Virology Breakout Sessions
Instructor Guide

The exercises in the sessions contained in this document should be performed in smaller break-out groups, so that questions or problems participants may have can be addressed.

On the pages that follow, you will work through scenarios related to laboratory diagnosis of influenza. Each scenario is preceded by specific instructions. Some scenarios will give you an opportunity to practice specimen collection methods; others will include discussion questions to answer. A numbered outline of the scenarios is provided below. Please complete the scenarios in the order in which they are listed.

1. State the purpose of different levels of laboratory testing

2. Determine a sampling and testing time schedule and investigation priorities for a suspected case of novel influenza

3. Fill in a laboratory form

4. Prioritize which laboratory tests to perform and determine when specimens should be sent to reference facilities
Activity 1: Purpose of Laboratory Testing
Instructor Guide

Instructions
Divide into groups of 4-8 people. Read the case study background information. Then work together as a group to develop a response to the questions that follow.

Time allotted: 15 minutes

Background
Countries may have existing protocols for laboratory specimen collection, packaging, storage, transport and testing and should follow their usual procedures. Respiratory virus detection depends on the collection of high-quality specimens, their rapid transport to the laboratory and appropriate storage before laboratory testing.

Question
In the context of severe respiratory illness, what is the purpose of a laboratory investigation? Consider investigations performed by
a. Basic clinical laboratories
b. Specialized clinical laboratories
c. National Influenza Centers (NIC’s)

Suggested Answers:

a. Basic laboratories can identify the general type (A or B) of influenza. This is useful for local, national, and global surveillance purposes.

b. Specialty laboratories identify the strain type. These would be laboratories with RT-PCR capabilities. Kits are available for identifying the strain types that are of highest interest.

c. Specialty laboratories and/or NIC’s can help with providing information on re-assortment and mutation of influenza viruses. Additionally, by analyzing strain data in conjunction with epidemiologic data, information can be gained on epidemiology and drug resistance of seasonal and novel or pandemic strains of influenza.
Activity 2: Sampling Timeframe and Investigation Priorities
Instructor Guide

Instructions
Read the case study background information. Then work together as a group to develop a response to each of the five discussion questions.

Time allotted: 1 hour

Scenario
A six-year-old girl presented to a district hospital with fever, cough, and sore throat on May 2nd. The hospital participates in the country’s program for sentinel surveillance of severe acute respiratory illness. The girl was admitted with a high fever and difficulty breathing. Her mother reported that the girl’s symptoms started on May 1st.

The physician is worried about the severity of the case, and considers influenza in the diagnosis because of recent public health concerns about the development of a novel influenza strain. As a hospital laboratorian, you advise the doctor on what specimens should be collected.

Questions
1. What type of specimens should be collected?

Suggested Answer: The optimal choice is a nasopharyngeal specimen (swab or aspirate). If it is not possible to collect a nasopharyngeal specimen (swab, aspirate), other options include a nasal wash, throat swab, or nasal swab, or a combination of these. An acute blood sample should also be taken.

2. When should the specimen be collected?
**Suggested Answer:** The respiratory and acute blood serum specimens should be collected as soon as possible. A convalescent sample should also be taken about 2 weeks after symptom onset. Often times, in the field, this is all that is possible and practical. However, if possible multiple sequential samples of all samples may be useful to better understand the natural history of the illness. The specimens collected may also depend on the status of the patient at hand.

3. If the girl had presented at the clinic 4 days after her symptoms began, would you change your choice of what specimens to collect?

**Suggested Answer:** No. Even though one is most likely to recover influenza virus when the sample is taken within 3 days of symptom onset, it is also possible to recover virus in samples taken at a later time.

4. How should the specimens be stored before they are sent to the laboratory?

**Suggested Answer:** The specimens should be stored at 4 °C (such as in a refrigerator) until they can be transported to the laboratory.

5. If there is a delay in sending respiratory and serum samples to the laboratory, what should you do with the samples?

**Suggested Answer:** Respiratory samples should be stored in a -70 °C freezer. If a -70 °C freezer cannot be located, keep samples in the refrigerator at – 4 °C. Do not put them in a normal freezer that has freeze-thaw cycles. Serum samples can be kept either in a -70 °C freezer or a -20 °C freezer. The most important point is to avoid freezing and thawing the samples multiple times.

