IMPLEMENTATION GUIDANCE

Influenza virologic surveillance is an essential function of all state health departments and requires partnership between the state PHL representatives, epidemiologists and influenza surveillance coordinators. At a minimum, virologic surveillance includes the ability to:

- Access a representative sample of clinical specimens from ILINet (ILI network) providers or other clinical primary care sources and clinical laboratories.

- Detect type and subtype influenza viruses from clinical specimens in a timely manner using standard laboratory methods.

- Report results to providers, epidemiologists, and CDC using standard electronic data systems.

- Rapidly refer unsubtypable influenza viruses to CDC to identify or rule out novel viruses.

- Routinely refer a subset of specimens (and viruses) to CDC or a CDC-designated laboratory for genetic and antigenic characterization and antiviral testing.

- Maintain the expertise, warm base (a minimum level of readiness or capacity), and surge capabilities necessary for pandemic response.

Detailed descriptions of the essential components for virologic surveillance were presented in the Requirements Intent section. In this section, suggestions and model practices are provided to assist state and local PH directors and laboratorians, epidemiologists and influenza surveillance coordinators operationalize the requirements. The model practices provided are based on experience with state virologic surveillance since the system's inception and expansion in the late 1990's, a series of right size project stakeholder meetings, a table-top exercise conducted in December 2012 testing the utility of the roadmap recommendations, and the data gathered through pilot projects conducted in four states during the 2012-2013 influenza season.

IMPLEMENTATION GUIDANCE

Sampling

A virologic surveillance sampling strategy should be implemented that will ensure year round access to an adequate number of representative clinical specimens to meet key surveillance objectives. Specimens should be obtained from ILINet providers and/or other clinical primary care sources and clinical laboratories. Feasibility and representativeness are the most important factors to consider when choosing specimen providers.

As discussed in the Sampling Requirements Intent section, the virologic surveillance landscape can be organized into five tiers based on where sampling and testing is performed. The five tiers of influenza surveillance are outlined in the Process Model in Appendix A. Since specimens are primarily obtained in the first tier and passed to subsequent tiers for testing, a sampling process takes place at each transfer point. The variation in sampling criteria throughout this sequential process complicates extrapolating the data from one testing tier and applying it to the population of another tier. The variability in sampling can greatly challenge national surveillance efforts where the data is aggregated from multiple states. The Roadmap sampling requirements are intended to apply a more consistent, standardized collection/sampling process to improve overall data confidence and representativeness.

State and Local Implementation Steps

1. **Establish a specimen provider network**

   A. ILINet or other specimen providers (Tier 1)

   - The primary care tier provides data and specimens for the influenza surveillance system. Specimen submitters may be ILINet sites or other primary care health care providers. The selected specimen submitters should be committed to collecting high quality specimens, and submitting the required number of samples in a timely manner and in accordance with jurisdictional criteria throughout the entire year. A number of states report that ILINet sites are generally not a reliable source of specimens, so alternate outpatient primary care sites have been recruited as specimen submitters.

   - Specimen provider recruitment and submission criteria should be established so that specimens submitted for virologic surveillance are representative of the diversity of the population as a whole or of specific targeted population as needed. The collective group of selected specimen providers should cover all age groups.

   - ILINet and other primary care health care providers may elect to test specimens using a point of care RIDT if one is available in their clinical setting. However, unscreened specimens are preferred for routine seasonal surveillance. If primary care submitters are using RIDT's for diagnostic purposes, a random mix of specimens, irrespective of the test result, should be sent to the PHL for surveillance purposes. This provides a better assessment of true positivity in the community and reduces potential bias introduced by screening with tests that have variable sensitivity and may not detect novel or drifted viruses (i.e., give false negative result).[8] Outside of influenza season, participating providers and clinical laboratories should submit all

RIDT positives to the PHL, in addition to unscreened samples from a subset of ILI patients from ILINet or other outpatient providers. In a rare/novel influenza event investigation, oversampling screened positives may be appropriate if the tests used are high performing with demonstrated reliability for detection of the virus of interest.

RIDT data and specimens contribute to Influenza Surveillance
The Iowa statewide influenza surveillance program collects data on the number of RIDTs performed and the percentage positive each week using a survey monkey tool. Additionally, during times of low prevalence, laboratories submit rapid test positive specimens to the State Hygienic Laboratory (SHL) for confirmatory testing using the CDC's real-time RT-PCR test panel. The RIDT survey data and the results of confirmatory testing are incorporated into the weekly influenza surveillance report compiled by the Iowa Department of Public Health. This report is widely distributed to public health officials, infection control practitioners, heath care providers and others to improve awareness about seasonal influenza activity and reliability of RIDT results.

- Provider compliance with specimen submission criteria may be enhanced by providing:
  - Clear instructions and submission forms customized for their site,
  - Cost-free specimen collection kits and shipping,
  - Guidance for optimum specimen collection,
  - Feedback and data to submitters, including influenza test results and/or aggregate results of testing for other respiratory pathogens if performed,
  - No cost training,
  - Certificates of recognition,
  - RIDT kits to incentivize specimen submission.

B. Clinical laboratory providers (Tier 2)
   In addition to the ILI/primary care provider network, virologic surveillance should include specimens from hospital/clinical laboratories to ensure that a subset of specimens represent more severe illness (inpatients, mortality, unusual cases) and outbreak sources. Many clinical laboratories also serve as reference laboratories for outpatient satellite clinics, and therefore may be a good source of ILI specimens for routine surveillance. Clinical laboratories will also be essential partners when responding to large scale outbreaks or a pandemic. The influenza surveillance coordinator, in collaboration with the PHL, should develop and disseminate policies and establish mechanisms to ensure submission of a subset of positive specimens and all unsubtypable influenza positives (if subtyping assays are used) from hospital/clinical laboratories performing influenza testing. If clinical laboratories are the primary resource for surveillance specimens, the specimens sent to the PHL may be overly representative of hospitalized patients (i.e., more severe cases). This may be mitigated by selecting sites that can provide specimens from both emergency room and inpatient settings and providing clear guidance on numbers and types of specimens to be submitted. Specimens from clinical laboratories should include both influenza positive and negative samples when possible.

SAMPLING

PHL testing of negative specimens will be useful to monitor the performance of test methods used in clinical laboratories and enhance likelihood of identifying novel viruses that may not be detected by commercial influenza assays. If data from clinical laboratory testing are being used for situational awareness, at a minimum ensure that the data are coming from sites that are performing high quality testing, and using sensitive methods such as rRT-PCR.

C. PHLs (Tier 3):

All state along with some local PHLs make up the third tier of influenza surveillance. These laboratories typically perform rRT-PCR testing to type and subtype influenza viruses in clinical specimens. Every PHL participating in virologic surveillance is responsible for testing clinical specimens submitted for surveillance purposes or epidemiologic investigations, and reporting data to CDC in a timely manner. PHLs are also required to submit representative clinical specimens and/or virus isolates to CDC (Tier 5) or a CDC-designated laboratory (Tier 4) for national surveillance purposes, including annual vaccine virus selection. PHLs performing virus culture should send both the original clinical material and the virus isolate to CDC or a CDC-designated laboratory. Providing the virus isolate along with the original clinical material allows for more rapid antigenic characterization at CDC. Original clinical material is requested so that viruses with potential for use as vaccine candidates can be grown under FDA regulated conditions for use in vaccine manufacturing. For example, the 2013-2014 vaccine was manufactured using viruses obtained through PHL testing in California, Texas and Massachusetts.

Laboratories should submit specimens and virus isolates based on annual CDC criteria and guidance which is sent to state PHL Directors and disseminated by APHL. In collaboration with APHL and CSTE, CDC also convenes teleconferences before and throughout the season as needed to update surveillance guidance. Participation in these teleconferences is strongly encouraged.

To enhance CDC's vaccine virus selection efforts, it is important to send recently collected specimens. Specimens submitted to CDC should be representative of the circulating influenza types/subtypes, geography, disease severity and age. Oversampling of low prevalence subtypes may be necessary to ensure that all circulating subtypes are represented in the samples sent to CDC. When available, viruses from particularly severe or unusual cases, and a sample of viruses isolated from outbreak investigations should also be represented in submissions to CDC. Examples to assist laboratory staff in selecting specimens are included in the Laboratory Testing Implementation Guidance section.

CDC may request additional viruses/specimens depending on circulating virus trends, vaccine virus selection and vaccine candidate development needs. CDC strongly recommends that PHLs subtype all, and at least 90%, of Influenza A positives. Unsubtypable[iv] viruses that may represent a novel subtype should be submitted to CDC within 24 hours of detection. These are ELC benchmarks.

[iv] Any influenza positive specimen that cannot be definitively typed and subtyped as a circulating seasonal influenza virus. Influenza positive specimens producing non-standard or inconclusive results as defined in the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Instructions for Use package insert.

2. **Determine appropriate sample sizes for each surveillance objective**

The need to characterize and improve the precision of the data that is provided through virologic surveillance was one of the principal drivers of the Right Size Influenza Virologic Surveillance Project. Implementing a statistical, systematic approach to determine the appropriate number of specimens to be collected and tested can be achieved by using sample size calculators. The calculators developed as right size virologic surveillance tools provide a statistical basis to estimate the number of specimens to be tested in order to provide a desired level of data confidence for situational awareness, rare/novel influenza event detection and rare/novel influenza event investigation (see Table 1). Conversely, these calculators can also be used to determine the confidence level of data derived from an existing sample of ILI patient specimens. These calculators were developed through input from CDC, APHL, state and local PHLs, epidemiology staff at stakeholder meetings, pilot sites and a tabletop exercise. In addition further development utilized academic researchers focusing on optimizing public health influenza surveillance.[22]

Table 1. Influenza Virologic Surveillance Right Size Objectives

Situational Awareness for Seasonal Influenza

Determine the beginning and end of the influenza season and monitor the prevalence and spread of influenza viruses throughout the year.

Rare/Novel Influenza Event Detection

Detect a rare/novel influenza virus among influenza positive surveillance specimens tested in all states at a low enough threshold for effective intervention and control measures. This objective relates to the initial detection of a rare/novel influenza virus which generally occurs as part of routine surveillance.

Rare/Novel Influenza Event Investigation

Determine the prevalence of the rare/novel influenza influenza virus (Rare+/Flu+) within a state following the initial detection of a rare/novel influenza influenza virus (i.e., "deep dive"); confirm that the prevalence of a rare event does not exceed a specific percent positivity. Investigation of a rare/novel influenza event is typically performed using enhanced, targeted surveillance.

Some state and local PHLs may need to test more or fewer specimens to achieve the same level of data confidence as another state or local jurisdiction with a larger or smaller population. Alternately, influenza surveillance coordinators may need to accept a lower confidence level or higher margin of error if the system does not have the capacity to collect or test the number of samples estimated by the calculators.

SAMPLING

Efficiency can be achieved using a sampling strategy that, where possible, provides sufficient specimens to address multiple surveillance objectives. For example, situational awareness and rare/novel influenza event detection rely on samples collected and tested for routine surveillance. At CDC, routine antiviral resistance surveillance testing currently uses the same samples submitted and tested for vaccine strain selection. Where differences are important, they should be addressed by the sampling strategy.

The sample size calculations are based on population size, desired level of confidence, margin of error and estimated or known prevalence or threshold for detection. More details on thresholds are provided in the Objectives: Thresholds and Representativeness section. State and local PHLs are encouraged to use sample size calculators or pre-calculated sample size tables (Appendix B) to achieve a more scientific, statistically based sample size that supports surveillance objectives. Sampling approaches should be established to prioritize collecting an adequate number of specimens for detection of rare/novel influenza events based on national thresholds, while at the same time providing sufficient number of representative specimens for overall situational awareness at the state level. For many states, the number of specimens to be tested during influenza season for each of these objectives is very similar. However, for smaller population states, the number of specimens necessary to achieve high confidence in situational awareness state level data will be much higher than the number of specimens needed to contribute to national rare/novel influenza event detection thresholds. Outside of influenza season, achieving a desired statistical confidence will not be possible in most states; therefore the focus of surveillance should shift to obtaining all specimens from clinical sites that have tested positive for influenza, or from patients with unusual respiratory illness, travel history or risk of exposure to animal-origin viruses.

Targeted surveillance may be useful to answer specific questions, especially when conducting an investigation if a rare/novel influenza event or new virus is detected. Therefore the surveillance program should have the capability to establish targeted surveillance of specific populations when needed. CDC will provide guidance to state epidemiologists and PHLs on the specific risk factors and need for enhanced surveillance (e.g., highly pathogenic avian influenza H5N1 risk factors, swine exposure). However, the current version of the rare/novel influenza event investigation calculator may not be useful in these situations, future editions of the Roadmap are expected to provide more options for targeted surveillance, addressing intentional and unintentional bias.

A. Calculator Inputs and Outputs:

The key variables in calculating sample size are described in Table 2. Understanding how these variables affect sample size and data confidence levels is important for generating valuable surveillance data.

Table 2. Key variables for calculating sample size.

