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Anti-D (IgM/IgG), monoclonal

(en) English

REF	Content
B054O8	- 1x 10 mL Anti-D (IgM/IgG), monoclonal
B08408	- 1x 1000 mL Anti-D (IgM/IgG), monoclonal

For professional in vitro diagnostic use only.

INTENDED USE

The DIALAB Anti-D reagent is a blood grouping reagent intended to be used to qualitatively determine the presence or absence of the Rh D antigen on the red cells of blood donors or patients requiring a blood transfusion when tested in accordance with the recommended techniques stated in this IFU.

DIAGNOSTIC SIGNIFICANCE

The Rh blood group system was discovered in 1940. The D antigen is the most clinically significant non-AB0 red blood cell antigen and has been implicated in causing Haemolytic Transfusion Reactions and Haemolytic Disease of the Newborn.

Anti-D	Phenotype	Caucasians %[3]	Afro-Americans %[3]
+	Rh D positive	83	92
0	Rh D negative	17	8

TEST PRINCIPLE

The reagents contain antibodies against the D antigen on human red cells and will cause direct agglutination (clumping) of human red cells that carry the D antigen and indirect agglutination of human red cells that are Category D^{VI} in the antiglobulin phase of testing. No agglutination (no clumping) generally indicates the absence of the D antigen on human red cells (see LIMITATIONS).

REAGENT COMPOSITION

The DIALAB Anti-D (IgM/IgG), monoclonal blood grouping reagent is a low protein, blended reagent containing a human monoclonal IgM and IgG anti-D, diluted in a phosphate buffer containing sodium chloride (0.9 g%), bovine albumin (2.0 g%) and macromolecular potentiators (1.5 g%). When typing patient samples, this reagent will directly agglutinate Rh D positive cells, including majority of variants (but not D^{VI}) and a high proportion of weak D (D^u) phenotypes when using the recommended techniques. 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. The reagent is supplied at optimal dilution for use on patient samples with all recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date see Vial Label.

IgM/IgG	Cell Line/Clone
IgM	RUM-1
IgG	MS-26

WEAKENED EXRPRESSION OF THE Rh D ANTIGEN:
The collective term D^u is widely used to describe red cells which have a weaker expression of the D antigen than normal. The term weak D denotes individuals with a reduced number of complete D antigen sites per red cell. The term partial D denotes individuals with missing D antigen epitopes. D^{VI} is a partial D category which misses most D epitopes. DIALAB Anti-D (IgM/IgG), monoclonal reagent will detect most examples of partial and weak D red cells by direct agglutination but will not detect D^{VI} cells. This reagent will detect D^{VI} and partial D cells in the IAT phase.

MATERIAL REQUIRED BUT NOT PROVIDED

- Anti-human globulin reagent, for example DIALAB Anti-HG reagent
- Applicator sticks
- Automatic plate reader
- Coombs cell washer
- Bio-Rad ID-Cards (LISS/Coombs) and (NaCl, enzyme test and cold agglutinins)
- Bio-Rad ID-Centrifuge
- Bio-Rad ID-CellStab or ID-Diluent 2
- Bio-Rad ID-Incubator equilibrated to 37ºC ± 2ºC
- Glass microscope slides or white card tiles
- Glass test tubes (10 x 75 mm or 12 x 75 mm)
- IgG sensitised red cell
- Microplate centrifuge
- Ortho BioVue System Cassettes (AHG/Coombs) and (Neutral).
- Ortho BioVue System Centrifuge
- Ortho BioVue System Heat Block equilibrated to 37ºC ± 2ºC
- Ortho 0.8% Red Cell Diluent
- Plate shaker
- PBS solution (pH 6.8 – 7.2) or Isotonic saline solution (pH 6.5 – 7.5)
- Positive (ideally R_1r) and negative (rr) control red cells
- Test tube centrifuge
- Validated "U" well microplates
- Volumetric pipettes
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.

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°C 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 ISO 23640:2015.

WARNINGS AND PRECAUTIONS

- The reagent is intended for *in vitro* diagnostic use only.
- If a reagent vessel 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 is 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 regent 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 reagent 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.

