ERYSCREEN®

ANTI-A, ANTI-B, ANTI-D (Rho) (IgM)
REAGENT COMBIPACK FOR ROUTINE BLOOD GROUPING AND TYPING

INTENDED USE
ERYSCREEN® reagent combipack consists of monoclonal blood grouping Anti-A, Anti-B, Anti-D (Rho) (IgM) reagents. These reagents can be used for *in vitro* determination of the presence or absence of A , B and D (Rho) antigen on human red blood cells.

SUMMARY
Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells or though EBV transformation. Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity. Since monoclonal antibodies only recognize one epitope, they generally have low cross reactivity with non-specific antigens. Their epitope specificity, limited cross reactivity, and long term yield make monoclonal antibodies attractive for use in many biological assays and application.
In blood transfusion the two most important blood group systems are the ABO system and the Rhesus system. The ABO system divides the blood groups into four major groups A, B, AB and O depending on the presence or absence of the A or B 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 the B antigen.
The second major blood group system is the Rhesus (Rho) system. D is the main antigen of the Rhesus system. Human red blood cells are classified as Rho (D) positive or Rho (D) negative depending upon the presence or absence of D (Rho) antigen on them. Approximately 85% of the Caucasian population are Rho (D) positive. The D^{u} phenotype is a variant of D (Rho) antigen and is recognised by performing the antiglobulin test.
About 60% of the D^{u}s, now classified as weak or partial D's, may react with Anti-D (Rho) (IgM) in slide tests and about 90% may be detected by the tube technique.

REAGENTS
Anti-A (Clone 11H5), Anti-B (Clone 6F9), and Anti-D(Rho) (IgM) (Clone P3 x 61) 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. These antibodies 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 antigen A, B and D (Rho) respectively.
Anti-D (Rho) (IgM) does not detect all the weak and partial D's. For the confirmation of negative reactions with Anti-D (Rho) (IgM) further testing with an incomplete Anti-D (Rho) of IgG class such as ERYCLONE® Anti-D (Rho) (IgG) is strongly recommended to confirm the presence or absence of weak/partial D's.
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°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 label provided it is not contaminated.

PRINCIPLE
Human red blood cells possessing A, B and D (Rho) antigen will agglutinate in the presence of antibody directed towards the antigen. Agglutination of red blood cells with Anti-A, Anti-B and Anti-D (Rho) (IgM) reagent 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-D (Rho) (IgM) reagent 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. Handle and dispose off reagents as potentially infectious.
5. 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 content.

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 reagents and samples to room temperature before testing.

Slide Test

1. Place one drop each of Anti-A , Anti-B and Anti-D (Rho) (IgM) reagent on a clean glass slide.
2. To each reagent drop, **add one small drop (50 µl) 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 each of Anti-A, Anti-B, Anti-D (Rho) (IgM) reagent into corresponding labeled test tubes.
3. Pipette into each of the test tubes, one drop (~50 µl) 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 alternative speed and time validated by the laboratory.
5. Gently resuspend the cell button, observing for agglutination macroscopically.

Microplate Method

ERYSCREEN® 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 and Anti-D (Rho) (IgM) 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 the respective red blood cell antigens. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination is a negative test result and indicates the absence of red blood cell antigens.
Cord cells heavily sensitized with Anti-D (Rho) may give a false negative tube test result.
All tests negative with Anti-D (Rho) (IgM) reagent (Rh negative) should be further tested for weak/partial D by D^u test procedure.

D^u TEST PROCEDURE

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of any incomplete Anti-D (Rho) (IgG class) reagent such as ERYCLONE® Anti-D (Rho) (IgG) into a labeled test tube.
3. Add to the test tube one drop (50 µl) of the 5% cell suspension and mix well. Incubate at 37°C for 15 minutes.
4. Wash the contents of the tube thoroughly, atleast three times, with isotonic saline and decant completely after the last wash.
5. Add two drops of ERYCLONE® Anti Human Globulin reagent and mix well.
6. Centrifuge for 1 minute at 1000 RPM (125 g) or 20 seconds at 3400 RPM (1000 g).
7. Very gently, resuspend the cell button, observing for agglutination macroscopically.

INTERPRETATION OF RESULTS

D^{u}Test Procedure

(a) Agglutination in the D^u test indicates presence of the D^u antigen (weak / partial D's). No agglutination in the D^u test indicates absence of the D^u antigen (weak / partial D's). Negative reactions obtained in D^u test must be validated:- add 50µl of Coomb's control cells to the reaction mixture. A positive reaction confirms the activity of the Coomb's reagent and validates the negative reaction before the addition of the Coomb's control cells.

(b) Mixed field agglutination in the D^u test on red cells from a recently delivered woman may indicate a mixture of maternal Rho (D) negative and fetal Rho (D) positive blood.

(c) Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for D^u antigen (weak / partial D's).

Note

1. It is strongly recommended that red cells with known ABO & Rh characteristics should be periodically run, preferably on a daily basis to validate the reagent performance.
2. The quality control of reagents is performed using immediate spin method for Anti-A ,Anti- B ,Anti- AB reagents.
3. The quality control of reagents is performed by incubating at 37 ºC for 30 minutes for Anti-D IgM reagent.
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 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 re-centrifuged 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 and Anti-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 and known Rho (D) characteristics such as Rho (D) positive and Rho (D) negative red cells 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^{0}C storage.
7. Do not interchange the dropper caps of the reagent vials of ERYSCREEN® Anti-A, Anti-B, Anti-D (Rho) combipack.
8. The label minimum titre claim is based on A_1 group cells for Anti-A reagent, B group cells for Anti-B reagent and Rho (D) positive red cells (phenotype : R_1r) for Anti-D (Rho)(IgM) reagent. This is based on the titration procedure as recommended by the manufacturer. Any deviation in test procedure could result in variable results.

PERFORMANCE CHARACTERISTICS

The performance of Anti-A, Anti-B and Anti-D (Rho) (IgM) complies with the common technical specifications of In-vitro diagnostic medical devices under the recommended methods. The performance of Anti-A, Anti-B and Anti-D (Rho)(IgM) was evaluated on over 3275 samples (from donors, clinical and neonates) drawn in the recommended anticoagulants. The evaluation demonstrated 100 % specificity of each reagent and 100% sensitivity of Anti-A and Anti-B reagent. The sensitivity of Anti-D(Rho)(IgM) reagent by slide test is 98.77% and by tube test it is 99.37% of reagent versus the expected results with common known Rhesus phenotypes.

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

(1) Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity. Nature, 256, 495-497. (2) Lee H. H., Rouger P., Germain C., Muller A & Salmon C. (1983). The production and standardisation of monoclonal antibodies as AB blood group typing reagents. Symposium of International Association of Biological Standardisation on monoclonal antibodies. (3) Human Blood Groups, by Geoff Daniels, 1st Ed., Blackwell Science, Oxford 1995. (4) HMSO, Guidelines for Blood Transfusion Services, 2nd Ed., 1994. (5) Quality Control of ABO and Rh blood grouping reagents " from NIB - INDIA. (6) Data on file: Tulip Diagnostics (P) Ltd.

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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
AN ISO 13485 Certified Company