Guidance for the implementation of the Influenza and SARS-CoV-2 Multiplex RT-PCR Assay into the influenza and COVID-19 integrated surveillance

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As part of the WHO's Global Influenza Surveillance and Response System (GISRS), National Influenza Centers (NIC) and National Public Health Laboratories in the Americas have been conducting influenza molecular detection and diagnosis for many years. With the COVID-19 emergence in early 2020, PAHO leveraged this robust and well-established laboratory network to quickly introduce a molecular testing protocol for SARS-CoV-2 throughout the Region.

Despite the low influenza virus circulation and detection since March 2020, PAHO has recommended to ensure influenza surveillance and to maintain the laboratory confirmation algorithms for sentinel surveillance of influenza-like illnesses (ILI) and severe acute respiratory infections (SARI) as well as unusual cases of respiratory diseases. Therefore, if influenza is suspected, routine influenza testing should be performed (including subtyping or genotyping) and notification must be continued.

The threat of influenza epidemics and pandemics persists. It is imperative for the GISRS to maintain meaningful surveillance of influenza worldwide and for countries to remain vigilant while adapting to meet COVID-19 surveillance objectives [1][1].

The World Health Organization (WHO) Collaborating Center (CC) for Surveillance, Epidemiology and Control of Influenza at US Centers for Disease Prevention and Control (CDC) developed in 2020 a multiplex molecular assay [2][2] to simultaneously detect influenza (types A and B) and SARS-CoV-2 viruses, which is now becoming available through the International Reagent Resources (IRR) facility.

This document is intended to summarize operational considerations for implementing Influenza and SARS-CoV-2 Multiplex PCR Assay into influenza and COVID-19 integrated surveillance. These considerations are not to replace the already existing recommendations of the GISRS for routine influenza surveillance, but to leverage existing national and sub-national influenza surveillance systems in the light of the multiplex assay implementation.

Operational consideration for SARI/ILI sentinel surveillance

In the context of the implementation of influenza and SARS-CoV-2 Multiplex Assay into routine influenza and COVID-19 surveillance, the following operational considerations should be contemplated:

- Available US-CDC influenza and SARS-CoV-2 multiplex kits could/should be ***prioritized for SARI/ILI sentinel surveillance***:
  - In any case, adequate specimens must be available for influenza A subtyping and influenza B genotyping.

  - NICs are required to send timely representative circulating seasonal influenza samples to WHO-CCs of the GISRS for further characterizations to inform decisions made at twice-yearly vaccine composition recommendation meetings.
  - Testing algorithm should be adapted for incorporating the multiplex testing.

- Currently, multiplex assay is ***not*** intended for universal COVID-19 surveillance because SARS-CoV-2 is still predominant:
  - Under a scenario of high or very high community transmission, SARS-CoV-2 confirmation may be prioritized when laboratory capacities are strained.
  - As influenza virus transmission is low, the importance of coinfection should be negligible.
  - If present, it might still be captured through sentinel surveillance.

Operational consideration for laboratories

The molecular detection of influenza viruses through real-time reverse transcription PCR (rRT-PCR) is the gold standard methodology for influenza laboratory diagnostic in all the GISRS laboratories [1] [3][1,3]. The International Reagent Resource (IRR) of the influenza WHO-CC at US-CDC, provides influenza molecular testing reagents and kits to the GISRS laboratories [4][4].

For SARS-CoV-2, molecular detection protocols were rapidly developed after sharing of the emergence SARS-CoV-2 sequencing data becoming available on WHO website [5][5]. PAHO has been supporting the implementation of SARS-CoV-2 molecular diagnostic, providing trainings and reagents since the development and release of the fist molecular assay for detecting SARS-CoV-2 [6][6].

Molecular assays are useful and powerful methodology for rapid virus detection in respiratory samples. In this regard, multiplex assays might allow considerable time and effort to be saved by simultaneously detecting more than one pathogen in a single reaction. Multiplex rRT-PCR assay formats also result on less consumption of reagents, consumables, and sample processing time.

