Objectives: Thresholds and Representativeness

In order to promote a more statistical, systematic approach to virologic surveillance, thresholds for the following key surveillance objectives have been established. The thresholds are necessary to "right-size" the virologic system – this number is used to calculate the number of specimens that should be tested to ensure adequate confidence in surveillance data as well as detection of novel viruses at a point where intervention can be effective. **In this context, a threshold is defined as the level which triggers some action.** The action may be as simple as defining a point in the influenza season, or initiating an investigation following detection of a novel virus as defined in the CDC's Interim Guidance on Use of Intervals, Triggers, and Actions for Novel Influenza A (H1N1) Response (ref).

Routine surveillance includes situational awareness, novel event detection and antiviral resistance monitoring. At a minimum, the system should be sized to achieve state level situational awareness needs, and national novel event and antiviral resistance thresholds. Efficiencies in the total number of specimens that need to be tested can be achieved by using a single sampling approach to address as many surveillance objectives as possible. The surveillance program should also have the capability to establish targeted surveillance of specific populations if needed.

1. Situational Awareness: Virologic surveillance provides confirmation of when and where influenza viruses are circulating to inform clinical decision making and public health interventions.
   a. Surveillance Objective: determine the beginning and end of the influenza season and monitor the prevalence and spread of influenza viruses throughout the year.
   b. Threshold: 10% prevalence of influenza positive specimens among total ILI specimens submitted to a public health laboratory or the total national system over a two week consecutive time period.

      While there is no specific threshold for action, the CDC has traditionally established the start of influenza season at a threshold of 10% positivity, calculated based on positivity of specimens submitted to the PHLs for testing by ILINet and other specimen providers, and the number of pre-screened positive influenza specimens received at PHLs. This value roughly corresponds to the CDC ILINet Seasonal Baseline where the percentage of outpatient visits for influenza like illness reaches 2.2%.

      The 10% positivity threshold has been selected for use in the right size situational awareness sample size calculation based on this historical precedent. Calculation of the sample size is made using assumptions regarding medically attended ILI (MA-ILI) based on historical data. State and local surveillance programs may use alternate criteria for declaring the start or end of the influenza season. Additionally, 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.

      In the past, ILI specimens tested in state public health laboratories were largely unscreened, i.e., not tested by the provider. Today, a significant portion of specimens submitted to public health laboratories may be prescreened positive for influenza by the

submitter, (i.e., tested positive using a commercially available influenza test), which can greatly alter the PHL positivity rate. The increased reliance on pre-screened specimens, and the higher sensitivity of PCR methods used more commonly in many clinical laboratories and in all state public health laboratories, may bias the influenza prevalence calculations, impacting the assessment of the scope or severity of the influenza season. Ideally, the percent positivity should be determined using specimens that have not been pre-screened to the greatest extent possible. If data from clinical laboratory testing is being used for situational awareness, at a minimum ensure that the data is coming from sites that are performing high quality testing, and using sensitive methods such as rRT-PCR. Future revisions to this threshold may be needed in the context of changing testing and specimen submission practices.

c. Representativeness: specimens submitted for routine virologic surveillance to inform community, state and national situational awareness should be broadly representative of the population as a whole (age, geography, risk groups, disease severity).

2. Novel event detection: Virologic surveillance detects the emergence of reassortant, animal origin or completely novel virus subtypes in humans. The initial detection of a novel virus is always laboratory-dependent, and may occur anywhere in the U.S. The sensitivity of the system to detect a novel virus relies on all states contributing at a reasonable level proportionate with their population.
   a. Surveillance Objective: Detect a novel influenza virus among influenza positive surveillance specimens tested in all states at a low enough threshold for effective intervention and control measures. Note: This objective relates to the initial detection of a novel virus, which generally occurs as part of routine surveillance. Investigation of a novel event (the deep-dive) after initial detection is a separate objective and is discussed in more detail in the Sampling Requirements and Implementation sections.
   b. Threshold: 0.5% (1/200); one novel virus among 200 influenza virus positive specimens aggregated *at the national surveillance level* over a one week period. A minimum threshold of 1/165 may be used for determining the sample size in states with limited testing capacity; this approximates the prevalence at which the 2009H1N1 influenza virus was detected. Application of a less sensitive threshold for detection (e.g. 1/150, 1/100) would mean that more novel viruses are circulating prior to detection and impair disease prevention and control efforts.

