Anti-A, Anti-B o Anti-AB

monoclonal

Reagents for the determination of ABO blood groups

SUMMARY

In the year 1900 Landsteiner discovered that human red blood cells could be classified in A, B, AB or O according to the presence (groups A, B, or AB) or absence (group O) of highly reactive antigens in its surface. He also demonstrated that there are antibodies (agglutinins) for A and B antigens and that one person's serum does not contain antibodies for the antigen present in his own red blood cells, but for the ones he does not have. Nowadays, subgroups of the A and B groups with different specificity have been identified. These observations demonstrated the significance of the ABO compatibility in the transfusion practice. Therefore, ABO blood grouping typification is the basic test on which all other pretransfusion tests are based.

PRINCIPLE

The patient red blood cells ar in contact with Anti-A, Anti-B or Anti-A,B monoclonal antibodies. The presence or absenceof agglutination of the tested erythrocytes with each reagent indicates the presence or absence of the corresponding antigens.

PROVIDED REAGENTS

Anti-A, Anti-B or Anti-A,B monoclonal: reagents prepared from monoclonal antibodies IgM class secreted by cellular lines of mouse hybridomas in a buffered solution containing < 1 g/l sodium azide as preservative. The involved clones and each product's color are detailed in the following table:

Product Name	Cell lines	Color
Anti-A	BIRMA-1	Blue
Anti-B	LB-2	Yellow
Anti-AB	BIRMA-1/ES-4/ES-15	Colorless

The antiserum is characterized by its high potency, avidity and specificity.

Since they are not from human origin, there is no risk of HIV, HBV or HCV infection.

NON-PROVIDED REAGENTS

The following may be required, according to the technique to be used:

- Saline solution
- Phosphate buffered saline solution (PBS) pH 7.0 ± 0.2
- Low ionic strength saline solution (LISS)

INSTRUCTIONS FOR USE

The Reagents are provided ready to use. Do not dilute.

STABILITY AND STORAGE INSTRUCTIONS

The Provided Reagents are stable in refrigerator (2-10ºC) until the expiration date shown on the box. Do not freeze. Extended storage periods at temperatures outside this range may increase the reagent reactivity loss.

Avoid repeated thermal changes and regulate the reagent's exposure to room temperature to the strictly necessary. In these usage and storage conditions, the reagent, after being opened, is stable until the expiration date shown on the box.

INSTABILITY OR DETERIORATION OF REAGENTS

Discard the reagent if contamination is observed. Even though sodium azide is added as bacteriostatic, it is recommended to visually inspect the reagent before use. Do not use in case of turbidity.

The reagent should not be used in the presence of precipitates or particles.

SAMPLE

Red blood cells or whole blood

a) Collection: blood should be aseptically obtained, with our without anticoagulant. For the slide technique, blood collected by digital puncture may be used. To avoid blood clotting when using this technique, mix with the reagent immediately. For cord blood tests, red blood cells should be previously washed with saline solution.

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

c) Known Interfering Substances: samples with hemolysis or microbial contamination should not be tested.

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 (2-10ºC). In case heparin or EDTA is used for collection, typification should be performed within 48 hours from collection. 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 from collection. The blood from donors may be tested until its expiration date.

REQUIRED MATERIAL (non-provided)

- Centrifuge
- Hemolysis tubes
- Slides
- Disposable mixing rods

WARNINGS

Reagents are 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 tab 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.

PROCEDURE

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

In the following techniques the red blood cells suspension to be tested should be prepared with saline, PBS or LISS.

I- SLIDE TECHNIQUE

Prepare a red blood cells suspension at 10%. A suspension at 35-45%, in plasma of the same patient or whole blood, may be used as an alternative.

1) Add 1 drop of red blood cells suspension to 1 drop of Reagent Anti-A, Anti-B or Anti-A,B monoclonal placed on a clean and labeled slide. The provided dropper dispenses a 50 $\pm$ 5 ul volume. The reagent:cell ratio should be maintained for all assay systems.

2) Mix the Reagent and blood cells with a disposable rod covering a circular area of 2 cm diameter and continually balance the slide for 2 minutes.

Observe the macroscopically visible agglutination up to 2 minutes. The observation may be simplified if a diffuse light source is used. Microscope should not be used.

The test should be interpreted within 2 minutes to avoid reagent drying due to evaporation. When weak agglutination is observed, the test should be repeated using the tube technique (by centrifugation). Two reagent volumes could be added to 1 sample volume to stress agglutination, not having the risk of obtaining false positive results.

