Appendix 1: National SARS-CoV-2 Strain Surveillance (NS3) Submissions to CDC for SARS-CoV-2 Positive Specimens

1. Beginning on Monday, April 12, 2021, CDC will accept SARS-CoV-2 positive specimens based on the revised guidance and processes below.

2. Please ship randomly selected SARS-CoV-2 positive specimens **on Monday** for overnight delivery to CDC on Tuesday. If Monday is an observed holiday, please ship on the next available business day (Tuesday). Please ship only on weekdays through Thursday.

3. Consult Appendix 2 for the recommended number of specimens to ship weekly or bi-weekly for your jurisdiction.

4. Acceptable specimen types for sequencing and potential virus characterization are the same as for the CDC SARS-CoV-2 diagnostic assays that were authorized by FDA under an EUA: upper and lower respiratory specimens, including nasopharyngeal, oropharyngeal, nasal mid-turbinate, and anterior nares (nasal swab) specimens. In addition, a nasopharyngeal wash/aspirate or nasal wash/aspirate specimen collected by a healthcare professional is acceptable, as is a naturally expectorated sputum. Acceptable specimens will be limited to those collected in media that allow for viral culture (e.g., PBS, VTM). Specimens collected in **Hologic Aptima buffer and Molecular Transport Media are excluded** from submission. For more information, see the interim specimen collection guidelines (available at https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html).

5. Considerations for selecting NS3 specimens:

    a. The quality of the specimen directly affects sequencing and virus culture success. Ideally, specimens should have an RT-PCR Ct value of ≤28. If Ct values are not available, specimens that are positive/strong positive for SARS-CoV-2 may be sent (avoid weakly positive samples).

    b. The time from specimen collection to sequence characterization has a large impact on our ability to quickly detect emerging variants. Please send specimens that have been collected within the last 7 days whenever possible. If the number of specimens collected within the last 7 days is insufficient to meet your jurisdiction's requested number for NS3 submission (Appendix 2), please send the most recent specimens possible (i.e., up to 14 days prior to shipment).

    c. Ideally, specimens should represent geographic, demographic (e.g., age), and clinical (e.g., disease severity or outcome) diversity from across the jurisdiction. This can be achieved through random selection of specimens collected within the last 7 days.

    d. Specimens that have not previously been sequenced are preferred to avoid duplication of sequence data submitted to public databases. However, specimens that have been sequenced by you or your partners can be sent to CDC for NS3 submissions if the number of unsequenced specimens is insufficient to meet your jurisdiction's requested number for NS3 submission (Appendix 2). Please enter "yes" in the Global File Accessioning Template (GFAT) form's *Previously Sequenced?* field and provide the GISAID and/or GenBank accession numbers (if available).

    e. Specimens can be stored at 2–8°C for no more than 72 hours from the time of collection. The 72-hour timeframe is a strict requirement for sequencing to be completed. Specimens that require storage longer than 72 hours must be frozen at ≤ -70°C. Prior to shipping, specimens should be frozen at ≤ -70°C and shipped on dry ice.

    f. Please submit original clinical specimens with at least 500 µL volume

6. Please use 1.0–2.0 mL O-ring screw cap microcentrifuge tubes labeled with the de-identified specimen ID. Please do not submit specimens in the thin, pre-barcoded tubes that were originally used in initiation of the NS3 program.

7. Please fill in the electronic Global File Accessioning Template (GFAT) form and NS3 Supplementary Form. Each specimen must be labeled with a unique identifier also included on both forms using the *SPHL Submitter Specimen ID* or the *Original Submitter Specimen ID* field (if no SPHL ID). Please fill out all GFAT fields for which you have data. Please note: **the fields highlighted in orange are required** for the processing of specimens and downstream uses of the sequence data for public health surveillance.

8. In the GFAT form, please select “National Surveillance System - Sequencing" in the *Event Name* field and “1771” in the *Event ID* field. Select “National SARS-CoV-2 Strain Surveillance (NS3)” in the *Reason for Submission* field of the NS3 Supplementary Information form.

9. Specimens should be packaged and shipped as Category B infectious substances, and all requirements for proper packaging and shipping should be observed (see https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety- ğuidelines.html#specimen).

