2024-2025 INFLUENZA SEASON: SURVEILLANCE FOR NOVEL INFLUENZA A AND SEASONAL INFLUENZA VIRUSES

As the multistate outbreak of highly pathogenic avian influenza (HPAI) A(H5) in dairy cows, poultry and other animals continues and routine fall/winter seasonal influenza activity approaches*, conducting surveillance for seasonal influenza viruses and monitoring for novel influenza A virus infections remain critical to inform public health actions.* CDC, in collaboration with STLT public health agencies, is employing a multi-faceted influenza surveillance strategy for the 2024-2025 season that will be modified as new information is learned or the situation changes in a way that warrants a revised approach.

The activities described below are aimed at identifying possible spread of HPAI A(H5) to and among people while monitoring seasonal influenza activity. These surveillance strategies encompass a range of activities beginning with symptom monitoring among those exposed to infected/potentially infected animals (specifically swine, cattle, poultry and other avian species) and extending outward to monitoring surveillance data from the general population. These activities are described at a high level, and where available, links to more detailed information are provided.

1. Identify human infections via symptom monitoring among workers and others with recent exposures to HPAI A(H5) infected animals on farms or other locations.
    - Partners: State and local public health; State Departments of Agriculture and Wildlife, CDC and USDA support as requested
    - Activities
        i. Consider conducting proactive outreach to poultry and livestock workers and farm owners in advance of a known exposure, in coordination with agriculture departments and other partners, to provide information about risk reduction and set expectations for symptom monitoring.
        ii. If possible, active symptom monitoring should involve daily contact between health department staff and exposed persons using a list of names and contact information provided by the farm. This daily contact can be made through various methods based on jurisdiction resources and preferences of the persons under monitoring (i.e., text based, phone, etc.). Ensure communication materials and methods are linguistically and culturally appropriate, such as documents available here.
        iii. If active monitoring is not possible (e.g., farms do not provide the names and contact information of exposed workers to public health), the health department could (1) provide information directly to the workers or the farm owners about self-monitoring and who to contact if symptoms occur so that any necessary testing can be arranged, or (2) work through an intermediary such as Department of Agriculture personnel, a facility veterinarian or a professional association to provide self-monitoring and symptom reporting information to farm workers.
        iv. Ensure the prompt availability of and access to testing for influenza and other respiratory viruses among exposed workers who present with acute respiratory infection (ARI) symptoms or conjunctivitis and who have had recent direct or close contact with animals potentially infected or confirmed to be infected with HPAI A(H5).
            - It is anticipated that with increased circulation of seasonal influenza and other respiratory viruses, the number of workers with signs and symptoms consistent with ARI will increase and specimen collection and testing will be critical in determining the cause of symptoms.
2. Conduct outreach and education to people who work with, exhibit and/or are exposed to animals and

related animal by-products (e.g., unpasteurized milk, cheese)

- Partners: State and local public health and departments of agriculture; CDC and USDA support as requested. Other potential partners include agricultural extension, trusted farmworker healthcare providers and veterinarians.
- Activities
    i. Develop or identify educational materials and methods specific to the target audience (e.g., linguistically and culturally appropriate documents, infographics and videos).
    ii. Disseminate information regarding risks, worker safety, infection prevention, resources available, symptoms to be aware of and whom to contact if symptoms develop.
        - Information for Specific Groups | Bird Flu | CDC
        - Avian Influenza Print Materials | Bird Flu | CDC

