Influenza Testing in Public Health Laboratories
Referral chart for WHAT Specimens and Viruses to send and WHERE to send them

Prepared September 22, 2010, replaces chart from November 2009

Please contact APHL at fluquestions@aphl.org if you have questions about information in this document.

Category and Purpose	What and when to send	Where to send	Required Form	CDC Contact
Virus Surveillance ("Virus Isolation Project") To provide comprehensive analyses of currently circulating influenza virus strains for vaccine strain selection and ACIP recommendations. CDC's analyses include: antigenic characterization (HAI), genetic analysis (sequencing) and tests for sensitivity to FDA-approved drugs.	• *What to send?* Representative seasonal A(H1N1), A(H3N2), B and 2009 A/H1N1 influenza virus isolates, plus the corresponding original clinical specimen (if available). **Note:** PHL's are encouraged to culture a subset of PCR positives if at all possible. • *How many and when to send?* Once every other week, up to **5** representative recent virus isolates, plus the corresponding original clinical specimens.	Virus Surveillance specimens should be sent to the State PHLs in **CA, IA or UT** depending on your designated region [1] • Do NOT send these viruses directly to CDC. **Note:** The 3 designated State PHLs are under APHL contract to grow large quantities of the virus isolates so that HAI and further analyses can be done more efficiently.	"The Influenza Specimens Submission Form" (can be accessed by clicking HERE). Please read the "Instructions for Filling-out the form" carefully.	Alexander Klimov, Ph.D. Chief, Virus Surveillance and Diagnosis Branch (VSDB), Influenza Division (ID), CDC Phone: 404-639-3387 Fax: 404-639-0080 Xiyan Xu, MD. Team Leader, Virus Surveillance Team, VSDB/ID Phone: 404-639-1657 Fax: 404-639-0080

[1] Designated Region. All the public health laboratories in the U.S. have been divided into 3 regions based on geographic locations. Where to ship specimens depends on your region AND on whether or not the samples are for **Virus Surveillance** or for **Antiviral Resistance Surveillance.** Please, be aware that the regional APHL contracted State Public Health Laboratories that you will send specimens to MAY BE DIFFERENT for Virus Surveillance versus Antiviral Resistance surveillance.

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Unsubtypables: Influenza A positive respiratory samples that cannot be subtyped using CDC rRT-PCR subtyping procedures A specimen is considered unsubtypable when the rRT-PCR result is positive for Inf A and negative for seasonal H1, 2009 A/H1N1 and H3. **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.	• Unsubtypable specimens should be retested using CDC's protocols as specified in the package inserts. • *What to send?* Original clinical specimens, IF (after retesting) specimens are **positive** for InfA (**Ct <35) but negative** with seasonal H1, 2009 A/H1 and H3 influenza targets. • *When to send?* As soon as possible.	CDC, Influenza Division **Note:** Please clearly label these specimens as "Influenza A, unsubtypable"	"The Influenza Specimens Submission Form" (can be accessed by clicking HERE.) Please read the "Instructions for Filling-out the form" carefully **Note: Please indicate specimen type on the WHO form in "Passage History" field.**	Stephen Lindstrom, Ph.D. Team Leader, Diagnostic Development Team VSDB/ID Phone: 404-639-1587 Fax: 404-639-0080

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Inconclusives: Specimens with inconclusive results **Note:** A specimen is only positive for influenza 2009 A/H1N1 if **all three** targets (InfA, pdm InfA and pdm H1) are positive in the CDC rRT-PCR 2009 A(H1N1) Flu Panel. A result is **inconclusive** if the test is positive for one of two pandemic 2009 A/H1N1 markers. Inconclusive test results with **high InfA Ct values (>35)** most likely indicate that the specimen contains a low virus titer near the limit of detection of the assay. **Note:** Increasing numbers of inconclusive test results may represent a problem with the performance of the primers and probes, a change in the virus, or a problem in your individual lab.	**Repeat testing** should be done using the CDC protocol as specified in the package insert. _____ 1. If, upon repeat testing, the specimen test results are still inconclusive with high InfA Ct values (>35), the sample should be **reported** as inconclusive. (You may report to your sender that the subtype could not be determined due to low viral titer). _____ 2. If, upon repeat testing, the test results are inconclusive for influenza 2009 A/H1N1 with strong InfA Ct values (<35), these should be sent to CDC for verification	CDC, Influenza Division **Note:** Please submit these specimens as "inconclusive for influenza 2009 A/H1N1."	"The Influenza Specimens Submission Form" (can be accessed by clicking HERE.) Please read the "Instructions for Filling-out the form" carefully **Note: Please indicate specimen type on the WHO form in "Passage History" field.**	Stephen Lindstrom, Ph.D. Team Leader, Diagnostic Development Team VSDB/ID Phone: 404-639-1587 Fax: 404-639-0080

