2026-27 INFLUENZA SEASON:

SURVEILLANCE FOR SEASONAL INFLUENZA AND NOVEL INFLUENZA A VIRUSES

Conducting surveillance to monitor seasonal influenza viruses and to detect novel influenza A virus infections remains critical to inform public health actions. As in previous years, CDC, in collaboration with State, Tribal, Local, and Territorial (STLT) public health agencies, is employing a multi-faceted surveillance strategy for seasonal influenza activity monitoring and novel influenza virus detection for the 2026-27 season. Much of this strategy includes activities that have been recommended and in place for many years prior to the recent animal outbreaks of highly pathogenic avian influenza (HPAI) A(H5) in the United States. CDC may modify the strategy 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 novel influenza A viruses, including HPAI A(H5), and the possible spread of these viruses 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 (e.g., swine, dairy cattle, poultry, wild birds, domestic cats, and other mammalian species) and extending outward to monitoring surveillance data from the general human population. These activities are described at a high level, and where available, internet links to more detailed information are provided.

1. Identify novel influenza A viral human infections via symptom monitoring among workers and others with recent exposures to HPAI A(H5) virus infected animals on farms or other locations.
    - Partners: State and local public health; State Departments of Agriculture and Wildlife; CDC and U.S. Department of Agriculture (USDA) support as requested
    - Activities
        i. Consider conducting proactive outreach to poultry and livestock workers, farm owners, and veterinarians in advance of a known exposure on the premises, in coordination with agriculture departments and other partners, to provide information about risk reduction and set expectations for symptom monitoring among people exposed to infected animals and their environment.
        ii. Monitor exposed workers for development of symptoms. If possible, active symptom monitoring should involve daily checks between health department staff and exposed persons using a list of names and contact information provided by the farm. If active monitoring is not possible, the health department could provide self-monitoring and symptom reporting information to farm workers or farm owners directly or by working through an intermediary such as Department of Agriculture personnel, a facility veterinarian, a professional association, or a contractor representative.
        iii. Ensure the prompt availability of and access to testing for influenza and other respiratory viruses among exposed workers who develop acute respiratory infection (ARI) symptoms or conjunctivitis and who have had recent direct or close contact with animals suspected or confirmed to be infected with HPAI A(H5) virus.
        iv. Testing of asymptomatic persons for HPAI A(H5) virus infection is not routinely recommended but may be considered in certain circumstances and in consultation with state and local health departments.

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 or cheese).
    - Partners: State and local public health authorities and departments of agriculture; CDC and USDA support as requested. Other potential partners include farmworker organizations, 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 and control, known signs and symptoms, resources available, and whom to contact if symptoms develop.
    - Information for Specific Groups | Bird Flu | CDC
    - Avian Influenza Print Materials | Bird Flu | CDC
    - Swine Flu Prevention and People Who Raise Pigs | Swine Flu | CDC

3. Conduct surveillance for novel influenza A virus infection among severely ill patients (e.g., hospitalized, including in intensive care unit (ICU)) by encouraging collection of lower respiratory tract specimens from patients with severe respiratory disease and subtyping influenza A positive specimens.
    - Partners: State and local public health authorities; 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 severe respiratory disease (irrespective of exposure history) and to relay the importance of determining the influenza A subtype for all influenza A positive specimens.
            - Encourage health care providers, hospital infection preventionists, and clinicians to consider asking questions about exposure to poultry, wild birds, dairy cattle, swine, raw animal products, and agricultural fairs and other events when collecting patient histories.
            - Encourage the collection of lower respiratory tract specimens (e.g., an endotracheal aspirate or bronchoalveolar lavage fluid).
        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 to develop or continue implementation of a process for subtyping of influenza A positive specimens from patients with severe respiratory disease, particularly those with relevant exposure history, either in the clinical laboratory or by shipping the specimen to the public health laboratory (PHL).
            - This would apply to all hospitalized patients, including in ICU; however, if volume, resources, or other reasons require prioritization, focus on those most critical, such as persons in the ICU.

4. Conduct surveillance for novel influenza A virus infections in the community.
    - Partners: State and local public health authorities; 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 Appendix A of 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. During the remainder of the season, jurisdictions should meet the 1 in 200 novel event detection goal.
            - Ask providers and clinical laboratories that have submitted specimens to the PHL during previous seasons to continue doing so this season.
            - Identify any additional providers or clinical laboratories that would be willing to submit specimens this season.
            - CDC recommends that commercial laboratories continue submission of influenza A positive specimens to PHLs for additional subtyping (see Appendix 1).