3. Conduct surveillance for novel influenza A virus infection among severely ill patients (e.g., hospitalized, including in ICU) by testing for influenza and subtyping influenza A positive specimens.
    - Partners: State and local public health; clinicians, hospitals, and hospital laboratories; CDC support as requested
    - Activities:
        i. Conduct outreach to providers to request influenza testing for hospitalized/ICU patients presenting with compatible illness (irrespective of exposure history) and to relay the importance of determining the influenza A subtype for all influenza A positive specimens.
        ii. Develop timely methods for identifying hospitalized patients who are influenza A positive. If possible, determine if the influenza A positive patient was in the ICU.
        iii. Recommend facilities have a process for subtyping of influenza A positive specimens from all severely ill persons, particularly those with relevant exposure history, either in the clinical laboratory or by shipping the specimen to the public health laboratory (PHL).
4. Conduct surveillance for novel influenza A virus infections in the community.
    - Partners: State and local public health; CDC support when possible and when requested
    - Activities
        i. Jurisdictions should plan to meet the 1 in 700 novel event detection goal as described in the Influenza Right Size Roadmap during periods of high influenza virus circulation through increasing the number of influenza positive specimens tested in PHLs. The number of influenza positive specimens tested each week using the CDC subtyping assay to meet this goal increases compared to goals during the summer, and ranges from 8 to 251 depending on the jurisdiction's population (see Influenza Right Size Roadmap, Appendix A). During the remainder of the season, jurisdictions should meet the 1 in 200 novel event detection goal.
            1. Ask providers and clinical laboratories that have submitted specimens to the PHL during previous seasons to continue doing so this season.
            2. Identify any additional providers or clinical laboratories that would be willing to submit specimens this season.
            3. CDC recommends that commercial laboratories continue submission of influenza A positive specimens to PHLs for additional subtyping (Appendix 1).
            4. Explore with HRSA what role rural health clinics might play in submitting specimens to PHLs.
        ii. PHLs should attempt to subtype at least 95% of influenza A positive specimens and submit influenza specimens for additional characterization to their designated National Influenza Reference Center (NIRC).
            1. For all PHLs, submit influenza positive specimens that meet the submission criteria outlined in Appendix 2: CDC-WHO Collaborating Center Guidance for Influenza Virus Surveillance for the 2024-2025 Influenza Season.
        iii. Conduct whole genome sequencing of additional influenza specimens.
            1. For states with a designated Influenza Sequencing Center (ISC) or NIRC, sequence up to 85 additional influenza specimens per month from their state that are not

submitted to the NIRC.
2. All influenza positive specimens submitted by states to a NIRC will undergo whole genome sequencing.

iv. Unexplained clusters of respiratory illness should be investigated, including collection of specimens for testing, to determine the pathogen(s) causing illness and identify likely routes of transmission.

5. Monitor influenza surveillance data for any unexpected patterns.
   - Partners: State and local public health, CDC
   - Activities
     i. State and local public health partners monitor data within their jurisdictions which may include systems listed in ii plus additional data/systems specific to a jurisdiction.
     ii. CDC analyzes the following data that are reported to CDC:
        1. Virologic data from 250 clinical laboratories (65,000-150,000 specimens tested weekly) and 90 PHLs (1,500-5,000 specimens tested weekly).
        2. Outpatient respiratory illness data reported to ILINet from more than 4,000 outpatient providers/EDs (approximately 2.5 million patient visits weekly).
        3. Emergency department visits with influenza as a discharge diagnosis reported to the National Syndromic Surveillance Program (NSSP)/ESSENCE.
        4. Hospitalization data from FluSurvNet sites which are operating year-round.
        5. The National Healthcare Safety Network's Hospitalization Surveillance that will include mandatory reporting from all hospitals beginning November 1, 2024.
        6. Mortality data from the National Vital Statistics Surveillance System that covers more than 99% of deaths occurring in the United States.
        7. Influenza concentrations identified in wastewater data.

     iii. Local data anomaly detection and investigation.
        1. Monitor data for anomalies in emergency department visits with influenza, influenza-like illness, or conjunctivitis as a discharge diagnosis and follow-up to identify the cause.
           - If anomalies are identified at CDC, other influenza data sources (e.g., laboratory, hospitalization, etc.) are reviewed for the area identified and results are shared with state/local public health partners who may have access to additional data in the affected area.
           - State/local public health partners often run their own anomaly detection algorithms and, when anomalies are identified, will review their influenza data and other relevant information (e.g., farms with HPAI A(H5) infected animals, milk producers, etc.) for the area identified.

     iv. Monitor for A(H5) detections in wastewater and review additional information to understand the source.
        1. CDC notifies state/local public health partners of the A(H5) detection if the testing occurs outside the justification's PHL.
        2. PHLs testing for A(H5) in wastewater will report the results to CDC.
        3. When an A(H5) detection occurs:
           - CDC reviews other data reported to CDC (e.g., influenza laboratory, emergency department hospitalization, etc.) for the area identified and shares results with state/local public health partners.
           - State/local public health partners review their influenza data and other relevant information (e.g., farms with HPAI A(H5) infected animals, milk producers, etc.) for the area identified.

