

## Technical Note

# Laboratory Diagnosis of Human Infection with Influenza A/H5

10 January 2023

### BACKGROUND

Since the beginning of 2022, multiple avian influenza outbreaks caused by Influenza A/H5 has been detected in countries of the Andean Region and North America. Avian influenza (AI) is a highly contagious viral disease that affects both domestic and wild birds and is caused by multiple subtypes (i.e. H5N1, H5N3, H5N8 etc.) whose genetic characteristics rapidly evolve<sup>1</sup>.

According to the severity of the disease in poultry (but not in humans), viruses are classified into two categories: low pathogenicity avian influenza (LPAI) that typically causes little or no clinical signs; and high pathogenicity avian influenza (HPAI) that can cause severe clinical signs and possible high mortality rates in birds<sup>1</sup>.

Although different species of birds are the natural hosts for avian influenza viruses, human infections can occur ranging from a mild illness to death.<sup>2</sup> Usually, these human infections of zoonotic influenza are acquired through direct contact with infected animals or contaminated environments, but human to human transmission is not sustained<sup>3</sup>.

However, the emerging and ongoing circulation of Influenza A/H5 in birds and poultry becomes a public health concern as influenza viruses have the potential to undergo mutations and reassortments that may result in increased transmissibility among humans. If these viruses acquire the capacity to spread easily among humans, either through adaptation or acquisition of certain genes from human influenza viruses, they could trigger an epidemic or a pandemic<sup>3</sup>.

In this sense, surveillance and early detection of Influenza viruses A/H5 infection in humans is crucial for identifying and coordinating response to zoonotic influenza outbreaks, as well as for potential pandemic preparedness. Additional characterization, as genomic sequencing, of the influenza A/H5 viruses detected in humans is key to early characterize influenza viruses with pandemic potential.

Considering the emergence and dissemination of Influenza A/H5 avian outbreaks to multiple countries in the Americas and the risk of zoonotic infection, it is important to be prepared to respond, and the laboratory tests that are used to detect influenza A/H5 in human samples and the testing algorithm recommendations should be established. Information on suspected case definition; specimen collection; reagents; testing algorithm and shipment; effective usage of global laboratory networking; and reporting of cases and test results can be found in this interim guidance.

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<sup>1</sup> WHOA (former OIE). Avian Influenza. Available at: <https://www.woah.org/en/disease/avian-influenza/>

<sup>2</sup> PAHO. Influenza at the Human-Animal Interface: PAHO Recommendations to Strengthen Intersectoral Work for Surveillance, Early Detection, and Investigation, 9 July 2020. Available at: <https://iris.paho.org/handle/10665.2/52563>

<sup>3</sup> WHO. Influenza virus Infections in humans. Available at: [https://cdn.who.int/media/docs/default-source/influenza/influenza\\_virus\\_infections\\_humans\\_oct\\_18.pdf](https://cdn.who.int/media/docs/default-source/influenza/influenza_virus_infections_humans_oct_18.pdf)

## LABORATORY DETECTION

### Sample collection in humans

Samples should be collected by trained personnel and considering all biosafety instructions including the use of personal protective equipment appropriate for respiratory viruses.

Recommended samples are the same type of samples used for influenza routine surveillance. Nasopharyngeal swab is the optimal specimen collection method for influenza testing. However, combined nasal and throat swab specimen or aspirate specimens can be collected.

Sterile Dacron/nylon swab should be used for sample collection. Cotton tipped and wooded swabs are not recommended since they interfere in the sample processing and inhibit molecular diagnostic reactions.

Swabs should be placed in viral transport media tube containing 3 mL of sterile viral transport medium and transported in the same tube with viral transport medium (VTM).

Sample collection is recommended within 4 days of symptoms onset for highest influenza virus yield and better detection. Sampling of asymptomatic contacts is not recommended, unless considered necessary according to national guidelines.

Samples should be kept refrigerated (4-8 °C) and sent to the laboratory (central, national or reference lab) where they should be processed within the first 24-72 hours from the collection.

If samples cannot be sent within this period, freezing at -70 °C (or less) is recommended until samples are shipped (ensuring the cold chain is maintained).

### Sample flow and Laboratory testing algorithm

In the Americas, all national influenza centers (NICs) and National Reference Laboratories (NRL) for human influenza inside the WHO Global Influenza Surveillance and Response System (GISRS), use molecular diagnostic protocols and reagents developed and validated by the WHO Collaborating Center at the US Centers for Disease Control (CDC).

