Influenza: Serologic Diagnosis and Epidemiology

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Note to Students
The fundamental purpose of all activities in health-care professions is to help other people. Like all behavior, helping behavior becomes more effective and natural with practice. This workbook enables you to practice by helping your fellow students to learn basic science. Your skill at helping your fellow students should relate to your ability to help your patients in the future.

This is a Patient-Oriented Problem-Learning ("POPS") workbook designed for four students. Before beginning this session, you should have (a) studied the objectives designed to prepare you for it, (b) taken the pretest, and (c) reviewed the topics listed at the end of the pretest. Now, each of you should take one of the four color-coded booklets and follow the directions in it. If your group has only three students, one of you should take two booklets. Leave the remainder of the workbook intact until you are given further instructions.

For additional information, SEE:
http://www.medinfo.ufl.edu/cme/flu/flu.html
http://www.cdc.gov/ncidod/diseases/flu/fluvirus.htm

A Patient-Oriented Problem-Solving (POPS) System
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Pretest Correct Answers

You have the answers to some of the 18 pretest questions, and other members of your group have the remainder. This arrangement is designed to encourage all members of your group to actively exchange ideas and concepts. First, study the answers in your booklet and then EXPLAIN them to your group. Please don't just read them to your classmates, and don't let your classmates read their answers to you. In explaining something to another person, most people gain a better understanding of it and often transmit a better understanding. The*pretest discussion and patient-oriented problem-solving parts of this activity are "open book".* Be sure to refer to textbooks, notes, and other written resources whenever questions arise.

You will probably want to make notes on your pretest to help you review questions that you missed. Avoid "collecting pages" for "later study and understanding". Learn the concepts*now* so that later you will only need to review them.

Reviewing pretest answers will probably take more time than any other part of this exercise. Some material needed to solve this problem is not found in many textbooks and therefore has been incorporated into the pretest answers.

4. **C** is correct. The titer is defined as the reciprocal of the highest dilution that still exhibits the phenomenon (in this case hemagglutination). This would be 64.

6. E is the incorrect answer. Influenza can be cultured from respiratory secretions, but results will not be available for several days. There is a rapid antigen detection system that can be performed on nasal or throat swabs; a result is available within one hour. An IgM titer to a specific pathogen can frequently occur and can be obtained, but a detection kit is not commercially available for anti-IgM antibodies against influenza viruses. Many infectious diseases can be diagnosed by a 4-fold change in IgG titers; the limitation of this method is that the convalescent serum is obtained about two weeks after the infection, so after processing, results are frequently not available until one month after the patient is seen!

10. **A** is correct. Note that there is no antibody and the virus has agglutinated the RBCs. The ability to visualize immunologic reactions and to depict them with simple diagrams is important*You should strive to be able to do this with any immunologic assay*.

14. **C** is correct. A rise in titer does not necessarily prove that that the patient was infected. He may have been immunized instead. Influenza immunizations stimulate the production of antibody but usually to lower titers and for shorter period than do infections.

18. Can't tell; therefore, **C** is correct. Although the data certainly demonstrate that Joe has had an influenza B infection, it is impossible to determine how long ago the infection occurred when only one titer is available. For this reason, the physician normally obtains an acute serum during an illness and a convalescent serum several weeks or a month afterward to see if there is a rise in titer. To make a serological diagnosis, one must observe at least a two-tube, or fourfold, increase between the acute and convalescent titer. The reason for the fourfold increase requirement is that when using twofold dilutions, a titer of 320 could *theoretically* be anywhere between 320 and 639, whereas a titer of 640 could be between 640 and 1,280. Thus, a true rise of 639 to 640 would be read as 320 to 640, ie, a twofold rise for less than1% change. In addition to this theoretical objection to relying on twofold titer increases, there is the inherent inaccuracy in reading titers. The fourfold or greater

titer rise between acute and convalescent sera can be used with many different assays systems (eg, complement fixation, bacterial agglutination, viral neutralization) to diagnose almost any infectious disease (eg, syphilis, brucellosis, polio).

