UK Health Security Agency

Guidance for the referral of influenza samples to the Respiratory Virus Unit, UK Health Security Agency, Colindale

2021 to 2022 season

Contents

Background

General principles

National surveillance of influenza depends on accurate and timely virological information indicating which influenza viruses are circulating and how closely related they are to seasonal vaccine components. Virological surveillance is achieved through the detailed analysis of samples which are taken as part of illness diagnosis within the NHS, with emphasis on unusual or severe illness, and samples taken specifically for surveillance purposes, usually via UK Health Security Agency (UKHSA) laboratories.

NHS laboratories can refer samples to the local UKHSA Public Health Laboratory (PHL) for diagnosis of influenza A and B infection, influenza A subtyping (only if subtyping is not available locally in the NHS laboratory) and the detection of H275Y mediated oseltamivir resistance in A(H1N1)pdm09 viruses. Influenza A subtyping should be performed in the local NHS laboratory if available. UKHSA laboratories will test for H275Y mediated oseltamivir resistance for individual patient management if not available locally, but will not routinely test all A(H1N1)pdm09 viruses.

The details of which UKHSA PHL and collaborating laboratories perform influenza A subtyping and H275Y mediated oseltamivir resistance in A(H1N1)pdm09 for their local NHS laboratories, is shown below in Table 1.

Table 1. UKHSA PHL and collaborating laboratories performing influenza A subtyping and H275Y mediated oseltamivir resistance in A(H1N1)pdm09

UKHSA and collaborating laboratories	Influenza A Subtyping (H1pdm09/H3) performed	H275Y (Oseltamivir sens/res) performed
Birmingham	Yes	Yes
Bristol	Yes	No
Cambridge	Yes	Yes
Manchester	Yes	Yes
Leeds	Yes	No
Newcastle	Yes	No

Some of these samples may then be referred to the reference laboratory (free of charge) for detailed virological surveillance. Testing for influenza performed by a UKHSA PHL for individual patient management will be charged for. No charge will be made by UKHSA PHLs performing subtyping of influenza A positive samples from severe (Intensive Therapy Unit - ITU/High Dependency Unit - HDU/ExtraCorporeal Membrane Oxygenation -ECMO) or fatal infections when this subtyping has not already been performed in the local laboratory.

UKHSA PHLs will refer influenza positive samples to the reference laboratory (Respiratory Virus Unit laboratory – RVU) at Colindale, as detailed below. NHS laboratories should not routinely refer samples directly to the RVU for influenza diagnosis, subtyping and H275Y resistance testing (as above) unless such services are unavailable regionally. RVU will charge for any service that is available regionally. More detail is provided in the testing to inform patient management section below.

Surveillance

Influenza strain surveillance informs the global vaccination programme and provides information for empirical antiviral choice, as well as informing pandemic early warning systems. Influenza strain surveillance is:

- UKHSA national virological data reported weekly, both nationally and internationally
- aggregate genetic and antigenic data submitted to the World Health Organisation (WHO) at the end of January and early September, as the UK evidence to guide the annual formulation of the influenza vaccine
- antiviral susceptibility surveillance undertaken at national level based on community samples, in addition to data and samples received from our regional UKHSA public health laboratories, and reported weekly to provide an estimate of the incidence of antiviral resistance

The RVU requests that influenza positive samples are submitted regularly throughout the season to ensure virological data is available for accurate weekly reporting. UK national virological surveillance data is regarded as Official National Statistics with some of the highest viewing figures of content on the government website. Your virus sample contributions are very valuable and contribute to the overall national picture of surveillance.

2021 to 2022 season

In the 2021 to 2022 season, influenza positive samples referred to RVU will be selected for detailed analysis using influenza genome sequencing. Antigenic characterisation will be performed on a subset of viruses isolated in cell culture. Selection for influenza genome sequencing will be based primarily on the PCR Ct value, with the exception of samples from certain settings, for example associated with severe illness or death (see Appendix 1). Reporting of results of genome sequencing, virus isolation, antigenic typing or antiviral susceptibility of influenza virus to referring laboratories will be done only for those samples where a specific request for characterisation has been made for patient management or for those samples where the reason for submission to RVU merits further characterisation for epidemiological purposes, for example as part of an outbreak. For typical influenza positive

samples, referring laboratories will receive a standard report with a link to the aggregated characterisation data in the weekly national influenza report.

The characterisation data is used to compare how similar the currently circulating influenza viruses are to the strains included in seasonal influenza vaccines, and to monitor for changes in circulating influenza viruses. The information obtained is an important component of the UK influenza surveillance. This will be reported in the weekly national joint flu/COVID reports.

Surveillance samples from UKHSA Public Health laboratories

The RVU at UKHSA Colindale requests the following influenza referrals detailed below, and summarised in Appendix 1, from regional UKHSA public health laboratories for surveillance purposes. No charge is made for processing these.

