MAYO CLINIC LABORATORIES

# Test Definition: COVNG

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Lineage, Clade, and Spike Gene Mutation Detection, Next-Generation Sequencing, Varies

## Overview

### Useful For

Distinguishing between persistent infection with the same viral strain and reinfection with a new viral strain in an individual with recurrent positive molecular test results for SARS-CoV-2

Detection and identification of vaccine-escape SARS-CoV-2 variants with spike (*S*) gene variant of interest

Detection and identification of SARS-CoV-2 variants containing *S* gene variants of interest that reduce the efficacy of vaccine-induced antibodies, convalescent plasma, and/or monoclonal antibody therapy for COVID-19

Detection and identification of SARS-CoV-2 variants containing RdRp codon mutations of interest that reduce the efficacy of nucleoside analogs used in the therapy of COVID-19

### Highlights

This test uses polymerase chain reaction (PCR) to amplify multiple SARS-CoV-2 genetic sequences covering 99.9% of the viral genome, followed by a next-generation sequencing assay with sequence analyses to determine the Pangolin lineage, Nextclade clade assignment, and alterations of the viral spike (S) protein and RdRp encoding codons in known SARS-CoV-2 RNA-positive respiratory tract specimens. Testing is more likely to be successful in positive specimens with PCR target cycle threshold values of 30.0 or less, or transcription-mediated amplification generated relative light units of 1200 or more.

### Method Name

Reverse Transcription Polymerase Chain Reaction (RT-PCR) followed by Next-Generation Sequencing

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

### Ordering Guidance

This test should only be requested on known SARS-CoV-2 RNA-positive upper or lower respiratory tract specimens, with polymerase chain reaction target cycle threshold value of to 30.0 or less or transcription-mediated amplification generated relative light units of 1200 or more.

This test **should not be used** to detect the presence or absence of SARS-CoV-2 in an individual, with or without symptoms or signs of COVID-19. For these cases, order COVOO / Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) RNA Detection, Varies.

## Shipping Instructions

Ship specimens refrigerated (if <72 hours from collection to arrive at Mayo Clinic Laboratories [MCL]) or frozen (if 72 hours or more from collection to arrive at MCL)

## Necessary Information

The following question must be answered at the time of test ordering:
Does the patient have a positive SARS-CoV-2, COVID19 polymerase chain reaction test result within the last 5 days? Answer "Yes" or "No".
**Note:** Test orders for submitted specimens with a "No" answer to this question will be canceled.

## Specimen Required

**Call 800-533-1710 to have this test added to a previously collected specimen that tested positive for SARS-CoV-2, COVID19 with COVOO, COVID, or COFLU. A new specimen would not be needed if there is sufficient specimen volume remaining.**

**Specimen Type:** Nasopharyngeal (NP), oropharyngeal (OP ie, throat), nasal mid-turbinate, or nares/nasal swab
**Supplies:** Swab, Sterile Polyester(T507)
**Collection Container/Tube:**
**Preferred**: Sterile polyester swab
**Acceptable**: Dacron-tipped swab with plastic shaft
**Submission Container/Tube:** Universal transport media, viral transport media, or equivalent (eg, Copan UTM-RT, BD VTM, MicroTest M4, M4-RT, M5). Media should not contain guanidine thiocyanate (GTC).
For more information on acceptable transport media, see
www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2
**Specimen Volume: Entire specimen with a minimum of 1.5 mL (maximum 3 mL) of transport media**
**Collection Instructions:**
1. Collect specimen by swabbing back and forth over nasal or pharyngeal mucosa surface to maximize recovery of cells.
2. NP and OP swab specimens may be combined at collection into a single vial of transport media but only 1 swab is required for analysis.
3. Swab must be placed into transport medium. Swab shaft should be broken or cut so that there is no obstruction to the sample or pressure on the media container cap.
4. Do **not** send in glass tubes, vacutainer tubes, or tubes with push caps.
5. Do **not** overfill with more than 3 mL total volume of media.

**Specimen Type:** Nasopharyngeal aspirate or nasal washings
**Container/Tube:** Sterile container
**Specimen Volume:** Minimum of 1.5 mL
**Additional Information: Do not aliquot** into viral transport media, glass tubes, vacutainer tubes, or tubes with push

caps.

**Specimen Type:** Nasopharyngeal aspirate or nasal washings, bronchoalveolar lavage (BAL) fluid, bronchial washings, endotracheal aspirate, sputum
**Container/Tube:** Sterile container
**Specimen Volume:** Minimum of 1.5 mL
**Additional Information: Do not aliquot** into viral transport media, glass tubes, vacutainer tubes, or tubes with push caps.