Appendix 1: Recommendations to commercial laboratories to increase submission of influenza A positive specimens to state and local PHLs for additional subtyping (including A(H5))

CDC, in coordination with STLT public health agencies, requests that commercial laboratories continue submissions of clinical specimens for additional influenza A subtyping testing to their jurisdictional PHL. Commercial laboratories are encouraged to communicate with the PHL of the patient's state of residence prior to submitting specimens to obtain the appropriate specimen submission form and any additional submission instructions. Specimens will be tested for surveillance purposes and patient specific reports might not be returned to the submitter.

CDC requests commercial laboratories continue to send the following specimens to PHLs as soon as possible for further testing and characterization.

1. Influenza A positive specimens that are unable to be subtyped by tests designed to provide an influenza subtyping result (e.g., Biofire) **and confirmed upon retest.**
2. Influenza A positive specimens that are subtype influenza A(H1) and not influenza A(H1)pdm09 on tests designed to provide an influenza subtyping result **and confirmed upon retest.**

For Awareness:

Performance characteristics for the CDC *in vitro* diagnostic reverse transcription real-time polymerase chain reaction (rRT-PCR) subtyping assays have been determined with the following human upper respiratory specimens from patients with signs and symptoms of respiratory infection and/or from viral culture:

1. nasopharyngeal swabs [NPS]
2. nasal swabs [NS]
3. throat swabs [TS]
4. nasal aspirates [NA]
5. nasal washes [NW]
6. dual nasopharyngeal/throat swabs [NPS/TS]

Performance characteristics for the CDC *in vitro* diagnostic reverse transcription real-time polymerase chain reaction (rRT-PCR) subtyping assays have been determined with the following human lower respiratory tract specimens from patients with signs and symptoms of respiratory infection:

1. bronchoalveolar lavage [BAL]
2. bronchial wash [BW]
3. tracheal aspirate [TA]
4. sputum and lung tissue

Additional Surveillance Activities:

Submissions of additional influenza A specimens that have not undergone influenza subtyping testing are greatly appreciated to ensure rapid detection of any human infections of A(H5). CDC, in collaboration with APHL, can help to determine an appropriate and feasible number of specimens each month in order to prioritize available laboratory resources. Please consider submission of specimens that meet the established assay cutoff of your testing method to identify positive specimens. CDC will continue to work with commercial laboratories and APHL to determine a process by which specimens will be submitted to state and local public health for additional subtype testing.

Appendix 2: CDC-WHO Collaborating Center Guidance for Influenza Virus Surveillance for the 2024-2025 Influenza Season

In this appendix, you will find guidance and instructions for surveillance and specimen submissions as follows:

- **National Influenza Virologic Surveillance Submissions (Routine Surveillance)**

For National Influenza Virologic Surveillance Submissions to CDC and NIRCs, please fill in the electronic Influenza Specimen Submission Form in its entirety to provide important metadata. Please email the electronic version of the Influenza Specimen Submission Form to the appropriate receiving laboratory and include a printed shipping manifest from the Influenza Specimen Submission Form (preset in excel template) in the shipping container.

Please contact Dr. Rebecca Kondor (rkondor@cdc.gov) and flusupport@cdc.gov if your laboratory observes a noticeable increase in specimen volume or positivity rate.

If there are any questions, please contact the CDC Influenza Division staff as listed below:

Rebecca Kondor, PhD
Lead, Genomic Analysis Team
VSDB/Influenza Division/NCIRD/CDC
Phone: 404-639-1371
Email: rkondor@cdc.gov

John Steel, PhD
Lead, Epidemic Virology and Vaccines
VSDB/Influenza Division/NCIRD/CDC
Phone: 404-718-7843
Email: jsteel@cdc.gov

National Influenza Virologic Surveillance Submission Guidance During the 2024-2025 Influenza Season
Please use the following virus surveillance specimen submission guidelines below:

1. Please send influenza virus positive specimens every two weeks to your designated NIRC (Table 1). Please do not ship surveillance specimens directly to CDC. We ask that all specimens sent to the NIRCs or CDC are SARS-CoV-2 negative using an FDA authorized or CLIA compliant real-time RT-PCR assay. This is an important safety consideration as samples submitted to NIRCs and/or CDC are subject to many tests (e.g., virus propagation). Furthermore, specimens that have mixed infections which are positive for influenza viruses and SARS-CoV-2 should not be submitted.
2. Specimen selection criteria for submission:
    a. Original clinical specimens positive for influenza and negative for SARS-CoV-2, which have been identified during the prior two weeks. Only specimens stored in either saline, VTM or UTM should be sent to your designated NIRC and not MTM since it inactivates the virus. Saliva is not a suitable specimen for influenza virus characterization.
        i. Ideally, specimens will be from the prior 7 days, but no older than 14 days.
    b. Specimens should have CT values of 28 or lower based on InfA or InfB tests using the CDC Flu SC2 Multiplex Assay or the CDC Flu rRT-PCR Dx Panel.
    c. Representative subtype/lineages (please see Table 2 for minimum weekly subtyping numbers):
        - 6 influenza A(H3N2) positive specimens
        - 4 influenza A(H1N1)pdm09 positive specimens
        - 4 influenza B/Victoria lineage* positive specimens