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**Patient Update**

Later that day, the doctor of the 6-year-old girl calls you again. He tells you that the girl’s mother has refused to allow nasal swabs to be collected from her child, and that she refuses to have more samples taken over the next several days. The doctor himself does not understand why so many specimens are necessary.

6. What do you tell the doctor?

**Suggested Answer:** Remind the doctor of the need to be vigilant for severe respiratory viruses. While the level of concern will change over time, it is important to correctly diagnose novel strains of influenza in order to contain the infection. The ability of a laboratory test to detect the virus will depend on the amount of virus present in the patient sample, the storage, handling, and shipping conditions of the
sample, and the accuracy and correct performance of the laboratory test. With so many potential problems, it is very important to take multiple samples on multiple days. The doctor may explain to the mother that the laboratory tests will help determine the best way to treat the child and improve her illness. The doctor should find a way to work through cultural or religious sensitivities if this becomes a problem (solution will vary depending on the situation).

May 3

On the morning of May 3, the health department issues an alert to area physicians, hospitals, and clinical laboratories. Several cases of severe respiratory illness testing positive for Influenza A have been reported in the last 36 hours. This amount of activity is unusual outside the normal influenza season. Physicians and laboratories are encouraged to participate in surveillance efforts for severe respiratory disease.

7. At the early stages of a potential influenza pandemic, how should specimen collection be prioritized?

**Suggested Answer:** At early stages of a pandemic, severe cases, unexplained clusters, and unexplained deaths should be prioritized for sampling and laboratory testing.

8. Does this change as the pandemic takes shape?

**Suggested Answer:** At all stages of a pandemic, prioritize sample collection from suspected deaths, ICU admissions, and hospitalizations. If infrastructure and resources do not allow testing of hospitalized cases, efforts should be made to select a representative sample of hospitalized cases for testing.

**Brainstorm**

What is the role of a sentinel surveillance site in testing specimens during “normal” times versus during times of increased concern or possible pandemic?

**Suggested Answer:** Whether or not there is a pandemic, sentinel surveillance sites should systematically test influenza-like-illness / acute respiratory illness (ILI/ARI), and severe acute respiratory illness (SARI).
Activity 3: Fill in a Laboratory Form
Instructor Guide

Instructions

The previous scenario is continued below. Please read the scenario, choose which of the provided 2 forms you should fill out, and then use the information provided to fill in the example laboratory specimen collection form. Note that the forms given in this exercise represent the minimal data requirements; each country and even specific surveillance sites may have additional items to include, and you may substitute your own country’s forms in this exercise. The identification numbers given in the scenario can be altered to reflect the identification number system used in your country.

Time allotted: 15 minutes

Scenario

A female child named Ayana presented to a local hospital with fever, cough, and sore throat on May 2, 2006. She was admitted with a high fever and difficulty breathing. The child lives with her family in Addis Ababa. Her mother reported that the girl’s symptoms started on May 1st. The mother also reported that several of their chickens had died one week ago. The child’s birth date is November 22, 2003.

The child was alert when a nasopharyngeal aspirate and blood sample were collected on May 2nd. The nasopharyngeal sample was given the number 730087 and the blood sample was given the unique number 730088. The child fully recovered, and a second blood sample was taken on May 20th. It was given the number 730889.

Facilitator: Give trainees about 15 minutes (or less) to fill in the form using the information given above. They should fill in the inpatient (SARI) form. Once everyone has completed the form, ask for volunteers to provide the answers for each question and check for agreement among the group.
**SARI Swab Form**

<table>
<thead>
<tr>
<th>ID Number:</th>
<th>Date of Symptom Onset:</th>
<th>Date of Form Completion:</th>
<th>Date of Specimen Collection:</th>
</tr>
</thead>
</table>

**IDENTIFICATION**

Patient Unique Identification Number:

OR

Sex:  Male ☐  Female ☐

Patient’s First Name:  Patient’s Last Name:

Date of Birth:  or  Age:  Years _____  Months (1-12) _____

Address:  Contact Telephone Number:

**PRE-EXISTING MEDICAL CONDITIONS**

☐ Heart Disease  ☐ Asthma  ☐ Chronic Lung Disease  ☐ Liver Disease  ☐ Pregnant  ☐ Diabetes

☐ Neuromuscular Dysfunction  ☐ Immune compromised  ☐ Other ________________________  ☐ Unknown

**VACCINES AND ANTIVIRALS**

Exposure to influenza antiviral drugs during the last 14 days?