Sentinel sites and/or decentralized laboratories should refer influenza A/H5 suspected cases samples to the NIC or NRL for testing (Figure 1)<sup>4</sup>.

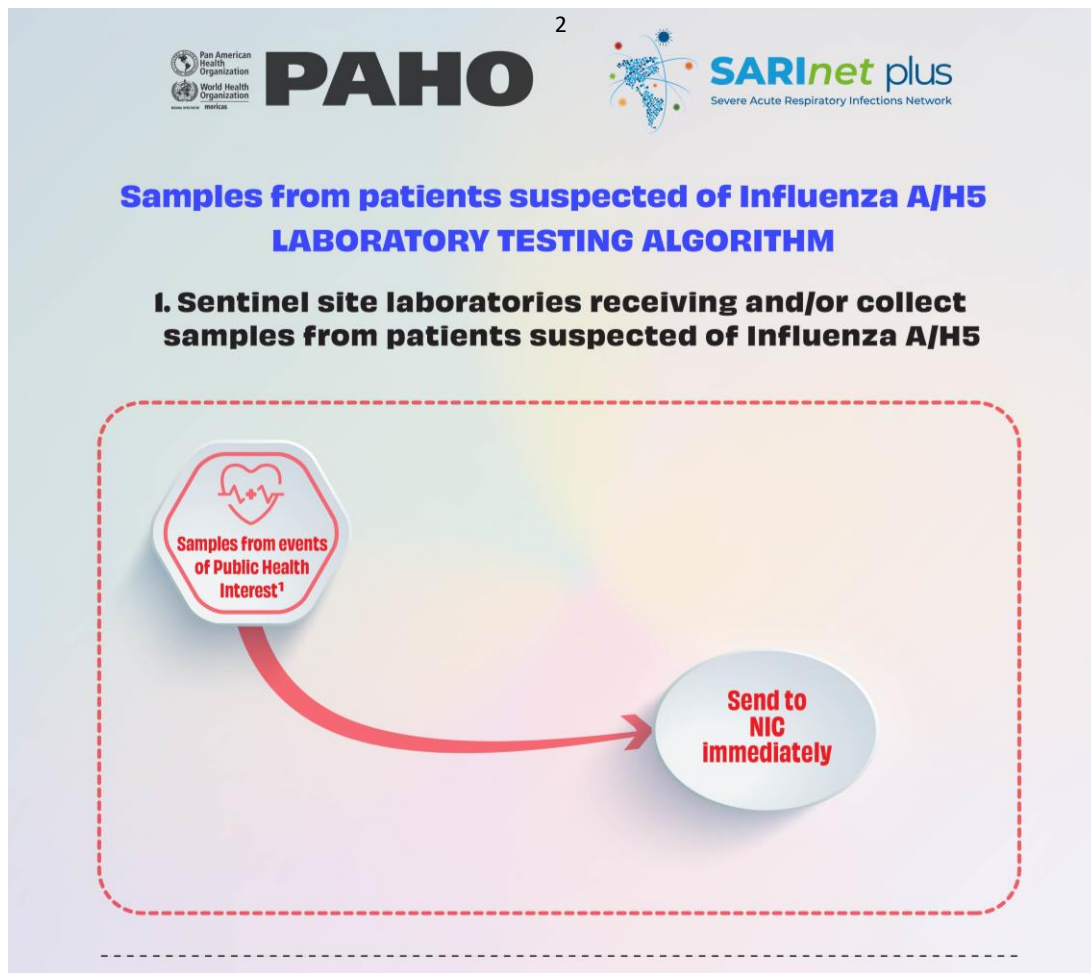
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<sup>4</sup> PAHO. Samples from patients suspected of Influenza A/H5 LABORATORY TESTING ALGORITHM. Available at: <https://www.paho.org/en/documents/samples-patients-suspected-influenza-ah5-laboratory-testing-algorithm>

Samples collected from suspected cases of human exposed to birds or humans infected with avian influenza A/H5 should be tested for influenza; influenza A positive samples should be subsequently subtyped directly for H5 (Figure 2)<sup>4</sup>.

