Public Health England

Protecting and improving the nation's health

Surveillance and Laboratory Testing of Influenza Neuraminidase Inhibitor Resistance

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Scope and background

Scope

This guidance summarises the current incidence and mechanisms of neuraminidase inhibitor (NAI) resistance in seasonal influenza viruses, and the availability of antiviral susceptibility testing in the UK. The UK national influenza antiviral susceptibility surveillance strategy and considerations for susceptibility testing in clinical situations are also described. This information is for laboratory scientists, clinicians and public health practitioners with responsibility for influenza virus diagnostic testing, the management of patients receiving antiviral prophylaxis or treatment, or investigation of influenza virus outbreaks. PHE guidance on antivirals for the treatment and prophylaxis of influenza is available.

Background

In the UK there are 2 NAIs approved for treatment and prophylaxis of influenza: oseltamivir (Tamiflu™) and zanamivir (Relenza™). Peramivir (Alpivab™), an intravenously administered NAI was approved for use by the EMA in 2018 (1), and has been in use in Japan, China, the Republic of Korea and the USA for several years. Laninamivir (Inavir™) is an inhaled, single dose NAI which is licenced in Japan. WHO global surveillance of human seasonal influenza viruses collected in 2016-2017 (13672 viruses) found >99% of viruses tested to be fully susceptible to all 4 NAIs (2).

The frequency of viruses with reduced susceptibility to NAIs been consistently low since publication of this global analysis began (2015/16: 0.8%, 2014/15: 0.5%; 2013/14: 1.9%; 2012/13: 0.6%) but 2016/17 has the lowest frequency observed to date at 0.2%, demonstrating that neuraminidase inhibitors remain suitable for treatment and prophylaxis of influenza virus infections.

Sporadic outbreaks of resistant virus have occurred, as well as global circulation of an oseltamivir resistant H275Y variant, showing that 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 (3-8).

Baloxavir marboxil (Xoflusa™) is an orally administered single dose cap dependent endonuclease inhibitor, which was licenced for use in Japan in May 2018, and the USA in October 2018 (9, 10). Further novel influenza antivirals targeting viral proteins or host factors are in late-phase clinical trials but not yet approved for use (11).

The PHE antiviral susceptibility surveillance strategy gathers data on all NAIs, but there is currently no susceptibility surveillance for non-NAI anti-influenza drugs. Clinical trials

did identify reduced susceptibility of viruses to baloxavir following treatment. Therefore there will be an expansion of surveillance testing to incorporate novel influenza antivirals as licensure is more widely achieved and usage increases (12).

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Diagnostic Testing of NAI Susceptibility

Whether performed regionally or by the Respiratory Virus Unit (RVU), PHE Colindale, susceptibility testing for patient management will 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 contacts of treated cases
- patients switching NAIs, particularly if they have received non-concurrent NAI treatments in the same illness episode
- patients who become influenza positive whilst, or shortly after, receiving NAI prophylaxis
- influenza positive contacts of confirmed NAI resistance cases
- 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 is appropriate for severely immunosuppressed patients, in whom resistant virus can transmit or 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.

Samples should be referred to RVU using the Influenza typing request form (E3) and ticking the resistance testing box
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.

What tests are available for determining NAI susceptibility?

H275Y 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 regional PHL.

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; therefore testing is not 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 whole genome sequencing on all samples when resistance testing is requested. This can take approximately 2 weeks to complete, but may take longer (refer to the VRD user manual).

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. Phenotypic testing is reserved for surveillance and characterisation of viruses with resistance SNPs.

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 the 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

The Respiratory Virus Unit conducts virological surveillance for NAI susceptibility using:

- genotypic susceptibility screening by analysis of full length neuraminidase sequencing generated by Illumina whole viral genome sequencing
- phenotypic susceptibility characterisation for oseltamivir and zanamivir of a subset of viruses using an enzyme inhibition assay (IC50)
- peramivir and laninamivir are also tested, but on a less frequent basis

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:
  - maintain a baseline of NAI susceptibility for week by week and year by year comparison
  - detect community transmission of NAI resistant variants in a timely manner

- analyse a proportion of samples from hospitalised influenza cases to:
  - monitor for resistant virus in potential cases (for which resistance testing was not requested)
  - 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 data

NAI susceptibility data, from surveillance and diagnostic testing, are reported locally, nationally and internationally, at regular intervals throughout the season.

