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ANTI-HUMAN GLOBULIN

Diagnostic reagent for the in-vitro qualitative determination of antibodies coating on human red blood cells.

REF: BS.1/AH01.010.0200	200 test	REF: BS.1/AH05.010.1000	1000 test
REF: BS.1/AH03.010.0600	600 test	REF: BS.1/AH06.010.1200	1200 test

CLINICAL SIGNIFICANCE [1,3]

Antibodies immunoglobulins may become attached to human red cells either in vivo (if the body produces an auto-antibody against a self-antigen located on its own red cells) or in vitro (during blood grouping tests or compatibility testing prior to transfusion). In 1945, Coombs, Mourant and Race described the use of anti-human globulin serum for detecting red cell-bound non-agglutinating antibodies. In 1957, Dacie et al showed that the antibodies present in antiglobulin sera were directed against certain components of complement. Anti-human globulin reagents detect non-agglutinating antibody molecules as well as molecules of complement attached to red cells following in vivo or in vitro antigen-antibody reactions.

METHOD PRINCIPLE [2]

When used by the recommended techniques, the reagents will react with immunoglobulins and/or complement attached to the red cell surface, resulting in agglutination (clumping) of adjacent sensitized cells. Cells not sensitized will not be agglutinated.

REAGENT COMPOSITION

Reagent: Anti-Human Globulin	
Antibodies Buffered Solution	Anti-IgG derived from rabbits with non-specific activity - Bovine Serum Albumin - Sodium Azide 0.1%

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R) contains sodium azide which is classified as dangerous substance for environment. 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.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Anti-Human Globulin reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Bioscien Anti-Human Globulin reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8˚C. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. Do not freeze.

Deterioration

The ***Bioscien*** Anti-Human Globulin reagent is normally clear, do not use if it is turbid or if a precipitate is present.

SPECIMEN COLLECTION AND PRESERVATION

Blood

Samples should be drawn aseptically into EDTA to prevent in vitro complement binding and tested within 24 hours. ACD, CPD or CPDA-1 may also be used in absence of EDTA. If only fully clotted specimen should be used do not refrigerate them before testing. All blood samples should be washed at least twice with PBS before being tested.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer
- Physiological saline: NaCl 0.9%, pH 7.0 ± 0.2 at 22ºC ± 1ºC.
- IgG sensitized red cells.
- Inert antibody Serum.
- Weak anti-D.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.
- Coombs cell washer.
- Low Ionic Strength Solution (LISS) .

ASSAY PROCEDURE

Many assay procedures can be described according the wide techniques varieties for the Anti-Human Globulin reagent testing.

Technique (1): Indirect test- Slide Method

1- Prepare in a test tube 2 - 4% suspension of red cells to be used in physiological saline.
2- Place in a glass test tube: 2 Volume of serum to be tested, 1 Volume of 3% red cell suspension and 1 volume of ***Bioscien*** 22% or 30% Bovine Albumin .
3- Mix well and incubate at 37 °C for 30 minutes.
4- Wash the cells 4 times with large volumes of physiological saline, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant the last wash.
5- Re-suspend the cells to a 3% suspension in physiological saline.
6- Mix in a clean tile or slide: one volume of ***Bioscien*** Anti-human globulin reagent and one volume of 3% suspension washed cells.
7- Allow to stand at room temperature for 5 minutes.
8- Rock the tile gently and examine for agglutination over a light source.

Technique (2): Indirect test- Tube Method

1- Prepare 2 - 4% suspension of red cells to be used in physiological saline.
2- Place in a glass test tube: 2 Volume of serum to be tested, 1 Volume of 3% red cell suspension and 1 volume of ***Bioscien*** 22% or 30% Bovine Albumin .
3- Mix well and incubate at 37 °C for 30 minutes.
4- Wash the cells 4 times with large volumes of physiological saline, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant the last wash.
5- Add 2 volumes of ***Bioscien*** Anti-human globulin reagent.
6- Mix well and centrifuge at 1000 rpm (100 RDF) for 1minute.
7- Gently resuspend the cell button and examine for agglutination, negative results can be checked microscopically.

Technique (3): Direct test- Slide Method

1- Wash the red cells to be tested 4 times with large volumes of physiological saline, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant the last wash.
2- Prepare a 3% suspension of washed red cells in physiological saline.
3- Mix on a clean tile or slide: 1 drop of ***Bioscien*** Anti Human Globulin reagent and 1 drop of 3% red cell suspension.
4- Allow to stand at room temperature for 5 minutes
5- Gently rock the tile and examine for agglutination over a light source.

Technique (4): Direct test- Tube Method

1- Wash the red cells to be tested 4 times with large volumes of physiological saline, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant the last wash.
2- Re-suspend the cell to 5% suspension in physiological saline
3- Place in a glass test tube: 2 Volume of ***Bioscien*** Anti Human Globulin reagent and 1 Volume of 5% red cell suspension.
4- Mix well and centrifuge at 1000 rpm (100 RDF) for 1minute.
5- Gently resuspend the cell button and examine for agglutination, negative results can be checked microscopically.

STABILITY OF THE REACTIONS

1. Read all tube and slide tests immediately.
2. Avoid delay in completing of washing steps, as it may result in dissociation of antigen-antibody complexes.
3. Slide tests should be interpreted within two minutes to ensure specificity and to avoid the false negative or weak positive reactions.

LIMITATIONS

1. Inadequate washing of red cells in the indirect antiglobulin techniques may neutralize the anti-human globulin reagent.
2. Following completion of the wash phase excess residual saline may dilute the antihuman globulin, reducing its potency.
3. A negative direct antiglobulin test result does not necessarily preclude clinical diagnosis of ABO Haemolytic Disease of the Newborn or Auto Immune Haemolytic Anaemia. It also does not necessarily rule out HDN, especially if ABO incompatibility is suspected.
4. 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.

QUALITY CONTROL

To ensure adequate quality control, it is recommended a positive control (weak Anti-D 0.1 IU/ml) and a negative control (an inert serum) be test in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results. For any questions or problems please contact ***Bioscien*** technical support.

PERFORMANCE CHARACTERISTICS

Anti-Human Globulin reagent have been characterized by all the procedures described in the insert. Prior to release, each lot of ***Bioscien*** Anti-Human Globulin is tested according the insert by all the techniques mentioned, against red cells coated with Anti-D to check suitable reactivity.

REFERENCES

1. Coombs RRA, Mourant AE, Race RR. A new test for the detection of weak and “incomplete” Rh antibodies. Brit J Exp Pathol. 1945; 26:255.
2. Wright MS, Issit PD. Anti-complement and the indirect antiglobulin test. Transfusion 1979; 19:688-694.
3. Howard JE, Winn LC, Gottlieb CE, Grumet FC, Garratty G, Petz LD. Clinical significance of the anticomplement components of anti-globulin antisera. Transfusion 1982: 22:269

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number	⚠	Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		