Surveillance and Laboratory Testing of Influenza Neuraminidase Inhibitor Resistance
About Public Health England

Public Health England exists to protect and improve the nation’s health and wellbeing, and reduce health inequalities. We do this through world-class science, knowledge and intelligence, advocacy, partnerships and the delivery of specialist public health services. We are an executive agency of the Department of Health, and are a distinct delivery organisation with operational autonomy to advise and support government, local authorities and the NHS in a professionally independent manner.

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Scope and background

Scope
This guidance summarises the incidence and mechanisms of neuraminidase inhibitor (NAI) resistance in seasonal influenza viruses, and the availability of NAI susceptibility testing in the UK. The UK national NAI susceptibility surveillance strategy and considerations for susceptibility testing in clinical situations are also described.

Background
This information is for laboratory scientists, clinicians and public health practitioners with responsibility for influenza virus diagnostic testing, the management of patients receiving NAI prophylaxis or treatment or investigation of influenza virus outbreaks. Public Health England (PHE) guidance on antivirals for the treatment and prophylaxis of influenza is available separately.

There are two NAIs approved for use in the UK for treatment and prophylaxis of influenza: oseltamivir (Tamiflu) and zanamivir (Relenza). Two further NAIs are licenced in Japan, laninamivir and peramivir; the latter is also approved in China, Republic of Korea and USA. Novel influenza antivirals targeting viral proteins or host factors are in late-phase clinical trials but not yet approved for use in the UK. The PHE NAI susceptibility surveillance strategy incorporates data on all NAIs, but there is currently no active susceptibility surveillance for non-NAI anti-influenza drugs.

WHO global surveillance of human seasonal influenza viruses collected in 2014-2015 found >99% of viruses tested to be fully susceptible to all NAIs. A small number of viruses with reduced susceptibility to one or more of the four NAIs were detected (approximately 0.5%); of note, this included a number of genetically similar influenza B viruses detected in China and Australia with the D197N NA mutation, known to be associated with reduced susceptibility to oseltamivir, zanamivir and peramivir. In the period 2014-15, the overall frequency of viruses with reduced susceptibility to NAIs was lower than that observed in 2013-14 (1.9%), but similar to the 2012-13 period (0.6%). The higher proportion in 2013-14 was due to genetically similar but epidemiologically unlinked clusters of oseltamivir resistant H275Y A(H1N1)pdm09 variant virus in the USA, Japan and China. Data for 2015-16 is undergoing analysis by the WHO GISRS antiviral working group (AVWG), but there has been no change in incidence of NAI resistance (WHO GISRS AVWG Meeting, Aug, 2016).

A robust NAI susceptibility surveillance strategy is critical to detect early potential emergence of NAI resistance, to ensure optimal advice on patient management and to maintain public health preparedness to respond to NAI resistant variant virus outbreaks.
Diagnostic testing of NAI susceptibility

Whether performed regionally or by the Respiratory Virus Unit (RVU), PHE Colindale, susceptibility testing for patient management may attract a charge. Nonetheless, in many cases resistance testing is critical to determine appropriate management (see Appendix 1). For further information on testing and charging by RVU refer to guidance on referral of influenza samples to Respiratory Virus Unit, PHE Colindale.

Which patients should be monitored for NAI resistance?

The requirement for susceptibility testing should be decided clinically, but maintain a high index of suspicion in the following risk groups (refer to PHE treatment guidance):

- severely immunosuppressed patients, particularly if NAI treated or a contact of treated cases
- patients who become influenza positive whilst, or shortly after, receiving NAI prophylaxis
- influenza positive contacts of confirmed NAI resistance cases
- patients switching NAIs, particularly if they have received non-concurrent NAI treatments in the same illness episode
- any patient who does not respond clinically or deteriorates during NAI therapy

When should NAI susceptibility testing be requested?

Susceptibility testing can be requested at any time during or after treatment. Genotypic testing (allelic discriminating RT-PCR; pyrosequencing) can identify low proportions of resistant virus. Testing prior to or early in treatment may be appropriate for severely immunosuppressed patients, in whom resistance can develop rapidly.