Relationship to Sample Size	
Confidence Level	This is the amount of certainty that the true prevalence is equivalent to the estimated prevalence. As this value increases the sample size also increases.
Margin of Error	This is the amount of error that can be tolerated. A 2% error would mean that the calculated prevalence may be plus or minus 2% from the true answer. As this value decreases the sample size increases.
Population	This is the population under surveillance. For routine influenza surveillance, this is the number of people in the state with ILI. As the population size increases the sample size increases.
Medically Attended ILI (MA-ILI)	This is the population of individuals with ILI who seek medical care. This is the subset of the population available for surveillance testing. This number is determined based on estimates that each person in the US visits an emergency room or physician in an ambulatory care setting 2.5 times per year, and that the percentage of ILI outpatient visits is ILI is 2.2% at CDC ILINet Seasonal Baseline – this number can be changed throughout the season as needed.
Expected Prevalence	In the calculators, this is the prevalence that the PHL expects to calculate or the level of detection the PHL wishes to achieve. For the purposes of calculating sample size, the expected prevalence refers to the prevalence of influenza positive (Flu+) specimens among the number of MA-ILI specimens tested. This is NOT the prevalence of disease in the community.
	Note that as the expected prevalence decreases, the sample size becomes smaller when the margin of error is held constant. This seems counter intuitive, but when the margin of error is scaled to align with the expected prevalence, the sample size should increase. For instance, a 5% margin of error is more appropriate for a predicted prevalence of 50% than a predicted prevalence of 1%. A more appropriate margin of error for a predicted prevalence of 1% may be 0.5%. Thus, it is important to scale the margin of error to the predicted prevalence.

B. Choosing an appropriate threshold, confidence level and error rate:

The number of samples to be tested will vary depending on the confidence level, margin of error, threshold, and assumptions used in the calculators. Selecting a lower confidence level increases the amount of uncertainty in the calculated prevalence but permits a smaller sample size which may be necessary if resources are limited. A higher margin of error means that more error can be tolerated. With input from the exercise participants and the evaluation of data from previous influenza seasons, the stakeholders identified optimal, mid-range and minimal confidence levels and error rates or thresholds for the two objectives that comprise routine surveillance. The ultimate goal is to have all jurisdictions participating in virologic surveillance at the optimal levels defined here. However, to accommodate differences in state and local resources, including the ability to acquire specimens from healthcare providers, alternate mid-range and minimal levels are provided. Additionally, options to supplement unscreened MA-ILI specimens with screened influenza positive specimens are provided, this reduces the total number of specimens a PHL needs to test to achieve the recommended thresholds. (Note: This may increase the risk of missing a rare/novel influenza virus if the commercial tests used have decreased sensitivity to detect the new virus).

Table 3. Recommended confidence level, margins of error and thresholds.

	Situational Awareness		Rare/Novel Influenza event Detection			
			High Season		Low Season	
	Confidence Level (%)	Margin of Error (%)	Confidence Level (%)	Threshold (%)	Confidence Level (%)	Threshold (%)
Optimal	95	5	95	1/700	95	1/200
Mid-range	90	5	95	1/600	95	1/165
Minimum	85	5	95	1/500	95	1/143

These parameters can be used in the online calculators to determine sample sizes for each state or jurisdiction. Pre-calculated sample sizes for each of the objectives using these confidence levels are provided in Appendix B. Depending on the surveillance priorities and capacity of the system overall, jurisdictions may choose to use the on-line calculators to vary the inputs to see how sample size is impacted.

C. Using the Influenza Virologic Surveillance Right Size Sample Size Calculators:

The influenza virologic surveillance right size sample size calculators are available as a web-based tool at http://www.aphl.org/aphlprograms/infectious/influenza/Pages/Influenza-Virologic-Right-Size-Sample-Size-Calculators.aspx. Basic information on the intent of the calculator for each of the key surveillance objectives, along with inputs and output examples is provided below. As is typical with most models, the Sample Size Calculators rely on certain assumptions regarding the population, or the expected prevalence (positivity rate). The assumptions used in each of these calculators are based on existing and/or historical ambulatory care and seasonal influenza data and are described below.

A more detailed "user guide" with instructions for using the web-based tools is provided with the Sample Size Calculators. Appendix B also includes tables with pre-calculated sample sizes for situational awareness and rare/novel influenza event detection covering a range of population sizes. States may opt to use these tables for quick reference or as an alternative to the on-line tool.

i. **Situational Awareness for Seasonal Influenza**

- **Surveillance Objective:** Determine the beginning and end of the influenza season and monitor the prevalence and spread of influenza viruses throughout the year.

- **Threshold:** The beginning of the influenza season is defined as the time when the prevalence of specimens testing positive for an influenza virus (Flu+) among specimens collected from patients with MA-ILI is at or above 10% over two consecutive weeks. This value roughly corresponds to the CDC ILINet Seasonal Baseline where the percentage of outpatient visits for ILI reaches 2.2%. Jurisdictions may choose to alter the percent positive used in the sample size calculator to more accurately determine the amount of testing needed throughout the season or assess the confidence level of the data provided.

- **Surveillance Question:** How many specimens from MA-ILI patients does the laboratory need to test in a given period (usually one week) to determine that the prevalence of Flu+ specimens among MA-ILI persons tested is X% (e.g., 10%) at a specified confidence level and error rate?

- **Assumptions:**

   - Each person in the US visits an emergency room or ambulatory primary care physician 2.5 times per year and 2.2% of medical visits are for ILI outside of influenza season ILINet baseline.[23,9]

   - The providers are randomly selecting patients with ILI for surveillance testing.

   - The specimens tested were either unscreened or submitted randomly irrespective of test result.

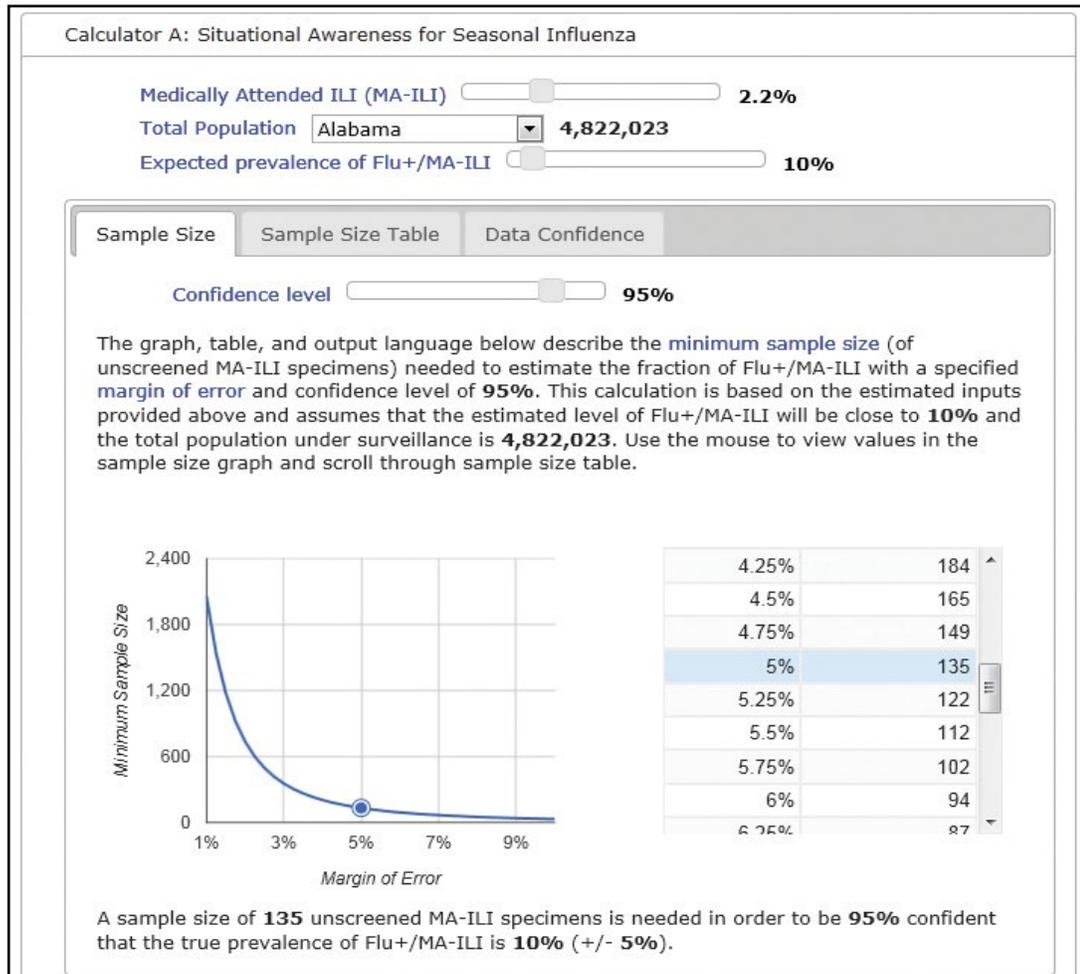   - Sampling is performed from a finite population (national or state level).

Figure 5. Screen shot of Situational Awareness sample size calculator demonstrating user inputs.

- **User Inputs (Figure 5):**

    ◦ **Total population:** The input is the total population under surveillance (e.g., state population). The calculator uses this number and the assumptions above to estimate the number of MA-ILI cases.

    ◦ **Estimated prevalence of MA-ILI:** Input based on ILINet data during the season. The default is 2.2%, which is the estimated ILINet seasonal baseline for the percentage of outpatient visits that are for ILI.

    ◦ **Expected prevalence of Flu+/MA-ILI:** Input the surveillance target. The default value is 10% for the beginning and end of the influenza season. Other percent positive values may be used based on jurisdictional preferences or seasonal variability in the prevalence of ILI or influenza.

SAMPLING

- ◦ **Confidence level:** The optimal level of for situational awareness is 95%, the minimum should be no less than 85%.

- ◦ **Margin of error:** An acceptable margin of error should be no greater than 5%.

- **Output example:** A sample size of 135 unscreened MA-ILI specimens is needed in order to be 95% confident that the true prevalence of Flu+/MA-ILI is 10% (+/- 5%).

- **Alternate calculation (sample power):** Determine the level of confidence and margin of error associated with the measured prevalence of influenza positives, given the sample size tested, i.e., what are the confidence and error rates associated with current sample size?

- **Alternate Output example:** If 100 MA-ILI specimens were tested and the estimated prevalence is 10%, the PHL can be 70 % (+/- 3%) confident that the true prevalence is 10%.

ii. **Detecting a Rare/Novel Influenza Event**

- **Surveillance Objective:** Detect a rare/novel influenza virus among influenza positive surveillance specimens tested in all states at a low enough threshold for effective intervention and control measures. This objective relates to the initial detection of a rare/novel influenza virus which generally occurs as part of routine surveillance. Investigation of a novel event after initial detection (the "deep-dive") is a separate objective and is discussed in more detail below.

- **National Threshold:** Different thresholds have been established for the high season (influenza positivity > 20%), and low season (influenza positivity < 20%). These thresholds represent achievable levels of detection based on review of virologic surveillance data from several recent influenza seasons.

  - ◦ **High Season:** 0.14% (1/700); one rare/novel influenza virus among 700 influenza virus positive specimens aggregated at the national surveillance level over a defined period. During influenza season sample sizes should be calculated based on weekly reporting to FluView. A minimum threshold of 0.2% (1/500) may be used for determining the sample size in states with limited testing capacity. Application of a less sensitive threshold for detection (e.g., below 1/500) would mean that more rare/novel influenza viruses are circulating prior to detection and would impair disease prevention and control efforts.

  - ◦ **Low season:** 0.5% (1/200); one rare/novel influenzal virus among 200 influenza virus positive specimens aggregated at the national surveillance level over a defined period. This approximates the prevalence at which the H1N1pdm2009 influenza virus was detected in April 2009. A minimum threshold of 0.6% (1/143) may be used for determining the sample size in states with limited testing capacity.

SAMPLING

- **Surveillance Question:** How many specimens does the PHL need to test to allow the national surveillance system to detect a novel virus at 0.14% prevalence with 95% confidence (aggregating testing data from all states)?

- **Assumptions:**

  - Specimens are collected randomly.

  - There is no correction for finite population size – this is a conservative assumption to prevent undersampling. Correcting for finite sample size requires accurately characterizing the surveillance population. In the case of a rare/novel influenza event, the size of the relevant population may be largely unknown. The sample size determined without correcting for a finite population size is always correct. If a sample size correction factor is improperly applied, the target population will be undersampled, resulting in an overestimate of the confidence level and underestimate of the error.

