

**ERYCLONE**[®]**ANTI-C3d MONOSPECIFIC COOMBS REAGENT FOR DIRECT AND INDIRECT ANTIGLOBULIN TEST****SUMMARY**

Generally antibodies involved in transfusion reactions are of two types namely the complete and incomplete, whereas the complete antibodies agglutinate red cells in saline medium, the incomplete type of antibodies sensitizes red cells without agglutination. Usually IgM class of antibodies and IgG₁ and IgG₃ type of IgG antibodies fix complement. Cell lysis, *in vivo*, is mediated through the complement system and the complement component C3b is further acted upon to produce C3d. In the direct antiglobulin tests, Anti-human globulin reagent is used to detect antibodies adsorbed to the red blood cells *in vivo*. After direct antiglobulin testing with a polyspecific reagent reveals globulins, Anti-C3d monospecific Coombs reagent is used to characterize the coating proteins.

In the indirect antiglobulin tests, Anti-human globulin reagent is used to detect antibodies adsorbed to red blood cells *in vitro*. Anti-C3d monospecific Coombs reagent is used in indirect antiglobulin testing to distinguish patterns of reactivity in a single serum containing complement binding and non-complement binding antibodies.

Anti-C3d monospecific Coombs reagent is useful in Direct and Indirect Antiglobulin test for identification of complement fixing antibodies.

PRESENTATION

REF	10181002
Pack	2 ml

REAGENT

ERYCLONE[®] Anti-C3d monospecific Coombs reagent is a ready to use reagent containing antibodies reactive with human complement component C3d. The anti complement antibodies are IgM class monoclonals and they impart the necessary sensitivity to the reagent.

Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and titre.

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.

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 antigen and antibody involved. ERYCLONE[®] Anti-C3d monospecific Coombs reagent would react with red cells sensitized with components of human complement involved and cause agglutination of the red blood cells.

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. ERYCLONE[®] Anti-C3d monospecific Coombs reagent is not from human source, hence contamination due to HBsAg and HIV is practically excluded.

SAMPLE COLLECTION AND STORAGE

No special preparation of 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 for testing purpose.

ADDITIONAL MATERIAL REQUIRED**For Direct Antiglobulin Test**

Test tubes (12 x 75 mm), Pasteur pipettes, centrifuge, isotonic saline, Complement coated control cells, optical aid.

For Indirect Antiglobulin Test

Test tubes (12 x 75 mm), Pasteur pipettes, centrifuge, Incubator (37°C), Isotonic saline, ERYBANK® Bovine Serum albumin, Reagent red blood cells for antibody detection and antibody identification, Complement coated control cells, optical aid.

PROCEDURE

Bring reagent to room temperature before testing.

Direct Antiglobulin Test

1. Prepare a 5% suspension of red blood 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 (1000 g).
4. Decant and repeat this washing at least thrice.
5. Add two drops of ERYCLONE® Anti-C3d monospecific Coombs 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.

Note: The sensitivity of complement / anti-complement reactions can be increased by incubation at room temperature for 5-10 minutes and then recentrifuged for observing the results.

Indirect Antiglobulin Test for antibody identification

1. Prepare 5% suspension of reagent red blood cells to be tested in isotonic saline.
2. Pipette two drops of serum to be tested in an appropriately labelled test tube.
3. Pipette one drop of 5% reagent red blood cell suspension and mix well.
4. If required, add two drops of ERYBANK® Bovine serum albumin reagent and mix well and incubate at 37°C for fifteen minutes.
5. If enhancement medium is not being used, incubate the tube at 37°C for 30 minutes.
6. After incubation wash the cells thoroughly with isotonic saline for minimum three times. Decant completely after last wash.
7. Place two drops of ERYCLONE® Anti-C3d monospecific Coombs reagent into the test tube containing the sedimented cells and mix well.
8. Centrifuge for one minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
9. Very gently resuspend the cell button and observe for agglutination macroscopically.

INTERPRETATION OF RESULTS

Direct Antiglobulin Test

Agglutination of red blood cells is a positive result and indicates presence of human complement component C3d on red blood cells.

No agglutination is a negative test result and indicates absence of human complement component C3d on red blood cells.

Indirect Antiglobulin Test

Agglutination of red blood cells is a positive result, which indicates the uptake of complement component C3d by the red blood cells, presumably as a result of antibody in the serum under test.

No agglutination of red blood cells is a negative result and indicates that there is no detectable concentration of C3d on the red blood cells.

REMARKS

1. ERYCLONE® Anti-C3d monospecific Coombs reagent does not contain antibodies to immunoglobulins. Thus it may be used in addition to, but not in place of polyspecific anti-human globulin reagent for antiglobulin tests.
2. Red blood cells showing a positive direct antiglobulin test cannot be used for the indirect antiglobulin test.
3. It is recommended that Anti-C3d monospecific Coombs reagent be tested from time to time preferably on a daily basis using Complement coated control cells as a positive control.
4. All glassware used in the test should be scrupulously clean dry and free from contamination with human serum.
5. Contaminated Bovine serum albumin, saline or glassware may inactivate Anti-C3d monospecific Coombs reagent.
6. Use of various drugs and certain diseases (such as megaloblastic anaemia) are known to be associated with a positive direct antiglobulin test.
7. ERYCLONE® Anti-C3d monospecific Coombs reagent cannot be used for D^u testing and for detecting haemolytic disease of newborn.
8. ERYCLONE® Anti-C3d monospecific Coombs reagent does not contain Anti-C4 activity.
9. 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. Blood Transfusion in Clinical Medicine, PL Mollison, CP Engelfriet, Marcela Contreras, 9th Edition, 1994 Blackwell Science Publications.
2. AABB Technical Manual, 13th Edition, 1999.
3. HMSO, Guidelines for the Blood Transfusion Services, 2nd Edition, 1994.
4. Data on File: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

	Temperature limitation		Manufacturer		Contains sufficient for <n> tests
	Use by		Consult Instructions for use		This side up
	Date of Manufacture		Catalogue Number		Authorised Representative in the European Community
	Batch Number/ Lot Number		<i>In vitro</i> Diagnostic Medical Device		

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EC REP

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