

Cold Agglutinin Titer

Objectives:

1. Perform a serial dilution to determine the amount of cold reacting antibody present in a patient specimen with the results obtained falling within ± 1 tube of instructor's value.
2. Properly dispense the correct amounts of diluent and red blood cells and transfer the necessary amount of serum from tube to tube, using precision and care.
3. Calculate the dilution of each tube in the serial dilution once all reagents and patient sample have been added.
4. Recognize the clumping of red blood cells as agglutination and properly interpret and record each tube as being positive or negative for agglutination according to the criteria in the procedure.
5. Recognize the endpoint for the test and correctly interpret and record the titer.
6. Appropriately record and report results as instructed.
7. List 2 limitations of the procedure and describe how the results will be affected..

Introduction:

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 the sixth week. 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. Test should be performed regularly, because an increase in titer throughout the duration of the illness is of greater clinical significance than a positive result on a single specimen and correlates with the severity of the infection..

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 all 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 on the patient's own red blood cells causing a falsely decrease titer. Should the sample be allowed to cool, it must be placed in a 37°C incubator for 30 minutes before removing the serum for testing. The binding of the anti-I to the I antigen is reversed by warming. Incubation at 37°C will cause the cold agglutinin to dissociate from the patient's cells.

Principle:

When serial dilutions of serum containing a cold agglutinin antibody with anti-I specificity are mixed with 3% group O adult red cells (which have the I antigen) and refrigerated, a positive reaction of agglutination will occur which indicates binding of the anti-I in the patient serum to the I antigens on the red blood cells. The end point is determined as the last tube demonstrating the agglutination, and the reciprocal of the dilution is reported as the titer.

Materials:

- | | | | |
|----|--------------------------------------|----|-------------------------------|
| 1. | Twelve (12) 12 x 75 Test tubes | 5. | Serological pipets |
| 2. | Test tube rack | 6. | Refrigerator |
| 3. | 0.85% Saline | 7. | 37°C waterbath |
| 4. | 3% Group O red blood cell suspension | 8. | Sharpie or water proof marker |

Procedure:

1. Number and label twelve 12x75 mm tubes with patient's initials. Place patient's full name on tube #1, and "**cell control**" on tube #12.
2. Place 0.3 mL of saline in each tube.
3. Use a clean serological pipet to add 0.3 mL patient's serum to the first tube. Mix thoroughly by raising and lowering the serum-saline solution three times in the pipet, taking care to avoid creating bubbles.
4. Using the same pipet, transfer 0.3 mL from tube #1 to tube #2. Again raise and lower to solution into the pipet three times to mix.
5. Continue to use the same pipet to repeat the procedure of transferring 0.3 mL from tube #2 to tube #3, then from 3 to #4, etc. through tube #11. After adding and mixing tube # 11, discard 0.3 mL. **NO serum goes into tube # 12.**
6. Use a clean serological pipet to add 0.3 mL of a 3% group O human red blood cell suspension to each tube.
5. Mix well by shaking the rack. Evaluate the fluid level in the tubes. If the titer process is performed correctly, the level will be the same in all tubes. **Show the tubes to your instructor.**
6. Incubate at 4°C (refrigerator temperature) for 15 minutes
7. After 15 minutes, remove the rack from the refrigerator and immediately centrifuge all tubes for 30 seconds.
8. Starting with tube #1, shake the tube **gently** to dislodge the cells from the bottom of the tube and read for macroscopic agglutination. Record the highest dilution in which agglutination is detected. Proceed with reading the tubes quickly and with little handling of the tubes as possible, as the reaction is reversible.
9. Tube # 12 is the negative control and its result must be NEGATIVE. A positive result in this tube invalidates the test result.
10. Incubate all positive tubes in a 37°C heat block for 15 minutes. Remove, spin and immediately read for agglutination. If still positive incubate an additional 15 minutes at 37C. They should be negative at this point. If still positive results are invalid.

Interpretation:

Read tests immediately on removal from the cold, spun and read 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”, proceed to reading 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 and a Blood Bank work up must be done to identify the antibody specificity.
4. The cell control (tube #12) must show no agglutination or the test is considered invalid and must be repeated. A positive result in tube #12 indicates spontaneous agglutination of the red cells. The test procedure must be repeated using a different cell preparation.

<u>Tube #</u>	<u>Dilution</u>	<u>Titer</u>
1	1:4	4
2	1:8	8
3	1:16	16
4	1:32	32
5	1:64	64
6	1:128	128
7	1:256	256
8	1:512	512
9	1:1024	1024
10	1:2048	2048
11	1:4096	4096

Name _____ Date _____

Cold Agglutinin Titer
Recording/Interpreting Results

1. In the chart below record the reactions on the “Observed Result” row for your visual observation of each tube after centrifugation and shaking has been performed. Record “A” for agglutination and “NA” for no agglutination.
2. After you have set up your tubes for the dilution procedure show them to your instructor *BEFORE* placing them in the refrigerator for incubation.
3. The last tube showing agglutination (A) is the endpoint.
4. The titer is determined as the reciprocal of the last tube showing agglutination.
5. The Cell Control is reported as “positive” or “negative”.

Patient's Name _____ Number _____												
Tube Number	1	2	3	4	5	6	7	8	9	10	11	12
Observed Result												
Endpoint _____												
Titer of endpoint _____												
Cell Control _____												

INSTRUCTOR USE ONLY

Skill	Possible Points	Points Awarded
1. Only asked questions that were NOT answered by procedure OR asked for clarification purposes only. Did NOT bother fellow students.	5	
2. Performed procedure according to written protocol AND verbal instructions given by instructor.	10	
3. Student results matched instructor results.	20	
4. Results reported out according to instructions given in procedure AND clerical errors corrected in appropriate manner.	5	
5. Organized and stayed on task.	5	

Cold Agglutinin Titer
Study Questions

Name _____ Date _____

Questions are worth one point, unless otherwise indicated.

1. Are the results valid? YES NO
2. What are cold agglutinins?
3. State the disease process cold agglutinins are most commonly associated.
4. State the genus and species of the organism which causes this disease.
5. List two other diseases which may cause the production of cold agglutinins.
6. Describe the proper collection and storage procedure for a cold agglutinin test.
7. Tubes which show positive results after incubation at 4C must be incubated at 37C. Explain the purpose of the 37°C incubation step including the expected outcome for a cold agglutinin. (2 points)
8. Explain the purpose and expected outcome of tube #12.
9. Why would a patient with a positive cold agglutinin test be retested?
10. Explain why the dilution of tube #1 is 1:4.
11. The tech mistakenly refrigerates a blood specimen for a cold agglutinin test. (2 points)
 - a. If the procedure is performed on this specimen, what effect would the improper storage have on the tests results?
 - b. Other than recollect and properly handle another sample, what could the tech correct the problem to obtain valid results?