**INTENDED USE**

BIOSCOT Anti-Human Globulin is a polyspecific IgG/C3d blood grouping reagent (cell line BRIC-8) which will detect sensitising (but not directly agglutinating) blood grouping antibodies. This reagent is designed for use by operators trained in serological techniques.

**APPLICATIONS**

**Indirect Antiglobulin Technique**
- a) In screening the serum of blood donors and patients for antibodies
- b) In compatibility testing prior to blood transfusion
- c) In red cell phenotyping
- d) In the identification and titration of antibodies found in sera or eluates.

**Direct Antiglobulin Technique**
- a) In the laboratory diagnosis of haemolytic anaemia
- b) In the laboratory diagnosis of haemolytic disease of the newborn
- c) In the investigation of suspected transfusion reactions
- d) In the investigation of those autoimmune disorders involving binding of immunoglobulin and/or complement to red blood cells.

**Note:**
The detection of some clinically significant antibodies which activate complement (usually within the Kidd system) is enhanced and occasionally only possible through the use of a polyspecific Anti-Human Globulin reagent rather than monospecific Anti-IgG. The importance of the red cell diluent/washing solution is often underestimated. Phosphate buffered saline (PBS) pH 6.8-7.2 is preferable to unbuffered normal ionic strength saline.

**PRINCIPLE OF THE REAGENT**

The addition of Anti-Human Globulin to thoroughly washed red cells which are coated with antibody (immunoglobulin) and/or fragments of the third component of the complement system (C3b, C3bi, C3dg or C3d) will generally result in clearly visible agglutination of the red blood cells. BIOSCOT Anti-Human Globulin (polyspecific) is a blend of selected dilutions of sera obtained from rabbits immunised with purified human IgG and murine monoclonal Anti-C3d (cell line BRIC-8). The reagent has been standardised to give optimal detection of human IgG (all four sub-classes) and C3 fragments bound to red cells in all the routine diagnostic applications where direct or indirect antiglobulin techniques are appropriate. The reagent will not agglutinate red cells coated with C4d fragments.

The reagent has been optimised for use as supplied by the recommended techniques without further dilution or additions.

The product is supplied filtered to 0.22 μm.
Indirect Antiglobulin Technique - Normal Ionic Strength Saline (NISS):
- Test tube
- Phosphate buffered saline
- Incubator 37°C
- Timer
- Centrifuge (1500 g)
- IgG sensitised red cells (Coombs control cells)

Indirect Antiglobulin Technique - Low Ionic strength saline (LISS):
- Test tube
- Phosphate buffered saline
- Low Isotonic Strength saline
- Incubator 37°C
- Timer
- Centrifuge (1500 g)
- IgG sensitised cells (Coombs control cells)

Direct Antiglobulin Technique:
- Test tube
- Phosphate buffered saline
- Timer
- Centrifuge
- IgG sensitised cells (Coombs control cells)

RECOMMENDED TECHNIQUES
The use of automated cell washers must be validated by the user.

1. INDIRECT ANTIGLOBULIN TECHNIQUE – Normal Ionic Strength Saline (NISS)
   1.1 To a clearly labelled clean glass test tube add 2 drops (80 μl) of the test serum.
   1.2 Add one drop (40 μl) of a 3-5% suspension of test red cells which have been washed three times and resuspended in PBS.
   1.3 Mix thoroughly and incubate at 37°C for 30-60 minutes.
   1.4 Wash the cells four times in PBS taking care to decant the washing fluid completely and resuspending the cell button after each wash. Decant the PBS completely after the last wash.
   1.5 Add 2 drops (80 μl) of BIOSCOT Anti-Human Globulin (polyspecific) to the dry cell button. Mix thoroughly and centrifuge at 1000 rcf for 20 seconds.
   1.6 Resuspend the cells by gentle agitation and read macroscopically. N.B.: vigorous agitation may disrupt weak agglutination.
   1.7 The validity of all negative antiglobulin tests should be confirmed by the addition of IgG sensitised red cells (Coombs control cells).

2. INDIRECT ANTIGLOBULIN TECHNIQUE – Low Ionic Strength Saline (LISS)
   The use of LISS test cell suspensions enables the incubation time to be reduced to 15 minutes. The sensitivity of the LISS antiglobulin technique is dependent on the use of an equal ratio of serum to red cell suspension. It is therefore, recommended that semi-automated pipettes are used for the addition of serum and cell suspension. Test red cells should be washed twice in PBS and once in LISS before being adjusted to a 3-5% suspension in LISS.
   2.1 To a clearly labelled clean glass tube add 1 drop (40 μl) of test serum.
   2.2 Add an equal volume (40 μl) of 3-5% suspension of the test cells in LISS.
   2.3 Mix thoroughly and incubate at 37°C for 15 minutes. Continue through stages 1.4 – 1.7 as specified in the indirect antiglobulin technique (NISS).

3. DIRECT ANTIGLOBULIN TECHNIQUE
   The direct antiglobulin technique is used to demonstrate in vivo adsorption of IgG and / or complement fragments to the red cells. The blood sample tested should be freshly drawn (less than 24 hours) and preferably collected into EDTA anticoagulant.
   3.1 Prepare a 3-5% suspension of test red cells in PBS.
   3.2 To a clearly labelled clean glass test tube add 1 drop (40 μl) of the cell suspension. Continue through stages 1.4 - 1.7 as specified in the indirect antiglobulin technique (NISS).

LIMITATIONS
Contamination with human serum and / or inadequate washing will neutralise Anti-Human Globulin.
Clotted blood samples should not be refrigerated prior to direct antiglobulin testing.
False positive or false negative results may occur through contamination of test materials or any deviation from the recommended techniques.

PERFORMANCE CHARACTERISTICS
Anti-Human Globulin (cell line BRIC-8) polyspecific human IgG/C3d reagent product code TS has been tested by each of the recommended techniques with donor, clinical and neonatal specimens collected in either EDTA or citrate anticoagulants. The total number of tests (n) and the sensitivity and specificity was calculated for each technique and is displayed below:

<table>
<thead>
<tr>
<th>Technique</th>
<th>Anti-Human Globulin Product Code TS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td>IAT (NISS)</td>
<td>0</td>
</tr>
<tr>
<td>IAT (LISS)</td>
<td>19</td>
</tr>
<tr>
<td>DAT</td>
<td>13</td>
</tr>
</tbody>
</table>

Abbreviations: IAT = Indirect Antiglobulin Test. DAT = Direct Antiglobulin Test. NISS = Normal Ionic Strength Saline. LISS = Low Ionic Strength Saline.

Definitions from the Common Technical Specifications (CTS):
Diagnostic Sensitivity: The probability that the device gives a positive result in the presence of the target marker.
Diagnostic Specificity: The probability that the device gives a negative result in the absence of the target marker.

REFERENCES
h) Bruce, M et al., A serious source of error in Antiglobulin Testing. Transfusion 26; 177-181 (1986).