Genomic Surveillance for SARS-CoV-2

In India

Indian SARS-CoV-2 Genomics Consortium

(INSACOG)

(Updated guidelines and SOPs)

(dated 15.07.2021)
1. Background

Globally, the SARS-CoV-2 virus has posed the biggest public health challenge of the century. However, India, has largely been able to keep the mortality low through effective diagnosis, appropriate treatment measures and contact tracing. In order to fully understand the spread and evolution of the SARS CoV-2 virus, and to tackle its future spread sequencing and analysing the genomic data of SARS CoV-2 is required. Any changes to the genetic code, or mutations in the SARS-CoV-2 virus, can be observed in the samples through genomic sequencing studies. Global experience has revealed the importance of genomic variants playing a key role in transmission and subsequent surges. The study of accumulation of mutations in the viral genomes enables us to compare virus samples and viral lineages in order to understand if local outbreaks are caused by transmission of single or multiple viral lineages. Analysis of SARS-CoV-2 genome sequences also allows us to study the evolution of the virus and assess whether these mutations influence transmission, clinical outcomes, severity, and their role in developing public health intervention measures and vaccines.

Indian SARS-CoV-2 Genomics Consortium (INSACOG) was established to expand Whole Genome Sequencing of SARS-CoV-2 across the nation, aiding our understanding of how the virus spreads and evolves. The Consortium had initially started with a network of ten regional genome sequencing laboratories spread across the country and has now been expanded to currently include 28 INSACOG Genome Sequencing Laboratories (IGSLs) that are mapped to the States and UTs for facilitating smooth flow of samples. The viral genome sequencing data generated by these IGSLs is analysed by the respective centres and sent to the National Centre for Disease Control (NCDC), Delhi for collation and integration. The Central Surveillance Unit (CSU) under Integrated Disease Surveillance Programme (IDSP) at the National Centre for Disease Control (NCDC) regularly collects data in a decentralized manner from various States/districts. This helps in correlating the data from the genome sequencing laboratories with the field data trends and study the linkages (if any) between the genomic variants and epidemiological trends based on COVID data generated by State and District Surveillance Units of IDSP. These correlations also enhance the understanding of unusual events like Vaccine breakthrough, suspected reinfections, super spreader events, outbreaks etc. The ultimate goal of this activity is to strengthen public health interventions across the country and breaking the chain of transmission. Linking this data with the IDSP epidemiological data and data on patient’s symptoms will allow us to better understand the viral infection dynamics, morbidity and mortality trends. Further, the data can be linked with host genomics, immunology, clinical outcomes and risk factors for a more comprehensive outlook.

Over the past few months many variants have been detected through the Whole Genome Sequencing activities undertaken by INSACOG. The information so obtained has been regularly shared with States/UTs to strengthen their public health response to the pandemic. A few of the
variants thus detected have contributed to surges across various regions and other variants are being monitored by INSACOG for their potential role in disease transmission dynamics. For this whole exercise to become more meaningful, it is necessary that timely clinical data and adequate number of RTPCR positive samples for genome sequencing are provided by the States/UTs to facilitate better analysis of the transmissibility and virulence of these variants.

2. Stakeholders
   1. State Governments - IDSP-SSUs and DSUs situated in States and Districts
   2. Sentinel Sites –Identified RT-PCR labs, secondary & tertiary care hospitals of States and UTs.
   3. INSACOG Genome Sequencing Labs
   4. Ministry of Health and Family Welfare - NCDC, ICMR
   5. Ministry of Science and Technology – DBT/DST/CSIR

3. Objectives of the Indian SARS-CoV-2 Genomics Consortium (INSACOG)

The overall aim of the Indian SARS-CoV-2 Genomics Consortium is to monitor the genomic variations in the SARS-CoV-2 on a regular basis through a multi-laboratory network. The mandate of INSACOG has evolved with time and the focus has shifted from primarily tracking variants among international passengers to early detection of variants that may emerge within the Country. In the present scenario, genomic surveillance of SARS-CoV-2 has the following objectives:

- Early detection of genomic variants of public health implication through sentinel surveillance
- To determine the genomic variants in unusual events/trends (Vaccine breakthrough, super-spreader events, high mortality/morbidity trend areas etc.)
- To correlate the genome surveillance data with epidemiological data
- To suggest public health actions based on the analysis of genomic and epidemiological surveillance data

