



Suero Anti-humano

(*poliespecífico*)

(Policlonal anti-IgG + monoclonal anti-C3d)
To perform Direct or Indirect Antiglobulin Tests (Coombs)

SUMMARY

The experiences described by Coombs, Mourant and Race in 1945 established that the Human Antiglobulin Tests were the best convenient detection methods for sensitizing, but not directly agglutinating, antibodies. Originally, the previous example referred to antigens from the Rh system. Later experiences proved the value of such tests in the detection of antibodies in almost every blood group of clinical significance, being currently used for the following cases:

Indirect Agglutination Test

- Donors and patients serum screening for irregular antibodies.
- Compatibility tests prior to transfusion.
- Red blood cells phenotype.
- Identification and titration of antibodies found in serum or eluates.

Direct Antiglobulin Test

- Laboratory diagnosis of hemolytic anemia and hemolytic disease of the newborn.
- Investigation of borderline reactions in a transfusion.
- Investigation of autoimmune diseases involving the fusion of immunoglobulins and/or red blood cells' complement fractions.

PRINCIPLE

The addition of **Suero Anti-humano (poliespecífico)** to red blood cells that are covered by immunoglobulins and/or complement fragments produces macroscopically visible red blood cells agglutination.

PROVIDED REAGENTS

Suero Anti-humano (poliespecífico): polyclonal anti-IgG (obtained from rabbits immunized with purified human IgG) and monoclonal anti-C3d mixture obtained from the culture of BRIC-8 cell line of murine hybridoma secretor of IgM. The reagent contains a green dye (not affecting the reagent properties) and < 1 g/l sodium azide as preservative.

NON-PROVIDED REAGENTS

- Reagents for blood group determination.
The following may be required according to the technique used:
- Saline solution.
- Phosphate buffer (PBS) pH 7.0 ± 0.2.
- Low ionic strength saline solution (LISS).

INSTRUCTIONS FOR USE

The reagent is ready to use. Do not dilute.

WARNINGS

The Provided Reagent is for "in vitro" diagnostic use and strictly intended for professional use by qualified personnel with proven immunohematology knowledge.

Do not use the reagent after the expiration date.

Azide may react with lead or copper pipes generating explosive compounds. When discarding the reagent, let run a copious tap water flow.

Employ the reagents following the ordinary work precautions used at the clinical chemistry lab.

Reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

The Provided Reagent is stable in refrigerator (2-10°C) until the expiration date shown on the box. Do not freeze nor expose to high temperatures.

INSTABILITY OR DETERIORATION OF REAGENTS

Discard the reagent whenever contamination is observed. The reagent should not be used in the presence of turbidity, precipitates or particles.

SAMPLE

Red blood cells

a) Collection: blood should be aseptically obtained, with or without anticoagulant.

b) Additives: the following may be used as anticoagulant: EDTA, heparin, ACD (citric acid, citrate, dextrose) or CPDA-1 (citrate, phosphate, dextrose, adenine). The use of Wiener lab.'s **Anticoagulante W** is recommended.

c) Known Interfering Substances: do not use contaminated samples or samples with intense hemolysis.

d) Stability and storage instructions: samples should be tested as soon as possible. If the test is not performed immediately, samples should be stored in refrigerator (2-10°C). In case heparin or EDTA is used for collection, typification should be performed within 48 hours. Samples collected with ACD, CPD or CPDA-1 may be tested within 35 days from collection. In case clots are used, typification should be performed within 7 days of sample collection. Clots must be refrigerated before performing the direct tests. Whenever direct antiglobulin tests are performed, blood should be preferably fresh (less than 24 hours from collection). Donor blood samples may be tested until their expiration date. Sera should be stored for no longer than 24 hours at 2-10°C or for 1 month at -20°C. Sera stored at -20°C or less for extended periods of time lose complement activity. Direct antiglobulin

tests should be performed on fresh erythrocytes collected with EDTA to avoid "in vitro" sensitization with complement factors.

REQUIRED MATERIAL (non-provided)

- Centrifuge
- Water bath at 37°C
- Hemolysis tubes

PROCEDURE

The reagent has been standardized according to the procedures detailed below. The usage performance with other techniques is not guaranteed.

I- INDIRECT ANTIGLOBULIN TEST (in normal ionic strength saline solution).

- 1) In a hemolysis tube place 2 drops of the serum to be tested.
- 2) Add 1 drop of the red blood cells suspension to be tested at 3%, washed 3 times and resuspended in PBS. The provided dropper dispenses a volume of 50 ± 5 μ l. The antiserum:cells ratio should be maintained for all assay systems.
- 3) Mix and incubate at 37°C for 30-60 minutes.
- 4) Wash blood cells with PBS 3 times. Make sure to remove as much saline solution as possible at the end of each washing step so as to obtain a "dry" cell button. Discard the supernatant after the last washing step.
- 5) Add 2 drops of **Suero Anti-humano (poliespecifico)** to the cell button. Mix thoroughly and centrifuge for 15 seconds at 1,100 g.
- 6) Gently stir the tube to remove the cell button and observe macroscopically. The observation may be simplified if a diffuse light source is used. Notice that too vigorous agitation could unbind weak agglutinations (false negative).
- 7) Negative results should be confirmed through sensitized red blood cells addition to weak IgG (Coombs control).

II- INDIRECT ANTIGLOBULIN TEST (in low ionic strength saline solution).

The use of such solution helps reduce the incubation time to 15 minutes.

The red blood cells should be washed 2 times with PBS and one time with LISS. Finally, using this solution prepare a red blood cells suspension at 3%.

