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SURVEILLANCE REPORT

Influenza Genomic Surveillance in Ontario: 2025–26 Early Season

Published: December 2025

Introduction

This report summarizes the results of influenza whole genome sequencing completed by Public Health Ontario (PHO) for the beginning of the 2025–26 influenza season. The 2024–25 season report can be found on PHO's website.

Highlights

- A total of 146 influenza A specimens from the early season were successfully sequenced (August 24, 2025 to November 15, 2025), representing 25.9% of specimens that tested positive for influenza at PHO or were referred to PHO for subtype testing.
- Overall, in the early season, the most common subclades were H1N1pdm09 subclade D.3.1 (clade 5a.2a.1) with 70 specimens (47.9%), and H3N2 subclade K (clade 2a.3a.1) with 56 specimens (38.4%).
    - The number of H3N2 subclade K specimens increased from 8 (36.4%) in September to 34 specimens (46.6%) for November 1 to 15.
- Within the H3N2 subtype, subclade K (clade 2a.3a.1) represented 82.9% of H3N2 specimens sequenced from November 1 to November 15.
    - The first H3N2 subclade K specimen was observed in September 2025 (through retrospective sampling). For details on additional H3N2 sequencing performed during the inter-season period (May 18, 2025 to August 23, 2025) (see Appendix A).
- Notably, Influenza A activity has increased in Ontario since November 15, 2025 (the end of the sampling period for specimens included in this report). Influenza A H3N2 is the dominant circulating subtype and represented 85.0% of specimens in week 49 (see Appendix B).
- No amino acid substitutions were identified that predict drug resistance to oseltamivir or baloxavir for specimens sequenced that met internal quality metrics.

Background

Two types of influenza virus are responsible for most human cases during the influenza season, influenza A and B. Influenza A can be further classified into subtypes (e.g., H1N1pdm09, seasonal H3N2) and influenza B can be further classified into lineages (e.g., 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, antiviral susceptibility, 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 2025–2026 influenza season, publicly funded vaccines available in Ontario are trivalent (influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1), influenza A H3N2 subclade J.2 (clade 2a.3a.1), and influenza B Victoria subclade C (clade 3a.2) vaccines.[2-4]

PHO performs routine testing for seasonal respiratory viruses for select population groups, including individuals exhibiting respiratory symptoms that are in a congregate living setting, part of an outbreak, or hospitalized.[5] In addition, PHO tests individuals attending physician offices that are part of the Sentinel Practitioner Surveillance Network (SPSN; see Technical Notes for additional information).[6]

PHO also subtypes influenza A specimens initially tested by other laboratories when requested and for enhanced H5N1 surveillance. As a result, PHO accumulated 51% of all influenza A positive specimens in Ontario available for sample selection.

To understand the diversity of the viruses circulating during the 2025–26 influenza season, PHO sequenced eligible specimens (Ct ≤ 27 and sufficient volume remaining) positive for influenza in the early 2025–26 season. This excludes specimens that were positive for more than one virus. Additionally, only the first positive specimen from an outbreak was selected for whole genome sequencing. Specimens that were part of the SPSN were also excluded. Sequences were processed using bioinformatics analyses and were assigned subtypes, lineages, clades, and subclades.

Results

Table 1: Number of Positive Influenza Specimens, Number and Percentage Sequenced, Public Health Ontario, August 24, 2025 to November 15, 2025

Month	Number of Positive Specimens	Number Sequenced	Percentage Sequenced
August 2025*	10	4	40.0%
September 2025	67	22	32.8%
October 2025	154	47	30.5%
November 2025*	332	73	22.0%
Total	**563**	**146**	**25.9%**

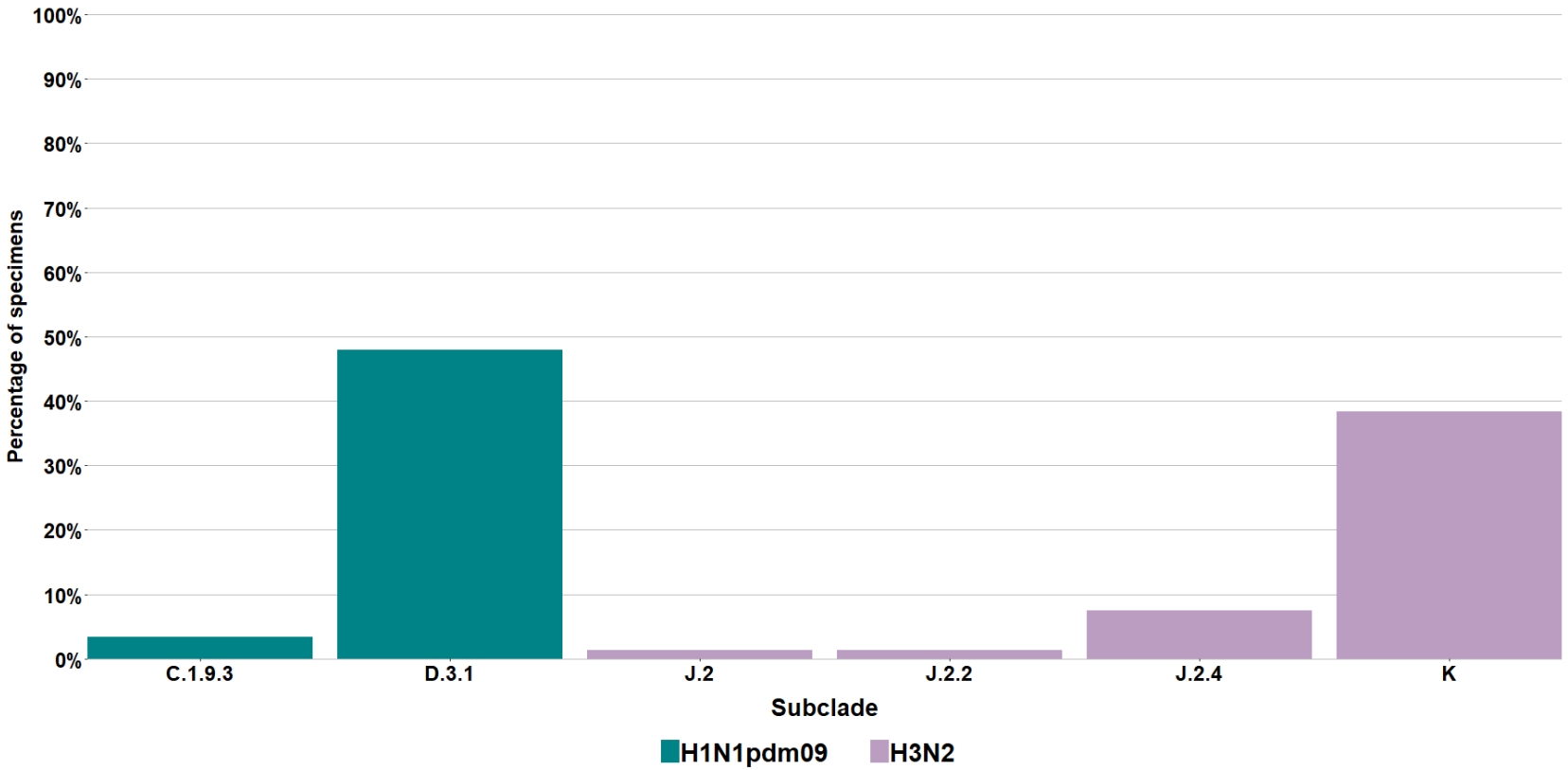
Note: *August (August 24 to 31) and November (November 1 to 15) 2025 are partial months. 'Positive specimens' includes specimens that tested positive for influenza at PHO or influenza A positive specimens referred to PHO for subtype testing. Of the 146 specimens sequenced, 4.1% (6/146) were outbreak-related. 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 Positive Influenza A Specimens, by Genetic Characterization, Public Health Ontario, August 24, 2025 to November 15, 2025

