PAHO Pan American Health Organization World Health Organization Americas

Guidance for SARS-CoV-2 samples selection for genomic characterization and surveillance

01 February 2021

Genomic sequencing has been an essential tool in generating virological data, driving the laboratory response, and better understanding the dispersal and evolutionary patterns of SARS-CoV-2 [1-2]. In addition to the characterization of the global circulation patterns, early detection of SARS-CoV-2 variants inside each country is critical to complement the epidemiological and virological surveillance.

PAHO/WHO has supported strengthening the in-country capacity to carry out genomic surveillance since March 2020 within the framework of the COVID-19 Genomic Surveillance Regional Network [3-4] and encourages Member States not only to participate in the Network but also to implement and enhance the sequencing capacities to timely upload sequences to the global platform GISAID [5].

A time series of samples is necessary to detect trends of change in genetic diversity and emerging variants. As well, appropriate number and representative samples, including unusual case samples, are important for generating reliable genomic sequencing data.

Because the success of genomic surveillance hinges on the sampling strategies as critical component for achieving high quality results, PAHO recommends appropriate selection of samples for sequencing, based on number of samples, time frames and representativeness.

SARS-CoV-2 sampling criteria

Representativeness criteria

In order to promote generation of more robust and legitimate data that reflects the genomic information of SARS-CoV-2 circulating inside the countries of the region, sampling based on representativeness is key for the genomic surveillance objectives.

For better representation of SARS-CoV-2 circulating in countries, the following **criteria for representativeness** are recommended:

- Regular surveillance in areas with high transmission:
  - Different age groups;
  - Different geographic locations within the country;
  - Different periods of time;
  - Different severity: mild, severe and fatal cases;
- Cases in areas with a significant increase of cases over a few weeks (not explained by relaxing of the 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.

Virologic characteristic of the clinical samples

Because SARS-CoV-2 has one of the largest RNA viral of about 30,000 nucleotide bases in length, good quality of sample and with high virus load is crucial for retrieving full genome sequence with high quality. Low quality of sample and RNA leads to failure of sequencing protocols. The ideal clinical samples are the ones that have been properly transported ensuring the cold chain maintenance and storage under ultra-low temperatures.

Because high RNA concentration and stability are bases for whole genome sequences of high quality, the recommended **clinical samples based on laboratory diagnostic** [6] are as follow:

- Samples with Ct values ≤ 30;
- Samples transported through an unbroken cold chain and storage under ultra-low temperatures conditions;
- Avoid multiple freeze-thaw cycles of the sample.

Time frame and number of SARS-CoV-2 positive samples

For better representativeness ideally, at least 50 SARS-CoV-2 samples per time frame (month) should be considered for better representativeness.

In the context of the emerging variants, the **recommended number of samples** for selection would be:

- At least 50 SARS-CoV-2 samples per time frame (month):
  - 24 samples from March to October 2020 (for last year information)
  - 50 samples from November 2020 to January 2021 (150 samples)
  - 50 samples from February onwards

SARS-CoV-2 Genomic Surveillance

The COVID-19 Genomic Surveillance Regional Network is open to all the countries of the Americas. For more information on the network, regional sequencing laboratories, samples shipping or any additional information, the PAHO Regional Office can be contacted at the emails leite@paho.org, ricoj@paho.org.

References

1. World Health Organization. Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). 2021 Feb 01. Available at: https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-

the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov)

2. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19. 2020 Mar 11. 2021 Feb 01. Available at: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020

3. Pan American Health Organization. COVID-19 Genomic Surveillance Regional Network. 2021 Feb 01. Available at: https://www.paho.org/en/topics/influenza/covid-19-genomic-surveillance-regional-network

4. Leite JA, Vicari A, Perez E, Siqueira M, Resende P, Motta F, et al. Implementation of a COVID-19 Genomic Surveillance Regional Network for Latin America and Caribbean Region. PLOSone 2021. Submitted

5. GISAID initiative. 2021 Feb 01. Available at: https://www.gisaid.org/

6. Pan American Health Organization. Laboratory Guidelines for the Detection and Diagnosis of COVID-19 Virus Infection (2020). 2021 Feb 01. Available at: https://iris.paho.org/handle/10665.2/52458