      Using the same detection threshold for identification of novel viruses at a state level (i.e., 1/200 among flu positive specimens tested in the state) would require a significantly larger sample size to achieve an adequate data confidence level. The resources and capacity are generally not adequate to test the number of specimens needed to generate statistically powerful novel event detection data at the state/local or even regional level.
   c. Representativeness:
      i. Routine Surveillance: Novel event detection is a component of routine virologic surveillance, specimens should be broadly representative of the population as a whole (age, geography, risk groups, disease severity).

ii. Targeted Surveillance: Detection of a novel virus that emerges within the U.S. may be enhanced with more targeted surveillance in specific populations or risk groups, based on the most current information of risk for novel virus emergence (e.g., returning travelers from high risk areas with ILI).

3. Vaccine Virus Selection: Virologic surveillance provides specimens to CDC for antigenic and genetic characterization to determine whether the circulating strains match the seasonal vaccine strains in "real time", and to inform annual vaccine virus selection. Submission of specimens should remain consistent throughout the season.
   a. Thresholds for the degree of difference between circulating viruses and vaccine strains are not defined here, as these criteria are more appropriately established seasonally by the WHO vaccine virus selection experts. Due to seasonal variability in subtype prevalence and the data and virus needs for vaccine virus selection and vaccine candidate development, CDC will provide specific guidance on specimen submission requirements at the beginning of the season, and may adjust submission requirements throughout the season as needed. Every PHL participating in virologic surveillance should submit specimens to CDC (or CDC-designated laboratory) in accordance with annual guidelines.
   b. Representativeness: Surveillance sampling strategies to ensure appropriate representativeness for vaccine virus selection should prioritize:
      - Timeliness – the most recent viruses.
      - Type and Subtype – viruses representing all circulating types and subtypes. Oversampling of less prevalent subtypes may be necessary to ensure an adequate number of viruses are available for antigenic and molecular characterization and vaccine candidate development.
      - Geographic – CDC should test viruses with sufficient diversity to be representative of the U.S. at a regional level; PHLs should ensure that specimens submitted to CDC are representative of the entire state.
      - Disease Severity –viruses representative of a range of disease severities (from outpatients to fatal cases). .
      - Age - age representativeness is not an important factor for vaccine virus selection.

4. Antiviral Resistance: Virologic surveillance testing to detect antiviral resistance is performed using molecular methods for detection of resistance markers AND phenotypic functional resistance testing, which requires viable virus. If surge antiviral resistance testing capacity is needed, genotypic testing (i.e., pyrosequencing) would be used to meet testing demand.
   a. Surveillance Objective: Detect antiviral resistance virus among influenza positive surveillance specimens tested across all states at a low enough threshold for effective intervention and control measures. Currently the majority of antiviral resistance surveillance testing is focused on oseltamivir and is performed at CDC on the same viruses that are submitted for vaccine virus selection. Some state PHLs perform pyrosequencing for molecular markers of antiviral resistance, these results should be reported to CDC for

inclusion in national surveillance FluView reports. National "percent resistance" is determined using all sources of data.

b. Thresholds:

- *National threshold:* detect oseltamivir resistance at or below 5% prevalence among each influenza A subtype or influenza B positive specimens tested at the national level. Seasonal oversampling of certain subtypes may be necessary if increased transmissibility or increasing numbers of resistant viruses are detected. Detection of antiviral resistance approaching or exceeding defined thresholds will initiate investigations and increased testing. Models may be used to assess sample size needed at other prevalence levels. These recommendations or thresholds may change over time depending on resistance trends, or if new viruses with resistance markers emerge. A sustained increase or an unexplained jump in number of resistant viruses may trigger an investigation and expanded testing. Confirmed, substantial increases in resistance may result in changes to clinical treatment guidance, depending on the overall influenza prevalence, resistant virus prevalence, and geographic/temporal spread.