### Specimen Minimum Volume

See Specimen Required

### Reject Due To

| | |
|---|---|
| Calcium alginate-tipped swab, wooden shaft swab, or swab collection tubes containing gel or charcoal additive<br>Transport media tubes containing the entire swab (shaft and knob attached)<br>Glass transport media tubes<br>Bloody specimen | Reject |
| Thawed | Cold OK; Warm reject |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Varies | Frozen (preferred) | 14 days | |
| | Refrigerated | 72 hours | |

## Clinical & Interpretive

### Clinical Information

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel, positive-sense, single-stranded RNA virus that causes COVID-19, and it contains an approximately 30,000 base pair long RNA genome that is prone to spontaneous genetic mutation. Worldwide scientific reports indicated emergence of specific variants of SARS-CoV-2 that are associated with increased transmissibility of this virus among susceptible humans, increased severity of disease, and/or reduced neutralization by vaccine-induced antibodies, therapeutic monoclonal antibodies, and convalescent plasma since December 2020.

A group of viruses within the same genus sharing the same distinctive set of mutations in the viral genome is called a variant. If enough mutations accumulate in a lineage, the viruses may evolve clear-cut differences in how they function. These lineages come to be known as strains. The United States SARS-CoV-2 inter-agency group, comprising the Department of Health and Human Services, Biomedical Advances Research and Development Authority, Centers for Disease Control and Prevention, Food and Drug Administration, National Institutes of Health, and Department of Defense, has developed a variant classification scheme that defines 3 classes of SARS-CoV-2 variants: variant of interest, variant of concern, and variant of high consequence ( www.cdc.gov/coronavirus/2019-ncov/variants/variant-surveillance.html). The relative proportions of these variants present among the reported COVID-19 infections at the US national and state levels are available at www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-proportions.html.

A SARS-CoV-2 variant of interest contains specific genetic mutations that are associated with predicted increase in transmissibility or disease severity, possible impact on serologic and/or molecular diagnostic assays, and changes to the ACE2 receptor binding domain that may result in reduced neutralization by antibodies generated from previous infection or vaccination and reduced efficacy of monoclonal antibody therapy. Current SARS-CoV-2 variants of interest in the US are the B.1.525, B.1.526, and P.2 lineages, all of which share the D614G codon mutation in the *S* gene of the virus, and this mutation is associated with increased transmission of this virus.

A SARS-CoV-2 variant of concern contains specific genetic mutations that are associated with increase in transmissibility, severe disease (increased hospitalization or death), failures of serologic and/or molecular diagnostic assays, and significant reduction in neutralization by antibodies generated from previous infection or vaccination, and reduced efficacy of monoclonal antibody therapy or vaccines. Current SARS-CoV-2 variants of interest in the US are the B.1.1.7, B.1.351, B.1.427, B.1.429, and P.1 lineages.

A SARS-CoV-2 variant of high consequence has clear evidence of significantly reduced effectiveness of current preventive measures, therapeutic agents, and medical interventions, when compared to previously circulating variants. At present, there are no such variants in US or globally.

### Reference Values

Not applicable

## Interpretation

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific *S* gene mutations are detected with this assay at a minimum 50% frequency for detecting codon substitutions in the viral sequence.

An "Inconclusive" result indicates that testing failed due to poor sequence quality resulting from the presence of inhibitory substances and/or low amount of virus (ie, polymerase chain reaction [PCR] target cycle threshold [Ct] value of >30.0) present in the submitted specimen. A new specimen should be collected and submitted for retesting if clinically necessary.

The table below indicates the clinical implications of known SARS-CoV-2 variants of interest and variants of concern:

| WHO label | SARS-CoV-2 PANGO lineage | Disease severity | Efficacy of convalescent plasma therapy | Efficacy of monoclonal antibodies(a) | Efficacy of Pfizer/BioNTech mRNA vaccine | Efficacy of Moderna mRNA vaccine | Efficacy of J and J adenovirus vaccine |
|---|---|---|---|---|---|---|---|
| Alpha | B.1.1.7 | No effect | Good | Good | Good | Good | Good |
| Beta | B.1.351 | Increased | Reduced | Reduced(b) | Reduced | Reduced | Good |
| Gamma | P.1 | Increased | Reduced | Reduced(b) | Reduced | Reduced | Reduced |
| Delta | AY……(c) | Increased | Reduced | Reduced(b) | Reduced | Reduced | Reduced |
| Delta | B.1.617.2 | Increased | Reduced | Reduced(b) | Reduced | Reduced | Reduced |
| Epsilon | B.1.427 | Unknown | Reduced | Reduced(d) | Reduced | Reduced | Reduced |
| Epsilon | B.1.429 | Unknown | Reduced | Reduced(d) | Reduced | Reduced | Reduced |
| Zeta | P.2 | Unknown | Reduced | Reduced(b) | Reduced | Reduced | Reduced |
| Eta | B.1.525 | Unknown | Reduced | Reduced(e) | Reduced | Reduced | Reduced |
| Theta | P.3 | Unknown | Reduced | Reduced(b) | Unknown | Unknown | Unknown |
| Iota | B.1.526 | Unknown | Reduced | Reduced(e) | Reduced | Reduced | Reduced |
| Kappa | B.1.617.1 | Unknown | Reduced | Reduced(b) | Reduced | Reduced | Reduced |
| Lambda | C.37 | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown |
| Mu | B.1.621 | Unknown | Reduced | Reduced(b) | Reduced | Reduced | Reduced |
| Omicron | B.1.1.529 | Increased | Reduced | Reduced(b) | Reduced | Reduced | Reduced |
| Omicron | BA……(c) | Increased | Reduced | Reduced(b) | Reduced | Reduced | Reduced |