Out of season and early in influenza season

All influenza positive samples from hospital and community sources. Samples from returning travellers must be sent together with details of travel history, as these out of season or early season travel associated influenza strains are of particular interest for surveillance purposes. RVU will notify laboratories when the early season is considered over.

Mid and late influenza season

Refer approximately 10% of influenza positive samples up to a maximum of 50 samples per week.

In the 10% of samples referred, include all influenza positive samples from categories A to G shown below and in Appendix 1, and complete the batch with samples randomly selected from category H. In the event that a laboratory has >50 samples per week in categories A to G, please ensure that the 50 samples referred includes all those in categories C and D below:

A. Patients with complicated influenza[1], including patients admitted to any area of the hospital. All ITU/HDU/ECMO and fatal cases should be included.
B. All influenza positive samples in which the oseltamivir resistance mutation 275Y has been detected.
C. Influenza A positive samples which cannot be subtyped as H3, (H1N1)pdm09, H5*, H7*, or that ARE subtyped as former seasonal H1 (if performed).
D. Influenza positive samples that have an unusual or unexpected PCR profile/Ct values for generic influenza A or specific subtyping.
E. Influenza positive samples where shedding of live attenuated influenza virus (LAIV) is suspected from the patients vaccination history or laboratory results[22]
F. Influenza positive samples from influenza only co-infections, or influenza and SARS CoV-2 co-infections, with influenza PCR Ct values ≤31.
G. Influenza positive samples from adults with a vaccination history for current season, with PCR Ct values ≤31.
H. Samples positive for influenza B, H3, or (H1N1)pdm09, with PCR Ct values ≤31

* Please make sure you urgently inform RVU of any H5/H7 positive samples before sending for confirmation due to the public health significance of these results.

See Guidance on the diagnosis and management of avian influenza.

It is recommended to send samples in regular batches if possible, except for those in categories A to E, which should be sent rapidly to RVU for investigation (see Acceptable referred samples below), and with the reason for sending, for example ECMO patient, clearly stated on the request form.

It is important that the current version of the typing of influenza strains request form should be used (E3). This has been updated to capture information on the SARS CoV-2 status of the referred sample, which will help guide the processing pathway of the sample for virus characterisation.

In addition, RVU will further investigate subtyped samples for which characterisation is required for an outbreak investigation. Subtyped samples from localised or unusual outbreaks should

[1] Complicated influenza: Influenza requiring hospital admission and/or with symptoms and signs of lower respiratory tract infection (hypoxaemia, dyspnoea, lung infiltrate), central nervous system involvement and/or a significant exacerbation of an underlying medical condition.

[2] Patients who have any positive result and a recent history of vaccination with LAIV or have been in contact with an individual who has recent history of vaccination with LAIV.

always be submitted and will be subject to enhanced testing including sequencing. There is no PCR Ct value cut-off for referring influenza samples from outbreaks (see Acceptable referred samples below). Please ensure the samples are clearly marked as from an outbreak, with details of the outbreak setting name or location and type, and the HPZone reference if known, using the E3 request form.

RVU will also perform enhanced analysis on influenza positive samples from children aged 2 to 17 years. Where influenza vaccination history is known, please include information on date of vaccination and type (inactivated or LAIV) on the referral form. There is no PCR Ct value cut-off for referring influenza samples from vaccinated children in this age group (see Acceptable referred samples below).

Surveillance samples from the NHS

NHS laboratories which perform their own subtyping and H275Y detection do not need to send their samples to their regional UKHSA public health laboratory routinely. For surveillance purposes, RVU requests that they only send influenza A positive samples from fatal cases and ITU/HDU/ECMO cases straight to RVU after subtyping and resistance testing have been performed. It is important that the current version of the typing of influenza strains request form should be used (E3) to capture information on the SARS CoV-2 status of the referred sample to help guide the processing pathway of the sample for virus characterisation.

NHS laboratories may refer subtyped influenza A and influenza B samples from treated patients directly to RVU if H275Y mediated resistance testing is not required for clinical reasons. Those laboratories performing subtyping but not antiviral resistance testing should do the same, except where the H275Y result is required for clinical reasons, in which case the sample should be referred to a regional UKHSA public health laboratory.

Surveillance samples submitted to RVU through these routes will not incur a charge for reference testing.

Testing to inform patient management

Please note RVU will charge for any service that is for immediate patient management.

To support clinical diagnosis and management, RVU can provide:

Influenza positive samples for antiviral susceptibility testing for clinical purposes

Clinical susceptibility testing will not be performed routinely in RVU. Laboratories with genuine suspicion of antiviral resistance must contact the RVU laboratory before sending samples for antiviral susceptibility testing. Antiviral (AV) susceptibility testing should be considered when there is clinical concern about AV treatment failure. The greatest risk of oseltamivir resistance is currently in immunocompromised patients with influenza A(H1N1)pdm09. Further recommendations can be found in the guidance on use of antiviral agents for the treatment and prophylaxis of seasonal influenza.