- **Options:** The rare/novel influenza event detection sample size calculation can be made based on a) the number of positives already identified as Flu+ by an RIDT or clinical laboratory or by the PHL, b) the number of MA-ILI specimens, or c) a combination of both. Although testing screened Flu+ specimens decreases the total number of specimens needed to meet the recommended threshold and confidence level, using only specimens that are screened Flu+ may reduce the sensitivity of the system to detect rare/novel influenza events because of the unknown sensitivity of commercial systems to detect novel or drifted viruses. Using a combination of Flu+ and MA-ILI specimens will moderate the potential loss in sensitivity, and allow PHLs with large populations to achieve statistical confidence with reasonable specimen numbers.
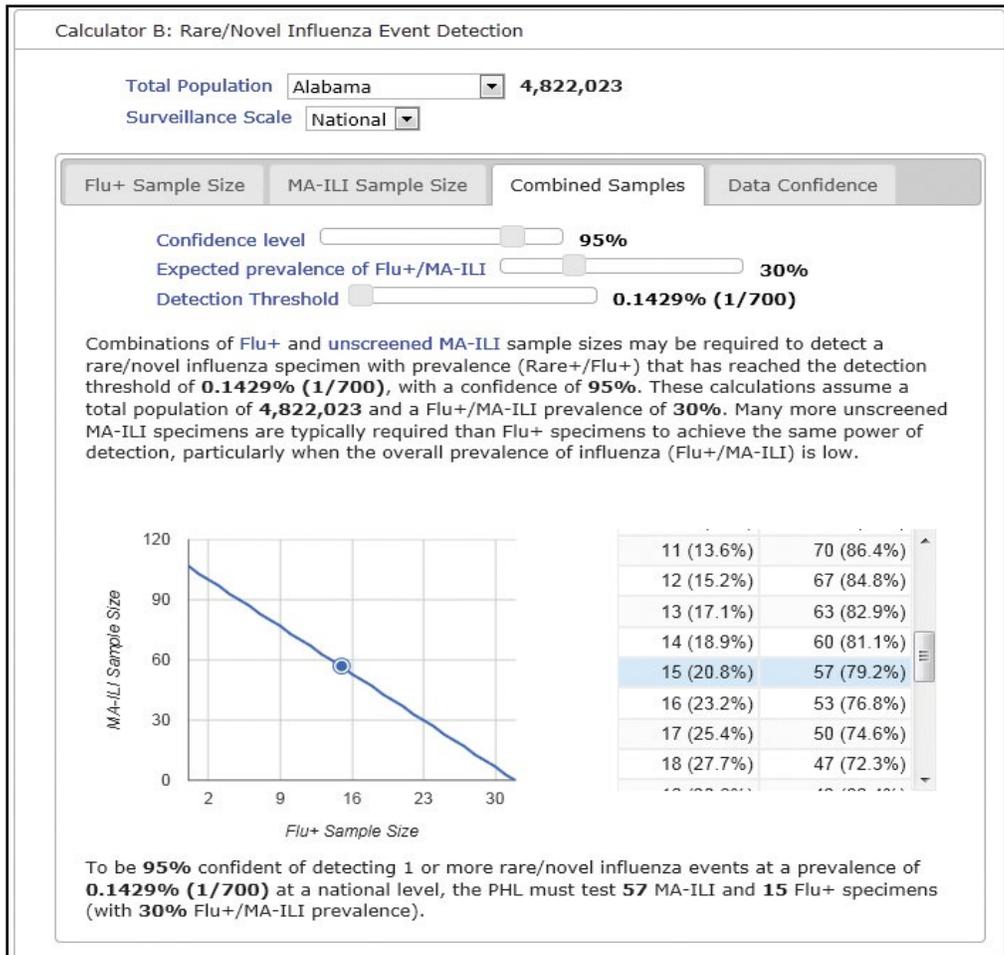
Figure 6. Screen shot of Novel Event Detection sample size calculator demonstrating user inputs.

- **User Inputs (Figure 6):**

  - **Total population:** The input is the total population under surveillance (e.g., state population). The calculator uses this number and the assumptions above to estimate the weeky number of MA-ILI cases.

  - **Surveillance scale:** The default is national, meaning that all states are contributing to a national surveillance effort proportional to their population size. The number of samples that a state PHL needs to test is apportioned based on population size. The calculator also provides the option for states to calculate the number of specimens to test for detection of a novel event at a specific threshold within their state, however, the sample size for an individual state at the same threshold (e.g., 1/200 or 1/700) will be significantly larger than that needed for the national threshold.

◦ **Confidence level:** The optimal level of confidence for rare/novel influenza event detection is 95%.

◦ **Expected prevalence of Flu+/MA-ILI:** This is an input when calculating the number of MA-ILI specimens needed, or the number of combined MA-ILI and Flu+ specimens. Use 10% positivity for seasonal baseline, and other percent positivity as needed throughout the year to reflect low or high season, or actual percent positivity. Pre-calculated sample size tables in Appendix B use 10% for low season and 30% for high season.

◦ **Detection threshold:** (Applicable for Rare/Novel Influenza Event Calculator, Combined Samples tab) The input is the desired detection prevalence of a rare influenza type among all influenza positive cases.

• **Output examples:**

◦ **Number of Flu+ specimens:** To be 95% confident of detecting 1 or more rare/novel influenza events at a prevalence of 1/700 at a national level, the PHL must test 32 Flu+ specimens.

◦ **Number of MA-ILI specimens:** To be 95% confident of detecting 1 or more rare/novel influenza events at a prevalence of 1/700 at a national level, the PHL must test 107 MA-ILI specimens.

◦ **Combined number of Flu+ and MA-ILI specimens:** To be 95% confident of detecting 1 or more rare/novel influenza events at a prevalence of 1/700 at a national level, the PHL must test 57 MA-ILI and 15 Flu+ specimens (using an estimated 80% MA-ILI and 20% Flu+ specimen type combination and 30% Flu+/MA-ILI prevalence).

◦ **Combined number of Flu+ and MA-ILI specimens (state level):** To be 95% confident of detecting 1 or more rare/novel influenza events at a prevalence of 1/700 (within the population under surveillance), the PHL must test 3809 MA-ILI and 952 Flu+ specimens (using an estimated 80% MA-ILI and 20% Flu+ specimen type combination and 30% Flu+/MA-ILI prevalence).

• **Alternate calculation (sample power):** Determine the level of confidence that a rare/novel influenza event can be detected at a given threshold, given the sample size tested.

◦ User Input: the number of Flu+ specimens tested and the number of MA-ILI specimens tested.

• **Alternate output:** If the laboratory tested 11 Flu+ and 26 MA-ILI specimens and estimated prevalence of Flu+/MA-ILI is 30%, the PHL can be 82% confident that the rare/novel influenza virus would be detected at a prevalence of 1/700.

iii. **Detecting/Monitoring Antiviral resistance**

• **Surveillance Objective:** Detect antiviral resistance virus among influenza positive surveillance specimens tested in all states at a low enough threshold for effective intervention and control measures.

- **National Threshold:** 5% prevalence of oseltamivir resistant viruses among positive specimens for each influenza A subtype or influenza B at the national level.

- **Surveillance Question:** How many of each influenza A subtype Flu+ or influenza B Flu+ specimens need to be tested for antiviral resistance to allow the national surveillance system to detect antiviral resistant viruses at or below a 5% prevalence with 95% confidence (aggregating testing data from all states)?

- Assumptions used in the calculator:

  - Specimens are collected randomly.

  - There is no correction for finite population size – this is a conservative assumption to prevent undersampling. Correcting for finite sample size requires accurately characterizing the surveillance population. In the case of a rare/novel influenza event, the size of the relevant population may be largely unknown. The sample size determined without correcting for a finite population size is always correct. If a sample size correction factor is improperly applied, the target population will be undersampled, resulting in an overestimate of the confidence level and underestimate of the error.

- **User Inputs:** The Flu+ tab of the rare/novel influenza event detection calculator can be used to determine sample size for this objective.

  - **Total population:** The input is the total population under surveillance (e.g., state population).

  - **Surveillance scale:** The default is national, representing the number of specimens that need to be tested by the state to detect antiviral resistance at a national aggregated threshold. The number of samples the state needs to test is apportioned based on population size. States wishing to calculate the number of specimens to test for detection of antiviral resistance at a specific threshold within their state can select their state, note that the sample size of an individual state will be significantly larger than that needed for the national threshold.

  - **Confidence level:** The optimal level of confidence for antiviral resistance is 95%, the minimum should be no less than 85%.

- **Output example**:

  - Number of Flu+ specimens: To be 95% confident of detecting antiviral resistant Influenza A H1N1pdm2009 viruses at a prevalence of 5% among influenza A H1N1pdm2009 positive specimens tested at the national level, the PHL must test or submit for antiviral resistance testing 1 Influenza A(H1N1) Flu+ specimens per week.

- **Alternate calculation (sample power)**: Determine the level of confidence that antiviral resistant viruses can be detected at a given threshold, given the sample size tested.

    ◦ **User Input:** The number of each influenza A subtype or influenza B Flu+ specimens tested, and the expected Flu+/MA-ILI specimens tested.

- **Alternate output**: If the CDC receives and tests 21 influenza A H1N1pdm2009 positive specimens, the national surveillance system can be 66% confident that antiviral resistant H1N1pdm2009 viruses would be detected at a prevalence of 5%.

iv. <u>**Rare/Novel Influenza Event Investigation**</u>

- **Surveillance Objective:** Determine the prevalence of the rare/novel influenza virus (Rare+/Flu+) within a state following the initial detection of a rare/novel influenza virus (i.e., "deep dive"); confirm that the prevalence of a rare/novel influenza event does not exceed a specific percent positivity. Investigation of a rare/novel influenza event is typically performed using enhanced, targeted surveillance.

- **Threshold:** There are no defined thresholds for rare/novel influenza event investigation, as specific situations and jurisdictional considerations may warrant different thresholds. Generally, investigations are undertaken to determine how much more of the rare/novel influenza virus is present in the community and identify source(s) of the new virus (i.e., animal-human, human-human). In general, if the rare/novel influenza event was detected at 1/700, the investigation threshold should be between 1-5%.

- **Surveillance Question:** Once a rare/novel influenza virus is detected, how many ILI specimens does the PHL need to test to determine that the true prevalence does not exceed a specified percent of Flu+ within the state or in the specific jurisdiction under investigation?

- **Assumptions:**

    ◦ Specimens are collected randomly. This is an assumption used in the calculator; however, in many rare/novel influenza event investigations targeted surveillance is applied based on the situation and appropriate epidemiologic criteria. Targeted surveillance intentionally biases the sample. Future iterations of the calculator will allow users to address bias in calculating sample sizes.

○ There is no correction for finite population size – this is a conservative assumption to prevent under sampling. Correcting for finite sample size requires accurately characterizing the surveillance population. In the case of a rare/novel influenza event investigation, the size of the relevant population may be largely unknown. The sample size determined without correcting for a finite population size is always correct. If a sample size correction factor is improperly applied, the target population will be under-sampled, resulting in an overestimate of the confidence level and underestimate of the error.

○ This calculator would be most relevant in a 2009 H1N1-like event, where the at-risk population group is unknown and a significant public health investigation for cases has been initiated. This assumption, however, results in very high sample sizes. This calculator may not be appropriate when targeted surveillance is a more efficient initial approach, such as the 2012 H3N2v summer surveillance scenario targeting visitors to state/county fairs.
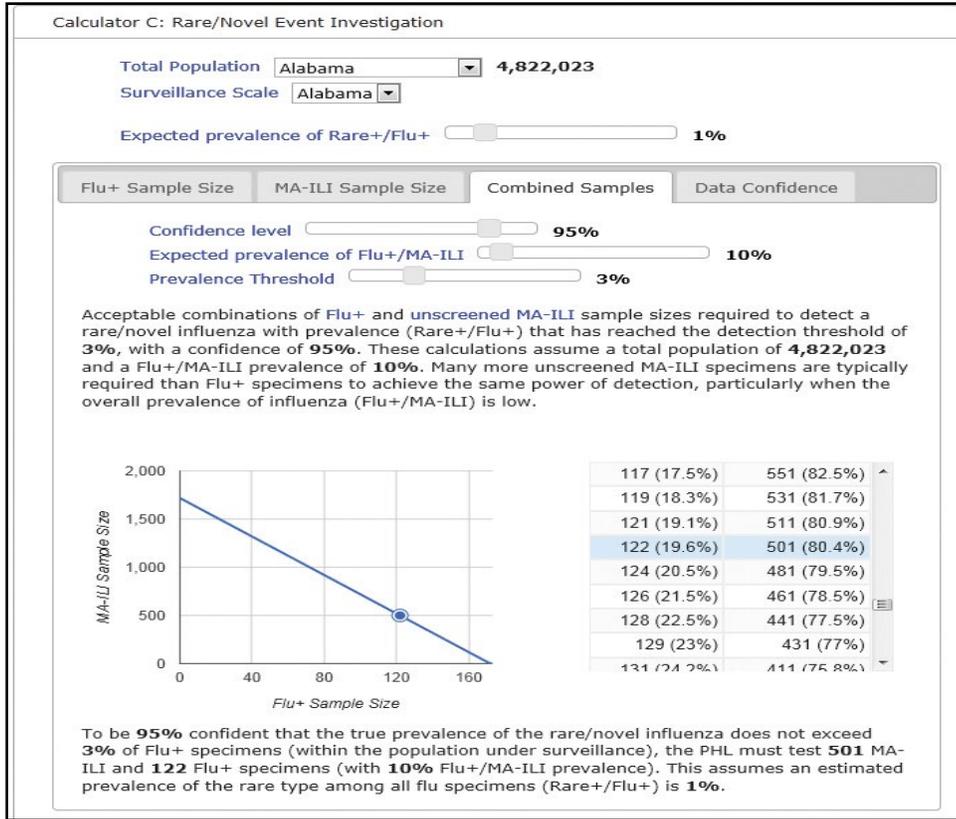
○ Asymmetrical distribution.

