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.

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). Conversly, 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.

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**			
	Confidence Level (%)	Margin of Error (%)	High Season		Low Season	
			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).

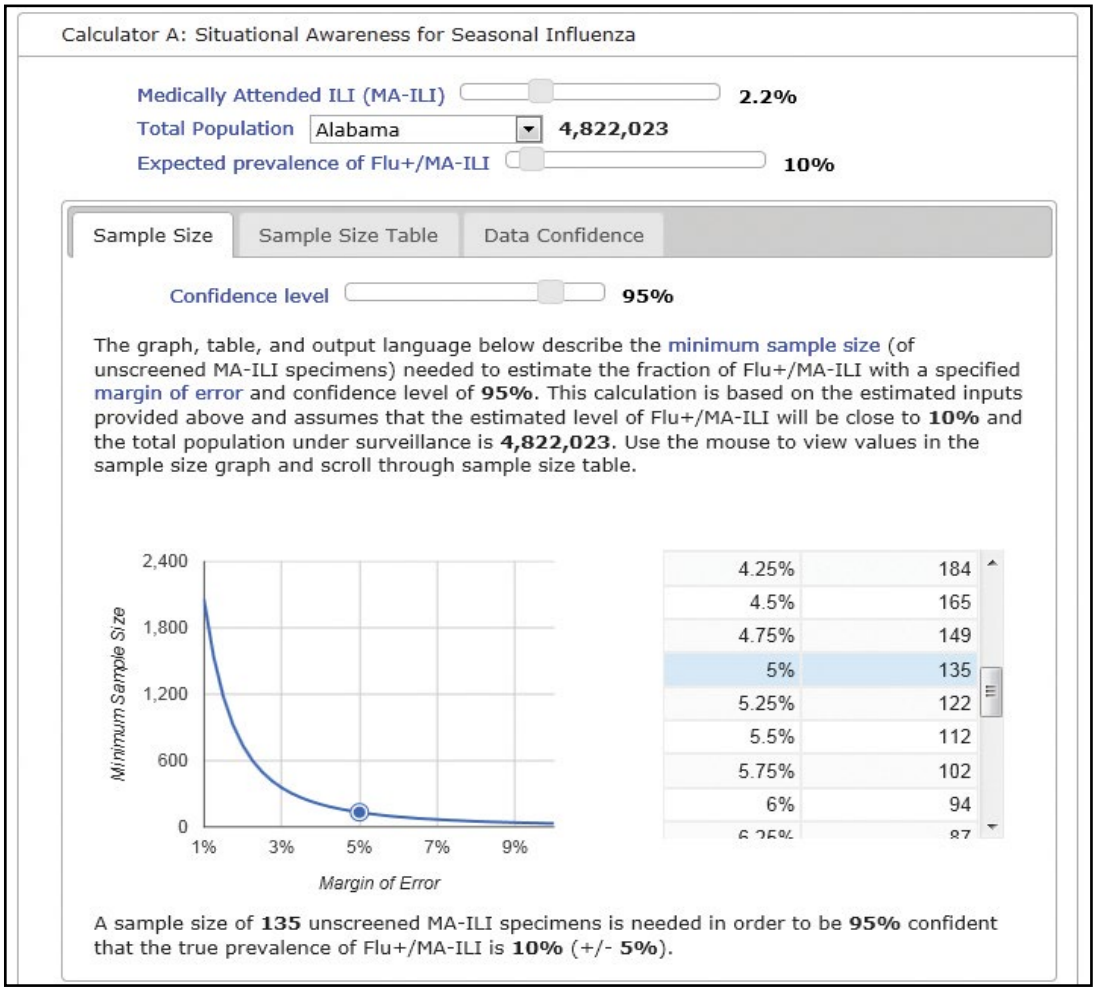

Figure 5. Screen shot of Situational Awareness sample size calculator demonstrating user inputs.

- **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.

- **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.

- **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.

