1. **What is MA-ILI and how do I know what to select for this variable?**

MA-ILI stands for “Medically Attended – Influenza like Illness (MA-ILI)”. This is the population of individuals with influenza like illness (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 ambulatory care setting 2.5 times per year, and that the percentage of outpatient visits that are for ILI is 2.2% at CDC ILINet Seasonal Baseline. This number can be changed throughout the season as needed.

To be the most accurate, this number should be calculated using jurisdiction-specific data, such as ILINet data. However, an alternate source for this number is the U.S. Outpatient ILINet data which can be found on CDC’s FluView under “Outpatient Illness Surveillance”.

2. **What does Flu+ represent and how do I know what to select for this variable? What constitutes influenza positive specimens (e.g. rRT-PCR, RIDTs) in the context of the calculators?**

Flu+ represents the number of specimens testing positive for an influenza virus among specimens collected from patients with MA-ILI. Each user can define what constitutes an influenza positive specimen for this variable, but this should be considered in how the output of the calculator is interpreted.

For Calculator A: Situational Awareness for Seasonal Influenza, the Flu+ number is used to determine an estimated proportion of Flu+/MA-ILI. Although the actual prevalence (Flu+/MA-ILI) may differ from the estimated prevalence, but this approximation still provides an important baseline for determining sample sizes.

For Calculators B and C, Flu+ is used to determine the estimated prevalence of a rare/novel influenza virus among all Flu+ specimens. Like Calculator A, the actual prevalence may differ from the estimated prevalence, but this approximation still provides an important baseline for determining sample sizes.

3. **Am I able to calculate a sample size for a special or subset population such as a city, county or region?**

Yes, under the Total Population dropdown list, select “Other” which is located at the bottom of the list. Once you select “Other”, an input field will appear to the right allowing you to type in a custom population size.
4. **What is a confidence level?**

This is the amount of certainty that the true prevalence is equivalent to the estimated prevalence. The higher the confidence level, the more confident the public health laboratory (PHL) can be that the true level of Flu+/MA-ILI in the PHL’s population falls within the estimated level of Flu+/MA-ILI. Intuitively, high confidence levels and small margins of error require many samples, while low confidence levels or large margins of error require fewer samples.

5. **What is margin of error?**

This is the amount of error that can be tolerated and when using laboratory samples to estimate Flu+/MA-ILI, the PHL will calculate an expected value plus or minus a margin of error. For example, the PHL might calculate 10% plus or minus 2%, which means that the Flu+/MA-ILI estimate will fall somewhere between 8% and 12%. The smaller the margin of error is the more precise the PHL’s estimate of Flu+/MA-ILI. Intuitively, high confidence levels and small margins of error require many samples, while low confidence levels or large margins of error require fewer samples.

6. **What should I select for confidence level?**

The percentage you select for confidence level depends on the surveillance question you are trying to address and the importance or value of having a high level of confidence. As you lower the confidence level, the sample size will decrease so the user will need to determine the balance between feasibility and desired confidence.

If you are trying to calculate a sample size to meet the thresholds listed in the Influenza Virologic Surveillance Right Size Roadmap, please refer to Table 3 in the Sampling Implementation Guidance section for the optimal, mid-range, and minimum values.

7. **What are the Data Confidence tabs and how/when should I use them?**

The data confidence tab allows the user to work in reverse from the other tabs. This means that if you already have a pre-specified sample size, you can use this tab to determine your level of confidence and margin of error.

8. **In the Data Confidence tabs on the calculators, what is the MA-ILI Sample Size?**

See question #7 for additional information on the Data Confidence tab. The MA-ILI sample size input field on this tab allows the user to input their specified sample size. From this number the calculator will provide outputs on confidence level and margin of error.
9. **Can I use non-PHL influenza testing data (e.g. clinical/commercial laboratory data) to reach my target sample size?**

Yes, you can use other testing data to meet your target sample size. That being said, you will need to consider the limitations of incorporating this data, particularly for a rare/novel influenza event. For instance, can the test data you are using detect the rare/novel influenza virus? How does the sensitivity and specificity of the test methods included in the alternate data compare to the PHL testing methods? It is important to ensure that the interpretation of the sample size takes these factors into consideration.

10. **My laboratory receives a lot of screened specimens that have already tested positive for influenza. How does this bias and affect my sample size calculations?**

Calculator A: Situational Awareness for Seasonal Influenza assumes that the specimens tested were either unscreened or submitted randomly irrespective of test result. This is important because the purpose of this calculator is to determine the true prevalence of influenza in the sample population.