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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 in the CDC rRT-PCR Flu Panel. A result is **inconclusive** for A/H5 if the test is positive for InfA as well as for one of two H5 markers.	• All samples that test presumptively positive or inconclusive influenza A/H5 Repeat testing should be performed on all samples that are inconclusive for influenza A/H5 using CDC's protocol as specified in the package insert. • *What to send?* Original clinical specimens, IF (after retesting) specimens are **positive** for InfA **and either or both H5a and H5b targets**. • *When to send?* As soon as possible. • *When to notify?* Notify CDC influenza Division immediately upon verification of presutive positive or inconclusive results for influenza A/H5	CDC, Influenza Division **Note:** Please submit these specimens as "presumptively positive for influenza A/H5" or "inconclusive for influenza A/H5" as appropriate	"The Influenza Specimens Submission Form" (can be accessed by clicking HERE.) Please read the "Instructions for Filling-out the form" carefully **Note: Please indicate specimen type on the WHO form in "Passage History" field.**	Stephen Lindstrom, Ph.D. Team Leader, Diagnostic Development Team VSDB/ID Phone: 404-639-1587 Fax: 404-639-0080

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Antiviral Resistance For Surveillance ("Pyrosequencing Project") To identify oseltamivir resistance in influenza 2009 A/H1N1 viruses caused by H275Y mutation in the neuraminidase. The regional PHLs will perform pyrosequencing. CDC performs confirmatory testing for antiviral resistance.	• *What to send?* influenza 2009 A/H1N1 original clinical materials only. • *How many and when to send?* Up to 5 every other week of those specimens confirmed by rRT-PCR to be positive for influenza 2009 A/H1N1 using the CDC reagent kit. Note[2] Exception [3] • *Qualifier.* Very important to submit strongly positive specimens (Inf A Ct values of 29 or less) for H275Y pyrosequencing.	Specimens to be sent September 2010: Depending on your region, send to the **CA, NY**, or **WI** Public Health Laboratory Do not send these directly to CDC unless testing is requested for clinical care (see below) **Note:** The 3 designated State PHLs are under APHL contract to perform antiviral resistance screening for H275Y mutation by pyrosequencing. These laboratories may be different from Virus Surveillance contract laboratories	"The Influenza Specimens Submission Form for Antiviral Resistance Surveillance Screening" (can be accessed by clicking here) Form should also be sent to the regional lab by email. Please read the "Instructions for Filling-out the form" carefully.	Larisa Gubareva, MD, Ph.D. Team Leader, Molecular Epidemiology Team VSDB/ID Phone: 404-639-3204 Fax: 404-639-0080
Antiviral resistance testing for clinical care • Antiviral resistance testing may be requested for patients that are not responding to antiviral treatment or who developed infection while on chemoprophylaxis. The testing information should be used for clinical care decisions.	• Clinicians can contact the state laboratory and CDC (*contact person/email pending)* to determine whether the patient specimen may be submitted for AVR testing . *Instructions are pending.* • Clinical specimen can be submitted for testing 2009 A/H1N1 viruses and results may be returned within 48 hours. Virus isolates are necessary for testing A(H3N2) and type B viruses and testing could take one week or longer.	• *Information pending*	• *Information pending*	Alicia Fry, MD, MPH Med Epidemiologist, Epidemiology and Prevention Branch, ID Phone 404 639-2680 Bb; 404 422-3155

[2] Note: Send 5 specimens to the regional laboratory even if your lab is performing pyrosequencing in-house.

[3] Exception. Laboratories having fewer than 10 confirmed positives of 2009 A/H1N1 specimens each week are advised to send half of their total number of positive specimens for Virus Surveillance and half for Antiviral Surveillance.