



LOW IONIC SALT SOLUTION FOR SEROLOGICAL APPLICATIONS

SUMMARY

The antigen-antibody interaction in blood group serology is dependant on antigen density, concentration of antibody, pH, ionic concentration of reaction medium and temperature. Reducing the ionic concentration of the reaction medium especially enhances the uptake of weak antibodies by the red blood cell antigens. Usage of low ionic salt solution is helpful in detection of weak antibodies during cross match techniques, antibody screening and antibody identification.

REAGENT

TULISS® is a buffered low ionic salt solution of appropriate sodium chloride molarity useful in serological applications such as antibody detection and cross match techniques.

REAGENT STORAGE AND STABILITY

Store the reagent at 2-8°C. DO NOT FREEZE. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE

In blood group serology the ionic concentration of reaction medium is largely dependant on the concentration of sodium and chloride ion contributed by isotonic saline. When optimum concentration of antibody is present, antigen-antibody interaction occurs even though the sodium and chloride ions are present in sufficient quantity. But when weak antibodies are present, sodium and chloride ions may interfere with binding of antibody to the antigens present on the red blood cell membrane. By lowering the ionic concentration of salt, the ionic strength is reduced which increases the rate of antibody uptake by red blood cells.

NOTE

(1) In vitro diagnostic reagent for laboratory and professional use only. (2) The reagent contains 0.1% sodium azide as a preservative. Avoid contamination with skin and mucosa. On disposal flush with large quantity of water. (3) Do not freeze or expose the reagent to elevated temperatures. After usage immediately replace the reagent vial back to 2-8°C. (4) Marked turbidity may indicate reagent deterioration or contamination, such reagent should not be used. Do not use the reagent beyond expiry date.

SAMPLE COLLECTION AND STORAGE

No special preparation is required prior to sample collection by approved techniques. Samples should be stored at 2-8°C, if not tested immediately. Do not use haemolysed samples. Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period: EDTA or Heparin-2 days, Sodium citrate or sodium oxalate-14 days, ACD or CPD-28 days.

ADDITIONAL MATERIAL REQUIRED

Test tubes (12x75 mm), Pasteur pipettes, laboratory centrifuge, incubator (37°C), isotonic saline/ isotonic buffered saline, donor red blood cells and recipient serum for cross match, reagent red blood cells for antibody detection, Anti-human Globulin reagent for cross match and antibody detection (Available from Tulip: ERYCLONE® Anti Human Globulin reagent Cat. No. 10180002, 10180005), optical aid.

PROCEDURE

Bring all the reagents to room temperature before testing.

INDIRECT ANTIGLOBULIN TEST FOR CROSS MATCH

Initial phase

(1) Wash donor red blood cells three times in isotonic saline. Decant the supernatant completely after last wash. (2) Finally wash the donor blood red cells with TULISS®. A final wash with TULISS® is recommended to reduce the effect of residual isotonic saline on the final ionic concentration of the test medium. (3) Prepare a 2-3% donor red blood cells suspension in TULISS®. (4) To an approximately labelled test tube add two drops of recipient serum. (5) Add two drops of TULISS® suspended donor red blood cells (6) Centrifuge for one minute at 1000 rpm (125g) or for 20 seconds at 3400 rpm (1000g). (7) First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically.

Incubation phase

(1) Incubate the tube containing the mixture of donor red blood cells and recipient serum at 37°C for 10 minutes, (2) First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically. (3) Proceed to the antiglobulin phase.

Antiglobulin phase

(1) Wash the mixture of donor red blood cells and recipient serum thoroughly with isotonic saline minimum for three times. Decant completely after the last wash. (2) Place two drops of Anti-human globulin reagent into the test tube and mix well. (3) Centrifuge for one minute at 1000 rpm (125g) or for 20 seconds at 3400 rpm (1000g). (4) Very gently, resuspend the cell button and observe for agglutination macroscopically.

FOR ANTIBODY DETECTION

Initial phase

(1) Wash red blood cells three times in isotonic saline. Decant the supernatant completely after last wash. (2) Finally wash the reagent red blood cells with TULISS®. A final wash with TULISS® is recommended to reduce the effect of residual isotonic saline on the final ionic concentration of the test medium. (3) Prepare a 2-3% reagent red blood cell suspension in TULISS®. (4) To an approximately labelled test tube add two drops of serum to be tested. (5) Add two drops of TULISS® suspended reagent red blood cells. (6) Centrifuge for one minute at 1000 rpm (125g) or for 20 seconds at 3400 rpm (1000g). (7) First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically.

Incubation Phase

(1) Incubate the tube containing the mixture of donor red blood cells and recipient serum at 37°C for 10 minutes, (2) First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically (3) Proceed to the antiglobulin phase.

Antiglobulin phase

(1) Wash the mixture of reagent red blood cells and serum thoroughly with isotonic saline minimum for three times. Decant completely after the last wash. (2) Place two drops of Anti-human Globulin reagent into the test tube and mix well. (3) Centrifuge for one minute at 1000 rpm (125g) or for 20 seconds at 3400 rpm (1000g). (4) Very gently, resuspend the cell button and observe for agglutination macroscopically

INTERPRETATION OF RESULTS

Crossmatch

In all phases of the compatibility test, if no agglutination or haemolysis is observed then the patient and donor may be considered to be compatible. If haemolysis or agglutination at any point till the completion of the antiglobulin phase is observed, the patient and donor are considered to be incompatible.

Antibody detection

Agglutination or haemolysis indicates that the antibody has reacted with the corresponding red blood cell antigen. No agglutination or haemolysis indicates the absence of corresponding antibody.

REMARKS

1. As under centrifugation or over centrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and the time required for achieving the desired results.
2. Erroneous results may also occur due to improper red blood cell concentration, improper incubation time or temperature while performing the test.
3. The ionic strength of the test system is dependant on the amount of serum used. Alteration of the ionic strength of LISS procedure by addition of excess human serum will increase the ionic strength and decrease the sensitivity of the test system.
4. The performance of TULISS® reagent should be periodically evaluated with a known LISS enhanced antibody and the corresponding antigen for positive result and red cell lacking the corresponding antigen for negative result.
5. To all negative test results after the antiglobulin test phase, one drop of Coombs control cells should be added. If Coombs control cells do not agglutinate then the test must be repeated.
6. Low ionic strength media have been used to enhance many antigen-antibody reactions. However not all antibodies are reactive in a LISS test system. Some weakly reactive IgM antibodies of ABO system may not be detected in the system employing low ionic strength media.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty for 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. Data on File: Tulip Diagnostics (P) Ltd.

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TULIP DIAGNOSTICS (P) LTD.

PLOT NOS. 92/96, PHASE II C, VERNA IND. EST.,
VERNA, GOA - 403 722, INDIA.
Website : www.tulipgroup.com

In vitro Diagnostic Reagent
NOT FOR MEDICINAL USE.
Store at 2-8°C. DO NOT FREEZE.
Preservative: 0.1% NaN₃

ISO 9001-2000, ISO 13485 (2003), NF EN ISO 13485 (2004)