratory Information Management System

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 November 23, 2024

Genetic Characterization	HA Antigenic Site Ca	HA Antigenic Site Cb	HA Antigenic Site Sa	HA Antigenic Site Sb	Total
H1N1pdm09	**97.5% (77/79)**	**2.5% (2/79)**	**5.1% (4/79)**	**1.3% (1/79)**	**98.7% (78/79)**
6B.1A.5a.2a	98.5% (66/67)	1.5% (1/67)	6.0% (4/67)	1.5% (1/67)	100% (67/67)
6B.1A.5a.2a.1	91.7% (11/12)	8.3% (1/12)	0.0% (0/12)	0.0% (0/12)	91.7% (11/12)
Total sequenced	**97.5% (77/79)**	**2.5% (2/79)**	**5.1% (4/79)**	**1.3% (1/79)**	**98.7% (78/79)**

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, 138, 139, 142, 169, 178, 223, 271 of the HA protein. Antigenic site Cb includes substitutions at positions 69, 73 of the HA protein. Antigenic site Sa includes substitutions at position 125 of the HA protein. Antigenic site Sb includes substitutions at position 195 of the HA protein.
Data sources: PHO Laboratory Information Management System

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 November 23, 2024

Genetic Characterization	HA Antigenic Site A	HA Antigenic Site B	HA Antigenic Site C	HA Antigenic Site D	HA Antigenic Site E	Total
H3N2	**95.7% (22/23)**	**0.0% (0/23)**	**95.7% (22/23)**	**95.7% (22/23)**	**21.7% (5/23)**	**100% (23/23)**
3C.2a1b.2a.2a.3a.1	95.7% (22/23)	0.0% (0/23)	95.7% (22/23)	95.7% (22/23)	21.7% (5/23)	100% (23/23)
Total sequenced	**95.7% (22/23)**	**0.0% (0/23)**	**95.7% (22/23)**	**95.7% (22/23)**	**21.7% (5/23)**	**100% (23/23)**

