Public Health England

Protecting and improving the nation's health

Guidance: Referral of influenza samples to Respiratory Virus Unit, PHE Colindale

1.0 General principles

National surveillance of influenza depends on accurate and timely virological information confirming 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 by PHE laboratories. NHS laboratories can refer samples to the local PHE Public Health Laboratory (PHL) for diagnosis of influenza A and B infection, influenza A subtyping and the detection of H275Y mediated oseltamivir resistance in A(H1N1)pdm09 viruses. Some of these samples may then be referred to the reference laboratory (free of charge) for detailed virological surveillance. Testing performed by a PHL for individual patient management will be charged for. No charge will be made by PHE PHLs for subtyping of influenza A positive samples from severe (ITU/HDU) or fatal infections when this subtyping has not already been performed in the local laboratory.

PHE public health laboratories will refer 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, the exception being samples requested for surveillance purposes from NHS laboratories performing their own influenza typing, subtyping and H275Y detection. More detail is provided in section 2.2 below.

2.0 Surveillance

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

PHE publications gateway number: 2016371
Published:

- PHE national virological data are reported weekly, both nationally and internationally
- PHE national virological surveillance data is regarded as Official National Statistics with some of the highest viewing figures of content on the PHE website
- Aggregate data are submitted internationally to the World Health Organisation (WHO) at the end of January, with genetic and antigenic data being the basis of data supplied from the UK to WHO as evidence to guide the annual formulation of the influenza vaccine
- Antiviral susceptibility surveillance is undertaken at national level based on community samples, in addition to data and samples received from our regional PHE public health laboratories, and is reported weekly to provide an estimate of the incidence of antiviral resistance

The RVU requests that samples are submitted regularly throughout the season to ensure virological data is available for accurate weekly reporting. Your virus sample contributions are very valuable and contribute to the overall national picture of surveillance.

2018/19 season

In the 2018/19 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 e.g. associated with severe illness or death (see sections 2.1 and 4.0, and 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, e.g. part of an outbreak.
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 flu reports. Available at: https://www.gov.uk/government/statistics/weekly-national-flu-reports

2.1 Surveillance samples from PHE Public Health laboratories

The RVU at PHE Colindale requests the following referrals detailed below, and summarised in Appendix 1 and 2, from regional PHE 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, including influenza positive samples from returning travellers (together with details of travel history), as these out of season/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-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-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-G, then 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 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 and/or specific subtyping.
E. Influenza positive samples where shedding of live attenuated influenza virus (LAIV) is suspected from the patients vaccination history and/or laboratory results[2]
F. Influenza positive samples from influenza co-infections, with 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.

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 (see section 4.0 below).

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

2.1.1. In addition, RVU will further investigate subtyped samples for which characterisation is required for an outbreak investigation. Samples from localised or unusual outbreaks should 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 section 4.0 below).

2.1.2. RVU will also perform enhanced analysis on influenza positive samples from children aged 2-17 years. Where 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 section 4.0 below).

2.2 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 PHE public health laboratory routinely. For surveillance purposes, RVU requests that they only send influenza positive samples from fatal cases and ITU/HDU cases straight to RVU after subtyping and resistance testing have been performed. 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 PHE public health laboratory.

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

3.0 Testing to inform patient management

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

RVU can provide the following services to support clinical diagnosis and management:

1. Influenza positive samples for antiviral (AV) susceptibility testing for clinical purposes. 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 PHE guidance on use of antiviral agents for the treatment and prophylaxis of seasonal influenza. Available at: https://www.gov.uk/government/publications/influenza-treatment-and-prophylaxis-using-anti-viral-agents

2. Influenza A positive samples for subtyping, should subtyping not be available locally or through the regional PHE Public Health Laboratory. RVU will charge for subtyping