Any influenza positive sample can be tested for resistance. However, samples with high typing/subtyping PCR Ct values are less likely to yield a result for NAI susceptibility testing.

References

What tests are available for determining NAI susceptibility?

**H275Y SNP detection assays for A(H1N1)pdm09 virus (real time RT-PCR)** are available at PHE Public Health laboratories (PHLs) and several laboratories in Scotland. As H275Y is the most frequently detected resistance mutation, rapid assays are available regionally. To investigate potential resistance in oseltamivir-treated A(H1N1)pdm09 infection, contact the relevant laboratory.

**Resistance SNP detection assays (pyrosequencing) for all seasonal influenza A subtypes and influenza B are available at RVU, Colindale PHE.** Resistance in influenza B and A(H3N2) is infrequent, as is zanamivir resistance in all influenza viruses. Resistance SNPs are diverse so testing is not yet available at regional PHE laboratories. RVU performs pyrosequencing assays (short range SNP sequencing) **on request only**, to rule out the most common resistance SNPs (see Appendix 2).

**Full length neuraminidase sequencing is performed at RVU to screen for all mutations that have been previously identified as causing NAI resistance.** RVU performs Illumina genome sequencing on all samples when resistance testing is requested. This can take approximately two weeks to complete.

**Phenotypic testing, performed by RVU confirms the role of novel mutations.** Phenotypic testing (enzyme inhibition assay) requires virus isolation, and therefore results cannot be obtained in a clinically relevant time frame for most cases.

**UK NAI susceptibility surveillance strategy**

The UK strategy for influenza NAI susceptibility surveillance is designed to both meet national needs and the requirements for reporting of NAI resistance to WHO. Any indication of increasing incidence of NAI resistance or circulation of NAI resistant variants in the community will be promptly communicated to the UK influenza laboratory network, via teleconferencing and/or briefing notes, with further cascading of information if appropriate for example if changes in use of antivirals is recommended.

**NAI susceptibility virological surveillance**

RVU tests a subset of material from all sources throughout the season, by whole virus genome sequencing (Illumina) and NA enzyme inhibition assay with oseltamivir and zanamivir. Peramivir and laninamivir are also tested, but on a less frequent basis.
Data from samples tested on the basis of clinical need forms part of the national surveillance dataset, but particular emphasis is placed on surveillance for community transmission or emergence in high risk populations. In the absence of known circulation of resistant variants, PHE will do the following:

- monitor susceptibility to oseltamivir and zanamivir in sentinel GP samples to
  o maintain a baseline of NAI susceptibility for week by week and year by year comparison
  o detect community transmission of NAI resistant variants in a timely manner
- analyse a proportion of samples from hospitalised influenza cases to:
  o monitor for resistant virus in potential cases (resistance testing was not requested)
  o detect increasing resistance frequency (potential accumulation of permissive or compensatory mutations)

**NAI susceptibility epidemiological surveillance**

The **goal of influenza antiviral susceptibility surveillance in the UK is to inform optimal clinical therapy and identify factors associated with resistance.** This will be achieved by:

- study of the epidemiology of NAI resistance in time, place and person, by age, gender and clinical risk factors
- study of the clinical features of NAI resistant influenza, in particular the use of antivirals and treatment outcome
- comparison of the demographic, epidemiological and clinical characteristics of virologically confirmed NAI resistant influenza to NAI susceptible influenza cases

**Surveillance indicators (total patients tested serves as denominator) used are:**

- weekly frequency of confirmed NAI resistant cases by virus sub-type and primary and secondary care
- cumulative number of NAI resistant influenza cases from week 40, by week, age group, region, immune compromise status, pre-sampling use of antivirals and mortality

**Reporting of NAI susceptibility surveillance data and investigation of resistant cases**

NAI susceptibility data, from surveillance and diagnostic testing, are reported locally, nationally and internationally, at regular intervals throughout the season.
Details of any resistant cases are referred by RVU to the Respiratory Diseases Department, PHE National Infection Service, for follow up and collation of clinical and epidemiological data. For resistance cases from all sources (community, outpatient, hospitalised, outbreak) a questionnaire is issued to the patient’s GP, to capture as many data as possible, including outcome (See Appendix 3).

