Anti-Human Globulin

IVD

Polyspecific, Anti-IgG+C3d for Coombs Test

PRODUCT CODE
SB007

INTENDED USE
Anti-Human Globulin Anti-IgG,+C3d; Polyspecific is used for the direct antiglobulin test to demonstrate the in-vivo coating of red blood cells with antibody molecules and/or complement components (such as autoantibodies, maternal antibodies in hemolytic disease of the newborn, alloantibodies against red blood cells in transfusion reactions).
Anti-Human Globulin Anti-IgG, C3d; Polyspecific is used for the indirect antiglobulin test to demonstrate the in-vitro coating of red blood cells with antibody molecules and/or complement components as in detection and identification of unexpected antibodies as well as cross match tests. Furthermore, blood group antigen typing (with the corresponding test reagent for the indirect antiglobulin-test) can be

PRINCIPLE
The test principle is a hemagglutination test. Anti-Human Globulin Anti IgG,+C3d; Polyspecific acts as a link between the antibody and/or complement coating of neighboring red blood cells and induces agglutination. Uncoated red blood cells will not agglutinate.

COMPOSITION
Polyspecific Anti-Human Globulin reagent contains anti-IgG derived from rabbits with non-specific activity removed by absorption and mouse monoclonal IgM anti-C3d, Clone BRIC-8. The antibodies are diluted in a buffered solution containing bovine albumin. Each reagent is supplied at optimal dilution, for use with all the recommended techniques stated below without need for further dilution or addition.

Reagent	Color	Dye Used
Anti-Human Globulin	Green	Patent Blue and Tartrazine

STORAGE AND STABILITY
Do not freeze. Reagent vials should be stored at 2-8°C on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity.

SAMPLE COLLECTION AND STORAGE
No special preparation of the patient is required prior to sample collection by approved techniques. Do not use hemolyzed samples.
For Direct Antiglobulin Test: Blood drawn into EDTA is preferred but oxalated, citrated or clotted whole blood may be used. The blood sample should be tested as soon as possible after collection and should not be stored.
For Indirect Antiglobulin Test: Serum, not more than 48 hours old, should be used. Donor units may be tested upto the end of their dating

PRECAUTIONS
1.The reagents are intended for in vitro diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagents past the expiration date (see Vial Label).
4. Do not use the reagents if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. The reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. The reagents contain < 0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
8. Materials used to produce the products were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved microbiological tests.
9. No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

RECOMMENDED TECHNIQUES:

A. DIRECT ANTIGLOBULIN TECHNIQUE (DAT)
This is a one-step test for demonstration of erythrocyte-bound antibodies (for example in hemolytic syndrome in the new born, hemolytic anemia)
1. Wash erythrocytes to be tested three times in isotonic saline and discard the supplement.
2. Prepare a 2-3% erythrocyte suspension in isotonic saline solution.
3. Place 1 drop of erythrocyte suspension into a tube.
4. Add 2 drops of coombs serum and mix.
5. Centrifuge at 1000g for 20 seconds or at a suitable alternative g force and time.
6. Gently shake the tube to dislodge the cell the bottom and observe macroscopically for agglutination.

B. INDIRECT ANTIGLOBULIN TECHNIQUE (IAT)
1) Indirect Antiglobulin Technique (NISS IAT)
1. Prepare a 2-3% suspension of washed test red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 2 volumes of test serum and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at 37ºC for 15 minutes.
4. Wash test red cells 4 times with PBS or Isotonic saline, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant saline after last wash.
5. Add 2 volumes of AHG to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
7. Gently resuspend red cell button and read macroscopically for agglutination

2). LISS Indirect Antiglobulin Technique (LISS IAT)
1. Prepare a 1.5-2% suspension of washed test red cells in LISS.
2. Place in a labelled test tube: 2 volumes of test serum and 2 volumes of test red cell suspension.
3. Mix thoroughly and incubate at 37ºC for 15 minutes.
4. Follow steps 4 to 7 of NISS IAT above.

INTERPRETATION OF TEST RESULTS
1. Positive: Agglutination of test red cells constitutes a positive test result and within the accepted limitations of the test procedure, indicates the presence of IgG and/or complement (C3d) on the test red cells.
2. Negative: No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of IgG and/or complement (C3d) on the test red cells.

LIMITATION
1. Red cells that have a positive DAT due to a coating of IgG cannot be typed by the Indirect Antiglobulin Techniques.
2. A positive DAT due to complement sensitization may not reflect in vivo complement fixation if test cells are from a refrigerated clotted specimen.
3. Inadequate washing of red cells in the indirect antiglobulin techniques may neutralize the anti-human globulin reagent.
4. Following completion of the wash phase excess residual saline may dilute the anti-human globulin, reducing its potency.
5. A negative direct antiglobulin test result does not necessarily preclude clinical diagnosis of ABO Hemolytic Disease of the Newborn or Auto Immune Hemolytic Anemia. It also does not necessarily rule out HDN, especially if ABO incompatibility is suspected.
6. False positive or false negative results may also occur due to: • Contamination of test materials • Improper storage, cell concentration, incubation time or temperature • Improper or excessive centrifugation

DISCLAIMER
1. The user is responsible for the performance of the reagents by any method other than those mentioned in the Recommended Techniques.
2. Any deviations from the Recommended Techniques should be validated prior to use.

BIBLIOGRAPHY

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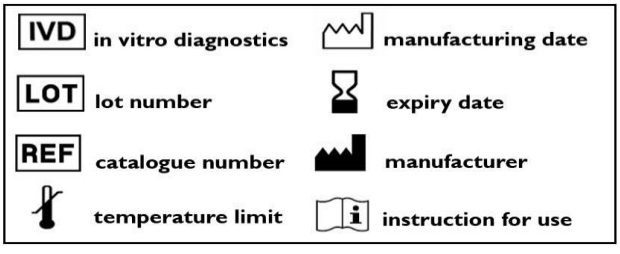
SYMBOL ON LABELS



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