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).
    - All PHLs should submit influenza positive specimens that meet the submission criteria outlined in Appendix 2.

iii. Conduct whole genome sequencing of additional influenza specimens (see Appendix 6).
    - 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.
    - 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 authorities; CDC
    - Activities
        i. State and local public health partners monitor data within their jurisdictions, which may include systems listed below plus additional data/systems specific to a jurisdiction.
        ii. CDC analyzes the following data that are reported to CDC:
            - Virologic data from approximately 260 clinical laboratories (approximately 40,000-150,000 specimens tested weekly) and approximately 90 PHLs (approximately 800-7,000 specimens tested weekly).
            - Outpatient respiratory illness data reported to ILINet from more than 4,000 outpatient providers/EDs (approximately 2.5 million patient visits weekly).
            - Emergency department visits with influenza as a discharge diagnosis reported to the National Syndromic Surveillance Program (NSSP)/ESSENCE.
            - Hospitalization data from FluSurv-Net sites.
            - The National Healthcare Safety Network's Hospitalization Surveillance that includes mandatory reporting from all hospitals.
            - Mortality data from the National Vital Statistics Surveillance System that covers more than 99% of deaths occurring in the U.S.
            - Detections of A(H5) and influenza A in wastewater.
        iii. Local data anomaly detection and investigation.
            - 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) 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) virus infected animals, milk producers) for the area identified.

Regarding A(H5) confirmatory testing, for state and local PHLs performing influenza A(H5) testing with CDC's 510(k) A(H5) IVD assay, CDC recommends:

1. When influenza A(H5) virus is detected in humans, the first three identified influenza A(H5) human cases by a PHL should be treated as presumptive positives and the PHL must send specimens from those three cases to CDC for confirmation.
2. Once three presumptive A(H5) positive patients have been confirmed A(H5) positive by CDC, subsequent A(H5) testing can be treated as confirmed at the PHL. This subsequent testing at PHLs should be performed according to the Instructions for Use (IFU) of CDC's 510(k) A(H5) IVD assay, including meeting clinical, epidemiologic, and public health response criteria for patient testing.
3. Positive A(H5) subtyping results from the PHL should be immediately reported to CDC by email to flusupport@cdc.gov and phone call to Influenza Division POC(s) (Marie Kirby: 404-718-7689) and/or CDC's Emergency Operations Center at 770-488-7100.
4. Due to the need for further virologic characterization, all influenza A(H5) positive samples (minimum of 500 µl or total amount available if less) should be sent to CDC within 24 hours of detection (or Monday following a Friday, Saturday, Sunday, or holiday detection) or as soon as possible.
5. Laboratories that have not yet validated conjunctival specimens should coordinate with CDC for A(H5) testing if clinical, epidemiologic, and public health response criteria for testing are met.

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 local public health point of contact and/or 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 to 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 A subtyping result (e.g., Biofire Respiratory Panel) **and confirmed upon retest.**
2. Influenza A positive specimens that are identified as subtype influenza A(H1) and not influenza A(H1)pdm09 on tests designed to provide an influenza A subtyping result **and confirmed upon retest.**

For Awareness:
Performance characteristics for the CDC *in vitro* diagnostic real-time reverse transcription polymerase chain reaction (rRT-PCR) subtyping assays have been determined with the following human upper respiratory and conjunctival swab 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. combined nasopharyngeal/throat swabs [NPS/TS]
7. **conjunctival swabs [CS] – for use with CDC's Influenza A/H5 subtyping kit only**

Performance characteristics for the CDC in vitro diagnostic 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

Appendix 2: CDC guidance for influenza virus surveillance during the 2026-27 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 National Influenza Reference Centers (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 InfluenzaVirusSurvei@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