Figure 7. Screen shot of Rare/Novel Influenza Event Investigation sample size calculator demonstrating user inputs.

- **User Inputs (Figure 7):**

  - **Total population:** The input is the total population under surveillance (e.g., state population). The calculator uses this number and the assumptions above to estimate the weekly number of MA-ILI cases.

  - **Surveillance scale:** State vs national. The default is state because the investigation of the rare/novel influenza event would usually occur locally.

  - **Expected prevalence of Rare+/Flu+:** This is the percent positivity of the rare/novel influenza event that the PHL wants to confirm has not been exceeded.

  - **Confidence level:** The optimal level of confidence is 95%, the minimum should be no less than 85%.

  - **Expected prevalence of Flu+/MA-ILI:** This is an input only for when calculating the number of MA-ILI needed, or the number of combined MA-ILI and Flu+ specimens. Use 10% positivity for seasonal baseline, other percent positivity as needed throughout the year.

- ○ **Prevalence threshold:** (Applicable for Rare/Novel Influenza Event Investigation Calculator, Combined Samples tab) This is an input for the prevalence of the rare/novel influenza type which is expected to be established with a specified level of confidence.

- **Output examples**:

  - ○ **Number of Flu+ specimens:** To be 95% confident that the actual prevalence of the rare/novel influenza virus does not exceed 1% (+/- 2%) of Flu+ specimens, the PHL must test 173 Flu+ specimens.

  - ○ **Number of MA-ILI specimens:** To be 95% confident that the actual prevalence of the rare/novel influenza virus does not exceed 1% (+/- 2%) of Flu+ specimens, the PHL must test 1721 MA-ILI specimens.

  - ○ **Combined number of Flu+ and MA-ILI specimens:** To be 95% confident that the true prevalence of the rare/novel influenza virus does not exceed 1% (+/- 2%) of Flu+ specimens (within the population under surveillance), the PHL must test 501 MA-ILI and 122 Flu+ specimens (using an estimated 80% MA-ILI and 20% Flu+ specimen type combination and 10% Flu+/MA-ILI prevalence).

- **Alternate calculation**: Determine the level of confidence given the sample size tested.

- **Alternate output**: If a combination of 75 Flu+ specimens and 300 unscreened MA-ILI specimens were tested, and the estimated prevalence of the rare/novel influenza virus among all influenza positive specimens (Rare+/Flu+) is 1%, the PHL can be 75% confident that the true prevalence does not exceed 2.04 (+/-1%). (This assumes that 10% of MA-ILI patients are Flu+).

3. **Establish policy for frequency of submissions**

    A. **Primary Care and clinical laboratory specimen submissions to PHL**

    The frequency of specimen submission for routine surveillance will vary depending on jurisdictional needs, and PHL capacity for specimen intake and processing. During influenza season it may be most convenient to ask providers to send specimens from the first few ILI patients they see each week. If the PHL prefers to receive specimens throughout the week, each provider may be asked to collect and send specimens on a different day. Specimens/viruses need to be submitted and tested in real time, not batched, in order to inform timely clinical management guidelines and ensure rapid detection of novel viruses. If specimens are being sent to the PHL for diagnostic testing (e.g., patient with high risk travel history, or unusual case presentation), these specimens should be transported promptly and not batched with surveillance specimens. Clinical laboratories that perform PCR testing with subtyping should immediately submit any specimens that produce unsubtypable test results to the PHL. Clinical laboratories should be notified of the most recent epidemiologic criteria for a potential rare/novel influenza event.

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B. **PHL Submission to CDC or a CDC-Designated Laboratory**

PHLS should submit specimens/viruses for routine surveillance year-round based on annual CDC criteria and guidance provided to state PHL Directors and disseminated by APHL. Routine surveillance specimens should be forwarded to CDC or a CDC-designated laboratory in a timely manner to provide real-time surveillance information. Ship routine surveillance specimens at least once every two weeks, this ensures that CDC can perform further characterization in time to guide international and domestic annual vaccine virus selection. Unsubtypable specimens, as defined in the RT-PCR package insert, require immediate action as they may reflect a novel virus with pandemic potential. These specimens are to be sent immediately to CDC for more comprehensive testing to ensure that appropriate interventions can be implemented if needed, and that CDC meets WHO international health regulations[v] for novel virus reporting.[24]

4. **Ensure samples are of acceptable quality**

Influenza surveillance coordinators and PHLs should provide instructions and training to specimen submitters to ensure that respiratory specimens are of high quality, properly collected, stored and transported.

A. **Specimen collection**

Respiratory tract specimens required for influenza diagnosis and identification are well-defined and include nasopharyngeal swabs and throat swabs, submitted separately or combined, nasopharyngeal aspirates, nasal washes, bronchoalveolar lavages, tracheal aspirates, bronchial washes and, following autopsy, respiratory tract tissues. The most appropriate specimen to collect depends upon the diagnostic test employed. This information will be provided by the test or reagent manufacturer and the laboratory performing the test. Additional resources can be found in clinical microbiology textbooks, and at the CDC website www.cdc.gov/flu/professionals/diagnosis/index.htm.

Diagnostic test results are only as good as the quality of the specimen. Specimen quality depends on proper collection technique and the amount of virus present at the source. The amount of virus shed in the upper respiratory tract declines over the course of the illness; therefore collecting specimens as close to symptom onset as possible is recommended. Optimally, specimens for virologic surveillance should be collected within 24-72 hours of symptom onset and no later than 5 days post onset of symptoms.

Specimen providers need to be trained in proper collection technique. It is ultimately the responsibility of the laboratory to ensure that specimens are properly collected. Descriptions of proper methods for specimen collection can be found in clinical textbooks, in product inserts and online. The most effective method, however, is demonstration by someone skilled in the

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[v] IHR Regulations: http://www.who.int/ihr/en/. State Parties to the IHR (2005) are required to immediately notify WHO of any laboratory confirmed case of a recent human infection caused by an influenza A virus with the potential to cause a pandemic. An influenza A virus is considered to have the potential to cause a pandemic if the virus has demonstrated the capacity to infect a human and if the hemagglutinin gene (or protein) is not a variant or mutated form of those, i.e., A/H1 or A/H3, circulating widely in the human population.

collection technique, followed by practice under observation. The Joint Commission Strategies for Improving Rapid Influenza Testing in Ambulatory Settings (SIRAS) website www.jointcommission.org/siras.aspx offers two free on-line courses, one for health care providers in ambulatory settings and one for specimen collectors.

B. **Specimen Handling**

Specimen quality also depends on proper handling of the specimen after collection. The laboratory, in coordination with the influenza surveillance coordinator, is responsible for providing information on proper specimen handling to specimen providers.

Specimens should be placed immediately into an acceptable viral transport medium in accordance with standard testing protocols or kit manufacturer recommendations and held at 2-8°C until testing is performed. Testing ideally should be performed as soon as possible. If a delay of more than 72 hours until specimens are tested is anticipated, specimens can be frozen at -70°C. However multiple freezing and thawing of specimens can adversely affect the test result and should be avoided whenever possible. Virus isolates and nucleic acid extracts also require special handling.

5. **Establish and support specimen transport systems**

Specimen transport is another critical component of influenza virologic surveillance. Specimen integrity must be maintained during transit. An effective and efficient process for specimen submission must account for the reliable and timely transport of specimens from clinical sites (providers) and clinical laboratories to the PHL and from the PHL to CDC or CDC-designated laboratories. Specimen transport must compy with US Department of Transportation and International Air Transport Association (IATA) regulations to ensure that specimens and infectious materials are properly packaged and safely shipped.[25,26] Timely and efficient transport of specimens is often quite costly, and must be adequately funded by the public health system for effective surveillance. Specimen collection and regulation compliant transport supplies, as well as courier/carrier costs, need to be covered. Providers and clinical laboratories should not be expected to assume these costs for routine surveillance testing.

In-state commercial couriers, healthcare system couriers, PHL-provided couriers or national carriers can be employed to transport specimens to the PHL. Redundancy in transport options is important to cover disruption of any particular method of transport and to provide maximum daily service. An interstate carrier is most often used for transport to CDC or the CDC-designated laboratories.

In special circumstances, direct shipment from the health care provider or clinical laboratory to the CDC may be warranted; however, this should be facilitated by the PHL to ensure proper handling and state epidemiologist engagement if case investigation is needed.

SAMPLING

6. **Recognize and Address Sampling Biases**

The influenza virologic surveillance system contains inherent biases due to the complexity of the sampling system and the use of different test methods in the different tiers (Appendix A). Sources of bias should be considered and addressed if possible when selecting specimen providers, selecting test methods, analyzing data and interpreting results.

A. **Specimen providers**: Specimen providers should represent the entire population under surveillance. Choose a mix of primary care health care providers representing all age groups (pediatrics, family practice, internal medicine and geriatrics). Specimen providers should be selected representing areas of diverse population density (urban, suburban, and rural).

B. **Unscreened vs. screened specimens**: Efforts should be made to limit sampling of screened (influenza positive) specimens. As previously discussed, unscreened specimens are preferred. If submitters are using RIDT's for diagnostic purposes, a random mix of positive and negative specimens, irrespective of RIDT results, should be submitted to the PHL for surveillance purposes.[7] At a minimum, data should differentiate screened from unscreened specimens. If screened specimens from clinical laboratories are the primary source of surveillance specimens, these may be overly representative of hospitalized patients (i.e., bias to severe cases). Data may not be representative of true prevalence of virus subtypes in the community. This may be mitigated by selecting sites that can provide specimens from both emergency room and inpatient settings and providing clear guidance on numbers and types of specimens to be submitted.

Laboratory Testing

Laboratory testing requires staff with expertise in the methods performed, adequate space with appropriate biosafety controls, well-maintained equipment, appropriate testing reagents and supplies, and reliable results reporting mechanisms. Essential equipment, materials, and reagents necessary to perform influenza virologic surveillance are listed in Appendix C: Laboratory Methods.

Influenza virologic testing methods are classified in the roadmap based on a stakeholder assessment of requirements that should be maintained and available at all PHLs involved in influenza surveillance (primary testing); or as additional influenza surveillance laboratory capabilities that may be maintained based on state and/or jurisdictional needs or provided through a shared services model. The laboratory must ensure quality for all influenza testing methodologies performed.

Primary Testing Method

PHLs performing virologic surveillance are expected to utilize molecular methods, such as real-time reverse transcriptase PCR (rRT-PCR), as the primary testing method for influenza detection and subtyping. This is an ELC benchmark. During rRT-PCR test processes, viral RNA is extracted from patient specimens, transcribed into DNA and then amplified. The product of the test reaction is detected in "real time" using labeled probes. Real-time RT-PCR can rapidly identify influenza A and B, distinguish influenza A subtypes, and offers the best performance characteristics (i.e., sensitivity, specificity) of all currently available testing methods. CDC, through the Influenza Reagent Resource (IRR), manufacturers and distributes the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (CDC Flu rRT-PCR Dx Panel) to all state and qualified local PHLs engaged in influenza surveillance testing to ensure widespread use of a nationally standardized protocol. The CDC also has the capability to rapidly adapt the assay to detect newly emerging viruses. The CDC Flu rRT-PCR Dx Panel is distributed under FDA special controls. The special controls require that all users of the diagnostic device be trained to perform and interpret the results by a competent instructor prior to use and that CDC limit distribution to only those users who have successfully completed a training course provided or approved by CDC.

If the PHL decides not to use the CDC Flu rRT-PCR Dx Panel for influenza surveillance testing but instead chooses an alternative molecular influenza testing methodology or platform, the PHL must ensure that the manufacturer of the assay monitors and updates assay performance as circulating viruses change over time and that the assay being utilized within the laboratory has been optimized to identify all currently circulating influenza viruses.

Influenza testing algorithms should be adopted in order to optimize testing efficiency and throughput. Laboratories should choose an algorithm that best fits their laboratory test flow, and surveillance needs as the influenza prevalence changes throughout the year, and ensures judicious use of CDC provided reagents. Algorithms should be continually reviewed by PHLs throughout the influenza season to ensure that the most efficient algorithm is being utilized. The direct material cost to CDC for each IVD CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, including Influenza A/B typing and subtyping reagents, enzymes, extraction kits and plastics, is approximately $14,000 (i.e., $20 for each specimen tested by the PHLs).

LABORATORY TESTING

Below are three common testing algorithms to consider.

- Using the 7-target algorithm, influenza typing and subtyping are performed on a single plate at the same time. This algorithm tests simultaneously for influenza A, B, H1, H3, A/H1pdm2009 and RNaseP (internal control).

- The Influenza A/B Reflex algorithm is a two-step process in which Influenza A/B typing (A, B, RNaseP) is performed first and the Influenza A subtyping (H1, H3, A/H1pdm2009) is performed only on Influenza A positive specimens.

- Surge algorithms can also be implemented if needed to accommodate high volume testing demand in an outbreak investigation or pandemic. This may include first screening all specimens associated with outbreak for the outbreak strain (e.g., A, H1pdm2009), followed by reflex testing of negative samples to detect other circulating influenza viruses.

