**METHOD SUMMARY**

<table>
<thead>
<tr>
<th>Validated Methods</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Volume</td>
<td>1 or 2</td>
</tr>
<tr>
<td>Cell Volume</td>
<td>J</td>
</tr>
<tr>
<td>Cell Concentration</td>
<td>3-5%</td>
</tr>
<tr>
<td>Incubation Time</td>
<td>5 mins</td>
</tr>
<tr>
<td>Temperature</td>
<td>Room Temp</td>
</tr>
<tr>
<td>Spin (Speed/Time)</td>
<td>Low for 20 secs</td>
</tr>
</tbody>
</table>

**REAGENT DESCRIPTION**

Epiclone™ Anti-C3d monospecific monoclonal reagent contains murine monoclonal antibodies specific for an epitope on the human C3d molecule. If correctly stored and used according to the recommended methods, Epiclone™ Anti-C3d will detect C3d present on red cells. The reagent contains Sodium Chloride, Bovine Albumin and Sodium Acide as a preservative. The reagent has been optimised for use without any further dilutions or additions. No colouring agent has been added. The clone used to produce this reagent is BRIC 8.

**STORAGE CONDITIONS**

Store at 2° to 8°C (Refrigerate. Do Not Freeze).

**PRINCIPLE OF THE REAGENT**

When IgG antibodies attach to their appropriate red cell antigens, they may fail to cause agglutination of the cells but remain firmly bound even when the cells are thoroughly washed in saline. This process is called red cell sensitisation. The presence of the bound antibody may then be detected by the addition of an Anti-Human Globulin (AHG) reagent. Certain antibody-antigen reactions can cause binding of complement components to the red cell. A Direct Antiglobulin Test (DAT) is used to determine in vivo coating of red cells, whilst an Indirect Antiglobulin Test (IAT), is used to detect antibodies (present in the serum) after in vitro adsorption onto red cells. Following the detection of a positive DAT, bioCSL's AHG Anti-IgG and Epiclone™ Anti-C3d reagents are used to identify whether IgG or complement (C3d) has sensitised the cells.

Epiclone™ Anti-C3d is a monospecific monoclonal reagent which will react only with red cells having C3d component attached to their surface. No reaction will be obtained with red cells sensitised with human immunoglobulins or with C4 complement component. It is designed to determine the presence of C3d on red cells which are agglutinated by Epiclone™ AHG Poly reagent.

Epiclone™ Anti-C3d reagent is designed for special applications in Antiglobulin testing. It MUST NOT be used as the routine reagent in compatibility and antibody detection tests, but may be used in addition to an AHG Polyspecific reagent for the classification of the type of globulin coating the red cells.

**BACKGROUND**

The Anti-Human Globulin (Coombs) Test was first described by Coombs, Mourant and Race in 1945, although the principle of antiglobulin reactions was reported by Morecki in 1908. Many immune antibodies may attach to their respective red cell surface antigens without causing direct agglutination of the red cells, but these antibodies can be detected by antiglobulin tests. The polyspecific Anti-Human Globulin reagent contains antibodies to both immunoglobulins (mainly IgG and IgM) and complement components (C3d) and is designed as the primary reagent for antibody detection, compatibility tests and investigation of auto-immune haemolytic anaemias.

Haemolytic transfusion reactions can be either immediate or delayed in nature. In Immediate Haemolytic Transfusion Reactions (IHTR), the red blood cells are destroyed by one of two mechanisms, either intravascular haemolysis or extravascular haemolysis. In both mechanisms, patient’s antibody binds to incompatible transfused red blood cells forming an antigen-antibody complex. Intravascular haemolysis occurs when complement is activated; resulting in haemoglobin, red blood cell stroma and intracellular enzymes being released from the lysed red blood cells. Extravascular haemolysis occurs when there is antigen-antibody formation on the red blood cells (sensitisation), with incomplete activation of complement. These sensitised red blood cells are then removed by the reticuloendothelial system. Delayed Haemolytic Transfusion Reactions (DHTR), can be caused by either secondary response to transfused red blood cells or primary alloimmunisation. Extravascular haemolysis is the mechanism of red blood cell destruction in both types of DHTR. Haemolytic Disease of the Foetus and Newborn (HDFN), occurs when maternal antibodies (IgG in class), directed towards foreign antigens on foetal red blood cells, cross the placenta and attach to the foetal red blood cells, causing their destruction. This leads to foetal anaemia of varying severity. In severe cases, an exchange transfusion may be required for foetal survival.

The complement system, denoted by the letter C, has been found to consist of a complex group of soluble serum proteins made up of at least nine components labelled sequentially C1 to C9. Activation of complement causes these proteins to follow a cascading pathway that may result in a range of effects. From a serological point of view, the end result is an active product capable of causing destruction of the red cells (called lysis). Reaction of the antigen and antibody leads to a change in the shape of the antibody, with exposure of a site on the Fc portion of the molecule capable of binding with the first component of complement C1. C1 has 3 parts Clq, C1r and C1s which act on C4 and in turn C2 which forms a complex, known as C3 convertase, that is capable of reacting with C3. C3 is then split into C3a and C3b with C3b fixing to the red cell surface. C3b reacts with C5 and subsequently C6, C7, C8 and C9 leading to cell membrane damage and lysis. Complement fixation does not always proceed through all of the steps to lysis and often stops after a few steps and leaves various complement components bound to the cell membrane, most often C3.