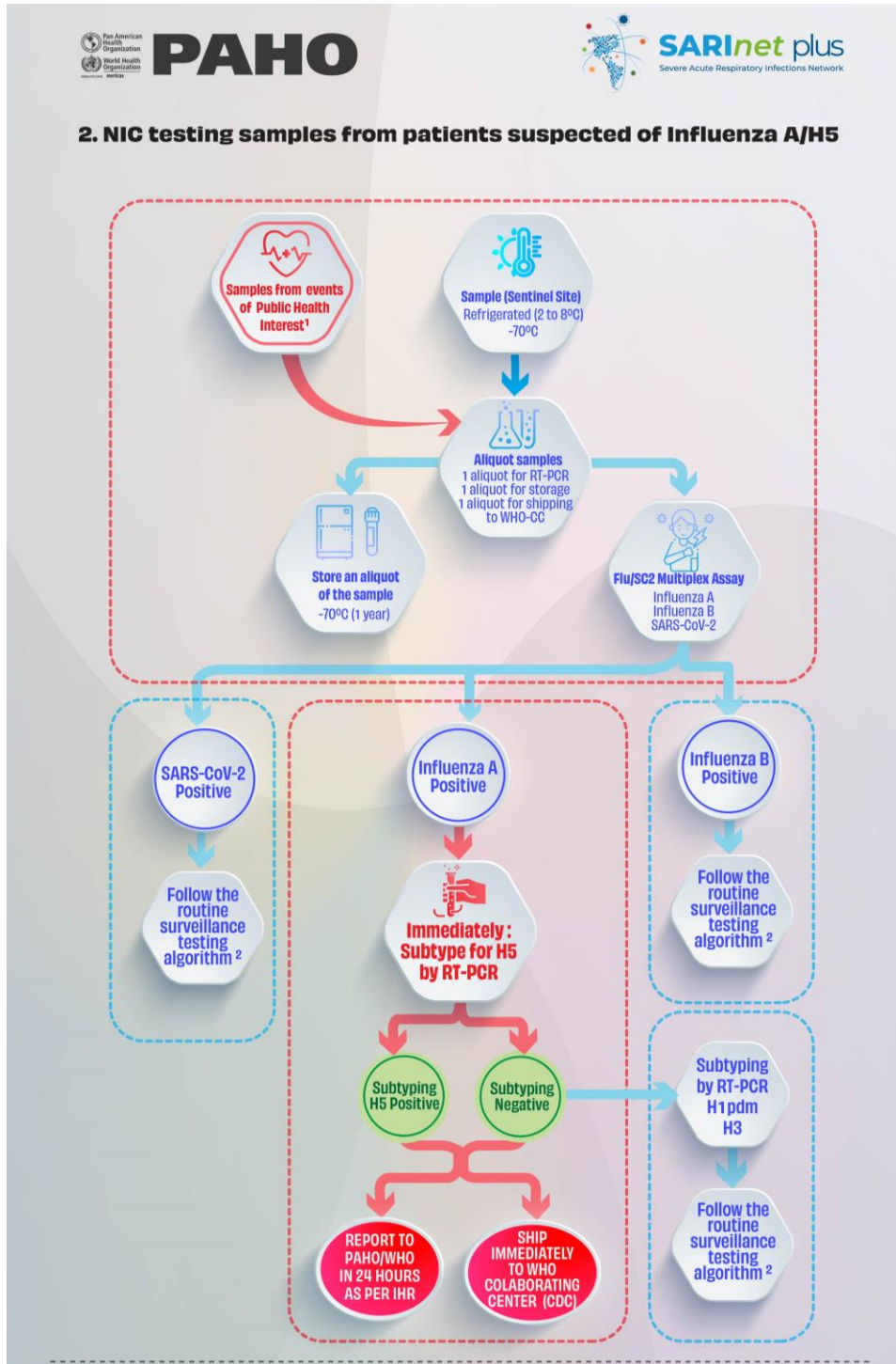
Figure 1

**Sample flow for samples of influenza A/H5 suspected cases at sentinel site and/or decentralized laboratories.**



<sup>4</sup> PAHO. Samples from patients suspected of Influenza A/H5 LABORATORY TESTING ALGORITHM. Available at: <https://www.paho.org/en/documents/samples-patients-suspected-influenza-ah5-laboratory-testing-algorithm>

Figure 2  
NIC testing samples from suspected cases of influenza A/H5



<sup>1</sup> Samples collected out of routine surveillance from event of public health interests. International Health Regulations 2005. Available at: <https://www.who.int/publications/i/item/9789241580496>

<sup>2</sup> Influenza and SARS-CoV-2 integrated surveillance laboratory testing algorithm. Available at: <https://www.paho.org/en/documents/influenza-and-sars-cov-2-integrated-surveillance-laboratory-testing-algorithm>

## Laboratory reagents

These CDC kits for real-time reverse transcription polymerase chain reaction (RT-PCR) detection of influenza viruses are available through the International Reagent Resource (IRR).