- 1) In a hemolysis tube place 1 drop of the serum to be tested.
- 2) Add 1 drop of red blood cells to test at 3% in LISS.
- 3) Mix and incubate at 37°C for 15 minutes in water bath.
- 4) Wash the red blood cells with PBS 3 times. Make sure to remove as much saline solution as possible at the end of each washing step so as to obtain a "dry" cell button. Discard the supernatant after the last washing step.
- 5) Add 2 drops of **Suero Anti-humano (poliespecifico)**

to the cell button. Mix thoroughly and centrifuge for 15 seconds at 1,000 g.

6) Gently stir the tube to remove the cell button and observe macroscopically. The observation may be simplified if a diffuse light source is used. Notice that too vigorous agitation could unbind weak agglutinations (false negative).

7) Negative results should be confirmed through sensitized red blood cell addition to weak IgG (Coombs control).

III- DIRECT ANTIGLOBULIN TEST

It is used to demonstrate the "in vivo" absorption of IgG and/or complement fractions in the red blood cells surface.

- 1) Prepare a red blood cell suspension to be tested in PBS at 3%.
- 2) In a hemolysis tube place 1 drop of this suspension.
- 3) Wash the red blood cells with PBS 3 times. Make sure to remove as much saline solution as possible at the end of each washing step so as to obtain a "dry" cell button. Discard the supernatant after the last washing step.
- 4) Add 2 drops of **Suero Anti-humano (poliespecifico)** to the cell button. Mix thoroughly and centrifuge for 15 seconds at 1,000 g.
- 5) Gently stir the tube to remove the cell button and observe macroscopically. The observation may be simplified if a diffuse light source is used. Notice that too vigorous agitation could unbind weak agglutinations (false negative).
- 6) Negative results should be confirmed through sensitized red blood cell addition to weak IgG (Coombs control).

INTERPRETATION OF RESULTS

The observation of agglutination (with any of the techniques used) in the presence of **Suero Anti-humano (poliespecifico)** indicates the presence of IgG or complement components in the red blood cell membrane; the reaction is positive. If no agglutination is observed, the reaction is negative.

QUALITY CONTROL METHOD

A quality control of the reagents should be performed at the beginning of each work day with red blood cells sensitized with IgG, for example, R1r red blood cells sensitized with weak Anti-Rh (D).

PROCEDURE LIMITATIONS

The following causes may lead to false positive and false negative reactions:

- Chemical or bacterial contamination of the sample and other materials used for the test.
- Inadequate washing of the cells. The washing steps should be completed as soon as possible and without interruption. Any delay in completing the washing or reading process may dissociate the antigen-antibody complex yielding weak

positive or negative results.

- Wash solution residues may dilute the reagent beyond the optimal work concentration. Therefore, after this process is finished, it is important to remove as much wash solution as possible.
- Inadequate incubation time or temperature.
- Inadequate centrifugation time or speed.
- Red blood cells inadequate concentration.
- Excessive agitation to detach agglutinated red blood cells.
- After the addition of human antiglobulin, read and centrifuge the assays immediately.

Positive erythrocytes by a direct antiglobulin test should not be used for an indirect antiglobulin test. The reagents have been standardized to detect human immunoglobulins and C3 fragments bound to the red blood cells. They are not suitable to detect antibodies from other sources.

WIENER LAB. PROVIDES

Vial x 10 ml (Cat. Nr. 1443156).

REFERENCES

- Coombs, R.R.A.; Mourant., A.E. and Race, R.R. - Lancet, ii:15, 1945.
- Garratty G, Petz LD. "The significance of red cell bound complement components in the development of standards and quality assurance for the anti-complement components of antiglobulin sera". Transfusion 19, 1976.
- Wright MS, Issitt PD. Anticomplement and the indirect antiglobulin test. Transfusion 19:688-694, 1979.
- Moore BPL. - Serological and immunological methods of the Canadian Red Cross Blood Transfusion Service. 8th ed. Toronto: Hunter Rose, 1980.
- Lachman PJ, Pangburn MK, Oldroyd RG. Breakdown of C3 after complement activation. J Exp Med 156:205-216, 1982.
- Howell P, Giles CM. - A detailed serological study of five anti-JK sera reacting by the antiglobulin technique. - Vox Sang 45:129-138, 1983.
- Issitt PD Applied blood group serology. 3rd ed. Miami: Montgomery Scientific, 1985.
- Voak D, Downie DM, Moore BPL et al. Quality control of anti-human globulin test: use of replicate test to improve performance. Bio Bull 1:41-52, 1986.
- ISBT/ICSH Working Party. International reference polyspecific antihuman globulin reagents. Vox Sang 53:241-247, 1987.
- Walker RH, ed. Technical manual. 11th ed. Bethesda: American Association of Blood Banks, 1993.

SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

 This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

 Authorized representative in the European Community

 "In vitro" diagnostic medical device

 Contains sufficient for <n> tests

 Use by

 Temperature limitation (store at)

 Do not freeze

 Biological risks

 Volume after reconstitution

 Contents

 Batch code

 Manufactured by:

 Harmful

 Corrosive / Caustic

 Irritant

 Consult instructions for use

 Calibrator

 Control

 Positive Control

 Negative Control

 Catalog number

 Wiener Laboratorios S.A.I.C.
Riobamba 2944
2000 - Rosario - Argentina
<http://www.wiener-lab.com.ar>
Dir. Téc.: Viviana E. Cétola
Biochemist
A.N.M.A.T. Registered product
Disp. N°: 2503/91



Wiener lab.

2000 Rosario - Argentina

UR110726