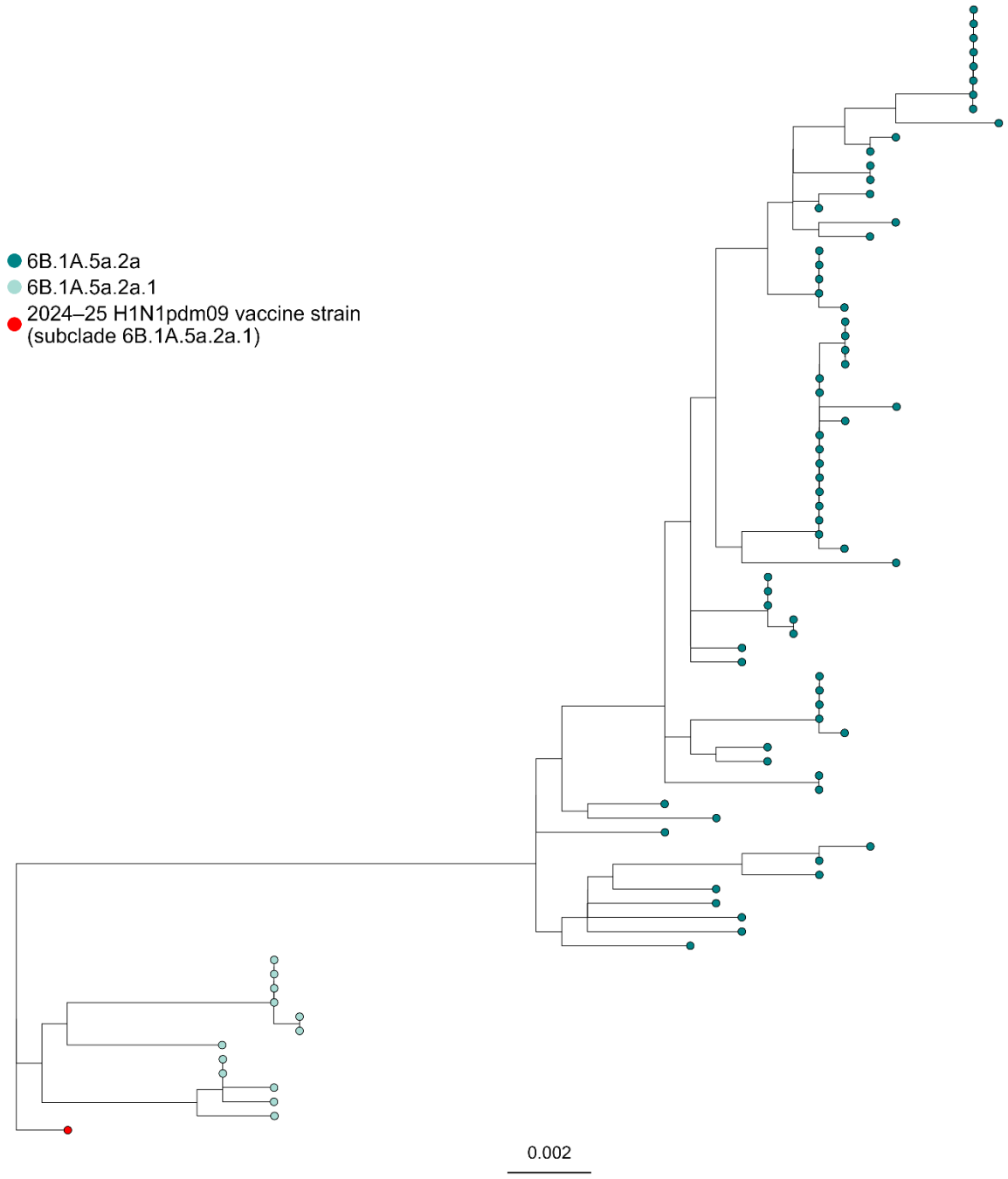
Note: The effect of the identified antigenic site substitutions on vaccine-induced immunity is unknown. This data is exploratory in nature and should be interpreted with caution. This data should not be used to directly inform clinical decisions. See Technical Notes for details. Antigenic site amino 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, 260, 262 of the HA protein. Antigenic site C includes substitutions at positions 48, 54, 276, 278 of the HA protein. Antigenic site D includes substitutions at position 96, 182, 207, 214 of the HA protein. Antigenic site E includes substitutions at positions 57, 63, 78, 94 of the HA protein.
Data sources: PHO Laboratory Information Management System

