MONOCLONAL ANTI–A (ABO/010), ANTI B (ABO/020) AND ANTI A,B (ABO/030) BLOOD GROUPING REAGENTS
For use by slide and tube methods

INTRODUCTION
In 1900, Landsteiner discovered that the serum of some individuals would agglutinate the red blood cells of others and that this phenomenon could be used to classify individuals into different blood group phenotypes. Four common phenotypes are recognised – O, A, B and AB. Subgroups of the A and B antigens have been identified.

The ABO phenotype for an individual is usually determined by the agglutination reactions of that individual's red cells with Anti–A, Anti–B and Anti–A,B antiserum (forward grouping). In testing blood samples from adults, confirmation of the ABO blood group can be provided by the reactions of the individual's serum with standard A and B red blood cell suspensions (reverse grouping).

WARNING AND PRECAUTIONS
Monoclonal antibodies exhibit a high degree of potency, avidity and specificity. When using such antibodies great care should be taken to avoid cross contamination.
Appropriate care should be taken in the disposal of this product.
Sodium Azide can be toxic if ingested and can react with lead and copper plumbing to form explosive compounds.
This reagent is for *in Vitro* use only.
The reagent should be stored at 2-8^{o}C, should not be used if turbid or if a precipitate is visible and should not be used beyond the stated expiry date.
UNDER NO CIRCUMSTANCES SHOULD THE PRODUCT BE DILUTED PRIOR TO USE.

PRINCIPLE
The test procedures recommended for this reagent are based upon the agglutination (clumping) of red blood cells which carry a specific antigen in the presence of a corresponding specific antibody.
These blood grouping reagents contain mouse monoclonal IgM antibodies. When used by the recommended techniques, these reagents will cause direct agglutination of red cells carrying the specific antigen.
The reagent has been optimised for use as supplied without further dilution or additions and by the recommended techniques.
Store at 2–8°C. Do not freeze. Prolonged storage at higher temperatures may result in accelerated loss of reagent activity.

ADVICE TO USERS
The results of red blood cells grouping should be confirmed by grouping the individual's serum with known A, and B red blood cells.
It is recommended that a positive control (ideally group A_1B cells) and a negative control (group A_1 cells) should be tested in parallel with each batch of tests.
The reagent has been characterised by the procedures recommended in this package insert and it's suitability for use in other techniques must be determined by the user.

SAMPLE COLLECTION
Blood samples should be withdrawn aseptically with or without the addition of anticoagulants. If testing the blood samples is delayed storage should be at 2–8°C.
Samples collected into EDTA or Heparin should be typed within 48 hours of withdrawal. Clotted samples may be tested up to fourteen days from the date of withdrawal. Samples collected into ACD, CPD OR CPDA–1 may be typed up to 35 days from the date of withdrawal.

RECOMMENDED TECHNIQUES
1. Slide Techniques

1.1 A 30–45% suspension of red blood cells in group homologous serum/plasma or PBS pH 7.0 may be used.

1.2 To one volume of reagent on a clean, labelled, glass slide add an equal volume of the test red cell suspension.

1.3 Mix the reagent and cells over an area about 2cm in diameter by gently and continually rocking the slide for 30 seconds. Incubate the test for 5 minutes at room temperature with occasional mixing.

1.4 Observe macroscopically for agglutination. This may be facilitated by use of a diffused light source.

1.5 Any weak of equivocal results should be repeated by tube technique with centrifugation. Alternatively repeat using two volumes of reagent to one volume of cells, which will often enhance weak reactions without risk of false positives.

2. Tube Technique – Immediate spin

2.1 To one volume of reagent in a labelled test tube, add an equal volume of a 2–3% suspension of test red blood cells in PBS at pH 7.0, or 1.5-2% in LISS.
2.2 Mix the test well.
2.3 Centrifuge at 1000g for 10 seconds or for a suitable alternative force and time.
2.4 Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.

3. Tube Technique – LISS

3.1 To one volume of reagent in a labelled test tube add an equal volume of a 1.5-2% suspension of test red blood cells in LISS.
3.2 Mix well and incubate at 18°–23°C for 15-20 minutes.
3.3 Centrifuge at 1000g for 10 seconds or for a suitable alternative force and time
3.4 Gently agitate the tube to dislodge the cells and examine macroscopically for agglutination.

QUALITY CONTROL

Quality control of reagents is essential and should be performed at the start of each days testing, with each series of groups performed and with single tests, e.g. an emergency compatibility test i.e.

Anti A)
Anti B) Should be tested with A_1, A_2, B and O cells.
Anti A,B)

GENERAL

1. Monoclonal ABO Blood Grouping Reagents will not detect crypt-antigens such as T, Tn or Cad.
2. Sub–optimal reactions may be obtained if the recommended incubation times are reduced.
3. Reactions with B sub-groups may be reduced in the tile technique.
3. Centrifugation. Please note that the recommended centrifugation conditions are stated as g. Please refer to your centrifuge manufacturer's instructions for equivalent rpm settings.
4. Quality Control of this reagent has been performed using WASHED cells.
5. The use of group AB serum as a negative control for purely monoclonal reagents is inappropriate. If desired a Monoclonal Control Reagent for this purpose is available.
6. All blood grouping reagents should be treated as potentially infectious. No known test methods can offer complete assurance that the reagents of human or animal origin are free from infectious agents. Appropriate care must be taken in the use and disposal of the container and its contents.
7. DO NOT FREEZE: Store at +2°C to +8°C. Storage at temperatures outside this range may result in an acceleration of the rate of loss of reactivity of the reagent.
8. Use droppers provided and protect from contamination. The reagent must be used as supplied, without dilution or addition.

TECHNICAL NOTES

The acquired B phenotype is seen very occasionally in group A patients and is caused by de-acetylation of the A antigen by bacterial enzymes, particularly those associated with intestinal infections. The Anti B reagent is derived from the cell line **LB2** does not recognise this “pseudo” antigen.

ABO antigens are not fully expressed at birth and, therefore, tests involving cord/neonatal red cells should be interpreted with particular care.

Slide tests are not recommended for detection of weak sub groups. All slide tests should be confirmed using tube tests.

Tests should be carried out using “tip and roll” procedure. Excessive agitation may disrupt weak agglutination and lead to false negative results.

The expression of certain red cell antigens may diminish in strength during storage, particularly in EDTA and clotted samples. Better results will be obtained from fresh samples.

REFERENCES

1. Widmann F.K. ed Technical Manual 10th Ed Washington DC, American Association of Blood Banks 1990, Chapter 11.
2. Race R. R and Sanger R. Blood Groups in Man, 6th Edition Oxford Blackwell Scientific Publishers 1975:178.
3. Issitt P.D. Applied Blood Group Serology 3rd Edition, Montgomery Scientific Publications, Miami, Florida, USA, 1985, Chapter 10.

PAB.V2
25/3/03

FOR *IN VITRO* DIAGNOSTIC USE ONLY