ANTI-A, ANTI-B, ANTI- A,B

MONOCLONAL BLOOD GROUPING ANTIBODIES FOR SLIDE AND TUBE TESTS

SUMMARY

Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells. Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity.

Human red blood cell antigens can be divided into four groups A, B, AB and O depending on the presence or absence of the corresponding antigens on the red blood cells.

Approximately 41% of the Caucasian population have the A Antigen, 9% have the B Antigen, 4% have both A and B antigens, while the remaining have neither the A nor the B antigen.

REAGENTS

Anti-A (Clone 11H5), Anti-B (Clone 6F9), and Anti-A,B (Clone 11H5+6F9+ES-15) are ready to use solutions of the respective specific antibodies of the immunoglobulin class IgM prepared from the corresponding supernatants of mouse hybridoma cell cultures. Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagent at 2-8^{0}C**.** DO NOT FREEZE.
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated.

PRINCIPLE

Human red blood cells possessing A and/or B antigen will agglutinate in the presence of antibody directed towards the antigen. Agglutination of red blood cells with Anti-A, Anti-B, Anti-A,B reagents is a positive test result and indicates the presence of the corresponding antigen.

Absence of agglutination of red blood cells with Anti-A, Anti-B, and Anti-A,B reagents is a negative test result and indicates the absence of the corresponding antigen.

PRECAUTIONS

1. In vitro diagnostic reagent for laboratory and professional use only. To be used by a qualified personnel. Not for medicinal use.
2. The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. MSDS available on request.
3. Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded.
4. Reagents are not from human source, hence contamination due to HBsAg, HIV and HCV is practically excluded.
5. It is necessary to use the dropper provided in the reagent vial to dispense a reagent drop.
6. It is advisable to wear gloves and safety spectacles and handle test specimens of human origin with caution.
7. Do not use damaged or leaking reagents.
8. Special protective measures, conditions for disposal and disinfection should be implemented in accordance with local regulations.

SAMPLE COLLECTION AND PREPARATION

No special preparation of the patient is required prior to sample collection by approved techniques. Sample should be stored at 2-8^{0}C if not tested immediately. For optimal results, freshly collected sample should be used. Anticoagulants like EDTA, CPD-A and Citrate can be used. Do not use haemolysed samples.

ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS

Slides (60 x 85 mm), Test tubes (12 x 75 mm), Micropipettes, Isotonic saline (0.9% NaCl), Centrifuge, Timer, Mixing sticks, test tube rack.

TEST PROCEDURE

Bring reagents and samples to room temperature before testing.

Slide Test

1. Place one drop of Anti-A, Anti-B, Anti-A,B reagents using the reagent vial dropper separately on a clean slide.
2. To each reagent drop, add 50µl of whole blood.
3. Mix well with a mixing stick uniformly over an area of approximately 2.5 cm^{2}.
4. Rock the slide gently, back and forth.
5. Observe for agglutination macroscopically at two minutes.

Tube test

1. Prepare a 2-3% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of Anti-A, Anti-B, Anti-A,B reagents using the reagent vial dropper into corresponding labeled test tubes.
3. Pipette into each of the test tube, 1 drop (~50µl) of the test red cell suspension and mix well.
4. Centrifuge for 1 minute at 1000 RPM (125g) or 20 seconds at 3400 RPM (1000g) or alternative speed and time validated by the laboratory.
5. Gently suspend the cell button, observing for agglutination macroscopically.

Microplate Method

The reagents are standardized for use in Microplate technique, however it is recommended that each laboratory should standardize and validate their own procedure using "U" bottom microplates. The below mentioned procedure is a guideline which should be considered while standardization and validation process of procedure because of the variation in methods and equipment used in various laboratories.

1. Prepare 2-3% suspension of the red cells to be tested in isotonic saline.
2. Add 1 volume of Anti-A, Anti-B, Anti-A,B reagents using the reagent vial dropper into appropriate microwells.
3. Add equal volume of test red cell suspension.
4. Mix the contents of the microplate, taking care to avoid cross-well contamination.
5. Centrifuge the microplate at low spin (400g for 30 secs). Or Centrifuge at a speed and time as per the standardization and validation process done using same centrifuge.
6. Re-suspend the red cells using a microplate shaker or manually.
7. Read and record results.

Note: Incubation of 10-15 mins at Room Temperature may be included to enhance the reactivity.

INTERPRETATION OF RESULTS

Slide and tube tests

Agglutination is a positive test result and indicates the presence of A and/or B antigen. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination is a negative test result and indicates the absence of A and/or B antigen.

Note

1. Results of forward grouping obtained by using Anti-A, Anti-B and Anti-A,B reagents should always be reconfirmed by performing reverse grouping with known red cells. If there is discordance, do not report the result and pursue blood identification in compliance with current recommendations and protocols or forward the sample to an expert laboratory.
2. It is strongly recommended that red cells with known ABO characteristics should be periodically run, preferably on a daily basis to validate the reagent performance.
3. The quality control of reagents is performed using immediate spin method for Anti-A ,Anti- B ,Anti- AB reagents.
4. The reagents are expected to exceed minimum specifications /acceptance criteria as per label claim for titre , specificity & avidity as laid down by transfusion medicine technical manual -2003 with reference to NIBSC -UK & WHO international; standards.

REMARKS

1. (a) Anti-A and Anti-A,B reagent reacts with Tn. Tn positive person must be excluded from donating blood as the occurrence of Tn is considered to be a symptom of a preleukaemic state and the red blood cells are polyagglutinable.
   (b) Anti-B is truly negative reacting with acquired B characteristics.
2. In the tube test procedure, it is recommended that tubes with negative reactions should be centrifuged once again (for the same centrifugation speed and time) and results read so that weak antigens are not overlooked.
3. As undercentrifugation or overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and determine the time required for achieving the desired results.
4. After usage the reagents should be immediately recapped and replaced to 2-8°C storage.
5. In certain cases (transfusion recipients, certain weak phenotypes A or B (A_3, B_3…), certain hemopathological modifications, mosaics or chimeras, etc.), an image of a double population may be observed.

PERFORMANCE CHARACTERISTICS

The performance of Anti-A, Anti-B, Anti-A,B complies with the common technical specifications of in-vitro diagnostic medical devices under the recommended methods.

The performance of Anti-A, Anti-B, Anti-A,B was evaluated on over 3275 samples (from donors, clinical and neonates) drawn in the recommended anticoagulants. The evaluation demonstrated 100% specificity and sensitivity of each reagent versus the expected results with common known phenotypes A_1, A_2, A_1B, A_2B, B and O.

WARRANTY

This product is designed to perform as described on the label and the package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

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0162-0164/0523/VER-04
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Manufactured by:

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In vitro Diagnostic Reagent
NOT FOR MEDICINAL USE.
Store at 2-8°C. DO NOT FREEZE.
Preservative: 0.1% NaN_3
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