

Module 5
Laboratory Diagnostics, Specimen Collection, and Biosafety Issues
Laboratory Practice Exercises

Facilitator Guide

General Instructions:

These exercises should be performed in smaller break-out groups, so that questions or problems trainees may have can be addressed. On the pages that follow, you will work through seven scenarios related to laboratory diagnosis of avian 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**, just as you would when working with a real suspected avian influenza case.

1. Determine a sampling time schedule for a suspected avian influenza case
2. Collect oropharyngeal specimens.
3. Fill in a laboratory form
4. Package specimens and label the package
5. Prioritize which laboratory tests to perform
6. Analyze laboratory data

1. Case Study: The Sampling Time Frame for a Suspect Avian Influenza Case

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: 15 minutes

Scenario

A six year-old girl presented to a local hospital with fever, cough, and sore throat on January 2nd. She was admitted with a high fever and difficulty breathing. Her mother reported that the girl's symptoms started on January 1st. The mother also reported that several of their chickens had died one week ago.

The doctor suspected avian influenza and called you for advice.

Questions:

1. What type of specimens should be collected?

Facilitator Answer: *The optimal choice is an oropharyngeal specimen (throat swab). A nasal swab could also be collected in the same VTM vial. An acute blood sample should also be taken.*

2. When should the specimens be collected?

Facilitator Answer: *The respiratory and acute blood serum specimens should be collected as soon as possible. A convalescent sample should also be taken about 3 weeks after 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?

Facilitator 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?

Facilitator 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 the samples to the laboratory, what should you do with the samples?

Facilitator 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. Serum samples can be kept either in a -70 °C freezer or a normal freezer (-20 °C). The most important point is to avoid freezing and thawing the samples multiple times.*

Update

You advise the doctor on what specimens should be collected. Later that day, the doctor 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. You must explain to the doctor why multiple samples are necessary, so he can explain this to the patient's mother. What do you tell him?

Facilitator Answer: *Explain to the doctor the status of avian influenza in your country (this will change over time) and the importance of correctly diagnosing avian 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).*

2. Practice clinical sample collection: throat swab

Instructions:

In the lecture, the instructor demonstrated how to perform respiratory specimen collection. You will now have the opportunity to perform these tasks wearing the appropriate personal protective equipment. **Each person in your group should practice the specimen collection procedures.** If you have an even number of people in your group, you may find it easiest to complete this activity in pairs.

Time allotted: 20 minutes

Facilitator/Participant Notes:

Technique (Adapted from the World Health Organization and American Public Health Laboratories guidelines for suspected H5N1 cases):

Clinical specimens should be collected as described below and added to transport medium. Nasal or nasopharyngeal swabs can be combined in the same vial of virus transport medium. When possible, the following information should be recorded: general patient information, type of specimens, date of collection, and contact information of person completing the form, etc.

Standard precautions should always be followed, and barrier protections applied whenever samples are obtained from patients.

Oropharyngeal swab specimen collection

1. Use only sterile Dacron or rayon swabs. Swab posterior oropharynx near tonsillar area, avoiding the tongue.
2. Place swab immediately into a sterile vial containing 2 mL of viral transport media. Cut off or break off the applicator shaft and close vial tightly..
3. Label vial with patient's name and number. **ALSO BE SURE TO RECORD ALL INFORMATION ON THE LABORATORY DATA FORM.**

3. Practice Scenario: Fill in a laboratory form

Instructions:

The previous scenario is continued below. Please read the scenario and then use the information provided to fill in the laboratory specimen form used by **your** country. The identification numbers given in the scenario can be altered to reflect the identification number system used in your country. **Note:** A sample specimen form has been included for your information.

Time allotted: 15 minutes

Scenario

A six-year-old girl named Zenab presented to Abassiya Hospital with fever, cough, and sore throat on January 2, 2006. She was admitted with a high fever and difficulty breathing. The child lives with her family in Cairo, Egypt. Her mother reported that the girl's symptoms started on January 1st. The mother also reported that several of their chickens had died one week ago. The child's birth date is November 22, 2000.

