Detection and diagnosis of SARS-CoV-2 in the context of the circulation of the Omicron variant of concern

30 November 2021

On November 26, 2021, WHO designated the B.1.1.529 lineage of the SARS-CoV-2 virus as a variant of concern (VOC) and assigned the name of Omicron to it, following the Greek alphabet.[1]

The decision was based on the large number of mutations identified throughout the genome and particularly in the *spike* (*S)* gene, some of which had already been described in other VOCs as responsible for an increased transmission capacity or potential evasion of the immune response. Preliminary data based on genomic information indicate a potential risk of reinfection by this variant. Likewise, the substantial increase in the detection of suspected cases of B.1.1.529 (based on the failure to detect the *S* gene in some RT-PCR protocols) in South Africa, the country that reported the first case B.1.1.529 in a sample collected on November 9, 2021 and reported on November 24, 2021, led WHO to make the decision to designate B.1.1.529 as a VOC as a precautionary measure.

In the context of the Omicron VOC circulation, PAHO/WHO laboratory response team recommends that Member States take into account the following considerations:

Laboratory Assays for the Diagnosis of SARS-CoV-2

Molecular Diagnosis (real-time RT-PCR)

To date, *recommended real-time RT-PCR tests for SARS-CoV-2 detection continue to detect Omicron as well as all other variants*:

- the reference protocol for molecular detection developed by the Institute of Virology Charité - Universitätsmedizin Berlin, Germany, recommended by PAHO for the universal surveillance of SARS-CoV-2[2]
- the influenza and SARS-CoV-2 multiplex protocol of the WHO Collaborating Center for the surveillance, epidemiology and control of influenza at the United States Centers for Disease Control and Prevention (CDC), which should be prioritized for the integrated sentinel surveillance of influenza-like illness (ILI) and severe acute respiratory infections (SARI).[3]
- In-house or commercial protocol that target the detection of other genes (*N*, *RdRP*, *Orf1*, etc.) have not been affected so far. However, those based on the detection of the *S* gene, must be reviewed as they might fail in the detection due to the high number of mutations.

Therefore, it is recommended to maintain the protocols already implemented for routine SARS-CoV-2 molecular detection.

Diagnosis using antigen detection (antigen-detecting rapid diagnostic tests, Ag-RDT)

Omicron VOC mutations are primarily concentrated in the *S* gene. Considering that the antigen tests recommended and included in the WHO Emergency Use List (EUL) have been developed based on the detection of the nucleocapsid protein (N) which is -in general- more conserved, there is no evidence at the moment to infer that the sensitivity and specificity of rapid antigen tests have changed. However, additional studies are being conducted to determine any possible impact on performance.

Therefore, and based on the information available to date, it is recommended to maintain the routine use of rapid antigen tests according to the protocols implemented in each Country.[4]

Detection of Omicron VOC

SARS-CoV-2 Genomic Surveillance

Considering that the identification and confirmation of the lineage of a circulating variant of SARS-CoV-2 is only possible through the phylogenetic analysis of the whole genomic sequence data, the COVID-19 Genomic Surveillance Regional Network (COVIGEN) of PAHO/WHO continues to work to strengthen the timely sequencing of SARS-CoV-2 positive samples. Sequencing should be increased by shipping samples to COVIGEN Reference Sequencing Laboratories and maintaining or increasing capacities in laboratories where sequencing platforms are already in place.[5]

Additional information on the COVID-19 Genomic Surveillance Regional Network (COVIGEN) can be found at: https://www.paho.org/en/topics/influenza-and-other-respiratory-viruses/covid-19-genomic-surveillance-regional-network

PAHO / WHO encourages laboratories to timely sequence SARS-CoV-2 positive samples selected according to established criteria and to share genetic information through GISAID.

General criteria for the selection of SARS-CoV-2 samples for genomic surveillance:[5]

Representativeness and epidemiological criteria:

- Different age groups
- Different geographic locations within the country
- Different periods of time
- Severity: mild, severe, and fatal cases
- Cases in areas with a significant increase of cases over a few weeks (not explained by relaxation of public health measures)
- Children in areas with increased incidence of pediatric disease
- Clusters of severe cases in people aged <60 years and without underlaying conditions
- Cases where reinfection is suspected
- Cases in fully-immunized people

- Cases with a history of travel to areas where different VOCs (including Omicron) circulate in the 14 days prior to the onset of symptoms

Virological characteristics and laboratory criteria:

- Samples with Ct values ≤ 25
- Samples transported through an unbroken cold chain and stored under ultra-low temperatures conditions (or at least -20 °C)
- Samples that have not gone through multiple freeze-thaw cycles

In parallel, PAHO/WHO is currently reviewing alternatives to complement the genomic surveillance of SARS-CoV-2 through the implementation or adaptation of real-time RT-PCR protocols that allow initial **screening** and early detection of the Omicron variant. These protocols would allow for the identification of mutations present in Omicron and the prioritization of the corresponding samples for whole genome sequencing (see algorithms in annex). The results of these protocols are not confirmatory and should always be interpreted in the context of the circulation of other lineages or variants that may present the same mutation.

