

Exercise 6

Laboratory Diagnostics Practice Exercises

General Instructions:

On the pages that follow, you will work through four exercises related to laboratory diagnosis of avian influenza A (H5N1). Each exercise is preceded by specific instructions. A list of the exercises is provided below.

1. The sampling time frame for a suspected avian influenza case
2. Prioritizing which laboratory tests to perform
3. Analysis of laboratory data
4. Interpretation of laboratory test results

Total time allotted: 1 hour

Part 1: The Sampling Time Frame for a Suspect Human Case of Avian Influenza H5N1

Objectives:

Determine what samples should be taken to test for avian influenza A (H5N1), as well as when these samples should be taken and how they should be stored.

Instructions:

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

Time allotted: 15 minutes

Scenario

Highly pathogenic avian influenza A (H5N1) has been reported among wild fowl in the United States. A 6-year-old girl presented to a local hospital in an area where the illness in wild birds has been reported. The girl presented 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 both she and the girl's father work on a poultry farm managing operation.

The doctor suspected avian influenza and called you for advice.

Question 1 – What type of specimens should be collected?

***Suggested Answer** – The optimal choice for a patient who is not intubated is the oropharyngeal swab or throat swab. If it is not possible to collect an oropharyngeal swab, other options include a nasal wash, nasopharyngeal swab, throat swab or nasal swab. An acute blood sample should also be taken. Note that a nasopharyngeal swab would be the best specimen to collect for human influenza, but an oropharyngeal swab would be the more likely way to detect avian influenza infection, when present. Lower respiratory tract specimens are often easier to collect in patients who are intubated.*

Question 2 – When should the specimens be collected?

***Suggested Answer** – The respiratory and acute blood serum specimens should be collected as soon as possible. All samples should be taken again in a few days,*

and more times, if possible. A second blood sample should be taken in about two weeks.

Question 3 – If the girl had presented at the hospital four 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 detect influenza virus when the sample is taken within three days of symptom onset, it is also possible to recover virus in samples taken at a later time.

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

Suggested Answer – If the specimens can be tested within 48 hours, they should be stored at 4°C (such as in a refrigerator) until they can be transported to the laboratory. Otherwise, samples should be immediately frozen, stored at -70°C, and transported on dry ice.

Question 5 – If there is a delay in sending the 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. **Serum** samples can be kept either in a -70 °C freezer or a normal freezer (-20 °C). 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. She states that she thinks there is very little likelihood that her child is infected with avian influenza. The doctor himself does not understand why so many specimens are necessary.

Question 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?

***Suggested Answer** – Explain to the doctor the status of avian influenza in the United States (this will change over time) and the importance of correctly diagnosing avian influenza in order to ensure appropriate treatment of the child and prevent further transmission to other people. 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).*

Part 2: Prioritizing which laboratory tests to perform

Objectives:

Determine which specimens should be collected and how they should be tested for suspect avian influenza.

Instructions:

Answer the following questions, using the previous scenario.

Time allotted: 10 minutes

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

Suggested Answer – Respiratory specimens are the most important because the presence of the virus can be directly measured. However, multiple samples (different types of respiratory samples as well as blood) should be collected on multiple days.

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

Suggested Answer – The answer to this question depends on the laboratory resources available in your area; please adapt the answer accordingly. Viral isolation is considered the most definitive, but this technique takes time and it requires enhanced BSL3 facilities. Molecular techniques and immunofluorescence are faster and do not require enhanced BSL3 facilities.

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

Suggested Answer – Disposable materials should be destroyed in the hospital incinerator.

Update

Having agreed to the testing of her sick child, the mother is now worried that her two other children may also get the illness and asks that these children be tested for avian influenza as well.

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

***Suggested Answer** – Under most circumstances, other family members or exposed persons would ONLY be tested if they have influenza symptoms. If they do not have symptoms, ask the family to carefully monitor the health status of the other children and family members. They should report to the doctor if they begin any coughing or respiratory symptoms. They would be tested at that time. However, with the first few cases of influenza A (H5N1) in the United States, serologic testing of close contacts may be done to look for antibody evidence of mild or asymptomatic infection.*

Question 5 – Will you recommend that the chickens on the farm where the parents work be tested?

***Suggested Answer** – At this point, there is no evidence that the chickens have avian influenza, so testing is not necessary. If the birds start displaying signs and symptoms of the disease, then they should be tested. However they should be closely monitored and any possible source of exposure of the chickens to wild waterfowl should be eliminated.*

Part 3: Analysis of laboratory data

Objectives:

Practice analyzing and reporting avian influenza laboratory data.