The child was alert when an oropharyngeal swab and blood sample were collected on January 2nd. The oropharyngeal 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 January 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. Once everyone has completed the form, ask for volunteers to provide the answers for each question. Check the answers against those provided below (if using the standard form provided), and correct any mistakes. A sample form has been included for your information.

The following information should be written on the laboratory form:

--Name, hospital name, town (Local names provided in the scenario above should be included here)

Sex: F

Birth date: November 22, 2000

Date of onset of illness: January 1, 2006

Under clinical specimens

For the oropharyngeal aspirate:

Unique ID: 730087

Type: nasopharyngeal aspirate

Date of collection: January 2nd, 2006

Clinical diagnosis: suspected avian influenza

Health status: signs and symptoms of pneumonia. Alert, not on ventilator.

For the first blood sample:

Unique ID: 730088

Type: blood

Date of collection: January 2nd, 2006

Clinical diagnosis: suspected avian influenza

Health status: signs and symptoms of pneumonia. Alert, not on ventilator.

For the second blood sample:

Unique ID: 730889

Type: blood

Date of collection: January 20, 2007

Clinical diagnosis: suspected avian influenza

Health status: healthy

4. Practice: Pack specimens and label the package

Instructions:

During the lecture, the instructor demonstrated how to package specimens and label a package. You will now have the opportunity to pack and label a specimen transport package. This procedure is country-specific, but some universal guidelines and a diagram are provided below:

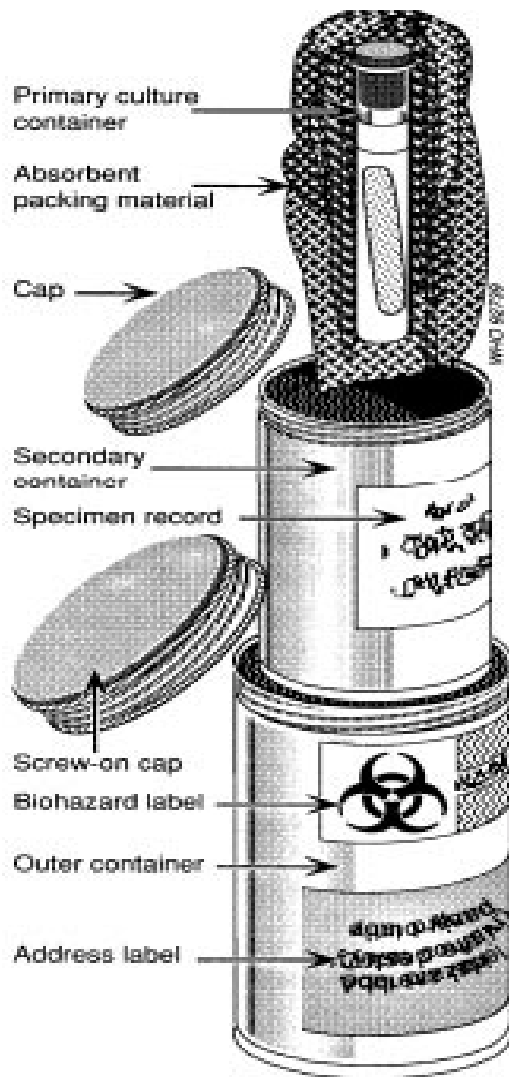
- Transport of specimens should comply with the WHO guidelines for the safe transport of infectious substances and diagnostic specimens
- The receiving laboratory should be notified before shipment of specimens in order to arrange for an import license for the specimens
- Follow the regulations of the country where the specimens are being transported
- Label the package to comply with international air transportation (IATA) regulations
- Make sure package is leak proof and contains less than 500 ml of liquid. There should be three layers, with absorbent material in each layer. The first or second packaging should be able to withstand a pressure differential of 95 Pa (0.95 bar)

Time allotted: 15 minutes

This information is taken directly from:

http://www.who.int/csr/disease/avian_influenza/guidelines/transport/en/index.html

Triple packaging system



5. Group Discussion: Prioritize which laboratory tests to perform

Instructions:

The previous scenario is repeated below. This time you will apply what you have learned in the lecture to answer the questions that follow.

