**METHOD SUMMARY**

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<th>Application</th>
<th>Tube</th>
<th>MTP*</th>
<th>BioVue™ (CAT)</th>
<th>ID-MTSTM (CAT)</th>
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<tbody>
<tr>
<td>Antibody Screen</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Antibody Identification</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<tr>
<td>CSL RAMPEG Autologous Adsorption Method</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Testing Eluates</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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*MTP Methods*  
Due to the wide variation in microplate methods and equipment, users should validate methods in routine use.

**PRODUCT DESCRIPTION**

CSL RAMPEG (Rapid Antibody Medium with Polyethylene Glycol) is a phosphate buffered low ionic glycine solution containing Polyethylene Glycol (PEG), used in the Indirect Antiglobulin Test (IAT). The product is manufactured to ensure optimum pH, osmolality and conductivity. It is serologically tested with selected blood group antibodies to ensure the correct specificity and sensitivity in antibody screening is achieved. CSL RAMPEG is not suitable for detection of IgM antibodies by direct (immediate spin) techniques. CSL RAMPEG provides a significant increase in test sensitivity to many clinically relevant IgG antibodies. It is commonly used for routine Antibody Screening, Antibody Identification, Investigation, Crossmatching and Testing Antibody Eluates. It can also enhance warm reacting antibodies and may be useful in detecting weak auto-antibodies. As the activity of CSL RAMPEG depends on correct pH, conductivity, molarity and potentiation concentration, care should be taken not to introduce saline or buffer solutions into test systems. CSL RAMPEG is provided ready to use without any further modifications or dilutions. CSL RAMPEG 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).

**PRINCIPLE OF THE PRODUCT**

CSL RAMPEG utilizes a combination of a low ionic environment and a high molecular weight potentiator to increase the rate of antibody uptake during test incubation. This enables a reduction in the incubation time from 30 to 10 minutes and also achieves a significant increase in test sensitivity. CSL RAMPEG is formulated to create these conditions by the addition method. CSL RAMPEG, when used by the recommended method (two drops serum or plasma + one drop red cells + two drops CSL RAMPEG), produces the optimum ionic and potentiation condition. No washing of the screening or treated cell suspension procedures are required prior to use. Users find the CSL RAMPEG method to be rapid, highly sensitive and have a low incidence of non-specific reactions. When used in accordance with the recommended method, CSL RAMPEG will give the sensitivity of tests for the detection of IgG antibodies by the Indirect Antiglobulin Test (IAT).

**BACKGROUND**

Polyethylene Glycol is a water soluble, linear, high molecular weight polymer. The addition of Polyethylene Glycol to an antibody screen test mixture effectively increases the relative concentrations of reactants and increases the rate of immune complex formation. This increases the sensitivity of the Indirect Antiglobulin Test (IAT) to clinically significant IgG antibodies. In 1987, Garity and Nance reported on the use of Polyethylene Glycol as a potentiator in immunohaematology testing. Many studies using Polyethylene Glycol report good results as an additive when antibody screening, testing eluates and when performing absorptions. The use of Polyethylene Glycol in the adsorption of autoantibodies from patient sera has been shown to eliminate the time taken to treat the alloreactive adsorbing cells and to also reduce the actual time of incubation.

Low and Messier found that when red cells were suspended in a low ionic 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. Low ionic strength reagents have also been shown to increase the rate at which blood group antibodies bind to their specific antigen. CSL RAMPEG uses Polyethylene Glycol suspended in a low ionic environment to combine both techniques. This provides a useful antibody screening additive reagent that enhances the sensitivity of the IAT.

Centrifugation of CSL RAMPEG and test serum should be avoided as non-specific aggregates may form that may reduce the rate of reagent or saline and increase the detection of antibodies. Direct agglutinins, such as IgM antibodies, may not be detected by the CSL RAMPEG method. For this reason, an expert panel of the International Society of Blood Transfusion has recommended that, when using the Polyethylene Glycol method for crossmatching, at least an immediate Spin test should be carried out before adding Polyethylene Glycol, in order to detect ABO incompatibility. Early publications recommend the use of non-specific Anti-IgG Anti-Human Globulin when using Polyethylene Glycol reagents to reduce non-specific reactions. There is wide variation in the incidence of these non-specific reactions between Anti-Human Globulin (AHG) reagents from different sources and from different batches. The use of AHG Anti-IgG is encouraged unless the polyspecific reagent has been proven to give acceptable specificity with CSL RAMPEG. Some publications report that Polyethylene Glycol can cause the precipitation of immunoglobulins (IgG), especially when serum Ig levels are high. This effect is related to the molecular weight of the Polyethylene Glycol used. CSL RAMPEG is formulated with a molecular weight Polyethylene Glycol that is shown not to cause this effect. As Polyethylene Glycol significantly increases the viscosity of the incubation mixture, care must be taken during the washing stage and automated cell washing systems should be validated to ensure the washing, декантирование and cell agglutination stages are sufficient to allow correct test performance.

**SPECIMEN COLLECTION AND PREPARATION**

Blood samples should be withdrawn aseptically to and 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 Celspresol™ 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 antigen-negative samples may result in failure to detect complement-dependent antibodies.

