Albumin was the first widely used additive solution for hemagglutination tests. Its major effect is to decrease the repulsive forces that keep red blood cells (RBCs) apart. This effect may enable some RBC antibodies, particularly those in the Rh blood group system, to directly agglutinate antigen-positive RBCs after 37°C incubation. The impact of albumin on antibody binding before detection by an indirect antiglobulin test (IAT) is minimal. Use of albumin in antibody identification may help with separation of RBC antibody specificities in a mixture when one or more antibodies demonstrate reactivity after 37°C incubation. Warm autoantibodies can show decreased reactivity in albumin IATs, allowing recognition of underlying alloantibodies.

**Key Words:** albumin, IAT, antibody identification

**Principle**

Albumin is added to serologic tests to overcome forces keeping red blood cells (RBCs) apart, and, in doing so, makes hemagglutination reactions more likely to occur. Use of albumin in antibody detection tests began as early as the 1940s before the advent of the antihuman globulin (AHG) phase of testing, when direct agglutination tests were the only available method for visualizing the antigen-antibody reaction. Initially, the reagent was used in albumin layering or albumin replacement methods. These methods were very sensitive when supporting hemagglutination but were not adopted widely in the United States. The albumin method used for routine tube testing in the 1970s and still in use today involves the simple addition of albumin to a RBC suspension/plasma mixture. Two theories as to the mechanism of albumin’s effect exist; one being that the albumin raises the dielectric constant of the test environment. The RBCs’ negative charge (zeta potential) is dispersed, causing the RBCs to come into close enough contact that an antibody molecule can bridge between two RBCs. Alternatively, the albumin molecule disrupts the water molecules associated with the RBC membrane, again allowing RBCs to be in closer proximity to allow for antibody crosslinking. In reality, each mechanism likely plays a role.

The practical effect of albumin’s impact on RBC crosslinking is that certain antibodies may show direct agglutination after a 37°C incubation using albumin that they would not show without its use. Rh system antibodies, in particular, can show increased reactivity after 37°C incubation in albumin tests. It has also been postulated, however, that the final concentration of albumin in the test environment (considering dilution by plasma and RBC diluent) is too low to significantly affect direct hemagglutination. Historically, some albumin preparations were formulated in a low-ionic environment, leading to a belief that albumin increased the speed of antibody binding. However, albumin alone does not increase the speed or amount of antibody that is bound to RBCs. As stated earlier, it only allows for closer proximity of the RBCs to occur, resulting in observable agglutination. This finding makes it necessary to incubate a test long enough to allow maximum antibody binding equilibrium. A 30- to 60-minute incubation in a normal ionic strength environment is required for adequate antibody binding. The reading after the 37°C incubation is followed by the AHG test.
Indications

The albumin-indirect antiglobulin test (IAT) is a method used in antibody identification studies. The increased tendency for 37°C agglutination when using albumin may allow recognition of antibody specificity before the AHG phase. This step is especially helpful when multiple alloantibodies are present; Rh system antibodies are most likely to react at this test phase. Newly developing antibodies may show increased reactivity in 37°C tests when compared with AHG tests using anti-IgG. Conversely, warm autoantibodies generally have decreased reactivity in albumin-IAT tests. This method may be used to avoid their reactivity while retaining the ability to detect clinically significant alloantibodies when proper incubation time is used.

Procedure

Place two drops of patient or donor plasma or serum into a properly labeled tube, followed by one drop of the 2–5 percent RBC suspension to be tested. If an immediate spin reading is desired, centrifuge at the calibrated time for a low-protein test, and gently resuspend while examining the tube for RBC agglutination. Add two drops of 22 percent bovine albumin to the tube. Incubate the tube at 37°C for 30–60 minutes or the incubation time specified in the manufacturer’s instructions. Centrifuge at the calibrated time for a high-protein test. If serum is being tested, the tube can be examined for hemolysis before beginning resuspension of the RBCs. Gently resuspend the RBC button while examining the tube for RBC agglutination. This step is the albumin 37°C reading. Wash the RBCs three to four times with large quantities of isotonic saline. Add one to two drops of antiglobulin reagent as specified by the manufacturer’s insert. Two drops of antiglobulin reagent generally produces a more sensitive test. Centrifuge at the calibrated time for an AHG reading. Gently resuspend the RBC button while examining the tube for RBC agglutination. Add IgG-coated reagent RBCs to all negative tests. Centrifuge, and read for agglutination. Tests that do not show agglutination or show agglutination that is weaker than expected per the manufacturer’s instructions indicate that inadequate washing has occurred and the test is invalid and must be repeated.

Quality Control

The albumin reagent should be tested for reactivity on each day of use, utilizing an antibody of known specificity and antigen-positive and -negative RBCs. The tests should give expected results at the AHG phase. If the antibody is known to show reactivity after 37°C incubation, this reactivity should be observed with the antigen-positive RBCs. Quality control of other components of the test system should be performed according to laboratory protocols.

Limitations

No single test method, including those using albumin enhancement, will detect all clinically significant alloantibodies. Insufficient incubation time is expected to decrease the sensitivity of the albumin-IAT.

The centrifuge used for the direct agglutination reading after 37°C incubation must have been calibrated for centrifugation of a high-protein test. The centrifugation time for this test is generally longer than the spin time for a low-protein test. Improper centrifugation may result in false results.

References


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