CE

ERYCLONE®

ANTI-HUMAN GLOBULIN REAGENT
FOR DIRECT AND INDIRECT ANTIGLOBULIN TESTS

SUMMARY

Generally antibodies involved in transfusion reactions are of two types, namely the complete and the incomplete, whereas the complete antibodies agglutinate red cells in saline medium, the incomplete type of antibody sensitizes red cells without agglutination. Usually IgM class of antibodies and IgG_1 and IgG_3 type of IgG antibodies fix complement. Cell lysis, *in vivo,* is mediated through the complement system and the complement component C_3b is further acted upon to produce C_3d.
In the direct antiglobulin tests, Anti-Human Globulin Reagent is used to detect antibodies adsorbed to the red blood cells *in vivo.*
In the indirect antiglobulin tests, Anti-Human Globulin Reagent is used to detect antibodies adsorbed to the red blood cells *in vitro.*
Anti-Human Globulin Reagent is useful for compatibility testing, antibody detection, antibody identification, umbilical cord red blood testing and detection of the D^u variant of the human red blood cell antigen D (Rho).

PRESENTATION

REF	10180010	10180610
ERYCLONE® Anti-Human Globulin	10 ml	6 x 10 ml
Packinsert	1	1

REAGENT

ERYCLONE® Anti-Human Globulin is a balanced ready to use blend of highly purified immunoglobulins. It contains Agglutinating sera for Human IgG and Agglutinating sera reactive with human complement components C_3d. The agglutinating sera which are specific for human complement components are of IgM class and they impart the necessary sensitivity to the reagent.
Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and titre.

REAGENT STORAGE AND STABILITY

(a) Store the reagent at 2-8°C. DO NOT FREEZE.
(b) 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

Normal human red blood cells, in presence of antibody directed towards the antigen they possess, may fail to agglutinate and become sensitized. This may be due to the particular nature of the antigen and agglutinating sera involved. ERYCLONE® Anti human globulin reagent would react with red cells sensitized with gamma globulins or components of human complement involved and cause agglutination of the red blood cells.

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 STORAGE

No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed samples.
For Direct Antiglobulin Test: Blood drawn into EDTA is preferred but oxalated, citrated or clotted whole blood may be used. The blood sample should be tested as soon as possible after collection and should not be stored.
For Indirect Antiglobulin Test: Serum, not more than 48 hours old, should be used. Donor units may be tested upto the end of their dating.

PREPARATION OF COOMBS CONTROL CELLS

1. Collect fresh O Rho (D) positive red blood cells preferably with citrate as an anticoagulant.
2. Wash 1ml of freshly collected O Rho (D) positive red blood cells with isotonic saline atleast three times.
3. After the third wash thoroughly decant the supernatant. To the cell button add 5 ml of AGTROL® Anti-D (IgG) reagent and gently resuspend the red blood cells.
4. Incubate the mixture at 37^0 for 15 minutes.
5. After incubation wash the sensitized red blood cells thoroughly atleast 4 to 5 times with isotonic saline.
6. Decant the supernatant thoroughly after the last wash. Resuspend the cell button gently with about 1-2 ml of AGTROL® red blood cell preserving solution. The complete resuspended red cells should be added back to the balance AGTROL® red blood cell preserving solution in the dropper vial. A stabilized suspension of 5% Coombs control cells is thus obtained. Label appropriately with the date of preparation.
7. Store the Coombs control cells at 2-8^0 C. **Use within 4 weeks of preparation**.

ADDITIONAL MATERIAL REQUIRED

For Direct Antiglobulin Test: Test tubes (12 x 75 mm), Pasteur pipettes, Centrifuge, Isotonic saline, Coombs control cells, Optical aid.

For Indirect Antiglobulin Test and Compatibility Test: Test tubes (12 x 75 mm), Pasteur pipettes, ERYCLONE® Bovine Serum Albumin, Centrifuge, Incubator (37^0C), Isotonic saline, Coombs control cells, Optical aid.

PROCEDURE

Bring reagent to room temperature before testing.

Direct Antiglobulin Test

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Pipette one drop of the cell suspension into a test tube.
3. Fill the tube with fresh isotonic saline and centrifuge for 30 seconds at 3400 RPM (1000g).
4. Decant and repeat this washing atleast thrice.
5. Add two drops of ERYCLONE® Anti-Human Globulin Reagent and mix well.
6. Centrifuge for one minute at 1000 rpm (125 g) or for 20 seconds at 3400 RPM (1000 g).
7. Very gently, resuspend the cell button observing for agglutination macroscopically.
8. To all negative antiglobulin tests add one drop of coombs control cells and observe for agglutination.

