INTRODUCTION

The ABO Blood Group System

In 1900, Landsteiner discovered that the serum of some individuals would agglutinate the red cells of others and that this phenomenon could be classified into different blood group phenotypes. Four common phenotypes are recognised – O, A, B and AB. Subgroups of the A and B antigens have since been identified.

The ABO phenotype of an individual is usually determined by the agglutination reactions of the individual’s red cells with Anti-A, Anti-B and Anti-A,B antisera (forward grouping). In testing blood samples from adults, confirmation of the ABO blood group can be provided by the reactions of the individual’s serum with standard A and B red cell suspensions (reverse grouping).

PRINCIPLE OF THE REAGENT

Anti-A (TL) monoclonal murine IgM blood grouping reagent contains antibody from the cell line BIRMA-1, Anti-B (TN) monoclonal murine IgM blood grouping reagent contains antibody from LB-2 and Anti-A,B (TM) monoclonal murine IgM blood grouping reagent contains antibodies from cell lines ES-4/ES-15. 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.

MATERIALS

Anti-A (cell line BIRMA-1) blood grouping reagent, Anti-B (cell line LB-2) reagent and Anti-A,B (cell lines ES-4/ES-15) blood grouping reagent are composed of monoclonal murine IgM antibodies in a buffer solution containing macromolecular chemical potentiators. These reagents contain 0.1% (w/v) sodium azide, bovine material and porcine material. These reagents contain the colourants:

- Anti-A Blue Patent Blue Violet
- Anti-B Yellow Tartrazine
- Anti-A,B None

PRECAUTIONS

1. The cell lines used to produce these reagents are of murine origin and have been tested and found to be negative for Mouse Antibody Production (MAP) viruses. 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 and 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. These 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 and porcine materials are obtained from USDA approved sources or from sources for which origin information is available. The donor animals for bovine material have been inspected and certified disease free and are deemed to have low TSE (Transmissible Spongiform Encephalopathy) risk.

6. These 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 control and a 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 these reagents. Only in typing the red cells of patients known to have auto antibodies or protein abnormalities is the use of a reagent control recommended. This should be tested in parallel with the reagents.

These reagents have been characterised by the procedures recommended in this package insert, their suitability for use in other techniques must be determined by the user.

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. 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, for example, storage at higher temperature or repeated freezing and thawing may result in false positive or false negative results.

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Rev 02
**Anti-A Monoclonal Reagent**  
**Anti-B Monoclonal Reagent**  
**Anti-A,B Monoclonal Reagent**

**MATERIALS REQUIRED BUT NOT PROVIDED**

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**RECOMMENDED TECHNIQUES**

1. **SLIDE TECHNIQUE**
   1. Prepare a 35-50% suspension of test red cells in autologous (or compatible) plasma, serum or in isotonic saline.
   2. Add one drop (40-50μl) of either Anti-A, Anti-B or Anti-A,B reagent to a clean, labelled microscope slide.
   3. Add one drop (40-50μl) of the suspension of test red cells.
   4. Mix the antiserum and cells over an area about 2cm in diameter by gently and continuously rocking the slide.
   5. Read macroscopically after 3 minutes. Do not confuse any drying of the mixture with agglutination.

2. **TUBE TECHNIQUE**
   1. 2.1 Prepare a 3-5% suspension of test red cells in isotonic saline.
   2. 2.2 Add 1 drop (40-50μl) of either Anti-A, Anti-B or Anti-A,B reagent to an appropriately labelled test tube.
   3. 2.3 Add 1 drop (40-50μl) of the suspension of test red cells.
   4. 2.4 Mix and centrifuge at 1000 rcf for 20 seconds.
   5. 2.5 Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.
   6. 2.6 Incubate weaker than expected reactions for 1 minute at room temperature and then re-spin.

3. **MICROPLATE TECHNIQUE**
   1. Prepare a 3-5% suspension of test red cells in isotonic saline.
   2. Add 1 drop (40-50μl) of Anti-A, Anti-B or Anti-A,B reagent to the appropriate test wells of a U well microplate.
   3. Add an equal volume (40-50μl) of the cell suspension to the appropriate test wells.
   4. Mix the contents of each well using manual means or a microplate shaker.
   5. Incubate the microplates at room temperature for 15-20 minutes.
   6. Centrifuge the microplates at 100 rcf for 40 seconds.
   7. Resuspend the red cells using the microplate shaker (as in 3).
   8. Read tests macroscopically or with an automated reader. The use of an automated plate reader must be validated by the customer.

**LIMITATIONS**

The results of red cell grouping should be confirmed by reverse grouping the individual’s serum with known A1 and B red cells. No recipient should be given AB blood unless the cells of the recipient are clearly positive with Anti-A and Anti- B and the recipient’s serum shown to give negative reactions with A1 and B cells (unless the recipient has been shown to be a subgroup of AB with Anti-A1 in the serum).

Anti-A blood grouping reagent product will not detect all examples of Aa cells.

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

**REFERENCES**


**Manufactured in the UK by:** Rapid Labs Ltd  
Unit 2•Hall Farm•Church Road•Little Bentley•Colchester•Essex CO7 8SD•U.K.  
Email: medical@rapidlabs.co.uk Website www.rapidlab.co.uk

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