If the resistant case is hospitalised, RVU will request that any further samples obtained are also forwarded for further resistance testing and characterisation.

If the resistant case has received treatment, pre-treatment samples, if available, should be sent to RVU, to investigate whether resistance occurred \textit{de novo} or was associated with treatment received.

The following information should be included on referral forms when resistance testing is requested, to aid the interpretation of results:

- NAI treatment start and end dates
- immune compromise status
- any other underlying health conditions or risk factors for severe disease
- details of patient contact with treated patients, and travel history

This information will be requested automatically by RVU if resistance is detected on any sample and relevant information has not been provided on the request form.

DA: devolved administrations; SMN: specialist microbiology network; LIMS: laboratory information management system; PII: patient identifying information
Appendix 1: NAI susceptibility testing availability

Appendix 2: Mechanisms of neuraminidase inhibitor resistance

Viruses achieve NAI resistance by mutation of amino acids in and around the neuraminidase enzyme active site. In most cases, these substitutions reduce the affinity of the NAI binding, and have a detrimental affect on viral fitness and/or transmissibility.
Influenza neuraminidases are divided by sequence and structure into three groups; influenza A group 1 (N1, N4, N5, N8) and group 2 (N2, N3, N6, N7, N9) and influenza B (Victoria and Yamagata). The binding pocket for the natural substrate and the NAIs differs in size and shape between these three groups and as such, each virus type/subtype generates resistance to NAIs by a different mechanism. Equally, since each of the NAIs differ in structure, and therefore by binding mechanism to the NA active site, the mechanism of resistance to each drug is different.

For more information on the mechanisms of NAI resistance, refer to the International Society for Influenza and Respiratory Viruses Antiviral Group’s website.

The table below gives details of the most frequently observed mutations and the resistance profile of these to the 4 NAIs. Due to differences in drug and viral structure, oseltamivir resistance occurs more frequently in A(H1N1)pdm09 strains than in influenza A(H3N2) and influenza B strains. Zanamivir resistance is uncommonly observed in all circulating influenza A and B strains.

<table>
<thead>
<tr>
<th>Amino Acid Change</th>
<th>Oseltamivir</th>
<th>Zanamivir</th>
<th>Peramivir</th>
<th>Laninamivir</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A(H1N1)pdm09</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H275Y</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>I223K/R/V</td>
<td>RS</td>
<td>S/RS</td>
<td>UNK</td>
<td>UNK</td>
</tr>
<tr>
<td><strong>A(H3N2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E119V</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>DEL 245-248</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>UNK</td>
</tr>
<tr>
<td>R292K</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>UNK</td>
</tr>
<tr>
<td>N294S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>UNK</td>
</tr>
<tr>
<td><strong>Influenza B (Victoria and Yamagata Lineages)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E105K</td>
<td>S</td>
<td>RS</td>
<td>R</td>
<td>RS</td>
</tr>
<tr>
<td>R150K</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>UNK</td>
</tr>
<tr>
<td>D197/N/E</td>
<td>RS</td>
<td>RS</td>
<td>RS</td>
<td>UNK</td>
</tr>
<tr>
<td>I221L/T/V/I</td>
<td>R</td>
<td>RS</td>
<td>RS</td>
<td>S</td>
</tr>
</tbody>
</table>

RS = reduced susceptibility, R = resistance, S = susceptible

Data summarised from the World Health Organization (WHO) Global Influenza Surveillance and Response System (GISRS) expert working group on surveillance of influenza antiviral susceptibility (WHO-AVWG), available here.