a. Based on in vitro (experimental) data only
b. Reduced efficacy of bamlanivimab, etesevimab, and casirivimab
c. All current Pango lineage designations that start with AY and BA are sublineages of the Delta and Omicron variants, respectively
d. Reduced efficacy of bamlanivimab
e. Reduced efficacy of bamlanivimab and casirivimab

Current antiviral drugs, such as remdesivir, that are used to treat COVID-19 are nucleoside analogs that inhibit viral RNA-dependent polymerase (RdRp) of coronaviruses (including SARS-CoV-2) and prevent viral replication. Genotypic mutations occurring alone or in combination in the RdRp-encoding region of the SARS-CoV-2 *nsp12* gene have been reported to be associated with resistance to these antiviral drugs. The RdRp codon mutations associated with such antiviral resistance are: F480L, D484Y, and V557L.

The table below indicates the clinical implications of known codon-substitutions in the SARS-CoV-2 spike protein (S) encoding region:

| **S codon mutation of interest** | **Effect on viral transmission and infectivity** | **Effect on severity of disease** | **Efficacy of convalescent plasma therapy** | **Efficacy of monoclonal antibodies*** | **Efficacy of vaccines** |
|---|---|---|---|---|---|
| H69-V70 deletion** | Increased | Unknown | No effect | No effect | No effect |
| G142D | Increased | Increased | Reduced | Reduced for bamlanivimab | Reduced |
| Y144 or Y145 deletion | Unknown | Unknown | No effect | No effect | No effect |
| E156-F157 deletion | Unknown | Unknown | Reduced | Reduced for bamlanivimab | Reduced |
| G158R | Unknown | Unknown | Reduced | Reduced for bamlanivimab | Reduced |
| Q409E | Unknown | Unknown | Unknown | Reduced for casirivimab | Unknown |
| K417E, K417N, K417R, K417T | Increased | Unknown | Unknown | Reduced for casirivimab, etesevimab | Unknown |
| D420N | Unknown | Unknown | Unknown | Reduced for etesevimab | Unknown |
| N439K | Unknown | Unknown | Unknown | Reduced for imdevimab | Unknown |
| K444N, K444Q | Unknown | Unknown | Unknown | Reduced for imdevimab | Unknown |
| K444T | Unknown | Unknown | Unknown | Reduced for casirivimab + imdevimab | Unknown |
| V445A | Unknown | Unknown | Unknown | Reduced for casirivimab + imdevimab | Unknown |
| G446V | Unknown | Unknown | Unknown | Reduced for imdevimab | Unknown |

| | | | | | |
|---|---|---|---|---|---|
| L452R | Increased | Unknown | Reduced | Reduced for bamlanivimab | Reduced |
| Y453F | Unknown | Unknown | Unknown | Reduced for casirivimab | Unknown |
| L455F | Unknown | Unknown | Unknown | Reduced for casirivimab | Unknown |
| N460K, N460S, N460T | Unknown | Unknown | Unknown | Reduced for etesevimab | Unknown |
| G476S | Unknown | Unknown | Unknown | Reduced for casirivimab | Unknown |
| S477N | Increased | Unknown | Unknown | Unknown | Unknown |
| V483A | Unknown | Unknown | Unknown | Reduced for bamlanivimab | Unknown |
| E484A | Unknown | Unknown | Reduced | Reduced excepted for sotrovimab | Reduced |
| E484K | Unknown | Unknown | Unknown | Reduced for bamlanivimab + etesivimab, casirivimab | Reduced |
| E484Q | Unknown | Unknown | Unknown | Reduced for bamlanivimab + etesivimab, casirivimab | Unknown |
| F486V | Unknown | Unknown | Unknown | Reduced for casirivimab | Unknown |
| F490S | Unknown | Unknown | Unknown | Reduced for bamlanivimab | Unknown |
| Q493E, Q493K | Unknown | Unknown | Unknown | Reduced for casirivimab | Unknown |
| Q493R | Unknown | Unknown | Unknown | Reduced for bamlanivimab + etesivimab | Unknown |
| S494P | Unknown | Unknown | Unknown | Reduced for bamlanivimab, casirivimab | Unknown |
| N501Y | Increased | Increased | Reduced | Reduced for etesevimab | Unknown |
| D614G | Increased | Unknown | No effect | No effect | No effect |
| Q677H, Q677P | Increased | Unknown | Unknown | Unknown | Unknown |

*Based on in vitro (experimental) data only.
**This dual codon mutation also causes failure of certain molecular detection assays.