The PHL is responsible for the timely referral of representative specimens (and viruses, if culture is performed) to CDC or a CDC-designated PHL for genetic and antigenic characterization throughout the year. PHLs should submit 1mL of original clinical material (a minimum volume of 300µl is required). To enhance CDC's vaccine virus selection efforts, it is important to routinely and consistently send recently collected specimens. Specimens submitted to CDC should be representative of the circulating influenza types/subtypes, geography, disease severity and age. Oversampling of low prevalence subtypes may be necessary to ensure that all circulating subtypes are represented in the samples sent to CDC. When available, viruses from particularly severe or unusual cases, and a sample of viruses isolated from outbreak investigations should also be represented in submissions to CDC. The two examples below illustrate the criteria that should be considered by the PHL when selecting the specimens that will be sent to CDC for routine national surveillance purposes.

At any time through the year, if the PHL identifies a specimen as unsubtypable following testing using all available targets (influenza A, B, H1, H3, H1pdm2009, etc), the laboratory must notify CDC immediately and refer the specimen to CDC within 24 hours of detection. Specific information about interpretation of inconclusive test results and referral requirements can be found in the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel package insert.

- In a two week period, the PHL testing yields 50 A/H3, 1 A/H1pdm2009 and 5 influenza B positive specimens. Send to CDC or the CDC-designated laboratory: the A/H1 specimen, 1 influenza B, and 3 A/H3 viruses that are representative of state geography and patient ages.

- In a two week period, the PHL testing yields 20 A/H3, one of which is from a patient who died, 12 A/H1pdm2009 viruses and 5 Influenza B viruses. Send to CDC or the CDC-designated laboratory: the A/H3 specimen from the patient who died, 2 other representative A/H3 specimens, 2 A/H1pdm2009 and 1 influenza B specimen.

Outside of influenza season, PHLs should follow CDC summer submission guidance and send all specimens that test positive by PCR to CDC for further characterization.

IMPORTANT REPORTING AND REFERRAL REQUIREMENT: At any time through the year, if the PHL identifies a specimen as unsubtypable following testing using all available targets (influenza A, B, H1, H3, H1pdm2009, etc), the laboratory must notify CDC immediately and refer the specimen to CDC within 24 hours of detection. Specific information about interpretation of inconclusive test results and referral requirements can be found in the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel package insert.

To ensure national and state coordination on virologic surveillance priorities, "Influenza Seasonal Kick off Teleconferences" are convened for PHLs, influenza coordinators, and state epidemiologists in the early fall each year. The main purpose of the teleconferences is to provide a situational update and surveillance guidance for the upcoming season, including expectations for submission of specimens to CDC. The guidance will identify how many specimens to submit throughout the season, where to submit the specimens, and the specimen submission form.

Additional Testing Methods

Additional testing methods include influenza virus culture, antiviral resistance testing, influenza hemagglutination inhibition (HAI), immunofluorescence testing, and serology. The methodology recommendations presented here focus specifically on advantages and disadvantages for influenza and may not apply to other virus assays. Each testing methodology listed has many advantages and disadvantages that will factor into jurisdictional test methodology decisions. Some of the advantages or disadvantages listed here are more applicable to national surveillance needs, but are included to help inform jurisdictional decisions.

- **Influenza Virus Culture**

  PHLs are encouraged to perform virus culture if they can sustain the appropriate level of staff expertise and the necessary resources. When PHLs submit virus isolates along with clinical material to CDC or the CDC-designated PHL, this improves the efficiency of the antigenic characterization process at CDC, so that information regarding influenza vaccine match or virus drift can be disseminated back to PHLs more quickly.

  At minimum this capability must be maintained at CDC, and at a subset of state PHLs. PHLs that do not have the capability or resources to perform high quality virus culture using consensus protocols may opt to utilize a shared services model instead, relying on the CDC or state PHLs that have been designated by CDC as national surveillance laboratories to provide virus culture.

  Advantages of Maintaining or Implementing Influenza Virus Culture:
  PHL:

  - Provides isolates for validation and verification of new or modified assays, and troubleshooting investigations.

  - Provides a back-up method to PCR.

  - Detects other respiratory viruses if additional cell lines are used.

  - Provides viruses required for phenotypic antiviral resistance testing.

CDC:

- Provides isolates for validation and verification of new or modified assays, and troubleshooting investigations.

- Provides viruses required for phenotypic and antigenic characterization. These are critical components of surveillance for vaccine virus strain selection, and development of the annual WHO kit distributed to domestic and international laboratories.

- Provides viruses that can be used to develop vaccine candidates.

Disadvantages of Influenza Virus Culture:
- Less rapid and sensitive than rRT-PCR and is not efficient in times of surge.

- Requires specialized expertise and capability to maintain high quality cell lines.

- Variable growth characteristics and sensitivity with influenza strains in different cell lines.

Minimal Implementation Considerations:
- Utilize standard reference methods such as those described in the "WHO Manual for the Laboratory Diagnosis and Virological Surveillance of Influenza".[27]

- Ensure sustainable expertise. Each laboratory must have a training plan in place for virus isolation methods and troubleshooting.

- Establish a backup plan for times when commercially available cells are not available.

- Perform an accurate cost analysis. Identify sustainable sources of funding.

- **Antiviral Resistance Testing (Pyrosequencing, Neuraminidase Inhibition)**
  Definitive antiviral resistance testing requires both phenotypic resistance testing of the virus using a neuraminidase inhibition (NAI) assay, and detection of genetic markers of drug resistance by pyrosequencing and/or sequencing. Both of these test methods are performed at CDC. Pyrosequencing is also performed at a subset of PHLs; this provides a cost-effective and efficient approach to expand antiviral resistance surveillance, providing data that can be used to inform patient management and treatment recommendations at the regional and national level.

  Advantages of Maintaining or Implementing Pyrosequencing:
  PHL and CDC:

  - Detects established and potential genetic markers of resistance.

  - Provides more rapid turn-around-time than NAI which requires propagated virus for testing.

  Disadvantages of Pyrosequencing:
  - Requires multiple assays to detect different genetic markers for each influenza subtype (e.g., H275Y is the marker for only A/H1pdm 2009).

◦ Requires supplemental testing by NAI for definitive confirmation of antiviral resistance.

◦ Requires specialized expertise to perform and interpret results.

◦ Requires specialized equipment and expensive reagents.

◦ Requires periodic revalidation of assays due to frequent genetic changes.

◦ Requires knowledge of molecular markers of resistance which may not be available for a novel virus and new NAIs.

Advantages of Maintaining or Implementing NAI:

 PHL and CDC:

◦ Detects resistance that is not caused by previously identified genetic markers of resistance.

◦ Detects resistance in novel viruses for which genetic markers of resistance are unknown.

◦ Detects resistance to a new NAI drug for which genetic markers of resistance are unknown.

Disadvantages of NAI:

◦ Requires specialized expertise to perform and interpret results.

◦ Requires specialized equipment and expensive reagents.

◦ Requires special formulations of antiviral drugs (e.g., oseltamivir carboxylate) not available outside of drug manufacturer.

Minimal Implementation Considerations**:**

◦ Perform an accurate cost analysis. Identify sustainable sources of funding.

◦ Laboratory must have the capability to:

□ Interpret and report test results appropriately for either virologic surveillance, or patient management.

□ Report all results to CDC in a timely manner for inclusion in national antiviral resistance surveillance data.

□ Revalidate assays when mutations occur or changes to the protocols are made.

□ Perform virus isolation if using NAI assay.

□ Submit all influenza viruses positive for any resistance marker to CDC in a timely manner for confirmatory testing.

LABORATORY TESTING

- **Respiratory Pathogen Assays**
  Molecular testing for other respiratory viruses, such as commercially available RVP assays or laboratory developed tests (LDTs), have become common in many clinical laboratories and are increasingly used in PHLs to provide jurisdictional and national information about other circulating respiratory pathogens. These data can aid in identifying other agents that cause influenza like community illnesses or outbreaks and provide reassurance that surveillance is not "missing" influenza during periods when influenza activity is low but other influenza like illnesses are prevalent.

  Advantages of Maintaining or Implementing Molecular Respiratory Pathogen Assays:
  PHL:

  - Provides data on circulating respiratory viruses that can be used for state, regional or national (NREVSS) surveillance or outbreak investigations.

  - Provides a more rapid, sensitive and specific alternative to respiratory virus culture.

  - Provides influenza surveillance partners with additional information about circulating viruses that may cause ILI, this may also help to incentivize partners to consistently contribute to flu surveillance.

  Disadvantages of Respiratory Pathogen Assays:
  - Cost.

  - Variable sensitivity and specificity across pathogens and commercial assays.

  - May lose sensitivity overtime, especially for influenza, due to changes in the viruses.

  Minimal Implementation Considerations:
  - Perform an accurate cost analysis. Identify sustainable sources of funding.

  - Determine how respiratory pathogen data will be used to supplement influenza surveillance data and/or diagnostic testing.

- **Influenza Hemagglutination Inhibition Test**
  Influenza hemagglutination inhibition (HI or HAI) test performed at CDC, using strain specific ferret antisera, remains a test of choice for antigenic characterization to monitor changes in circulating influenza viruses and inform influenza vaccine virus selection. HAI testing using the WHO kit reagents that are provided by CDC to the PHLs can be used to detect and identify influenza viruses, but not for monitoring antigenic drift of influenza viruses.

  Advantages for Maintaining or Implementing Influenza HAI test:
  PHL:

  - Identifies influenza type, influenza A subtype, and influenza B lineage using the Influenza WHO kit reagents provided by CDC.

  - Facilitates and helps to ensure more efficient detection of antigenic variants.

CDC:

- ◦ Provides information on antigenic changes that may impact vaccine effectiveness, these data are critical to inform annual vaccine virus selection.

Disadvantages of Influenza HAI test:

- ◦ Requires significant expertise and specialized reagents (e.g., turkey red blood cells).

- ◦ Requires virus culture.

- ◦ Time consuming.

- ◦ Influenza type and subtype can more rapidly be determined in PHLs using rRT-PCR.

Minimal Implementation Considerations:

- ◦ Laboratory must have the capability to:

  - □ Interpret and report test results appropriately for virologic surveillance.

  - □ Perform virus isolation.

  - □ Obtain red blood cells from appropriate species (e.g., turkey).

- ◦ Perform an accurate cost analysis. Identify sustainable sources of funding.

- **Influenza Serologic testing**
  Serologic testing is neither a surveillance nor rapid diagnostic testing tool but is currently used primarily by CDC and academic institutions for vaccine effectiveness studies, annual vaccine strain selection, research purposes or retrospective seroprevalence studies. Serologic testing to detect influenza virus antibodies may be performed using a variety of methods, including hemagglutination inhibition, enzyme-linked immunosorbent assay and microneutralization.

  Advantages of Maintaining or Implementing Influenza Serologic Testing:
  PHL and CDC:

  - ◦ Provides serology data to inform virologic strain selection.

  - ◦ Provides serological diagnosis and retrospective seroprevalence data.

  Disadvantages of Influenza Serologic Testing:

  - ◦ Requires capability to implement assay as a laboratory-developed test, there are no FDA cleared methods for influenza serology.

  - ◦ Requires both a high level of expertise and specialized reagents.

  - ◦ Interpreting results is difficult and patient serologic responses may be cross-reactive.

LABORATORY TESTING

Minimal Implementation Considerations:

- ◦ Perform an accurate cost analysis. Identify sustainable sources of funding.

- ◦ Maintain staff with capability to interpret serologic test results in the context of surveillance.

- **Direct Specimen Immunofluorescence**
Direct specimen immunofluorescence using direct fluourescent antibody (DFA) testing methods involve testing clinical material taken directly from the patient. These cell preparations are tested using fluorescent-labeled antibodies, which are visible when examined using an immunofluorescence microscope. DFA is primarily a diagnostic tool.

Advantages of Maintaining and Implementing DFA:

 PHL:

- ◦ Provides the ability to produce results within an hour of specimen receipt.

Disadvantages of DFA:

- ◦ Requires specialized expertise and fluorescent microscopes.

- ◦ Results are subjective and dependent on the individual reader's expertise.

- ◦ Not as accurate as rRT-PCR.

- ◦ Sensitivity and specificity of reagents may vary in response to current strains.

Minimal Implementation Considerations:

- ◦ Assess the necessity of maintaining this as a diagnostic tool.

- ◦ Perform an accurate cost analysis, identify sustainable sources of funding.

- **RIDTs**
Currently available RIDTs for the detection of influenza viruses employ a variety of methods, including enzyme-linked immunosorbent assays, immunochromatographic lateral flow immunoassays, and membrane-based immunoassays. In addition to differences in methodologies, these tests also have varying requirements for specimen collection and handling.

Advantages of Maintaining and Implementing RIDTs:

 PHL:

- ◦ Produce rapid results for patient care, often within fifteen to thirty minutes.

- ◦ Have widespread commercial availability.

- ◦ Require minimal training, can be performed in non-laboratory settings.

Disadvantages of RIDTs:

- ◦ Significantly less sensitive and specific than rRT-PCR assays.

- ◦ Potentially less reliable when new virus strains emerge.