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 November 23, 2024

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

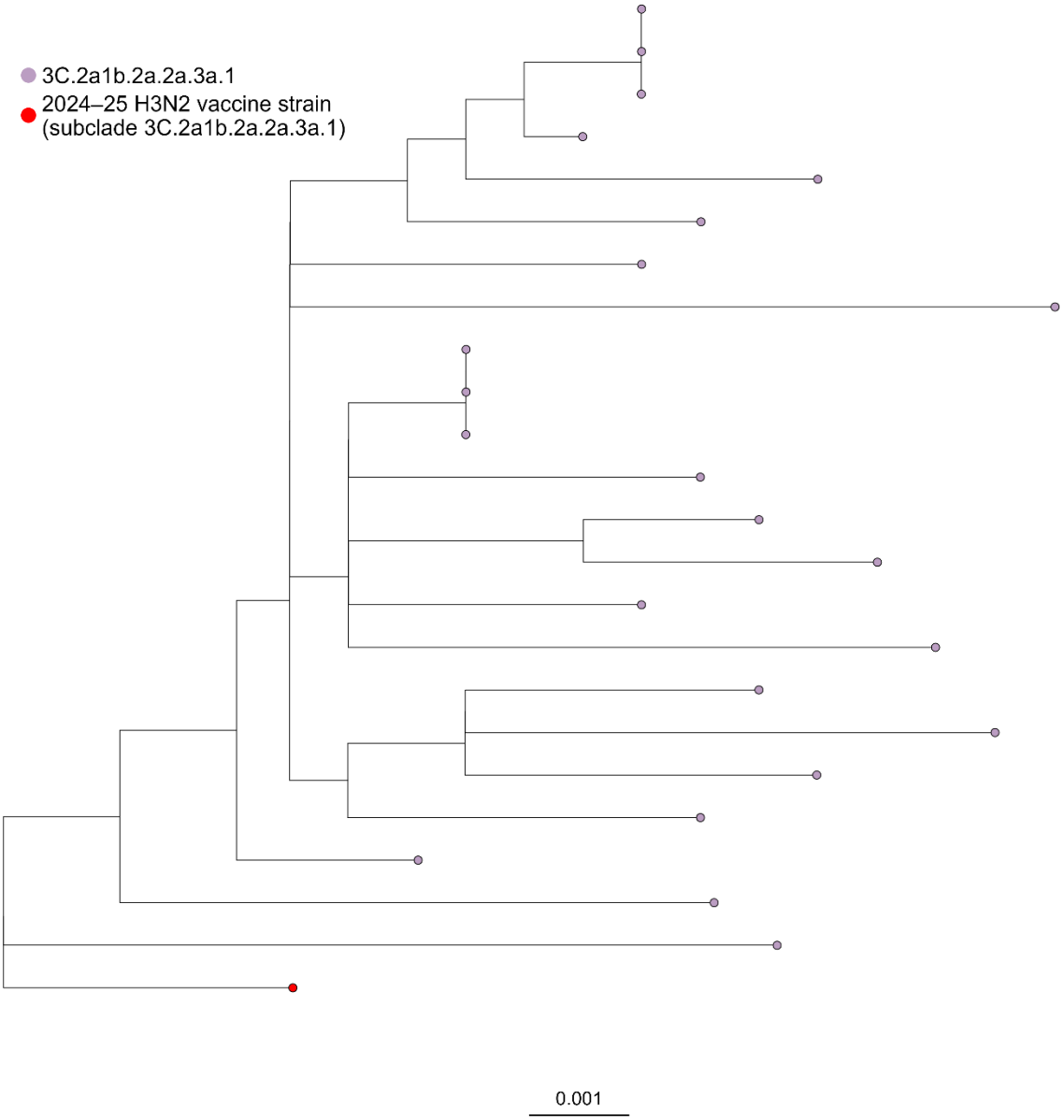
Note: The effect of the identified antigenic site substitutions on vaccine-induced immunity is unknown. This data is exploratory in nature and should be interpreted with caution. This data should not be used to directly inform clinical decisions. See Technical Notes for details. Antigenic site amino 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, 129 of the HA protein. Antigenic site 190-helix includes substitutions at position 194, 196, 199 of the HA protein.
Data sources: PHO Laboratory Information Management System

Figure 3a: Phylogenetic Tree of Positive Influenza A H1N1pdm09 Specimens, Public Health Ontario, September 1, 2024 to November 23, 2024



