APHL/CDC Respiratory Virus Surveillance 2024-2025 Season Kick-Off

September 18, 2024

Call Summary

Contact for Questions or Suggestions
APHL Infectious Disease Contact: infectious.diseases@aphl.org
APHL influenza contact: fluquestions@aphl.org

Quick Links to Respiratory Season Guidance:

- Influenza Surveillance Guidance
- SARS-CoV-2 Surveillance Guidance
- RSV Surveillance Guidance

Influenza Updates: Guidance and Situational Awareness (*Alicia Budd, CDC*)
The 2023-2024 influenza season was classified as moderately severe activity was similar to pre-COVID-19 seasons. The season saw influenza activity increase in November, peak in late December nationally and then stay at an elevated level for a few weeks before finally starting to decline; summer levels of circulation began in May. Percent positivity from clinical laboratories has been less than 1% nationally for each of the last twelve weeks.

Looking at the 2023-2024 season as a whole, states reported testing more than 130,000 specimens for influenza, of which 40,000 were positive. Of the positive specimens, 31,000 were influenza A viruses and 9,000 were influenza B viruses. Eighty four percent of the influenza A viruses were subtyped and the predominant virus was H1N1pdm09. Eighty seven percent of the influenza B viruses were lineage tested and all were B/Victoria.

H5 novel influenza activity has remained steady at 14 cases since March. Most cases occurred during the summer period with 4 cases occurring after exposure to dairy cows, 9 after poultry exposure and the most recent case had no immediate known animal exposure. There are a lot of interesting points about this last case, but in particular, it was detected via routine influenza surveillance, which demonstrates the robustness and value of the work public health laboratories do.

In addition to H5, we had a number of variant virus infections this season. There were 7 total, 6 of which were during the summer. There were 4 H1N2v, 2 H3N2v and 1 H1N1v detections. For states that have not identified a variant yet, there are no longer international health regulation (IHR) requirements for this calendar year since all variant viruses are covered. CDC is putting together a summary of all this information and will post it to the influenza website in the next couple of weeks.

Internationally, in the southern hemisphere, H3N2 viruses have been predominant in most countries during their summer 2024 season with the main exception being South Africa, which is seeing predominantly H1 viruses. Timing and levels of activity are back to pre-COVID-19 levels in most countries. In the northern hemisphere, temperate countries are seeing low levels of activity similar to the US while some countries in the tropical regions are still seeing some elevated activity.

Building off the enhanced summer surveillance strategy, the following changes were made in the 2024-2025 influenza surveillance guidance document:

- The document describes activities aimed at detecting possible spread of H5 to or among people within the context of monitoring for seasonal activity. This approach to H5 surveillance looks at widening circles of the population: it starts with targeted surveillance among agricultural workers and exposed people then moves out to H5 infected animals, people who are exposed to animals in general, people who are severely ill, the general population and finally anomalies in general influenza surveillance data.
- In the summer guidance there was a specific mention of agricultural fairs. Since we are coming out of that season, some of those specific references were removed, however, this new document still focuses on people who have agricultural exposure.
- The summer document also stressed the importance of arranging subtyping of any influenza A positive that was from a severely ill patient. With increasing influenza activity, it may not be able to subtype every influenza A virus from a severely ill patient, but it is important to do so in those individuals who have a relevant exposure history.
- The final change from the summer strategy has to do with novel event detection goals. During the summer CDC requested jurisdictions strive to meet their 1:200 novel event detection goal so that CDC could maintain good visibility on influenza A subtypes that were circulating. Now that we are moving towards a period with expected higher levels of influenza virus circulation, we want to make sure jurisdictions move toward their 1:700 goal (See Table 2. Weekly Number of Influenza Positive Specimens Recommended for Influenza A Subtyping/B Lineage Testing).

Finally, the format of FluView has changed; CDC had to make updates to be in compliance with their new web template.

Influenza Updates: Guidance and Situational Awareness (*Becky Kondor and Marie Kirby, CDC*)
All of this season's influenza guidance can be found on the APHL National Influenza Surveillance Guidance website. The seasonal virologic surveillance guidance is very similar to last year; CDC is still recommending laboratories send influenza virus positive specimens every two weeks to the NIRCs. Within the guidance document, there is a table that describes the right-size goals as well as the criteria for clinical specimens (specimens should be negative for SARS-CoV-2). The breakdown of specimens to be sent every two weeks to NIRCs is as follows:

- 6 influenza A(H3N2)
- 4 influenza A(H1N1)pdm09
- 4 influenza B/Victoria lineage (or 4 influenza B non-lineage typed if you don't use the lineage assay)

Of note: CDC has not seen any B/Yamagata specimens since spring 2020. CDC still recommends public health laboratories perform B lineage testing and if any B/Yamagata's are detected they should be sent to CDC. Due to the changing environment with B/Yamagata and B/Victoria circulation and fluctuating availability of In Vitro Diagnostics (IVD) kits, CDC recommends laboratories move from using the influenza B lineage under CLIA diagnostics to the research use only (RUO) surveillance centric assay.

