

CDC-APHL Respiratory Syncytial Virus (RSV) Genomic Surveillance Guide for Public Health Laboratories

CDC and APHL are seeking continued support from public health laboratories through routine participation in the establishment of systematic and ongoing molecular surveillance for RSV in the United States. Moreover, surveillance specimens submitted to CDC are critical to analyze national and global circulating RSV trends, increase publicly available viral sequence data, and expand the representative repository of viruses available for further characterization. As a leading cause of hospitalizations among young children, this data is critical for monitoring severe disease burden. RSV vaccines became available for the first time in 2023 to protect those at increased risk for severe disease, including adults aged 60 years and older and infants (protected through maternal vaccination). In addition, a monoclonal antibody immunization option became available to protect infants and young children. Ongoing RSV surveillance will also help assess the effectiveness of these prevention measures.

Target Surveillance Specimen Submissions

Public health laboratories are encouraged to submit a maximum of 10 RSV-positive specimens each quarter (i.e., four shipments of 10 specimens each for 40 specimens total annually) to CDC for sequencing. Specimen selection and submission instructions are included in [Appendix 1](#). Shipping costs will be paid for by APHL for up to four shipments annually. Shipping guidance is included in [Appendix 2](#). RSV is classified into two circulating subgroups A and B. Participating laboratories are not required to have RSV subgroup assays. However, laboratories interested in implementing RSV subgroup real-time RT-PCR assays should contact the CDC Coronavirus and Other Respiratory Viruses Division, Laboratory Branch via sarsseqshipping@cdc.gov.

Specimen Diversity

For each shipment, please submit RSV-positive specimens that are representative of your seasonal activity including geography, time, severity, outbreak-associated and a mix of RSV A/B subgroups, if you perform subgroup testing.

Please only submit one representative specimen per outbreak. As a reminder, if your state needs assistance with any RSV-related or suspected outbreak, CDC is available to provide assistance, support and technical consultation, as appropriate.

Data Sharing of RSV Genetic Sequences

CDC and APHL will share results of further surveillance testing with the submitting laboratory director when these data are available. RSV genetic sequence data will be made publicly available in GenBank and/or to the submitting laboratory upon completion of aggregate analysis (usually done once or twice a year).

Appendix 1: RSV Specimen Selection for Submission to CDC for Genomic Surveillance

1. Specimen Selection

- a. Submit a maximum of 10 RSV-positive specimens per quarter to CDC for further testing.
- b. The quality of the specimen directly affects sequencing and virus culture success.
 - i. Ideally, specimens should have an RT-PCR Ct value of ≤ 32 .
- c. Submit original clinical specimens with at least 500 μL volume and no more than 1 mL unless confirmed beforehand.
- d. Acceptable specimen types include upper and lower respiratory specimens: NP or OP swabs, oral swab, throat swab, anterior nasal swabs in viral transport media (VTM) or universal transport media (UTM), nasal aspirates, nasal washes, bronchoalveolar lavage (BAL) in saline, other lower respiratory tract aspirates and virus isolates obtained from clinical specimens.

1. Specimen Labeling

- e. All specimen containers should be properly labeled with a unique identifier and securely sealed to avoid spillage and breakage. Each specimen must have the unique SPHL Submitter Specimen ID on the tube that may be cross-referenced.
- f. For all specimen submissions to CDC, each specimen shipment must be accompanied by a completed [GFAT \(Global File Accessioning Template\)](#). Additional instructions on completing the GFAT can be found in [Appendix 2](#) and [Appendix 3](#).
 - i. If you need assistance accessing the current GFAT, it can be provided upon request.
 - ii. Include the appropriate CDC Event ID: 4023.

Appendix 2: RSV Specimen Shipping Instructions

1. Notifications:

- a. Prior to sending any specimen shipments to the CDC, the following CDC personnel should be contacted, with the completed GFAT and any additional documentation, for review and approval:
 - Molecular Investigation and Surveillance Team Lead, Lydia Atherton, ibz1@cdc.gov
 - CDC Coronavirus and Other Respiratory Viruses Laboratory Branch Sequencing, sarsseqshipping@cdc.gov
 - APHL, Infectious Disease Program, Infectious.Diseases@aphl.org

2. Global File Accessioning Template (GFAT):

- a. Please fill in the electronic [Global File Accessioning Template \(GFAT\) form](#). GFATs need to be reviewed PRIOR to shipment and one GFAT per shipment per pathogen (i.e. separate GFAT for SARS-CoV-2 specimens than RSV specimens).
- b. Each specimen must be labeled with a unique identifier also included on the GFAT using the SPHL Submitter Specimen ID or the Original Submitter Specimen ID field (if no SPHL ID) (See Table 1 in [Appendix 3](#) for more information).
- c. Please fill out all GFAT fields for which you have data.
- d. The fields highlighted in [Appendix 3](#) are required or requested for the processing of specimens and downstream uses of the sequence data for public health surveillance.
 - i. The GFAT file has hidden columns; do not fill out or unhide.
 - ii. For missing data, please leave the column blank.
- e. Do not include Personally Identifiable Information including “Patient Names, Birthdates”.
- f. In the GFAT form, please select or enter “HRSV - Specimens” in the CDC Event Name field and “4023” in the CDC Event ID field.
- g. Delete any empty rows from the GFAT spreadsheet in excess of total number of specimens submitted.
- h. If you are submitting from an institution or address that is not present in the GFAT please contact sarsseqshipping@cdc.gov. We can either expand the list in the GFAT to include it there, or we may already have it in our system.
- i. Email the GFAT form along with tracking information to sarsseqshipping@cdc.gov.
- j. Please include a printed manifest of your specimens with your shipment.