4. Genomic Surveillance Strategy:

The current genomic surveillance is based on a three-pronged strategy that focusses on:

4.1. International Travellers
4.2. Regular on-going surveillance in the community (through Sentinel Sites) &
4.3. Event based surveillance in special case scenarios
4.4. Other general considerations

The salient features of the three-pronged strategy are:
4.1 International Travellers:
   i. The aim is to detect the entry of new variants/mutants in India from other countries.
   ii. The details of the Countries, Points of Entry and Variants to be included in this approach shall be decided on the basis of the available information and the guidance released by the WHO under the provisions of the International Health Regulations (IHR 2005) from time to time.
   iii. Guidance for Epidemiological Surveillance and Response in the context of new variant of SARS-CoV-2 virus detected in other Countries shall be made available on the MoHFW website as and when the need arises.
   iv. This shall involve, inter alia, screening & testing of international travellers followed by sequencing a fixed percentage of the positive cases thus detected.

4.2 Regular on-going surveillance in the community through Sentinel Sites:
   i. This is based on the WHO document “Operational Considerations to expedite Genomic Sequencing Component of GISRS surveillance of SARS CoV-2” that has been adapted taking into consideration the specific requirements of the country and the present sequencing capacity of the laboratories in the country.
   ii. The States and UTs are required to identify adequate number of Sentinel Sites ensuring representation across geographical territory (coverage of at-least 80% of the districts to be ensured) as well as the clinical spectrum of cases reported from the State/UT.
   iii. The sentinel sites can either be the RT-PCR labs or secondary & tertiary care hospitals managing COVID cases.
   iv. The sentinel surveillance methodology requires that ideally each sentinel site sends at-least 15 samples every 15 days to the identified IGSL as per standard specimen collection procedure depicted in Annexure-1.
   v. However, this methodology may be customised by the States/UTs to suit local conditions. States are encouraged to refer samples in numbers that reflect the field situation and are in-sync with the sequencing capacity of the designated IGSL mapped to the State/UT.

4.3 Event based surveillance in special case scenarios:
   i. The IDSP network captures unusual COVID-19 events such as suspected vaccine breakthrough, super-spreader events, clusters of cases with high mortality and/or morbidity and reports to the concerned DSUs for verification of these events
   ii. Upon verification, a detailed epidemiological investigation including instituting genomic analysis of the samples collected from such events is to be carried out by the concerned SSU/DSU in consultation with the CSU
   iii. The number of samples to be sent for sequencing in such events shall be determined by the investigating team and can be 100% for events such as vaccine breakthrough and suspected reinfections.
   iv. The Central Surveillance Unit – IDSP will provide technical assistance to the States/UTs in this regard.

4.4 Other General Considerations of the Strategy:
i. The relevant case details of any sample detected with the new variant which is found to be significant from public health perspective will be communicated directly to NCDC (Director), NCDC being the Nodal Unit along with the members of INSACOG SCAG.

ii. Data privacy is to be ensured with respect to personal identifiers as well as findings that may have national or international public health implications.

iii. Submission of genomic data to international databases e.g. GISAID etc shall not be undertaken before submitting the summary findings to NCDC.

iv. The database of all the samples sent for genome sequencing will be maintained in the special surveillance module of IHIP-INSACOG portal. Further, clinical and outcome details of all such samples must also be stored, updated and shared with CSU-IDSP, NCDC for establishment of clinic-epidemiological correlation. Individual SSUs/DSUs must also proactively review this data.

v. The States will be intimated in case of identification of any new variant of public health concern after discussion with the technical experts for further epidemiological analysis and planning response strategies.

vi. All the genomic sequencing data will be maintained in National database at three sites:
   a. NIBMG, Kalyani,
   b. IGIB, New Delhi and
   c. NIV, Pune.

vii. The central database shall be accessible to all the contributing IGSLs.

viii. The genome sequence data will be shared with international organisations only after it has been shared with NCDC.

5. Organisational structure:

A. **Centre level:** A Nodal Unit has been created at NCDC, New Delhi with officers from Division of Bio-technology, Epidemiology and Central Surveillance Unit. This unit will act as a pivot and coordinate with the respective State/district surveillance units and plan the transportation of samples to the designated IGSL. Samples can also be transported by Sentinel Sites directly to sequencing labs. This unit at NCDC, New Delhi will also act as the Nodal National Hub for all Regional Hubs as detailed below.