For Calculators B and C, there is the option to supplement unscreened MA-ILI specimens with screened influenza positive specimens by using the Combined Specimens tab. 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 rare/novel influenza virus). If you are using a mix of screened influenza positive specimens and unscreened specimens, select the Combined Samples tab and then select the appropriate inputs. A graph and table will be generated that will allow you to determine the number of influenza positive screened specimens and unscreened specimens you would need to meet your selected inputs at a blend level (combination of Flu+ and unscreened MA-ILI specimens) that reflects your available specimens.

11. **For the Rare/Novel Influenza Event Detection and Investigation calculators, what should I select for the surveillance scale?**

The surveillance scale input allows the user to indicate whether surveillance is being conducted at the national or state/regional level.

For Calculator B: Rare/Novel Influenza Event Detection, 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 rare/novel influenza 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.
For Calculator C: Rare/Novel Influenza Event Investigation, the default is state, meaning that all states are able to calculate the number of specimens to test for an investigation of a rare/novel influenza event at a specific threshold within their state. A national scale means 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. Note: 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. Also note that Calculator C relates to a scenario in which the user is determining the number of specimens needed once a rare/novel influenza event has already been detected.

12. When calculating sample size, does it include both Influenza A and B samples? Just Influenza A samples? All Subtypes?

The MA-ILI specimens used to calculate the sample size will depend on the scenario you are using when calculating sample sizes. For example if you want to know the prevalence of influenza circulating in your jurisdiction then you would include both influenza A and influenza B specimens. However, if you just wanted to know the prevalence of only H3N2v cases in your jurisdiction then you would only include influenza A, H3N2v specimens. In many cases, the sample size will include both influenza A and influenza B samples.

Specifically, for calculators B and C when selecting national for the surveillance scale, the expectation is that the sample size will include all types (A and B) and all influenza A subtypes. This will help minimize bias among the specimens submitted to detect a rare/novel influenza virus. However, when selecting state for the surveillance scale, you could adjust the types of specimens depending on the scenario you are using.

13. What is the time frame for the calculators? Are the sample sizes what need to be collected daily? Weekly?

Calculator A: Situational Awareness for Seasonal Influenza assumes that the samples sizes listed are being collected on a weekly basis.

Calculators B and C do not have a timeframe built into them. The timeframe for the calculators will depend on the scenario you are using when calculating the sample sizes. In many cases, this will be weekly or bi-weekly.

14. How were the thresholds developed?

The thresholds have been discussed and determined based on stakeholder input at the March 2012 stakeholder meeting in Atlanta. Historical data (from past influenza seasons) was also examined to determine the thresholds.
15. Are we testing enough?

It is important to review the entire roadmap document, specifically the requirements section, to determine areas where your system/jurisdiction meets the requirements and where there are gaps within your system/jurisdiction. Within the roadmap, the Sampling Implementation Guidance section provides additional details on how to use the sample size calculators to help guide the amount of testing that your jurisdiction could achieve for both national surveillance and your own jurisdiction surveillance objectives. The calculators are meant to be a tool to help guide your influenza virologic surveillance activities but should not be considered the absolute answer for sampling. If you want to get a quick feel for how your current testing volumes compare to the roadmap thresholds, you can look at the quick glance tables in Appendix B of the Influenza Virologic Surveillance Right Size Roadmap (page 100).

16. What if we can’t meet the recommended thresholds listed in the Roadmap?

We recognize that not every jurisdiction is meeting the thresholds at this time; we aren’t even meeting them at a national level for every threshold yet. However, we see these as a goal to work towards and to help justify future resources. That being said, in some cases you may never be able to reach the recommended thresholds.

However, you can use the sampling section and the sample size calculators to look at other ways you may be able to meet the recommended thresholds such as lower confidence levels and higher margins of error. This can then be turned in to an advocacy piece to explain what you are accomplishing and what is needed to meet desired thresholds for influenza virologic surveillance.

17. How did we come up with these numbers?

Please consult the Influenza Virologic Surveillance Right Size Sample Size Calculator User Guide for additional information on the specific equations and assumptions used for the sample size calculators. If you have additional questions on the statistics behind the calculators after consulting the User Guide, please contact fluquestions@aphl.org.

18. Does it matter if our specimens are screened before arriving at the PHL when we calculate sample size? Can we use screened and unscreened specimens to calculate sample size?