Instructions:

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

Time allotted: 15 minutes

Background

As a result of an outbreak investigation of avian influenza, respiratory specimens were collected from 20 different people who worked around dead chickens in a major poultry farming area. 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 dead 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	-

Question 1 – How would you report the results?

***Suggested 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 dead 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 dead chickens.

Question 2 – What is the incidence of infection in the population tested?

***Suggested Answer** – Incidence = # testing positive / total # people = 5 / 20 = 25%*

Question 3 – What is the attack rate of infection by exposure to dead chickens?

***Suggested Answer** – Attack rate = # testing positive with exposure / total # exposed = 3 positive exposed / 5 exposed = 60%*

Question 4 – Is the infection more common in children or adults?

***Suggested Answer**– The infection appears to be slightly more common in children – 3 out of 5 children (< 18 yrs old) tested positive, and 2 out of 15 adults (>= age 18) tested positive. However, the sample size is too small to make any generalizations.*

Part 4: Interpretation of laboratory test results

Objectives:

Examine and interpret the results from several different laboratory tests for avian influenza.

Instructions:

Three rounds of laboratory test results from patients with suspected avian influenza are presented below. Examine the results given, and provide the interpretation or each sample in the table.

Time allotted: 20 minutes

***Facilitator** – Allow the students about 15 minutes to complete their responses. They may work with a partner or in small groups, and may consult the lecture slides to form their answers. After most people are finished, ask for volunteers to provide the answers, and tell them whether they are correct. If answers are incorrect, provide and explain the correct answers.*

Laboratory Test 1

Here are the results from six different real-time RT-PCR assays. Individual tests were used to determine if the clinical sample contained A, A/H1, A/H3, A/H5 and B viruses. Interpret the results for each sample in the far right column of the table.

	A	H1	H3	H5	B	Positive Control	Negative Control	Interpretation?
Sample A	-	-	-	-	+	+	-	
Sample B	+	+	-	-	-	+	-	
Sample C	+	-	-	+	-	+	-	
Sample D	-	-	-	-	-	-	-	

Question 1 – How would you interpret these results?

Suggested Answer –

Sample A *Influenza B (not avian)*

Sample B *Influenza A H1 (probably a human strain)*

Sample C *Influenza A, H5 (avian influenza)*

Sample D *Inconclusive*

Laboratory Test 2

Below are the results of the haemagglutination inhibition test, which determines if antibodies present in serum are specific to avian influenza. Paired samples (acute and convalescent) were taken for each of three subjects. Paired samples will show if the subject has had a recent infection because antibodies to the infection will increase greatly between the first sample and the second. The relative concentrations of antibodies specific to avian influenza in the acute and convalescent phases are presented below. Determine whether there is evidence that any of the subjects were exposed to avian influenza and note your interpretation of the test results in the far right column of the table.

	Acute	Convalescent	Interpretation?
Subject A	high	high	
Subject B	low	high	
Subject C	low	low	

Question 2 – How would you interpret these test results?

Suggested Answer –

Subject A *Evidence of past exposure to H5 influenza*

Subject B Evidence that clinical disease was caused by H5 influenza

Subject C No evidence of H5 infection

Laboratory Test 3

A nasopharyngeal aspirate from a suspected avian influenza case was tested using a commercially available rapid influenza test for influenza A. The result was positive.

Question 3 – How would you interpret the results? Are any other tests necessary?

***Suggested Answer** – A positive result for a rapid test for influenza A means that influenza A is present, but doesn't provide information about the influenza A subtype. This influenza A virus could be a human strain or an avian strain. Therefore, additional testing is needed. Molecular testing, immunofluorescence, or viral culture could be used to further test the specimen. However RT-PCR is the recommended test because culture of influenza A (H5N1) viruses can only be done in enhanced BSL-3 laboratories. These laboratories require special equipment and procedures. In addition, any attempt to culture influenza A (H5N1) cannot be undertaken without prior authorizations.*

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Updated Interim Guidance for Laboratory Testing of Persons with Suspected Infection with Avian Influenza A (H5N1) Virus in the United States

This update provides revised interim guidance for testing of suspected human cases of avian influenza A (H5N1) in the United States and is based on the current state of knowledge regarding human infection with H5N1 viruses. The epidemiology of H5N1 human infections has not changed significantly since February 2004. Therefore, CDC recommends that H5N1 surveillance in the United States remain at the enhanced level first established at that time. However, this revised interim guidance provides an updated case definition of a suspected H5N1 human case for the purpose of determining when testing should be undertaken and also provides more detailed information on laboratory testing. Effective surveillance will continue to rely on health-care providers obtaining information regarding international travel and other exposure risks from persons with specified respiratory symptoms as detailed in the recommendations below. This guidance will be updated as the epidemiology of H5N1 changes. Note: CDC is revising its interim guidance for infection control precautions for avian influenza A (H5N1). These will be issued as soon as they are available.