B. **Regional level:** It is proposed that the ten identified IGSL will serve as the regional hub laboratories for genome sequencing of the relevant region. These ten IGSLs are supported with 18 Satellite labs for genome sequencing activities (Annexure 2). These satellite labs will be sharing the genome sequencing data with respective hub IGSLs which in turn will update the same on IHIP-INSACOG portal. The Country will be divided into six regions for clearly defining the sample collection/transportation flow, as below:
<table>
<thead>
<tr>
<th>Regional Hub</th>
<th>List of Hub Labs</th>
<th>State/UT(s)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>East and North East</strong></td>
<td>1. DBT- National Institute of Biomedical Genomics (NIBMG), Kalyani (near Kolkata) Estimated sequencing capacity – 5000 per month</td>
<td>Andaman &amp; Nicobar Islands, West Bengal, Bihar, Jharkhand, Assam, Tripura, Meghalaya, Manipur, Arunachal Pradesh, Sikkim, Nagaland, Mizoram Odisha, Chhattisgarh, Sikkim</td>
</tr>
<tr>
<td></td>
<td>2. DBT-Institute of Life Sciences, (ILS) Bhubaneshwar Estimated sequencing capacity – 1200 per month</td>
<td></td>
</tr>
<tr>
<td><strong>West</strong></td>
<td>3. ICMR-National Institute of Virology (NIV), 4. DBT-National Centre for Cell Science, Pune Estimated sequencing capacity – 1200 per month</td>
<td>Goa, Maharashtra, Gujarat, western part of MP, UT of Dadar and Nagar Haveli and Daman and Diu</td>
</tr>
<tr>
<td><strong>South</strong></td>
<td>5. CSIR-Centre for Cellular and Molecular Biology (CCMB) and 6. DBT-Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad Estimated sequencing capacity – 5000 per month at CCMB and 1200 at CDFD</td>
<td>Andhra Pradesh, Telangana, Goa (northern part of Karnataka)</td>
</tr>
<tr>
<td></td>
<td>7. DBT InSTEM/NCBS, Bengaluru Estimated sequencing capacity – 1200 per month 8. NIMHANS, National Institute of Mental Health and Neuro Sciences Hospital (NIMHANS), Hosur Road, Bangalore</td>
<td>Karnataka, Tamil Nadu, Puducherry</td>
</tr>
<tr>
<td><strong>Central</strong></td>
<td>9. CSIR-Institute of Genomics and Integrative Biology (IGIB), Delhi Estimated sequencing capacity – 10,000 per month</td>
<td>Rajasthan, Punjab, Haryana and western part of UP. Kerala samples will be sequenced at IGIB</td>
</tr>
<tr>
<td><strong>North &amp; Central</strong></td>
<td>10. NCDC, Delhi - Division of Bio-technology, Epidemiology and Central Surveillance Unit Estimated sequencing capacity – 3,000 per month</td>
<td>Eastern part of MP, Uttarakhand, Chandigarh, Delhi, Haryana, Himachal Pd., Ladakh, J&amp;K &amp; Punjab</td>
</tr>
</tbody>
</table>

*: States/UTs may plan and indicate the practical feasibility to CSU. CSU will further fine tune the regional linkages with IGSLs.

NCDC based on a continuous and dynamic assessment exercise would readjust the mapping of states, parts of states to IGSLs.
6. **Flow of Information:**

6.1 All the Sentinel Sites and IGSLs have been provided username and passwords for the INSACOG WGS Surveillance module of IDSP-IHIP.

6.2 The Sentinel Sites will send adequate number of samples for genome sequencing to the mapped IGSLs (10 Hub IGSLs and 18 Satellite IGSLs) – list placed at Annexure 2, and will update the details of the samples on the INSACOG WGS Surveillance module of IDSP-IHIP including the name of IGSL to which samples are being sent.

6.3 The IGSL will share the genome sequencing results (annotated data) with NCDC, Delhi for further analysis and compilation (taking help from NIBMG and IGIB sequencing analysis team).

6.4 The analysis reports will be sent periodically to the MoHFW and also shared with the relevant IGSL and CSU/SSU for necessary action.
7. Key steps in Implementation of the genomic surveillance strategy:

7.1 Each State and UT is to designate a WGS nodal officer who will be the focal point for coordination of WGS related activities between the State, Sentinel Sites, IGSLs and NCDC. The State nodal officer is responsible for overall coordination from sending of the samples to the designated IGSLs/satellite labs along with updating of data for vaccination status of the patient, travel history, and outcome on IHIP WGS portal.