  If there is an increase in influenza antiviral resistance outside of the U.S., the right size virologic surveillance thresholds may be lowered, targeted surveillance may be implemented, or additional samples may be tested to increase the confidence and decrease the error in detecting a 5% prevalence of resistant viruses.
- *State and local thresholds*: Using the same antiviral resistance detection threshold at a regional or state/local level would require would require a significantly larger sample size to achieve an adequate data confidence level. Although some jurisdictions may wish to report antiviral resistance surveillance data at the local/state level to help inform local provider's clinical management decisions, the resources and capacity are generally not adequate to test the number of specimens needed to generate statistically powerful antiviral resistance testing data at the state/local or even regional level. State and local laboratories choosing to perform antiviral resistance testing are encouraged to utilize sample size models to assess statistical confidence of prevalence rates generated from PHL testing. All PHLs performing pyrosequencing are required to report test results to CDC in a timely manner to be incorporated into national surveillance data.

c. Representativeness:

All surveillance samples submitted to CDC (or CDC-designated laboratory) for antigenic characterization are tested for antiviral surveillance. Surveillance sampling strategies to ensure appropriate representativeness for monitoring antiviral resistance should prioritize:

- Timeliness – recent specimens provide the most valuable data. Testing early and peak season specimens is especially important to monitor changes in antiviral resistance profiles. (Note: Surveillance testing is generally not sufficiently timely for individual patient treatment decisions. Individual results are not reported. CDC does provide diagnostic testing on a case specific basis. Contact xxx for more information).

- Subtype – viruses representing all circulating subtypes should be tested. Oversampling of certain subtypes may be recommended based on seasonal criteria or emergence of resistant viruses.
- Geographic – sufficient diversity to be representative of the US.
- Disease Severity – submit a mix of both outpatient and inpatient specimens.
- Outbreaks/clusters – will be investigated to evaluate geographic spread and drug exposure.
- Age - Age representativeness is not considered to be an important factor for this surveillance objective.

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 Requirements 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 described 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**

   The primary care tier provides data and specimens for the influenza surveillance system. Selected ILINet providers and/or other primary care sites send respiratory specimens to a public health laboratory according to jurisdictional sampling criteria. ILINet and other clinicians may elect to test specimens using a point of care rapid influenza diagnostic test (RIDT) if one is available in their clinical setting. Hospitalized patients, as well as patients seen by non-ILINet health care providers may have specimens tested by fluorescent antibody, virus culture, or molecular methods in a clinical/commercial laboratory. State and local public health laboratories make up the third tier of influenza surveillance. These laboratories typically perform rRT-PCR testing to type and subtype influenza viruses in clinical specimens.

   A. ILINet or other specimen providers (Tier 1)
      - 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 good source of specimens, so alternate 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.

- 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 pre-screening with tests that have variable sensitivity, and may not detect novel or drifted viruses (i.e., give false negative result)[1]. Outside of influenza season, all RIDT positives should be submitted to the PHL. In a novel event investigation, oversampling pre-screened positives may be appropriate if the tests used are high performing with demonstrated reliability for detection of the virus of interest.
- 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,
  - feedback and data to submitters, including influenza test results and/or aggregate results of testing for other respiratory pathogens if performed,
  - training.
  - Some PHLs and/or surveillance coordinators have also elected to provide RIDT kits to incentivize specimen submission.

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.

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 labs also serve as reference labs 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

[1] Balish A, Garten R, Klimov A, Villanueva J. Analytical detection of influenza A(H3N2)v and other A variant viruses from the USA by rapid influenza diagnostic tests. Influenza Other Respi Viruses 2012;September 18 [Epub ahead of print].

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). Specimens from clinical laboratories should include both influenza positive and negative samples. 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.

C. Public Health Laboratories (Tier 3):

Every public health laboratory 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 CDC-designated laboratory (Tier 4) for national surveillance purposes, including annual vaccine virus selection. Viruses from these specimens may also be used to manufacture seasonal influenza vaccines. The 2013-2014 vaccine will contain A/California/7/2009pdm09-like(2009 H1N1) virus, A/Texas/50/2012(H3N2) and B/Massachusetts/2/2012-like (B/Yamagata lineage) virus.