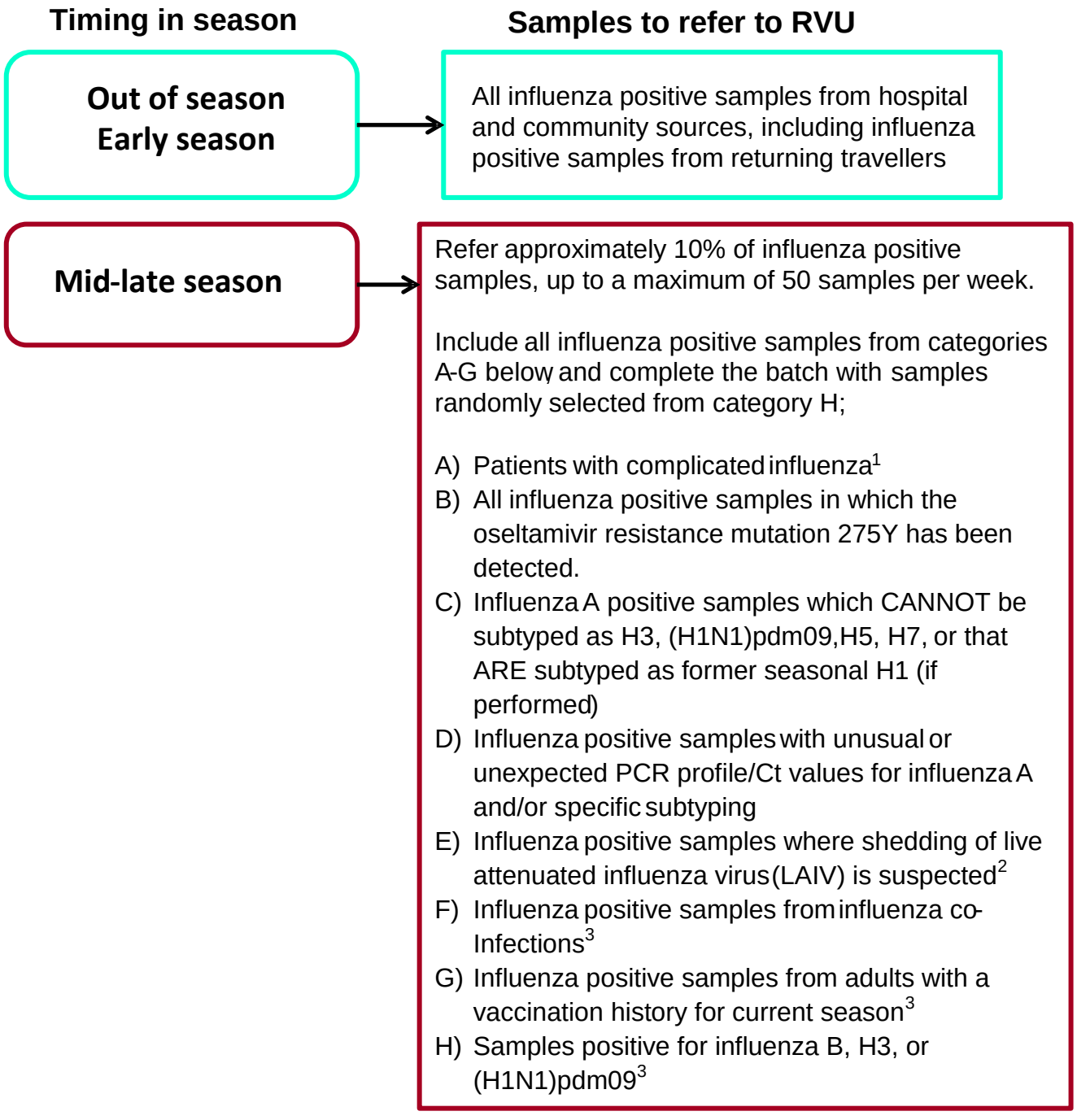
influenza A positive samples sent directly to the reference laboratory where subtyping is available locally or through the regional PHE Public Health Laboratory.

4.0 Acceptable referred samples

- RVU accepts original samples or culture isolates. **Please do not send extracts or samples in lysis buffer.** Extracts and samples referred in lysis buffer 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-E (see section 2.1 and 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-E (see section 2.1 and 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-E in section 2.1 above**
  - **In any cases where there is urgent clinical need**
  - **From vaccinated children aged 2-17 years (section 2.1.2)**
  - **From influenza confirmed outbreaks (section 2.1.1)**

It is recommended to send samples in regular batches if possible, except for those in categories A–E in section 2.1, which should be sent rapidly to RVU for investigation.