Genetic Characterization	2025–26 Season (August 24, 2025 – November 15, 2025)
H1N1pdm09	**75 (51.4%)**
C.1.9.3 (5a.2a)	5 (3.4%)
D.3.1 (5a.2a.1)	70 (47.9%)
H3N2	**71 (48.6%)**
J.2 (2a.3a.1)	2 (1.4%)
J.2.2 (2a.3a.1)	2 (1.4%)
J.2.4 (2a.3a.1)	11 (7.5%)
K (2a.3a.1)	56 (38.4%)
Total Sequenced	**146 (100%)**

Note: Genetic characterization is formatted as 'subclade (clade)'. Date was assigned based on the earliest date available for the specimen. Results may not be representative of Ontario overall. Influenza subclade nomenclature is dynamic and may be revised as additional information (e.g. mutational patterns) becomes available. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1), influenza A H3N2 subclade J.2 (clade 2a.3a.1), and influenza B Victoria subclade C (clade 3a.2).[4]

Figure 1: Percentage of Positive Influenza A Specimens, by Genetic Characterization, Public Health Ontario, August 24, 2025 to November 15, 2025



Note: Results may not be representative of Ontario overall. Influenza subclade nomenclature is dynamic and may be revised as additional information (e.g. mutational patterns) becomes available. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1), influenza A H3N2 subclade J.2 (clade 2a.3a.1), and influenza B Victoria subclade C (clade 3a.2).[4]

Table 3a: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Month, Public Health Ontario, August 24, 2025 to November 15, 2025

Genetic Characterization	August 2025*	September 2025	October 2025	November 2025*	Total
H1N1pdm09	**3 (75.0%)**	**12 (54.5%)**	**28 (59.6%)**	**32 (43.8%)**	**75 (51.4%)**
C.1.9.3	0 (0.0%)	4 (18.2%)	1 (2.1%)	0 (0.0%)	5 (3.4%)
D.3.1	3 (75.0%)	8 (36.4%)	27 (57.4%)	32 (43.8%)	70 (47.9%)
H3N2	**1 (25.0%)**	**10 (45.5%)**	**19 (40.4%)**	**41 (56.2%)**	**71 (48.6%)**
J.2	0 (0.0%)	1 (4.5%)	0 (0.0%)	1 (1.4%)	2 (1.4%)
J.2.2	0 (0.0%)	0 (0.0%)	1 (2.1%)	1 (1.4%)	2 (1.4%)
J.2.4	1 (25.0%)	1 (4.5%)	4 (8.5%)	5 (6.8%)	11 (7.5%)
K	0 (0.0%)	8 (36.4%)	14 (29.8%)	34 (46.6%)	56 (38.4%)
Total Sequenced	**4 (100%)**	**22 (100%)**	**47 (100%)**	**73 (100%)**	**146 (100%)**

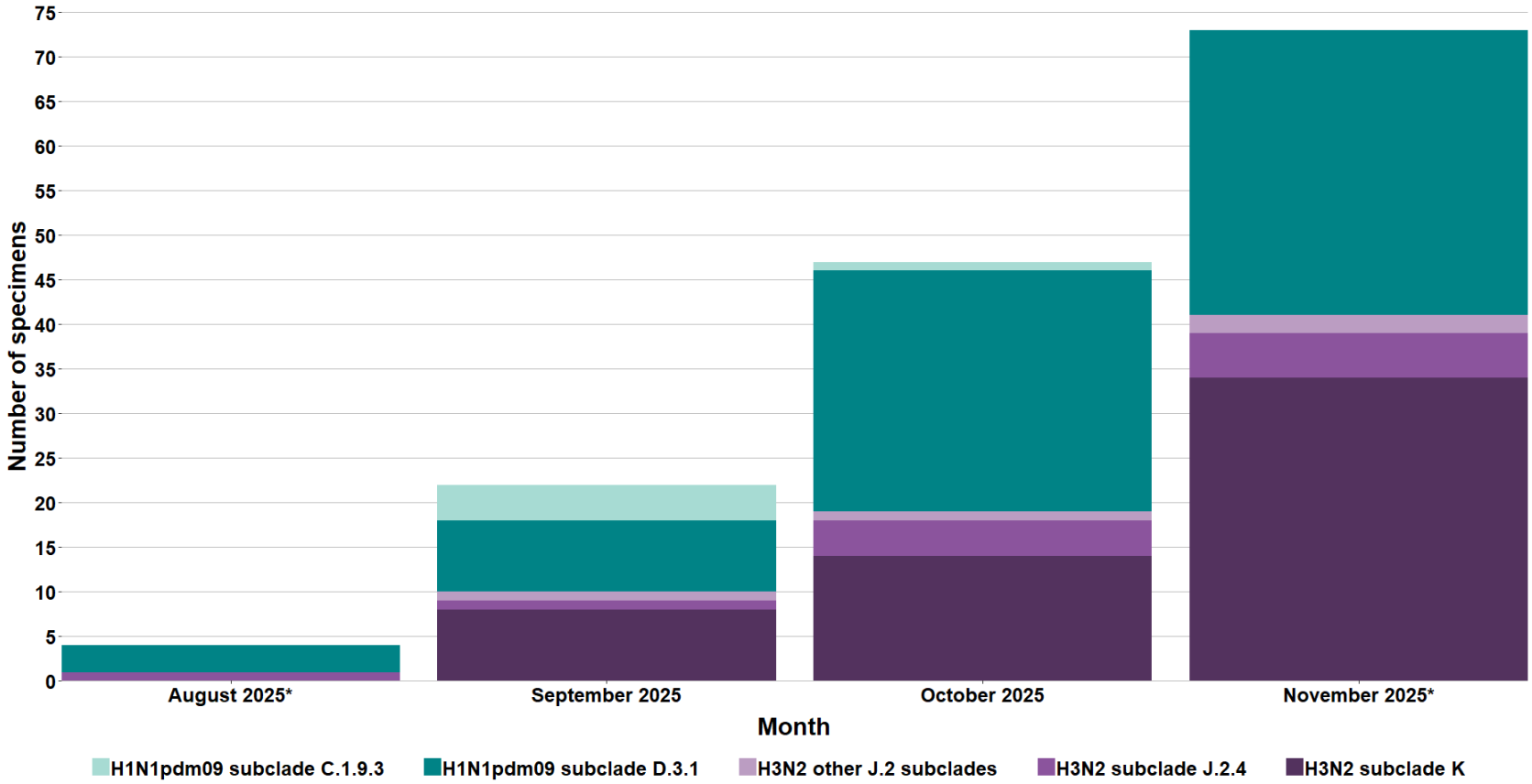
Note: *August (August 24 to 31) and November (November 1 to 15) 2025 are partial months. Month was assigned based on earliest date available for a specimen. Results may not be representative of Ontario overall. Influenza subclade nomenclature is dynamic and may be revised as additional information (e.g. mutational patterns) becomes available. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1), influenza A H3N2 subclade J.2 (clade 2a.3a.1), and influenza B Victoria subclade C (clade 3a.2).[4]

