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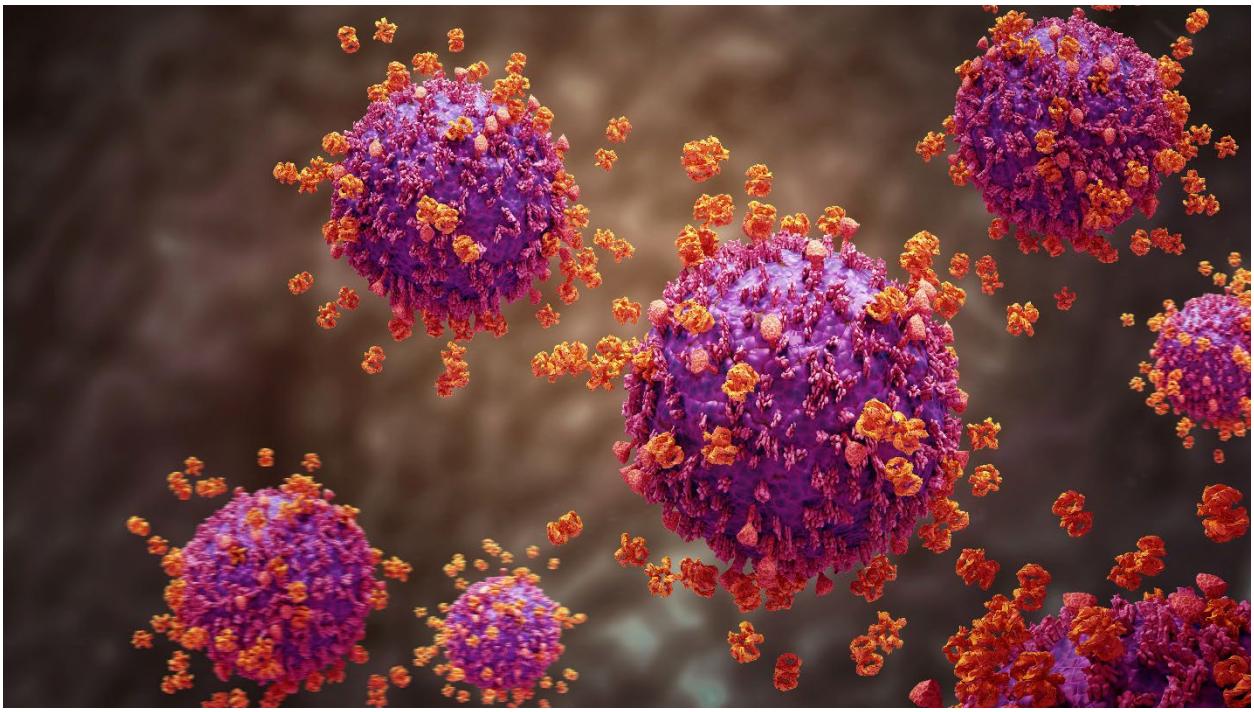
EVIDENCE BRIEF

Timing of Antiviral Susceptibility Testing During Influenza Outbreaks



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Key Messages

- There are no published, peer-reviewed literature identified in the Public Health Ontario search that specifically discussed the optimal time to test for antiviral susceptibility in individuals who developed influenza while on antiviral *prophylaxis*.
- Literature was available on the emergence of reduced antiviral susceptibility in influenza infected individuals while on *treatment* with antivirals, with evidence suggesting antiviral susceptibility may develop in as little as 2 days following the initiation of treatment.
- Guidance regarding antiviral susceptibility testing varied by jurisdiction, with most recommendations relying on expert opinions. Guidance from the United States and England recommend considering antiviral susceptibility testing if a person develops influenza at any time while on prophylaxis, while the guidance reviewed from Canadian provinces suggests timelines between 3 and 5 days.
- Relevant published and grey literature support the consideration of antiviral susceptibility testing in those who test positive for influenza after initiation and/or completion of influenza prophylaxis, with stronger consideration given as the duration of prophylaxis increases and not sooner than 24 hours after initiation.

Issue and Research Question

The identification of influenza strains with decreased antiviral susceptibility has been relatively uncommon in Canada. For example, during the 2024–25 season, just eight out of the 1,438 influenza specimens selected for testing demonstrated reduced susceptibility to oseltamivir, with all eight specimens being influenza A (H1N1).[1] In Ontario, the Influenza Genomic Surveillance data identified no influenza A (H1N1) specimens with genetic markers of resistance in the 2024–25 early influenza season and only 1.5% of specimens in the 2023–24 season.[2,3] Antiviral resistance is typically associated with the H275Y mutation, which can be detected through polymerase chain reaction (PCR) testing, and is associated with oseltamivir (but not zanamivir) resistance in influenza A H1N1pdm09 viruses.[4,5]