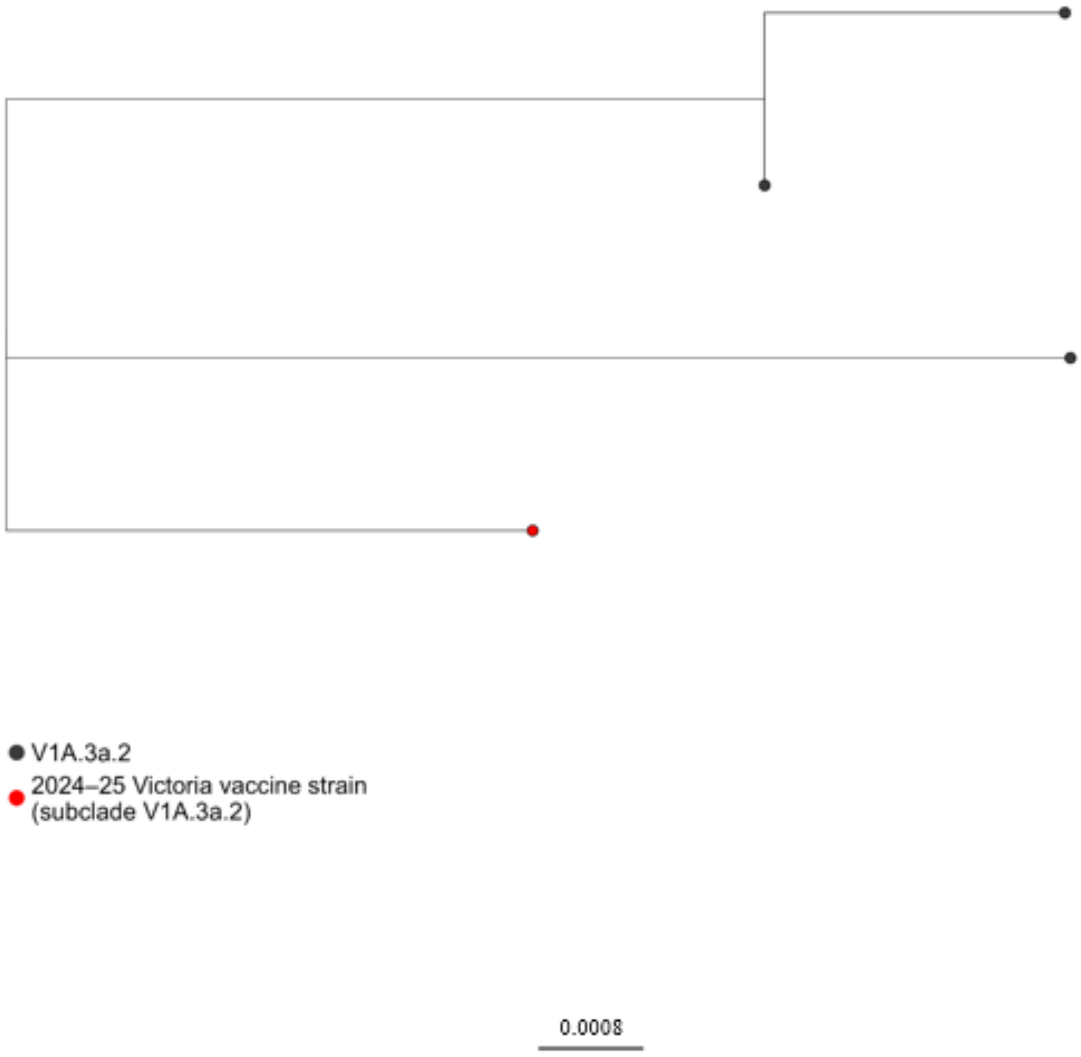
Note: Each circle represents a separate specimen. Results may not be representative of Ontario overall. The maximum likelihood phylogenetic tree was generated based on the HA region of the influenza genome using the IQ-TREE GTR model with 100 bootstrap replicates. Identical sequences are retained in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

Figure 3b: Phylogenetic Tree of Positive Influenza A H3N2 Specimens, Public Health Ontario, September 1, 2024 to November 23, 2024



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).
Data sources: PHO Laboratory Information Management System

Figure 3c: Phylogenetic Tree of Positive Influenza B Victoria Specimens, Public Health Ontario, September 1, 2024 to November 23, 2024



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).
Data sources: PHO Laboratory Information Management System

Table 4: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Age Group, Public Health Ontario, September 1, 2024 to November 23, 2024

Genetic Characterization	0–4 Years	5–19 Years	20–64 Years	65 Years and Over	Total
H1N1pdm09	**11 (91.7%)**	**8 (88.9%)**	**19 (67.9%)**	**41 (77.4%)**	**79 (77.5%)**
6B.1A.5a.2a	10 (83.3%)	7 (77.8%)	17 (60.7%)	33 (62.3%)	67 (65.7%)
6B.1A.5a.2a.1	1 (8.3%)	1 (11.1%)	2 (7.1%)	8 (15.1%)	12 (11.8%)
H3N2	**1 (8.3%)**	**1 (11.1%)**	**9 (32.1%)**	**12 (22.6%)**	**23 (22.5%)**
3C.2a1b.2a.2a.3a.1	1 (8.3%)	1 (11.1%)	9 (32.1%)	12 (22.6%)	23 (22.5%)
Total sequenced	**12 (100%)**	**9 (100%)**	**28 (100%)**	**53 (100%)**	**102 (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]
Data sources: PHO Laboratory Information Management System