        **All B/Yamagata lineage positive specimens should be submitted following the guidance for diagnostic specimens. If B genotyping is not performed, please submit only 4 influenza B positive specimens.*
    d. Ideally send 0.5 mL of original clinical specimen; if 0.5 mL is not available, submit no less than 0.3 mL.
    e. Additional considerations for selecting specimens to send to the NIRC: when possible, send specimens from patients of varying ages, disease severity and location within the jurisdiction. When choosing among specimens that meet the above criteria, prioritize those for which level of care (inpatient/outpatient) is known or that were systematically collected as part of the surveillance enhancement.
3. Please follow instructions to complete the electronic Influenza Specimen Submission Form and provide important metadata.
    a. Email the electronic version of the Influenza Specimen Submission Form to the address for your designated NIRC (Table 1).
    b. Print a shipping manifest from the Influenza Specimen Submission Form (preset in Excel template) and include it in the shipping container.

Timely submission of original clinical specimens every two weeks is critical. Do not wait to ship specimens even if you only have a few specimens available that meet the requirements detailed above. In order to meet specific needs (e.g., obtain egg isolates) or to achieve our virus surveillance goals, we may send a special request that deviates from this guidance. Additionally, it will be helpful to coordinate with any clinical laboratory partners to ensure they are submitting influenza positive specimens to you for surveillance purposes. It is especially important to obtain specimens early in the influenza season when fewer viruses are circulating because they are very important for development of future vaccines.

Table 1. Designated National Influenza Surveillance Reference Centers and Shipping Addresses

NIRC*	Shipping Address	**Specimen Submission Laboratories** All public health laboratories (state, county, or city) in the following states/territories:
California Department of Public Health, Viral and Rickettsial Disease Laboratory	CDPH/VRDL Attn: Estela Saguar/Mimi Reyes-Martin/Hugo Guevara 850 Marina Bay Parkway, B262 Richmond, CA 94804 Phone: (510) 307-8497 Email: CDPHVirusIsolationProject@cdph.ca.gov	Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, New Mexico, Nevada, Oregon, Texas, US Affiliated Pacific Islands*, Utah, Washington, Wyoming **via Guam and/or Hawaii*
New York State Department of Health (Wadsworth Center)	David Axelrod Institute Attn: Laboratory of Viral Diseases 120 New Scotland Ave Albany, NY 12208 Tel: (518) 474-4177 Email: fluNYS@health.ny.gov	Connecticut, Delaware, District of Columbia, Florida, Georgia, Massachusetts, Maryland, Maine, North Carolina, New Hampshire, New Jersey, New York, Pennsylvania, Puerto Rico, Rhode Island, South Carolina, US Virgin Islands, Virginia, Vermont, West Virginia
Wisconsin State Laboratory of Hygiene	Wisconsin State Laboratory of Hygiene Attn: Communicable Disease Division (PO Box 7904) Virology Laboratory 2601 Agriculture Drive Madison, WI 53718 Phone: (800) 862-1013 Email: virus@slh.wisc.edu	Alabama, Arkansas, Iowa, Illinois, Indiana, Louisiana, Kansas, Kentucky, Michigan, Minnesota, Missouri, Mississippi, North Dakota, Nebraska, Ohio, Oklahoma, South Dakota, Tennessee, Wisconsin

