

LABORATORY GUIDANCE

SARS-CoV-2 Whole Genome Sequencing and Analysis Request

11/12/2021

Introduction

As SARS-CoV-2 cases fluctuate, the Ontario COVID-19 Genomics Network (OCGN) changes the proportional sampling strategy for representative surveillance to guide sequencing efforts in Ontario. This ensures the Network can maintain maximum utilization of its capacity while keeping optimal testing turnaround time. When proportional sampling is less than 100% of eligible specimens, Public Health Units (PHU) may request sequencing of additional specimens that were not selected for representative surveillance in order to support their response.

These may include requests for sequencing for the purpose of outbreak investigation, re-infection investigations, vaccine breakthrough, etc. Currently, OCGN labs report VOC lineages by name (e.g. B.1.1.7) on the clinical report through their lab information systems and the Ontario Laboratory Information System (OLIS); VOC sub-lineages are reported according to the parent lineage. Non-VOC lineages are reported as “Variant of Concern not detected” due to changing classifications of non-VOC lineage designations as the virus keeps evolving.

In addition to sequencing, PHUs may request genomic analysis for outbreak specimens. There are two types of analyses and reports – lineage-only, and in-depth analysis, as described below. Requests for analysis can be made at the time of request for sequencing if testing has not been completed, or later for specimens already sequenced.

PHUs should not approach individual labs with requests for WGS testing or questions about specimens’ eligibility criteria before their request is approved. This document summarizes the process for requesting whole genome sequencing (WGS) of SARS-CoV-2 positive specimens and the intake process for the required information and specimens for this purpose.

Request for Whole Genome Sequencing

All requests for sequencing are made through the Incident and Outbreak Response Team (IORT) at PHO’s (Public Health Ontario’s) Laboratory. Requests should include **a brief epidemiologic summary**. Analysis could be requested at the same time, see below and process flowchart for summary.

Request for Analysis - Overview

There are two main types of Outbreak-related WGS analysis requests:

1. **Requests for a lineage-only report (Simple COVID-19 Outbreak WGS Report)** – This report is useful if multiple introductions are suspected and different lineages/sub-lineages are circulating in the community. Sequencing additional specimens from an outbreak may reveal multiple lineages that would indicate multiple introductions. The simple outbreak report will include the named lineages/sub-lineages for all specimens listed regardless of VOC status to help identify potential multiple introductions. A sample Simple Outbreak Report is shown in Appendix A.
2. **Requests for in-depth genomic analysis (In-depth COVID-19 Outbreak WGS Report)** – these are requested in cases where the PHU is investigating the outbreak for specimen relatedness to assess the possibility of multiple introductions. Note that in-depth analysis for individual outbreaks cannot conclusively determine if involved lineages are associated with vaccine breakthrough or increased severity. A sample In-depth Outbreak Report is shown in Appendix B.

For each of these requests, the specimens can either be at PHO's laboratory due to being tested by PHO for COVID-19 PCR or WGS, or the specimens could be with another COVID-19 testing lab.

Requests for simple and in-depth outbreak reports should be emailed to the Incident and Outbreak Response Team's (IORT) intake email address iort@oahpp.ca for approval by the Outbreak Adjudication Group. The Outbreak Adjudication Group consists of OCGN members who will review the appropriateness of the request. Please do not coordinate transfer of samples or contact testing laboratories prior to approval by the Outbreak Adjudication Group.

The questions below should be addressed in the email for every request for analysis, whether the PHU is requesting lineage-only report or in-depth genomic analysis:

- What is the rationale for the request; what scientific question/hypothesis does the PHU wish to answer?
- Provide a detailed epidemiologic summary
- How will the genomic analysis support the PHU (e.g. how will this impact outbreak management, will this be shared with external stakeholders, is it expected to garner media attention)
- Is this a time sensitive request?

Upon approval, IORT will assign a WGS-request ID to each request for tracking purposes and provide the requestor with the COVID-19 WGS Request Form (Appendix C). Requestors will then be asked to complete the form and confirm specimens' eligibility and availability.

Completed request forms will be uploaded to IORT's secure SharePoint collaboration site. IORT will review the completed form and assign a testing laboratory in consultation with the OCGN. The requestor will be asked to arrange for specimens to be transferred to the sequencing laboratory.