10. Please include a printed manifest of your specimens with your shipment.

11. Email the GFAT and NS3 Supplementary forms along with tracking information to sarsseqshipping@cdc.gov.

12. If possible, please ship specimens every Monday for overnight delivery to CDC using the following address:

    ATTN: STATT Lab: Unit 66 TRL
    Centers for Disease Control and Prevention
    1600 Clifton Road, NE
    Atlanta, Georgia, 30333
    Telephone: 404-639-3931
    Email: sarsseqshipping@cdc.gov

Appendix 2: Weekly Target Number of National SARS-CoV-2 Strain Surveillance (NS3) Specimens Requested for Submission to CDC by Jurisdiction

Jurisdictions are encouraged to submit specimens weekly, and to prioritize those specimens collected in the previous 7 days. If submitting every two weeks, please submit twice the number of specimens listed for your jurisdiction (collected in the 7 days before shipment).

Abbr	Jurisdiction	No of weekly specimens*
AK	Alaska	9
AL	Alabama	11
AR	Arkansas	10
AS	American Samoa	5
AZ	Arizona	14
CA	California	35
CHI	Chicago	12
CO	Colorado	12
CT	Connecticut	11
DC	District of Columbia	10
DE	Delaware	9
FL	Florida	26
FM	Micronesia	5
GA	Georgia	17
GU	Guam	5
HI	Hawaii	9
HOU	Houston	9
IA	Iowa	10
ID	Idaho	9
IL	Illinois	17
IN	Indiana	13
KS	Kansas	10
KY	Kentucky	11
LA	Louisiana	11
LAC	Los Angeles County	19
MA	Massachusetts	14
MD	Maryland	13
ME	Maine	9
MH	Marshall Islands	5
MI	Michigan	16
MN	Minnesota	13
MO	Missouri	13
MP	Northern Marianas	5
MS	Mississippi	10
MT	Montana	9
NC	North Carolina	16
ND	North Dakota	9
NE	Nebraska	9
NH	New Hampshire	9
NJ	New Jersey	16
NM	New Mexico	10
NV	Nevada	10
NY	New York	18
NYC	New York City	18
OH	Ohio	17
OK	Oklahoma	10
OR	Oregon	11
PA	Pennsylvania	18
PHL	Philadelphia	7
PR	Puerto Rico	10
PW	Palau	5
RI	Rhode Island	9
SC	South Carolina	12
SD	South Dakota	9
TN	Tennessee	13
TX	Texas	33
UT	Utah	10
VA	Virginia	16
VI	Virgin Islands	5
VT	Vermont	9
WA	Washington	14
WI	Wisconsin	13
WV	West Virginia	9
WY	Wyoming	9
	TOTAL	770

*Based on population – Minimum number of five (5) specimens per week by jurisdiction.

Appendix 3: Enhanced Surveillance for SARS-CoV-2

Since the emergence of SARS-CoV-2, national and global sequencing efforts have identified changes in the SARS-CoV-2 genetic code resulting from transmission and evolution in humans and animals. These changes can affect many aspects of our response including transmission, diagnostics, therapeutics, and vaccines. Therefore, we may ask that additional specimens be sent to CDC for a defined period of time (short-term, interim) to address variants of interest, variants of concern, or other specified viral classifications. The criteria for submitting enhanced surveillance specimens to CDC is listed in the table below and are expected to be continually updated to address gaps in our understanding. The general submission guidelines for any enhanced surveillance specimens are provided in Section 1 below, and specific details about particular variants are listed in Section 2. Information about potential vaccine breakthrough cases is listed in Section 3. We will keep you informed by updating the enhanced surveillance guidelines and communications through APHL.