Influenza Subtyping/Lineage determination options:
Subtyping/lineage determination is important to meet novel virus detection goals and right-size submission recommendations. While we recommend laboratories to strive to meet the ELC goals for influenza A subtyping (95% of specimens), to meet this goal, specimens must be tested at your PHL using the CDC Influenza PCR subtyping/lineage assays as they are the only tests we fully understand the performance characteristic of and trust to identify an inconclusive or potentially novel influenza virus. Please continue to use the CDC Influenza A subtyping (Ver 2, FluIVD03-06), or its replacement product (Ver 3, FluIVD03-10), and Influenza B Lineage Genotyping (Ver 1.1, FluIVD03-7) kits that are available through IRR to determine the subtype and lineage. Additionally, the data from the CDC Flu A Subtyping and Flu B Lineage kits can be considered diagnostic if run under CLIA compliant conditions for reporting an influenza diagnostic result or as RUO to meet surveillance goals if using an extraction process which has not been CLIA validated for reporting influenza subtyping as a diagnostic result. Therefore, subtyping/lineage determination can be conducted using nucleic acid extracted from approved platforms for either influenza diagnostic assays or SARS-CoV-2 diagnostic assays. However, we don't recommend using specimens stored in MTM which inactivates viruses. For the 2024-2025 influenza season, CDC recommends continuing to perform influenza B lineage determination using the IVD kit, FluIVD03-7. However, when a given laboratory's current B lineage determination IVD kit is depleted, CDC recommends that laboratories begin using RUO kits for influenza B lineage determination available in IRR. Because of changing epidemiology and exclusion of influenza B/Yamagata in seasonal vaccines used in the USA, use of RUO kits will meet surveillance reporting requirements. RUO results are considered for surveillance purposes and should be reported to CDC; however, they cannot be reported as diagnostic results to physicians or healthcare.

Table 2 indicates the minimum recommended number of specimens to subtype/lineage test every week for each US state/territory to meet right size novel virus detection goals (Influenza Right Size Roadmap 2nd Edition (aphl.org)) for the peak of influenza season. We understand that these goals may not be achievable when there is very limited influenza virus circulation, but please make efforts to subtype weekly or biweekly. You may wish to subtype/lineage test more viruses than this to provide the information you need to understand influenza activity in your state.

Table 2. Weekly Number of Influenza Positive Specimens Recommended for Influenza A Subtyping/B Lineage Testing

State	N	State	N	State	N	State	N
Alabama	39	Indiana	43	New Hampshire	9	Texas	182
Alaska	5	Iowa	20	New Jersey	57	U.S. Virgin Islands	1
Arizona	46	Kansas	19	New Mexico	14	Utah	21
Arkansas	20	Kentucky	29	New York	124	Vermont	4
California	251	Louisiana	30	North Carolina	66	Virginia	54
Colorado	37	Maine	9	North Dakota	5	Washington	48
Connecticut	23	Maryland	39	Ohio	75	West Virginia	12
Delaware	7	Massachusetts	44	Oklahoma	25	Wisconsin	37
District of Columbia	5	Michigan	64	Oregon	27	Wyoming	4
Florida	135	Minnesota	36	Pennsylvania	82		
Georgia	67	Mississippi	19	Puerto Rico	21		
Guam	2	Missouri	39	Rhode Island	7		
Hawaii	10	Montana	7	South Carolina	33		
Idaho	12	Nebraska	13	South Dakota	6		
Illinois	81	Nevada	20	Tennessee	43		

Appendix 3: CDC-WHO Collaborating Center Guidance for Influenza Antiviral Resistance Surveillance During the 2024-2025 Influenza Season

In this appendix, you will find detailed guidance and instructions for influenza antiviral resistance surveillance and specimen submissions as follows:

- **Influenza In-State Antiviral Resistance Surveillance [Referral Chart 1]**

CDC no longer performs diagnostic testing for influenza antiviral resistance.

If you have any questions regarding influenza antiviral resistance testing, please contact Dr. Larisa V. Gubareva at LGubareva@cdc.gov or send an email to fluantiviral@cdc.gov.