Table 3b: Number and Percentage of Positive Influenza A H3N2 Specimens, by Genetic Characterization and Month, Public Health Ontario, August 24, 2025 to November 15, 2025

Genetic Characterization	August 2025*	September 2025	October 2025	November 2025*	Total
H3N2	**1 (100%)**	**10 (100%)**	**19 (100%)**	**41 (100%)**	**71 (100%)**
J.2	0 (0.0%)	1 (10.0%)	0 (0.0%)	1 (2.4%)	2 (2.8%)
J.2.2	0 (0.0%)	0 (0.0%)	1 (5.3%)	1 (2.4%)	2 (2.8%)
J.2.4	1 (100%)	1 (10.0%)	4 (21.1%)	5 (12.2%)	11 (15.5%)
K	0 (0.0%)	8 (80.0%)	14 (73.7%)	34 (82.9%)	56 (78.9%)
Total Sequenced	**1 (100%)**	**10 (100%)**	**19 (100%)**	**41 (100%)**	**71 (100%)**

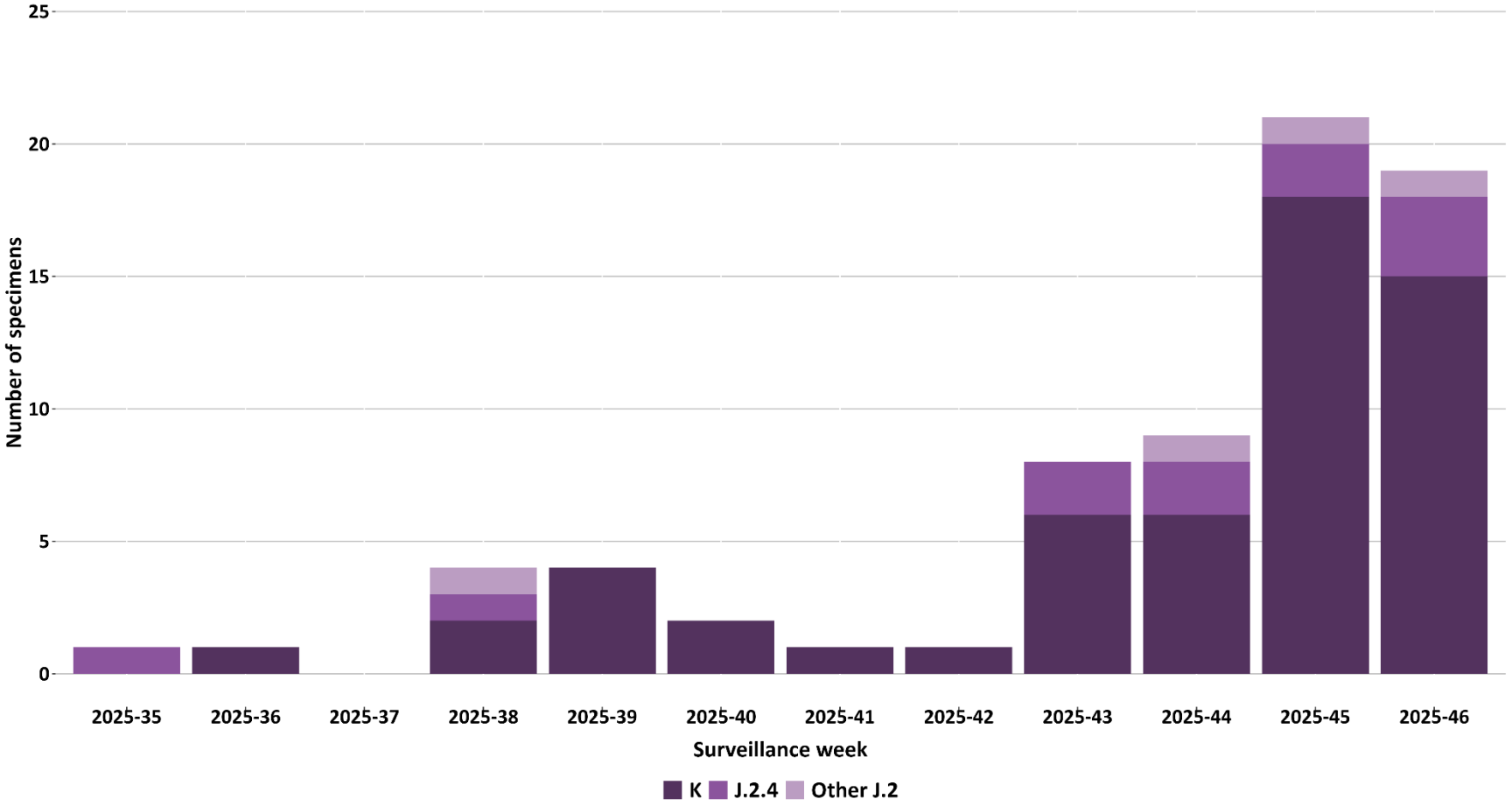
Note: *August (August 24 to 31) and November (November 1 to 15) 2025 are partial months. Month was assigned based on earliest date available for a specimen. Results may not be representative of Ontario overall. Influenza subclade nomenclature is dynamic and may be revised as additional information (e.g. mutational patterns) becomes available. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1), influenza A H3N2 subclade J.2 (clade 2a.3a.1), and influenza B Victoria subclade C (clade 3a.2).[4]