Testing Prerequisites

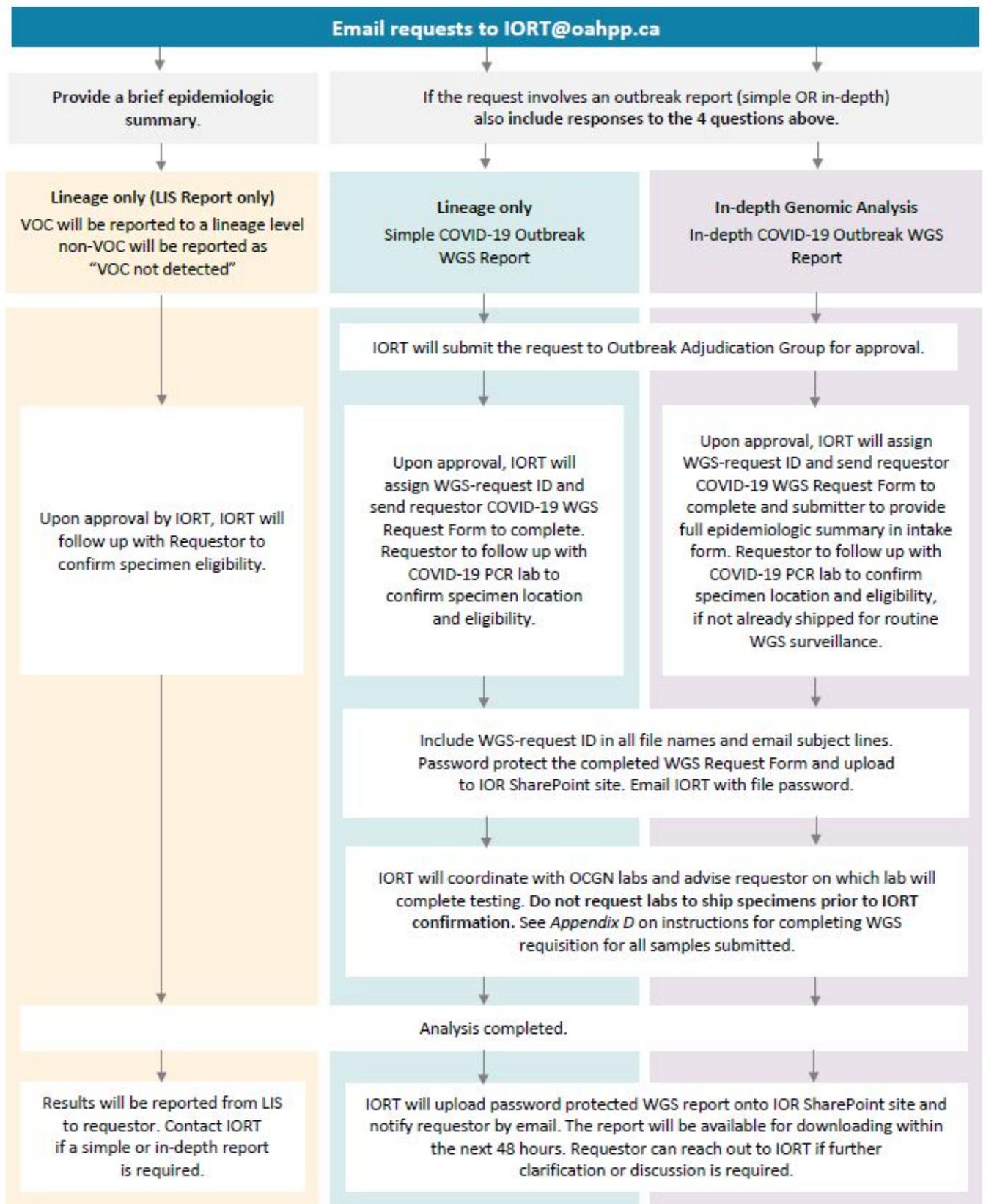
Specimen Eligibility

1. Specimen Volume: 1ml (0.5 ml as absolute minimum)
2. Ct \leq 30 from the SARS-CoV-2 (COVID-19) -PCR test

Data Transfer

To be able to collect the required information to execute WGS requests for outbreak specimens, it is strongly advised that PHUs use the Incident and Outbreak Response Collaboration SharePoint Site (IOR Site). This secure method facilitates the transfer of Personal Health Information (PHI) between the PHU and Public Health Ontario's laboratory as well as between the laboratory and other testing labs.

Request Process



Appendix A – Simple Outbreak Report

SARS-CoV-2 WGS Lineage Report

[Date]

Requestor:

WGS Request ID:

Outbreak Number(s):

Request Description:

Summary:

- X of X SARS-CoV-2 specimens requested for whole genome sequencing and bioinformatics analysis were successfully sequenced.
- X lineages were associated with the outbreak(s). Of the X cases, X were [lineage (WHO label)] and X were [lineage (WHO label)].

