Selection of clinical specimens for virus isolation and of viruses for shipment from National Influenza Centres to WHO Collaborating Centres

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Global influenza virological surveillance relies on antigenic characterization (Haemagglutination Inhibition (HI) and Virus Neutralization (VN) assays) supported by gene sequencing (predominantly for HA and NA) of influenza viruses, the results of which form the foundation of influenza epidemic/pandemic risk assessment and selection/development of seasonal/pandemic vaccine viruses. In this whole process, virus isolates are essential. Virus isolates are also required to monitor influenza antiviral drug resistance using phenotypic assays. Therefore performing virus isolation has been one of the Terms of Reference for National Influenza Centres (NICs)¹ of the Global Influenza Surveillance Network (GISN).

In recent years molecular technology has been widely used, particularly during the pandemic A(H1N1) 2009 and the number of laboratories performing virus isolation has decreased. To ensure that in future, sufficient and representative viruses are shared with the GISN, this document aims to help laboratories to select clinical specimens for virus isolation and to determine which virus isolates should shared with the GISN through shipment to a WHO Collaborating Centre (CC)². These recommendations also describe how to combine the use of polymerase chain reaction (PCR) and virus isolation. Details on optimal specimen storage and transport are also described.

Principles of selection of specimens for virus isolation:

- PCR can be used for rapid detection of influenza in clinical specimens.
- Depending on the numbers of positive specimens, taking into consideration the epidemiological and clinical information available and relevant biosafety requirements, all or a proportion of those positive specimens can then be selected for virus isolation.

A recommended way of combining the use of PCR and virus isolation is illustrated in Figure 1, taking into consideration of advantages of each technology, needs for global influenza virological surveillance and practicality of NICs.

Recommendations to NICs for selection of clinical specimens/virus isolates to send to a WHO CC on Influenza:

NICs are strongly recommended to perform virus isolation wherever possible and to send, in a timely manner, representative virus isolates to one of the WHO CCs for further characterization. Where laboratories do not have the capacity to perform virus isolation, or as requested by the WHO CC, those labs may send clinical specimens.

The criteria for selection of specimens and/or virus isolates to send to a WHO CC are:

- approximately five representative recent (collected within 4-6 weeks) specimens and/or virus isolates of each of the following groups:
  - from different age groups (e.g. from children, adults and elderly)
  - from different settings and geographical locations in the whole country
  - from different phases of progression of influenza activity e.g. beginning, middle and end of an epidemic;

- all viruses that yield lower titres than expected in the HI test with the WHO reference kit or influenza-specific sheep or ferret antiserum;

- any virus that is unsubtypable i.e. influenza A positives which cannot be identified as subtypes known to be circulating should be sent to a WHO CC as quickly as possible;

- a subset of clinical specimens/virus isolates from severe cases and unusual outbreaks (not more than five per outbreak);

- specifically for antiviral susceptibility monitoring:
  - representative samples collected from patients on antiviral treatment;
  - samples from immunocompromised patients who may be on antiviral treatment and have long-term virus shedding or treatment failure;
  - representative samples from viruses known to be resistant to an antiviral drug;
  - viruses showing intermediate IC50s for any drug in phenotypic testing (i.e. those that have elevated IC50s compared to the normal ‘susceptible’ range but below that of ‘resistance’ in tests as performed in their laboratory).

It is recommended that shipments to WHO CCs are sent 2 to 4 times a year in a timely manner to include the most recent clinical specimens/virus isolates, and taking into consideration the timing of the two WHO vaccine strain selection consultations in February and September each year.

- Specimens from early in a season are important if antigenic variants are emerging as this will allow generation of the required antigenic characterization reagents (notably ferret antisera) in a timely manner. Samples received before the third week of January and before the third week of August can be characterized in time for the February and September meetings respectively of the same year.

- During periods of enhanced surveillance such as during a pandemic, samples should be sent more often based on WHO CC recommendations.

- For those NICs with RT-PCR capacity only, it is recommended that representative positive clinical specimens are selected based on the above described criteria and sent to CCs.

**Specimen storage and transport**

- ensure holding of specimens at room/fridge (4°C) temperature are kept to a minimum time;
- aliquot specimens to prevent repeated freeze/thaw cycles (can result in the loss of 1 log of infectivity [virus viability] and consequently loss of RNA integrity per cycle);
- ensure specimens are frozen at -70/80°C, NOT -20°C;
- use of proper cryovials for specimen storage and transport on dry ice, to prevent poor sealing of specimen tubes;
• storage at -70/80°C of a number of original clinical specimens which test positive for influenza for at least 2 years is desirable, but is dependent on national legislation and is not mandatory. Freezer capacity also plays a critical limiting factor in storage of specimens.

Further specimen storage and transport advice can be found below:


http://www.who.int/entity/influenza/gisrs_laboratory/logistic_activities/FAQInfluenzaLogistics.pdf
Fig. 1 Selection of specimens for virus isolation and shipment of viruses to WHO CCs by NICs

Clinical specimens

Aliquots of the specimen  e.g. 1 aliquot for PCR; 1 aliquot for virus isolation, 1 aliquot for stock

Extraction of nucleic acids

Perform PCR

POSITIVE*

NEGATIVE

5%

UNTYPED A**

Virus Isolation

Send to WHO CCs***

*Selection of positives chosen according to criteria in document

**Untyped A’s by PCR should be sent to a WHO CC if circumstances are unusual

***Selection of isolates chosen according to criteria in document