

## Laboratory Testing

**Laboratory Testing Requirements:** Detect, type, subtype, and characterize influenza viruses from clinical specimens in a timely manner using reliable laboratory methods.

1. Utilize molecular detection and subtyping methods (e.g., rRT-PCR) for influenza virologic surveillance.
2. Maintain instrumentation, personnel, expertise and adequate capacity to test the volume of specimens needed to achieve national, state and local surveillance objectives.
3. Ensure that staff members are knowledgeable in general principles of virology, molecular biology and surveillance, as well as appropriate specimen collection, handling, and transport methods.
4. Notify CDC immediately and ship unsubtypeable influenza A viruses to CDC within 24 hours of detection to rule-out novel viruses.
5. Routinely refer a representative subset of specimens (and viruses) to CDC or CDC- designated laboratory for genetic and antigenic characterization.
6. Maintain capability to rapidly adopt new molecular test methods or test modifications if a new influenza virus with pandemic potential emerges or when new technology provides improvements to virologic surveillance.
7. Maintain additional influenza testing capabilities (as defined in this document) as appropriate for the jurisdiction, or utilize shared services models to ensure access to testing.
8. CDC: Identify, characterize, and rapidly conduct risk assessments of emerging novel influenza viruses; develop, deploy and evaluate CDC assays to assure optimum performance; utilize sequencing methods; evaluate new technologies; and develop technical standards and guidance for virologic surveillance.

### Requirement Intent

Influenza virologic surveillance, by definition, requires laboratories with the capability and capacity to detect, type, subtype and characterize circulating and emerging viruses. The introduction and widespread adoption of molecular methods has reduced the need to maintain classic virologic capabilities in every PHL. The essential components of laboratory testing described below takes into consideration the role of new technologies, the changing landscape of virology expertise in PHLs and the expected availability of national, state and local fiscal resources.

On the basis of a stakeholder assessment, the roadmap classifies virologic testing components into 1) **primary testing**: requirements that should be maintained and available at all PHLs involved in influenza surveillance, or 2) **additional testing**: additional surveillance testing capabilities that may be maintained based on jurisdictional needs and resources, or provided through a shared services model.

## Primary Testing Method

PHLs performing virologic surveillance are expected to utilize molecular methods, such as rRT-PCR, as the primary method for influenza detection and subtyping. This is an ELC benchmark. Influenza rRT-PCR testing provides rapid, sensitive and accurate detection and identification of influenza viruses for routine influenza surveillance, outbreak detection and pandemic response. The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (CDC Flu rRT-PCR Dx Panel) is an FDA-cleared *in vitro* diagnostic assay that is manufactured and distributed by CDC to all qualified state and local PHLs engaged in influenza virologic surveillance testing. Although PHLs have the option to use commercial rRT-PCR assays, there are specific benefits to utilizing the CDC assay and CDC supplied reagents. This is a nationally recognized reference method and allows for standardization of influenza testing across all PHLs. The assay detects current influenza strains, is continually assessed and updated as needed to detect strain variations, and should detect novel viruses. The assay also allows for higher throughput testing algorithms to support outbreak and pandemic response. This method is consistent with the laboratory workforce's increasing proficiency in molecular testing methods. However, all PHLs engaged in influenza surveillance should also have staff knowledgeable in general principles of virology and surveillance, and appropriate specimen collection, handling, and transport methods.

## Additional Testing Methods

There are additional testing methods that may be used to support influenza virologic surveillance. These include influenza virus culture, antiviral resistance testing of influenza viruses, influenza hemagglutination inhibition (HAI), immunofluorescence identification of influenza viruses and serology testing. Each of these methods has distinct purposes, advantages and disadvantages for both national and state surveillance. The determination to use any of these methods in PHLs should be made based on state and jurisdictional needs, detailed cost analysis, and identification of sustainable funding source (see Financial Resources Requirements Intent and Implementation Guidance sections).

Virus isolation has the advantage of producing quantities of virus sufficient for full antigenic characterization for determining vaccine match and conducting antiviral resistance testing.<sup>5</sup> However, influenza virus culture is less sensitive and specific than rRT-PCR, and there are vast variations in the sensitivity of different cell culture lines, the growth characteristics of influenza virus strains and PHL practices and expertise. In addition, influenza virus culture is less rapid than influenza rRT-PCR and less adaptable to sudden surges in specimen numbers. Influenza virus culture must be maintained at CDC and a subset of PHLs.

