

Mechanized blood grouping: a hospital trial using an 8-channel grouping machine

G. C. JENKINS, R. G. FEWELL, M. J. LLOYD, JEAN BROWN, J. JUDD¹, R. S. LANE¹, AND W. J. JENKINS¹

From the London Hospital, Whitechapel, London and the Regional Blood Transfusion Centre¹, Brentwood, Essex

SYNOPSIS ABO and Rh (D) groups of 6403 blood samples were assessed on an 8/9-channel auto-analyser in the Serology Department of the London Hospital; the results were independently checked at the Regional Blood Transfusion Centre, Brentwood, using the routine methods for grouping donor blood. Results of this comparative study are given and instances are described in which anomalous results or incorrect groupings occurred; the possible causes are discussed.

The 8/9-channel automated blood group analyser is evaluated in terms of routine hospital laboratory practice.

Samples of blood from a patient population present a far more varied and difficult challenge than those taken from a healthy *donor* population; therefore, conditions which apply within a hospital laboratory are quite different from those in a regional blood transfusion centre. Most centres in the United Kingdom have adopted automated blood grouping systems and a number of trials have led to improvements in the techniques. Technicon Instruments Ltd have designed an eight-channel blood grouping system which may be used in the transfusion departments of large hospitals.

This report is a comparative account of results obtained from approximately 6400 consecutive blood samples studied by automated techniques at The London Hospital and at the Regional Blood Transfusion Centre in Brentwood. Modifications to the operation of the grouping system introduced during the study are described; suggestions are made for further improvements for its use within a hospital transfusion laboratory.

Materials and Methods

AUTOMATED BLOOD GROUPING

Equipment

The Technicon blood grouping machine under assessment was similar to that described by Scott and Priest (1967). Red cells were treated with bro-

melin and their agglutination was further enhanced by the use of methyl cellulose (Marsh *et al*, 1968) and bovine albumin in 1.3% saline.

Cells were tested with anti-A, anti-B, anti-A + B, anti-D, and group AB 'inert' serum, while plasma was tested against group A, B, and O red cells: 120 specimens were sampled per hour with a wash sample ratio of 1:4.

Collection of Samples

The machine sampler held 81 13 mm tubes (Labco Ltd). Initially, specimens consisted of 4 ml whole blood mixed with 1 ml of acid-citrate-dextrose (ACD) solution but, after some 3000 samples had been tested, the anticoagulant was changed to 1 mg EDTA per ml of whole blood and the sample tray was modified by placing a Perspex disc on its underside, thus accommodating conventional 4 ml EDTA sample containers used in routine haematological departments. Avoiding separate sampling for blood grouping greatly facilitated blood collection and EDTA did not influence the results in any way.

Reagents

The Centre supplied routine antisera having the following titres in saline: anti-A > 1:120 against group A, red cells; anti-B > 1:64 against group B red cells; anti-A + B > 1:128 against group A₁ red cells and > 1:64 against group B red cells. The titres of anti-D sera were above 1:512 against albumin-suspended Rh(D) positive red cells.

Anti-A, anti-B, anti-A + B, and group AB inert

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Antiserum	Dye	Concentration of dye in neat serum	Colour
Anti-A	Cortical tartrazine S	0.13% (w/v)	Yellow
Anti-B	Cortical carmosine	0.08% (w/v)	Red
Anti-A + B	Brilliant blue FCF	0.08% (w/v)	Blue
Anti-D	Cortical tartrazine S and brilliant blue FCFO	0.75% (w/v) 0.75% (w/v)	Green

Table I Dyes and their concentrations used for identification of antisera

sera were diluted 1:10 in a solution containing 2 parts 1% methyl cellulose, 1 part 30% bovine albumin, and 24 parts 1.3% saline. Anti-D was diluted 1:12 in a solution containing 4 parts 1% methyl cellulose, 1 part 30% bovine albumin, and 17 parts 1.3% saline. It was found convenient to prepare 80 ml of each antiserum at a time since this was sufficient to test 300 specimens.

Each antiserum was identified in the reaction coils and subsequently on the filter paper by colouring the neat serum with a dye (see table I). Preliminary studies at the Centre together with results from tests on more than 100 000 donor samples had shown that dyes neither affect serological behaviour nor adversely affect storage of antisera at -25°C (table I).

Reagent red cells collected into ACD were obtained from the Centre. Groups A₁ Rh(D) negative, B Rh(D) positive, and O Rh(D) positive (R₁ R₂-CDe/cDE, Kell positive) red cells were prepared daily at a concentration of 5% in a solution containing 2 parts 1% methyl cellulose, 1 part 30% bovine albumin, and 26 parts 0.2% bromelin solution.

Bromelin solution was prepared daily by agitating 2 g of bromelin powder in 100 ml 1.3% saline for 15 min at room temperature. The solution was centrifuged and the supernatant was diluted in 1 litre with 1.3% saline. The addition of EDTA (dipotassium salt), 2 g/l, to both the bromelin and wash solutions stabilized the enzyme and retarded fibrin deposition within the machine.