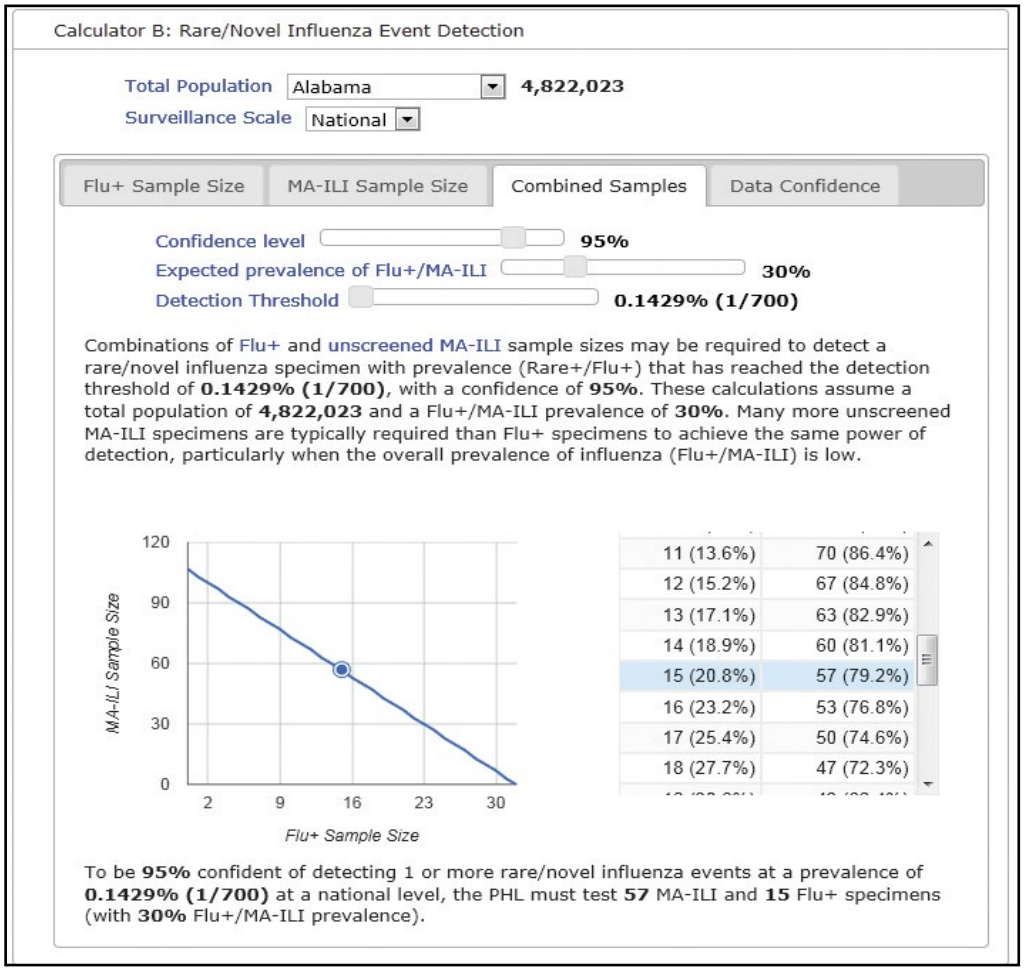

Figure 6. Screen shot of Novel Event Detection sample size calculator demonstrating user inputs.

- **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. **<u>Detecting/Monitoring Antiviral resistance</u>**

- **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. **Rare/Novel Influenza Event Investigation**

- **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.

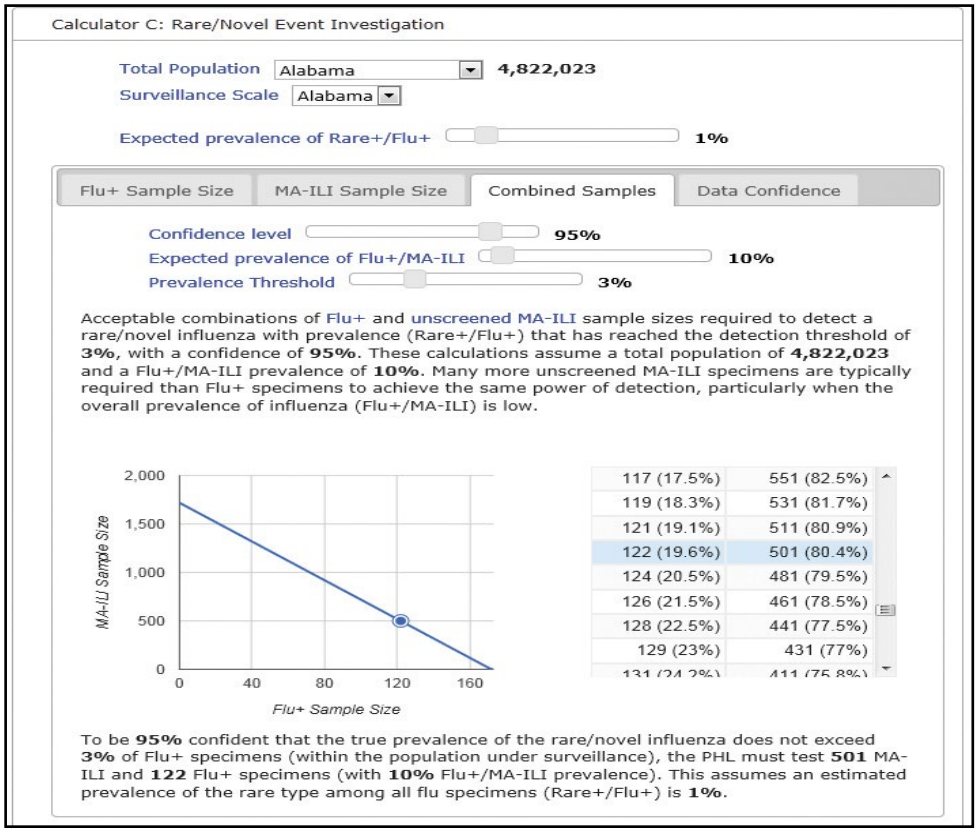

Figure 7. Screen shot of Rare/Novel Influenza Event Investigation sample size calculator demonstrating user inputs.

- **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.

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

[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.

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.