SPECIMEN COLLECTION AND STORAGE

Blood samples can be collected into EDTA, citrate, CPDA anticoagulants or as a clotted sample. The samples should be tested as soon as possible following collection. If a delay in testing should occur, store the samples at 2-8°C. Samples displaying gross haemolysis or microbial contamination should not be used for testing. Blood samples showing evidence of lysis may give unreliable results. It is preferable (but not essential) to wash all blood samples with PBS or Isotonic saline before being tested.

TEST PROCEDURE

NOT CATEGORY D^{VI}:

A. Tube Technique

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic Saline.
2. Place in a labelled test tube: 1 volume of DIALAB Anti-D (IgM/IgG), monoclonal reagent and 1 volume of red cell suspension.
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.
5. Any tubes, which show a negative or questionable result (which can happen with D^u or weak D samples), should be incubated for 15 minutes at room temperature.
6. Following incubation, repeat steps 3 and 4.

B. Bio-Rad-ID Technique (NaCl, enzyme test and cold agglutinins cards)

1. Prepare a 0.8% suspension of red cells in an ID-CellStab or ID-Diluent 2.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50 µL of red cell suspension and 25 µL of DIALAB Anti-D (IgM/IgG), monoclonal reagent.
4. Centrifuge the ID-Card(s) in a Bio-Rad gel card centrifuge.
5. Read macroscopically for agglutination.

C. Ortho BioVue Technique (Neutral cards)

1. Prepare a 0.8% suspension of red cells in 0.8% Ortho Red Cell Diluent.
2. Remove aluminium foil from as many reaction chambers as needed.
3. Place in appropriate reaction chamber: 50 µL of red cell suspension and 40 µL of DIALAB Anti-D (IgM/IgG), monoclonal reagent.
4. Centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
5. Read macroscopically for agglutination.

D. Microplate Technique, using "U" wells

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic Saline.
2. Place in the appropriate well: 1 volume DIALAB Anti-D (IgM/IgG), monoclonal reagent and 1 volume test red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination.
4. Incubate at room temperature for 15 minutes (time dependant on user).
5. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
6. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker.
7. Read macroscopically or with a validated automatic reader.
8. Any weak reactions should be repeated by the tube technique.

E. Slide Technique

1. Prepare a 35-45% suspension of red cells in serum, plasma or PBS or Isotonic solution or use anti-coagulated whole blood (in it's own plasma).
2. Place on a labelled glass slide: 1 volume of DIALAB Anti-D (IgM/IgG), monoclonal reagent and 1 volume of red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 1 minute period, maintaining slide at room temperature.
5. Read macroscopically after 1 minute over a diffuse light and do not mistake fibrin strands as agglutination.
6. Any weak reactions should be repeated by the tube technique.

TO DETECT CATEGORY D^{VI}:

A. Indirect Antiglobulin Technique (IAT)

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume of DIALAB Anti-D (IgM/IgG), monoclonal and 1 volume of red cell suspension.
3. Mix thoroughly and incubate at 37°C for 15 minutes.
4. Wash red cells at least once 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.

5. Add 2 drops of Anti-HG reagent or anti-IgG to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf for a suitable alternative time and force.
7. Resuspend each cell button and read macroscopically.
8. Confirm validity of all negative reactions with IgG sensitised red cells.

B. Bio-Rad-ID Techniques (LISS/Coombs cards)

1. Prepare 0.8% suspension of red cells in ID-CellStab or ID-Diluent 2.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50 µL of red cell suspension and 25 µL of DIALAB Anti-D (IgM/IgG), monoclonal.
4. Incubate the ID-Card(s) for 15 minutes at 37°C.
5. Centrifuge the ID-Card(s) in a Bio-Rad gel card centrifuge.
6. Read macroscopically for agglutination.

C. Ortho BioVue Technique (AHG/Coombs cards)

1. Prepare a 0.8% suspension of red cells in 0.8% Ortho Red Cell Diluent.
2. Remove aluminium foil from as many reaction chambers as needed.
3. Place in appropriate reaction chamber: 50 µL of test red cell suspension and 40 µL of DIALAB Anti-D (IgM/IgG), monoclonal.
4. Incubate the cassette(s) for 15 minutes at 37°C.
5. Centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
6. Read macroscopically for agglutination.