Amino acid changes in bold represent the SNPs for which RVU will, on request, perform rapid screens for diagnostic specimens; the screens are based on the frequency of detection (published case reports and UK testing) and the resistance profile for NAIs used in the UK.
Appendix 3: NAI Resistance follow up questionnaire

IN STRICT MEDICAL CONFIDENCE: INFLUENZA ANTIVIRAL QUESTIONNAIRE

Public Health England, Enhanced influenza surveillance

Patient name: Date of birth:  
NHS Number: PHE Ref:  

1. Gender: ☐ Male ☐ Female (Please tick as appropriate) 

2. Ethnicity:  
   White: ☐ British ☐ Irish ☐ Other  
   Mixed: ☐ White and Black Caribbean ☐ White and Black African  
          ☐ White and Asian ☐ Other mixed  
   Asian/Asian British: ☐ Indian ☐ Pakistani  
                        ☐ Bangladeshi ☐ Other Asian  
   Black or Black British: ☐ Black Caribbean ☐ Black African ☐ Other Black  
                          ☐ Chinese/Other: ☐ Chinese ☐ Other ☐ Unknown  

3. If female, is the patient pregnant? ☐ Yes ☐ No  
   If yes, EDD: ………./……../……….

4. Date of onset of first symptoms: ………./……../……….

5. Any recent foreign travel history and which country if travelled? ………………………………………..

6. Was a sample collected for laboratory confirmation of influenza? ☐ Yes ☐ No  
   If yes, date of sample: ………./……../……….
   Result: ☐ Influenza A/H1N1 (2009) ☐ Influenza A/H3N2 ☐ Influenza B  
            ☐ Influenza (other), please specify________  
            ☐ Negative

7. Did the patient take antivirals? ☐ Yes ☐ No ☐ Unknown  
   If yes, which: ☐ Oseltamivir (Tamiflu) ☐ Zanamivir (Relenza)  
   If yes, what date started: ………./……../……….

8. Has the patient received the following influenza vaccines?

8.1 Seasonal influenza vaccine for 2016/17: ☐ Yes ☐ No ☐ Unknown  
   If yes, date of vaccination: ………./……../……….
   Batch no: ………..Manufacturer: …………..

8.2 Seasonal influenza vaccine for 2015/16: ☐ Yes ☐ No ☐ Unknown  
   If yes, date of vaccination: ………./……../……….
   Batch no: ………..Manufacturer: …………..
8.3 Seasonal influenza vaccine for 2014/15: □ Yes □ No □ Unknown

If yes, date of vaccination: ........../........../........... Batch no: ............... Manufacturer: .................

9. Was patient admitted to hospital? □ Yes □ No □ Unknown

If yes, which hospital: ................. Name of consultant: .................

Date of Admission: ........../........../...........

10. Was the patient admitted to ITU? □ Yes □ No

11. Please list complications during admission: □ Viral pneumonia □ ARDS □ Shock

□ Secondary bacterial pneumonia □ Renal Failure □ Encephalitis □ Other: ..............

If patient had secondary bacterial pneumonia, list organism if known: ..............

12. Did the patient require mechanical ventilation? □ Yes □ No

13. Date of death: ........../........../...........

Cause of death: ..................................................

Contribution of influenza to death as listed on the death certificate:
□ Underlying/primary □ Contributing/secondary □ No contribution to death □ Unknown

14. Did a clinician verify this death as being associated with influenza? □ Yes □ No

15. Was a post-mortem performed? □ Yes □ No □ Unknown

If yes, what were the results? ..................................................

16. Is the patient in any of the following clinical risk groups for severe influenza?

a. Chronic respiratory disease, excluding asthma □ Yes □ No

b. Chronic heart disease □ Yes □ No

c. Chronic renal disease □ Yes □ No

d. Chronic liver disease □ Yes □ No

e. Chronic neurological disease □ Yes □ No

f. Diabetes requiring insulin or oral hypoglycaemic □ Yes □ No

g. Obesity □ Yes □ No

h. Immunosuppression (due to disease) □ Yes □ No

i. Immunosuppression (due to treatment) □ Yes □ No

j. Asthma, requiring treatment within last 3 yrs □ Yes □ No

If YES to j: Does patient require continuous or repeated use of inhaled or systemic steroids or had previous exacerbations requiring hospitalisation? □ Yes □ No

k. Any other relevant medical condition? □ Yes □ No

If YES to any of above, please describe ..................................................

Any other comments: ............................................................................

COMPLETED BY:

Name of doctor: ........................................ GP Telephone: .........................

Address: .................................................. Date ____/____/____