7.2 The nodal officer is also responsible for identification of adequate number of sentinel sites (Minimum of 10 for larger States) in consultation with their respective State Govt./UT Administration. These sentinel sites must adequately represent geographical and clinical spectrum of the cases in the respective State/UTs.

7.3 A nodal officer for each sentinel site needs to be designated. The user credentials for these sentinel sites to be shared by the State Nodal Officer. The list of sentinel sites along with the details of the nodal officer for each site has to be communicated to IDSP, NCDC.

7.4 Adequate number of samples (At least 15 per fortnight) from each of the sentinel site should flow towards the designated IGSL. (Relaxation in this criterion of 15 per fortnight may be considered when the daily new cases reported from the Sentinel Site are less than 1) In such a case, all RTPCR positive samples in the given fortnight may be sent for WGS.

7.5 The data of the samples sent to IGSLs to be entered in the IHIP-INSACOG portal only using the credentials. The user guide for data entry is placed at Annexure – 3.

7.6 The sentinel sites or District Surveillance Units have to enter the travel history, vaccination history and outcome details of the cases sent for WGS, and review this data proactively.

7.7 IGSLs will have to enter the results of the WGS directly on to the IHIP-INSACOG portal using their credentials which is provided by the State nodal officer. The details of results updation on the portal are depicted in Annexure-4.

7.8 In case of any variant detected by the IGSLs and which may be of concern, such as having immune escape capacity or lowering immune response in the host etc., a technical discussion with the SCAG group members of INSACOG would determine for the actions to be taken at the field level. CSU-NCDC will communicate these findings to the concerned States/UTs.

8. Rapid Response Strategy:

8.1 A Rapid Response Team (RRT) will be formed in each State/UT by the Health Department. The team will comprise of a clinician, a microbiologist and a member from Medical College (preferably from Community Medicine Department). As soon as any mutation is detected and conveyed to the State/UT, the RRT will be deployed by the State/UT to the site, where it will investigate the mutant based on following aspects:

i. Contact tracing of case in which the mutation is detected
ii. Epidemiological aspects of the mutant detected with respect to number of cases, deaths in the community etc.
iii. Clinical spectrum of the positive case to detect any change in the severity or mortality.
iv. Samples of all members in the family in which a variant has been detected and their contacts need to be collected and sent for WGS to the mapped IGSL. This activity should be overseen by the Rapid Response Team.
v. Take necessary containment measures in the area in conjunction with the district administration
vi. Provide daily status reports to NCDC

8.2 Review by States/UTs

Regular reviews of the whole genome sequencing activity are to be held by Additional Chief/Principal/Secretary (Health)/MD (NHM) for the following activities:

- Identification of adequate numbers of sentinel sites for the State/UT, ensuring geographical, demographic, and clinical spectrum representativeness
- Ensuring that adequate number of samples are sent for WGS to the designated laboratories
- Reviewing correlation of mutants/VOCs with the epidemiological and clinical data available with the State/UTs

9. Standard Operating Procedure for various Stakeholders:

9.1 Sentinel Sites:

i. The sentinel sites are required to send 15 samples per fortnight to the tagged IGSLs
ii. The sentinel sites can send samples directly to the satellite lab linked to their identified Hub lab (Details annexed)
iii. The sample referral form (Metadata) of every sample sent for WGS has to be filled up by the Sentinel Site online on the IHIP portal (Username, passwords have been provided to all the sentinel sites)
iv. The results would be made available to the sentinel sites only through the online referral & reporting system on IHIP
v. The sample referral form is required to be filled up as completely as possible (including the sections on clinical severity, vaccination, travel history and outcome, if available) before sending to the IGSLs

9.2 Hub IGSLs:

i. All the Hub IGSLs have been provided credentials for reporting the WGS results on the IHIP portal
ii. The Hub IGSLs are expected to provide technical support to the satellite labs enabling them to function independently in the future
iii. The Hub IGSLs are also required to enter the results of the satellite laboratories till the time the INSACOG recognizes them as independent IGSL (akin to Hub IGSL) for WGS & data entry
iv. The sample wise results updated by Hub IGSLs shall be immediately visible to the respective sentinel site as well as the concerned IDSP SSU/DSU
v. All the IGSLs (Hub & Satellite) have also been provided login credentials (as sentinel sites) for their own sample referral for internal WGS (3-5% of internally tested RT-PCR positive samples)

