PRINCIPLE OF THE PRODUCT

The low ionic environment created by CSL RAM is designed to increase the rate of antibody uptake during test incubation, thus enhancing reactivity. This enables a reduction in the incubation time from 30 to 10 minutes and also achieves a significant increase in test sensitivity. CSL RAM is formulated to create these conditions by the additive method. CSL RAM, when used by the recommended method (two drops serum or plasma + one drop red cells + two drops CSL RAM), produces the optimal ionic conditions. No washing of the screening cells or complicated cell suspension procedures are required prior to use. Users find the LISS additive method to be rapid, sensitive and have a very low incidence of non-specific reactions.

BACKGROUND

Low and Messeter found that when red cells were suspended in a low ionic strength solution, the incubation time of the cells and antibody-containing serum could be significantly reduced when performing the Indirect Antiglobulin Test (IAT), without a loss of sensitivity.

Although low ionic strength solutions have been used for many years by automated techniques, reports of non-specific agglutination have led to a reluctance to use low ionic strength media in manual tests. Low and Messeter showed that specific non-specific reactions were not obtained, providing the ionic strength was not below 0.03M and the solution was buffered to pH 6.7.

Moore and Mollison confirmed these earlier findings and found that C3 and C4 reactions were also enhanced in addition to those of IgG. They also reported that the Indirect Antiglobulin Test using a low ionic strength solution was more sensitive in detecting low affinity antibodies. Many scientists have added to the evidence of the advantages of using low ionic strength solution in the indirect antiglobulin phase of compatibility testing.

Low ionic solutions may be formulated for use as a suspending medium or as an additive solution. When used in accordance with recommended methods, both types will provide an incubation mixture concentration of 0.06 - 0.09M. The LISS suspension method requires washing of test red cells and resuspension to the correct cell concentration. LISS additive methods have become more commonly used due to the lack of a cell resuspension requirement and convenience.

METHOD SUMMARY

<table>
<thead>
<tr>
<th>Application</th>
<th>Tube</th>
<th>MTP*</th>
<th>BioVueSM (CAT)**</th>
<th>ID-MTS™ (CAT)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Screen</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Antibody Identification</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Crossmatching</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

* MTP Methods
  Due to the wide variation in microplate methods and equipment, users should validate methods in routine use.

** CAT Methods
  Refer to the test method recommended by the manufacturer of Column Agglutination Technology (CAT) Systems.

PRODUCT DESCRIPTION

CSL RAM (Rapid Antibody Medium) is a phosphate buffered low ionic glycine solution. The product is manufactured to ensure optimum pH, osmolarity and conductivity. It is serologically tested with selected blood group antibodies to ensure the correct specificity and sensitivity in antibody screening is achieved. CSL RAM is designed for Tube methods, is suitable for Column Agglutination Technology (CAT) Systems, and is validated for direct addition into Ortho-Clinical Diagnostics BioVueSM (BioVue™). As the activity of CSL RAM depends on correct pH, conductivity and osmolarity, care should be taken not to introduce saline or buffer solutions into test systems. CSL RAM is prepared ready to use without any further modifications or dilutions. CSL RAM will retain optimum sensitivity until the expiry date, providing there is no contamination with foreign solutions, fungi or bacteria.

STORAGE CONDITIONS

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

RECOMMENDED METHODS

Tube Method – For Antibody Screen, Antibody Identification or Crossmatching

Low Ionic Strength Additive Method

A commonly used CSL RAM low ionic strength additive method is:

1. Prepare a 3-5% suspension of test red cells in buffered or unbuffered isotonic saline, or in CSL Celpresol™.
2. Add 2 drops of test serum to an appropriately labelled, clean glass test tube (10x75mm or 12x72mm).
3. Add 1 drop of the suspension of test red cells or Reagent Red Blood Cells. Gently, mix well.
4. Add 2 drops of CSL RAM (Rapid Antibody Medium) (See note 2 and 3). Gently, mix well.
5. Incubate at 37°C for 10 minutes.
6. Centrifuge at low speed (500rcf) for 15 to 20 seconds.
7. Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination. Record results.
8. Wash the cells 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.
9. To the ‘dry’ button of cells remaining after the fourth wash, add 1 or 2 drops of CSL Epiclone™AHG Poly or CSL AHG Anti-IgG**
10. Mix well and centrifuge at low speed (500rcf) for 15 to 20 seconds.
11. Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination. Record results.
12. Add 1 drop of CSL AHG Control Cells 3% to all negative test tubes to validate the results.
13. Repeat steps 10 and 11.

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

** CSL Epiclone™ AHG Poly and CSL AHG Anti-IgG reagents are 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.
1. It is preferable to use glass test tubes rather than plastic for two reasons: (a) To ensure standard drop volumes. Moore and Mollison point out that plastic tubes attract drops by electrostatic force and drop volume can vary by greater than ±40% of the mean value.
(b) To ensure rapid warming of reagents to 37°C. Plastic tubes are less efficient than glass at heat transfer. To obtain optimum results, it is essential that the incubation mixture remains at 37°C for at least 7 minutes.
2. A four drop technique is sometimes used in an attempt to improve the sensitivity of a 30 minute normal ionic strength test. Unlike most other blood group serology tests, the addition of extra serum to a LISS-additive technic may actually decrease sensitivity by raising the ionic strength of the incubation mixture. It is therefore most important to use the correct ratio of serum, cells and CSL RAM.
3. The cells should not be suspended directly in CSL RAM, as this may result in non-specific aggregation due to the very low ionic strength of the product. Therefore, the order of addition of sample and product is important.
4. When using this method for crossmatching, the test may be read following Step 5 to detect ABO incompatibility.