Main actions for Member States when the Omicron variant is suspected or identified

- Consider the validation of the Omicron variant identification results by the National Public Health Laboratory for the surveillance of COVID-19 of the Ministry of Health before notifying the finding through the official channels of the International Health Regulations (IHR).
- Immediately report initial cases/clusters associated with Omicron variant infection to PAHO/WHO through the IHR mechanism.
- Submit complete or partial genome sequences and associated metadata to a public database, such as GISAID.

Laboratory algorithms

Considering that the diagnostic protocols and sequencing recommendations have not changed, the recommended laboratory algorithms for the detection and diagnosis of SARS-CoV-2[2,4] and for integrated sentinel surveillance of ILI/SARI[3] should remain unchanged. Proposed algorithms for the use of screening RT-PCRs for Omicron and other variants are included in the Annex.

References

1. World Health Organization. Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern. 26 November 2021. Available at: https://www.who.int/news-room/statements/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern

2. Pan American Health Organization. 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

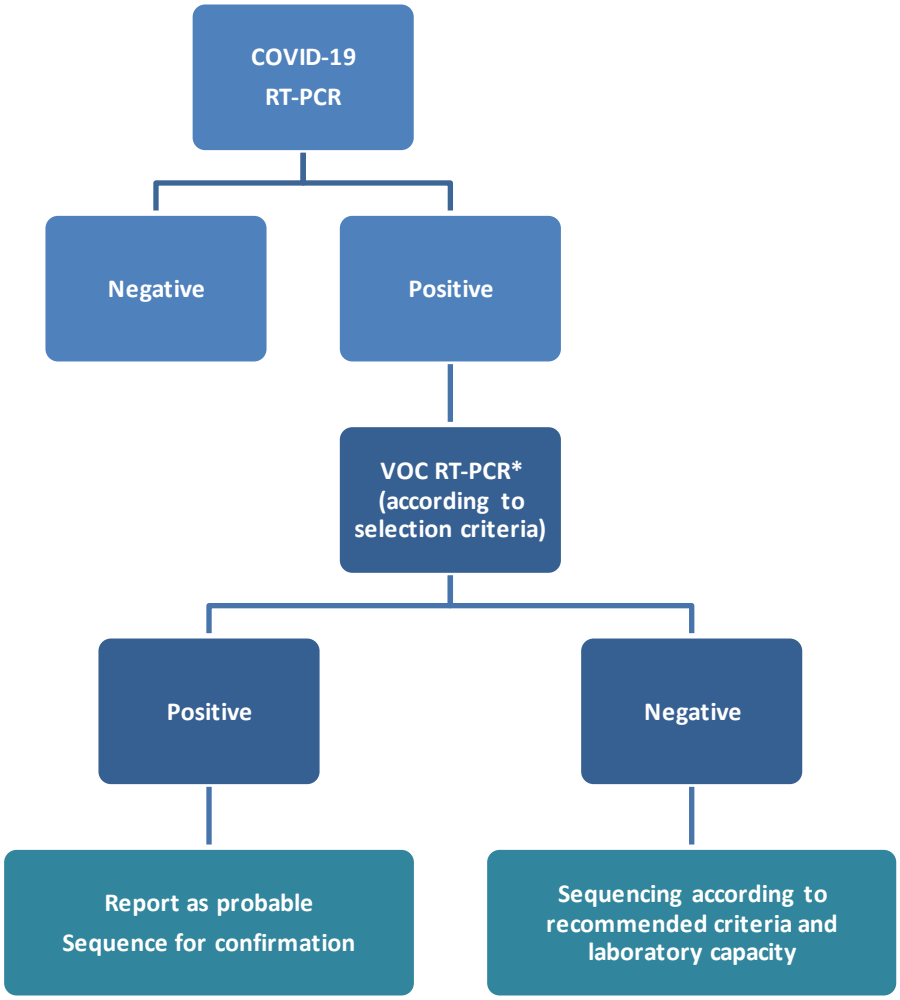
3. Pan American Health Organization. Guidance for the implementation of the Influenza and SARS-CoV-2 Multiplex RT-PCR Assay into the influenza and COVID-19 integrated surveillance. 19 April 2021. Available at: https://www.paho.org/en/documents/guidance-implementation-influenza-and-sars-cov-2-multiplex-rt-pcr-assay-influenza-and

4. Pan American Health Organization. Implementation of COVID-19 rapid antigen detection test – Pilot. 27 October 2020. Available at: https://www.paho.org/en/documents/implementation-covid-19-rapid-antigen-detection-test-pilot

5. Pan American Health Organization. Guidance for SARS-CoV-2 samples selection for genomic characterization and surveillance. 9 February 2021. Available at: https://www.paho.org/en/documents/guidance-sars-cov-2-samples-selection-genomic-characterization-and-surveillance