Influenza A positive samples for subtyping

RVU can provide influenza A positive samples for subtyping, should subtyping not be available locally nor through the regional UKHSA Public Health Laboratory. RVU will charge for subtyping influenza A positive samples sent directly to the reference laboratory where subtyping is available either locally or through the regional UKHSA Public Health Laboratory.

Acceptable referred samples

A checklist to aid sample referral is provided in Appendix 2.

RVU accepts original samples or culture isolates. Please do not send extracts or samples in lysis buffer unless in categories A to E (see Appendix 1). Extracts and samples referred in lysis buffer in categories F to H will be stored without testing if received, with a report sent to the referring laboratory to indicate this.

Please do not refer samples with Ct values >31, except for those samples in categories A to E (see Appendix 1). Samples with a Ct value >31 are unlikely to be successfully sequenced or cultured, so no characterisation can be performed.

Please do not refer samples which have been tested with an assay that does not generate PCR Ct values, except for those samples in categories A to E (see Appendix 1).

However, samples with Ct values >31 or where no PCR Ct value has been generated by the assay used, will be accepted in order to process for genetic characterisation:

- in categories A to E in Appendix 1
- in any cases where there is urgent clinical need
- from vaccinated children aged 2 to 17 years
- from influenza confirmed outbreaks

It is recommended to send samples in regular batches if possible, except for those in categories A–E, which should be sent rapidly to RVU for investigation. The current version of the typing of influenza strains request form should be used (E3).

Appendices

Appendix 1: Surveillance samples – referrals requested from UKHSA public health laboratories to RVU

Timing in season	Samples to refer to RVU
Out of season and early season	All influenza positive samples from hospital and community sources, including influenza positive samples from returning travellers
Mid-late season	Refer approximately 10% influenza positive samples, up to a maximum of 50 samples per week. Include all influenza positive samples from categories A to G below, and complete the batch with samples randomly selected from category H
	A) Patients with complicated influenza[1]
	B) All influenza positive samples in which the oseltamivir resistance mutation 275Y has been detected
	C) Influenza A positive samples which CANNOT be subtyped as H3, H1pdm09, H5, H7 or that ARE subtyped as former seasonal H1 (if performed)
	D) Influenza positive samples with unusual or unexpected PCR profile/Ct values for influenza A or specific subtyping
	E) Influenza positive samples where shedding of live attenuated influenza vaccine (LAIV) virus is suspected[2]
	F) Influenza positive samples from influenza co-infections, or influenza/SARS CoV-2 co-infections[3]
	G) Influenza positive samples from adults with a known vaccination history for the current season[3]
	H) Samples positive for influenza B, H3 or H1pdm09[3]

[1]Including patients admitted to any area of the hospital. All ITU/HDU and fatal cases should be included. Complicated influenza: influenza requiring hospital admission or with symptoms and signs of lower respiratory tract infection (hypoxaemia, dyspnoea, lung infiltrate), central nervous system involvement or significant exacerbation of an underlying medical condition.
[2]Patients who have any positive result and a recent history of vaccination with LAIV or have been in contact with an individual who has recent history of vaccination with LAIV.
[3]With Ct values ≤31.
It is recommended to send samples in regular batches if possible, except for those in categories A to E, which should be sent rapidly to RVU for investigation

Appendix 2: Checklist for referral of influenza samples to RVU

Has the relevant section in the guidance been checked for sample referral?

- See section UKHSA public health laboratories
- See section for NHS laboratories
- See section for testing to inform patient management

Is the current version of the Typing of Influenza Strains E3 referral form being used?

Has all relevant patient information been included on the E3 referral form?

- Include details of ICU/HDU/ECMO or fatal case

Is the sample an acceptable referred sample type?

- See guidance for acceptable referred samples
- Include reference number, sample type, date of collection and date sent to PHE

Has the relevant safety information been provided on the E3 form?

- Do you suspect that the patient is infected with a Hazard group 3 or 4 pathogen?
- Is there foreign travel history?

Have the sending laboratory influenza and SARS CoV-2 (if performed) results been provided?

- Influenza type or subtype? If SARS CoV-2 tested for, positive or negative?
- Assay used and Ct values where generated MUST be included

If antiviral susceptibility testing requested, has this been discussed with the reference laboratory before sending sample?

- If no, call reference laboratory first to discuss

Is the referred sample from an influenza outbreak?

- If yes, provide details of the name or type of setting and HPZone reference if known, on the E3 referral form

Has the patient received the current season flu vaccine?

Has the patient received antivirals in the past 14 days?

- If yes, provide details on the E3 referral form

About the UK Health Security Agency

The UK Health Security Agency is an executive agency, sponsored by the Department of Health and Social Care.

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