9.3 Satellite IGSLs:
   i. Each satellite IGSL is linked to a designated Hub laboratory
   ii. The sentinel sites can send samples directly to the satellite lab
   iii. The satellite labs are required to share the sequencing results with the Hub IGSLs for updating the same on portal
   iv. The satellite labs have to ensure that all relevant information pertaining to WGS is shared with the concerned Hub IGSL
   v. The satellite labs shall continuously strive to update their technical skills as per the guidance from the Hub laboratories / INSACOG.

9.4 IDSP DSUs:
   i. District Surveillance Officer (DSO) is responsible for coordination of sending samples from the sentinel sites to the designated IGSLs or satellite labs.
   ii. DSO is responsible for updating of the data with respect to vaccination status, travel history and outcome of the patients for whom the samples have been sent for WGS.
   iii. Provide continuous support and constructive feedback in a timely manner and also update the records of samples sent by sentinel sites including clinical and outcome data of each sample sent for WGS..
Annexure 1

Specimen Collection, Packaging and Transport Guidelines for SARS-CoV-2 positive samples for genome sequencing

To be used for genome sequencing of RT-PCR positive samples by the laboratory personnel from Government or private health authorities/ hospitals.

Purpose: Specimen packaging and transport of clinical specimens to IGSL for genome sequencing.

Sample collection:

a) From the identified Sentinel Sites as per the strategy
b) From Non-Sentinel Sites (Labs/Hospitals) in case of unusual events such as vaccine breakthrough, super-spreader events, high mortality/morbidity trend areas etc.

Data sheet:

The Sentinel Sites will submit the sample referral form on the INSACOG WGS Surveillance module of IDSP-IHIP portal.

Roles and Responsibilities: The Sentinel Site will collect, package & transport SARS-CoV-2 positive samples.
Only those samples which are positive for SARS-CoV-2 by RT PCR preferably with a Ct value of 25 or less should be packaged & transported.

After carrying out the RT-PCR test the remaining samples (within 72 hours of collection, stored at 2-8°), which are RT-PCR positive (Ct value <25), will be transported in VTM with cool pack (4-8 degree) or in ice.

Alternatively, remaining RNA samples may be stored and aliquoted in the 1.5 ml microcentrifuge tubes followed by proper labelling and sealing with the parafilm (stored at -70 degree Celsius). RNA placed together in plastic/ cardboard cryo-box and packed in the thermocol box with dry ice should be shipped to the respective IGSL for sequencing.

Samples should be packaged and transported with all biosafety precautions and should be accompanied with line list and details of samples including the Ct values of all the target genes detected in standard triple packaging.
The packaging consists of three layers as follows.

1. **Primary receptacle:** A labelled primary watertight, leak-proof receptacle containing the specimen. The receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage.

2. **Secondary receptacle:** A second durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material must be used to cushion multiple primary receptacles.

3. **Outer shipping package:** The secondary receptacle is placed in an outer shipping package which protects it and its content from outside influences such as physical damage and water while in transit

Personal protective equipment (apron, hand gloves, face shield, N95 Masks etc.) need to be used and all biosafety precautions should be followed while carrying out sample packaging and transport.
Annexure 2

List of INSACOG Genome Sequencing Laboratories (IGSL) – Hub and Satellite Labs

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>IGSL</th>
<th>State</th>
<th>Type of Lab (Hub/ Satellite)</th>
<th>Name of Hub Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NIBMG, Kalyani</td>
<td>West Bengal</td>
<td>Hub</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>ILS, Bhubaneshwar</td>
<td>Odisha</td>
<td>Hub</td>
<td>N/A</td>
</tr>
<tr>
<td>3</td>
<td>IGIB, New Delhi</td>
<td>Delhi</td>
<td>Hub</td>
<td>N/A</td>
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<td>4</td>
<td>NCDC, New Delhi</td>
<td>Delhi</td>
<td>Hub</td>
<td>N/A</td>
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<tr>
<td>5</td>
<td>CCMB, Hyderabad</td>
<td>Telangana</td>
<td>Hub</td>
<td>N/A</td>
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<td>NCCS, Pune</td>
<td>Maharashtra</td>
<td>Hub</td>
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<td>Hub</td>
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