Test Definition: COVNG

SARS-CoV-2 Lineage, Clade, S Mut, V

Overview

Useful For

Distinguishing between persistent infection with the same viral strain and re-infection with a new viral strain in an individual with recurrent positive molecular test results for severe acute respiratory syndrome coronavirus 2 (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 severe acute respiratory syndrome coronavirus 2 (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

No

Specimen

Specimen Type

Varies

Ordering Guidance

This test should only be requested on known severe acute respiratory syndrome coronavirus 2 (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 coronavirus disease 2019 (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 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 severe acute respiratory syndrome coronavirus 2019 (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 severe acute respiratory syndrome coronavirus 2019 (**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, 10 per package (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.

Reject Due To

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

Specimen Minimum Volume

See Specimen Required

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Frozen (preferred)	14 days	
	Refrigerated		

Clinical & Interpretive

Clinical Information

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel, positive-sense, single-stranded RNA virus that causes coronavirus disease of 2019 (COVID-19), and it contains 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 (US) 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/cases-updates/variant-surveillance/variant-info.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 (RBD) 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	Unknow	Unknown

						n	
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	Unknow n	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	Reduced

				bamlanivimab	
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	Unknown

				casirivimab	
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 severe acute respiratory syndrome coronavirus 2 (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 Feb 17;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 severe acute respiratory syndrome coronavirus 2 (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

Specimen Retention Time

30 days

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test 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	Reporting Name	LOINC®
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