The influenza virologic surveillance system contains inherent biases due to the complexity of the sampling system; however, at this time, the calculators do not take into account sampling bias. Unscreened specimens are preferred and efforts should be made to limit sampling of screened (influenza positive) specimens. 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 screened from unscreened specimens. For additional information, please refer Sampling Implementation Guidance section (pg. 66).
When using Calculator A: Situational Awareness for Seasonal Influenza, a combination of Flu+ (screened) and unscreened MA-ILI samples can be used. However, as mentioned above, unscreened specimens are preference. If those samples are not available, a random mix of positive and negative specimens should be used.

For Calculators B and C, the 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.

19. Are the sample size outputs on the various calculators for my jurisdiction what I need to submit to CDC for national surveillance?

The sample size outputs from the three calculators are the amount that needs to be tested to answer specific surveillance questions and this time is unrelated to the number of specimens that need to be submitted to CDC. Please continue to report and submit specimens in accordance with the annual CDC specimen submission guidelines. For more information on the specific surveillance questions please refer to the Influenza Virologic Surveillance Right Size Sample Size Calculator User Guide.

20. Under the surveillance objective for Calculator C: Rare/Novel Influenza Event Investigation what does percent positivity refer to?

The surveillance objective for Calculator C: Rare/Novel Influenza Event Investigation is to determine the prevalence of the rare/novel influenza virus (Rare+/ Flu+) within a state following detection of a rare/novel influenza virus (i.e. “deep dive”) and confirm that the prevalence of a rare/novel influenza event does not exceed a specific percent positivity. Percent positivity refers to the expected prevalence of Rare+/Flu+ that the PHL wants to confirm has not been exceeded.

21. What are the optimal sample size target values?

With input from the pilot study and table top exercise participants as well as 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 (please see table below). 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 is provided and this reduces the total number of specimens a PHL needs to test to achieve the recommended thresholds. However, it’s important to note that supplementing with screened influenza positive specimens may increase the risk of missing a rare/novel influenza virus if the commercial tests used have decreased sensitivity to detect the new virus. The number of samples to be tested will vary depending on the confidence level, margin of error, threshold and assumptions used in the calculators. For more information, please refer Sampling Implementation Guidance section.

Recommended Confidence levels, Margin of Errors, and Thresholds:

<table>
<thead>
<tr>
<th>Situational Awareness</th>
<th>Rare/Novel Influenza Event Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High Season</td>
</tr>
<tr>
<td></td>
<td>Confidence Level (%)</td>
</tr>
<tr>
<td>Optimal</td>
<td>95</td>
</tr>
<tr>
<td>Mid-range</td>
<td>90</td>
</tr>
<tr>
<td>Minimum</td>
<td>85</td>
</tr>
</tbody>
</table>

22. How can PHLs acquire funding to ensure that they are meeting the recommended sample sizes?

An optimal influenza surveillance system requires adequate resources to support all essential elements defined in this roadmap document. Implementation of the right size virologic surveillance guidelines will help CDC, PHLs and surveillance programs maximize available resources, redirect resources as necessary and build new capacity as needed for optimal surveillance. Collaborative planning, grant proposal development and funding allocation between influenza surveillance programs and PHLs is essential to ensure all involved parties have an understanding of the costs associated with all aspects of influenza surveillance and that all virologic surveillance requirements are adequately resourced. For additional information, resources, and funding tools, please refer to the Finance Resources Requirement and Implementation Guidance sections.
Additionally, it is important that states establish and maintain partnerships and networks among PHLs, clinicians, state epidemiologist/influenza surveillance coordinator, clinical laboratories, RIDT sites, CDC and manufacturers to help maintain influenza surveillance. For additional information, please refer to the Partnerships and Communication Requirement and Implementation Guidance sections.

At this time, there is no additional funding specifically to help states reach the Roadmap thresholds. However, this Roadmap is meant to be a resource that allows us to demonstrate what is needed for an effective surveillance system at the state and national level and then use that to justify future funding requests at both the state and federal level. There may be other ways to tweak your system and reallocate current resources to help you reach the Roadmap objectives.

23. How have PHLs eliminated sampling biases?

The influenza virologic surveillance system contains inherent biases due to the complexity of the sampling system; however, at this time, the calculators do not take into account sampling bias. PHLs should consider the sources of bias and if possible address them, when selecting specimen providers, selecting test methods, analyzing data and interpreting results. For additional information on recognizing and addressing sampling biases, please refer Sampling Implementation Guidance section (pg. 66).

Helpful Links:

Influenza Virologic Surveillance Right Size Roadmap Website

Influenza Virologic Surveillance Right Size Sample Size Calculators

Influenza Virologic Surveillance Right Size Sample Size Calculator User Guide