NOTE:
- Read all tube and microplate tests immediately after centrifugation.
- Complete washing steps without interruption and centrifuge and read tests immediately after addition of anti-human globulin because delays may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.
- Slide tests should be interpreted after a maximum of one minute to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
- Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

INTERPRETATION OF RESULTS

1. **Positive:** Agglutination of the red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the D antigen on the test red cells.
2. **Negative:** No agglutination of the red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of the D antigen on the test red cells.
3. Test results of cells that are agglutinated using the reagent negative control shall be excluded, as the agglutination is most probably caused by the effect of the macromolecular potentiators in the reagent on sensitised cells.

QUALITY CONTROL AND CALIBRATION

- It is recommended a positive control (ideally R_1r cells), a negative control (ideally rr cells) and a reagent negative control (e.g. DIALAB Anti-D Negative Control) be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
- When typing red cells from a patient who is diagnosed with a disease that causes the red cells to become coated with antibody or other proteins (such as HDN, AIHA), it is important to test the patient's red cells using DIALAB's Anti-D Negative Control. Tests must be considered invalid if red cells are agglutinated using DIALAB's Anti-D Negative Control.
- Test samples for category D^{VI} determination by the Indirect Antiglobulin Test, Coombs Bio-Rad-ID and Coombs Ortho BioVue Techniques only.
- Weak and variant D antigens are poorly detected by gel card, microtitre plate and slide techniques. It is recommended that weak and partial variants are tested using the tube test technique.
- The antiglobulin tube technique can only be considered valid if all negative tests react positively with IgG sensitised red cells.
- In the chapter TEST PROCEDURE one volume is approximately 50 µL when using the vial dropper provided with the 10 mL vial.
- The use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
- The user must determine the suitability of the reagents for use in other techniques.

PERFORMANCE CHARACTERISTICS

- Prior to release, each lot of DIALAB Anti-D (IgM/IgG), monoclonal reagent was tested using the recommended test methods listed in this IFU. 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' and the 'Common Technical Specifications'.
- The Quality Control of the reagent was performed using red cells with phenotypes that were verified by a blood transfusion centre and had been washed with PBS or Isotonic saline prior to use.
- Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.

TRACEABILITY

The potency of the reagent has been tested against the following minimum potency reference standard obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-D reference 99/836.

EXPECTED VALUES

Expected values are shown in the table in the chapter DIAGNOSTIC SIGNIFICANCE.

LIMITATIONS

- The user is responsible for the performance of the reagents by any method other than those mentioned in the chapter TEST PROCEDURE.
- Any deviations from the TEST PROCEDURE should be validated prior to use.
- DIALAB Anti-D (IgM/IgG), monoclonal is not suitable for use with enzyme treated cells or cells suspended in LISS.
- The use of solutions for making red cell suspensions other than those described in the TEST PROCEDURE section in the document must be validated prior to use. Some solutions may give rise to false positive or false negative reactions.
- Stored blood may give weaker reactions than fresh blood.
- False positive agglutination may be seen when testing IgG sensitised cells.
- 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
  - Deviation from the recommended techniques

WASTE MANAGEMENT

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

LITERATURE

1. Issitt PD. Applied Blood Group Serology, 3rd Edition, Montgomery Scientific, Miami, 1985, Chapter 10.
2. AABB Technical Manual, 16th Edition, AABB 2008.
3. Marion E. Reid and Christine Lomas-Francis, Blood Group Antigens and Antibodies, SBB Books, New York 2007; Page 192.
4. Jones J, Scott ML, Voak D. Monoclonal anti-D specificity and Rh D structure: criteria for selection of monoclonal anti-D reagents for routine typing of patients and donors. Transfusion Medicine 1995. **5**, 171-184
5. Guidelines for the Blood Transfusion Service in the United Kingdom. 6th Edition 2002. The Stationary Office.
6. British Committee for Standards in Haematology, Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of new techniques for blood grouping, antibody screening and cross matching. Transfusion Medicine, 1995, **5**, 145-150.

USED SYMBOLS

Symbol	Description
Cont.	Content

IVD