National Influenza Virologic Surveillance Submission Guidance During the 2026-27 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 rRT-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 collected during the prior three weeks. Only specimens stored in saline, viral transport media (VTM), or universal transport media (UTM) should be sent to your designated NIRC and not molecular transport media (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 not older than 21 days.
    b. Specimens should have cycle threshold (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):
        - 4 influenza A(H3N2) positive specimens
        - 4 influenza A(H1N1)pdm09 positive specimens
        - 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.
    f. Please do not ship specimens positive for multiple influenza viruses (e.g., H3N2 and H1N1pdm09 coinfections).
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. 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 **either directly to CDPH/VRDL or 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 determination options:
Subtyping determination is important to meet novel virus detection goals and right-size submission recommendations. While we recommend laboratories 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 assays as they are the only tests we fully understand the performance characteristic of and trust to identify an inconclusive or potentially novel influenza A virus. Please continue to use the CDC Influenza A subtyping Ver 4, FluIVD03-12 kits that are available through IRR to determine subtype. Data from the CDC Flu A Subtyping can be considered diagnostic if run under CLIA compliant conditions for reporting an influenza diagnostic result or as research use only (RUO) to meet surveillance goals if using an extraction process which has not been CLIA validated for reporting influenza A subtyping as a diagnostic result. Subtyping 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 2026-27 influenza season, CDC will stop distribution of the influenza B genotyping kit. Because of changing epidemiology and exclusion of influenza B/Yamagata in seasonal vaccines used in the U.S., sequencing of influenza B positive specimens by the NIRC and ISC submissions will be sufficient for surveillance. PHLs should continue to submit influenza B positive specimens to their designated NIRC for additional virus characterization and sequencing.

Table 2 indicates the minimum recommended number of specimens to subtype test every week for each US state/territory to meet right size novel virus detection goals (Influenza Right Size Roadmap) 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 test weekly or biweekly. You may wish to subtype 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 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 guidance for influenza antiviral resistance surveillance during the 2026-27 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 (NGS-based) To identify potential drug resistance in influenza A and B viruses conferred by mutations in the neuraminidase and polymerase acidic protein (PA). 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 equal or below 28 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** (NGS-based).	Larisa V. Gubareva, MD, PhD Team Lead, Molecular Epidemiology Team VSDB/ID Phone: 404-639-3204 Email: fluantiviral@cdc.gov Email: LGubareva@cdc.gov

Appendix 4: CDC guidance for influenza virus diagnostic submission during the 2026-27 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, and A subtyping assays. Please notify CDC **IMMEDIATELY** (flusupport@cdc.gov) if you observe any presumptive positive or inconclusive results from the influenza A(H5) or influenza A(H7) testing. 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.
- If a laboratory is performing A(H5) subtyping with an internally developed Laboratory Developed Test (LDT), the algorithm should include testing influenza A positive samples for seasonal A(H1) and A(H3) viruses before (or in parallel with) A(H5) testing.
- When influenza A(H5) virus is detected in humans, the first three influenza A(H5) identified patient cases by a PHL should be treated as presumptive positives and the PHL must send specimens from those three cases to CDC for confirmation.
- Once three presumptive positive patients have been confirmed A(H5) positive by CDC, subsequent A(H5) testing can be treated as confirmed at the PHL. This subsequent testing at PHLs should be performed according to the IFU of CDC's 510(k) A(H5) IVD assay.
- Conjunctival swabs are an approved specimen type for the FDA approved 510(k) CDC A(H5) assay. PHLs will still need to follow guidance for internal policies on how to validate this specimen type for use in diagnostic programs. Conjunctival swabs are not an approved specimen type for other CDC influenza assays.
    - Laboratories that have not yet validated conjunctival specimens should coordinate with CDC for A(H5) testing if clinical, epidemiologic, and public health response criteria for testing are met.
- PHLs may run conjunctival swab specimens stored/shipped in approved media for the CDC A(H5) assay with or without a paired respiratory specimen but collection and testing of paired respiratory specimens are encouraged in patients exhibiting respiratory-based symptoms.
- Specimens should be sent to CDC under typical shipping protocols.
- Any unsubtypable results from the CDC A(H5) assay in which seasonal subtypes have already been ruled out should be shipped to CDC immediately.
- Any presumptive positive or confirmatory 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 test request form via CSTOR: 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 in CSTOR.
    - Include patient's name and date of birth in CSTOR.
    - Please be sure to label the specimen with two identifiers, including patient name and specimen ID.
    - 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 Kit **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. • Continue to send InfB positive specimens to NIRC for sequencing/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 test request form in CSTOR:** 1) CDC Specimen Test Order and Reporting (CSTOR) | Submitting Specimens to CDC | Infectious Diseases Laboratories | CDC is required for all diagnostic submissions when results can be reported back to a patient or healthcare provider. information**:** • **Reason for Submission:** CDC-10421 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 and specimen ID ☐ 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, confirmatory or inconclusive results A specimen is only **presumptively positive or confirmed** for influenza A(H5) if all three targets (InfA, H5a and H5b) are positive. Note: When influenza A(H5) virus is detected in humans, the first three (3) identified cases by a PHL should be treated as presumptive positive and the PHL must send specimens from those three cases to CDC for confirmation. A result is **inconclusive** for A(H5) if the test is positive for InfA and has only one of the two A(H5) markers testing positive. **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 or confirmatory 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 Test Order and Reporting (CSTOR) | Submitting Specimens to CDC | Infectious Diseases Laboratories | CDC • **Reason for Submission:** CDC-10421 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 guidance for National Influenza Reference Centers (NIRCs) during the 2026-27 influenza season