Time allotted: 20 minutes

Scenario

A six-year-old girl named Rana presented to Alexandria Hospital with fever, cough, and sore throat on January 2, 2006. She was admitted with a high fever and difficulty breathing. The child lives with her family in Agamy, Alexandria Governorate, Egypt. Her mother reported that the girl's symptoms started on January 1st. The mother also reported that several of their chickens had died one week ago. The child's birth date is November 22, 2000.

The child was alert when an oropharyngeal swab and blood sample was collected on January 2nd. The oropharyngeal 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 January 20th. It was given the number 730889.

Questions:

1. What type of specimen is most important for the diagnosis of avian influenza?

Facilitator Answer: Respiratory specimens are the most important because the presence of the virus can be directly measured. However, multiple samples (respiratory, blood) should be collected on multiple days.

2. What particular test would you use for the respiratory samples?

Facilitator Answer: The answer to this question depends on the laboratory resources available in your area or country; please adapt the answer accordingly. Viral isolation is considered the most definitive, but this technique takes time and it requires BSL3 facilities. Molecular techniques such as Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR) are faster and do not require BSL3 facilities.

3. How should the hospital dispose of the materials used to collect the sample?

Facilitator Answer: Disposable materials should be destroyed in the hospital incinerator. If no incinerator is available, one should be constructed of a metal drum, or a pit should be dug to burn the materials.

Update

The mother is worried that her 2 other children may also get the illness, and asks that these children be tested for avian influenza as well.

4. Should you recommend that samples be taken from the other children in the household?

Facilitator Answer: *No. Samples should be taken from suspected cases only. Contacts should be followed-up daily during the next 7 days, and checked for the appearance of signs/symptoms of AI. If, any develops, then the contact should be considered as suspected, and therefore specimen should be taken at that time.*

5. Will you recommend that the family chickens be tested?

Facilitator Answer: *The chickens should be tested. You should coordinate with the appropriate veterinary department and / or environmental health department to take samples from chickens, chicken coops, and in and around the home.*

6. Problem Solving Exercise: Analyze laboratory data

Instructions:

Read the information given below and review the data table provided. Then answer the questions that follow.

Time allotted: 20 minutes

Background

As a result of an outbreak investigation of avian influenza, respiratory specimens were collected from 20 different people exposed to chickens in a small village. The respiratory specimens were tested for the presence of the H5 virus using real-time RT-PCR. Test results are summarized in the table below.

Subject	Contact with chickens?	Age	Test Result
1	no	55	-
2	yes	10	+
3	yes	23	-
4	no	65	-
5	no	22	-
6	no	45	+
7	no	5	+
8	yes	10	+
9	no	45	-
10	no	70	-
11	no	23	-
12	no	30	-
13	no	32	-
14	no	17	-
15	no	21	-
16	yes	55	-
17	yes	41	+
18	no	80	-
19	no	21	-
20	no	11	-

Questions:

1. How would you report the results?

Facilitator Answer: *One way to report the results is as they are listed above. However, this is difficult to read. A summary report that shows the people who have contact with chickens and whether or not they tested positive might be most useful. Providing an age range of those testing positive would also be helpful. See the example given below:*

Number of people tested: 20

Number of people testing positive: 5 (age range 5 years – 45 years)

Of those testing positive, 3 (60%) had contact with chickens.

2. What is the prevalence of infection?

Prevalence = # testing positive / total # people = 5 / 20 = 25%

3. What is the prevalence of infection by exposure to chickens?

Prevalence = # testing positive with exposure / total # exposed = 3 positive exposed / 5 exposed = 60%

4. Is the infection more common in children or adults?

The infection is more common in children:

- 3 out of 4 children tested positive: 3 / 4 = 75%

- 2 out of 16 adults tested positive: 2 / 16 = 12.5%

Field Data Collection Form

General patient information

Name:
Address:
Country:
County:
City/town/village:

Tracking record number

Date of Birth (dd/mm/yyyy):
Sex: M [] F []
Nationality:
Occupation:

Date of onset of illness (dd/mm/yyyy):

Clinical specimens

Unique ID No.	Type	Date of collection	Clinical diagnosis	Health status when specimens collected	Remarks

Name of person completing form: _____

Institutional affiliation: _____

Contact details: _____

Date(dd/mm/yyyy): ___/___/___