National and state level variant proportions are available on CDC's website:
CDC COVID Data Tracker

Brief description of enhanced surveillance efforts* (details below in Section 2)

Reason for Submission in Supplemental Form	Selection criteria**	Specimen submission - GFAT Event ID	Variant Case Notification by Jurisdiction to CDC
ES21-01 – SARS-CoV-2 S gene target failure	• As of April 12, 2021, CDC is no longer requesting case notifications or submission of specimens of B.1.1.7 variants confirmed by jurisdictions.	Closed	Closed
ES21-02 – B.1.351 Lineage (20H/501Y.V2 or B.1.351)	• As of April 12, 2021, CDC is no longer requesting case notifications or submission of specimens of B.1.351 variants confirmed by jurisdictions.	Closed	Closed
ES21-03 - Additional variants	• Special circumstances with prior approval from CDC	1850	Open
ES21-04 - Vaccine Breakthroughs	• Positive EUA diagnostic test result ≥ 14 days after completion of FDA authorized vaccination series • See Section 3 below for inclusion and exclusion criteria	1890	Open
ES21-05 - P1 lineage	• As of April 12, 2021, CDC is no longer requesting case notifications or submission of specimens of P.1 variants confirmed by jurisdictions.	Closed	Closed
*No special reporting or submission of specimens are needed at this time for newly classified variants of interest or variants of concern (i.e., B.1.427, B.1.429, B.1.525, B.1.526, P.2). If this guidance changes, including when new variants of interest are classified, CDC will notify partners of changes to reporting and specimen submission guidelines.			
**Please submit original clinical specimens with at least 300 µL volume and ideally Ct value ≤ 28 by RT-PCR.			

Section 1. Submission of specimens for Enhanced Surveillance

1. If a variant is identified based on criteria in Appendix 3 and detailed descriptions below, please notify CDC by emailing eocevent506@cdc.gov. Please include the number of variant specimens and information about the specimens including relevant sequence, clinical, and epidemiology data.

2. Acceptable specimen types for sequencing and potential virus characterization are the same as for regular NS3 surveillance described in Appendix 1.

3. Specimens can be stored at 2–8°C for no more than 72 hours from the time of collection. The 72-hour timeframe is a strict requirement for sequencing to be completed. Specimens that require storage longer than 72 hours must be frozen at ≤ -70°C. Prior to shipping, specimens should be frozen at ≤ -70°C and shipped on dry ice.

4. Please submit original clinical specimens with at least 300 µL volume.

5. Acceptable specimens will be limited to those collected in media that allows for viral culture (e.g., PBS, VTM). Specimens collected in Hologic Aptima buffer and Molecular Transport Media are excluded from submission.

6. Sequencing and virus culture success are directly impacted by the quantity and quality of the specimen. Acceptable specimens will be limited to those with Ct values ≤28. If Ct values are not available, specimens that are positive for SARS-CoV-2 can be sent.

7. Please use 1.0–2.0 mL O-ring screw cap microcentrifuge tubes labeled with the de-identified specimen ID. Please **do not** submit specimens in the thin pre-barcoded tubes that were used in the early period of this program.

8. Please fill in the electronic Global File Accessioning Template (GFAT) form and NS3 Supplementary Form. Each specimen must be labeled with a unique identifier on both forms using the *SPHL Specimen ID* or *Original Submitter Specimen ID* (if no SPHL ID).

9. Please indicate “Emerging Variants” for the *Event Name* and “1850” for the *Event ID* in the GFAT form (see Table above).

10. Use the “Reason for Submission” field in the NS3 Supplementary Form to indicate *Enhanced Surveillance* by selecting “ES21-XX” (where “XX” is the number in the table above for the appropriate reason for submission).

11. Specimens should be packaged and shipped as Category B, and all requirements for proper packaging and shipping should be observed (see https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.).

12. Please include a printed manifest of your specimens with your shipment.

13. Email the GFAT and NS3 Supplementary Form along with tracking information to sarsseqshipping@cdc.gov.

14. Please ship specimens weekly (preferred) or bi-weekly, Monday through Thursday, for overnight delivery to CDC using the following address:

    ATTN: STATT Lab: Unit 66 TRL
    Centers for Disease Control and Prevention

1600 Clifton Road, NE
Atlanta, Georgia, 30333
Telephone: 404-639-3931
Email: sarssegshipping@cdc.gov

Section 2. Enhanced Surveillance – Emerging Variants (Updated 4/7/2021)

I. ES21-01: SARS-CoV-2 positive Spike Gene Target Failure (SGTF) - CLOSED

As of April 12, 2021, CDC is no longer requesting case notifications or submission of specimens of B.1.1.7 variants.