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 in 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 CSL Celpresol™ at 2° to 8°C for up to 42 days.

For compatibility testing and antibody screening, serum from freshly clotted blood should be used. The use of stored serum or plasma from anticoagulated samples may result in failure to detect complement-dependent antibodies.

**CAT Methods

When used in accordance with recommended methods, both types will provide an incubation mixture concentration of 0.06 - 0.09M. The LISS suspension method requires washing of test red cells and resuspension to the correct cell concentration. LISS additive methods have become more commonly used due to the lack of a cell resuspension requirement and convenience.
1. Prepare a 3-5% suspension of test red cells in buffered or unbuffered isotonic saline, or in CSL Celpresol™.
2. Appropriately label either a BioVue™ AHG Polyospecific or BioVue™ AHG Anti-IgG cassette.
3. Add 40µL of test serum or plasma to the appropriate reaction chamber.
4. Add 10µL of the suspension of test red cells or Reagent Red Blood Cells to the appropriate reaction chamber.
5. Add 40µL of CSL RAM to the appropriate test reaction chamber.
6. Observe that the contents of the reaction chambers are combined. If necessary, tap gently. The contents must stay within the reaction chamber and not fall through to the column to obtain valid results.
7. Incubate in the BioVue™ System Incubator at 37 ±1°C for 15 minutes.
8. Centrifuge the cassette in the BioVue™ System Centrifuge at the automatic preset speed setting of the centrifuge.
9. Read both front and back sides of the individual columns for agglutination and/or haemolysis under illumination. Record results.

**INTERPRETATION OF RESULTS**

A positive reaction is indicated by agglutination of the test cells in the presence of CSL Epiclone™ AHG Poly or CSL AHG Anti-IgG. A positive reaction in the Indirect Antiglobulin Test (IAT) indicates the presence of either human immunoglobulin (IgG) or human complement (C3d) on the red cells following incubation with serum. In the case of testing unknown serum, this means that an antibody directed at an antigen on the test cells is present in the serum. When testing unknown cells against a phenotyping reagent that requires the use of an IAT technique, the presence of the appropriate antigen on those cells is indicated, provided a Direct Antiglobulin Test (DAT), on the same cells or an auto control test performed in parallel with the test, gives a negative reaction.

Some laboratory scientists prefer to interpret antiglobulin reactions microscopically. Whilst this practice may lend additional sensitivity to the test, it can also be a potential source of misleading positive reactions, the predominant cause of which is the propensity of red cells to adsorb complement during storage at refrigerator temperatures. Accordingly, it is recommended that antiglobulin tests be read with the use of a hand lens or a concave mirror and a suitable source of illumination. Stronger magnification, such as microscopes, may be used for investigative tests, but are not recommended for routine tests.

**CONTROLS**

The Antiglobulin Test is an exceedingly delicate procedure and the presence of even minute amounts of free human protein can result in neutralisation of the Anti-Human Globulin (AHG) reagent. The washing of cells should be carried out with great care and the quality and cleanliness of saline and glassware should be maintained. The inclusion of both positive and negative controls is essential with every batch of tests and as a final check on the adequacy of washing and on the presence of the AHG reagent.

Following the interpretation of each test, one drop of sensitised cells (eg. CSL AHG Control Cells 3%) should be added to all negative tests and the mixtures re-examined to ensure that agglutination occurs. This control is based on the principle that in a truly negative test the Anti-Human Globulin reagent will have remained unconsumed after exposure to the particular washed cell suspension and therefore agglutinate the AHG Control Cells. Its use safeguards against error caused by imperfect technique, poor washing and inadequately reactive antiglobulin reagents.

**LIMITATIONS OF PROCEDURE**

1. It is imperative that the correct ratio of serum, cells and CSL RAM is used in the test and that reagents are added to the tube in the specified order. Direct contact of red cells and CSL RAM may cause non-specific aggregation.
2. Red cells may be damaged by prolonged exposure to a low ionic environment.
3. Variations in time and temperature of incubation, time and speed of centrifugation and reaction reading technique may cause imprecision in results.
4. Some samples of cold reacting antibodies may not be optimally active by the recommended test method.
5. The cell button obtained following centrifugation in Step 6 may vary in appearance to that usually seen in Saline or Albumin tests. Interpretation should be made only after gentle, but complete, resuspension of the cell button.
6. As the procedure depends on the ionicity of the incubation mixture, false results may occur when testing eluates.
7. High concentrations of protein in patient samples may cause autoagglutination or rouleaux.
8. IgM antibodies may not be detected by the CSL RAM method.
9. CSL RAM is not a resuspension medium unlike LISS. It should be used only as an additive following the methods detailed above.

Discrepant 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. Red cells that have a positive Direct Antiglobulin Test (DAT).
6. Other deviation from the recommended test methods.

**REFERENCES**