

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. 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?

Question 2 – When should the specimens be collected?

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?

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

Question 5 – If there is a delay in sending the samples to the laboratory, what should you do with the samples?

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?

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?

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

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

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?

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

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?

Question 2 – What is the incidence of infection?

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

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

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 of each sample in the table.

Time allotted: 20 minutes

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?

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?

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?

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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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