Indirect Antiglobulin Test

MAJOR CROSS MATCH PROCEDURE

Initial Phase

1. Label two test tubes as A (for albumin) and B (for saline), depending upon the number of donors to be cross matched, as many pairs of such labelled tubes would be required.
2. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
3. Pipette two drops of recipient serum in both the labelled test tubes.
4. Pipette one drop of donor red cells in both the labelled test tubes and mix well.
5. Only to the albumin tube (A) add two drops of ERYBANK® Bovine Serum Albumin reagent and mix well.
6. Centrifuge both the tubes for one minute at 1000 RPM (125g) or for 20 seconds at 3400 RPM (1000 g).
7. First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically.
8. Proceed to incubation phase.

Incubation Phase

1. Incubate the saline tube at room temperature and the albumin tube at 37^0C for fifteen minutes.
2. First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically.
3. Proceed to the antiglobulin phase.

Antiglobulin Phase

1. Only the albumin tubes (A) are tested in the antiglobulin phase.
2. Wash the mixture of red blood cells and serum thoroughly with isotonic saline for minimum of three times. Decant completely after the last wash.
3. Place two drops of ERYCLONE® Anti-Human Globulin Reagent into the test tubes containing the sedimented cells and mix well.
4. Centrifuge for one minute at 1000 RPM (125 g) or for 20 seconds at 3400 RPM (1000 g).
5. Very gently, resuspend the cell button and observe for agglutination macroscopically.

INTERPRETATION OF RESULTS

Direct Antiglobulin Phase

Agglutination of red blood cells is a positive test result and indicates presence of human IgG or components of complement on the red blood cells.

No agglutination is a negative test result and indicates absence of human IgG or components of complement on the red blood cells.

Indirect Antiglobulin Phase
In all phases of the compatibility test, if no agglutination or haemolysis is observed then the patient and the donor may be considered compatible. If haemolysis or agglutination at any point till the completion of the antiglobulin phase is observed, the patient and the donor are considered incompatible.

PERFORMANCE CHARACTERISTICS
Over 500 tests were conducted on a panel of random samples (Blood donors, patients and neonates) drawn on the recommended anticoagulants using the Tulip IVDMD intended for erythrocytic Resus D (RH1) phenotyping in the indirect antiglobulin test combined with **ERYCLONE® Anti-Human Globulin, ERYCLONE® Anti-D IgG and RHOFINAL® Anti-D (IgM+IgG)** showed 100% reactivity and specificity vis-a-vis common Resus D (RH1) phenotypes.

REMARKS
1. If plasma is used in the indirect antiglobulin test, the complement dependent antibodies may not be detected due to the absence of calcium.
2. To all negative test results, after the antiglobulin test phase, one drop of Coombs control cells should be added. If Coombs control cells do not agglutinate then the compatibility test must be repeated.
3. In the indirect antiglobulin test procedure an auto control tube (individual's cells in his own serum) should be run.
4. Red blood cells showing a positive direct antiglobulin test cannot be used for the indirect antiglobulin test.
5. It is recommended that Anti-IgG activity of the Anti-Human Globulin Reagent be tested from time to time preferably on a daily basis using Coombs control cells as a positive control.
6. All glassware used in the test should be scrupulously clean dry and free from contamination with human serum.
7. Contaminated Bovine Serum Albumin, saline or glassware may inactivate Anti-Human Globulin Reagent.
8. Use of various drugs and certain diseases (such as megaloblastic anaemia) are known to be associated with a positive direct antiglobulin test.
9. Cord cells obtained from a newborn exhibiting haemolytic disease of the newborn, especially due to ABO incompatibility may give false negative results.
10. ERYCLONE®Anti-Human Globulin Reagent does not contain Anti-C_4 and is free from Anti-T activity.
11. As undercentrifugation or overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and the time required for achieving the desired results.

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.

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, lst Ed., Blackwell Science, Oxford 1995.
4. HMSO, Guidelines for the Blood Transfusion Services., 2nd Ed., 1994.
5. Data on File: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

	Temperature limitation		Manufacturer		Contains sufficient for <n> tests
	Use by		Consult Instructions for use	PS	Production Site
	Date of Manufacture	REF	Catalogue Number		This side up
LOT	Batch Number/ Lot Number	IVD	*In vitro* Diagnostic Medical Device	EC REP	Authorised Representative in the European Community

TULIP DIAGNOSTICS (P) LTD.

PS

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