From a clinical point of view, the complement fraction of serological interest is C3b; a complex of C3d, C4b and C3c. When the immune reaction has occurred in vivo (in the patient), C3d is usually the component that remains attached to the cell. Therefore, AHG Polyspecific reagents need to contain Anti-C3d which will detect only the clinically relevant C3d complement component on red cells following both in vivo and in vitro immune red cell reactions, and not C4b and other non-clinically relevant components or trace amounts of C3 that may normally be found on stored red cells. Users of AHG may choose a polyspecific reagent to allow detection of IgG and complement sensitised cells, and usually will have available Anti-IgG and Anti-C3d monospecific reagents to assist in investigating samples found positive with the polyspecific reagent. An exception to this general rule, are users of EDTA plasma, who may choose monospecific Anti-IgG as their primary reagent. This is because EDTA, a commonly used anticoagulant, prevents complement activation. However, Polyspecific AHG is also suitable for use with EDTA plasma samples.

**SPECIMEN COLLECTION AND PREPARATION**

Blood samples should be withdrawn aseptically with or without the addition of anticoagulants. Tests should be performed as soon as possible after collection of the sample. If testing the blood samples is delayed, samples should be stored between 2° to 8°C. Samples collected into EDTA or Heparin may be tested up to 7 days from the date of withdrawal provided storage has been at 2° to 8°C. Clotted samples may be tested up to 14 days from the date of withdrawal provided storage has been at 2° to 8°C.

Samples collected into Citrate may be tested up to 42 days from the date of withdrawal provided storage has been at 2° to 8°C. Cells may also be stored in Celpresol™ at 2° to 8°C for up to 42 days.

For Direct Antiglobulin Tests (DAT), blood collected into an anticoagulant (EDTA) is preferred and tests should preferably be performed within 24 hours of collection. EDTA is the recommended anticoagulant, as this will prevent complement sensitisation in vitro.

**RECOMMENDED METHOD**

Epiclone™ Anti-C3d reagent is recommended for use by the tube method.

**Tube Method**

**Direct Antiglobulin Test**

The Direct Antiglobulin Test (DAT), is a one-stage procedure for the detection of in vivo red cell sensitisation by antibodies and/or complement as found in Haemolytic Disease of the Foetus and Newborn (HDFN) and certain auto-immune conditions.

1. Appropriately label 2 separate, clean glass test tubes (10x75mm or 12x75mm).
2. Prepare a 3-5% suspension of test red cells in buffered or unbuffered isotonic saline, or in Celpresol™.
3. Place 1 drop of the suspension of test red cells into each tube.
4. Wash the cells in both tubes with 4 changes of isotonic saline, ensuring that the saline is decanted completely after each wash and that the cells are completely resuspended between washes.
5. To the 'dry' button of cells remaining after the fourth wash, in the first tube, add 1 or 2 drops of Epiclone™ Anti-C3d™.
6. To the 'dry' button of cells remaining after the fourth wash, in the second tube, add 2 drops of isotonic saline (the purpose of this second tube is to check that a positive result in the first is genuinely due to a reaction between the reagent and the C3d coating the cells, as distinct from saline agglutination which has not been dispersed by the washing or aggregation due to Wharton’s jelly).
7. Mix well and incubate at room temperature for 5 minutes.
8. Centrifuge at low speed (500rcf) for 15 to 20 seconds.
9. Gently agitate the tube to dislodge the red cells and examine for agglutination. Read and record results.

Notes: *Epiclone™ Anti-C3d reagent is validated for either 1 or 2 drop methods. Users may find that the 2 drop method provides a higher final liquid volume and easier reaction reading with the “tip and roll” technique.

* Or centrifuge at a speed and time appropriate for the centrifuge in use.

**INTERPRETATION OF RESULTS**
A positive reaction is indicated by agglutination of the test cells in the presence of Epiclone™ Anti-C3d.

A positive reaction in the DAT, accompanied by a negative saline blank, indicates that human complement (C3d) has been adsorbed to the cells from the patient’s own serum, with the proviso that cold auto-antibodies may bind complement as a result of cooling after the blood sample has been withdrawn from the patient. Precautions are necessary when dealing with patients in whom detectable cold auto-antibody is present. A positive DAT reaction indicates sensitisation of the patient’s cells in vivo.

**CONTROLS**
DAT tests should be controlled using a suitable positive control cell in addition to the saline control.

**LIMITATIONS OF PROCEDURE**
Epiclone™ Anti-C3d is not a polyspecific Anti-Human Globulin reagent, and as such should not be used as a routine reagent in Direct or Indirect Antiglobulin Tests.

False results may occur due to:
1. Incorrect technique.
2. Presence of gross rouleaux.
3. Use of aged blood samples, reagents or supplementary materials.
4. Contaminated blood samples, reagents or supplementary materials.
5. Other deviation from the recommended test methods.
6. Incorrect concentrations of red cells or expired reagents.
7. Incorrect reading of results.

**PRECAUTIONS**
1. For in vitro diagnostic use only.
2. The material from which this product was derived was found to be non-reactive for specified markers for HIV 1 and 2, Hepatitis B and C, HTLV and Syphilis by currently approved methods. However no known method can assure that products derived from human blood will not transmit infectious agents.
3. Sodium Azide 0.1% w/v is added as a preservative. Users should be aware of the toxicity and cumulative explosive nature of Sodium Azide and take appropriate precautions when handling and discarding this reagent.
4. This product should be clear; turbidity may indicate bacterial contamination. The reagent should not be used if a precipitate or particles are present.
5. The bovine material used is from an approved source free of Bovine Spongiform Encephalopathy (BSE).

**REFERENCES**