Influenza In-State Antiviral Resistance Surveillance Specimen Referral Chart 1

Category and Purpose	What and when to send	Where to send	CDC Contact
Antiviral Resistance Surveillance (Pyrosequencing or NGS) To identify drug resistance in influenza A and B viruses conferred by mutations in the neuraminidase. Laboratories may submit specimens to the reference center (New York State Department of Health, **NYSDOH**). **Note:** **ALWAYS SUBMIT TO NATIONAL INFLUENZA VIRUS SURVEILLANCE FIRST, PRIOR TO SUBMITTING ADDITIONAL VIRUSES TO ANTIVIRAL RESISTANCE SURVEILLANCE.**	What to send? • Original clinical specimens only • Positive for influenza A(H1N1)pdm09, A(H3N2) and type B viruses • Ct values less than 29 How many and when to send? • Up to 5 specimens every other week. Do **NOT** submit aliquots from the same specimen for both Antiviral Resistance Surveillance and National Influenza Virus Surveillance. Please submit **DIFFERENT** specimens.	**Designated Laboratory for Influenza Antiviral Resistance Surveillance**: Wadsworth - **NYSDOH** David Axelrod Institute 120 New Scotland Ave Albany, NY 12208 Phone:(518) 408-2007 Email: fluNYS@health.ny.gov Complete the Influenza Specimen Submission Form and indicate the following specific information**:** • **Reason for Submission**: Antiviral Surveillance **Notes:** Send completed Influenza Specimen Submission Form and tracking information electronically to fluNYS@health.ny.gov. Include a hard copy of the form in the shipment. The reference center at **NYSDOH** receives clinical specimens submitted 1) for National Influenza Virus Surveillance, and 2) to perform antiviral testing on additional (and different) clinical specimens. Note: please indicate clearly on the Influenza Specimen Submission Form whether **Reason for Submission** is **Surveillance** or **Antiviral Surveillance** (pyrosequencing/NGS).	Larisa V. Gubareva, MD, PhD Team Lead, Molecular Epidemiology Team VSDB/ID Phone: 404- 639-3204 Fax: 404-639-2350 Email: fluantiviral@cdc.gov Email: LGubareva@cdc.gov

Appendix 4: CDC-WHO Collaborating Center Guidance for Influenza Virus Diagnostic Submissions During the 2024-2025 Influenza Season

For Influenza Diagnostic Submissions to CDC, please contact Dr. Marie Kirby or flusupport@cdc.gov. It is very important that you rapidly contact CDC, at these addresses, if your laboratory identifies specimens that are atypical or have non-standard results. Atypical or negative subtyping results could represent a variant influenza A virus or other novel influenza A viruses with pandemic potential.

In particular, please send specimens with non-standard test results as detailed in the instructions for use of the CDC Flu SC2 Multiplex, A/B Typing, A subtyping assays and B genotyping assays. Please send any inconclusive results or presumptive positive results from the A(H5) or A(H7) assays and notify CDC **IMMEDIATELY** (flusupport@cdc.gov).

More information on non-standard test results is included in Referral Charts 2 and 3.

Reminders:

- The A(H5) assay should not be performed unless the patient meets clinical, epidemiologic, or public health criteria for testing suspected specimens.
- PHL may run conjunctival swab specimens in the CDC A(H5) assay as long as they are paired with approved respiratory specimens and stored/shipped in approved media for the assay. CDC is approved to run conjunctival swab specimens with this assay as a CLIA validated laboratory developed test.
- If presumptive positive for a novel virus, per CDC's FDA approved instructions for use (IFU), they can be sent to CDC as a diagnostic specimen under typical diagnostic shipping protocols.
- Any unsubtypable results from CDC A(H5) assay should be shipped to CDC immediately.
- Any presumptive positive results from the CDC A(H5) assay should be shipped to CDC immediately.
- Any inconclusive test results from the CDC A(H5) assay should be shipped to CDC immediately.

To submit viruses for diagnosis, please fill out the following forms and send along with your submission:
CDC Specimen Submission Form, CDC **50.34** (Required for CLIA reporting) or you can use CDC Specimen Test Order and Reporting (CSTOR) | Submitting Specimens to CDC | Infectious Diseases Laboratories | CDC

Additionally, please include:

- **Reason for Submission:** Diagnosis
- **If Clinical Specimen**: Indicate specimen type
- **Type/Subtype:** Inconclusive
- **Comments:**
    - Assign CDC-10421 Influenza Molecular Detection in Clinical Specimens for diagnostic submissions.
    - Provide any relevant rRT-PCR data in the comments section of the 50.34/CSTOR.
    - Include patient's name and date of birth on the CDC 50.34/CSTOR.
    - Please be sure to label the specimen with two identifiers, including patient name.
    - Samples must be received frozen.
    - Please include a hard copy of the 50.34 form in the package with submissions.

Shipping Address
Marie Kirby, PhD
Centers for Disease Control and Prevention
Influenza Division, H23-6
c/o STAT (unit 198)
1600 Clifton Rd, NE
Atlanta, GA 30329

If there are any questions about an influenza diagnosis or the CDC diagnostic assays, please contact Dr. Marie Kirby or flusupport@cdc.gov.

