INTENDED USE

BIOSCOT Anti-D (cell line MS-201) and Anti-D (cell line RUM-1) are monoclonal human IgM blood grouping reagents which will detect the D antigen when tested according to the slide, tube, microplate and column techniques. These reagents are designed for use by operators trained in serological techniques.

INTRODUCTION

The Rh Blood Group System

The observations of Levine and Stetson in 1939 and Landsteiner and Weiner in 1940 provided the basis for current understanding of the clinical significance and laboratory detection of Anti-D. Approximately 15% of Caucasians lack the RhD antigen and are easily stimulated by an RhD positive pregnancy or blood transfusion to produce anti-D. This may cause haemolytic disease of the newborn or severe haemolytic transfusion reactions.

Weak and Partial D

The term weak D, denotes individuals with a reduced number of entire D antigen sites per red cell. The term partial D, signifies individuals with missing D epitopes. D category VI (D®) is the partial D category which lacks the most D epitopes. BIOSCOT Anti-D reagent, product code GG, will detect most examples of weak D and partial D red cells by direct agglutination but will not detect D®. BIOSCOT TP and GG Anti-D reagents are recommended as particularly suitable for grouping patients. The slide and microplate techniques are not recommended for the detection of weak or partial D cells.

PRINCIPLE OF THE REAGENT

When used by the recommended techniques these reagents will cause agglutination (clumping) of red cells carrying the specific antigen (positive test). Lack of agglutination of the red cells demonstrates the absence of the specific antigen (negative test).

These reagents have been optimised for use by the recommended techniques without further dilution or additions.

The products are supplied filtered to 0.22 μm.

MATERIALS

Product code TP Anti-D blood grouping reagent contains antibody from cell line MS-201. Product code GG Anti-D blood grouping reagent contains antibody from cell line RUM-1. The reagents are composed of monoclonal human IgM antibodies in a buffered solution containing macromolecular chemical potentiators. The reagents contain 0.1% (w/v) sodium azide and bovine material. Each vial (10 mL) contains sufficient material for approximately 250 - 400 tests.

PRECAUTIONS

1. All blood products should be treated as potentially infectious. The human donors or the cell lines used to produce these reagents have been tested and found to be negative for Anti-HIV, Anti-HCV, HBsAg, EBV and Mouse Antibody Production (MAP) viruses. No known tests can guarantee that any product derived from human blood is free from infectious agents. Care must be taken in the use and disposal of each container and its contents.

2. These reagents contain 0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead or copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.

3. These products should be clear. Turbidity may indicate bacterial contamination. The reagents should not be used if a precipitate, fibrin gel or particles are present.

4. These reagents are for professional in vitro diagnostic use only.

5. The bovine material is obtained from USDA approved sources or from sources for which origin information is available. The donor animals have been inspected and certified disease free and are deemed to have low TSE (Transmissible Spongiform Encephalopathy) risk.

6. The products should be disposed of either by overnight immersion in disinfectants at appropriate concentrations or by autoclaving.

ADVICE TO USERS

It is recommended that a positive and negative control should be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show the expected reactions.

It is not required to use a reagent control in parallel with all tests using this reagent. Only in typing the red cells of patients known to have auto-antibodies or protein abnormalities is the use of a reagent control such as BIOSCOT Monoclonal Control (product code: TT) recommended. This should be tested in parallel with the reagent.

These reagents have been characterised by the procedures recommended in these instructions for use; their suitability for use in other techniques must be determined by the user.

In the event of changes in analytical performance of the device or damage to the packaging, please contact the Quality Assurance department at Millipore (UK) Ltd.

STORAGE

Store the opened / unopened products at 2-8°C until the expiry date detailed on the product label.

Failure to store the products at the correct temperature, for example, storage at higher temperature or repeated freezing and thawing may result in accelerated loss of reagent activity.

SPECIMEN COLLECTION

No special preparation of the patient is required prior to specimen collection. Blood should be collected by an approved phlebotomy technique into citrate, EDTA anticoagulants or as a clotted sample. The specimen should be tested as soon as possible following collection. If a delay in testing should occur, store the specimen at 2-8°C. Specimens displaying gross haemolysis or microbial contamination should not be tested with this reagent. Failure to store the specimens at the correct temperature may result in false positive or false negative results.

MATERIALS REQUIRED BUT NOT PROVIDED

- Slide Technique:
  • Microscope slide
  • Isotonic saline or compatible plasma/serum
  • Timer

- Microplate Technique:
  • U well microplate
  • Isotonic saline
  • Timer
  • Centrifuge (1000 rcf)
  • Microplate shaker
  • Microplate reader (optional)

- Ortho BioVue System:
  • Ortho BioVue® Neutral Cassettes
  • Isotonic saline, phosphate buffered saline (PBS) or Ortho® 0.8% Red Cell Diluent
  • Micropettes capable of delivering 10, 40 and 50 μL
  • Timer
  • Centrifuge suitable for Ortho BioVue Cassettes
  • Reader (optional)

- Tube Technique:
  • Test tube
  • Isotonic saline
  • 37°C Incubator
  • Timer
  • Centrifuge (1000 rcf)

- DiaMed ID-Card (Bio-Rad):
  • ID-Card “NaCl, enzyme test and cold agglutinins”
  • Isotonic saline, phosphate buffered saline (PBS) or ID-Diluent 2
  • Micropettes capable of delivering 10, 25 and 50 μL
  • Reader (optional)
RECOMMENDED TECHNIQUE

1. SLIDE TECHNIQUE
1.1. Prepare a 35-50% suspension of test red cells in autologous (or compatible) plasma, serum or in isotonic saline.
1.2. Add 1 drop (40 μL) of Anti-D reagent to a clean, labelled microscope slide.
1.3. Add 1 drop (40 μL) of the suspension of test red cells.
1.4. Mix the antiserum and cells over an area about 2 cm in diameter by gently and continuously rocking the slide. Read macroscopically after 2 minutes. Do not confuse any drying of the mixture with agglutination.