☐ None  ☐ Yes, patient  ☐ Yes, household contact  ☐ Unknown

If Yes, name of antiviral:

Vaccination for influenza in current season?

☐ Yes  ☐ No  ☐ Unknown

**SARI CASE CRITERIA**

Measured fever of > 38 degrees?

☐ Yes  ☐ No  ☐ Unknown

Cough?

☐ Yes  ☐ No  ☐ Unknown

Sore throat?

☐ Yes  ☐ No  ☐ Unknown

Shortness of breath or difficulty breathing?

☐ Yes  ☐ No  ☐ Unknown

Requiring hospitalization?

☐ Yes  ☐ No  ☐ Unknown

**REPORTING INFORMATION**

Name/Unique ID Number of Reporting Doctor:  Telephone Number:

Name/Unique ID Number of Person Completing Form:  Signature:

Send one copy of this form to the confirmatory laboratory with the specimen and one copy to the national surveillance centre. The original form should be kept at the surveillance site.
# Outpatient Swab Form

**CASE CLASSIFICATION**: □ ARI □ ILI

<table>
<thead>
<tr>
<th>ID Number:</th>
<th>Date of Symptom Onset:</th>
<th>Date of Form Completion:</th>
<th>Date of Specimen Collection:</th>
</tr>
</thead>
</table>

**IDENTIFICATION**

- Patient Unique Identification Number:  
- OR  
  - Sex: □ Male □ Female

- Patient’s First Name: 
- Patient’s Last Name: 
- Date of Birth: or 
  - Age: Years _____ Months (1-12)____

**VACCINES AND ANTIVIRALS**

- Exposure to influenza antiviral drugs during the last 14 days? □ None □ Yes, patient □ Yes, household contact □ Unknown  
  - If Yes, name of antiviral: 
  - Vaccination for influenza in current season? □ Yes □ No □ Unknown

**REPORTING INFORMATION**

- Name/Unique Id Number of Reporting Doctor: 
- Telephone Number: 
- Name/Unique ID Number of Person Completing Form: 
- Signature: 

Send one copy of this form to the confirmatory laboratory with the specimen and one copy to the national surveillance centre. The original form should be kept at the surveillance site.
May 3 Update

Additional cases of influenza illness continue to be reported within your sentinel site and from the community. Other sites in your sentinel surveillance system are reviewing their records to be sure that recent severe cases of respiratory illness have been adequately tested. There is an urgent need for definitive laboratory test results about the influenza strain that has been detected at your sentinel site and in your community.

Questions

1. What are the primary methods for detection and sub-typing of influenza viruses in respiratory specimens?

_Suggested Answer:_ Detection and subtyping of influenza viruses in respiratory specimens has relied for decades upon the isolation of influenza viruses in eggs and later cell culture followed by haemagglutination inhibition assay (HAI) using the WHO CDC reagent kit.

More recently, reverse transcriptase PCR (RT-PCR) or real-time RT-PCR have been used as the method of choice for detection and subtyping.

2. What are the advantages and disadvantages of each method?

_Suggested Answer:_

Instructions

The previous scenario is continued below. Read the information about the scenario given below. Then work together as a group to develop a response to the questions that follow.

Time allotted: 1 hour
HAI –
- **Advantages:** Many viruses are available to WHO for vaccine strain selection.
- **Disadvantages:** Virus isolation is performed on clinical specimens before the influenza virus and subtype has been identified. Techniques used to detect and type influenza viruses in clinical specimens include immunofluorescence assay (IFA) or ELISA and this initial screening can be used to select specimens for virus isolation. However, both assays have a relatively low sensitivity. In addition, IFA requires living cells and thus specimens should be refrigerated and processed preferably within 24 hours of specimen collection.

RT-PCR –
- **Advantages:** Can be used to detect low amounts of influenza virus in clinical specimens: this high sensitivity allows virus isolation to be performed only on those specimens for which the influenza type and subtype has been determined, reducing the risk of isolating potentially novel strains of influenza in a BSL-2 laboratory, as with virus isolation and detection methods. Can be performed rapidly. Has also been shown to be more cost-effective.
- **Real-time RT-PCR has additional advantages, in that it is quicker, quantitative, and may provide fewer false-positive results.**
- **Disadvantages:** Technology may not be available at all sentinel sites or even in all countries.

