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 A nor B antigen.

REAGENTS
Anti-A, Anti-B, and Anti-A, B are ready to use reagents prepared from supernatants of mouse hybridoma cell cultures. These antibodies of the immunoglobulin class IgM are a mixture of several monoclonal antibodies of the same specificity but having the capability of recognising different epitopes of the human red blood cell antigens A and B. Each batch of reagent undergoes quality control at various stages of manufacture for its specificity, avidity and performance.

REAGENT STORAGE AND STABILITY
1. Store the reagent at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagents is as per the expiry mentioned on the reagent vial label.

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.

NOTE
1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
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 sources, hence contamination due to HBsAg and HIV is practically excluded.

SAMPLE COLLECTION AND PREPARATION
No special preparation of the patient is required prior to sample collection by approved techniques. Samples should be stored at 2-8°C if not tested immediately. Do not use haemolysed samples. Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period:

EDTA or HEPARIN : 2 days
Sodium citrate or sodium oxalate : 14 days
ACD or CPD : 28 days

ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS
Glass slides (60 x 85 mm), Test tubes (12 x 75 mm), Pasteur pipettes, Isotonic saline, Centrifuge, Timer, Mixing sticks.

TEST PROCEDURE
Bring reagent and samples to room temperature before testing.
Slide Test
1. Place one drop of reagent Anti-A or Anti-B or Anti-A, B on a clean glass slide.
2. To each reagent drop, **add one small drop of whole blood**.
3. Mix well with a mixing stick uniformly over an area of approximately 2.5 cm².
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 reagent Anti-A, Anti-B, Anti-A, B into correspondingly labeled test tubes.
3. Pipette into each of the test tubes, one drop of the test red cell suspension and mix well.
4. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g) or incubate at room temperature for 20-30 minutes.
5. Gently resuspend the cell button, observing for agglutination macroscopically.

**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.

**REMARKS**
1. (a) Anti-A, Anti-B, Anti-A, B reagents do not show a reaction with crypt antigens (T, Tn, Tk activated cells).
(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 recentrifuged and results read again after 5 minutes 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. Results of forward grouping obtained by using Anti-A, Anti-B, Anti-A, B reagents should always be reconfirmed by performing reverse grouping with known red cells.
5. It is strongly recommended that red cells with known ABO characteristics should be occasionally run, preferably on a daily basis so as to control reagent performance and validate test results.
6. After usage the reagents should be immediately recapped and replaced to 2-8°C storage.
7. The label minimum titre claim is based on A group cells for Anti-A reagent, B group cells for Anti-B reagent and A, B cells for Anti-A, B reagent. This is based on titration procedure as recommended by the manufacturer. Any deviation in test procedure could result in variable results.

**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**
5. Data on file: Tulip Diagnostics (P) Ltd.