While infrequent, influenza outbreaks in congregate care settings involving influenza strains with decreased antiviral susceptibility have been documented in the literature,[6-10] and have occurred in Ontario. Outbreak management guidance can delay antiviral susceptibility testing until several days after antiviral prophylaxis begins. For example, Ontario guidance suggests that antiviral susceptibility testing (and testing for other respiratory viruses) can be considered in individuals who develop symptoms after 4 or more days of antiviral prophylaxis. Similarly, the Ontario guidance suggests that new cases of influenza-like illness in an outbreak area occurring after 4 days of antiviral prophylaxis use may indicate a circulating strain of influenza which has decreased antiviral susceptibility.[11] However, this approach may limit the ability to detect resistance early and respond promptly, which can be detrimental in congregate settings where transmission can occur rapidly. Despite the relative rarity of such events, a recent outbreak in Ontario highlighted the importance of rapid identification and management of cases and outbreaks involving influenza strains with decreased antiviral susceptibility.

This evidence review summarizes the available literature on the timing of antiviral susceptibility testing during influenza outbreaks, aiming to inform public health strategies and improve outbreak response in congregate settings.

Methods

Public Health Ontario (PHO) Library Services performed two literature searches of three databases (MEDLINE, Embase and CINAHL) to identify peer-reviewed publications on timing of antiviral susceptibility testing. The initial search was conducted on February 27, 2025, for articles published between 2015 to January 2025, followed by a second search conducted on June 27, 2025, to capture new or updated articles of interest.

The search was limited to articles published in English and included primary studies, reviews and editorials; animal studies were excluded. Title and abstract screening and full text screening were done by five reviewers independently using Covidence, and relevant information was extracted from each article.

PHO Library Services also developed a search strategy for identifying grey literature that was executed in March 2025. The search contained a list of targeted resources from key international public health organizations. No jurisdictional limits were applied to this search strategy.

In addition, a rapid jurisdictional scan was undertaken, reviewing relevant guidance documents from selected jurisdictions and public health organizations, including Canada, the US Centers for Disease Control and Prevention (CDC), the World Health Organization (WHO), Australia, and the UK Health Security Agency (UKSHA).

Main Findings

A total of 495 articles were identified through both literature searches, of which only eight met the inclusion criteria and were reviewed in more detail.[6-9,12-15] Two additional review articles were identified through hand search.[10,16]

Antiviral Susceptibility Testing in Newly Symptomatic Individuals Receiving Prophylaxis

None of the included studies addressed specific timing for when antiviral susceptibility testing should occur in individuals who developed influenza or influenza-like illness while on antiviral prophylaxis (e.g., at 72- or 96-hours post initiation).

Antiviral Susceptibility Testing After Treatment Initiation

All included articles reported on when antiviral susceptibility testing was performed during treatment and the timing of when resistance was first detected. Notably, none of the articles reported on outbreaks in congregate living settings (e.g., long-term care). Instead, most presented findings from hospital settings while one article reported on outpatient clinics. For these reasons, the available evidence can only provide indirect insights relevant to congregate living settings.

The time at which samples were collected post treatment initiation varied greatly between studies. As a result, the time of antiviral susceptibility detection varied between studies, with some reporting detection two to three days after treatment began and others reporting detection around 12 days after treatment initiation.

The largest study, conducted by Lina et al., collected samples from 1,207 patients who were treated with oseltamivir for influenza A H1N1pdm09 or H3N2 on days 1, 3, 6 and 10 in a prospective observational study.[6] Decreased antiviral susceptibility emerged in 43 patients (3.56%) and was first detected within 2 days of the initiation of treatment.

Holmes et al. offered similar insights, finding that decreased susceptibility during treatment usually develops 3–4 days post-infection, if at all.[10] However, they do not address the specific timing of decreased antiviral susceptibility relative to the initiation of antiviral treatment. In a summary article, Lampejo, states that resistant variants can emerge and be transmitted within 1–2 days of treatment. Although the published evidence supporting this statement is unclear, the article suggests that resistance testing can be performed at any time prior to, during or after antiviral treatment.[16]

Antiviral Susceptibility Testing Guidance for Newly Symptomatic Individuals Receiving Prophylaxis

The rapid jurisdictional scan included a review of relevant guidance documents from British Columbia, Quebec, CDC, England, Australia, and New Zealand. Guidance for timing of antiviral susceptibility testing in individuals who developed influenza while on antiviral prophylaxis varied across jurisdictions. Some provided default timeframes for testing ranging between 72 hours to 5 days, while others recommended testing only in the event of breakthrough cases or offered no guidance, citing limited evidence to support testing recommendations.