Laboratories should submit specimens/viruses 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 pre-season and throughout the season as needed to update surveillance guidance. Participation in these teleconferences is strongly encouraged.

PHLs performing virus culture should send both the original clinical material and the virus isolate to CDC (or CDC-designated laboratory). Providing the virus isolate along with the original clinical material allows for more rapid antigenic characterization at CDC. Maintaining virus culture requires specialized cell lines and well trained staff with sufficient expertise to perform high quality testing. PHLs opting not to perform virus culture should submit original clinical specimens with a minimum volume of xx mls.

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. The two examples below demonstrate the criteria that should be considered by the PHL when selecting the specimens that will be sent to CDC for routine national surveillance purposes.

- In a two week period, the PHL testing yields 50 A/H3, 1 A/2009H1, and 5 Influenza B positive specimens. Send to CDC or the CDC-designated laboratory: the A/H1 specimen, one Flu 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/2009H1 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/2009 H 1 and one Flu B specimen.

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[2] viruses that may represent a novel subtype should be submitted to CDC within 24 hours of detection.

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 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 specific level of data confidence for situational awareness, novel event detection and novel event investigation. In addition, these calculators can also be used to determine the confidence level of data derived from an existing sample of ILI patient specimens.

Some state and local public health laboratories 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.

Efficiencies in the total number of specimens that need to be tested can be achieved by using a single sampling approach to address as many surveillance objectives as possible. For example, situational awareness and novel event detection rely on samples collected and tested for routine surveillance. At the national level, routine antiviral resistance surveillance testing currently uses the same samples as vaccine strain selection testing. 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 public health laboratories are encouraged to use sample size calculators or pre-calculated sample size tables to achieve a more scientific, statistically based sample size that supports surveillance objectives. Specimen sampling approaches should be established to prioritize collecting an adequate number of specimens for detection of novel events based on national thresholds, while at

[2] 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.

the same time providing sufficient number of representative for overall situational awareness at the state level. For many states, the number of samples to be tested for each of these objectives is very similar. However, for smaller states, the number of samples necessary to achieve high confidence in situational awareness state level data will be much higher than the number of samples needed to contribute to national novel event detection thresholds. Outside of influenza season, achieving statistical confidence will not be possible in most states; 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 novel event or new virus is detected. Therefore the surveillance program should have the capability to establish targeted surveillance of specific populations if needed. CDC will provide guidance to state epidemiologists and PHLs on the specific risk factors and need for enhanced surveillance (e.g., HPAI H5N1 risk factors, swine exposure). However, the current version of the novel event investigation calculator may not be useful in these situations, future of 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 the table below. Understanding how these variables affect sample size and data confidence levels is important for generating valuable surveillance data.

	Relationship to Sample Size
Confidence Level	The confidence level is the amount of uncertainty 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 also increases.
Population	This is the population under surveillance. For routine influenza surveillance, this is the number of people in your state with ILI. As the population size increases the sample size increases.
Medically attended ILI (MA-ILI)	The population of individuals with influenza-like illness 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 generalist physician 2 times per year, and that r the percentage of outpatient visits that are for influenza like illness is 2.2% at CDC ILINet Seasonal Baseline).

Expected Prevalence	In the calculators, this is the prevalence that you expect to calculate or the level of detection you wish to achieve. For the purposes of calculating sample size, the expected prevalence refers to the prevalence of influenza positive specimens among the number of 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 you scale your margin of error to align with your 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 your margin of error to your predicted prevalence.

b. Choosing an appropriate confidence level and error rate:

The number of samples to be tested will vary depending on the Confidence Level and Margin of Error used in the calculators. Selecting a lower confidence level increases the amount of uncertainty in the calculated prevalence. A higher margin of error means that more error can be tolerated. Stakeholders and exercise participants identified optimal, mid-range and minimal confidence levels and error rates or thresholds for the two objectives that comprise routine surveillance.