Minimal Implementation Considerations:

- ◦ Determine how RIDT data will be used to supplement influenza surveillance data and/or diagnostic testing.

- ◦ Establish policies for reflex/ confirmatory testing.

- ◦ Perform an accurate cost analysis. Identify sustainable sources of funding.

Considerations for Maintaining or Implementing Influenza Testing Methodologies

In addition to considering the advantages and disadvantages of the various test methods, below is a set of questions that can be used as a decision tool in deliberations among the laboratory director, senior infectious disease laboratory staff, epidemiologists, influenza surveillance coordinator, and clinical laboratory partners.

1. Which influenza tests are most important to maintain?

2. For what purpose is the influenza test(s) needed?

3. How will the test results contribute to influenza virologic surveillance data within the state?

4. How is the influenza test methodology in question currently funded? Is this funding mechanism sustainable?

5. Is there an expectation, either through official policy or relations with the local clinical community, that the laboratory maintain capacity to perform some or all of the "additional" influenza tests?

Performance Accuracy of RIDT's

RIDTs may be used to help with diagnostic and treatment decisions for patients in clinical settings, such as whether to prescribe antiviral medications. However, due to the limited sensitivities and predictive values of RIDTs, negative results of RIDTs do not exclude influenza virus infection and influenza should still be considered in a patient if clinical suspicion is high based upon history, signs, symptoms and clinical examination.[31]

Reported sensitivities of RIDTs range from 10-80% compared to viral culture or rRT-PCR. Specificities of RIDTs are approximately 90-95% (range 85-100%). RIDT users whould be especially aware of the potential limitations of these tests to detect novel influenza viruses.[8,32]

rRT-PCR to confirm results of an RIDT are recommended when:

- patient tests negative by RIDT when community influenza activity is high and laboratory confirmation of influenza is desired.

- patient tests positive by RIDT, the community prevalence of influenza is low and a false positive result is a consideration.

- patient has had recent close exposure to pigs or poultry or other animals and novel influenza A virus infection is possible.

6. If yes, what are the reasons for this expectation? For example, is there a perceived need to maintain "traditional methods" in the PHL as the capability to perform culture and HAI declines in the clinical laboratory sector?

7. Is the necessary expertise available in the PHL and is it sustainable? Does the laboratory have an experienced microbiologist/virologist that understands the basic virology, epidemiology, pathogenesis of influenza and other respiratory pathogens to enable test result and surveillance data interpretation, and surveillance capability decisions?

8. Does to laboratory have access to and utilize appropriate consensus protocols and testing materials (e.g., MDCK cell lines, antiviral drugs, monoclonal fluorescent antibodies)?

9. If an influenza test method, such as culture, is eliminated, do decision makers understand that implementing testing on an as needed basis would be extremely difficult due to loss of expertise, length of time needed to acquire testing supplies, and time to perform CLIA required validation studies?

10. Is the influenza methodology that is deemed necessary available from another source, such as CDC, a PHL shared services site, or a hospital/academic laboratory? What are the concerns with and road blocks to accessing alternate sources of testing?

11. Do the resources (funding, staffing, supply costs) needed to maintain the additional test methods adversely impact the capacity to perform rRT-PCR and test the number of specimens necessary for routine surveillance, or other essential laboratory functions?

12. If the IRR was unable to continue providing either some (i.e., ancillary) or all the reagents needed for rRT-PCR, how would this affect the decision to maintain "additional" testing methods?

13. Does the laboratory meet the minimal considerations listed for each test method that will be implemented or maintained?

Data Management

PHL Data for Surveillance

The data generated through PHL reporting are published each week through CDC's FluView. Therefore, timely and accurate reporting of laboratory surveillance data using electronic data systems is of the utmost importance. For PHLs, the main method of electronic reporting is through PHLIP. PHLIP is an effort to achieve interoperability between different types of systems in order to exchange information in a useful and meaningful way. PHLIP utilizes an HL7 messaging standard to facilitate data exchange, allowing for harmonization of laboratory test results using standard vocabularies and terminology, including LOINC and SNOMED. PHLs can find information regarding implementation of HL7 messaging for CDC Flu rRT-PCR Dx Panels, including applicable LOINC test codes and SNOMED result codes at www.cdc.gov/flu/professionals/diagnosis/rtpcr-test-kits.htm. Additional information about LOINC, SNOMED and HL7 can be found at:.

- LOINC – www.loinc.org,

- SNOMED – www.ihtsdo.org,

- HL7 – www.hl7.org.

The use of electronic data systems that provide data in real time and comply with national standards is a requirement to achieve right size virologic surveillance. The real time data elements (as found in the HL7 PHLIP message guide) available at each PHL may vary; some PHLs receive considerably more epidemiologic and specimen collection information than other PHLs. The minimum the PHLs should provide is:

- Specimen identifier and unique patient identifier,

- State where specimen was collected,

- Date of birth of patient and/or age plus unit (years, weeks, months, days),

- Specimen collection date,

- Specimen received date,

- Performed test method,

- Test result.

The PHLs that already have PHLIP capability should consider reporting additional information. The additional information to include, if available, is as follows:

- Current influenza vaccination status,

- Antiviral treatment,

- Patient location at time of testing (inpatient, outpatient, long-term care facility),

- Travel information,

- Patient death information,

- Additional geographic information (e.g.,county, city, zip),

- Whether specimen was related to an outbreak,

- Whether specimen was sent to CDC and if so, ID included with CDC specimen,

- Date of illness onset.

APHL's Informatics Program has a Technical Assistance Team available to assist PHLs with PHLIP implementation. The Technical Assistance Team provides tools and human resources to assist PHLs, public health agencies, and other data exchange partners in understanding, navigating and accomplishing the task of sending electronic data using simple, effective, standards-based methods. For more information about the Technical Assistant Team, visit the APHL Informatics website and refer to frequently asked questions about technical assistance teams.

Non-PHL Data for Surveillance

As resources for PHL testing decrease, the value of alternate data sources becomes increasingly important. State or local public health departments and laboratories are encouraged to explore options to collect and incorporate influenza testing data from non-PHL sources. The supplemental data can help increase the confidence in the surveillance data within the state. This data could include rapid influenza diagnostic testing (RIDT) data and/or data from clinical and commercial laboratories within a jurisdiction. A number of other sources of virologic surveillance data may be available to augment both state and national surveillance. Data from these sites may be transmitted electronically as specimen level records or in aggregate by a simpler method. Regardless of whether specimen level or aggregate data is received, necessary data elements include:

- Date or week of specimen collection, receipt or test

- Total number of tests performed and influenza positives by:

  - Type,

  - Subtype (if available),

  - Age group.

Potential Alternate sources of local, state and national virologic surveillance data include:

- Clinical sites including RIDT sites and clinical laboratories. A number of epidemiologists and influenza coordinators have initiated laboratory test reporting from selected facilities in their states. These data back are used by the influenza coordinators to monitor influenza activity. Providing the supplemental surveillance data to clinicians is a useful resource to guide patient management decisions.

DATA MANAGEMENT

- Commercial laboratories. A significant number of non-hospital laboratory testing is performed by a small number of commercial laboratories (e.g., Quest Diagnostics, LabCorp, ARUP Laboratories, and Mayo Medical Laboratories). Surveillance programs could obtain data from these laboratories on specimens tested from within their jurisdiction.

- Electronic Laboratory Reporting (ELR) for Meaningful Use. As part of the Affordable Care Act and related activities, inpatient and outpatient healthcare facilities have been provided monetary incentives to implement electronic health records. Along with these incentives, facilities are required to report notifiable diseases, syndromic surveillance, and vaccine registry data using automated electronic messaging standards (e.g., HL7, LOINC, SNOMED). For states that have required reporting of influenza laboratory results, health departments may be able to use these electronic messages to augment virologic surveillance.

Alternate data sources for CDC to supplement national surveillance data:

- Commercial laboratories. A significant number of non-hospital laboratory tests are performed by a small number of commercial laboratories (e.g., Quest Diagnostics, LabCorp, ARUP Laboratories, and Mayo Medical Laboratories). These laboratories provide a significant amount of influenza testing for hospitals, and physician offices around the country.

- Federally Qualified Health Centers (FQHCs). As of 2010, over 1,100 of these clinics operate under the supervision of the Health Resources and Services Administration (HRSA) to provide care to the medically underserved.[28] With the Affordable Care Act (ACA), the numbers and patient volumes are anticipated to increase.

- Department of Veterans' Affairs (VA). There are 1,766 VA facilities[29], although influenza laboratory testing is only available at a subset of these.

- Department of Defense (DoD). Data available from DoD includes virologic data collected among military personnel around the globe and laboratory test information from military and non-military facilities that care for military dependents, notably through TriCare Insurance.

- Centers for Medicare and Medicaid Services (CMS). Since 2009, data on influenza testing has been made available on a more real-time basis utilizing the CMS reimbursement exchange data repository.

- Vendors of Electronic Health Records (EHR) and Laboratory Information Management Systems (LIMS). Vendors and large users of EHRs and LIMS may be a source of influenza testing information. Examples of these include GE Healthcare, Cerner, Sunquest, HCA, and others.

- Diagnostic Device Manufacturers. An increasing trend in diagnostic testing is the use of mobile communications from test devices to cloud-based web services to allow ease of access of information to patients, doctors, and insurers. This capability also allows automated messaging of de-identified results from influenza test devices to a cloud where public health entities can access the information for monitoring influenza in their jurisdictions.

DATA MANAGEMENT

Considerations for Data Management

1. Are you currently utilizing PHLIP to report influenza virologic surveillance to CDC? If not, have you contacted APHL or CDC to start the process?

2. If currently utilizing PHLIP, what data elements are being sent? Have you explored incorporating additional information fields listed above?

3. Have you identified the potential sources of bias in your virologic surveillance data? What changes could be made in your system to reduce the impact of bias?

4. Does your influenza surveillance system incorporate virologic data from healthcare providers utilizing RIDTs? If yes, how is this data collected from rapid test sites? Do you collect both the number positive and the total number tested (denominator data)? Is the data collected currently reflected within your jurisdictional surveillance data?

5. Does your influenza surveillance system incorporate virologic data from clinical/commericial laboratories? If yes, how is this data collected from the laboratories? Are the number positive and the total number tested collected (denominator data)?

6. How stable and reliable is the data received? How often is data from alternate sources received (e.g., clinical, commercial, physician office laboratories)?

7. If no alternate data is collected and incorporated into your surveillance data, would it be possible to collect the data in the future?

8. What are the challenges to collecting alternate data?

9. What is the plan for incorporating new data sources into your influenza surveillance data?

10. What resources are required to collect non-public health laboratory testing data?

Partnerships & Communication

It is important that states establish and maintain partnerships and networks among PHLs, clinicians, state epidemiologist/influenza surveillance coordinator, clinical laboratories, RIDT sites, CDC and manufacturers. Many states already have existing partnership and communication networks for both influenza surveillance and other activities such as Laboratory Response Network (LRN) and APHL's Lab System Improvement Program (L-SIP).[14,17] Professional organizations such as APHL and CSTE provide programmatic and technical support to member states and facilitate communications among CDC, PHLs, and epidemiologists. Improvements to influenza surveillance can be made by leveraging existing partnerships and communication networks for influenza surveillance, LRN and other laboratory-based surveillance activities. For example, contact databases that already exist for LRN can be enhanced to include laboratories that perform influenza testing without creating an entire new system. Many states have established courier services to transport specimens for LRN, newborn screening and other programs. These may be leveraged to improve access to specimens for influenza surveillance. Additional examples related to key partnerships, provided through stakeholder input and pilot site activities, are described below.

> **Collaboration between Epidemiologists and Laboratorians at the Washington State Department of Health**
>
> Every August, epidemiologists and laboratorians at the Washington State Department of Health (DOH) meet in person to discuss virologic surveillance plans for the upcoming influenza season. They discuss criteria for influenza testing at the PHLs, plans for engaging sentinel providers and laboratories and changes to specimen submission instructions. Written instructions for submitting specimens to PHL for influenza testing are revised collaboratively.
>
> Healthcare providers and local health jurisdiction staff who want to submit non-routine specimens for influenza testing, including specimens from patients with suspected novel influenza and those in outbreaks, are asked to contact a DOH influenza epidemiologist prior to submission. The epidemiologist reviews the request and informs the laboratory staff about the estimated arrival time and priority status of the specimens. If critical specimens do not arrive at the PHL by the expected time a laboratorian will contact the submitter to determine the whereabouts of the specimens. This system ensures that epi and lab partners have access to timely information regarding status of high priority specimens.

The most important partnership for effective virologic surveillance is the relationship between the ***PHL and epidemiology/influenza coordinators***. Examples of ways to optimize epidemiology-laboratory (epi-lab) partnerships:

- Conduct regular in-person epi-lab meetings to establish seasonal virologic surveillance strategies, determine appropriate sample sizes, allocate funds and regularly assess the effectiveness of the surveillance system.

- Collaborate in grant writing, monitor grant activities, identify and address problems and gaps and coordinate outbreak response.

- Establish consensus protocols for sharing influenza testing data. Examples include releasing laboratory data to a secure portal that epidemiologists can access or providing epidemiologists access to selected views in the laboratory databases.