Surveillance for antiviral susceptibility is like previous years. States that want to submit extra samples for testing can send them to the New York State Department of Health. As a reminder, these must be original clinical specimens that have already been through the CDC subtyping and B lineage assays. States can send up to 5 specimens every other week.

There is one update of note to the recommended submissions for diagnostics specimens guidance regarding the IVD level B genotyping kit. The kit that most laboratories currently have expires in October. CDC is in the process of extending that expiration date and will communicate this information once it is approved via APHL and IRR. Once the IVD level kit is exhausted, laboratories can request RUO kits through IRR. The difference in the IVD vs. RUO kits is that you will not report back B genotyping to patients; laboratories can still use the Flu SC2 multiplex assay or A/B typing kit to diagnose influenza B positivity but will not send Vic or Yam results when using the RUO kit.

A general reminder: the H5 assay should not be performed unless the patient meets the clinical and epidemiological criteria. Currently, laboratories can test conjunctival swabs using the H5 kit as long as they are paired with an approved respiratory specimen and stored and shipped in media approved for the H5 assay. Conjunctival swabs are not an approved specimen type for the H5 assay but are allowed to be tested under enforcement discretion. If your laboratory has a presumptive positive for a novel or variant virus you can ship them to CDC as a diagnostic specimen under typical diagnostic shipping protocols. If your laboratory has any questions or gets an abnormal result, you can reach out to flusupport@cdc.gov for additional assistance.

Also regarding the H5 assay: right now many laboratories have lot #220307 in their laboratories, which has a faulty H5B analyte. If your laboratory is testing a specimen for H5, you can use this lot, but the results interpretation of that specimen should be made using the guidelines from the April letter. With lot #220307 if you get a positive for H5, it is considered presumptive. Laboratories should reach out to CDC in this instance so that they can receive the specimen for confirmation testing. Once laboratories have used all of their current test kits, they can request a new kit from IRR. The new lot that is now shipping (lot #220303-1) contains the full complement of analytes; laboratories can go back to the IFU results interpretation guidelines.

Finally, CDC has been working on converting the Flu SC2 multiplex assay to 510k; CDC is currently working with FDA on this and hopes to start the necessary experiments this calendar year. CDC is also evaluating the KingFisher Apex Dx, MagNA Pure 24 and EZ2 Connect MDx extraction instruments currently.

SARS-CoV-2 and RSV Testing and Guidance (*Natalie Thornburg, Laboratory Branch/Coronavirus and Other Respiratory Viruses Division (CORVD)/CDC*)

New VPD Funds Available

There are new funds available through the ELC Program J Enhanced Surveillance for Vaccine Preventable Disease (VPD) and Respiratory Diseases. CDC added $22 million in additional funds following the original award date. The intention is for those dollars to be used for:

- Supporting influenza H5N1 efforts
- Supporting increases in respiratory specimen submissions for SARS-CoV-2 genomic surveillance and influenza surveillance
- Supporting SARS-CoV-2 genomic surveillance and sequencing efforts

Because funding was pushed out quickly, they may have been awarded in other budget lines and redirections may or may not be required. If you have questions about any workplan or budget changes that may be required, reach out to your ELC Program Officer.

2024-2025 Respiratory Syncytial Virus (RSV) Surveillance Guidance

The RSV surveillance guidance document has been updated for this year. Last year's guidance was a pilot for RSV surveillance and this year we are transitioning to routine RSV surveillance. Last year several vaccine products were approved for older adults, pregnant persons, as well as some

monoclonal antibody prophylactics for young infants. This molecular surveillance program has been established for RSV to ensure there are not any escape substitutions in circulating viruses. RSV surveillance does not need the same level of surveillance as SARS-CoV-2 because RSV does not have the same mutation rate, so CDC is requesting very modest numbers for surveillance.

This year, CDC is requesting submission of up to 10 RSV positive specimens each quarter (up to 40 annually).

