ERYCLONE®

ANTI C+ D+ E

MONOCLONAL Rh TYPING ANTIBODIES FOR SLIDE AND TUBE TESTS

SUMMARY

The Rh blood group system consists of forty-five antigens, which are expressed on human red blood cells. Besides the Rho (D) antigen other important antigens of Rh system are C (rh'), E (rh"), c (hr'), e (hr").

Approximately 85% of Caucasian population is Rho(D) positive. The D^{u} phenotype is a traditional definition to describe weak / partial D's. Approximately 70% of Caucasian populations have the C antigen, 30% have the E antigen, 80% have the c antigen and 90% have the e antigen.

ERYCLONE® Anti-C+D+E reagent is useful for determination of probable Rh genotype of an individual, for selection of donors who become immunized with Rh antigens during pregnancy or transfusion, in pre-transfusion testing and prediction of haemolytic disease of the newborn.

REAGENTS

ERYCLONE® Anti-C+D+E is a ready to use reagent blend prepared from cell culture supernatant of respective human cell lines. 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.

PRINCIPLE

Human red blood cells possessing the C and / or D and / or E antigen will agglutinate in the presence of antibody directed towards the antigen. Agglutination of red blood cells with ERYCLONE® Anti-C+D+E is a positive result and indicates the presence of C and / or D and / or E antigen. No agglutination is a negative result and indicates the absence of corresponding antigen. All negative test results should be further tested for D^{u} (weak / partial D's) by performing the D^{u}test procedure, as described later.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use. Not for medicinal use.
2. ERYCLONE® Anti-C+D+E reagent is not from human source, hence contamination due to HBsAg and HIV is practically excluded.
3. The reagent contains 0.1% sodium azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. Extreme turbidity may indicate microbial contamination or denaturation of the protein due to thermal damage. Such reagents should be discarded.

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^{0}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

Glass slides (60 x 85 mm), Test tubes (12 x 75 mm), Pasteur pipettes, Isotonic saline, Centrifuge, Timer, Mixing slicks, ERYCLONE® Anti human Globulin reagent.

TEST PROCEDURE

Bring reagent and samples to room temperature before testing.

Slide test

1. Place one drop of ERYCLONE® Anti-C+D+E on a clean glass slide.
2. Pipette one equal drop of whole blood on the slide.
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 5% suspension of red cells to be tested in isotonic saline or buffered isotonic saline.
2. Place one drop of ERYCLONE® Anti-C+D+E reagent into labelled test tubes.
3. Pipette into each of the test tubes, one drop of the 5% cell suspension and mix well.
4. Centrifuge for one minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
5. Gently resuspend the cell button observing for agglutination macroscopically.

D^{u} Test Procedure

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline or isotonic buffered saline.
2. Place one drop of ERYCLONE® Anti-C+D+E reagent into a labelled test tube.
3. Add to the test tube one drop of the cell suspension, mix well and incubate at 37^{0}C for 15 minutes.
4. Wash the contents of the tube atleast three times, with isotonic saline or isotonic buffered 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 and observe for agglutination macroscopically.

INTERPRETATION OF RESULTS

Slide and Tube Tests

1. Agglutination is a positive result and indicates the presence of C and / or D and / or E antigen. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination is a negative result and indicates the absence of C, D and E antigen.
2. Cord cells heavily sensitized with Anti-C or Anti-D or Anti-E may give a false negative immediate spin test result.

D^{u} Test Procedure

1. Agglutination with the reagent and no agglutination with the control indicates the presence of D^{u} antigen (weak / partial D's). No agglutination with reagent and agglutination with control indicates the absence of D^{u} antigen (weak / partial D's).
2. 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.
3. Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for D^{u} antigen (weak / partial D's).

REMARKS

1. As undercentrifugation or overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and time required for achieving the desired results.
2. In the tube test procedure, it is recommended that tubes with negative reactions should be centrifuged and results read again after 5 minutes so that weak antigens are not overlooked.
3. A positive control known to possess C, D and E antigen and a negative control (known red blood cells lacking the respective antigen) should be tested preferably on a daily basis so as to control reagent performance and validation of test results.
4. After usage the reagents must be immediately recapped and replaced at 2-8^{0}C storage.

WARRANTY

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

BIBLIOGRAPHY

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4. AABB Technical Manual, 13th edition.
5. HMSO Guidelines for the Blood Transfusion Service (1994), 2nd edition.
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ERYCLONE® Anti - C+D+E	
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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
ISO 9001-2008, ISO 13485 (2003), NF EN ISO 13485 (2004)