Table 5: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Setting, Public Health Ontario, September 1, 2024 to November 23, 2024

Genetic Characterization	Intensive Care Unit	Hospital/Emergency Department	Congregate Living	Ambulatory or No Setting Reported	Total
H1N1pdm09	**0 (0.0%)**	**47 (82.5%)**	**8 (72.7%)**	**24 (70.6%)**	**79 (77.5%)**
6B.1A.5a.2a	0 (0.0%)	40 (70.2%)	8 (72.7%)	19 (55.9%)	67 (65.7%)
6B.1A.5a.2a.1	0 (0.0%)	7 (12.3%)	0 (0.0%)	5 (14.7%)	12 (11.8%)
H3N2	**0 (0.0%)**	**10 (17.5%)**	**3 (27.3%)**	**10 (29.4%)**	**23 (22.5%)**
3C.2a1b.2a.2a.3a.1	0 (0.0%)	10 (17.5%)	3 (27.3%)	10 (29.4%)	23 (22.5%)
Total sequenced	**0 (0.0%)**	**57 (100%)**	**11 (100%)**	**34 (100%)**	**102 (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 33% 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]
Data sources: PHO Laboratory Information Management System

Table 6: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Region, Public Health Ontario, September 1, 2024 to November 23, 2024

Genetic Characterization	Northern	Eastern	Central East	Toronto	South West	Central West	Total
H1N1pdm09	**2 (100%)**	**3 (75.0%)**	**23 (76.7%)**	**16 (69.6%)**	**7 (63.6%)**	**28 (87.5%)**	**79 (77.5%)**
6B.1A.5a.2a	2 (100%)	2 (50.0%)	18 (60.0%)	12 (52.2%)	7 (63.6%)	26 (81.2%)	67 (65.7%)
6B.1A.5a.2a.1	0 (0.0%)	1 (25.0%)	5 (16.7%)	4 (17.4%)	0 (0.0%)	2 (6.3%)	12 (11.8%)
H3N2	**0 (0.0%)**	**1 (25.0%)**	**7 (23.3%)**	**7 (30.4%)**	**4 (36.4%)**	**4 (12.5%)**	**23 (22.5%)**
3C.2a1b.2a.2a.3a.1	0 (0.0%)	1 (25.0%)	7 (23.3%)	7 (30.4%)	4 (36.4%)	4 (12.5%)	23 (22.5%)
Total sequenced	**2 (100%)**	**4 (100%)**	**30 (100%)**	**23 (100%)**	**11 (100%)**	**32 (100%)**	**102 (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]
Data sources: PHO Laboratory Information Management System

Table 7: Number and Percentage of H1N1pdm09 Specimens with Amino Acid Substitution H275Y Associated with Oseltamivir Resistance, among Positive Influenza A Specimens by Genetic Characterization, Public Health Ontario, September 1, 2024 to November 23, 2024

Genetic Characterization	Amino Acid Substitution H275Y
H1N1pdm09	**0.0% (0/79)**
6B.1A.5a.2a	0.0% (0/67)
6B.1A.5a.2a.1	0.0% (0/12)
Total sequenced	**0.0% (0/79)**

Note: H275Y substitution has been associated with oseltamivir resistance in influenza A H1N1pdm09 viruses.[9] See Technical Notes for details. 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.
Data sources: PHO Laboratory Information Management System

Technical Notes

Data Sources

Public Health Ontario (PHO)

- Data were extracted from the PHO Laboratory Information Management System on December 11, 2024 at approximately 1:00 p.m.
- Bioinformatics processing of data by the Biocomputing Centre were completed on December 11, 2024 at approximately 1:00 pm.