Figure 2a: Number of Positive Influenza A Specimens Sequenced, by Genetic Characterization and Month, Public Health Ontario, August 24, 2025 to November 15, 2025



Note: *August (August 24 to 31) and November (November 1 to 15) 2025 are partial months. Month was assigned based on earliest date available for a specimen. Results may not be representative of Ontario overall. Influenza subclade nomenclature is dynamic and may be revised as additional information (e.g. mutational patterns) becomes available. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1), influenza A H3N2 subclade J.2 (clade 2a.3a.1), and influenza B Victoria subclade C (clade 3a.2).[4]

Figure 2b: Number of Positive Influenza A H3N2 Specimens Sequenced, by Genetic Characterization and Week, Public Health Ontario, August 24, 2025 to November 15, 2025



Note: Week was assigned based on earliest date available for a specimen. Results may not be representative of Ontario overall. Influenza subclade nomenclature is dynamic and may be revised as additional information (e.g. mutational patterns) becomes available. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1), influenza A H3N2 subclade J.2 (clade 2a.3a.1), and influenza B Victoria subclade C (clade 3a.2).[4]

Table 4: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Age Group, Public Health Ontario, August 24, 2025 to November 15, 2025

Genetic Characterization	0–4 Years	5–19 Years	20–64 Years	65 Years and Over	Total
H1N1pdm09	**10 (62.5%)**	**8 (34.8%)**	**21 (67.7%)**	**36 (47.4%)**	**75 (51.4%)**
C.1.9.3	1 (6.2%)	0 (0.0%)	1 (3.2%)	3 (3.9%)	5 (3.4%)
D.3.1	9 (56.2%)	8 (34.8%)	20 (64.5%)	33 (43.4%)	70 (47.9%)
H3N2	**6 (37.5%)**	**15 (65.2%)**	**10 (32.3%)**	**40 (52.6%)**	**71 (48.6%)**
J.2	0 (0.0%)	1 (4.3%)	0 (0.0%)	1 (1.3%)	2 (1.4%)
J.2.2	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (2.6%)	2 (1.4%)
J.2.4	1 (6.2%)	3 (13.0%)	3 (9.7%)	4 (5.3%)	11 (7.5%)
K	5 (31.2%)	11 (47.8%)	7 (22.6%)	33 (43.4%)	56 (38.4%)
Total Sequenced	**16 (100%)**	**23 (100%)**	**31 (100%)**	**76 (100%)**	**146 (100%)**

Note: Age was assigned based on the birth date provided; excludes specimens with missing birth dates. Results may not be representative of Ontario overall. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1), influenza A H3N2 subclade J.2 (clade 2a.3a.1), and influenza B Victoria subclade C (clade 3a.2).[4]

Table 5: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Setting, Public Health Ontario, August 24, 2025 to November 15, 2025

Genetic Characterization	Intensive Care Unit	Hospital/ Emergency Department	Congregate Living	Ambulatory or No Setting Reported	Total
H1N1pdm09	**0 (0.0%)**	**41 (67.2%)**	**11 (40.7%)**	**23 (40.4%)**	**75 (51.4%)**
C.1.9.3	0 (0.0%)	2 (3.3%)	1 (3.7%)	2 (3.5%)	5 (3.4%)
D.3.1	0 (0.0%)	39 (63.9%)	10 (37.0%)	21 (36.8%)	70 (47.9%)
H3N2	**1 (100%)**	**20 (32.8%)**	**16 (59.3%)**	**34 (59.6%)**	**71 (48.6%)**
J.2	0 (0.0%)	1 (1.6%)	0 (0.0%)	1 (1.8%)	2 (1.4%)
J.2.2	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (3.5%)	2 (1.4%)
J.2.4	1 (100%)	3 (4.9%)	2 (7.4%)	5 (8.8%)	11 (7.5%)
K	0 (0.0%)	16 (26.2%)	14 (51.9%)	26 (45.6%)	56 (38.4%)
Total Sequenced	**1 (100%)**	**61 (100%)**	**27 (100%)**	**57 (100%)**	**146 (100%)**

Note: 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 34% of influenza A specimens are missing information on setting and are grouped into 'Ambulatory or no setting reported' category. Results may not be representative of Ontario overall. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1), influenza A H3N2 subclade J.2 (clade 2a.3a.1), and influenza B Victoria subclade C (clade 3a.2).[4]

Table 6: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Region, Public Health Ontario, August 24, 2025 to November 15, 2025

Genetic Characterization	Northern	Eastern	Central East	Toronto	South West	Central West	Total
H1N1pdm09	**2 (100%)**	**2 (40.0%)**	**28 (68.3%)**	**17 (38.6%)**	**11 (36.7%)**	**15 (62.5%)**	**75 (51.4%)**
C.1.9.3	0 (0.0%)	0 (0.0%)	1 (2.4%)	3 (6.8%)	0 (0.0%)	1 (4.2%)	5 (3.4%)
D.3.1	2 (100%)	2 (40.0%)	27 (65.9%)	14 (31.8%)	11 (36.7%)	14 (58.3%)	70 (47.9%)
H3N2	**0 (0.0%)**	**3 (60.0%)**	**13 (31.7%)**	**27 (61.4%)**	**19 (63.3%)**	**9 (37.5%)**	**71 (48.6%)**
J.2	0 (0.0%)	0 (0.0%)	1 (2.4%)	0 (0.0%)	0 (0.0%)	1 (4.2%)	2 (1.4%)
J.2.2	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (6.7%)	0 (0.0%)	2 (1.4%)
J.2.4	0 (0.0%)	0 (0.0%)	3 (7.3%)	3 (6.8%)	3 (10.0%)	2 (8.3%)	11 (7.5%)
K	0 (0.0%)	3 (60.0%)	9 (22.0%)	24 (54.5%)	14 (46.7%)	6 (25.0%)	56 (38.4%)
Total Sequenced	**2 (100%)**	**5 (100%)**	**41 (100%)**	**44 (100%)**	**30 (100%)**	**24 (100%)**	**146 (100%)**

Note: 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. Results may not be representative of Ontario overall. The genetic subclades included in this season's influenza vaccine are influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1), influenza A H3N2 subclade J.2 (clade 2a.3a.1), and influenza B Victoria subclade C (clade 3a.2).[4]