Specimen Selection:

- Ideally Ct value ≤ 28. If using an assay that does not have Ct values, CDC will still accept those specimens.
- Original clinical specimens with at least 500 µL, no more than 1 mL
- Upper and lower respiratory specimens acceptable

Specimen Labeling:

- No PII
- Unique SPHL specimen ID on the tube
- Each specimen on GFAT (can be provided upon request)
- Event ID 4023
- Specific instructions on how to fill out GFAT are in the RSV Guidance document

Please notify CDC and APHL prior to shipping RSV specimens to CDC:

- Lydia Atherton: ibz1@cdc.gov
- sarsseqshipping@cdc.gov
- infectious.diseases@aphl.org

Storage and shipping requirements:

- Prior to shipping, specimens can be stored at:
  - 2-8°C for no more than 72 hours
  - Frozen at ≤ -15°C for longer term storage
- Specimens should be shipped on dry ice standard overnight to CDC Monday through Wednesday to avoid weekend delivery.
- Shipping costs are covered by APHL and billing instructions are included in the RSV Guidance document

2024-2025 SARS-CoV-2 Surveillance Guidance

The National SARS-CoV-2 Strain Surveillance (NS3) guidance and the new GFAT form are available on APHL's NS3 webpage.

There are three goals for SARS-CoV-2 surveillance.

1. The first goal is phenotypic or viral characterization. CDC needs to determine how antigenically similar the viruses that are currently circulating are to the vaccine composition. To accomplish this goal, CDC needs SARS-CoV-2 specimens from PHLs to generate viral isolates for animal inoculation and antigenic cartography and neutralization studies with human post vaccine sera. This data is used by WHO, FDA VRBPAC, and CDC ACIP for vaccine reformulation recommendations. Specimens should be shipped to CDC (NS3 program) for sequencing, isolation and further characterization.
2. The second goal is national genetic surveillance to conduct population-level molecular epidemiology/virus monitoring and novel variant detection. To meet national genetic surveillance

goals, CDC needs approximately 3000 sequences bi-weekly. These sequences can be generated by PHLs, partners, or by CDC (using specimens that were shipped to NS3 program). Sequences must be high quality, with good coverage over the spike gene, and timely.

3. The third goal is jurisdictional detection of rare variants. This target is optional, and not necessary to achieve national surveillance goals, but was provided should jurisdictions want the ability to detect a novel variant circulating in their jurisdiction at 3% with a confidence interval of 95%. This number is independent of state population, which can be confusing, but is based off of the Texas calculator, and the sensitivity is about the number of sequences generated and not based on population.

These goals are year-round and do not vary based on transmission levels. During times of lower transmission, there is often more diversity, and most SARS-CoV-2 variants emerge during this time. During times of high transmission, there can be more uniformity so there is no need to increase surveillance numbers. It is acknowledged that there are times when these goals are difficult to attain and no punitive actions will be taken if goals are not met, but efforts should be made to work towards reaching those goals.

Table 1 in the SARS-CoV-2 surveillance guidance document lists the bi-weekly jurisdictional targets for each of the 3 surveillance goals.

1. Target numbers to achieve surveillance goal 1 (phenotypic/viral characterization) are listed in the CDC NS3 Viral Characterization column. These shipping targets are scaled to population and range of 5-35 (and rounded to 5).
2. Target numbers to achieve surveillance goal 2 (national genetic surveillance) are listed in the National Surveillance column. These sequencing targets are scaled to population with a range of 5-90 (not rounded). These numbers can be reached by performing sequencing at PHLs, partner laboratories or by shipping specimens to CDC's NS3 program for sequencing. Sequence data should meet established quality thresholds listed in the guidance document.
3. Target numbers to achieve the optional surveillance goal 3 (jurisdictional emerging lineage variant detection) are listed in the Jurisdictional Surveillance column. This sequencing target is invariant to population size and is 90 for every jurisdiction. Numbers tested above this target will further enhance variant detection within the jurisdiction.

The target numbers for each goal in Table 1 are not additive. Three hypothetical jurisdictional scenarios are provided to help illustrate the new guidance.

1. SCENARIO 1: Small jurisdiction with no sequencing capacity or partners would like to detect rare lineages in their jurisdiction, and they do have access to positive SARS-CoV-2 specimens.

NS3 Shipping Goal:	National Sequencing Goal:	Jurisdictional Sequencing Goal:
5	5	90

   - Jurisdiction would ship a total of 90 specimens to CDC. CDC would sequence and exceed national sequencing goals, but meet the jurisdictional sequencing goal.
   - Each specimen contributes to the 3 different goals.
   - Note they did not need 100 specimens to reach all the goals.

2. SCENARIO 2: Medium sized jurisdiction with sequencing capacity does NOT desire the ability to detect rare lineages in their jurisdiction.