Table 1. Example: Lineage results for cases associated with Outbreak 0000-2021-0000

| Case | CCM ID | Sample ID | Collection Date (YYYY-MM-DD) | Pango Lineage (WHO Label) |
|------|-----------|------------|------------------------------|---------------------------|
| 1 | 123456789 | 21CXXXXXXX | 2021-08-27 | AY.4 (Delta) |
| 2 | 123456789 | 21CXXXXXXX | 2021-08-29 | AY.12 (Delta) |
| 3 | 123456789 | 21CXXXXXXX | 2021-08-31 | B.1.621 (Mu) |
| 4 | 123456789 | 21CXXXXXXX | 2021-08-31 | B.1.617.2 (Delta) |
| 5 | 123456789 | 21CXXXXXXX | 2021-09-01 | Not determined |

Notes: 'Not determined' includes samples with a Ct>30, genome coverage of <90% or an excess variants flag. Cases with identical lineages do not imply transmission and should be interpreted in the context of epidemiological information.

Data source: PHO Laboratory Information Management System [extracted: YYYY-MM-DD], PHO Laboratory SARS-CoV-2 Whole Genome Sequencing Database [extracted: YYYY-MM-DD], [OCGN Lab] [extracted: YYYY-MM-DD]

Technical notes, Appendix A

- The Ontario COVID-19 Genomics Network is a network of laboratories in Ontario that perform SARS-COV-2 whole genome sequencing.
- Lineage is assigned using the Phylogenetic Assignment of Named Global Outbreak Lineages (pangolin) tool, a software for predicting SARS-CoV-2 genome sequences to global lineages. Lineages reported using pangolin X, pangoLEARN X, pango designations X.
- Lineage nomenclature is dynamic. Pango lineage naming and assignment may change as more samples are sequenced and analyzed globally.

- Samples that generated high quality data are those with a genome completeness of $\geq 90\%$, partial data are those with a genome completeness of 50%-89%, and samples not successfully sequenced are those with a genome completeness of $< 50\%$.
- Transmission cannot be ruled in or out based on the lineage designation as lineages are predicted and can be influenced by the quality and genome completeness of the sequenced sample, and therefore should be interpreted in the context of epidemiological information.

For further questions, please contact the Incident and Outbreak Response team at IORT@oahpp.ca.

Appendix B – In-depth Outbreak Report

SARS-CoV-2 WGS In-depth Genomic Analysis Report

[Date]

Requestor:

WGS Request ID:

Outbreak Number(s):

Request Description:

Summary:

- Of the X SARS-CoV-2 specimens requested for whole genome sequencing and bioinformatics analysis, X samples generated high-quality data, X samples generated partial data and X samples were not successfully sequenced. X samples were unavailable for sequencing.
- There were X SARS-CoV-2 lineages associated with the outbreak(s). Of the X cases, X were [lineage (WHO label)] and X were [lineage (WHO label)].
- Within X lineage, there were X distinct genomic clusters and within Y lineage, there were X distinct genomic clusters.
- [Other summary statements depending on the request details.]
- Results should be interpreted in the context of epidemiological information. Related or identical genetic sequences do not imply transmission

Table 2. Example: Lineage and genomic cluster for cases associated with outbreak 0000-2021-0000

| Case | Sample ID | Collection Date (YYYY-MM-DD) | Name | DOB (YYYY-MM-DD) | Genome Completeness | Pango Lineage (WHO Label) | Genomic Cluster | Other Epidemiological Information |
|------|------------|------------------------------|-------------|------------------|---------------------|---------------------------|-----------------|-----------------------------------|
| 1 | 21CXXXXXXX | 2021-01-01 | Bob Black | 2020-01-01 | 0.96 | B.617.2 (Delta) | A | Apt#101 |
| 2 | 21CXXXXXXX | 2021-01-03 | Wendy Black | 2020-01-01 | 0.85 | AY.3 (Delta) | A | Apt#101 |
| 3 | 21CXXXXXXX | 2021-01-16 | John Black | 2020-01-01 | 0.96 | B.617.2 (Delta) | A.1 | Apt#101 |
| 4 | 21CXXXXXXX | 2021-01-08 | Jane Red | 2020-01-01 | 0.91 | B.617.2 (Delta) | B | Apt#504 |
| 5 | 21CXXXXXXX | 2021-01-12 | Susan Green | 2020-01-01 | Failed QC | Failed QC | | Apt#615 |

Notes: 'Failed QC' includes samples that were not successfully sequenced (genome completeness<50%). A genomic cluster refers to a group of SARS-CoV-2 sequences that are genetically related by 2 mutations or less. Sequences that belong to a sub-cluster (e.g. A.1) are considered genetically related (1-2 mutations) to the parent cluster (e.g. A). Cluster designations, are arbitrary letters assigned to delineate genetic clusters containing related sequences and are specific to this WGS request.