Annex: Algorithms for molecular screening

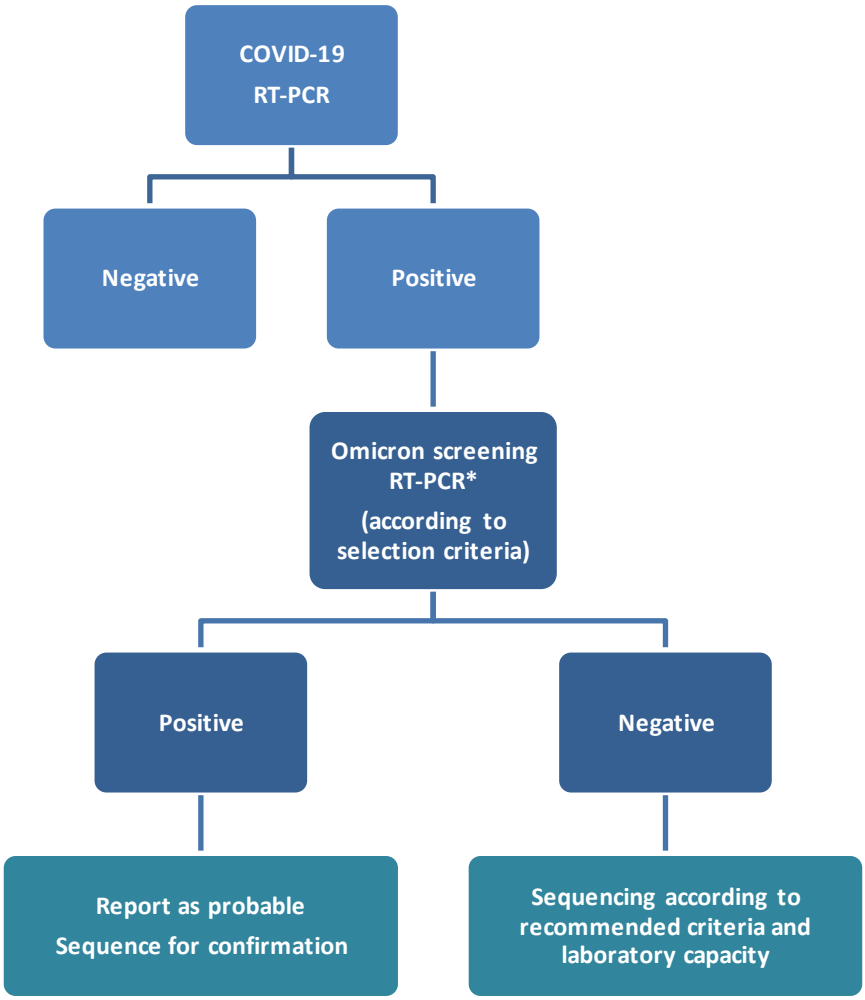
Algorithm for screening of variants of concern (including Omicron) by real-time RT-PCR, using the protocol PAHO previously recommended by PAHO for VOCs



* Naveca et al. protocol: detects a 3 amino acids deletion (S106/G107/F108) in the NSP6 protein. This deletion is present in Alpha, Beta and Gamma VOCs, as well as in the variant of interest (VOI) Lambda. In the case of Omicron, a similar deletion of 3 amino acids (L105/S106/G107) generates a sequence with a single nucleotide difference with the sequence detected by the RT-PCR probe in the other variants mentioned above. Therefore, to ensure a more efficient detection, the original probe sequence will be adjusted. The results must be interpreted in the light of the variants known to circulate in the country and that could be detected by this RT-PCR. For example, in the context of a predominance of Delta and the absence/low circulation of Alpha, Beta, Gamma and Lambda, the presence of the deletion suggests the detection of Omicron.

Reference: Naveca et al., COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and P.1 emergence, *Nat Med* **27,** 1230–1238 (2021), https://doi.org/10.1038/s41591-021-01378-7

Generic algorithm for screening of variants of concern (including Omicron) by real-time RT-PCR, using other protocols



* Note: Different screening protocols may exist for Omicron and other VOCs. In all cases, the mutation detected and its potential presence in other variants or lineages must be taken into account (see Table).

Characteristic mutations in the S protein for variants of concern and interest

VOC/VOI	69/70 deletion	Position 417	Position 484	Position 501	Position 681
GISAID reference sequence	*No*	*K*	*E*	*N*	*P*
Alpha	**Yes**	K	E	**Y**	**H**
Beta	No	**N**	**K**	**Y**	P
Gamma	No	**T**	**K**	**Y**	P
Delta	No	K	E	N	**R**
Lambda	No	K	E	N	P
Mu	No	K	**K**	**Y**	**H**
Omicron	**Yes**	**N***	**A**	**Y**	**H**

* Note: K417N mutation present in approximately 50% of the Omicron sequences available to date.

This table does not include all mutations present in the S protein. The mutations listed are the most common in each variant. However, some sequences of the same variant may have accumulated additional mutations (eg, Alpha with 484K or Delta with 417N).