

Technical Monograph No. 2a**Problems associated with *ABO* Blood Grouping**

In the *ABO* system anti-A reacts (agglutinates) specifically with *A* substance on the red cell surface and anti-B agglutinates *B* red cells. A group *A* person has anti-B in their serum and a group *B* person has anti-A. Group *O* individuals have both anti-A and anti-B in their serum and a group *AB* person has neither anti-A nor anti-B. To obtain an accurate group certain criteria need to be observed. *In vitro* agglutination by these antibodies is optimal with cells suspended in isotonic saline at about 5°C since the strength of the reaction decreases as the temperature of testing is raised. For convenience, *ABO* testing is performed at room temperature (18-24°C). At lower temperatures non-specific cold agglutinins interfere with reverse grouping and at temperatures above 30°C, the activity of the reagents and the isoagglutinins are lessened.

This factor is particularly important in hot climates. A clotted sample of blood should be used for blood grouping. The blood sample must be allowed to completely clot. This can be difficult in coagulopathy cases but adding a few drops of thrombin can speed up the clotting process. After centrifugation of the sample, the serum can be removed, labelled and used for the reverse group. Free cells from the bottom of the tube are used for the cell group. For slide grouping and the tube method - immediate spin then a 30-45% cell suspension in autologous serum/plasma or PBS should be used. A 1.5-3% cell suspension is adequate for tube grouping. However, the instruction for use included in the product should always be followed.

The cell concentration for slide grouping should be sufficient so that individual cells can be discerned microscopically on the slide and the light source should not be obliterated by too concentrated a suspension. If the patient is anaemic the normal colour of the cell suspension will not be achieved. Nevertheless the normal number of cells should still be present. A normal haemoglobin range of 130-180g/l for males and 115-165g/l for females is not usually encountered when transfusions are required. Severe red cell depletion when less than 2.0×10^{12} cells/l are present should still not affect the concentration required for grouping since more cells can be added to achieve the correct concentration. It is particularly important to centrifuge and decant the saline from the suspension at least once. Fresh saline can then be added to achieve the correct concentration of cells for the technique being employed. This washing process will obviate extraneous material and reduce problems in microscopic interpretation. In emergency situations, a direct dilution in saline can be used but care must be taken that the difference between agglutination, clotting and rouleaux are clearly understood. In clotting strands of fibrinogen are clearly visible. Rouleaux can be seen in severe rheumatoid and jaundice cases where the cells are arranged in stacks like rolls of coins. The pH and conductivity of the isotonic saline also affect the potency of reaction. It is particularly important in warm climates to prevent bacterial contamination of any of the grouping components by storing them at 2-8°C.

It is only by following the recommendations of the reagent manufacturer that reliable blood grouping will be achieved. It is recommended that appropriate controls are used daily or with every batch of tests. From the pattern of results obtained with any individual's red cells and serum their blood group can be determined. Any discrepancy between the red cell group and the reverse group demands that further testing should be done to establish the actual group of an individual before any mistakes occur.