Incidentally, the amount of the rise in titer depends on factors such as age, health, and immunological capacity of the patient plus the immunogenicity of the virus.

When your group has completed its discussion of the pretest, you should have an understanding of the principles involved in hemagglutination and hemagglutination inhibition. If you are still unclear about some of these principles, be sure to consult textbooks or ask members of your group.

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Instructions for the Clinical Problem

In the remainder of this package, you are to use your knowledge of hemagglutination inhibition to answer questions pertaining to an infectious disease epidemic.

Each of you has a complete copy of the same problem but only part of the data. After you have read the problem, analyze your own data and try to decide the antibody titer each patient has to each virus and, also, which patients were infected with which viruses. Then pool your knowledge with that of your groupmates and answer the questions.

The Problem

There is an epidemic of upper respiratory disease at the Loving Care Orphanage. Of the 123 children in the orphanage, three became ill during the last week of February. During the first week of March, 53 more children became ill. “Flu shots” have never been used at this institution.

On March 5, a blood sample was taken from each of 12 children who were randomly selected from the 123 children at the Loving Care Orphanage. On March 26, another blood sample was drawn from the same 12 children.

Serum from these blood samples was assayed for antibody to two different viruses using the hemagglutination inhibition (HI) assay. Each person in your group has different data. The data from one fourth of these assays are given on the last page of your booklet; there are extra copies of your data for you to give to your colleagues when you discuss it.

Your goal is to decide which influenza virus (A or B) is causing the epidemic. Begin by having each person determine the HI titer for the assays in his or her booklet. The group should then answer the questions on the “Group Question Sheet”. Answering the questions will require you to collaborate with one another and compare data.

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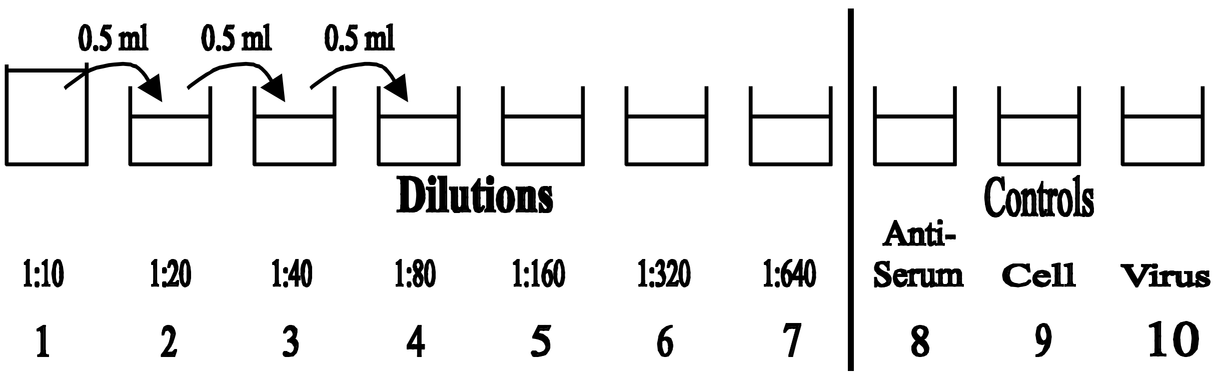
Review of Hemagglutination Inhibition (HI) Test

If a hemagglutinin (eg, influenza virus) is mixed with its homologous antibody in suitable amounts and erythrocytes are then added, hemagglutination does not occur. Using this property, one can test the potency of an antiserum to a virus hemagglutinin by measuring the minimum amount of antiserum (ie, highest dilution of antiserum) necessary to completely inhibit hemagglutination.

The procedure is as follows:

Serial dilutions of serum

Prepare serial twofold dilutions of the serum to be tested for antibody to virus ranging from 1:10 to 1:640 in 0.5 ml amounts as shown below.