Current Situation:

The avian influenza A (H5N1) epizootic (animal outbreak) in Asia has expanded to wild birds and/or poultry in parts of Europe, the Near East and Africa. Sporadic human infections with H5N1 continue to be reported and have most recently occurred in China, Egypt, Indonesia, Azerbaijan, Cambodia and Djibouti. In addition, rare instances of probable human-to-human transmission associated with H5N1 viruses have occurred, most recently in a family cluster in Indonesia. So far, however, the spread of H5N1 virus from person to person has been rare, inefficient and unsustainable. The total number of confirmed human cases of H5N1 reported as of June 7, 2006, has reached 225. The case fatality rate for these reported cases continues to be approximately 50 percent. As of this date, H5N1 has not been identified among animals or humans in the United States.

The epizootic in Asia and parts of Europe, the Near East and Africa is not expected to diminish significantly in the short term, and it is likely that H5N1 infection among birds has become enzootic in certain areas. It is expected that human infections resulting from direct contact with infected poultry will continue to occur in affected countries. Since no sustained human-to-human transmission of influenza H5N1 has been documented anywhere in the world, the current phase of alert, based on the World Health Organization (WHO) global influenza preparedness plan, remains at Phase 3 (Pandemic Alert).^{*} In addition, no evidence for genetic reassortment between human and avian influenza A virus genes has been found. Nevertheless, this expanding epizootic continues to pose an important and growing public health threat. CDC is in communication with WHO and other national and international agencies and continues to monitor the situation closely.

Reporting and Testing Guidelines:

CDC recommends maintaining the enhanced surveillance efforts practiced currently by state and local health departments, hospitals and clinicians to identify patients at increased risk for avian influenza A (H5N1). Guidance for enhanced surveillance was first described in a HAN update issued on February 3, 2004, and most recently updated on February 4, 2005.

Testing for avian influenza A (H5N1) virus infection is recommended for:

A patient who has an illness that:

- requires hospitalization or is fatal; AND
- has or had a documented temperature of $\geq 38^{\circ}\text{C}$ ($\geq 100.4^{\circ}\text{F}$); AND
- has radiographically confirmed pneumonia, acute respiratory distress syndrome (ARDS), or other severe respiratory illness for which an alternate diagnosis has not been established; AND
- has at least one of the following potential exposures within 10 days of symptom onset:

A) History of travel to a country with influenza H5N1 documented in poultry, wild birds, and/or humans,[†] AND had at least one of the following potential exposures during travel:

- direct contact with (e.g., touching) sick or dead domestic poultry;
- direct contact with surfaces contaminated with poultry feces;
- consumption of raw or incompletely cooked poultry or poultry products;
- direct contact with sick or dead wild birds suspected or confirmed to have influenza H5N1;
- close contact (approach within 1 meter [approx. 3 feet]) of a person who was hospitalized or died due to a severe unexplained respiratory illness;

B) Close contact (approach within 1 meter [approx. 3 feet]) of an ill patient who was confirmed or suspected to have H5N1;

C) Worked with live influenza H5N1 virus in a laboratory.

Testing for avian influenza A (H5N1) virus infection can be considered on a case-by-case basis, in consultation with local and state health departments, for:

- A patient with mild or atypical disease[‡] (hospitalized or ambulatory) who has one of the exposures listed above (criteria A, B, or C); OR
- A patient with severe or fatal respiratory disease whose epidemiological information is uncertain, unavailable or otherwise suspicious but does not meet the criteria above (examples include: a returned traveler from an influenza H5N1-affected country whose exposures are unclear or suspicious, a person who had contact with sick or well-appearing poultry, etc.)

Clinicians should contact their local or state health department as soon as possible to report any suspected human case of influenza H5N1 in the United States.

Specimen Collection and Testing Guidelines:

- Oropharyngeal swab specimens and lower respiratory tract specimens (e.g., bronchoalveolar lavage or tracheal aspirates) are preferred because they appear to contain the highest quantity of virus for influenza H5N1 detection, as determined on the basis of available data. Nasal or nasopharyngeal swab specimens are acceptable, but may contain less virus and therefore not be optimal specimens for virus detection.
- Detection of influenza H5N1 is more likely from specimens collected within the first three days of illness onset. If possible, serial specimens should be obtained over several days from the same patient.