In the influenza surveillance and specimen submission guidance sent to state PHLs (referred to as originating lab) for 2026-27, 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:

- 4 influenza A(H3N2) positive specimens
- 4 influenza A(H1N1)pdm09 positive specimens
- 4 influenza B 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:

- 2 influenza A(H3N2) positive specimens
- 2 influenza A(H1N1)pdm09 positive specimens
- 2 influenza B positive specimens

NGS of isolates – only the first 100 isolates should be sequenced by NGS. After which, the subsequent isolates should be stored at NIRC and sequenced on an "as needed" basis.

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 stored at the NIRC and sequenced on an "as needed" basis.**
        - 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.

Appendix 6: CDC guidance to U.S. laboratories performing next generation sequencing of influenza virus genomes

CDC Contact
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1. Influenza Genome Sequence Quality

These recommendations assume that raw sequence reads have been produced on either Illumina or Oxford Nanopore Technologies using CDC's Multi-segment Reverse Transcription PCR protocol.

Every batch of PCR should include a no-template reaction to serve as a negative control, which is carried through library preparation and barcoding. After sequencing, if a negative control has >= 1000 reads and 1% of those reads match influenza, that is considered a failed negative, and you must go back to PCR and generate cleaner amplicons.

Influenza viruses, type A and B, are the primary viruses causing seasonal epidemics. These types are genetically distinct and do not reassort gene segments. Within the A type, highly divergent subtypes exist with nomenclature based on the hemagglutinin (HA) gene segment. On average, influenza generates a mutation with each replication, leading to the high level of genetic diversity observed. Standard reference-based alignment of raw sequence reads against a reference genetically distant from the sequenced specimen can lead to assembly errors which could mask real variants or create false variants. Assembly pipelines which use "reference-filling" for areas with low read coverage could yield artificial gene segment mutations or viral genomes. For these reasons, CDC recommends using an assembler which does not use "reference-filling", such as the Iterative Refinement Meta Assembler (IRMA) to assemble reads and call a consensus genome. IRMA also generates an amended consensus, that calls the IUPAC code for an ambiguous base-call when a minor allele is >= 20% frequency and meets minimum coverage. Calling a mixed base adds information to a single consensus sequence by reflecting a site under active selection within a single infection.

Following consensus sequence generation with an assembly method that can handle influenza viruses' high variability, additional sequence checks can be performed to assure accuracy. These quality checks include minimum per-base and median coverages, minimum reference length assembled, frame-shift detection and maximum number of minor variants detected per gene segment. In high quality influenza sequences, CDC observes <= 5 minor variants with allele frequencies >= 5% per gene segment. Higher counts can indicate sample contamination or co-infection with more than one influenza virus, either of which lead to erroneous consensus calling.

CDC provides a genome assembly and QC pipeline called Mira, which can be installed locally and operated through a graphical interface. For laboratories with a bioinformatician, consider running the command-line version: Mira-nf. Mira runs IRMA on demultiplexed fastqs from Illumina or Oxford Nanopore Technologies sequencers to generate an amended consensus, translates the nucleic acid sequences into the multiple open reading frames' amino acid sequences, and then automatically flags sequences that do not meet CDC quality metrics (outlined in Table 3).