In the context of the implementation of Influenza and SARS-CoV-2 Multiplex Assay into routine influenza and COVID-19 laboratory surveillance, the following operational considerations should be contemplated:

Clinical samples collection, storage and transport

- Upper respiratory tract specimens are suitable for molecular detection of influenza and SARS-CoV-2. However, nasopharyngeal and oropharyngeal swabs remain the specimen type of choice for influenza and SARS-CoV-2 molecular detection[2].

- Proper specimen collection is important for the laboratory diagnosis since specimen that is not collected correctly may lead to false or inconclusive test results.

- Influenza virus should be viable for shipping to WHO-CC at US CDC and virus isolation. Therefore, it is important to the use of the right volume (3 mL) proper samples collection media (virus transport media or PBS buffer).
- For conserving virus integrity, samples should be transported immediately to the laboratory. The cold chain must be maintained at all times of sample transportation.
- At laboratory, samples can be stored at refrigeration temperature (4°C) for up to 72 hours. After this period, specimens should be kept frozen at -70°C or below.
- Samples should not be repeatedly frozen and thawed, since this results in viral degradation, reducing detectability and viability for further characterization and isolation.

Multiplex assay approach

- The CDC influenza and SARS-CoV-2 multiplex assay simultaneously detect SARS-CoV-2 and influenza viruses A and B in one single reaction.
- A limit number of kits are available to GISRS laboratories through the CDC's IRR.
- The CDC influenza and SARS-CoV-2 multiplex assay instructions for use [2][2] and the primers and probes sequence information [7][7] is publicly available for reference at CDC webpage.
- It is important to ensure the quality of the process, including sensitivity and specificity for the targeted virus; therefore, positive and negative controls must be included in each test run, as well as the internal RP marker.
- The CDC influenza and SARS-CoV-2 multiplex assay consist of 4 molecular markers: InfA, InfB, SC2 and RP, that must be analyzed for testing results.
- Interpretation and results should be as follow[2]:

Marker				Interpretation	Result
InfA	InfB	SC2	RP		
+	-	-	+ or -	Influenza A RNA detected	Positive for Influenza A
-	+	-	+ or -	Influenza B RNA detected	Positive for Influenza B
-	-	+	+ or -	SARS-CoV-2 RNA detected	Positive for COVID-19
-	-	-	+	No influenza/SARS-CoV-2 RNA detected	Negative
-	-	-	-	Invalid results	Invalid

* If more than one marker for SARS-CoV-2 or influenza A and B are positive, it is indicative of coinfection.

Testing algorithm using influenza and SARS-CoV-2 multiplex

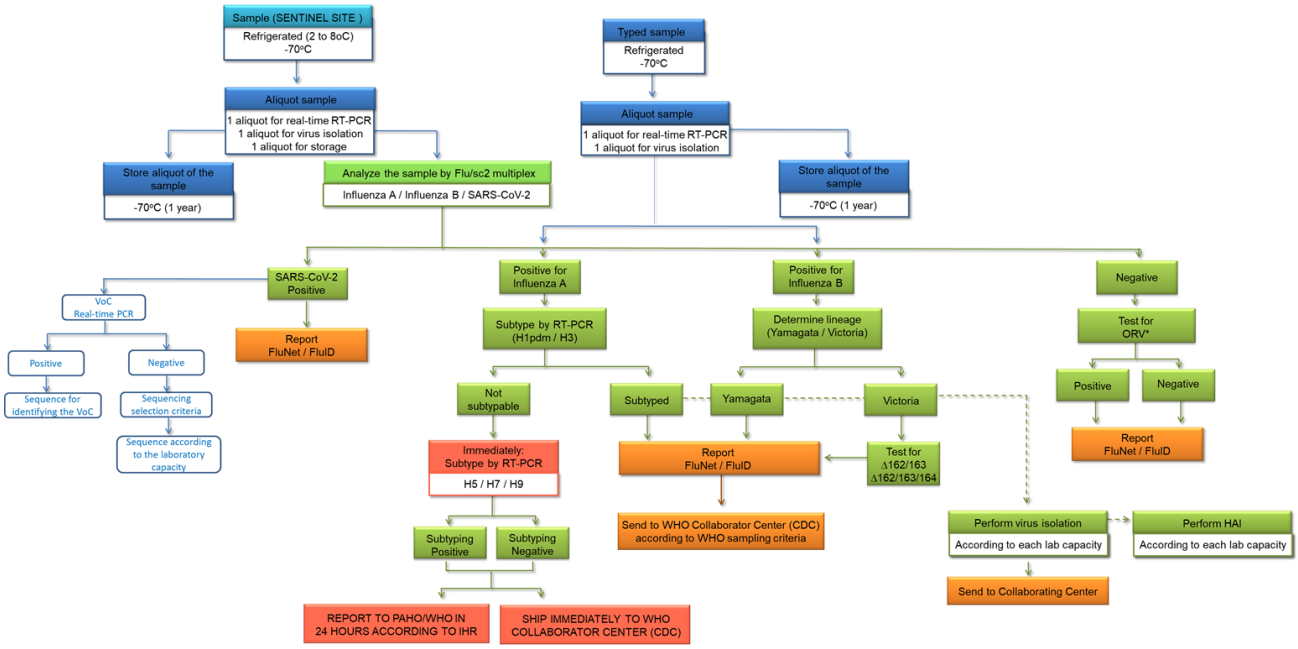
- ***SARI/ILI Sentinel sites samples <u>not tested</u> for influenza virus or SARS-CoV-2*** may be tested using influenza and SARS-CoV-2 multiplex (Figure 1). Influenza A positive samples should be subtyped and influenza B positive samples should be genotyped following the established influenza testing algorithm.

