BLOOD GROUPING
ANTI A, ANTI B, ANTI A+B MONOCLONAL
Tube, Slide, Diamed-ID and Microplate tests

IVD

Qualitative test for determination of A and/or B antigens on human red blood cells.

PACKAGING

Anti A	**Ref. 101-0518**	**Cont.: 10 mL**
Anti B	**Ref. 101-0519**	**Cont.: 10 mL**
Anti A+B	**Ref. 101-0520**	**Cont.: 10 mL**

Store at 2-8ºC

CLINICAL SIGNIFICANCE

In 1900, Landsteiner discovered the serum of some people would agglutinate the red cells of others. Four common phenotypes are now recognised: O, A, B and AB. Subgroups of A and B have since been identified.

Forward Group			Reverse Group				ABO Phenotype	Caucasians %
A	B	A,B	A_1	A_2	B	O		
+	0	+	0	0	+	0	**A**	42
0	+	+	+	+	0	0	**B**	10
0	0	0	+	+	+	0	**O**	44
+	+	+	0	0	0	0	**AB**	4

PRINCIPLE

The reagents will cause direct agglutination (clumping) of test red cells that carry the corresponding ABO antigen. No agglutination generally indicates the absence of the corresponding ABO antigen (see **Limitations**).

REAGENTS

Monoclonal IgM ABO blood grouping reagents contain mouse monoclonal antibodies diluted in a phosphate buffer containing sodium chloride, EDTA and bovine albumin. Each reagent is supplied at optimal dilution for use with all the recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see **Vial Label**.

Product	Cell Line/Clone	Colour	Dye Used
Anti-A	9113D10	Blue	Patent Blue
Anti-B	9621A8	Yellow	Tartrazine
Anti-A,B	152D12 + 9113D10	Colourless	None

STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8º C on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity.

REAGENTS AND MATERIALS REQUIRED

1. Glass test tubes (10 x 75 mm or 12 x 75 mm).
2. Test tube centrifuge.
3. Volumetric pipettes.
4. Glass microscope slides.
5. Applicator sticks.
6. Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22ºC ± 1ºC.
7. Positive (ideally group A_2B) and negative (group O) control red cells.
8. DiaMed ID-Cards (Neutral).
9. DiaMed ID-Centrifuge.
10. DiaMed ID-Diluent: e.g. ID-CellStab.
11. Plate shaker.
12. Automatic plate reader.
13. Validated "U" well microplates.
14. Microplate centrifuge.

SAMPLE

Blood samples drawn with or without anticoagulant may be used for antigen typing. If testing is delayed then store specimens at 2-8º C. EDTA and citrate samples should be typed within 48 hours. Samples collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS before being tested. Blood samples showing evidence of lysis may give unreliable results.

PRECAUTIONS

1. The reagents are intended for *in vitro* diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagents past the expiration date (see **Vial Label**).
4. Do not use the reagents if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. The reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. The reagents contain < 0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
8. No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.
9. For information on disposal of the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

NOTES

1. It is recommended a positive control and a negative control be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. When typing red cells from a patient it is important that a reagent negative control is included since the macromolecular potentiators in the reagent may cause false positive reactions with IgG coated cells.
3. Blood specimens of weak A or B subgroups (e.g Ax) may give rise to false negative or weak reactions when tested using slides, microtitre plates or gel cards. It is advisable to re-test weak subgroups using the tube technique.
4. Individuals older than six months should have their ABO blood-grouping results confirmed by testing their serum or plasma against known group A_1 and B cells before their ABO blood group can be confirmed.
5. In the procedures here detailed one volume is approximately 40µl when using the vial dropper provided.
6. The use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
7. The user must determine the suitability of the reagents for use in other techniques.

PROCEDURE

A. Tube Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume of Anti-ABO reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at room temperature for 1 minute.
4. Centrifuge all tubes for 10 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination
6. Any tubes, which show a negative or questionable result, should be incubated for 15 minutes at room temperature.
7. Following incubation, repeat steps 4 and 5.

B. Slide Technique

1. Prepare a 35-45 % suspension of test red cells in serum, plasma or PBS.
2. Place on a labelled glass slide: 1 volume of Anti-ABO reagent and 1 volume of test red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2-minute period, maintaining slide at room temperature.
5. Read macroscopically after 2 minutes over a diffuse light and do not mistake fibrin strands as agglutination.
6. Any weak reactions should be repeated by the tube technique.

C. DiaMed-ID Micro Typing Technique

1. Prepare a 0.8% suspension of washed test red cells in an ID-Diluent.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50µl of test red cell suspension and 25µl of Anti-ABO reagent.
4. Centrifuge the ID-Card(s) for 10 minutes at 90 rcf or for a suitable alternative time and force.
5. Read macroscopically for agglutination.

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D. Microplate Technique, using "U" wells

1. Prepare a 2-3 % suspension of washed test red cells in PBS.
2. Place in the appropriate well: 1 volume Anti-ABO reagent and 1 volume test red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination.
4. Incubate at room temperature for 15 minutes (time dependant on user).
5. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
6. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker
7. Read macroscopically or with a validated automatic reader.
8. Any weak reactions should be repeated by the tube technique.

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the appropriate ABO antigen on the test red cells.
2. **Negative:** No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of the appropriate ABO antigen on the test red cells.
3. **Discrepancies:** If the results obtained with reverse group don't correlate with forward group, further investigation is required.
4. Test results of cells that are agglutinated using the reagent negative control shall be excluded, as the agglutination is most probably caused by the effect of the macromolecular potentiators in the reagent on sensitised cells.

Stability of the reactions

1. Read all tube and microplate tests straight after centrifugation.
2. Slide tests should be interpreted within two minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
3. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. When using Monoclonal Anti-A,B, blood specimens of weak A or B subgroups (e.g Ax) may give rise to false negative or weak reactions when tested using slides, microtitre plates or gel cards. It is advisable to re-test weak subgroups using the tube technique.
3. Monoclonal Anti-A and monoclonal Anti-B are not validated to detect Ax and A3 or Bx and B3 antigens resp and we therefore do not claim reactivity of the monoclonal Anti-A or Anti-B reagent against these weak A and B sub-groups.
4. Stored blood may give weaker reactions than fresh blood.
5. False positive or false negative results may also occur due to:

- Contamination of test materials
- Improper storage, cell concentration, incubation time or temperature
- Improper or excessive centrifugation
- Deviation from the recommended techniques.
- Cord samples contaminated with Wharton's jelly

6. The user is responsible for the performance of the reagents by any method other than those here mentioned.
7. Any deviations from the techniques here recommended should be validated prior to use[9].

PERFORMANCE CHARACTERISTICS

1. The reagents have been characterised by all the procedures here mentioned.
2. Prior to release, each lot of Chronolab Monoclonal Anti-A, Anti-B and Anti-A,B is tested by the techniques here recommended against a panel of antigen-positive red cells to ensure suitable reactivity.
3. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
4. Chronolab Anti-B does not react with "Acquired-B" red cells.
5. Chronolab Monoclonal ABO reagents do not detect crypt antigens such as T, Tn or Cad.
6. The potency of the reagents has been tested against the following minimum potency reference standards obtained from National Institute of Biological Standards and Controls (NIBSC):
   - Anti-A reference standard 88/722 And / Or
   - Anti-B reference standard 88/724
7. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS prior to use.
8. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

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