Interval	Reported To	Reported By	Data Provided	Issued Via
Daily	Sending laboratory	RVU	Case based	LIMS
Weekly	DataMart	SMN, RVU	Aggregated	Closed email group
Weekly	PHE Flu report	RVU, DAs	Aggregated	Public web-based
Weekly	FluNews Europe	RVU, DAs	Case based: no PII	Public web-based
Annually	WHO vaccine selection group	RVU, DAs	Aggregated	International expert group: invitation only

DA: Devolved Administrations; SMN: Specialist Microbiology Network; LIMS: Laboratory Information Management System; PII: Patient Identifying Information

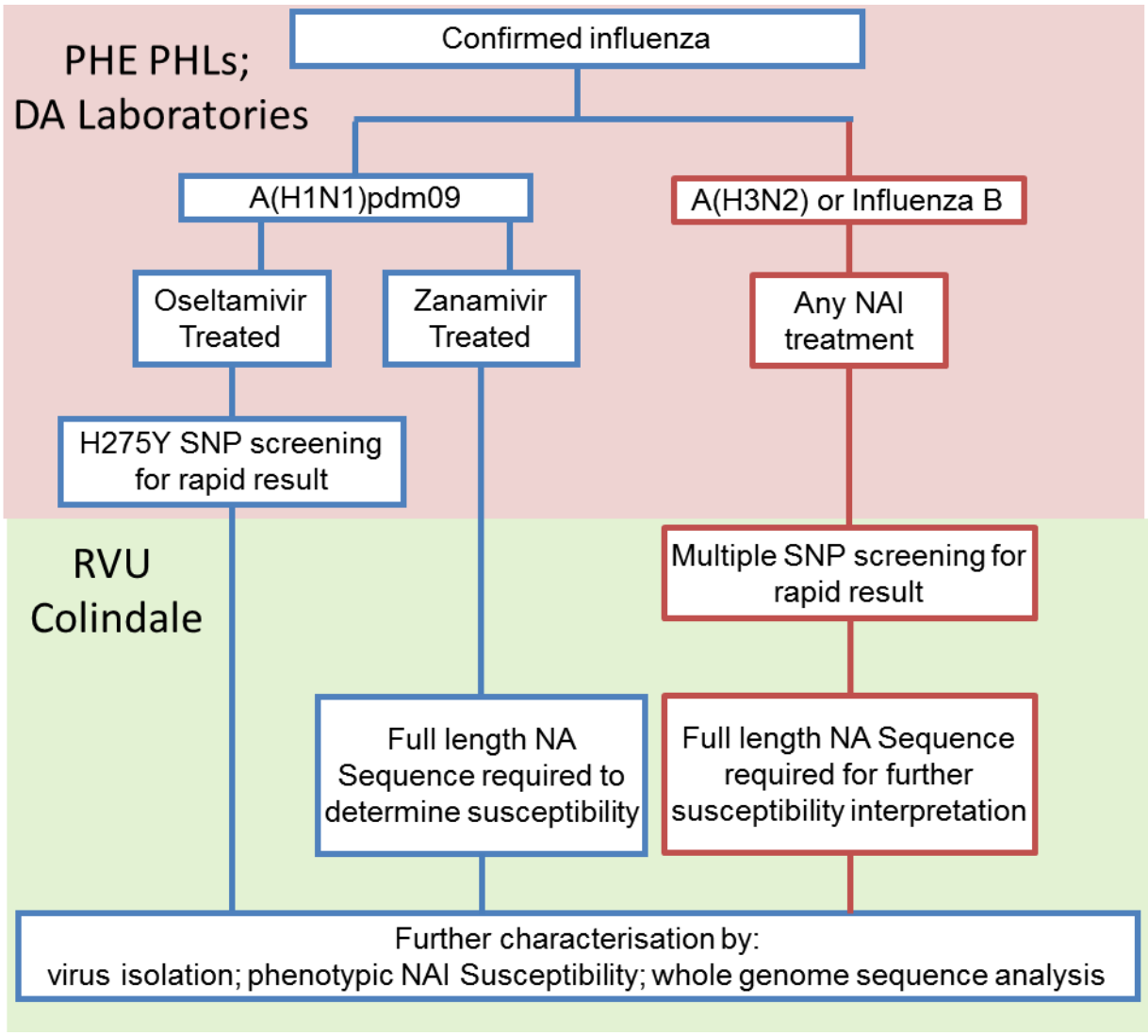
Investigation of confirmed resistant cases

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).

For resistance cases referred from local or regional microbiology laboratories, RVU will also issue the questionnaire to the clinical virologist or treating physician to capture information not included on the sample referral form rapidly:

- if the resistant case has received treatment:
  - pre-treatment samples, if available, should be sent to RVU to investigate whether resistance is *de novo* or associated with treatment received
- if the resistant case remains hospitalised:
  - further influenza positive samples obtained should be forwarded to RVU for further resistance testing and characterisation
  - follow up testing from a resistant case after identification will not be charged

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 effect on viral fitness and/or transmissibility. Influenza neuraminidases are divided by sequence and structure into 3 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 3 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. These data are 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), availabble here.

Amino acid changes in bold represent the SNPs for which RVU can perform rapid screens for diagnostic specimens; selected based on the frequency of detection (published case reports and UK testing) and the NAIs used in the UK.

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

RS= Reduced susceptibility, R=Resistance, S=Susceptible

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 2018/19: ☐Yes ☐No ☐Unknown

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

8.2 Seasonal influenza vaccine for 2017/18: ☐Yes ☐No ☐Unknown

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

8.3 Seasonal influenza vaccine for 2016/17: ☐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...../...../..... C*ause 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 ____/_____/____