Appendix 1. Surveillance samples: referrals requested from PHE Public Health Laboratories to RVU



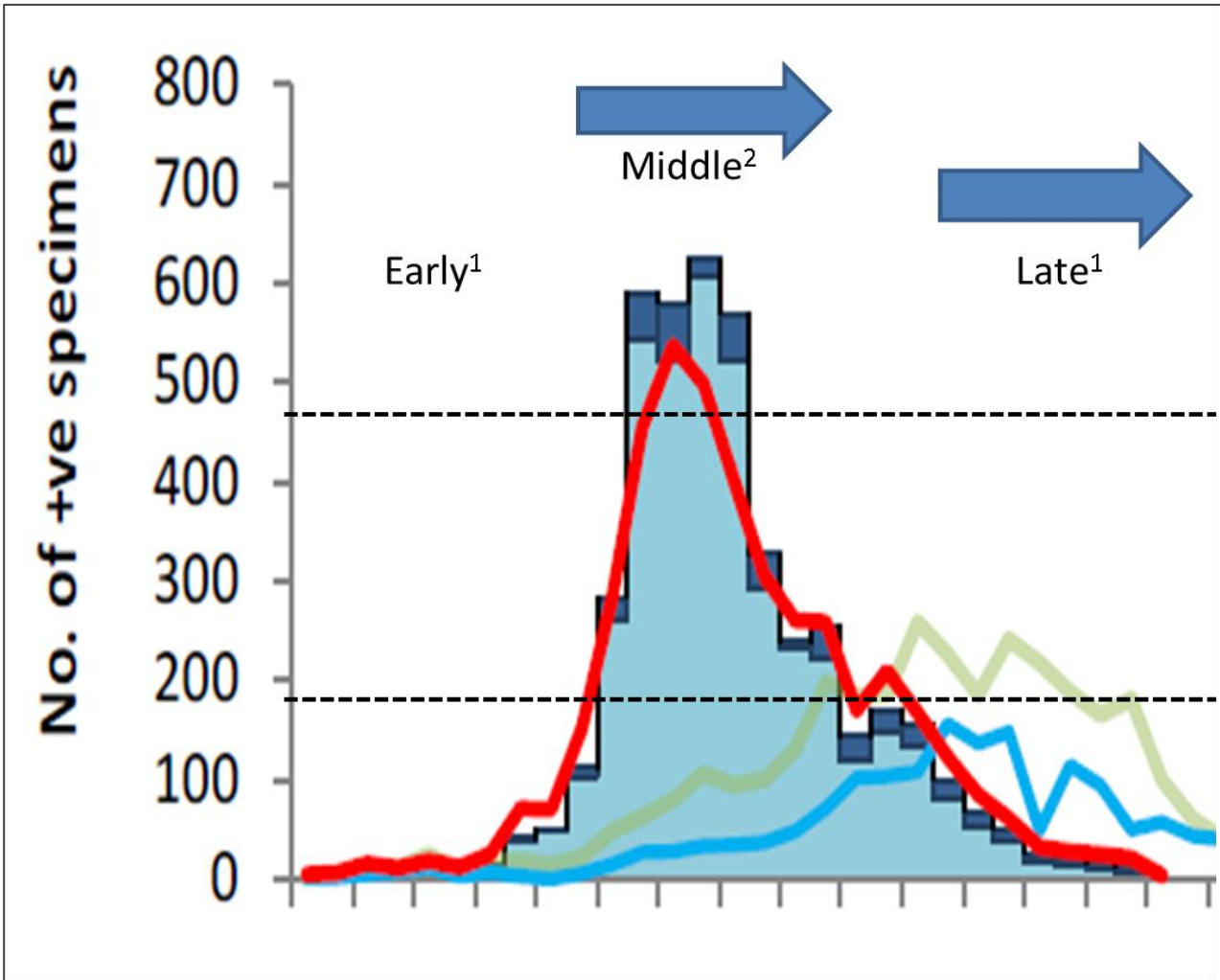
[1] Including patients admitted to any area of the hospital. All ITU/HDU and fatal cases should be included. Complicated influenza: Influenza requiring hospital admission and/or with symptoms and signs of lower respiratory tract infection (hypoxaemia, dyspnoea, lung infiltrate), central nervous system involvement and/or a significant exacerbation of an underlying medical condition

[2] From the patients vaccination history and/or laboratory results. Patients who have recent history of vaccination with LAIV or have been in contact with an individual who has recent history of vaccination with LAIV and are positive for influenza

[3] With Ct values ≤31

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.

Appendix 2. Schematic of sample referral from PHE public health laboratories to RVU during the influenza season



[1]Early and late in season; send all influenza positive samples to RVU
[2]Mid-season; send 10% influenza positive samples (max 50/week) according to section 2.1