### Cautions

The ability to amplify the SARS-CoV-2 target sequences and detect *S* gene mutations with this assay is limited in specimens with viral polymerase chain reaction target cycle threshold values of greater than 30.0 (ca. <50,000 copies/mL).

Viral variants present at less than 50% of the total viral population in a given clinical specimen will not be detected with this assay, as the nucleic acid substitution detection threshold is set at 50%.

### Clinical Reference

1. Lauring AS, Hodcroft EB: Genetic variants of SARS-CoV-2-what do they mean? JAMA. 2021 Feb 9;325(6):529-531. doi: 10.1001/jama.2020.27124
2. Walensky RP, Walke HT, Fauci AS: SARS-CoV-2 variants of concern in the United States-challenges and opportunities. JAMA. 2021 Mar 16;325(11):1037-1038. doi: 10.1001/jama.2021.2294
3. Centers for Disease Control and Prevention: SARS-CoV-2 variant classifications and definitions. Available at www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html
4. World Health Organization: Weekly epidemiological update on COVID-19 - 8 June 2021. Available at www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---8-june-2021
5. Scripps Research: SARS-CoV-2 (hCoV-19) Mutation Situation Reports. Available at https://outbreak.info/situation-reports
6. European Centre for Disease Prevention and Control. SARS-CoV-2 variants of concern. ECDC; June 24, 2021. Accessed June 28, 2021. Available at www.ecdc.europa.eu/en/covid-19/variants-concern

## Performance

### Method Description

This test utilizes the commercially available Ion AmpliSeq SARS-CoV-2 Research Panel, a next-generation sequencing assay based on a sequencing by synthesis method. The assay amplifies 237 sequences ranging from 125 to 275 base pairs in length covering 99% of the SARS-CoV-2 genome. Clinical respiratory tract specimens undergo manual total nucleic acid extraction and purification using MagMAX Viral / Pathogen Nucleic Acid Isolation Kit. The eluate undergoes automated reverse-transcription-polymerase chain reaction of viral sequences, DNA library preparation (including enzymatic shearing, adapter ligation, purification, normalization), DNA template preparation, and sequencing on the automated Genexus integrated sequencer. No-template control and a positive SARS-CoV-2 control are included in each assay run for quality control purposes. Viral sequence data are assembled using the Iterative Refinement Meta-Assembler (IRMA) application (50% base substitution frequency threshold) to generate unamended plurality consensus sequences of SARS-CoV-2 for analysis with the latest versions of the web-based application tools: https://pangolin.cog-uk.io/ for SARS-CoV-2 lineage assignment; https://clades.nextstrain.org/ for viral clade assignment

and phylogenetic analysis; https://covdb.stanford.edu/sierra/sars2/by-sequences/ for codon mutation calling, in comparison to wild-type reference sequence of Wuhan-Hu-1, lineage B, clade 19A.(Package insert: Ion AmpliSeq SARS-CoV-2 Research Panel. Life Technologies Corporation; rev. B.0, publ. no. MAN0019278, 10/08/2020)

### PDF Report
No

### Day(s) Performed
Varies

### Report Available
2 to 10 days

### Specimen Retention Time
30 days

### Performing Laboratory Location
Rochester

## Fees & Codes

### Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

### Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

### CPT Code Information
87913

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| COVNG | SARS-CoV-2 Lineage, Clade, S Mut, V | 96894-1 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 614373 | SARS-CoV-2 PANGO lineage | 96895-8 |
| 614421 | SARS-CoV-2 Nextstrain clade | 96896-6 |

| | | |
|---|---|---|
| 614374 | S codon mutations of interest | 96751-3 |
| 614501 | S mutations of unknown significance | 96751-3 |
| CVNGS | SARS-CoV-2 Specimen Source | 31208-2 |
| CVNGR | Patient Race | 72826-1 |
| CVNGE | Patient Ethnicity | 69490-1 |
| CVPOS | Recent Positive PCR Result within 5 days? | 86955-2 |
| 616432 | RdRp codon mutations of interest | 99314-7 |
| 616433 | RdRp mutations of unknown significance | 99314-7 |