Table 7a: Number and Percentage of Positive Influenza A H1N1pdm09 Specimens with Any Antigenic Site Amino Acid Substitutions, by Genetic Characterization, Public Health Ontario, August 24, 2025 to November 15, 2025

Genetic Characterization	HA Antigenic Site Ca	HA Antigenic Site Cb	HA Antigenic Site Sa	HA Antigenic Site Sb	Total
H1N1pdm09	**62.7% (47/75)**	**0.0% (0/75)**	**1.3% (1/75)**	**1.3% (1/75)**	**65.3% (49/75)**
C.1.9.3	100% (5/5)	0.0% (0/5)	0.0% (0/5)	0.0% (0/5)	100% (5/5)
D.3.1	60.0% (42/70)	0.0% (0/70)	1.4% (1/70)	1.4% (1/70)	62.9% (44/70)
Total Sequenced	**62.7% (47/75)**	**0.0% (0/75)**	**1.3% (1/75)**	**1.3% (1/75)**	**65.3% (49/75)**

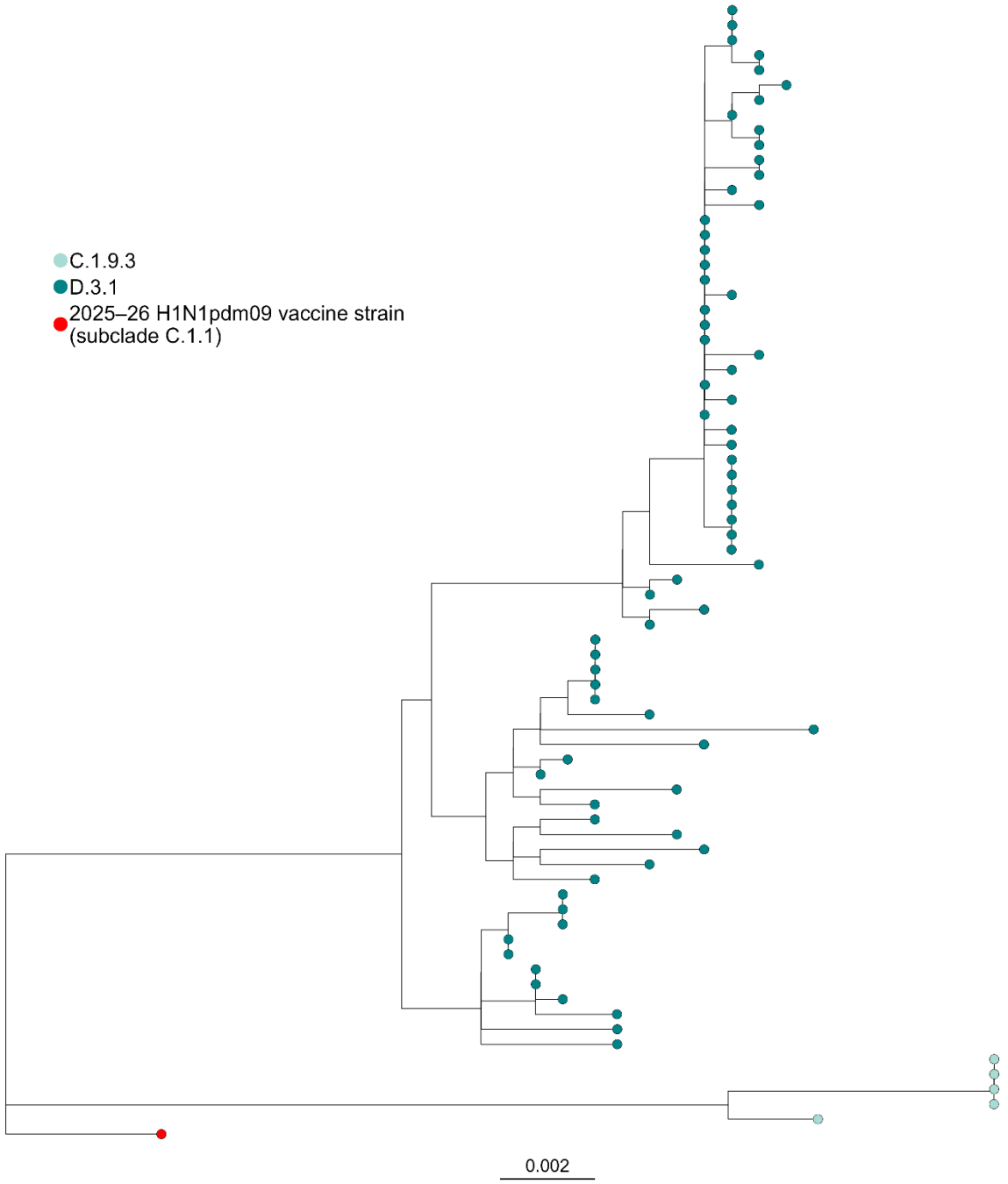
Note: The effect of the identified antigenic site substitutions on vaccine-induced immunity is unknown. This data is exploratory in nature and should be interpreted with caution. This data should not be used to directly inform clinical decisions or infer impacts on vaccine-induced immunity. See Technical Notes for details. Antigenic site amino acid substitutions were identified relative to the strain included in the 2025–26 influenza vaccine (influenza A H1N1pdm09 subclade C.1.1 (clade 5a.2a.1)).[4] Sequenced viruses may have substitutions at more than one position within the antigenic site. Antigenic site Ca includes substitutions at positions 139, 140, 142, 166, 168, 205 of the hemagglutinin (HA) protein. No substitution was observed at antigenic site Cb. Antigenic site Sa includes substitutions at position 121 of the HA protein. Antigenic site Sb includes substitutions at position 185 of the HA protein.