- Bronchoalveolar lavage is considered to be a high-risk aerosol-generating procedure. Therefore, infection control precautions should include the use of gloves, gown, goggles or face shield, and a fit-tested respirator with an N-95 or higher rated filter. A loose-fitting powered air-purifying respirator (PAPR) may be used if fit-testing is not possible (for example, if the person has a beard). Detailed guidance on infection control precautions for health-care workers caring for suspected influenza H5N1 patients is available.]]
- Swabs used for specimen collection should have a Dacron tip and an aluminum or plastic shaft. Swabs with calcium alginate or cotton tips and wooden shafts are not recommended.§ Specimens should be placed at 4°C immediately after collection.
- For reverse-transcriptase polymerase chain reaction (RT-PCR) analysis, nucleic acid extraction lysis buffer can be added to specimens (for virus inactivation and RNA stabilization), after which specimens can be stored and shipped at 4°C. Otherwise, specimens should be frozen at or below -70°C and shipped on dry ice. For viral isolation, specimens can be stored and shipped at 4°C. If specimens are not expected to be inoculated into culture within two days, they should be frozen at or below -70°C and shipped on dry ice. Avoid repeated freeze/thaw cycles.
- Influenza H5N1-specific RT-PCR testing conducted under Biosafety Level 2 conditions is the preferred method for diagnosis. All state public health laboratories, several local public-health laboratories, and CDC are able to perform influenza H5N1 RT-PCR testing, and are the recommended sites for initial diagnosis.
- Viral culture should NOT be attempted on specimens from patients suspected to have influenza H5N1, unless conducted under Biosafety Level 3 conditions with enhancements.
- Commercial rapid influenza antigen testing in the evaluation of suspected influenza H5N1 cases should be interpreted with caution. Clinicians should be aware that these tests have relatively low sensitivities, and a negative result would not exclude a diagnosis of influenza H5N1. In addition, a positive result does not distinguish between seasonal and avian influenza A viruses.
- Serologic testing for influenza H5N1-specific antibody, using appropriately timed specimens, can be considered if other influenza H5N1 diagnostic testing methods are unsuccessful (for example, due to delays in respiratory specimen collection). Paired serum specimens from the same patient are required for influenza H5N1 diagnosis: one sample should be tested within the first week of illness, and a second sample should be tested 2-4 weeks later. A demonstrated rise in the H5N1-specific antibody level is required for a diagnosis of H5N1 infection. Currently, the microneutralization assay, which requires live virus, is the recommended test for measuring H5N1-specific antibody. Any work with live wild-type highly pathogenic influenza H5N1 viruses must be conducted in a USDA-approved Biosafety Level 3 enhanced containment facility. Visit <http://www.cdc.gov/flu/h2n2bsl3.htm> for more information about procedures and facilities recommended for manipulating highly pathogenic avian influenza viruses.

Laboratory testing results positive for influenza A (H5N1) in the United States should be confirmed at CDC, which has been designated as a WHO H5 Reference Laboratory. Before sending specimens, state and local health departments should contact CDC's on-call epidemiologist at (404) 639-3747 or (404) 639-3591 (Monday – Friday, 8:30 AM - 5:00 PM) or (770) 488-7100 (all other times).

Travel Health Notice:

CDC has not recommended that the general public avoid travel to any of the countries affected by H5N1. However, CDC does recommend that travelers to these countries avoid poultry farms and

bird markets or other places where live poultry are raised or kept. For details about other ways to reduce the risk of infection, see http://www.cdc.gov/travel/other/avian_influenza_se_asia_2005.htm.

More Information:

Department of Health and Human Services at www.pandemicflu.gov
World Health Organization at
World Organization for Animal Health (OIE) at http://www.oie.int/eng/en_index.htm

*For the current WHO Pandemic Phase, see
http://www.who.int/csr/disease/avian_influenza/phase/en/index.html.

† For a listing of influenza H5N1-affected countries, visit the CDC website at <http://www.cdc.gov/flu/avian/outbreaks/current.htm>; the OIE website at http://www.oie.int/eng/en_index.htm; and the WHO website at http://www.who.int/csr/disease/avian_influenza/en/.

‡ For example, a patient with respiratory illness and fever who does not require hospitalization or a patient with significant neurologic or gastrointestinal symptoms in the absence of respiratory disease.

|| Interim recommendations for infection control in health-care facilities caring for patients with known or suspected avian influenza are available at <http://www.cdc.gov/flu/avian/professional/infect-control.htm>.

§ Specimens can be transported in viral transport media, Hanks balanced salt solution, cell culture medium, tryptose-phosphate broth, veal infusion broth or sucrose-phosphate buffer. Transport media should be supplemented with protein, such as bovine serum albumin or gelatin, to a concentration of 0.5% to 1%.

Information regarding Laboratory Biosafety Level Criteria can be found at <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm>.

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