Thermal amplitude test

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The thermal amplitude test is performed to determine the reactivity of a cold autoantibody at varying temperatures: 4°C, 22°C, 30°C, and 37°C. Cold autoantibodies that are reactive at temperatures greater than 30°C have the potential to be clinically significant regardless of the antibody titer. Cold autoantibodies that are reactive at temperatures less than 30°C are not considered to be clinically significant. ***Immunohematology* 2013;29:49–50.**

Key Words: thermal amplitude, cold agglutinin disease, cold autoantibody, titer, clinical significance

Principle

Cold autoantibodies and agglutinins are naturally occurring in most people, and incidental cold agglutinins are seen in normal healthy blood donors at low titers (<1:64).[1] These benign cold autoantibodies are reactive at 4°C and have little or no reactivity at temperatures greater than 30°C. Cold autoantibodies may become clinically significant, resulting in red blood cell (RBC) hemolysis, if their titer increases or if they become reactive at temperatures greater than 30°C.[2] Cold agglutinin disease (CAD) is hemolytic anemia secondary to cold autoantibodies and is responsible for approximately 13 to 15 percent of total cases of autoimmune hemolytic anemia (AIHA).[3] It is characterized by immunoglobulin (Ig) M (and, rarely, IgG) autoantibodies, which cause agglutination of RBCs in vitro at room temperature and can cause hemolysis in vivo if the antibody is reactive at temperatures greater than 30°C and present at sufficient titers.[2,4] These autoantibodies are usually directed against I, i, and related RBC antigens.[1] The autoantibodies reactive at temperatures greater than 30°C may cause RBC hemolysis through activation of the complement pathway, leading to formation of membrane attack complex on the RBC membrane and intravascular hemolysis, or they may cause extravascular hemolysis via phagocytosis of C3b-coated RBCs by macrophages.[2] Combined cold and warm AIHA (mixed AIHA) occurs when a patient has both warm AIHA and CAD.

Indications

Benign cold autoantibodies may be detected in the serum of many normal individuals. Pathologic cold autoantibodies capable of causing intravascular or extravascular hemolysis and CAD are characterized as having reactivity at cold temperatures (4°C–18°C) and warm temperatures (30°C–37°C) and are of high titer (>1:512 at 4°C).[5–7] Both benign and pathologic cold autoantibodies may be seen in patients with warm AIHA. Patients with clinical and laboratory evidence of immune-mediated hemolytic anemia and a detectable cold autoantibody need additional evaluation to determine the significance of this cold autoantibody. The thermal amplitude of the cold autoantibody, defined as the highest temperature at which the autoantibody binds the RBC antigen, most accurately predicts severity of the disease.[2,6,7]

Reagents/Supplies

Reagents	Supplies
▪ Two examples of a 3%–5% saline suspension of Group O (I+) washed RBCs ▪ RBCs chosen should lack any RBC antigens for which the patient has known antibodies ▪ 0.9% saline	▪ Water baths at 37°C, 30°C, and 22°C ▪ 10 × 75-mm or 12 × 75-mm test tubes ▪ Tube rack ▪ Transfer pipettes ▪ Calibrated thermometer ▪ Calibrated centrifuge

RBC = red blood cell.

Procedural Steps

- Warm RBCs and patient sample to 37°C separately
- Mix patient sample and RBCs
- Incubate mixture at 37°C for 1 hour
- Centrifuge the mixture and return to 37°C for 5 minutes
- Read for agglutination
- Incubate mixture at 30°C for 1 hour
- Centrifuge the mixture and return to 30°C for 5 minutes
- Read for agglutination
- Incubate mixture at 22°C for 1 hour
- Centrifuge mixture and return to 22°C for 5 minutes
- Read for agglutination
- Interpret results

RBC = red blood cell.

Procedure

Obtain an appropriate sample for testing (Tables 1, 2). The patient's sample should be collected and maintained at 37°C until the serum and the RBCs can be separated. This is done to avoid in vitro autoadsorption. Alternatively, an EDTA plasma sample can be used if it is warmed to 37°C with repeated mixing and then separated for testing.[5] Before performing the thermal amplitude test, the patient's sample should be evaluated for antibodies to clinically significant antigens, and appropriate antibody identification or exclusion should be performed. Prepare two different examples of washed 3 to 5 percent saline suspensions of group O adult (I+) RBCs. If the patient has an identified antibody to an RBC antigen, the cells selected should lack this antigen to prevent falsely positive results. Label two tubes appropriately. Warm the tubes, serum, and RBCs separately until they reach 37°C (about 5–10 minutes). For each RBC suspension to be tested, place one drop of RBCs and three drops of serum into each of the warmed test tubes. Mix well and incubate at 37°C for 1 hour. Centrifuge according to the appropriate time and speed based on current calibrations for serologic agglutination testing for the centrifuge. Return the tubes to the 37°C water bath for 5 minutes before reading. Examine and grade the tubes for agglutination and record results. Transfer the tubes to a 30°C water bath and incubate for 1 hour. Remove the tubes and centrifuge. Place the centrifuged tubes back into the 30°C water bath for 5 minutes before reading the results. Examine and grade the tubes for agglutination and record results. Last, transfer the tubes to a 22°C water bath and allow them to incubate for 1 hour. Centrifuge the tubes and return them to 22°C for 5 minutes. After the tubes have incubated, examine and grade them for agglutination. Record the results.

An alternative to using centrifugation is to use the settling method in which the 37°C and 30°C incubations are extended to 2 hours each and the tubes are then examined for agglutination without centrifugation. The tubes undergoing room tempera-ture incubation can be centrifuged after 30 minutes or 1 hour at 22°C.[8]

Interpret the results. If agglutination is present at 22°C but not at 30°C, then the antibody has limited thermal amplitude. If agglutination is observed at 30°C but not 37°C, then the antibody may have the potential to be clinically significant. If agglutination is observed at 37°C, then the antibody should be considered clinically significant.[9]

In addition, the literature suggests that if no reactivity is observed at 30°C and the patient has hemolytic anemia, then an additional test at 30°C using 30 percent albumin may be helpful to further distinguish a clinically significant antibody capable of causing RBC destruction from a clinically insignificant cold autoantibody.[8,10]

Limitations

Proper sample collection is critical to obtain valid results for this test. Improper sample collection can lead to in vitro autoadsorption of the cold autoantibody and falsely negative results. Choosing antigen-negative RBCs for patients with known antibodies is critical to preventing falsely positive results.

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