Data source: Laboratory Information Management System [extracted: YYYY-MM-DD], PHO Laboratory SARS-CoV-2 Whole Genome Sequencing Database [extracted: YYYY-MM-DD], [OCGN Lab] [extracted: YYYY-MM-DD]

Phylogenetic analysis and/or mutation profile and notes: [include where appropriate]

Technical notes

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- Lineage is assigned using the Phylogenetic Assignment of Named Global Outbreak Lineages (pangolin) tool, a software for predicting SARS-CoV-2 genome sequences to global lineages. Lineages reported using pangolin X, pangolearn X, pango_designations X.
- Lineage nomenclature is dynamic. Pango lineage naming and assignment may change as more samples are sequenced and analyzed globally.
- Samples that generated high quality data are those with a genome completeness of $\geq 90\%$, partial data are those with a genome completeness of 50%-89%, and samples not successfully sequenced are those with a genome completeness of $< 50\%$.
- Transmission cannot be ruled in or out based on the lineage designation as lineages are predicted and can be influenced by the quality and genome completeness of the sequenced sample, and therefore should be interpreted in the context of epidemiological information.

For further questions, please contact the Incident and Outbreak Response team at iort@oahpp.ca.

Appendix C – Outbreak Data Collection Form

WGS_Request_Id (Assigned by PHO*): _____

*If this is an addition to a previous request please provide the Id that was assigned by PHO

Submitter Information

Health Unit/Institution: _____

Address: _____

City: _____

Postal Code: _____

Contact Person: _____

Email: _____

Phone: _____

Fax: _____

Date (yyyy-mm-dd): _____

| Patient Information | | | | | Specimen Information | | | | | | Reason for Request | | | | | |
|---------------------|------------------------------|----------------|-----|--------|----------------------|--------------------|----------------------------|------------------------------|--------------------------|----------|--------------------|-----|--------------|--------------|---------------|------------------------|
| No. | Name (Last name, first name) | Healthcard No. | DOB | Gender | No. | Testing Laboratory | Lab/Ware No./ Specimen No. | Collection Date (yyyy-mm-dd) | Report Date (yyyy-mm-dd) | CT value | Specimen Type | No. | Outbreak No. | Inquiry Type | Facility Type | Additional Information |
| | | | | | | | | | | | | | | | | |
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Appendix D – Guide to Completing WGS Requisition Form

For each sample sent to the OCGN lab for testing, an individual WGS requisition must be completed. For the latest version of the WGS requisition, please see this [link](#). Please complete the following on the requisition.

- **Section 2:** Patient information including first and last names, date of birth and health card number
- **Section 2:** Outbreak or Investigation Number
- **Section 3:** Clinician information including name, CPSO and address
- **Section 4:** Enter one SARS-CoV-2 gene target Ct value if multiple targets were tested. Choose the lowest Ct value (must be ≤ 30).
- **Section 5:** WGS ID for the request

For Simple Lineage Report: Please check off Special request approved by PHO and Other. Indicate that this is for OB Specimen for Lineage or Lineage Request Only. See example below

For In-Depth WGS Analysis Report: Please check off Special request approved by PHO and Outbreak specimens for in-depth analysis. See example below.

| 5 - Special Requests (For non routine requests only) |
|--|
| If none of the indications for SARS-CoV-2 whole genome sequencing are met, contact PHO Laboratory Customer Service Centre at 1-877- 604-4567 or 416-235-6556 for testing approval for "Special request" prior to submission. |
| <input checked="" type="checkbox"/> Special request approved by Public Health Ontario: ¹⁴ |
| <input type="checkbox"/> Suspected reinfection |
| <input checked="" type="checkbox"/> Outbreak specimens for in-depth analysis |
| <input type="checkbox"/> Other (Specify): |
| WGS ID assigned: ¹⁵ COVID-WGS-12345 |
| Non-PHO lab specimen ID (if applicable): |
| Provide details: |
| Approving PHO Laboratory microbiologist: |

| 5 - Special Requests (For non routine requests only) |
|--|
| If none of the indications for SARS-CoV-2 whole genome sequencing are met, contact PHO Laboratory Customer Service Centre at 1-877- 604-4567 or 416-235-6556 for testing approval for "Special request" prior to submission. |
| <input checked="" type="checkbox"/> Special request approved by Public Health Ontario: ¹⁴ |
| <input type="checkbox"/> Suspected reinfection |
| <input type="checkbox"/> Outbreak specimens for in-depth analysis |
| <input checked="" type="checkbox"/> Other (Specify): OB Specimen for lineage |
| WGS ID assigned: ¹⁵ COVID-WGS-12345 |
| Non-PHO lab specimen ID (if applicable): |
| Provide details: |
| Approving PHO Laboratory microbiologist: |

Citation

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For Further Information

For more information, contact iort@oahpp.ca.

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