Both the Provincial Infection Control Network of British Columbia (PICNet) and the Institut national de santé publique du Québec recommend testing for antiviral susceptibility when patients in long-term home and care settings develop influenza after receiving prophylaxis with oseltamivir.[17,18] In British Columbia, testing is considered when new cases of influenza emerge 4–5 days post implementation of infection prevention and control (IPAC) measures including prophylaxis,[17] and in Quebec, testing is considered if a patient develops influenza after 72 hours on prophylaxis.[18] In contrast, the Association of Medical Microbiology and Infectious Disease Canada foundation does not specify timelines for when susceptibility testing should be considered in the case of breakthrough influenza.[19]

Outside of Canada, the Infectious Disease Society of America (IDSA) and Public Health England recommend considering testing for antiviral susceptibility whenever a patient develops influenza while on, or shortly after receiving prophylaxis.[20,21] Similarly, the United States CDC recommends having a high index of suspicion whenever a patient develops influenza anytime after starting antiviral prophylaxis.[22] The IDSA notes that there is poor evidence to support their recommendation and that it is based on the opinions of respected authorities.[20]

Guidance on antiviral use from Australia and New Zealand does not provide specific recommendations regarding susceptibility testing in cases of influenza infection among individuals currently receiving or who have recently received antiviral prophylaxis.[23,24]

Discussion and Conclusions

Overall, there were no published, peer-reviewed literature identified that specifically discussed the timing of antiviral susceptibility testing in individuals who developed influenza or influenza-like illness while on antiviral prophylaxis. The available literature focused only on the emergence of reduced antiviral susceptibility in influenza infected individuals while on treatment with antivirals. Evidence from these observational studies suggest that decreased antiviral susceptibility may develop in as little as 2 days following the initiation of treatment.

Guidance on when to conduct susceptibility testing varies across jurisdictions. International guidance from the United States and England recommend considering antiviral susceptibility testing if a person develops influenza at any time while on prophylaxis. In contrast, guidance from Canadian provinces suggests considering testing after 3 and 5 days of prophylaxis. This variation in guidance reflects the lack of consensus in the literature and underscores the need for more research.

Overall, the reviewed literature did not provide conclusive evidence on the appropriate time for conducting antiviral susceptibility testing in newly symptomatic individuals receiving oseltamivir prophylaxis.

Implications for Practice

Individuals receiving antiviral prophylaxis found to be infected with a strain of influenza with decreased resistance may have either acquired the resistant strain from a previous case (i.e., acquired resistance) or develop decreased susceptibility during prophylactic treatment (i.e., emergent resistance). Both patterns of acquisition are considered rare as influenza strains with decreased antiviral susceptibility are uncommon in Ontario and Canada.

Among individuals with acquired resistance, symptoms may develop within the typical incubation period of 1–4 days following exposure.[16] On the other hand, the timeline for developing decreased susceptibility (i.e., emergent resistance) in those receiving oseltamivir prophylaxis is unclear. In the absence of evidence specific to oseltamivir prophylaxis, research related to the development of decreased susceptibility while receiving oseltamivir treatment is instructive. For individuals diagnosed with an influenza infection and subsequently started on oseltamivir treatment, there is limited evidence suggesting that decreased susceptibility may develop as early as 2 days following the start of treatment.

Overall, the potential for clinical disease in an individual receiving oseltamivir prophylaxis due to a strain of influenza with decreased antiviral susceptibility may be as low as 1–2 days following exposure or the initiation of antiviral prophylaxis.

Antiviral susceptibility testing may be considered for individuals who test positive for influenza after the initiation or completion of oseltamivir prophylaxis. Testing is generally not recommended within the first 24 hours of starting prophylaxis, as the likelihood of acquired or emergent resistance during this period is extremely low, except in cases involving close contact with a confirmed case of influenza with reduced antiviral susceptibility. The rationale for testing strengthens as the duration of prophylaxis increases or if multiple individuals in an outbreak setting develop influenza while on prophylaxis. These circumstances may warrant further investigation to assess potential resistance patterns and inform public health response.

While information on the antiviral susceptibility of circulating influenza viruses has the potential to inform public health responses, it remains unclear to what extent early susceptibility testing could impact outbreak outcomes. Moreover, expanded susceptibility testing could identify outbreaks with mixed susceptibility patterns, for which guidance is lacking in terms of antiviral treatment and prophylaxis. These are important implementation considerations, but they fall outside the scope of the stated objective of this evidence brief. Regional inequities may also have practice implications for testing. For instance, in rural and remote areas with limited courier service or batched specimen transportation, changes in testing practices may not result in earlier identification of influenza strains with decreased antiviral susceptibility due to delays in specimen arrival at the laboratory.

Specification and Limitations

This Evidence Brief present key findings from the best available evidence. Its purpose is to investigate a research question in a timely manner to help inform decision making. This report presents key findings, based on a systemic search, screen and extraction of the best available evidence near the time of publication. There may be relevant studies that are not included, and these may alter the conclusions drawn from the report.

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