Antiviral resistance testing is necessary to monitor the presence and level of antiviral resistance in circulating influenza viruses. These data inform patient management and treatment recommendations as well as national antiviral stockpile policies. Definitive antiviral resistance testing requires both phenotypic resistance testing, using a neuraminidase inhibition (NAI) assay (requires cultured viruses), and detection of genetic changes (drug resistance markers) by pyrosequencing and/or sequencing. Both of these test methods are available at CDC and a subset of PHLs. Performing pyrosequencing at a subset of PHLs provides a cost-effective and efficient approach to expanded surveillance screening. Supporting a limited number of testing sites allows for efficient updates to methods for viral mutations and training to develop the extensive expertise required to perform and interpret the test results and limits costs.

Influenza hemagglutination inhibition (HAI) testing remains a cornerstone of antigenic characterization for influenza vaccine strain selection. CDC maintains HAI expertise for antigenic characterization of

influenza virus culture isolates using specialized antisera. HAI tests using the WHO kit reagents that are provided to PHLs can be used to determine influenza type and influenza A subtype, but are not a reliable indicator of influenza strain or strain changes. Results of influenza HAI tests using WHO kit reagents may be over-interpreted as indicators of vaccine effectiveness and circulating strains. In addition, influenza HAI testing requires frequent practice to maintain expertise and proficiency in test performance and result interpretation.

Immunofluorescent antibody identification of influenza viruses is used in some PHLs for confirmation and identification of influenza viruses that have grown in cell cultures. Immunofluorescence (IF) testing is not as specific as rRT-PCR testing and requires a fluorescent microscope and staff with specialized expertise. However, influenza IF testing has the potential to identify novel strains of influenza grown in culture that evade detection by rRT-PCR.

Serologic testing is neither a routine surveillance nor diagnostic tool; it is currently used primarily by CDC or academic institutions for research purposes or retrospective seroprevalence studies. Testing requires staff with a high level of expertise and specialized reagents, there are currently no FDA cleared influenza serology tests.

Rapid influenza diagnostic tests (RIDTs) for the detection and identification of influenza viruses are used by many sentinel surveillance providers, other primary care sites, emergency departments as well as physician office and clinical laboratories. However, these tests are significantly less sensitive and specific than molecular assays.<sup>6,7</sup> RIDTs may also be less reliable when new virus strains emerge. If PHLs choose to use RIDTs to serve diagnostic needs for selected patient populations, they should be aware of the limitations of these tests (e.g., the considerable variability of the positive and negative predictive values depending upon the prevalence of influenza in the community) and should follow guidelines provided by CDC (currently at [www.cdc.gov/flu/professionals/diagnosis/rapidlab.htm](http://www.cdc.gov/flu/professionals/diagnosis/rapidlab.htm)). If an important clinical decision will be affected by either a positive or negative rapid test result, the rapid test result should be confirmed by another test, such as rRT-PCR. Even if the PHL does not perform RIDTs, virology staff should maintain awareness of the performance characteristics of currently available RIDTs that may be used within their jurisdiction in order to provide seasonal guidance to clinicians, clinical laboratories and surveillance coordinators. PHLs or surveillance coordinators may also collect data from clinical sites using RIDTs to provide additional seasonal situational awareness data.

Direct specimen immunofluorescence using a direct fluorescent antibody method (DFA) involves testing clinical material taken directly from the patient and using fluorescent-labeled antibodies to detect influenza antigens that may be present. DFA can be performed quickly but is not as accurate as rRT-PCR testing and requires significant expertise and expensive fluorescent microscopes. In addition, results can be very subjective and are dependent on the individual reader's expertise.

Expanded testing for other respiratory viruses using molecular respiratory virus panels (RVPs) has become common in many clinical laboratories and is increasingly used in PHLs to provide jurisdictional and national information about circulating viruses that are associated with acute respiratory illness. While detection and identification of non-influenza respiratory viruses is not a component of national influenza virologic surveillance, data from these assays can aid in identifying the cause of non-influenza community illnesses or outbreaks. If surveillance for other respiratory viruses is performed to meet jurisdictional needs and resources are available, it is recommended that PHLs consider adopting molecular RVPs to replace less sensitive viral culture.