NS3 Shipping Goal:	National Sequencing Goal:	Jurisdictional Sequencing Goal:
10	64	90

   - Jurisdiction would sequence 64 specimens, upload to public repository and tag as baseline.

- Jurisdiction would still ship 10 of those 64 to CDC for viral characterization and indicate on GFAT that they have already been sequenced (CDC will not upload duplicate sequences from those 10 specimens to sequence repositories).
- Disregard jurisdictional goal of 90.

3. SCENARIO 3: Large jurisdiction with sequencing capacity would like the ability to have higher sensitivity to detect rare lineages in their jurisdiction.

NS3 Shipping Goal:	National Sequencing Goal:	Jurisdictional Sequencing Goal:
35	90	90

- Jurisdiction would sequence >90 specimens (number that meets the needs they have determined), upload to public repository and tag as baseline.
- Jurisdiction would still ship 35 of those specimens to CDC for viral characterization, and indicate on GFAT that they have already been sequenced
- All three goals were met.

The new SARS-CoV-2 national surveillance guidance document includes data quality recommendations for sequences:

- Collection date – uploaded within 21 days of sample collection
- Depth of coverage – 20X per nucleotide to call a base at a position (<20X use "N")
- Base representation – ambiguous nucleotide calls at 25-75% should be marked
- Complete spike gene coverage at 20X per base
- Primer sequences MUST be trimmed prior to consensus generation
- >27,700 unambiguous bases, represented as a single contig

These recommendations are guidelines. If sequence data does not meet these quality thresholds, they can still be uploaded to public repositories, but they will not be counted towards the national sequencing goal or used in Nowcast analysis. If you see an abnormal number of lower quality sequences, CDC can assist in troubleshooting.

Other details included in the updated SARS-CoV-2 surveillance guidance include:

- Sequence tagging and BioProject accessioning instructions
- Database naming convention instructions
- Technical assistance and instructions on categorizing sequences as "baseline" surveillance
- Shipping instructions
- GFAT column descriptions

Shipping Problems:

CDC has had 19 NS3 shipments arrive at CDC thawed. The cause appears to be that some are taking longer than overnight to be delivered, but some that did arrive this summer overnight were still thawed.

CDC is asking laboratories to look take the following actions:

- Look at the guidance closely and make sure the shipping address is correct
- Send CDC a tracking number with the GFAT
- Use a lot of dry ice, especially in the summer

Contact information:

Natalie Thornburg, PhD – Chief Laboratory Branch: nax@cdc.gov
Clinton Paden, PhD – Bioinformatics Lead: fep2@cdc.gov
Lydia Atherton, PhD – Sequencing Lead: ibz1@cdc.gov

Peter Cook, PhD – Specimen Metadata Lead: ooj4@cdc.gov
Questions: sarsseqshipping@cdc.gov

Data Reporting Updates (*Krista Kniss, Epidemiology and Prevention Branch/ID/CDC*)

There is no change in APHL's Public Health Laboratory Interoperability Project (PHLIP) reporting procedures for this season. If your laboratory is sending PHLIP messages, please continue to do that. CDC and APHL are working to upgrade all remining PHLIP 2.3.1 PHLs to the 2.5.1 feed. Ten PHLs are still using 2.3.1, seven of which are already engaged in the upgrade. If your PHL is still using 2.3.1 and not yet engaged in the upgrade, please try to progress toward upgrading messages to 2.5.1 specifications.

Request that PHLs map influenza H5 assays to PHLIP messages and if you have not yet done that please reach out to the PHLIP Technical Assistance (TA) team to get that moving. Six laboratories have not done the validation for that assay yet, but it is very important to get that data mapped and coming to CDC.

Watch for emails for error reports from CDC's data messaging and brokering (DMB) system and make any corrections to messages that have failed. Contact CDC/APHL if you are planning a LIMS upgrade or bringing on new assays that will be sent via PHLIP (e.g., new RPP panel or additional tests for influenza, SARS-CoV-2 or other respiratory virus pathogens).

CDC will be mailing out season packets along with certificates; follow the instructions and provide contact updates if needed. Please be on the look out for this packet, or reach out to CDC if you do not receive this.

International Reagent Resource (*Vic Veguilla, CDC*)

Domestic Distribution (September 1, 2023 – August 31, 2024)

Molecular Kit Category	Sum of Quantity Approved
Multiplex (FluSC2)	714 kits
Seasonal (A subtype, A/B typing)	237 kits
Non-Seasonal (A/H5, A.H7)	173 kits
B-lineage	134 kits
Total	1258 kits

Kit Updates:

- H5 Kit
  - New lot (220303-1) for FR-1719 is now available. CDC advises laboratories to continue using lot 220307 with the current interpretation (exclude the Hb5 component per field correction) until inventory is depleted.
  - If a specimen is positive for InfA and H5b, that is considered a presumptive positive and should be sent to CDC for confirmatory testing. When labs receive the new lot of H5 kit 220303-1, laboratories can return to the normal test interpretation (include the H5b component per IFU).
- B lineage kit:
  - Undergoing continued expiration date extension (lot 221122).