Table 3. CDC Influenza Genome Assembly Quality Metrics

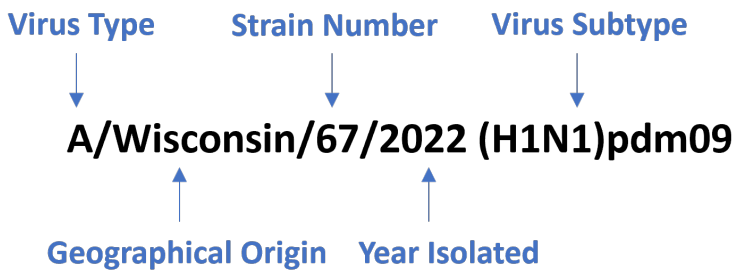
Metric	Illumina	Oxford Nanopore
Negative control	<1% reads matching flu and <1000 reads matching flu	<1% reads matching flu
Read length	>= 125 bp (150 cycle chemistry)	>= 150 bp
Insertion frequency for consensus incorporation	>= 25%	>= 75%
Deletion frequency for consensus incorporation	>= 60%	>= 75%
Minimum coverage to call single base	>= 30x	>= 50x
Minimum mean base quality score per read	>= 30	>= 10
Maximum count minor alleles >= 5% frequency	10	10
Minimum reference length coverage	>= 90%	>= 90%
No premature stop codons	True	True

2. Influenza virus naming

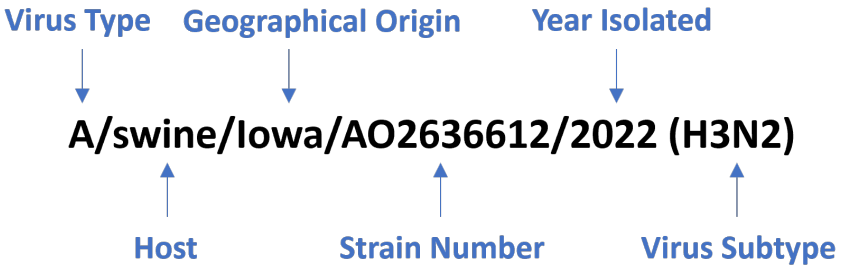
CDC follows an internationally accepted naming convention strategy for influenza viruses. This guidance states that all strain names include:

- Virus type (A, B, C)
- Host of origin, if not human (duck, chicken, seal, horse, etc.)
- Geographical origin
    - This can be country, state, province, or city. The most discrete location decreases the likelihood of name duplication. For viruses collected in the U.S., CDC uses the state or territory.
- Strain number
    - This has typically been a left-zero-padded-integer of varying lengths but can include other information.
- Year collected
    - This is the year that the sample was taken from the patient.
- For influenza A viruses, the virus subtype (HA and NA description) is provided in parenthesis, i.e. (H1N1) or (H3N2)
    - Human viruses from the HA lineage responsible for the 2009 H1N1 pandemic have “pdm09” after their subtype.
    - Human strains with known source of infection from an animal host like swine are considered variants and a ‘v’ is added to the name, such as (H3N2)v.

Example strain name of human seasonal virus:



Example strain name of swine virus:



Additional details for the strain number field:

- Each lab performing sequencing should create a lab identifier that includes the two-letter code for the state followed by the characters to represent their institute, such as PHL (Public Health Laboratory) or SPHL (State Public Health Laboratory).
- This code will be the first part of the strain number, followed by a hyphen (-) and then a 4-digit number. The 4-digit number should be associated with your laboratory identifier for that specimen and not include personally identifiable information. A key exists at the PHL that contains the metadata for that submission tied to the strain number field. CDC may contact your laboratory if they observe that this genome has significant or novel characteristics and will request sharing of the sample material and/or further epidemiological details. Maintaining this datalink internally in your laboratory is critical for public health responses.
    - The lab identifier must be as short as possible. Downstream analysis applications may truncate characters when used as identifiers in sequence analysis.
    - Notify CDC of the convention they will use for your naming by emailing idseqsupport@cdc.gov.
        - Example: A/Wisconsin/WISPHL-0023/2024

Considerations for the surveillance efforts put in place by the CDC:

- Specimens arriving through the NIRCs or directly submitted to CDC without a strain designation are assigned a strain number, sequentially, in the order that they are received. This *does not include* information about the submitting laboratory.
    - As an example, the first sample received in the year 2025 by CDC through their established network from Wisconsin would be named "A/Wisconsin/01/2025".
    - Sequences from NIRC in-state or ISCs are assigned strain designations with either "NIRC-IS" or "ISC" added to the strain number fields such as "A/Wisconsin/NIRC-IS-1078/2024".
- PHLs that are sequencing and submitting the resulting consensus sequences to a public database should not send that same specimen to the CDC or their NIRC to meet their submission goals.
- A PHL that has sent a specimen to CDC should not submit that sequence data to the sequence databases, as it is impossible to identify duplications based on the strain name alone.
    - Example scenario:
      Georgia submits to the NIRC according to CDC submission guidelines but wants to sequence

additional/different specimens above those guidelines and submit them to one of the public databases. These submissions that are not sent to the NIRCS or CDC could be named as below.
A/Georgia/GAPHL-4216/2023
A/Georgia/GAPHL-4217/2023
A/Georgia/GAPHL-4219/2023

- A key should be kept at the PHL that contains the metadata for their submissions tied to the strain number field. None of these specimens should be sent to the NIRC or CDC, as they will also be sequenced, and we do not want duplication in any of the databases.

3. Sequence submission to public databases
It is recommended that U.S laboratories submit influenza genome consensus sequences which meet the QC standards described in this document to both NCBI's GenBank and GISAID repositories. It is critical to distinguish between surveillance samples and those from outbreaks or research collections. Surveillance samples should represent random sampling of influenza positive samples derived from networks established to meet CDC right-size guidance. It is recommended that surveillance as the sample strategy be included in the meta data for these specimens.

NCBI Bioprojects
When submitting to NCBI, you should first create Bioprojects for your laboratory for each seasonal influenza subtype (H1, H3, B-vic). When you create your Bioprojects, you will be asked if they should be linked with an Umbrella Bioproject. Please link your institution's Bioprojects to the following Umbrella Bioprojects, based on identified subtype:

Influenza subtype	Bioproject name	Bioproject Accession
B – vic	United States Genomic Surveillance - Influenza B-vic	PRJNA998891
A – H3	United States Genomic Surveillance - Influenza H3	PRJNA998890
A – H1	United States Genomic Surveillance - Influenza H1	PRJNA998889

Influenza sequence data to be linked to these CDC Umbrella Bioprojects must be obtained from surveillance samples, representing random sampling. The Umbrella Bioprojects are for baseline surveillance, and PHLs should not link non-surveillance sequence data (e.g., outbreak investigations, special studies).

NCBI Sequence Read Archive
It is also useful to submit fastq data to SRA to provide the global research and surveillance community access to read-level sequence data. CDC recommends that you only submit sequence reads which match to influenza and request that NCBI-SRA perform human read scrubbing on your submission. This request must be done by email. When using IRMA, raw reads matching influenza are available in IRMA's output at:

```
<sample-id>/intermediate/4-ASSEMBLE_SSW/reads.tar.gz
```

When using Mira, it will automatically remove unneeded intermediate files that IRMA produces and zip up the remaining files that include logs, plurality consensus fastas, iteration-round-1 (F1) bam files and the influenza matched reads. You will need to unzip this archive in Mira's run folder on the command line:

```
tar -xzf irma_allconsensus_bam.tar.gz

# The raw influenza matching reads per sample can now be found as above
in:
IRMA/<sample-id>/intermediate/4-ASSEMBLE_SSW/reads.tar.gz
```

Metadata
Sample metadata is critical in analyzing sequence data. Without certain pieces of metadata, the sequence will not be included in CDC-based analyses for situational awareness and vaccine strain selection recommendations to the WHO.

Required:
- Strain name
- Subtype
- Collection location
- Collection date
- Host
- Segment ID per segment
    - HA
    - NA
    - PB2
    - PB1
    - PA
    - NP
    - M
    - NS
- Originating lab ID (GISAID)
- Authors

Strongly recommended:
- Assembly methods
    - If using Mira, please write that with the version number, i.e. “Mira v1.1.3”. Otherwise, please provide as much detail as possible for all bioinformatic tools used.
    - In GISAID, the most common assembly methods are available in a drop-down field of the submission template. This data could also be added in the “Notes” field.
    - This can be added to SRA and linked to other NCBI records or in GenBank as a structured comment.
- Sequencing platform
    - Illumina, Oxford Nanopore, etc.
    - This can be added to SRA and linked to other NCBI records or in GenBank as a structured comment.

SeqSender
CDC provides a command line tool called SeqSender that prepares submissions of sequences, metadata, and sequence reads for NCBI’s GenBank, Biosample and SRA in a single operation.