Public Health Ontario's Influenza Whole Genome Sequencing Strategy

- Due to low case counts in the beginning of the season, Public Health Ontario used convenience sampling to select all eligible specimens (Ct ≤ 27 and sufficient volume remaining) for whole genome sequencing. This excludes specimens that are positive for more than one virus.
- Only the first specimen from an outbreak was selected for whole genome sequencing. Multiple specimens from the same outbreak were not selected. Specimens tested as part of the Sentinel Practitioner Surveillance Network (SPSN) were excluded.
- Only upper respiratory specimens (e.g. nasopharyngeal or throat swabs) were included. All other specimen sources were excluded.
- 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 (2.14.0) 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 (ED), symptomatic hospitalized patients (ward and ICU/CCU), symptomatic residents in institutional settings (non-outbreak), specimens from the first four symptomatic individuals (including healthcare workers/staff) in an outbreak that requests 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 residents and healthcare workers/staff in institutional settings in an outbreak after the first four specimens are tested for SARS-CoV-2 and MRVP. FLUVID is also performed on symptomatic adult individuals seen in the Emergency Department (ED) who are at risk for severe disease/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)[8] 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 H3N2, influenza A H1N1pdm09, and influenza B.
  - A 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.

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.[18]
- 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 is considered a clinically relevant amino acid substitution associated with oseltamivir resistance in influenza A H1N1pdm09 viruses.[9] The effect of other substitutions (including those in H3N2 viruses) on antiviral 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.

- Numbers and proportions may not align with the Ontario Respiratory Virus Tool as specimens referred to PHO for subtyping were included. In addition, only specimens eligible (Ct ≤ 27, sufficient volume remaining, and first specimen from an outbreak) were included.

- PHO conducts approximately 31% of influenza testing in Ontario. The majority of individuals tested at PHO are over 65 years of age and reside in congregate living settings. Further, 44.9% of positive specimens were sequenced during the current season. Biases may introduced due 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 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 includes 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 includes Haliburton, Kawartha, Pine Ridge District Health Unit (HKPR), Peterborough Public Health, Durham Region Health Department, Simcoe Muskoka District Health Unit, York Region Public Health, Peel Public Health;
  - Toronto includes Toronto Public Health;
  - Central West includes 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, City of Haldimand-Norfolk Health Unit;
  - South West includes Grey Bruce Health Unit, Huron Perth Public Health, Southwestern Public Health, Middlesex-London Health Unit, Lambton Public Health, Chatham-Kent Public Health, Windsor-Essex County Health Unit
- Age was assigned based on the birth date provided and the specimen collection or login date.
- Patient setting is missing for approximately 33% of influenza specimens. Therefore, results by patient setting should be interpreted with caution.

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Appendix A: Jurisdictional Comparison

Supplementary Table 1: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Jurisdiction, May 19, 2024 to November 24, 2024

Genetic Characterization	Ontario (September 1 – November 23	Canada (September 1 – November 23)	United States of America (May 19 – November 23)	United Kingdom (August 26 – November 24)
H1N1pdm09	**79 (77.5%)**	**54 (79.4%)**	**381 (45.0%)**	**169 (80.1%)**
6B.1A.5a.2a	67 (65.7%)	21 (30.9%)	171 (20.2%)	160 (75.8%)
6B.1A.5a.2a.1	12 (11.8%)	33 (48.5%)	210 (24.8%)	9 (4.3%)
H3N2	**23 (22.5%)**	**14 (20.6%)**	**466 (55.0%)**	**42 (19.9%)**
3C.2a1b.2a.2a.3a	0 (0.0%)	0 (0.0%)	3 (0.4%)	0 (0.0%)
3C.2a1b.2a.2a.3a.1	23 (22.5%)	14 (20.6%)	463 (54.7%)	42 (19.9%)
Total sequenced	**102 (100%)**	**68 (100%)**	**847 (100%)**	**211 (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]

Supplementary Table 2: Number and Percentage of Positive Influenza B Specimens, by Genetic Characterization and Jurisdiction, May 19, 2024 to November 24, 2024

Genetic Characterization	Ontario (September 1 – November 23)	Canada (September 1 – November 23)	United States of America (May 19 – November 23)	United Kingdom (August 26 – November 24)
Victoria	**3 (100%)**	**1 (100%)**	**83 (100%)**	**24 (100%)**
V1A.3a.2	3 (100%)	1 (100%)	83 (100%)	24 (100%)
Total sequenced	**3 (100%)**	**1 (100%)**	**83 (100%)**	**24 (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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