Kit Distribution Updates:

- Distribution for FluSC2 Multiplex Assay will be the same as last year. CDC recommends that PHLs use the FluSC2 assay as the primary assay for influenza surveillance.
- CDC's Influenza Division is continuing to provide ancillary reagents to support the weekly influenza subtyping goals for novel virus detection, like last year. The ancillaries are proportional to the number of test kits available to each lab. States can submit orders for the new influenza season from September 1, 2024 through August 31, 2025.
- Additional enzymes can be purchased directly from commercial manufacturers.

IRR Reminders

- Website Functionality
    - A feature exists for automatic notifications for items that are Temporarily Out of Stock (TOS) or items with an important banner (i.e., products undergoing expiration extension). Sign up for product notifications, to receive emails when an item is back in stock (for TOS items) or when there has been an update to a product (i.e., the new lot is available, or the expiration has been extended again).
- Tips for Ordering
    - Please align your orders with immediate testing needs to ensure receipt of material with the longest expiration dates – this is especially true with ancillaries.
    - When placing an order on the IRR website, **please use the order comments to provide a justification for the items you are requesting** (i.e., technical issues with product, need a new lot, experiencing an increase in number of cases, etc.). This helps IRR to properly route the request and expedite approvals for the order.
    - Who can order ancillaries?
        - Only the state's main PHL will be able to submit orders for the enzymes – with a few exceptions based on the large specimen number required to meet their right size novel virus detection goals.
        - CDC defers subsequent allocation from this reserve to local or branch laboratories at the discretion of their main state PHL.
    - If you have questions about items in IRR's catalog, need a status update on an order, or need to add an additional user to your PHL, please visit the Contact Us page or the updated FAQs of the IRR website.

Questions and Answers (Q&A)

Q: Is there a plan to add the QuantStudio 5 as an acceptable PCR platform for the SC2 and any other assays where QS has not been added yet?
A: CDC has been working with FDA on this. There are no updates at this time. As updates become available, APHL will alert public health laboratories.

Q: Is there a general timeline as to when the additional extraction platforms will be approved?
A: There is no update on additional extraction platforms yet.

Q: Can you clarify whether IVD B genotyping kits will still be available? Or will all kits be RUO now?
A: Laboratories can continue to use the IVD kits they have for B lineage testing, however new B lineage IVD kits from IRR will not be available. Only RUO kits will be available from IRR for B genotyping.

Q: It was mentioned to only ship Monday-Wednesday, with no Thursday / Friday shipments - is this for SARS and RSV?
A: Only ship Monday – Wednesday for RSV. SARS specimens should be shipped every other Monday for overnight delivery on Tuesday. If Monday is an observed holiday, please ship on the next available business day.

Q: Is there an update on the replacement thermocycler for the ABI 7500 fast dx?
A: CDC has been working with FDA on this. There are no updates at this time. As updates become available, APHL will alert public health laboratories.

Q: Regarding the complete S gene coverage requirement for SC2 sequencing- What is considered complete (e.g. 100% coverage)? Some lineages using the artic primer scheme may show a drop out region, resulting in non-complete S gene coverage. Could the "complete" S gene coverage requirement result in biases in the reported lineage proportions?
A: Complete coverage is considered 100% of the spike gene. The intention is to ensure all of the primer schemes currently in use actually cover the target. We need high quality spike coverage. If you submit sequences that have primer dropout, that information can be useful to determine if a widely used primer scheme is having issues.

Q: Is there a point of contact for influenza sequencing? We are very motivated to start routine sequencing, but would like support from CDC on best practices and analysis.
A: Please reach out to idseqsupport@cdc.gov for support. APHL and CDC are currently working on developing resources for laboratories interested in influenza sequencing.

Q: Is there a chemistry difference in the influenza B IVD and RUO typing kits?
A: There is no difference in chemistry between these kits; it's more about quality control and the manner in which they are manufactured. The kits are exactly the same; the paperwork and level of scrutiny is what differentiates the IVD and RUO kits.

Q: For samples that do not have complete S2 coverage, would CDC accept another method? For example: hybrid capture or molecular inversion probes?
A: All of those methods could work.