CORRELATION OF RESULTS

The results of each line output were recorded by a technician at The London Hospital, who added his

No. tested	Correct Group	Group recorded by Machine			
		O	A	B	AB
2845	O	2844	—	—	1
2586	A	—	2584	—	2
699	B	—	—	699	—
273	AB	—	—	38	235

Table II Anomalous ABO cell groups

No. tested	Correct Group	Group recorded by Machine			
		O	A	B	AB
2845	O	2831	1	2	11
2586	A	—	2552	—	34
699	B	—	—	693	6
273	AB	—	3	7	263

Table III Anomalous ABO plasma groups

own interpretation of the blood groups. These results, together with an aliquot of each blood sample, were sent to the Centre for confirmation. At the Centre, the samples were tested by the routine methods used for all blood donor samples. Discrepancies between the laboratories were further investigated at the Centre by conventional manual techniques.

Results

ABO ANOMALIES

Of a total of 6403 samples, there were 41 anomalous ABO cell groupings and 64 anomalous plasma groupings (see tables II and III).

One group O cell sample typed as AB with anti-A, anti-B, and anti-A + B but gave a positive reaction with AB serum: further investigation revealed that the patient had auto-immune haemolytic anaemia (AIHA). Two group A cell samples typed as AB, one due to AIHA (AB serum control positive) the other due to possible misreading of the cell group since a weak result was recorded with anti-B. Thirty-eight group AB cell samples grouped as B in the cell group (negative with anti-A) and these were subsequently grouped as A₂B.

Fifty-one plasma groups were incorrectly grouped as AB, presumably due either to failure of the machine to sample correctly or to weak anti-A/anti-B in the patient's plasma. Ten group AB plasma samples gave anomalous results: seven grouped as B, attributed to the presence of anti-A; three grouped as A, due to misreading or to the presence of irregular antibodies.

Eight samples were grouped incorrectly by both cell and plasma tests. Five group A₂B samples with anti A₁ in the plasma were grouped as B. One group A sample was grouped as AB, giving an apparent weak result with anti-B in the cell group together with a negative result in the plasma against B cells. Subsequent re-examination of the results showed that no reaction was obtained with anti-B serum. Two group AB samples were grouped as A due to weak reactions with anti-B in the cell group and misreading in the plasma group.

No. tested	Correct Group	Group recorded by Machine	
		D positive	D negative
5356	D positive	5350	6
1016	D negative	6	1010
31	D ^u	3	28

Table IV Rh (D) grouping

RH(D)-GROUPING

Analysis of the Rh(D)-grouping results is shown in table IV. Of 6403 samples, 1010 were grouped correctly as D-negative and 5350 correctly as D-positive. Of 31 samples later identified as D^u, 28 grouped as D-negative and 3 grouped D-positive; 6 D-positive samples were also grouped as D-negative.

Six D-negative samples were grouped as D-positive; one of these was from a case of AIHA (AB serum control positive), and subsequent examination of the other five results showed them to be D-negative; the results had been either read or transcribed incorrectly.

Discussion

The purpose of this study was to examine the performance and test the reliability of the Technicon eight-channel analyser in hospital blood bank practice. Results showed that particular attention must be given to the choice of reagents used in the instrument, to the reading, transcription, and interpretation of reactions in order to achieve the high degree of accuracy demanded of blood grouping procedures.

Of all ABO groups, 1.6% resulted in easily recognizable anomalies between cell and plasma tests requiring further investigation by manual techniques. This is not a great inconvenience, but it could cause a dangerous delay in the supply of compatible blood for urgent transfusion. A more serious problem arose when wrong ABO groups were not associated with evident anomalies, for example, eight group A₂B patients, of whom five had anti-A₁ in their plasma and thus were incorrectly grouped. In this situation, if a nine-channel analyser is used, group A₂ cells may be included, thus aiding the interpretation of results.

While it is disappointing that 90% of D^u samples were incorrectly grouped as D-negative and that six D-positive samples were wrongly grouped as D-negative, it is extremely serious to note that five D-negative samples were grouped as D-positive in spite of the correct behaviour of the machine. These five samples were incorrectly read on the filter paper, underlining the need for rigid control of the interpretation and transcription of results. Loading of the sample tray should be checked by an independent worker before the testing of samples, and reactions on the filter paper should be read by the instrument operator *and* by an independent worker. The protocols and their interpretation should then be checked against the filter paper.

The reagents supplied by the Centre were the same as those used regularly in routine grouping of blood donor samples in a 15-channel analyser. The anti-A, anti-B, and high-titre group O sera were obtained from unimmunized donors but selected on the basis of their avidity and titre against appropriate cells. Within the transfusion service, when selecting donors, it is known that, using such reagents, reactions with weak group A and AB sub-types are often below the sensitivity of machines, and precautions include the grouping of all apparent group B samples by a more sensitive manual technique. It is suggested that similar precautions should be taken in hospital laboratories.

The authors are conscious of the danger of contamination of the filter paper by hepatitis B antigen. Technicians handling the paper should be aware of this hazard and should use plastic gloves, finally disposing of the paper by incineration.

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References

- Marsh, W. L., Nichols, M., and Jenkins, W. J. (1968). Automated detection of blood group antibodies. *J. med. Lab. Technol.*, **25**, 335-342.
- Scott, G. L. and Priest, C. J. (1967). Experiences with automatic equipment for routine blood-group serology. *Brit. J. Haemat.*, **13**, Suppl., 40-49.