**RECOMMENDED METHODS**

**Tube Method – For Antibody Screen, Antibody Identification or Crossmatching**

**Low Ionic Strength Additive Method**

A commonly used CSL RAMPEG Low Ionic Strength Additive method is:

1. Prepare a 5-15% suspension of test red cells in buffered or unbuffered isotonic saline, or in CSL Celspresol™.
2. Add 2 drops of test serum to an appropriately labelled, clean glass test tube (10x75mm or 12x75mm).
3. Add 1 drop of the suspension of test red cells or Reagent Red Blood Cells, gently mix well.
4. Centrifuge at low speed (500rcf) for 15 to 20 seconds*.
5. Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination. Record results.
6. Add 2 drops of CSL RAMPEG (Rapid Antibody Medium with Polyethylene Glycol) (see note 2 and 3). Gently, mix well.
7. Incubate at 37°C for 10 to 15 minutes (see note 4).
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 (see note 5).
9. To ensure a good number 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 Mellon point out that glass 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.
2. To obtain optimum results, it is essential that the incubation mixture remains at 37° for 37° at a minimum.

b) To ensure rapid warming of reagents to 37°C. Plastic tubes are less efficient than glass at heating. To obtain optimum results, it is essential that the incubation mixture remains at 37° for 37° at a minimum.

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 drops to a LISS additive technique 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 RAMPEG.
3. The cells should not be suspended directly in CSL RAMPEG, 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. An examination for haemolysis at this point may be carried out and if present must be investigated.
Agglutination may not be read at this stage as Polyethylene Glycol causes red cells to aggregate.
5. If using an automated cell washer, it is recommended that 1 manual wash step is performed prior to automated washing, as it has been noted that some automated cell washers fail to inject wash saline with sufficient force to thoroughly mix the viscous CSL RAMPEG-serum mixture. This may lead to non-specific agglutination of red cells and/or failure of CSL AHG Control Cells 3% to validate. An initial manual wash adequately resuspends the sample in the serum mixture, preventing non-specific aggregation and subsequent neutralisation of the Anti-Human Globulin reagent.
6. When using this method for crossmatching, the test may be read following Step 5 to detect ABO incompatibility.

**CSL RAMPEG Autologous Adsorption Method**

CSL RAMPEG may be used for performing autologous adsorption procedures without pre-treatment of the patient’s red cells. It is simpler and less time consuming than other common methods such as ZAP. CSL RAMPEG is more successful than using Polyethylene Glycol only reagents. Note that no method is optimal for all antibodies and the success of this method depends on the specificity of the antibody under test.

1. Centrifuge patient sample for 10 minutes at 1000g and separate the plasma and red cells.
2. Mix 1 volume of patient red cells with 1 volume of plasma and 1 volume of CSL RAMPEG.
3. Incubate for 15 minutes at 37°C.
4. Centrifuge for 7 minutes at 1000g.
5. Remove supernatant and test with an appropriate antibody detection method.

**INTERPRETATION OF RESULTS**

A positive reaction may be 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 refrigeration 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 sensitivity to the test, it can also be a potential source of misleading positive reactions, the predominant cause of which is that antiglobulin tests be read at this stage as Polyethylene Glycol causes red cells to agglutinate.

**CONCLUSIONS**

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 potency 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 and negative controls is essential with every batch of tests and as a final check on the adequacy of washing and on the potency of the AHG reagent.

**LIMITATIONS OF PROCEDURE**

1. The sensitivity that the direct ratio of serum, cells and CSL RAMPEG is used in the test and that reagents are added to the tube in the specified order. Direct contact of red cells and CSL RAMPEG may cause non-specific aggregation.
2. Centrifugation of CSL RAMPEG and test serum should be avoided as non-specific aggregates may form that are difficult to disperse and may cause non-specific results.
3. Red cells may be damaged by prolonged exposure to a low ionic environment.
4. Variations in time and temperature of incubation, time and speed of centrifugation and reaction reading technique may cause discrepancies in results.
5. Some samples of cold reacting antibodies may not be optimally active by the recommended test method.

6. The cell button obtained following centrifugation in Step 4 may vary in appearance to that usually seen in Saline or Albumin tests. Interpretation should be made only after full, but complete, resuspension of the cell button.
7. High concentrations of protein in patient samples may cause autoagglutination or rouleaux.
8. Anti-IgM antibodies may not be detected by the CSL RAMPEG method.
9. CSL RAMPEG is not a resuspension medium unlike LISS. It should be used only as an additive following the method detailed above.

**DISCREPANT RESULTS**

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.
7. Incorrect concentrations of red cells.
8. Incorrect reading of results (ie. failure to detect haemolysis, etc).

**PRECAUTIONS**

1. For in vitro diagnostic use only.
2. The material from which this product is derived is from non-human sources, there is no risk of HIV or HBsAg infection. However, good laboratory practice requires safe handling procedures are used.
3. Thrombosed 0.01% w/v has been added to retard fungal and bacterial contamination. Users should take appropriate precautions when handling and discarding this product.
4. Bacterial or fungal contamination may occur as a result of incorrect storage of open containers. Take care to avoid contamination. The product should not be used if a precipitate or particles are present.
5. As the batch of CSL RAMPEG depends on correct pH, conductance and ionotropy, care should be taken not to introduce saline or buffer solutions.

**REFERENCES**


**Full Name of file:**

**Date last compiled:**

**Date last verifying:**

**Actioned Approval:**

02380000E

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E3

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