Diagnostic Specimen – Seasonal and Variant Influenza Referral Chart 2

Category and Purpose	What and when to send	Where to send	CDC Contact
Respiratory Specimens with Inconclusive results using the CDC Influenza A Subtyping or Influenza B Lineage Kits **See the CDC Flu rRT-PCR Dx Panel package insert for detailed descriptions of inconclusive results.** **Specimens with non-standard test results that suggest a potential novel influenza virus should be sent to CDC.** **All H3N2v presumptive positive clinical samples should be sent to CDC.** **Note**: Unsubtypable results may represent changes in the circulating viruses, introduction of a new virus, a problem with the performance of the primers and probes, or a problem in your individual laboratory.	If, upon repeat testing using the CDC protocol as specified in the package insert, specimen test results are: • Influenza A unsubtypable with InfA Ct value <35, notify CDC **IMMEDIATELY** (flusupport@cdc.gov) and send the clinical specimen to CDC **IMMEDIATELY** for further characterization • Presumptive positive A/H3v similar to those circulating in swine, notify CDC **IMMEDIATELY** (flusupport@cdc.gov) and send the clinical specimen to CDC **IMMEDIATELY** for further characterization. • Inconclusive indicating possible variant influenza A virus similar to those circulating in swine, notify CDC **IMMEDIATELY** (flusupport@cdc.gov) and send the clinical specimen to CDC **IMMEDIATELY** for further characterization. • Inconclusive influenza B viruses that are unable to be genotyped, send the clinical specimen to CDC for further characterization. • All influenza B genotype results of B/Yamagata-lineage, send the clinical specimen to CDC for further characterization. **Note:** Influenza A unsubtypable with InfA Ct value >35, the sample may be reported as inconclusive. • Report may indicate that the subtype could not be determined due to low viral titer. • These specimens do not need to be sent to CDC for verification following consultation with CDC.	Ship to: Marie Kirby Ph.D. Centers for Disease Control and Prevention Influenza Division, H23-6 c/o STAT (unit 198) 1600 Clifton Rd, NE Atlanta, GA 30329 **Complete one of the following forms:** 1) **CDC Specimen Submission Form, CDC 50.34** or CDC Specimen Test Order and Reporting (CSTOR) | Submitting Specimens to CDC | Infectious Diseases Laboratories | CDC which are required for all diagnostic submissions when results can be reported back to a patient or healthcare provider. information**:** • **Reason for Submission:** Influenza Molecular Detection in Clinical Specimens • **If Clinical Specimen**: Indicate specimen type • **Type/Subtype:** Inconclusive • **Comments:** ☐ Provide relevant rRT-PCR data ☐ Include patient's name and DOB ☐ Please be sure to label the specimen with two identifiers, including patient name ☐ Samples must be received frozen	Marie Kirby, Ph.D. Team Lead, Genomics and Diagnostics Team VSDB/ID Phone: 404-718-7689 Email: flusupport@cdc.gov Email: pbi0@cdc.gov **Note:** Send completed form(s) and tracking information electronically to flusupport@cdc.gov. Include hard copies of both forms in the shipment.

Diagnostic Specimen - Suspect A(H5) and A(H) (Eurasian Lineage) Cases Referral Chart 3