A variant of interest referred to as B.1.1.7 or 20I/501Y.V1 emerged and disseminated in the United Kingdom throughout Fall and Winter 2020. This lineage has many substitutions throughout the genome and the S proteins typically share the following amino acid changes: 69-70 deletion, Y144 deletion, and the following substitutions: N501Y, A570D, P681H, T716I, S982A, 1118H. There are other virus lineages that have also independently evolved the deletion of amino acids 69-70.

The deletion of the nucleotides encoding amino acids 69 and 70 impacts some diagnostic assays resulting in SGTF. For example, the Thermo Fisher TaqPath and Linea COVID-19 Assay Kit S-gene probes fail to bind to the genome of 69-70 del variants efficiently and can be used to identify potential B.1.1.7 lineage viruses.

II. ES21-02: B.1.351 Lineage (20H/501Y.V2 or B.1.351) - CLOSED

As of April 12, 2021, CDC is no longer requesting case notifications or submission of specimens of B.1.351 variants.

A variant of interest emerged and spread rapidly in South Africa and around the globe. The lineage of this variant is currently referred to as B.1.351, or 20H/501Y.V2. This lineage has many substitutions throughout the genome, and the S proteins typically share the following amino acid changes: L18F, D80A, D215G, R246I, K417N, E484K, N501Y, A701V. Many of these viruses have deletion of residues 241-243 based on Wuhan-Hu-1 S protein. At this time, no EUA assays specifically detect B.1.351 variants.

III. ES21-03 Additional Variants

This category is reserved for special circumstances and should only be used with prior approval from the CDC (eocevent506@cdc.gov). If you have identified viruses with some characteristics matching any of the other variants described in this Appendix (e.g., E484K, N501Y, or deletions in the S protein) through sequencing or other lab-developed tests, please contact CDC to discuss options for submission and characterization.

IV. ES21-05 P.1 lineage - CLOSED

As of April 12, 2021, CDC is no longer requesting case notifications or submission of specimens of P.1 variants.

The P.1 variant is a branch off the B.1.1.28 lineage that was first reported by the National Institute of Infectious Diseases (NIID) in Japan in travelers from Brazil, identified during routine screening at Haneda airport outside Tokyo. As of January 25, 2021, this variant was identified in the United States.

There is evidence to suggest that some of the mutations in the P.1 variant might affect its transmissibility and antigenic profile, which could affect the ability of antibodies generated through a previous natural infection or through vaccination to recognize and neutralize the virus. Typical changes in the S protein of P.1 variants include: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T10271

Section 3. Enhanced Surveillance - Potential Vaccine Breakthroughs (Updated 4/7/2021)

I. ES21-04 Vaccine Breakthroughs

CDC is currently defining suspect vaccine breakthrough cases as a U.S. resident who has SARS-CoV-2 RNA or antigen detected on a respiratory specimen collected ≥ 14 days after completing the full series of an FDA-authorized SARS-CoV-2 vaccine (e.g., two doses of the Moderna or Pfizer vaccine, one dose of Johnson and Johnson vaccine). Acceptable specimens are the same as those listed above in Appendix 1.

A case will be excluded from further investigation if: 1) the patient received a COVID-19 vaccine that is not authorized by FDA; 2) the respiratory specimen that was positive for SARS-CoV-2 RNA or antigen was collected < 14 days after completing vaccination; or 3) the patient had a previous positive test for SARS-CoV-2 RNA or antigen on a specimen collected < 45 days prior to the most recent positive test and prior to vaccination or < 14 days after completing the full vaccination series.

1. On Monday, January 25, 2021, CDC began accepting potential vaccine breakthrough specimens based on the guidance below.

2. Please send up to twenty (20) potential vaccine breakthrough specimens weekly as previously described in Section 1. If convenient, these can be included with NS3 specimen shipment to CDC but please indicate which specimens are being submitted for this purpose:
    a. Please indicate “ES21-04 - Vaccine Breakthrough” in the *Reason for Submission* field of the NS3 Supplementary Form.
    b. On the GFAT, indicate “Vaccine Breakthroughs” for *Event Name* and “1890” for *CDC EVENT ID*.

3. Potential vaccine breakthrough specimens that have been sequenced by you or your partners should be sent to CDC **as described previously in Section 1**. Please select “yes” in the *Previously Sequenced?* field of the NS3 Supplementary Form and provide the GenBank and/or GISAID accession numbers if available.