	Situational Awareness		**Novel event Detection**	
	Confidence Level	Margin of Error	Confidence Level	Threshold
Optimal	95	5	95	1/200
Mid-range	90	5	95	1/165
Minimum	85	5	90	1/165

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. **Assumptions:** 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 these calculators are based on existing and/or historical ambulatory care and seasonal influenza data.

d. **Using the Sample Size Calculators:** The virologic surveillance sample size calculators are available as a web-based tool at www.to be determined.org. Basic information on the intent of the calculator for each of the key surveillance objectives, along with inputs and output examples is provided here. A more detailed "user guide" with instructions for using the web-based tools is provided in Appendix B. The appendix also includes tables with pre-calculated sample sizes for

situational awareness and novel 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- Flu^+/medically attended ILI (MA-ILI)**

- **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 Flu^+ among 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 influenza like illness 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% at a specified confidence level and error rate?
- **Assumptions:** Each person in the US visits an emergency room or generalist physician 2 times per year. 2.2% of medical visits are for ILI outside of influenza season ILINet baseline) (ref). The providers are randomly selecting patients with ILI for surveillance testing. The specimens tested were either not pre-screened, or submitted randomly irrespective of test result.
- **User Inputs:**
  - 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.
  - 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 influenza like illness.
  - Expected prevalence of Flu+/MA-ILI: Input your 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.
  - Specified confidence level: The optimal level of confidence for situational awareness is 95%, the minimum should be no less than 85%.
  - Acceptable margin of error: An acceptable margin of error should be no greater than 5%.
- **Output example:** A sample size of 132 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 flu 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%, you can be 70 % +/- 3% that the true prevalence is 10%.

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

- **Surveillance Objective:** Detect a rare event/novel influenza virus among influenza positive surveillance specimens tested in all states at a low enough threshold for effective intervention and control measures.
- **Threshold:** State surveillance systems should collectively test a sufficient number of specimens to detect a novel influenza virus at a prevalence threshold of 0.5% (1/200) Novel Flu+/Total Flu+ specimens at the aggregated national level. At a minimum, a threshold of 1/165 may be used for determining the sample size in states with limited resources.
- **Surveillance Question:** How many specimens does the PHL need to test to allow the national surveillance system to detect a rare/novel virus at 0.5% 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 novel 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 under-sampled, resulting in an overestimate of the confidence level and underestimate of the error.
- **Options:** The novel event detection sample size calculation can be made based on a) the number of positives already identified as Flu+ by a clinical laboratory or the PHL, b) the number of MA-ILI specimens, or c) a combination of both. Although testing prescreened Flu+ specimens decreases the total number of specimens needed to meet the recommended threshold and confidence level, using only specimens that are Flu+ received from clinical laboratories may reduce the sensitivity of the system to detect novel 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.
- **User Inputs:**
  - Population-: The input is the total population under surveillance (e.g., state population).
  - 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 needs to tests 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 1/200 threshold will be significantly larger than that needed for the national threshold.
    - Specified confidence level: The optimal level of confidence for novel event detection is 95% at 1/200, the minimum should be no less than 90% at 1/165.
    - Expected 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, other percent positivity as needed throughout the year.
- **Output examples:**
    - Number of Flu+ specimens: To be 95% confident of detecting 1 or more rare events at a prevalence of 1/200 at a national level, the PHL must test 11 Flu+ specimens per week.
    - Number of MA-ILI specimens: To be 95% confident of detecting 1 or more rare events at a prevalence of 1/200 at a national level, the PHL must test 108 MA-ILI specimens per week.
    - Combined number of Flu+ and MA-ILI specimens: To be 95% confident of detecting 1 or more rare events at a prevalence of 1/200 at a national level, the PHL must test 58 MA-ILI and 5 Flu+ specimens per week.
    - Combined number of Flu+ and MA-ILI specimens (state level): To be 95% confident of detecting 1 or more rare events at a prevalence of 1/200 at a state level, the PHL must test 257 MA-ILI and 411 Flu+ specimens per week.
- **Alternate calculation (sample power):** Determine the level of confidence that a novel 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 x Flu+ and y MA-ILI specimens, you can be 70% confident that the novel virus would be detected at a prevalence of 1/200.