Begin with 1.0 ml of a 1:10 dilution of serum in well 1 and 0.5 ml of saline in wells 2 through 7. Remove 0.5 ml from well 1, place it in well 2, and mix. You now have 1.0 ml of a 1:20 dilution in well 2. Next, take 0.5 ml of the 1:20 dilution and mix it with the 0.5 ml of saline in well 3. This gives 1.0 ml of a 1:40 dilution of serum in well 3 and leaves 0.5 ml of a 1:20 dilution in well 2. Repeat this procedure for wells 4, 5, 6, and 7 to give serial twofold dilutions in the row of wells. Ask your colleagues how to do serial fivefold dilutions. (Answer: Transfer 0.1 ml into 0.4 ml.)

Addition of virus

Add 0.2 ml of the hemagglutinin (virus) suspension to each well, mix thoroughly, and incubate for 30 minutes at 37°C.

Addition of RBCs

Add 0.2 ml of washed chicken erythrocytes to each well and incubate for one hour at 37°C.

Controls

Three controls - virus, cells, and serum - are also set up.

1. The *virus control* is made by mixing virus with red blood cells (RBCs) to check the hemagglutination capacity of the virus, ensuring no false results due to faulty virus.

2. *The serum control* is set up by mixing a 1:10 dilution of antiserum with RBCs to see if "serum agglutinins" (ie, antibody in the human serum to chicken RBCs) are present. These agglutinins would give false hemagglutinations if present in high enough titer. Routinely, antisera are absorbed with chicken RBCs before being serially diluted in order to remove these interfering agglutinins. If this step is improperly performed, hemagglutination due to this antibody can be confused with viral hemagglutination, but the "serum control" will detect the error. You will see examples of this technical error in some of the data.

3. Finally, a *cell control* is set up to determine whether or not the cells hemagglutinate when they are alone in saline and therefore give invalid results.

After the incubation period, the presence or absence of hemagglutination in each well should be recorded. *The highest dilution of serum causing complete inhibition of hemagglutination is taken as the endpoint.* The reciprocal of the highest dilution exhibiting HI is the titer.

Data Sheet – Hemagglutination Inhibition Assay

Influenza B Virus

PATIENT	AGE (yrs)	DATE	DILUTION OF SERUM 1:10	1:20	1:40	1:80	1:160	1:320	1:640	CONTROLS Serum + Cells	Saline + Cells	Virus + Cells
I	8	3/5	⊙	⊙	⊙	⊙	⊙	◍	◍	⊙	⊙	◍
		3/26	⊙	⊙	⊙	⊙	◍	◍	◍	⊙	⊙	◍
J	9	3/5	⊙	◍	◍	◍	◍	◍	◍	⊙	⊙	◍
		3/26	⊙	⊙	⊙	⊙	◍	◍	◍	⊙	⊙	◍
K	9	3/5	◍	◍	◍	◍	◍	◍	◍	⊙	⊙	◍
		3/26	◍	◍	◍	◍	◍	◍	◍	⊙	⊙	◍

Influenza A Virus

PATIENT	AGE (yrs)	DATE	DILUTION OF SERUM 1:10	1:20	1:40	1:80	1:160	1:320	1:640	CONTROLS Serum + Cells	Saline + Cells	Virus + Cells
I	8	3/5	⊙	⊙	◍	◍	◍	◍	◍	⊙	⊙	◍
		3/26	⊙	⊙	⊙	◍	◍	◍	◍	⊙	⊙	◍
J	9	3/5	⊙	◍	◍	◍	◍	◍	◍	⊙	⊙	◍
		3/26	⊙	⊙	◍	◍	◍	◍	◍	⊙	⊙	◍
K*	9	3/5	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙
		3/26	⊙	⊙	◍	◍	◍	◍	◍	⊙	⊙	◍

*IMPORTANT: In preparing the HI test on four year old patient E, influenza B and A on 3/26, the technician ran out of chicken erythrocytes and had to switch to a new batch of cells. This was the only test using these cells.