II- TUBE TECHNIQUE (by centrifugation)

1) Add 1 drop of red blood cells suspension at 3-5% to 1 drop of Reagent Anti-A, Anti-B or Anti-A,B monoclonal placed on a hemolysis tube.

2) Mix and centrifuge for 20 seconds at 1,000 g.

3) Stir the tube to detach the cells and examine macroscopically the presence or absence of agglutination. The observation may be simplified if a diffuse light source is used.

III- TUBE TECHNIQUE (by sedimentation)

1) Add 1 drop of red blood cells suspension at 3-5% to 1 drop of Reagent Anti-A, Anti-B or Anti-A,B monoclonal placed on a hemolysis tube.

2) Mix and incubate for 60 minutes at room temperature.

3) Stir the tube to detach the cells and examine macroscopically the agglutination. The observation may be simplified if a diffuse light source is used. Read the tube tests immediately and interpret the results without delays.

INTERPRETATION OF RESULTS

Slide technique

Positive reaction: the erythrocytes agglutinate within seconds and stay agglutinated when the plate is balanced. It indicates the presence of the corresponding erythrocyte antigen.

Negative reaction: if no agglutination is observed after 2 minutes, it indicates the absence of the corresponding antigen.

Tube technique

Reading: gently tap the tube to detach the sediment and ma-croscopically examine the presence or absence of aggluti-nation.

Negative reaction: if no agglutination is observed after 2 minutes, it indicates the absence of the corresponding antigen. The red blood cell resuspension is homogeneous.

Positive reaction: the erythrocytes agglutinate within seconds and stay agglutinated after resuspension. It indicates the presence of the corresponding erythrocyte antigen.

In case of doubt, it is recommended to wait for 2 minutes.

The obtained results in the red blood cell tests (direct), except the ones using newborn blood samples, should be confirmed by a serum test (reverse), using A_1, A_2, B and O typified ery-throcytes. The expected profiles are shown in the following table:

Direct test (Red blood cells)			Reverse test (Serum)				Group
Anti-A	Anti-B	Anti-AB	Cell A_1	Cell A_2	Cell B	Cell O	
-	-	-	+	+	+	-	O
+	-	+	-	-	+	-	A
-	+	+	+	+	-	-	B
+	+	+	-	-	-	-	AB

Any discrepancy between the direct and reverse tests should be investigated and determined before reporting the blood group.

QUALITY CONTROL METHOD

The quality control method should be performed assaying typified red blood cells. The reagent quality control process is essential and should be performed at the begining of each work day with A_1, A_2, B and O red blood cells.

PROCEDURE LIMITATIONS

Erroneous results may be obserced in the following conditions:

- Test material contamination.
- Incorrect incubation time.
- Recommended incubation time reduction.
- Newborns: the A and B antigens are not fully expressed

in the newborn. Therefore, it should be handled with care, mostly on premature babies.
- Anti-A and Anti-B reactivity may be reduced and eventually disappear from serum or plasma of very young patients, elder adults or immunosuppressed individuals.
- Leukemia or other harmful disorders.
- Recently transfused patients with important quantities of non-identical blood group may show a mixed field serological reaction.
- False positive reactions: some problems were observed caused by the typification of the ABO blood groups due to antibodies that react with drugs, colorants, chemical compounds or with colloidal silica particles released by bad-quality glasses.
- Cold agglutinins: in the presence of cold agglutinins, the red blood cells washing step performed 4-6 times with saline solution, is generally enough to obtain appropriate cells for typification. A heating process at 37°C for 10 minutes before the above mentioned washing is rarely required. A drop of these cells with 2 drops of saline solution is placed as control. Agglutination should not be produced.
- Slide tests are not recommended for weak subgroup determination.
- If the tube technique is used, excessive agitation may disintegrate weak agglutinations and produce false negative results.
- It is important to use the recommended g force during cen-trifugation, since an excessive centrifugation may produce difficulties for cell button resuspension and an insufficient centrifugation may cause easily disintegrating agglutinations.
- The intensity of some erythrocyte antigen expression may decrease during storage, especially in clotted samples or samples collected with EDTA. Optimal results are obtained with fresh samples.

WIENER LAB. PROVIDES

Anti-A: vial x 10 ml (Cat. Nr. 1443152).
Anti-B: vial x 10 ml (Cat. Nr. 1443154).
Anti-AB: vial x 10 ml (Cat. Nr. 1443153).

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Symbols

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

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

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — 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. Nº: 1076/89 (Anti-A)
Disp. Nº: 1073/89 (Anti-B)
Disp. Nº: 1071/89 (Anti-A,B)

Wiener lab.

2000 Rosario - Argentina

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