Table 7b: Number and Percentage of Positive Influenza A H3N2 Specimens with Any Antigenic Site Amino Acid Substitutions, by Genetic Characterization, Public Health Ontario, August 24, 2025 to November 15, 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% (71/71)**	**95.8% (68/71)**	**1.4% (1/71)**	**83.1% (59/71)**	**4.2% (3/71)**	**100% (71/71)**
J.2	100% (2/2)	50.0% (1/2)	0.0% (0/2)	0.0% (0/2)	50.0% (1/2)	100% (2/2)
J.2.2	100% (2/2)	0.0% (0/2)	0.0% (0/2)	100% (2/2)	100% (2/2)	100% (2/2)
J.2.4	100% (11/11)	100% (11/11)	0.0% (0/11)	9.1% (1/11)	0.0% (0/11)	100% (11/11)
K	100% (56/56)	100% (56/56)	1.8% (1/56)	100% (56/56)	0.0% (0/56)	100% (56/56)
Total Sequenced	**100% (71/71)**	**95.8% (68/71)**	**1.4% (1/71)**	**83.1% (59/71)**	**4.2% (3/71)**	**100% (71/71)**

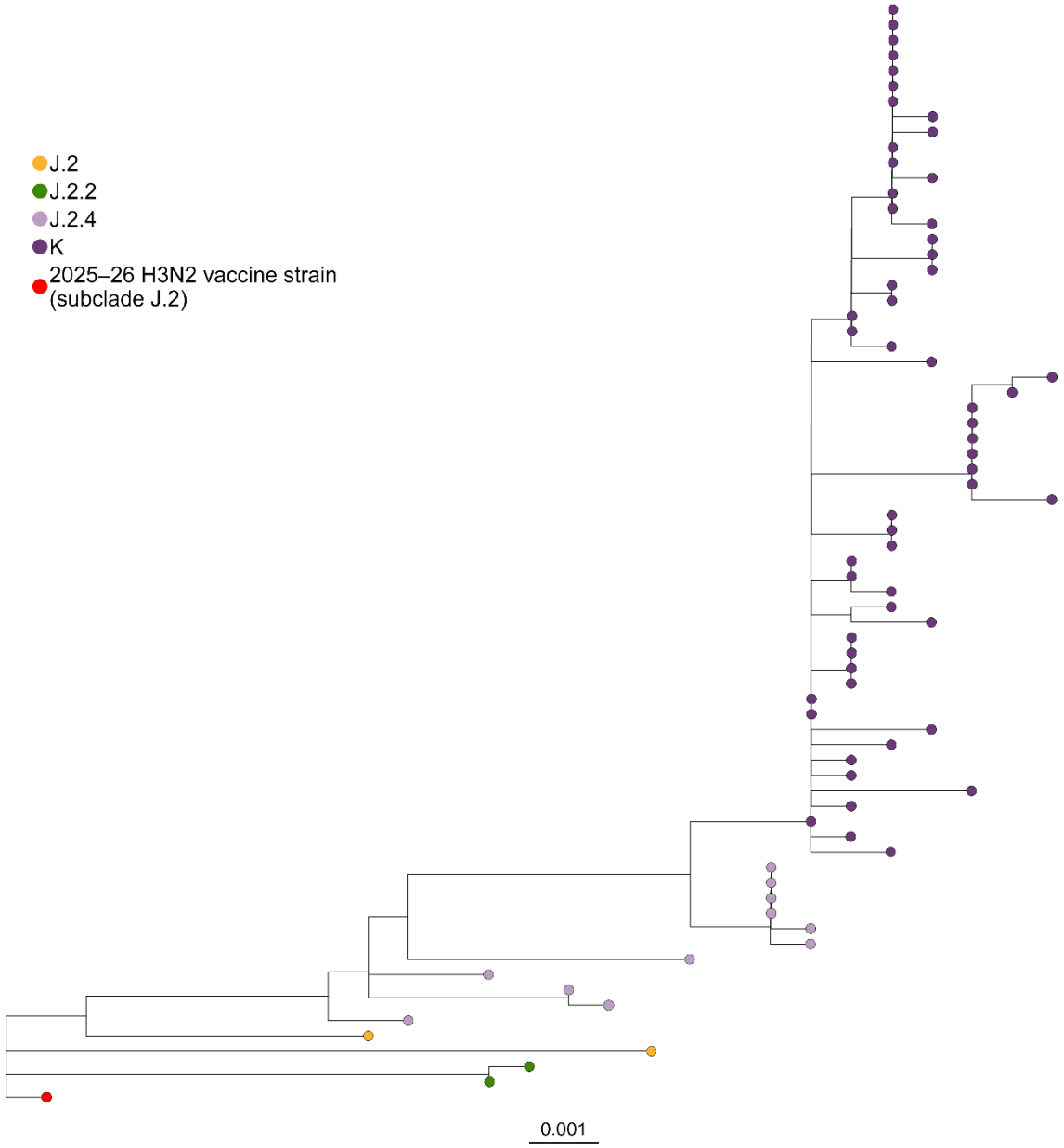
Note: The effect of the identified antigenic site substitutions on vaccine-induced immunity is unknown. This data is exploratory in nature and should be interpreted with caution. This data should not be used to directly inform clinical decisions. See Technical Notes for details. Antigenic site amino acid substitutions were identified relative to the strain included in the 2025–26 influenza vaccine (influenza A H3N2 subclade J.2 (clade 2a.3a.1)).[4] Sequenced viruses may have substitutions at more than one position within the antigenic site. Antigenic site A includes substitutions at positions 124, 135, 144, 145 of the HA protein. Antigenic site B includes substitutions at positions 158, 160, 189 of the HA protein. Antigenic site C includes substitutions at position 278 of the HA protein. Antigenic site D includes substitutions at positions 117, 171, 173, 244 of the HA protein. Antigenic site E includes substitutions at positions 62, 67, 78 of the HA protein.

Figure 3a: Phylogenetic Tree of Positive Influenza A H1N1pdm09 Specimens, Public Health Ontario, August 24, 2025 to November 15, 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/Wisconsin/67/2022 H1N1pdm09-like virus (EPI_ISL_15928563).

Figure 3b: Phylogenetic Tree of Positive Influenza A H3N2 Specimens, Public Health Ontario, August 24, 2025 to November 15, 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/District_Of_Columbia/27/2023 H3N2-like virus (EPI_ISL_18937823).

Table 8: Number and Percentage of Positive H1N1pdm09 Specimens with Amino Acid Substitution H275Y Associated with Oseltamivir Resistance, by Genetic Characterization, Public Health Ontario, August 24, 2025 to November 15, 2025

Genetic Characterization	Amino Acid Substitution H275Y
H1N1pdm09	**0.0% (0/75)**
C.1.9.3	0.0% (0/5)
D.3.1	0.0% (0/70)
Total Sequenced	**0.0% (0/75)**

Note: H275Y substitution has been associated with oseltamivir resistance in influenza A H1N1pdm09 viruses.[7] 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 9: Number and Percentage of Positive Influenza A Specimens with Amino Acid Substitution I38T Associated with Baloxavir Resistance, by Genetic Characterization, Public Health Ontario, August 24, 2025 to November 15, 2025

Genetic Characterization	Amino Acid Substitution I38T
H1N1pdm09	**0.0% (0/34)**
C.1.9.3	0.0% (0/2)
D.3.1	0.0% (0/32)
H3N2	**0.0% (0/62)**
J.2	0.0% (0/2)
J.2.2	0.0% (0/2)
J.2.4	0.0% (0/10)
K	0.0% (0/48)
Total Sequenced	**0.0% (0/96)**

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.[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.

