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Anti-HG, polyspecific/monoclonal

(en) English

REF	Content
B05181	- 1x 10 mL Anti-HG, polyspecific/monoclonal

For professional in vitro diagnostic use only.

INTENDED USE

These reagents are polyspecific blood grouping reagents intended to be used to qualitatively detect the presence or absence of sensitising IgG antibodies (all 4 subclasses) and complement factors C3d and C3b on human red cells when tested in accordance with the recommended techniques stated in this IFU.

DIAGNOSTIC SIGNIFICANCE

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.

TEST PRINCIPLE

When used by the recommended techniques, the reagents will react with IgG immunoglobulins and/or C3 complement factors (C3d and C3b) attached to the red cell surface, resulting in agglutination (clumping) of adjacent sensitised cells. Cells not sensitised will not be agglutinated (See LIMITATIONS).

REAGENT COMPOSITION

DIALAB Anti-HG, polyspecific/monoclonal 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. The reagents do not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitisation or an allergic reaction by the user. Each reagent is supplied at optimal dilution, for use with all the recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date see **Vial Label.**

Reagent	Colour	Dye Used
Anti-HG, polyspecific/monoclonal	Green	Patent Blue and Tartrazine

MATERIAL REQUIRED BUT NOT PROVIDED

- Coombs cell washer
- Glass test tubes (10 x 75 mm or 12 x 75 mm)
- IgG sensitised red cells
- Inert antibody (inert AB serum)
- Low Ionic Strength Solution (LISS): Containing 0.03 M NaCl, 0.003 M Na_2HPO_4: NaH_2PO_4 buffer pH 6.7 at 22ºC ± 1ºC and 0.24 M glycine
- PBS solution (pH 6.8 – 7.2) or Isotonic saline solution (pH 6.5 – 7.5)
- Volumetric pipettes
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC
- Weak Anti-D

REAGENT PREPARATION

The reagent is ready to use.
Before use, let the reagent warm up to room temperature. As soon as the reagent has been used, put the reagent back in storage at 2-8 °C.

STORAGE AND STABILITY

Reagent vials should be stored at 2-8 ºC on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. This reagent has undergone transportation stability studies at 37°C and -25°C as described in document EN 23640:2015.

WARNINGS AND PRECAUTIONS

- State hazards regarding storage, use or disposal.
- The reagent is intended for in vitro diagnostic use only.
- If a reagent vial is cracked or leaking, discard the contents immediately.
- Do not use the reagent past the expiration date (see Vial Label).
- Do not use the reagent if a precipitate is present.
- Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
- The reagent has been filtered through a 0.2 µm capsule to reduce the bio-burden, but are not supplied sterile. 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.
- The reagent contains < 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.
- 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.
- 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.
- In the TEST PROCEDURE one volume is approximately 50 µL when using the vial dropper provided.
- Use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagents are in use. User must determine the suitability of the reagents for use in other techniques.

SPECIMEN COLLECTION AND STORAGE

This test can be used with fresh human whole blood samples.

Samples should be drawn aseptically into EDTA to prevent in vitro complement binding and tested as soon as possible. If EDTA is unavailable, samples drawn into ACD, CPD or CPDA-1 are preferable to clotted ones. If only clotted samples are available, do not refrigerate them before testing.

TEST PROCEDURE

A. Direct Antiglobulin Technique (DAT)

1. Wash 1 volume of test red cells (2-3% suspension in PBS or Isotonic saline) 4 times with PBS or Isotonic saline, taking care to decant saline between washes and resuspend each cell button after each wash. Completely decant saline after last wash.
2. Add 2 volumes of DIALAB Anti-Human Globulin to each dry cell button.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
4. Gently resuspend red cell button and read macroscopically for agglutination.

B. 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 37º C for 15 minutes.
4. Wash test red cells 4 times with PBS, 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 DIALAB Anti-Human Globulin 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 each cell button and read macroscopically for agglutination.

C. 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.

NOTE: Washing steps should be completed without interruption and tests centrifuged and read immediately after addition of the reagent. Delays may result in dissociation of antigen-antibody complexes, causing false negative or weak positive results.

INTERPRETATION OF RESULTS

- **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/C3b) on the test red cells.
- **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/C3b) on the test red cells.

NOTE: Caution should be exercised in the interpretation of results of tests performed at temperatures other than those RECOMMENDED.

QUALITY CONTROL AND CALIBRATION

- It is recommended a positive control (weak Anti-D < 0.1 IU/mL) and a negative control (an inert serum) to be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
- The antiglobulin techniques can only be considered valid if all negative tests react positively with IgG sensitised red cells.

PERFORMANCE CHARACTERISTICS

- Prior to release, each lot of the reagents were tested using the recommended test methods listed in this IFU against red cells coated with Anti-D, Anti-K and Anti-FyA to check suitable reactivity. The tests complied with the test requirements as stated in the current version/issue of the 'Guidelines for the Blood Transfusion Services in the United Kingdom'.
- Anti-C3d potency is demonstrated in tests employing cells coated with C3d and C3b.
- The presence of contaminating heterospecific agglutinins or antibodies to C4d has been excluded in tests employing red cells of all AB0 groups and cells coated with C4d.
- The reactivity of any Anti-IgM, Anti-IgA or Anti-light chain components that might be present has not been established.

TRACEABILITY

The anti-IgG and anti-C3d potencies have been tested against the following minimum potency reference standard obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-AHG reference standard 96/666.

EXPECTED VALUES

The Quality Control of the reagents was performed using red cells with phenotypes that were verified by a UK blood transfusion centre and had been washed with PBS or Isotonic saline prior to use.

LIMITATIONS

- The user is responsible for the performance of the reagent by any method other than those mentioned in the TEST PROCEDURE.
- Any deviations from the TEST PROCEDURE should be validated prior to use.
- Red cells that have a positive DAT due to a coating of IgG cannot be typed by the Indirect Antiglobulin Techniques.
- A positive DAT due to complement sensitisation may not reflect in vivo complement fixation if test cells are from a refrigerated clotted specimen.
- Inadequate washing of red cells in the indirect antiglobulin techniques may neutralise the anti-human globulin reagent.
- Following completion of the wash phase excess residual saline may dilute the anti-human globulin, reducing its potency.
- A negative direct antiglobulin test result does not necessarily preclude clinical diagnosis of ABO Haemolytic Disease of the New-born or Auto Immune Haemolytic Anaemia. It also does not necessarily rule out HDN, especially if ABO incompatibility is suspected.

- 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

WASTE MANAGEMENT

For information on disposal of the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

LITERATURE

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USED SYMBOLS

Symbol	Description
Cont.	Content

CE 0123 IVD 2°C 8°C