2. TUBE TECHNIQUE
2.1. Prepare a 3-5% suspension of test red cells in isotonic saline.
2.2. Add 1 drop (40 μL) of Anti-D reagent to an appropriately labelled test tube.
2.3. Add 1 drop (40 μL) of the suspension of test red cells.
2.4. Mix and centrifuge at 1000 rcf for 20 seconds.
2.5. Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.
2.6. Incubate all negative or weakly positive tests at 37°C for 5 minutes and repeat steps 2.4 and 2.5. This may enhance the reaction strength in typing red cells of weak or partial D phenotypes.

3. MICROPLATE TECHNIQUE
3.1. Prepare a 3-5% suspension of test red cells in isotonic saline.
3.2. Add 1 drop (40 μL) of Anti-D reagent to the appropriate test wells of a microplate.
3.3. Add an equal volume (40 μL) of the test cell suspension to the appropriate test wells.
3.4. Mix the contents of each well using manual means or a microplate shaker.
3.5. Incubate the microplate at ambient temperature for 15-20 minutes.
3.6. Centrifuge the microplate at 100 rcf for 40 seconds.
3.7. Resuspend the red cells by manual means or using the microplate shaker.
3.8. Read tests macroscopically or with a reader. The use of a plate reader must be validated by the user.

4. DIAMED ID CARD (BIORAD)
4.1. Prepare a 3-5% suspension of test red cells in isotonic saline/PBS or a 0.8% suspension in ID-Diluent 2.
4.2. Add 10 μL of 3-5% or 50 μL of 0.8% suspension of test red cells to the appropriate microtube of the ID-card.
4.3. Add 25 μL of Anti-D reagent to the appropriate microtube.
4.4. Mix gently and centrifuge the ID-card at the speed and time recommended in the ID-card manufacturer’s instructions.
4.5. Read macroscopically or with a reader. The use of a reader must be validated by the user.

5. ORTHO BIOVUE SYSTEM
5.1. Prepare a 3-5% suspension of test red cells in isotonic saline/PBS or a 0.8% suspension in Ortho Red Cell Diluent.
5.2. Add 10 μL of 3-5% or 50 μL of 0.8% suspension of test red cells to the appropriate Ortho BioVue cassette reaction chamber.
5.3. Add 40 μL of Anti-D reagent to the appropriate reaction chamber.
5.4. Mix gently and centrifuge the cassette at the speed and time recommended in the cassette manufacturer’s instructions.
5.5. Read macroscopically or with a reader. The use of a reader must be validated by the user.

LIMITATIONS
BIOSCOT Anti-D reagent, product code TP, will detect some examples of D weak and partial D red cells by direct agglutination but will not detect D0 phenotypes (as required by the UKBTS and BCSH Guidelines). This product should not be used for the D typing of donor red cells. Anti-D reagent, product code GG, will detect most examples of D weak and partial D red cells but will not detect D0 phenotypes. Users wishing to detect D0 phenotypes should use BIOSCOT Anti-D IgM/IgG blood grouping reagent – product code BM.

The slide and microplate techniques are not recommended for the detection of weak or partial D cells.

Red cells that have a positive direct antiglobulin test (DAT) may produce false positive results. The use of BIOSCOT Monoclonal Control Reagent (product code TT) is recommended for detection of such potentially false positive results.

Rigid polystyrene microplates are generally more suitable than those made from PVC. Each batch of microplates should be evaluated in the user’s system prior to acceptance as suitable for routine usage.

The concentration of cells used in the microplate technique is critical. Weak suspensions may result in monolayering of the cell button after centrifugation. Strong suspensions may mask weak results. The degree of agitation after centrifugation is critical. Excessive agitation may weaken the reaction sufficiently to give false negative results. Optimal agitation time and speed must be identified and validated.

Incorrect handling or storage of the ID-cards or Ortho BioVue cassettes could lead to incorrect results. Cards and cassettes should be stored and handled according to the manufacturer’s instructions.

False positive and false negative results may occur through contamination of test materials or any deviation from the recommended techniques.

PERFORMANCE CHARACTERISTICS

Anti-D (cell line MS-201) monoclonal blood grouping reagent TP and Anti-D (cell line RUM-1) monoclonal blood grouping reagent GG have been tested by each of the recommended techniques with donor, clinical and neonatal specimens. The sample population represented all major RhD phenotypes. The total number of tests (n), and the calculated sensitivity and specificity for each technique are displayed below.

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<tr>
<th>TECHNIQUE</th>
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<tr>
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<td>SENSITIVITY</td>
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<td>Tube</td>
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<td>Slide</td>
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<tr>
<td>DiaMed ID-Card (Bio-Rad)</td>
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<tr>
<td>Ortho BioVue System</td>
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<td>281</td>
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Definitions from the Common Technical Specifications (CTS):
Diagnostic Sensitivity: The probability that the device gives a positive result in the presence of the target marker.
Diagnostic Specificity: The probability that the device gives a negative result in the absence of the target marker.

REFERENCES

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