Laboratory 11 Cold Agglutinin Titer Case Study  
MLAB 1335 Immunology

Cold Agglutinin Titer Case Study

Objectives

1. Review the cold agglutinin titer case study.
2. Calculate the dilution of each tube in the serial dilution.
3. Interpret the endpoint for the test accurately.
4. Record the titer accurately.
5. List two (2) limitations of the procedure and describe how the result may be affected.
6. Use lecture notes, textbook, and laboratory information to answer the study questions.

Cold Agglutinins

Serology testing for cold agglutinins are commonly requested in suspected cases of primary atypical pneumonia, where this rapid screening test has proven useful. Cold agglutinin antibodies are found in the serum of approximately 55% of the patients with primary atypical pneumonia, a respiratory disease caused by *Mycoplasma pneumoniae*. These antibodies cause agglutination of adult red blood cells at 4°C, but not at normal body temperature (37°C). Cold agglutinin antibody levels are often detectable by the end of the first or second week of the disease, increasing to their maximum by the second to fourth week and decreased or absent by six to eight weeks. In *M. pneumoniae*, a positive correlation exists between the level of cold agglutinin antibodies and the severity of the disease, the extent of pulmonary involvement and duration of illness. Extremely high titers are sometimes found in cases of hemolytic anemia. A fourfold or greater rise in titer from paired sera (where one sample is taken early in the disease and another sample is drawn several days or a week later) is significant of acute disease. Cold agglutinins may also be produced by other diseases including liver disorders, chronic sepsis, acquired hemolytic anemia, leishmaniasis, and black water fever. Most of these diseases have symptoms that easily distinguish them from those of primary atypical pneumonia.

Most cold agglutinins have a specificity for the “I” antigen found on most adult human red cells. The specimen must be kept warm until the serum containing the antibody can be separated from the patient’s red cells which contain the “I” antigen. Proper handling requires the blood be collected in tubes kept warm from the moment of collection until the physical separation of the serum from the cells. If the specimen is allowed to cool, the antibody may attach to the "I" antigens causing a falsely decrease titer. Should this happen to the sample, it must be placed in a 37°C incubator for 30 minutes before removing the serum for testing. The incubation at 37°C will cause the cold agglutinin to dissociate from the patient’s cells.

Approximately 75% of patients have a cold agglutinin titer of at least 1:32 before the end of the second week of the illness. The titer disappears after 6-8 weeks. This is not a specific test for the bacterial infection, but when a cold agglutinin titer is > 1:64, there is a high probability that the cold agglutinins are due to *M. pneumoniae*.

Principle of the titer procedure

When serial dilutions of serum containing a cold agglutinin antibody with anti-I specificity are mixed with 1% group O adult red cells and refrigerated, a positive reaction of agglutination will occur in those tubes containing sufficient antibody. The endpoint is determined as the last tube demonstrating the agglutination, and the reciprocal of the dilution is reported as the titer.
The test is read immediately upon centrifugation and in numerical order. A positive test will result in a cell button on the bottom of the tube that is difficult to dislodge by gentle shaking. Large or small clumps will be seen while gently shaking. As soon as a tube has been determined as being “positive”, read the next tube. The last tube showing agglutination is the endpoint of the test. The titer is reported out as the reciprocal of the last dilution showing a positive result.

Limitations of the Procedure

1. Dispensing incorrect quantities of diluent or red blood cell solution or transferring more or less than the required amount of diluted serum will adversely affect the outcome of this test, resulting in a falsely increased or decreased titer.

2. The technique for shaking the tubes to detect agglutination is critical. Harsh shaking may cause weak or fragile agglutinates to break apart, resulting in a false negative result in the tube and a false decrease in the reported titer.

3. The reaction between a true cold agglutinin and the red blood cells is reversible. To prove the presence of a true cold agglutinin, all tubes showing agglutination at 4°C must be negative after incubation at 37°C for 15 - 30 minutes. If agglutination remains, the antibody involved is not a true cold agglutinin.

4. Tube #12 is the cell control and must demonstrate a negative reaction. A positive result in tube #12 indicates spontaneous agglutination of the red cells. The test procedure must be repeated using a different cell suspension.

5. Test should be performed regularly, because an increase in titer throughout the duration of the illness is of greater significance than a positive result on a single specimen.

6. A fourfold or greater rise in the titer of cold agglutinins is suggestive of a recent *Mycoplasma pneumoniae* infection.

<table>
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<th>Tube Number</th>
<th>Dilution</th>
<th>Titer</th>
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</tr>
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<td>11</td>
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Case Study

A 70-year old female was admitted to the hospital through the emergency room with pallor, headache, cough, weakness, fatigue, and fever. The cough, she stated, has been worsening over the last two weeks. The patient also stated that her energy level has not been very high over the last week. After a chest x-ray, it was determined the patient has pneumonia.

The family physician, Dr. Spokane suspects *Mycoplasma pneumoniae*. Dr. Spokane suspects the patient is in her second to third week of the disease and thinks the cold agglutinin titer may be high and orders this test to be performed ASAP. The laboratory draws a large red top tube, keeps it at 37°C until the blood clots, and then spins it down to perform the titer. The results are below. Complete the dilution factor row.

### Results of the titer

<table>
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<th>3</th>
<th>4</th>
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<th>6</th>
<th>7</th>
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</table>

*Tube 12 = control (no serum added)*

1. Based on the results of this titer, is the patient likely to have an infection caused by *Mycoplasma pneumoniae*?  
   Yes _____   No _____

2. What should Dr. Spokane order in about 4 weeks on the patient and why?
Cold Agglutinin Titer Study Questions

Questions are worth one point each, unless otherwise indicated. ____/12 points

1. Define cold agglutinins.

2. In what disease process are cold agglutinins most commonly encountered and what organism causes this disease? (2 points)

3. List two other diseases in which cold agglutinins may be produced. (2 points)

4. Explain the purpose of the 37°C incubation step including the expected outcome for a cold agglutinin. (2 points)

5. Explain the purpose and expected outcome of tube #12. (2 points)

6. Why would a patient with a positive cold agglutinin test be retested?

7. What was the titer of the case study patient from the previous page? (2 points)