3. At the current, relatively low level of infection in the population, which cases should be prioritized for testing at a sentinel surveillance site? If the infection becomes widespread, do the priorities change?

**Suggested Answer:** It is recommended that testing occur for unexplained deaths or events, and for the early case specimens that signal a change in the virus. During the initial stages, testing should be extensive enough to determine the new locations where the virus is appearing, and to be able to gather epidemiologic data on confirmed cases. However, when given a choice between quantity and quality of specimens, a higher quality of specimen is always more desirable than higher quantity.

Once the infection becomes widespread in the community or in the larger population, it is no longer feasible nor desirable to test every potential case. Clinical specimens should be collected from all SARI deaths, where possible, a high proportion of SARI patients and a systematic sample of ILI patients to monitor the shape and progress of the epidemic. These specimens can be processed in sentinel site laboratories, but further analyses may require their transport to additional laboratories. Ideally, specimens would be tested for evidence of influenza viruses by reverse transcription-PCR (RT-PCR).
Later that day...

Your laboratory has RT-PCR capability and has confirmed the presence of influenza A from the specimens of the 6-year-old girl and several other SARI patients. However, further RT-PCR to determine the strain type does not yield any conclusive results, and you suspect that the specimens contain a novel strain of influenza A. Your hospital director wants to make an announcement, but you suggest that the specimens you have tested should be sent to a WHO Reference Laboratory first.

4. When should specimens be sent to a WHO Reference laboratory? Which specimens should be sent to WHO collaborating centers?

**Suggested Answer:**

- **WHO Reference Laboratories** are set up for influenza A(H5N1) and novel emerging infections such as the novel influenza A(H1N1). It is appropriate to send a specimen of a novel virus to the Reference Laboratory, or to send a specimen from an unexplained death when the laboratories in-country are not capable of identifying the pathogen. Note that Reference Laboratories should not be sent numerous specimens after a novel strain has been confirmed. All countries should dispatch immediately to a WHO CC or WHO H5 Reference Laboratory, using the WHO Global Shipping Project, the following:
  - the first specimens found positive for pandemic (H1N1) 2009, for confirmation and further virus identification and strain characterization; as infections with pandemic (H1N1) 2009 become more widespread, additional specimens (or isolated viruses where available) should be shared from a selection of cases to monitor any changes in the virus.

- **All samples that signal a change** should be sent to the collaborating centers (CC), along with a representative sample of seasonal specimens. All countries should forward the following to a WHO CC for virus strain characterization and vaccine strain selection:
  - Representative seasonal influenza viruses A(H3N2), A(H1N1) and B, isolated from cases of SARI and ILI should be sent at the beginning of the influenza season, during the peak of the epidemic, and towards the end of the season.
  - Any viruses that react poorly with standard WHO reagents in HAI assays
  - All specimens containing influenza A for which the influenza subtype was not identified
  - All specimens suspected of containing avian influenza or other novel influenza viruses

- **Clinical specimens** should be accompanied by the swab form as well as an itemized list of contents enclosed between the secondary packaging and the outer packaging.
Specimen Shipment Debate

Your hospital director concedes that the specimen may be sent to the WHO Reference Laboratory. However, there is no funding at the hospital for the urgent shipment of biological specimens. He asks you who will pay for this service. And, the director tells you, he would still like to make an announcement about the results from your laboratory.

5. What do you tell the hospital director about specimen shipment costs?

*Suggested Answer:* There is a global fund, the WHO Global Shipping Project, for shipment of specimens to H5 Reference Laboratories (for specimens noted in the first bullet of Answer #6). The cost of shipment will be paid by World Courier. If the laboratory is not familiar with the procedure, they can check the Sentinel Surveillance guidelines for more information.

6. Do you need to wait until results are available from WHO to announce the findings?

*Suggested Answer:* For the first identification of a novel strain within your country, you should wait until the findings have been verified by a reference laboratory. The WHO laboratory will determine the strain type, and if the infection is widespread this information will assist in making test kits for country-level laboratories. It may be a good idea to wait for confirmation from WHO for the first tests even using the test kits. This ensures that your assays are working properly.

If the pandemic strain was already verified by WHO in your area, would you need to wait for results from WHO Collaborating Centers to confirm a case?

*Suggested Answer:* If the assays are validated and the Minister of Health also decides that those results are valid, then you can confirm the case and make the announcement. At this point, the Collaborating Centers would mostly be doing confirmation as test kits are working well and providing reproducible results.