3. Storage and Shipping Conditions:

- a. Prior to shipping, specimens can be stored at 2–8°C for no more than 72 hours, and/or frozen ($\leq -15^{\circ}\text{C}$) for longer term storage.

4. Shipping Instructions

- a. APHL is able to cover the FedEx overnight shipping costs for public health laboratories sending RSV specimens on dry ice to CDC.
- b. Specimens should be shipped overnight on dry ice to CDC Monday through Wednesdays to avoid weekend delivery.
 - i. If you need to ship specimens on an alternate weekday communicate that with the above CDC personnel prior to shipping.
- c. To request a shipping label, please contact APHL, Infectious Disease Program, Infectious.Diseases@aphl.org at least **72 hours in advance** to allow time for the creation of the shipping label.
- d. If preparing your own shipping label, please use the address below and include your laboratory's return address. This dry ice shipment should be a **standard overnight shipment**.

ATTN: CDC-APHL RSV Sequencing
STAT Lab: Unit 66 COORS
Centers for Disease Control and Prevention
1600 Clifton Rd NE
Atlanta, GA, 30329
Telephone: 404-639-3931
Email: sarsseqshipping@cdc.gov

Appendix 3: RSV GFAT Column Description of Required and Requested Fields

- Additional requested and required columns have been added to account for data that may have been collected using the supplementary form. The NS3 Supplementary form is no longer required. Please fill out the requested columns instead.
- Periodic adjustments to the GFAT structure will occur, the GFAT column letters are provided to help identify the appropriate column in the GFAT file but may be incorrect in newer versions. The column names can be used to search for the updated column.
- Column description of required and requested fields detailed below are provided based on the GFAT version 6 template, effective date of September 16, 2024.

Table 1: GFAT columns for all RSV Specimens				
GFAT Column	Column Name	Requested/ Required	Action	Examples
D	Origin	Required	No action needed; Pre-selected as “human”	
E	Test Order Name	Required	No action needed; Pre-selected as “Respiratory Virus (Non-Influenza) Special Study”	
F	Suspected Agent	Required	Select “RSV - Human respiratory syncytial virus”	
G	Date Sent to CDC	Required	Enter date of shipment to CDC (MM/DD/YYYY)	
H	At CDC, bring to the attention to	Required	No action needed; Pre-selected as “STATT Lab: Unit 66 COORS”	
P	Patient Age	Required	Numeric, Blank	3
Q	Age Units	Required	Enter Years, Months, Days	Years
AC	Specimen collected date	Required	Enter date of collection (MM/DD/YYYY)	
AE	Material submitted	Required	No action needed, Pre-selected as “Primary Specimen”	
AF	Specimen source (Type)	Required	Select the appropriate specimen source type	Anterior Nasal Swab, etc.
AH	Specimen Source Site	Required	Select the appropriate specimen source site	Nose
AL	Transport medium/Specimen preservative	Required	Select the appropriate transport medium/specimen preservation	Viral transport media, universal transport media, sterile saline, etc.
AN	SPHL Submitter	Required	Select SPHL Submitter ID that matches institution	SPHL-0000001

BD	SPHL Submitter Specimen ID	Required	Enter a unique SPHL submitter specimen ID	
DX	Immunization 1	Requested	Enter RSV vaccine name and manufacturer	Arexvy;GlaxoSmith
DY	Immunization 1, Date Received	Requested	Enter date of RSV vaccination (MM/DD/YYYY)	
EF	Previous Laboratory Results	Required	<p>Include Diagnostic PCR results using the following pattern with semi-colons as the delimiter.</p> <p>If multiple targets, or multiple results sets are available – provide only a single assay and the lowest target available.</p> <ul style="list-style-type: none"> • Assay;Target;Ct value <p>If only a Ct value is known, just include it as a single number.</p> <ul style="list-style-type: none"> • 24.5 <p>If an assay only generates a Positive or Negative value, only include that</p> <ul style="list-style-type: none"> • Positive <p>Information not in one of the 4 available formats for data. If your data is not able to fit one of these formats, contact sarsseqshipping@cdc.gov</p>	SARS-CoV-2/Flu A/B/RSV;RSV;Ct 24.4
FD	Alpha Numeric 01	Required	Enter “Yes” or “No” to indicate this specimen has been or will be sequenced locally	
FW	CDC Event ID	Required	No action needed; Pre-selected as “4023”	
FX	Event Name	Required	No action needed; Pre-selected as “HRSV - Specimens”	

Table 2: CDC Event Identifiers

Event ID	Event Name
4023	HRSV - Specimens