Respiratory Syncytial Virus (RSV) laboratory diagnosis
Technical note

24 January 2023

Background

Together with influenza and SARS-CoV-2, Respiratory Syncytial Virus (RSV) is a respiratory virus that can cause severe acute respiratory infection (SARI), with a particular impact on children and infants under one year of age. RSV is one of the most prevalent respiratory pathogens to infect children worldwide and usually causes mild, cold-like symptoms and often causes severe disease including deaths, particularly in very young children[1]. Infections in healthy children and adults are generally less severe than among infants and older adults with certain medical conditions[2] . Recently, RSV infections have increased significantly in the Americas region, Canada, Mexico, Brazil, Uruguay, and the United States. RSV circulation in most subregions of the Americas has reached pre-pandemic seasonal circulation levels since early 2022, with activity increases during mild 2022 (June) and peaked during October-November[3]. The World Health Organization (WHO) efforts are addressed towards integrating RSV to the existing influenza surveillance platform, the WHO Global Influenza Surveillance and Response System (GISRS) to acknowledge a better understanding of the burden of disease in different populations without negatively impacting the current influenza surveillance network[4].

Laboratory testing

Influenza and SARS-CoV-2 testing should be prioritized as part of Influenza and SARS-CoV-2 integrated surveillance. RSV molecular testing should be used as a differential diagnostic after influenza and SARS-CoV-2 have been ruled out, as per testing algorithm recommended by PAHO[5].

Result reporting

RSV results should be weekly reported through FluNet report.

PAHO encourages laboratories to timely subtyping and sequence RSV positive samples and share genetic information through the global platform GISAID. In addition, countries implementing sequencing should follow the guidelines of the RSV reference laboratories following the WHO RSV Sequencing Strategy.

For additional information on RSV or diagnostic reagents, please contact PAHO Regional Office at flu@paho.org

[1] WHO. WHO Strategy for the Global Respiratory Syncytial Virus Surveillance based on Influenza Surveillance. Geneva: World Health Organization; 2019. Available at who-rsv-surveillance-strategy-phase-26mar2021.-final.pdf

[2] CDC. Respiratory Syncytial Virus Infection (RSV). 2022. [Last Reviewed: October 28, 2022]. Available at https://www.cdc.gov/rsv/index.html

[3] PAHO. Epidemiological Update - Influenza and other respiratory viruses - 13 November 2022. Available at https://www.paho.org/en/documents/epidemiological-update-influenza-and-other-respiratory-viruses-13-november-2022

[4] WHO. WHO strategy to pilot global respiratory syncytial virus surveillance based on the Global Influenza Surveillance and Response System (GISRS). 2017. Available at https://apps.who.int/iris/handle/10665/259853

[5] PAHO. Integrated Influenza Laboratory testing algorithm available at https://www.paho.org/en/documents/influenza-and-sars-cov-2-integrated-surveillance-laboratory-testing-algorithm

RSV molecular detection protocol[6]

Reaction Master Mix

For the master mix preparation calculate the amount of each reagent to be added as follows:

Reagent	qScriptTM One-Step qRT-PCR Kit	AgPath-IDTM One-Step RT-PCR Kit
Nuclease-free water	N x 5.5 µL	N x 5.0 µL
Forward primer	N x 0.5 µL	N x 0.5 µL
Reverse primer	N x 0.5 µL	N x 0.5 µL
Probe	N x 0.5 µL	N x 0.5 µL
2X PCR Master Mix	N x 12.5 µL	N x 12.5 µL
Enzyme Mix	N x 0.5 µL	N x 1.0 µL
Total Volume	**N x 20 µL**	**N x 20 µL**

Note: The controls must be included in each run.

RT-PCR amplification conditions

Select the cycling protocol on the instrument:

	AgPath-IDTM One-Step RT-PCR Kit	qScriptTM One-Step qRT-PCR Kit
Reverse transcription:	45°C for 10 min	50°C for 10 min
PCR inicial denaturation:	**95°C for 10 min**	**95°C for 5 min**
PCR amplification (45 cycles)	95°C for 15 sec 55°C for 60 sec*	95°C for 15 sec 55°C for 60 sec*

* Fluorescence data (FAM) should be collected during the 55°C incubation step
Detector: FAM | Quencher: None | Passive Reference: None | Sample Volume: 25 µl

Result interpretation:

HRSV	RP	Interpretation	Report
+	+ or -	RSV RNA detected	Positive for RSV
-	+	RSV RNA not detected	Negative
-	-	Invalid result	Invalid

[6] Wang et al. Duplex real-time RT-PCR assay for detection and subgroup-specific identification of human respiratory syncytial virus. J. Virol Methods. 2019. Available at https://doi.org/10.1016/j.jviromet.2019.113676