Technical Notes

Data Sources

Public Health Ontario (PHO)

- Data were extracted from the PHO Laboratory Information Management System on November 28, 2025 at approximately 3:00 p.m.
- Bioinformatics processing of data by the Biocomputing Centre were completed on November 28, 2025 at approximately 4:30 pm.

Public Health Ontario's Influenza Whole Genome Sequencing Strategy

- For the early season report, Public Health Ontario selected all eligible specimens (Ct ≤ 27 and sufficient volume remaining) since the beginning of the season for whole genome sequencing.
- 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).
- 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 (3.10.1), bwa (0.7.17), bedtools (2.31.1), bcftools (1.22), ivar (1.4.3), emboss (6.6.0), and BLAST (2.17.0).[9-17] Clade and subclade were assigned with Nextclade (3.18.0) and Nextclade dataset (2025-11-04--15-46-13Z).[18-19]
- Phylogenetic trees were created using IQ-TREE (3.0.1).[20]

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 patients who meets one of the following criteria:
  - Hospitalized patients requiring intensive care
  - Hospitalized admitted patients that are
    - Children < 18 years who are at risk of complications, in the presence of community-acquired pneumonia, or
    - immunocompromised or immunosuppressed, or
    - pregnant
  - Patients/residents who are part of a hospital or PHU-declared respiratory outbreaks. (Only the first four symptomatic patients in a respiratory outbreak (subsequent patients will be tested by FLUVID).

- FLUVID testing is offered to individuals exhibiting respiratory symptoms that meet at least one of the following eligibility criteria:
  - Residents in congregate living setting (long-term care, retirement home, and correctional facilities) that are not in an outbreak
  - Admitted patients in a hospital or residents of congregate living setting that is in an outbreak beyond the first four specimens tested by MRVP
  - Adults admitted to the hospital
  - Staff from congregate living setting that are part of an outbreak.
- Individuals attending physician offices that are part of the Sentinel Practitioner Surveillance Network (SPSN)[6] 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).
  - 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.[21]
- 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 are 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.[7] 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.[8] 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 51% 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, H1N1pdm09 and H3N2 subtype proportions sequenced may not align with the Ontario Respiratory Virus Tool and 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.
- Influenza subclade nomenclature is dynamic and may be revised as additional information (e.g. mutational patterns) becomes available.
- Age was assigned based on the birth date provided and the specimen collection or login date.
- Patient setting is missing for approximately 34% of influenza specimens. Therefore, results by patient setting should be interpreted with caution.
- 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 Public Health (formerly Porcupine Health Unit and Timiskaming 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 Southeast Public Health (formerly Hastings and Prince Edward Counties Health Unit, Kingston, Frontenac and Lennox and Addington Health Unit, and Leeds, Grenville and Lanark District Health Unit);
- Central East includes Durham Region Health Department, Lakelands Public Health (formerly Haliburton, Kawartha, Pine Ridge District Health Unit and Peterborough County-City 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 Public Health (formerly Brant County Health Unit and Haldimand-Norfolk Health Unit), Halton Region Public Health, Niagara Region Public Health, Region of Waterloo Public Health and Emergency Services, and Wellington-Dufferin-Guelph Public Health.

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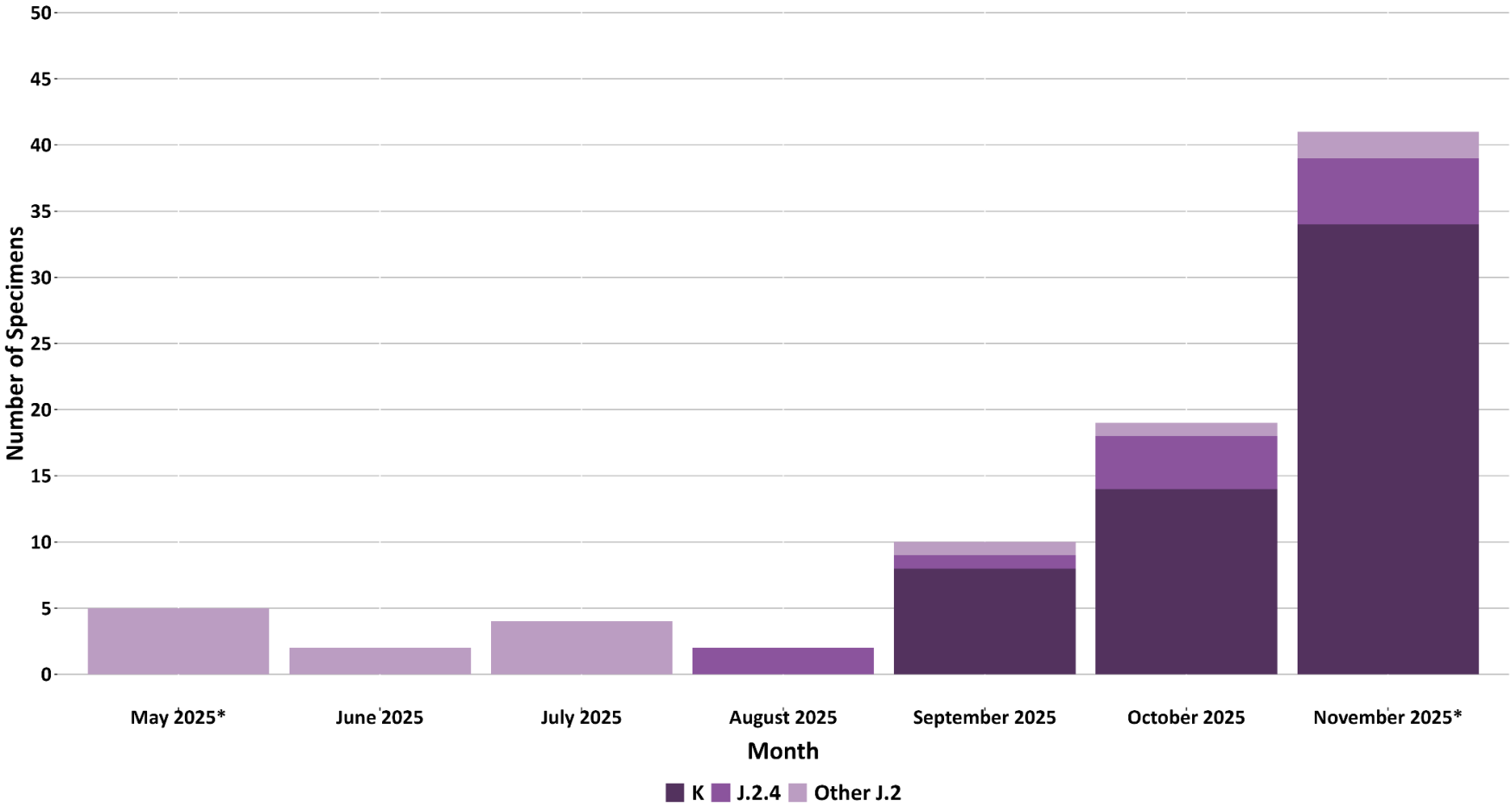
Appendix A: Influenza A H3N2 Specimens by Genetic Characterization and Time Period

