ERYGEN-AC

A panel of 3% Reagent Red Blood Cells for quality control of anti-human globulin procedures

SUMMARY
The antiglobulin test, which is also referred to as the anti-human globulin test (AHG) or the Coombs test, is the cornerstone of detecting clinically significant unexpected antibodies that have coated cells either *in vivo* or *in vitro*. Erythrocytes sensitized with Anti-D (IgG) monoclonal also known as Coombs Control Cells are used as positive controls in anti-human globulin testing. Erythrocytes sensitized with Anti-D (IgG) monoclonal should be used with anti-human globulins containing anti-IgG. The binding of anti-IgG in anti-human globulin, to IgG molecules attached to red cells results in agglutination.

REAGENT
ERYGEN-AC is a reagent set for laboratory use only.
ERYGEN-AC panel of 3% reagent red blood cells comprise of human erythrocytes of group O Rho (D) positive cells sensitized with Anti-D (IgG) monoclonal.
The reagent red blood cells are suspended in isotonic medium to which a Red Cell Preserving solution is added to preserve the red cell integrity and antigenicity.

ADDITIONAL MATERIAL REQUIRED
Test tubes (12x75 mm or 10x75 mm), Physiological saline, Optical aid, Centrifuge (calibrated for 1000 RPM and/or 3400 RPM) and pipettes.

PRINCIPLE
Red cells coated with complement or IgG antibodies do not agglutinate directly when centrifuged. These cells are said to be sensitized with IgG or complement. In order for agglutination to occur, an additional antibody, which reacts with the Fc portion of the IgG antibody, or with the C_3b or C_3d component of complement, must be added to the system. This will form a "bridge" between the antibodies or complement coating the red cells, causing agglutination. Agglutination of the IgG-sensitized erythrocytes indicates that the anti-human globulin has been added to the test and that its anti-IgG component is active.

NOTE
1. *In vitro* diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. Indications of deterioration are notable hemolysis (which may be caused by microbial contamination or improper handling), darkening of cells or spontaneous clumping. Such reagents should be discarded.
3. The reactivity of the product may diminish slightly during the dating period.
4. Ensure that the centrifuge used for testing is properly calibrated for specific test procedures.
5. Ensure that the cells are firmly packed and the negative control cells can be resuspended easily.

Caution: All blood related products should be treated as potentially infectious. **ERYGEN-AC** reagents are derived from donors found negative for HIV, HBsAg, HCV and Syphilis. However, absence of infectious agents in products derived from human blood cannot be guaranteed by any test method.

SAMPLE COLLECTION AND PREPARATION
No special preparation of the patient is required prior to sample collection by approved techniques. Serum collected from freshly clotted blood may be used for optimum results. Serum samples, if not tested immediately may be frozen at - 20° to - 70° C or stored at 2-8°C for not more than 48 hours.

TEST PROCEDURE
1. Bring the kit to room temperature before testing.
2. Gently resuspend the Coombs control cells by repeated inversion of the **ERYGEN-AC** vial.
3. After examining the antiglobulin tests for agglutination, add one drop of Coombs Control Cells to each negative or questionable reaction. Shake to mix well.
4. Centrifuge for 60 seconds at approximately 1000 RPM or for 20 seconds at 3400 RPM
5. Gently resuspend the cells and examine immediately for macroscopic agglutination. Record results accordingly.

INTERPRETATION OF RESULTS
1. Agglutination of the Coombs Control Cells indicates that anti-human globulin was added and that its anti-IgG component was active. Therefore, if performed properly, the original antiglobulin test is valid.
2. Very weak agglutination of the Coombs Control Cells may be due to an insufficiently reactive anti-human globulin or inadequate washing of the test cells. The original antiglobulin test may be invalid and should be repeated.
3. No agglutination of the Coombs Control Cells usually indicates that either the test cells were inadequately washed or anti-human globulin was omitted from the test or that the anti-IgG in the serum was accidentally neutralized. The original antiglobulin test is therefore invalid and should be repeated.

LIMITATIONS
1. In all serological tests, factors such as contaminated materials, improper incubation time / temperature, improper centrifugation or improper interpretation of agglutination pattern may be the cause of false test results.
2. Anti-human globulin lacking antibodies to human IgG will not react with Coombs Control Cells.

False positive results may occur if:
1. Coombs Control Cells or anti-human globulins are contaminated by microbial organisms.
2. Cells are overcentrifuged.

False negative results may occur if:

1. Coombs Control Cells are improperly stored and hence there is loss in reactivity.
2. Cells are improperly centrifuged.
3. The resuspension technique applied is too vigorous to preserve agglutination of weakly sensitized erythrocytes.

SPECIFIC PERFORMANCE CHARACTERISTICS

Each lot of Coombs Control Cells are carefully sensitized and standardized to provide a positive agglutination control for anti-human globulins containing anti-IgG when used as outlined in the procedure. Sufficient testing is performed on each lot of material to assure its reactivity through the dating period.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

1. Mollison P.L., Blood Transfusion in Clinical Medicine, 9th Edition, Oxford: Blackwell Scientific Publications 1993: Chapter 8.
2. Technical Manual of the American Association of Blood Banks, 12th Edition: 1996: Chapter 11.
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