iii. **<u>Rare/Novel Event Investigation</u>**

- **Surveillance Objective:** Determine the prevalence of the novel virus (Novel Flu+/Total Flu+) within a state following detection of a novel influenza virus; c*onfirm that the prevalence of a rare event does not* exceed a specific percent positivity. Investigation of a novel event is typically performed using enhanced, targeted surveillance.
- ***Threshold:*** There are no defined thresholds for novel event investigation, as specific situations and jurisdictional considerations may warrant different thresholds. In general, if the novel event was detected at 1/200, the investigation threshold should be between 1-5%.

- **Surveillance Question:** Once a novel 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*+?*
- **Assumptions:**
  - Specimens are collected randomly.
  - 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 novel 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 an H1N1 type event, where the at-risk population group is unknown. 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
- **User Inputs:**
  - Population: The input is the total population under surveillance (e.g., state population).
  - Surveillance scale: STATE vs national. The default is state because the investigation of the novel event would occur locally.
  - Expected prevalence of Rare+/Flu+: This is the percent positivity of the rare event that you want to confirm has not been exceeded.
  - Specified confidence level: The optimal level of confidence is 95%, the minimum should be no less than 85%.
  - Expected 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.
- **Output examples:**
  - Number of Flu+ specimens: To be 95% confident that the true prevalence of the novel virus does not exceed 1% +/- 2% of Flu+ specimens, the PHL must test 478 Flu+ specimens per week.
  - Number of MA-ILI specimens: To be 95% confident that the true prevalence of the novel virus does not exceed 1% +/- 2% of Flu+ specimens, the PHL must test 4778 MA-ILI specimens per week.

- Combined number of Flu+ and MA-ILI specimens: To be 95% confident that the true prevalence of the novel virus does not exceed 1% +/- 2% of Flu+ specimens, the PHL must test 533 MA-ILI and 359 Flu+ specimens per week.

- **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 novel virus among all flu positive specimens (Rare+/Flu+) is **1%**, you can be 75% confidence that the true prevalence does not exceed 1% +/-1%. (This assumes that **10%** of MA-ILI+ patients are Flu+).

iv. **<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.
- **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 or influenza B Flu+ specimens does the laboratory need to test 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)?
- **User Inputs: T**he Flu+ tab of the novel event detection calculator can be used to determine sample size for this objective.
  - 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.
  - Specified confidence level: The optimal level of confidence for antiviral resistance is 95%, the minimum should be no less than 85%.
  - Expected 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.
- **Output example:**
  - Number of Flu+ specimens: To be 95% confident of detecting antiviral resistant Influenza A 2009H1N1 viruses at a prevalence of 5% among influenza A 2009H1N1

positive specimens tested at the national level, the PHL must test x 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 Flu+ specimens tested and the number of MA-ILI specimens tested.
- **Alternate output:** If the laboratory tested x Flu+ and y MA-ILI specimens, you can be 70% confident that the antiviral resistant viruses would be detected at a prevalence of 5%.

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. If specimens are being sent to the PHL for diagnostic testing (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.

   b. PH Labs submission to CDC or CDC-designated laboratory

   Laboratories 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 section. 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[3] for novel virus reporting.

[3] 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.

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/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 within24-72 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 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 4-8°C until testing is performed. Testing ideally should be performed as soon as possible. If a delay of more than 2-3 days until specimens are tested is anticipated, specimens can be frozen at -70C. 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. 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. It is not reasonable to expect the providers and clinical labs to assume these costs for surveillance testing.

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 labs to the PHL and from the PHL to CDC or CDC-designated laboratories. Specimen transport must comply with a variety of federal (DOT, USPS) and international (IATA) regulations that ensure that specimens and infectious materials are properly packaged and safely shipped (ref). 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 whenever possible 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. 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: 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. prescreened specimens: efforts should be made to limit sampling of prescreened (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. At a minimum, data should differentiate pre-screened from unscreened specimens. If prescreened 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.