Table A1: Number and Percentage of Positive Influenza A H3N2 Specimens, by Genetic Characterization and Time Period, Public Health Ontario, May 18, 2025 to November 15, 2025

Genetic Characterization	2025-26 Inter-season (May 18, 2025 – August 23, 2025)	2025-26 Early Season (August 24, 2025 – November 15, 2025)
H3N2	**12 (100%)**	**71 (100%)**
J.2	3 (25.0%)	2 (2.8%)
J.2.2	2 (16.7%)	2 (2.8%)
J.2.3	5 (41.7%)	0 (0.0%)
J.2.4	1 (8.3%)	11 (15.5%)
J.2.5	1 (8.3%)	0 (0.0%)
K	0 (0.0%)	56 (78.9%)
Total Sequenced	**12 (100%)**	**71 (100%)**

Note: Includes all influenza A(H3N2) samples tested at PHO that were eligible for sequencing and had sufficient volume. Date was assigned based on the earliest date available for the specimen. Influenza subclade nomenclature is dynamic and may be revised as additional information (e.g. mutational patterns) becomes available. The influenza A H3N2 vaccine component included in the 2025–26 northern hemisphere influenza season belongs to genetic subclade J.2 (clade 2a.3a.1).4

Figure A1: Number of Positive Influenza A H3N2 Specimens, by Genetic Characterization and Month, for the Inter-Season and Early Season, Public Health Ontario, May 18, 2025 to November 15, 2025



Note: *May and November are partial months. Inter-season time period includes May 18 to August 23, 2025, and early season time period includes August 24 to November 15, 2025. Month was assigned based on earliest date available for a specimen. The influenza A H3N2 vaccine component included in the 2025-26 northern hemisphere influenza season belongs to genetic subclade J.2 (clade 2a.3a.1)

Appendix B: Influenza A Specimens by Subtype

Table B1: Number and Percentage of Influenza A Specimens by Week and Subtype, Ontario Laboratory Information System, August 24, 2025 to November 29, 2025

Surveillance Week	H1N1pdm09	H3N2	Total
Week 35 (August 24 – August 30)	8 (72.7%)	3 (27.3%)	**11 (100%)**
Week 36 (August 31 – September 6)	7 (41.2%)	10 (58.8%)	**17 (100%)**
Week 37 (September 7 – September 13)	5 (55.6%)	4 (44.4%)	**9 (100%)**
Week 38 (September 14 – September 20)	11 (44.0%)	14 (56.0%)	**25 (100%)**
Week 39 (September 21 – September 27)	5 (38.5%)	8 (61.5%)	**13 (100%)**
Week 40 (September 28 – October 4)	9 (69.2%)	4 (30.8%)	**13 (100%)**
Week 41 (October 5 – October 11)	13 (61.9%)	8 (38.1%)	**21 (100%)**
Week 42 (October 12 – October 18)	10 (62.5%)	6 (37.5%)	**16 (100%)**
Week 43 (October 19 – October 25)	24 (63.2%)	14 (36.8%)	**38 (100%)**
Week 44 (October 26 – November 1)	36 (45.6%)	43 (54.4%)	**79 (100%)**
Week 45 (November 2 – November 8)	49 (36.0%)	87 (64.0%)	**136 (100%)**
Week 46 (November 9 – November 15)	104 (34.3%)	199 (65.7%)	**303 (100%)**
Week 47 (November 16 – November 22)	126 (26.1%)	357 (73.9%)	**483 (100%)**
Week 48 (November 23 – November 29)	172 (17.5%)	810 (82.5%)	**982 (100%)**
Week 49 (November 30 – December 6)	151 (15.0%)	855 (85.0%)	**1,006 (100%)**
Total	**730 (23.2%)**	**2,422 (76.8%)**	**3,152 (100%)**

Note: Data are shown at the specimen level. Includes all influenza A positive specimens that were successfully subtyped in Ontario. Overall, 59.3% of influenza A positive specimens were not subtyped or unsuccessfully subtyped.
Data source: Ontario Laboratory Information System data received by Public Health Ontario

Appendix C: Jurisdictional Comparison of Influenza A Specimens by Genetic Characterization

Table C1: Number and Percentage of Positive Influenza A Specimens, by Genetic Characterization and Jurisdiction, May 18, 2025 to November 16, 2025

Genetic Characterization	Ontario (August 24 – November 15)	Canada (September 1 – November 15)	United States of America (May 18 – November 15)	United Kingdom (September 29 – November 16)
H1N1pdm09	**75 (51.4%)**	**36 (58.1%)**	**293 (66.7%)**	**63 (22.0%)**
C.1.9.3	5 (3.4%)	1 (1.6%)	7 (1.6%)	1 (0.3%)
D.1	0 (0.0%)	0 (0.0%)	2 (0.5%)	0 (0.0%)
D.3.1	70 (47.9%)	35 (56.5%)	284 (64.7%)	62 (21.6%)
H3N2	**71 (48.6%)**	**26 (41.9%)**	**146 (33.3%)**	**224 (78.0%)**
J.2	2 (1.4%)	1 (1.6%)	15 (3.4%)	7 (2.4%)
J.2.2	2 (1.4%)	2 (3.2%)	9 (2.1%)	0 (0.0%)
J.2.3	0 (0.0%)	0 (0.0%)	19 (4.3%)	0 (0.0%)
J.2.4	11 (7.5%)	5 (8.1%)	21 (4.8%)	10 (3.5%)
J.2.5	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.3%)
K	56 (38.4%)	18 (29.0%)	82 (18.7%)	206 (71.8%)
Total sequenced	**146 (100%)**	**62 (100%)**	**439 (100%)**	**287 (100%)**

Note: 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[22], UK Health Security Agency[23]

Citation

Ontario Agency for Health Protection and Promotion (Public Health Ontario). Influenza genomic surveillance in Ontario: 2025–26 early season. Toronto, ON: King's Printer for Ontario; 2025.

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