For Influenza detection and Influenza A/H5 subtyping, the following kit and controls for molecular detection are available:

- Influenza SARS-CoV-2 Multiplex Assay (RUO) (500 reactions) (Catalog No. FluSC2PPB-RUO), dried primers and probes
- Influenza SARS-CoV-2 Multiplex Assay Positive Controls Kit (RUO) (500 reactions) (Catalog No. FluSC2PC-RUO)
- CDC Real-Time RT-PCR Influenza Virus A/H5 (Asian Lineage) Subtyping Panel (VER 4) (RUO) (Catalog No. FluRUO-13)
- CDC Influenza A/H5N1 (Asian Lineage) Real-Time RT-PCR Positive Control with Human Cell Material (RUO) (Catalog No. VA2715)

## Results interpretation

The markers (targets) of the CDC kits for influenza A/H5 subtype detection are: INFA (M), H5a (HA), H5b (HA), RP.

When using the CDC influenza A/H5 subtyping kit:

- Samples positive for INFA, H5a and H5b markers are **positive samples for influenza A/H5**.
- Samples positives for only one H5 marker are **presumptive influenza A/H5**.

In both cases, samples should be referred to a WHO Collaborator Center for further characterization or confirmation in the case of presumptive results. Nevertheless, a positive sample for influenza A/H5 (both markers positive) should be reported immediately.

Currently, PAHO is working to support member states on preparedness and response to Influenza A/H5. For additional support please contact at [flu@paho.org](mailto:flu@paho.org)

## Sample shipping

The US Centers for Disease Control (CDC) is the WHO Collaborator Center in the Americas Region for receiving human samples positive for Influenza A/H5.

All shipping human samples to WHO Collaborating center at US-CD, outside the country and by air must ensure compliance with all international standards (International Air Transport Association - IATA). Special documents are required for transportation to the United States other than documents for routine shipment of seasonal influenza samples.

It is important to note that the samples should **not** be sent as routine influenza samples to CDC.

Animal samples should be sent to WHO Collaborating Center at St. Jude Children's Hospital. Special documents are necessary for transportation to US documents and must ensure compliance with all international standards.

For logistic and shipping information for human or avian influenza A/H5 samples, PAHO should be contacted at [flu@paho.org](mailto:flu@paho.org).

## Genomic sequencing

Submission of a positive sample for influenza A/H5, animal or human, to the appropriate WHO Collaborating Centre should be prioritized for antigenic and genomic characterization.

For laboratories that have sequencing capacity, in addition to sending the positive sample to the Collaborating Center, it is encouraged to sequence the sample to generate genomic sequencing data and to upload the sequences in a timely manner to the GISAID global platform.

The sequences upload to GISAID requires the use of the nomenclature recommended by the WHO<sup>5</sup> :

- The format for humans is:  
[influenza type]/[region]/[internal reference number]/[year of collection]  
Ex: A/Wisconsin/2145/2001
- For all other animal hosts:  
[Influenza Type]/[Host]/[Region]/[Internal Reference Number]/[Year of Collection]  
Ex: A/chicken/Rostov/864/2007

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<sup>9</sup> OPAS. Nota Técnica: Nomenclatura de Virus Influenza. Disponível em: <https://www.paho.org/pt/documentos/nota-tecnica-nomenclatura-do-virus-influenza>

## GUIDANCE FOR NATIONAL AUTHORITIES

PAHO/WHO reiterates to Member States the need to maintain influenza virus surveillance and for immediate shipping of human influenza samples to the WHO Collaborating Center at US-CDC.

Since information on circulation avian influenza A/H5 are important for the human zoonotic influenza vaccine composition and for generating data for preparedness and response, countries are encouraged to share animal influenza samples with WHO Collaborating Center at St. Jude Children’s Hospital. St. Jude WHO Collaborating Centers is focused exclusively on the threat to humans from influenza viruses of animals.

### Notification of cases in humans

1. A **confirmed positive case** of human influenza A/H5 infection should be **reported immediately** via two channels—the WHO International Health Regulations (IHR) Regional Contact Point (via the IHR National Focal Point: [ihr@paho.org](mailto:ihr@paho.org)) and the GISRS managed by PAHO and WHO (via [flu@paho.org](mailto:flu@paho.org)). The report should include all available results from the epidemiologic case investigation and the virologic characteristics of the virus.
2. A **suspected** case of human influenza A/H5 infection, should be **reported immediately** to the GISRS and information about the suspected case should be shared with the IHR National Focal Point, given it is an unusual event. The report should include all available results from the epidemiologic case investigation and the virologic characteristics of the virus.