PHL-Epidemiology-Clinician-Academic Partnerships

- Provide strategic communication from state epidemiologists and PHLs to clinicians and clinical laboratories as needed to increase awareness when targeted surveillance is needed to identify emerging viruses or characterize outbreaks.

- Provide education programs to clinicians, especially on the utility of rapid point of care tests, and provide training on specimen collection, handling and transport to ILINet and other surveillance specimen submitters. Training may be achieved through on-site presentations, teleconferences, mailings, and on-line training courses.

  ◦ The Joint Commission Strategies for Improving Rapid Influenza Testing in Ambulatory Settings (SIRAS) offers two free on-line courses one for health care providers in ambulatory settings and one for specimen collectors.

- Collaborate with clinicians and academic researchers on studies to increase understanding of influenza infection and epidemiology.

Value of Epidemiology, PHL and Clinician Partnerships

Successful influenza virologic surveillance programs are not built overnight and cannot be sustained without proper care. Strong relationships between state epidemiology, PHL, and clinical partners are crucial to ensuring quality and consistent data and specimens for influenza virologic surveillance. Clinicians with a keen interest in public health who can help grow and foster surveillance efforts in the community and among their colleagues can be an enormous asset. Establishment of a strong network of providers who will submit timely and quality specimens requires dedicated resources to provide encouragement, feedback and guidance. When dedicated staff routinely work with submitters on appropriate reporting, specimen collection and submission, specimens are more likely to be of higher quality and improve the virus detection abilities at PHLs. Virologic surveillance efforts cannot be a one way street. Giving back to providers who participate serves as a reminder of the importance of their contributions. Providing incentives can be as simple as ensuring timely feedback of results and findings or as advanced as offering additional testing of negative samples for other respiratory pathogens.

Source: Unpublished communications with Influenza Incidence Surveillance Project (IISP) participants

PARTNERSHIPS & COMMUNICATION

PHL-Clinical Laboratory/Testing Site –Influenza Surveillance Coordinator Partnerships

Strong partnerships and communications with clinical laboratories and influenza testing sites are important to obtaining quality and consistent data and specimens. Listed below are some examples for enhancing the effectiveness of these relationships.

- Access alternate data sources to supplement influenza surveillance, as described in the Data Management Implementation Guidance section. Commercial, web-based survey instruments are available at little to no cost (e.g., SurveyMonkey™, SurveyGizmo) to collect testing data from partners. Some of the tools can provide participants with an identifying login and be pre-filled with participant information to ease the burden and increase participation rates. The data collected can include information on a variety of agents and test methods. Data can be downloaded to a spreadsheet for analysis. These reports provide data from thousands of tests performed by clinical laboratories and test sites throughout the influenza season. In addition, a number of clinical laboratories perform their influenza testing as part of a respiratory virus molecular panel; access to this data allows for a more complete picture of circulating respiratory pathogens.

- Establish collaborative relationships with specimen providers to ensure and/or improve the quality and consistency of specimen submissions as outlined in the Sampling Requirements Intent and Implementation Guidance sections. For more information on implementation for establishing specimen provider networks, please reference the sampling implementation guidance.

  ◦ In addition to the standard communication methods, some states distribute a "handbook" containing instructions, forms and summary data of laboratory surveillance needs in their jurisdiction. The Wisconsin Laboratory-Based Surveillance Plan is one such example.

- Promote the value of participating in the surveillance system, provide incentives when permissible. Incentives do not need to be monetary; they can be test kits, training, certificates of appreciation, attendance at state conferences and reference books, as well as the "added value" of improved surveillance data that can be used to improve clinical management recommendations.

- Provide specimen collection and shipping supplies and courier service to virologic surveillance participants. Most health care providers and clinical laboratories will not be able to absorb the cost of surveillance supplies or shipping.

- Provide timely updates to specimen providers via email, web or fax. Clinical laboratory partners and PHLs both benefit from exchanges of information related to current influenza activity, commercial test shortages, and emerging disease threats (e.g., 2013 novel coronavirus and H7N9).

- Provide workshops, teleconferences/webinars and educational materials related to influenza surveillance, proper specimen collection and use of RIDT.

- Provide proficiency assessment challenges or exercises related to influenza testing and specimen packaging if budget permits. These exercises may be coordinated with other state/local preparedness activities.

PARTNERSHIPS & COMMUNICATION

PHL and CDC Partnerships

Collaboration between the PHLs and CDC's Influenza Division is imperative for effective virologic surveillance. The CDC Influenza Division, especially the Virus Surveillance and Diagnosis Branch, provides vital support to the PHLs and relies on data and specimens submitted by the PHLs. Coordination between the CDC and PHLs is often facilitated by APHL.

- CDC provides didactic and hands-on training to PHLs in test methods and national teleconferences to share surveillance guidance for laboratory testing and influenza coordinator activities.

- CDC provides technical support to PHLs including assistance with assay troubleshooting and interpreting unusual results.

- CDC provides testing reagents and materials through the IRR along with updates to the assay and implementation support when novel viruses emerge (e.g., H3N2v, H7N9).

- CDC partners with PHLs to complete the necessary validation studies for regulatory approval of new assays.

- CDC provides guidance to assist states develop emergency outbreak and pandemic response plans and provides essential support in actual response situations.

Considerations for Building Effective Partnerships and Communications

1. Has your influenza surveillance program and PHL identified the appropriate contacts among public health, clinician and laboratory partners within your jurisdiction?

2. If so, do you routinely and collaboratively review the list of contacts to ensure that all key partners are included (e.g., identify new partners, update for staffing changes, etc).

3. Does your laboratory maintain a database of current contact information and influenza testing capabilities for identified laboratories within your jurisdiction?

   - Some SPHLs maintain multiple separate databases – one for the LRN, one for a statewide laboratory network for surveillance, etc. while some SPHLs have added influenza testing sites and included influenza testing capability, surveillance participation, etc. to the existing LRN database.

4. Does your influenza surveillance program and PHL designate one or more staff members to coordinate outreach activities (e.g., network or surveillance coordinator/manager/advisor)?

5. Do you maintain a communication plan that identifies and links system partners?

6. Do you collaborate with other laboratories and rapid influenza testing sites to acquire virologic testing result data and specimens for further virologic testing?

7. Do you have a method to collect influenza testing data from clinical laboratories/testing sites (e.g., survey tool, fax or web portal)?

8. Does your laboratory and/or influenza surveillance program maintain the capability to exchange information and data via email, fax or other electronic tools with laboratories within your jurisdiction?

9. Do you provide reports on the current status of circulating influenza types and subtypes, and other respiratory viruses if available, via website, newsletter or other means?

10. Do you provide an end of year summary to all stakeholders about the influenza season? Do you provide individualized reports to participating laboratories and/or providers?

11. Do you relay the importance of receiving specimens for confirmatory testing, subtyping and identification of unsubtypable specimens to your clinical partners especially when the threat of a novel virus is high (e.g., H3N2v, H7N9)?

12. Does your influenza surveillance program and/or laboratory provide teleconferences, webinars or in-person training and outreach to clinician and laboratory surveillance partners and potential partners?

13. Is there a mechanism for feedback and corrective action to providers who incorrectly or inappropriately send specimens to the PHL (e.g., improperly shipped, incorrect form, incomplete information, sent dry swab not in media)?

PARTNERSHIPS & COMMUNICATION

QUALITY MANAGEMENT SYSTEMS

Quality Management Systems

National Surveillance System Quality Monitoring

The ultimate value of virologic surveillance data is dependent on the quality of specimens, laboratory procedures and data analysis. CDC and state/local jurisdictions should establish performance metrics and monitor essential components of the national influenza virologic surveillance system to ensure quality and make improvements as needed. Listed in this section are key components that should be routinely assessed, but it should be noted that each quality management system will vary and jurisdictions need not be limited by this list. It is likely that existing data sources can be leveraged to assess the quality of many surveillance components.

State/Local Quality Management Responsibilities

At the state and local level, quality management systems need to monitor both internal performance and performance in meeting national surveillance requirements including those defined in this document. As discussed previously, influenza virologic surveillance systems are complex and vary across jurisdictions; quality management systems will likewise need to be tailored to each system. Regardless of the assessment mechanism(s), it is recommended that states have some method to evaluate the following elements related to influenza virologic surveillance and make adjustments and improvements as needed.

- Compliance with ELC, PHEP and other cooperative agreement and grant benchmarks for all epidemiology and laboratory components of the surveillance system.

New Hampshire PHLs Influenza Quality Monitoring, 2013

When it became clear that the 2012-2013 influenza season was ramping up to be the busiest since the 2009 pandemic, staff at the NH PHLs realized they needed to closely monitor influenza submissions in order to ensure resources were appropriately allocated to meet the goals of the surveillance program. This was achieved by building a simple "Daily Flu Data" report in the laboratory LIMS, which effectively extracted all data associated with influenza specimens received from the start of the season in October 2012.

The influenza report was run daily and data was dumped into an Excel spreadsheet. Once populated in the spreadsheet, the data could be manipulated in a number of ways: specimens received by date, specimens received by provider, specimens received by county, etc. This manipulation allowed the team to quickly see if submissions were increasing or decreasing and if the PHL was were obtaining representative samples from across the state. They were able to use this tool to reach out to health care providers and encourage additional submissions from providers in underrepresented areas of the state, while informing others that their submissions had exceeded surveillance needs. By storing the spreadsheet in a shared folder on the network, all staff who needed to use the information were able to access it quickly and conveniently.

- Specimen submissions through the provider networks including consistency, quality and number. Timely electronic transmission of specimen-level data. The PHLIP system is the preferred method of reporting.

- Percentage of influenza test results received by CDC from the PHL within two weeks of the test date.

- Capability to provide year-round molecular testing for the detection, typing and subtyping of seasonal influenza viruses and detection of novel influenza viruses.

- Systematic submission of representative influenza positive clinical materials and/or viral isolates for national virologic in accordance with annual CDC specimen submission guidance.

- Rapid referrals of all unsubtypable influenza A viruses to CDC.

- Proficiency in PCR methods for influenza virus detection, typing, and subtyping. The laboratory must operate in compliance with the Clinical Laboratory Improvement Amendment(CLIA) 88' Requirements, which include participating in an external/blinded proficiency test for each assay. CDC provides a quality assessment panel to PHLs at least one time per year which helps PHLs fulfill this CLIA requirement. Participation in this CDC assessment also provides data that helps CDC assess and address training needs.

- Usage of IRR-provided reagents, materials and other resources used for national surveillance in comparison to the number of specimens tested and reported to CDC. IRR reagents are provided to PHLs to support testing for national surveillance. Prior authorization from CDC is needed if IRR-provided materials are needed to support special studies.

- Staff expertise to perform each influenza test method used at the PHL. Every PHL should have a competency assurance policy that addresses initial training, assay update training and cross-training to ensure continuity of operations in a surge event such as the 2009 H1N1 pandemic.

- Staff expertise and ability to adopt influenza assay revisions, add additional testing markers or adopt assay interpretation updates. The detection of novel or variant viruses may result in new assay components or modified interpretation guidelines.

- Maintenance of an influenza specimen repository that can be utilized for assay verification and validation and competency testing as needed. Store a subset of positive and negative specimens containing a mix of influenza types and subtypes at -70°C.

CDC Quality Management Responsibilities:
- Cross-reference PHL influenza testing data reported to CDC against virologic specimen submissions to CDC and CDC-designated laboratories.

QUALITY MANAGEMENT SYSTEMS

• Monitor national surveillance data for timeliness, adequate testing and specimen submissions numbers and representativeness to ensure the system is able to effectively inform situational awareness and vaccine virus selection efforts. When needed, provide targeted communications to PHLs that are not consistently complying with specimen submission expectations or to request additional specimens as needed. Targeted communications help reduce confusion about specimen requirements and focus attention on key gaps or special needs.

• Monitor IRR reagent ordering history in relation to testing reported to CDC. Targeted follow-up to PHLs can be an effective method for addressing excessive reagent ordering which may be due to oversampling or unrecognized technical problems. When technical problems are identified, CDC and the PHL should collaborate to implement appropriate solutions as needed.

Considerations for Establishing and Maintaining Quality Management Systems

1. Does your laboratory and surveillance program have mechanisms in place to monitor compliance with grant/cooperative agreement benchmarks and deliverables?

   • Example: Leadership should meet regularly to review grant line items, identify issues and document progress. LIMS and tracking spreadsheets can be used to document and verify deliverables are being met.

2. Does your laboratory and/or surveillance program have processes for monitoring the quality, quantity, consistency, representativeness and timeliness of specimen submissions from specimen providers?

   • Example: Influenza coordinator and PHL may regularly review specimen submission data for quality indicators such as number of specimens rejected for poor quality, number of inconclusive test results, etc.

   • Example: Influenza coordinator and PHL may regularly review number of specimens received compared to number designated by sample size calculators. Sampling may be adjusted as appropriate.

3. Does your laboratory have mechanisms in place to ensure that representative specimens are being submitted to CDC or CDC-designated laboratories in accordance with annual specimen submission guidance and other CDC requests?

