# HPA Guidance for Neuraminidase Inhibitor Resistance testing of Influenza B and A(H3N2) viruses

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**Version 1.0**

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Microbiology Services. HPA Guidance for Neuraminidase Inhibitor Resistance (NAI) testing of Influenza B and A(H3N2) viruses. Version 1. Health Protection Agency 2012

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## Background

Oseltamivir resistance can be generated relatively easily in A(H1N1)pdm09 strains, in contrast to influenza A(H3N2) and influenza B strains, although resistance can be a particular problem in the immunocompromised. Zanamivir resistance is uncommonly observed in all circulating influenza A and B strains (see appendix). HPA public health laboratories within the specialist microbiology network can detect the common major oseltamivir resistance mutation (H275Y) of A(H1N1)pdm09 using locally available assays. Neuraminidase inhibitor (NAI) resistance testing of all other influenza A and B strains is available within the HPA only at microbiology services Colindale RVU.

The clinical settings for suspicion of NAI resistance in influenza A(H3N2) and influenza B viruses remain the same as those associated with NAI resistance in A (H1N1) (see laboratory testing guidance) but the likelihood is significantly lower.

Clinical guidance recommends sampling at 5 days if the patient is deteriorating, to monitor for continued viral shedding, important for infection control, irrespective of subtype. Such retesting may allow changes in viral load to be considered in the overall evaluation of patient response to treatment, with caution over comparing sample types. Testing for NAI resistance in A(H1N1)pdm09 infections is recommended at this stage, but is not strictly necessary in all A(H3N2) or influenza B patients who remain virus positive at day 5 of treatment.

As a guide, give consideration to testing A(H3N2) and influenza B for antiviral resistance in the following specific clinical circumstances

1. **If there has been unsatisfactory clinical response to NAI treatment after 10 days, and non-viral causes are unlikely, and influenza virus remains detectable at a significant level (Ct level indicating a reliable positive result).**
2. **In any patient who has been on any NAI therapy for greater than 1 month.**

**Note: Samples with Ct values of >= 34 are unlikely to yield a result for NAI resistance testing and should only be sent following discussion with RVU staff**

*Please note that all positive influenza A or B samples received by the RVU for NAI resistance testing performed as part of clinical management will attract a charge.*

## Surveillance for Antiviral Resistance

Surveillance testing will be done by the reference laboratory at MS Colindale regularly on an appropriate subset of material arriving in the reference laboratory, from all sources throughout the season, with particular emphasis on looking for evidence of community transmission of resistance or emergence in high risk populations. This is *not charged for,* and sample results will be reported to the sending laboratory, although not necessarily in a clinically relevant time frame. Any change to the baseline data (i.e. detection of viruses with altered NAI susceptibility) will be communicated to the network promptly.

## Appendix 1: Significance of Influenza Type and Subtype

The clinical groups at highest risk of developing NAI resistance are similar irrespective of influenza type and subtype. However, the incidence of NAI resistance in randomised clinical trials and observational studies has been significantly lower with influenza A (H3N2) and influenza B infection than with viruses containing the N1 neuraminidase (A(H1N1)pdm09 and former seasonal H1N1) [1] and possibly H5N1. These studies also show that children and severely immunocompromised patients are at higher risk of NAI resistance emergence, likely to be due to higher viral load and prolonged shedding. Overall incidence in these studies of oseltamivir resistance in any subtype was 2.6%, and 0% for zanamivir resistance.

Fundamental differences in enzyme structure dictates that NAI resistance is less likely to occur in influenza B NA and A N2, compared with A N1, without causing significant loss of enzyme function and therefore viral fitness, unless multiple compensatory mutations are also introduced [2, 3]. Differences in the chemical structure of oseltamivir and zanamivir and their binding into the enzyme active site also determine that zanamivir resistance is less likely, without causing significant impairment of enzyme function and therefore viral fitness [4].

There are several differences considering NAI resistance in A(H3N2) and influenza B versus A(H1N1) viruses;

1. The mechanism of NAI resistance in A (H3N2) and influenza B viruses is complex, with several mutations having been described, but each one infrequently [5]. It is likely that there are several mutations which would confer NAI resistance or altered susceptibility which have not yet been identified in influenza B NA and A N2

**Note: As mutations in A N2 and B NA virus associated with NAI resistance are currently rare and varied, rapid single snp screening assays would not be effective, in contrast to the situation with H275Y in A N1.**

2. Most of the mutations detected in a clinical setting have no impact on zanamivir sensitivity.
3. Most case reports of A (H3N2) and Influenza B NAI resistance show resistant virus emergence only after prolonged treatment of >10 days, in many cases >1 month [1].
4. *In vitro* and *in vivo* studies show that A (H3N2) and Influenza B NAI resistant viruses are less able to transmit and have lower replicative capacity [6].
5. Several case studies, in which oseltamivir resistance in A (H3N2) virus did arise, show that the resistant variant disappeared after oseltamivir therapy was stopped [7].

**Note: This is in contrast to A N1, where the H275Y mutation is known to persist after cessation of treatment.**

6. Untargeted surveillance has detected relatively few NAI resistant A (H3N2) and Influenza B viruses [8] and the clinical significance of minor alterations in *in vitro* susceptibility is not clear.

## References


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