RHOFINAL®

Anti-D (Rho)
(IgM + IgG)

MONOCLONAL BLOOD TYPING 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 or are derived from a human B cell line through EBV transformation. 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 cells are classified as Rho (D) positive and 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 traditional definition to describe the weak / partial D's that can be detected with Anti-D (Rho) (IgM+IgG).
About 60% of the D^{u} (weak / partial D's) may react with Anti-D (Rho) (IgM+IgG) in slide tests and about 90% may be detected by the tube technique.

REAGENT

RHOFINAL® Anti-D (Rho) (IgM + IgG) is a ready to use reagent, prepared from supernatants of cell cultures with antibody producing B lymphocytes obtained through EBV transformation and is a blend of Aggluinating sera of the immunoglobulin class IgM and IgG (Clone P3 x 61+ NaTH119+LOR15C9). These Agglutinating sera are of same specificity but having the capability of recognising different epitopes of the human red blood cell antigen D (Rho).
RHOFINAL® Anti-D (Rho) (IgM + IgG) is a blend of Agglutinating sera of IgM and IgG class which agglutinates the D(Rho) antigen, a characteristic which accords versatility to the reagent. It gives an avid saline reacting slide / tube test reagent the capability of detecting D^{u} (weak/partial D's) in the Anti Human Globulin Phase.
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 cell possessing D antigen will agglutinate in the presence of the Agglutinating sera directed towards the antigen. Agglutination of red blood cells with **RHOFINAL®** Anti-D (Rho) (IgM + IgG) reagent is a positive test result and indicates the presence of the D (Rho) antigen. No agglutination with **RHOFINAL®** Anti-D (Rho) (IgM + IgG) reagent is a negative test result and indicates absence of D (Rho) 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.

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), Test tube rack, Micropipettes, Isotonic saline (0.9% NaCl), Centrifuge, Timer, Mixing sticks, **ERYCLONE®** Anti-Human Globulin (Coombs) reagent.

TEST PROCEDURE

Bring reagents and samples to room temperature before testing.

Slide Test

1. Place one drop of **RHOFINAL®** Anti-D (Rho) (IgM + IgG) reagent on a clean slide.
2. Pipette 50µl whole blood on the slide.
3. Mix well the reagent and blood sample 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 the end of two minutes.

Tube Test

1. Prepare a 5% cell suspension of the red cells to be tested in isotonic saline.
2. Place one drop **RHOFINAL®** Anti-D (Rho) (IgM + IgG) reagent into a labeled test tube.
3. Pipette 1 drop (~50µl) of test red cell suspension into the test tube and mix well.
4. Centrifuge for one minute at 125 g or 20 seconds at 1000 g or alternative speed and time validated by the laboratory.
5. Gently resuspend the cell button observing for agglutination macroscopically.

Microplate Method

RHOFINAL® is 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 **RHOFINAL®** Anti-D (IgG+IgM) reagent 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.

D^{u} TEST PROCEDURE

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of **RHOFINAL®** Anti-D (Rho) (IgM + IgG) reagent into a labeled test tube.
3. Add to the test tube 50µl of cell suspension under test, mix well and 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 100µl 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

a) Agglutination with the **RHOFINAL®** Anti-D (Rho) (IgM + IgG) is a positive test result and indicates the presence of D (Rho) antigen. Do not interpret peripheral drying or fibrin strands as agglutination.

b) No agglutination with **RHOFINAL®** Anti-D (Rho) (IgM + IgG) is a negative test result and indicates the absence of D (Rho) antigen.

c) It is strongly recommended that as a routine quality control measure with known Rho (D) positive and Rho (D) negative red cells be occasionally run, preferably on a daily basis to validate reagent performance.

D^{u} Test Procedure

(a) Agglutination with reagent indicates the presence of D^{u} antigen (weak / partial D's). (b) No agglutination with reagent indicates the absence of D^{u} antigen (Absence of 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. (c) 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. (d) Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for D^{u} antigen (Presence of weak / partial D's).

Note

1. It is strongly recommended that red cells with known Rh characteristics should be periodically run, preferably on a daily basis to validate the reagent performance.
2. The quality control of reagents is performed by incubating at 37° C for 30 minutes for Anti-D IgM, Anti-D IgM+IgG reagents.
3. 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. As undercentrifugation and overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and the time required of achieving the results.
2. After usage, the reagents should be immediately recapped and replaced to 2-8°C storage.
3. Cord Cells heavily sensitized with Anti-D (Rho) may give false negative result in immediate spin test.
4. False positive reactions may occur if the test subject has cold agglutinins.
5. **RHOFINAL®** Anti-D (Rho) (IgM + IgG) have the feature of recognizing certain rare antigen motif of type (RoHar) and may thus yield discordant results with polyclonal reagents that may or may not recognize them.
6. **RHOFINAL®** Anti-D (Rho) (IgM + IgG) enables the screening for weak Rh red blood cells in the D^{u} test procedure with coomb's reagent.
7. The tests conducted on particular phenotypes, while satisfactory, cannot ensure recognition of all weak or variant subjects, due to variability of antigen motifs.

PERFORMANCE CHARACTERISTICS

The performance of **RHOFINAL®** Anti-D (Rho) (IgM + IgG) complies with the common technical specifications of in-vitro diagnostic medical devices under the recommended methods.

The performance of **RHOFINAL®** Anti-D (Rho) (IgM + IgG) was evaluated on over 3275 samples (from donors, clinical and neonates) drawn in the recommended anticoagulants. The evaluation demonstrated 100% specificity. The sensitivity of the reagent by slide test is 99% and by tube test it is 99.53% of reagent versus the expected results with common known Rhesus phenotypes.

WARRANTY

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

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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

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