Category and Purpose	What and when to send	Where to send	CDC Contact
A(H5N1): Specimens with presumptive positive or inconclusive results A specimen is only **presumptively positive** for influenza A(H5) if all three targets (InfA, H5a and H5b) are positive. A result is **inconclusive** for A(H5) if the test is positive for InfA and has only one of the two H5 markers testing positive. Note: If using lot 220307, refer to acknowledgement form sent in April 2024 for updated interpretation guidance. **A/H7 (Eurasian Lineage): Specimens with presumptive positive or inconclusive results** A specimen is only **"Influenza A Detected; Subtype Eurasian H7 detected"** if both targets (InfA and EuH7) are positive. A result is **inconclusive** for A/H7 (Eurasian lineage) if the test is positive for EuH7 and is negative for InfA. **Note:** Testing with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H5) or A(H7) (Eurasian Lineage) Assay should only be performed when the patient meets clinical and epidemiologic criteria for testing suspect specimens.	If specimen test results are presumptive positive for A(H5) or A(H7), notify CDC **IMMEDIATELY** (flusupport@cdc.gov) and send the clinical specimen to CDC **IMMEDIATELY** for further characterization. Repeat testing should be done on all samples that are inconclusive for influenza A(H5) or A(H7) (Eurasian Lineage) using the CDC protocol as specified in the package insert. If, upon repeat testing, specimens are either 1) positive for InfA and for either or both H5a and H5b targets, or 2) positive for InfA and EuH7, these should be sent to CDC **IMMEDIATELY** for verification. What to send? Original clinical specimens When to send? **IMMEDIATELY** When to notify? Notify CDC influenza Division **IMMEDIATELY** (flusupport@cdc.gov) upon verification of presumptive positive or inconclusive results for influenza A(H5) or detection of influenza A(H7) (Eurasian Lineage).	Ship to: Marie Kirby Ph.D. Centers for Disease Control and Prevention Influenza Division, H23-6 c/o STAT (unit 198) 1600 Clifton Rd, NE Atlanta, GA 30329 **Complete one of the following forms:** 1) **CDC Specimen Submission Form, CDC 50.34** or CDC Specimen Test Order and Reporting (CSTOR) | Submitting Specimens to CDC | Infectious Diseases Laboratories | CDC • **Reason for Submission:** Influenza Molecular Detection in Clinical Specimens • **If Clinical Specimen**: Indicate specimen type • **Type/Subtype:** Inconclusive • **Comments:** ☐ Provide relevant rRT-PCR data ☐ Include patient's name and DOB ☐ Please be sure to label the specimen with two identifiers, including patient name ☐ Samples must be received frozen	Marie Kirby, Ph.D. Team Lead, Genomics and Diagnostics Team VSDB/ID Phone: 404-718-7689 Email: flusupport@cdc.gov Email: pbi0@cdc.gov **Note:** Send completed form(s) and tracking information electronically to flusupport@cdc.gov. Include hard copies of both forms in the shipment.

Appendix 5: CDC-WHO Collaborating Center Guidance for National Influenza Reference Centers (NIRCs) During the 2024-2025 Influenza Season

In the influenza surveillance and specimen submission guidance sent to state PHLs (referred to as originating lab) for 2024-2025, influenza positive specimens are to be submitted to NIRCs every two weeks which meet the stated specimen selection criteria and with the following representative subtype/lineages:

- 6 influenza A(H3N2) positive specimens
- 4 influenza A(H1N1)pdm09 positive specimens
- 4 influenza B/Victoria lineage positive specimens

All specimens received at the NIRC will continue to undergo NGS, however not all specimens will undergo isolation. Each NIRC should randomly select specimens from each originating lab package and follow the isolation right-size number. Any specimens received from the originating lab above the isolation right-size number do not undergo isolation.

NIRC isolation right-size number:

- 3 influenza A(H3N2) positive specimens
- 2 influenza A(H1N1)pdm09 positive specimens
- 2 influenza B/Victoria lineage positive specimens

NGS of isolates – only the first 100 isolates should be sequenced by NGS. After which, the subsequent isolates should be sent in the ISA tube to CDC.

Shipment to CDC should occur after the NIRC has completed the isolation workflows for selected specimens from the originating lab package/state PHL. The shipment will include all RCV tubes, as well as the additional aliquots for specimens with successful isolation results bundled by the originating lab(s)/state PHL.

For example:

1) Specimens NOT chosen for isolation:
    - ISR Aliquot – NGS performed @ NIRC
    - RCV (ORIGINAL) – remaining tube sent to CDC
    - Data fields would appear as follows: Inoculation Date: null; Grow TC?: null; Passage: null; HA titer: null
2) Specimens chosen for isolation:
    - ISR Aliquot – NGS performed @ NIRC
    - RCV (ORIGINAL) – remaining tube sent to CDC
    - Successful Isolates
        - **ISA tube – NGS performed @ NIRC for first 100 isolates, after which the ISA tube of subsequent isolates should be sent to CDC**
        - REF tube – sent to CDC
        - MET tube – sent to CDC
        - RPS tube – sent to CDC
        - RHI – scintillation vial – sent to CDC
    - Data fields would appear as follows: Inoculation Date: Filled in; Grow TC?: Filled in; Passage History: Filled in, if successful; HA titer: Filled in, if successful.

Once received and accessioned at CDC, the date received from contract lab will be filled in signifying that NIRC workflows have been completed.

Additional Sequencing for NIRC Surveillance: The NIRC may be reimbursed for up to 500 additional specimens from their jurisdiction (beyond right size submission to NIRC) per season. This is approximately 85 specimens per month during the influenza season. NIRCs should select specimens that are representative of what are typically selected for NIRC submissions.