- ***SARI/ILI Sentinel sites samples <u>primarily tested</u> for influenza virus***, should <u>not</u> be re-tested using influenza and SARS-CoV-2 multiplex. Otherwise, testing should go directly to subtyping of influenza A positive samples and genotyping of influenza B positive samples (Figure 1) and follow the routine subjacent steps for the influenza testing algorithm.

- ***Samples primarily tested for SARS-CoV-2*** should <u>not</u> be re-tested with the multiplex assay for influenza diagnostic. Singleplex strategy should be used for this purpose.

- ***Unusual SARI samples*** that test negative for influenza and SARS-CoV-2 should be tested other respiratory viruses. Unusual cases should be notified through International Health Regulation.

Figure 1: Influenza testing algorithm using US-CDC influenza and SARS-CoV-2 multiplex assay.



Influenza and COVID-19 surveillance data reporting

- FluNet weekly reporting is part of the National Influenza Centers terms of reference [8][8,] and this should be continue done through the existing regional platform.
- For sentinel surveillance reporting, PAHO/WHO request to Member States to report through FluNet (virological data) and FluID (epidemiological data) databases on a weekly basis to flu@paho.org
- Influenza testing data: continue reporting aggregated influenza surveillance data on a weekly basis to the regional level. At a minimum, this should be the number of samples from all sources processed for influenza testing, the number of samples positive for influenza and the number of samples tested and/or samples negative for influenza. Wherever possible, this data should be disaggregated by source (sentinel and non-sentinel).
- COVID-19 testing data: countries are requested to report weekly-aggregated COVID-19 results in the same format and frequency as they have been reporting influenza surveillance data. Virologic data (such as the number of samples testing positive and negative for COVID-19) from cases sampled in existing sentinel and non-sentinel or syndromic surveillance systems should be reported on a weekly basis to the regional level.
- PAHO will continue to include the reported data to WHO FluMart regional and global platform; and coordinates the exchange of reported data with WHO headquarters. Therefore, timely and complete reporting to PAHO ensures compliance with what is expected by WHO.

References

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6 - Pan American Health Organization (PAHO). Laboratory Guidelines for the Detection and Diagnosis of COVID-19 Virus Infection, 8 July 2020. Available at: https://www.paho.org/en/documents/laboratory-guidelines-detection-and-diagnosis-covid-19-virus-infection.

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8 - World Health Organization (WHO). Terms of Reference for National Influenza Centers of the Global Influenza Surveillance and Response System. 2017. Available at: https://www.who.int/influenza/gisrs_laboratory/national_influenza_centres/tor_nic.pdf.