   • Example: Use LIMS and tracking spreadsheets to monitor the timeliness of influenza surveillance testing and submissions to CDC or CDC-designated laboratories. Regularly check to ensure specimens submitted to CDC are representative of the influenza activity in your jurisdiction (see examples in Sampling Implementation) and current CDC guide-lines. Verify that shipment quantities and frequencies are in compliance with the CDC guidelines.

Surge Capacity for Influenza Surveillance, Novel Event Investigation and Outbreak Events

The virologic surveillance system should be flexible and scalable for rapid, effective response to support initial diagnostic needs and case counts in rare/novel influenza event investigations and enhanced surveillance in outbreak and pandemic scenarios. Pre-event and during an event, communication and coordination between epidemiology and laboratory leadership will be essential to develop, refine and change the strategy for virologic surge sampling and testing.

Pre-event:

- Ensure that PHL representatives are included in state preparedness and pandemic planning activities. Address the role and resource needs of the PHL in state/jurisdictional pandemic plans. Pandemic Planning Information can be found at: www.cdc.gov/phpr/coopagreement.htm, www.cdc.gov/flu/pandemic-resources/tools/index.htm.

- Utilize the APHL Infectious Disease Planning and Response Framework Checklist to identify key partners and preparedness activities, including validation of new testing methodologies, biosafety, regulatory requirements, training, information dissemination, specimen collection and transport guidance.[21]

- Develop and maintain a laboratory pandemic surge plan that is integrated into a laboratory wide Continuity of Operations Plans (COOP). The surge plan should address:

    ◦ Communication/coordination with epidemiologists for specimen triage,

    ◦ Algorithm changes to improve efficiency and throughput or to meet specific surveillance needs,

    ◦ Resources (e.g., staff, cross-training, equipment, space, reagents and consumable supplies),

    ◦ Biosafety considerations for working with novel viruses,

    ◦ Options to mitigate the capacity gaps and bottlenecks identified in the *APHL-CDC Influenza Laboratory Resource and Process Modeling Project* report provided to participating states by APHL/CDC.[20]

- Establish mechanisms to determine and implement a sampling strategy for investigation following detection of a rare/novel influenza event. Consider the potential scenarios that may define sampling approaches, such as the need to identify additional cases and detect person-to-person transmission. Consider targeted surveillance options including clinical severity criteria, exposure risk, number of hospitalized cases/deaths and other event specific needs.

- Establish criteria for specimen triage and decision points for performing diagnostic testing and/

SURGE CAPACITY

or expanding virologic surveillance testing. Draft scenario specific scale up and ramp down criteria that can be quickly applied when a rare/novel influenza event or outbreak occurs.

- Define laboratory testing algorithms that may be implemented to accommodate the influx of surveillance and diagnostic specimens.

- Periodically assess laboratory contingency and crisis surge capacity, as defined in Surge Requirements Intent section. Laboratory capacity modeling has been conducted in over 35 PHLs using a model developed by APHL and CDC.[20] These models estimated baseline capacity, identified likely sources of bottlenecks in a surge event and evaluated the impact of various changes on overall throughput. Utilizing a surge algorithm with surge resources (staff, equipment, etc.) that are expected to be available to the laboratory during emergency periods of high testing demand provided a capacity increase of 127% compared to the Influenza A/B Typing Assay with reflex Influenza A Subtyping algorithm and baseline resources. The implementation of a super surge process strategy, which included changing from the Influenza A/B Typing with full Influenza A Subtyping Panel baseline to an Influenza A/B Typing only testing algorithm, along with the addition of staff and equipment, could increase national aggregate PHL daily capacity from approximately 5,000 specimens to approximately 14,250 specimens – an estimated change of 185%.

- Utilize sample size calculators to estimate the number of samples to be collected and tested for various rare/novel influenza event investigation scenarios. Compare laboratory surge capacity to likely sample size expectations so that both epidemiology and laboratory leaders understand capacity gaps, if any. Collaboratively explore strategies to reduce sample size or increase capacity.

- Identify and address expectations to support diagnostic testing needs, including potential support to assist clinical laboratories validate tests for the new virus.

Event:

- Use sample size calculators to determine the appropriate sample size for the investigation, based on the scenario, acceptable confidence level and error rate. Sustaining testing to provide daily case counts will not be possible and states should consider use of sample size calculators to adjust testing volumes as necessary to answer key surveillance objectives as the event evolves.

- Develop, refine and change state/local and/or CDC guidance based on the latest information as needed dependent on the specific event:
  - Defined surveillance/investigation objectives,
  - Targeted sampling approaches,
  - Initial virus detection reporting criteria (laboratory to epidemiology),
  - Ramp up/ Ramp down criteria.

- Revise testing algorithms to improve efficiency and throughput or meet specific surveillance needs.

- Communicate closely with health department leadership; participate in state health department emergency operations.

- Provide timely specimen collection, testing and biosafety guidance to clinical laboratories and clinicians.

Detailed guidance on pandemic response is beyond the scope of this document. During a large scale event, CDC, CSTE and APHL will coordinate to provide timely direction and support. It is important that information disseminated by CDC, state health officials, and APHL to PHL directors is disseminated to the laboratory staff. Management and technical staff should participate in CDC/APHL conference calls to obtain pertinent recommendations.

SURGE CAPACITY

Financial Resources

Every state will need to determine how to achieve influenza surveillance goals to meet national and state needs. Federal resources (funding, reagents) distributed to states need to be directed principally to activities that support overall national priorities. State/local capabilities beyond those recommended as essential to meet national virologic surveillance goals will require securing sustainable state or jurisdictional funding. When optimizing services and justifying budget requests, PHLs and surveillance programs should work cooperatively to address:

- Essential elements defined in this roadmap for national surveillance.

- Options for shared services among PHLs.

- Scalability of the surveillance system based on available resources.

- State/local specific influenza surveillance expectations or operational issues.

Cost Accounting

Optimizing resources and justifying funding requests will require better cost accounting at the national, state and local level. Results from the 2011 Right Size Influenza Virologic Surveillance Landscape survey showed that 35% of state PHLs (16 out of 45 SPHLS) were unable to provide accurate estimates of influenza testing costs or were only able to provide rough estimates. There are many advantages to effective cost accounting including, but not limited to:

- Identify true cost of virologic surveillance.

- Plan and allocate resources for each influenza season.

- Justify surveillance program and laboratory testing budgets.

- Assess which surveillance components are covered by various funding sources (e.g., federal vs state funds).

- Calculate the cost the PHL absorbs beyond the actual state or federal funds provided.

- Ensure PHLs and programs are good stewards of existing resources.

- Determine and justify the most efficient testing algorithm for various scenarios (see Laboratory Testing Implementation Guidance section for additional information on testing algorithms).

- Write grant proposals.

- Characterize impacts of funding reductions.

The cost of performing influenza surveillance testing varies across jurisdictions. While there is no standard method that can be applied across all jurisdictions to assess costs, at a minimum a cost analyses should include four areas related to influenza surveillance (unpublished APHL internal report): labor, consumable materials, equipment and overhead/miscellaneous.

- **Labor** – Including laboratory, epidemiology/influenza coordinator, and information technology staff salaries, fringe/benefits costs and capabilities.

- **Consumable Materials** – Including material costs for specimen collection materials (if provided to the specimen submitters by the surveillance program or laboratory), submitter incentives (if provided), reagents and testing kits used for extraction and rRT-PCR processes as well as consumables both directly and indirectly associated with PCR testing. If the laboratory is performing any additional tests as defined in the Laboratory Testing Requirements Intent and Implementation Guidance sections, costs per test should be determined for these consumables as well.

- **Equipment** – Including acquisition, service/maintenance and depreciation costs for all equipment used for influenza testing.

- **Overhead & Miscellaneous** – Including costs associated with facilities, surcharges, utilities, transportation of specimens to and from the laboratory, maintaining sentinel provider networks (e.g., provider communication tools), information technology support, training and travel.

It may also be helpful to reference CMS Medicare/Medicaid CPT codes and fee schedules when performing cost analysis. The federal standards for clinical diagnostic reimbursement for testing can help estimate laboratory costs for surveillance testing as well as serve as a comparator to true cost accounting. The CMS fee schedule will vary across states and PHLs should reference the most recent CPT fee schedule for their state. Listed below are some of the relevant CPT codes for influenza surveillance testing that may assist with determining laboratory costs.

- CDC Flu rRT-PCR Dx Panel: 87501 x 2 if assay uses separate wells for Flu A and Flu B.

- If a laboratory uses a commercial multiplex test, reference the 87502 code x 1 for up to two analytes.

- Influenza subtyping: 87503 x number of analytes (e.g., influenza A(H1N1), influenza A(H3N2) and influenza A(H1N1)pdm).

- Virus culture: 87252.

- Virus culture by shell vial: 87254.

- Immunofluorescent identification (if culture positive) and hemadsorption: 87253.

Allocating Available Funds

These questions address suggested processes for cost analysis and coordination needed to optimize funding allocation among those involved in influenza surveillance within the state.

1. Do you have a routine meeting or other process for all involved parties to discuss grant development, planning, fund allocation, and deliverable/benchmark monitoring?

2. Do you have a process to determine how much it costs your jurisdiction to perform influenza virologic surveillance?

   • Example: Perform a detailed cost analysis for both surveillance program and laboratory components. See the cost accounting sub-section above for some helpful tips.

3. Do you have a method or process for equitably allocating funds across program and laboratory elements?

   • Example: Appropriate representatives of the laboratory and surveillance program meet at the beginning of each season and periodically throughout season to discuss allocation of funds and monitor expenditures throughout the season.

4. Do you have a method or process to collaboratively address funding and resource reductions?

5. Are ELC, PHEP, and quality management benchmarks considered in prioritization of funding allocation?

Table 4 is provided as a tool to facilitate funding allocation discussions and to help identify potential funding gaps. Use of this table, or a similar state developed tool, can help elucidate the actual costs of influenza surveillance and provide a basis for discussion and priority setting. This table can be modified to fit a jurisdiction's funding sources and surveillance components. Depending on the level of detail desired, this table can be completed by listing dollar amounts, percentages or simply using checkmarks to indicate which surveillance components are funded by each of these sources in the jurisdiction.

Table 4. Resource Allocation Tracking Table

	ELC	PHEP	State	Other: _____
Influenza Surveillance coordinator				
Epidemiology staff for influenza				
Laboratory staff for Influenza				
Laboratory testing reagents, supplies not provided by CDC)				
LIMS/electronic reporting/IT support				
Specimen collection supplies (e.g., VTM, swabs)				
Specimen transport (e.g., shipping boxes, courier or commercial carrier costs)				
Sentinel provider incentives				
Equipment & equipment maintenance costs, including service contracts				
Laboratory overhead (e.g., travel/training, autoclave/waste, printing/publications/education/press releases)				
Supporting local capacity (e.g., local PHLs and programs)				
Other: _____				
Other: _____				

FINANCIAL RESOURCES

Resource Justification

As previously mentioned, state/local capabilities beyond those recommended as essential to meet national virologic surveillance goals will require securing alternate, supplemental, sustainable state or local funds. As federal and state funds to support influenza surveillance decline in this post-pandemic period, it may be necessary to explore options for alternate non-traditional funding sources such as research grants or academic partnerships for special studies. Justifying resource needs requires an accurate estimate of surveillance system costs and funding needs/gaps.

Funding Fact Sheet Tool for States

Fact sheets and success/impact stories are useful tools when requesting additional funds and resources to meet surveillance requirements. Appendix D provides a funding justification "fact sheet" template that can be modified and used as a tool by public health laboratory leaders to highlight a specific jurisdiction's program impact and funding needs for influenza virologic surveillance. To see other example fact sheets, please visit http://www.aphl.org/policy/facts/Pages/default.aspx.

Intended Use: Public health laboratory leaders can customize this document using the editable version of Appendix D located at http://www.aphl.org/aphlprograms/infectious/influenza/Documents/ID_2013July_Editable-Funding-Fact-Sheet.docx. This is intended to be used to highlight surveillance program successes, impact, and funding needs to non-public health audiences such as policy makers and other government officials.

Instructions for Use: To create a jurisdiction-specific fact sheet, go to http://www.aphl.org/aphlprograms/infectious/influenza/Documents/ID_2013July_Editable-Funding-Fact-Sheet.docx for an editable version of Appendix D that users can modify to highlight their own program's success and funding needs. For example, the current template includes a story for the 2009 H1N1 pandemic to provide an example of a captivating story structure. Users should replace this story with a jurisdiction-specific story. Finding a story that is both recent and has major impact on the specific jurisdiction will improve the reception by target readers.

Users will notice that the fact sheet uses basic, non-scientific language; the level of technical detail included should be tailored to the target audience. In the example language in Appendix D, the target audience would be a lay person that has no prior knowledge of influenza testing, surveillance, or public health laboratories. For example, the term such as "influenza" is replaced with "flu," a widely recognized, colloquial reference.

The template also includes a box to highlight funding needs. It is recommended that this box only include the funding needs being requested of the specific target audience. In some jurisdictions this fact sheet is more useful for promoting impact and success, in which case the funding needs box can be deleted. Lastly, keep the fact sheet focused on a specific topic and/or request. The recommended maximum length is approximately 900 words or two pages to allow for printing on both sides of a single sheet.