

Automated blood group serology

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Although automation was introduced into biochemistry in 1957 (Skeggs, 1957), it was not until 1963 that McNeil, Helrick, and Ferrari (1963) first described a mechanized technique for ABO grouping. This was followed a few months later by a method employing the AutoAnalyzer continuous flow principle by which up to 40 samples per hour could be both ABO and Rh (D) grouped (Sturgeon, Cedergren, and McQuiston, 1963).

During the succeeding six years developments have been such that blood samples can now be ABO grouped by machine, Rh typed, screened for irregular blood group antibodies, and some of the subtypes determined at a rate of one sample per 30 seconds, or 120 samples per hour. This is accomplished by a multichannel machine. There is also available a single-channel AutoAnalyzer for use in screening and quantitation.

THE MULTICHANNEL MACHINE

The principle of the machine is both simple and basic. Antigen, on the red cells, and antibody, in the plasma or serum, are brought together by dipping probes (Fig. 1) and allowed to interact in a mixing coil. In a manual technique the red cells and the test serum are mixed for 60 minutes at room temperature before being examined for agglutination. The automated procedure devised by Rosenfield and Haber (1965) uses bromelin to induce red cell agglutination and polyvinylpyrrolidone (PVP) to accelerate red cell contact by rouleaux formation, thus effecting agglutination within three to five minutes. Recently, Marsh, Nichols, and Jenkins (1968) have substituted methyl cellulose 0.3% for PVP as a more sensitive rouleaux-inducing agent. Methyl cellulose is less expensive than PVP and has the additional advantage of reducing sample cross contamination. The latter is further minimized by the introduction of an air bubble after the reagents merge, so that each test sample is broken down into about six discrete segments before passage through the delay coil.

Each sample will spend about eight minutes in the coil and saline is then added to disperse the rouleaux.

True agglutinates remain intact and are decanted at a T-junction to be ejected on to a roll of absorbent paper. Plasma containing haemolysins agglutinates the test cells because of the anticomplementary nature of the citrate and the bromelin.

The multichannel machines available are equipped with nine or 15 channels and occupy about 10 feet of bench space. There must be free access to both sides and at one end for servicing and minor adjustment. The test samples are approximately 4 ml of blood in 1 ml of acid-citrate-dextrose anticoagulant, and must be free from clots to prevent blocking of probes or pump lines.

When first received in February 1967 our machine had nine channels. We have since added three additional lines. As a result each sample may be tested as follows.

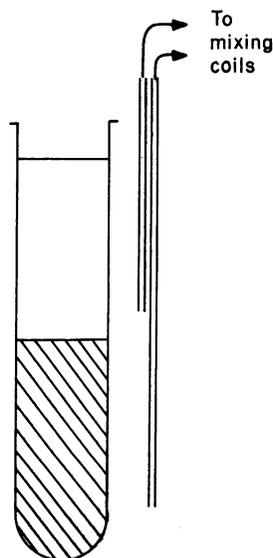


FIG. 1. One limb of the sampling probe dips into the donor plasma, the other into the red cells. The aspirated plasma and red cell samples are then passed to the mixing coils.

TEST PROCEDURE

RED CELLS After mixing in a coil with 0.2% bromelin the red cells are subdivided into seven channels to react with anti-A, anti-A₁, anti-B, anti-A + B (group O serum), anti-D, anti-C + D + E, and a spare (at 37° or 12°C).

PLASMA Each plasma sample is subdivided into five channels to react with the following bromelin-treated test cells: group A₁, group A₂, group B, group O (pooled R₁R₁, R₂R₂, and R₁R₂), and group A₁B (to detect 'dangerous universal donors').

ANTISERA The sensitivity of the system is such that anti-A and anti-B sera normally used for manual grouping can be used diluted 1:8, and only 15 ml of each is required for 500 groupings. Anti-D sera too weak for manual grouping are similarly satisfactory. The enhanced sensitivity has enabled us to replace human anti-A by an anti-A reagent prepared from the albumin gland of snails or snail eggs (Tovey and Lockyer, 1968); the anti-A₁ is obtained from *Dolichos biflorus*.

READING The deposited cell-serum mixtures are read from the paper and the sample numbers and results are marked against each, which then serves as a permanent record. The number of tests read per day by one individual is limited to 300 or 400 in order to avoid errors due to fatigue.

CLEANING After use the apparatus is washed through with Pyroneg at 70°C and the tubing is left filled with this solution overnight. Before use it is washed through with saline to which is added a few drops of Tween 20. Once a month the tubing is cleansed with a dilute solution of chromic acid followed by Tween saline.

MAINTENANCE Pump tubing has to be replaced two or three times a year (approximate cost £15). Over a period of 22 months, during which the machine has been in daily use, there has been only one major breakdown. This resulted from a fault developing in a micro-switch in the sampling unit and the machine was out of action for about 24 hours until this was detected and replaced.

STAFF One experienced technician (part-time) and one junior technician can operate the apparatus at a rate of 600 samples in eight hours, including the preparation of reagents, recording of results, cleaning, and maintenance. Six to eight technicians would be needed to test the same number of samples manually. Not only does this result in a financial saving sufficient to offset the cost of the machine within three to four years, but the offloading of dull, repetitive work is of immense importance to the technical staff.

RESULTS

The use of the apparatus may be divided into the three following phases.

1 During the first three months 10,854 random donor samples were grouped by machine and by hand. No discrepancies were discovered.

2 Grouping by machine was limited to samples from donors whose ABO and Rh group had been determined at the time of a previous donation. This enabled confirmatory manual grouping to be discontinued, since the results could be checked against the previous manual grouping. During this period it was realized that knowledge of the previous grouping might bias the reading of the results. It was therefore decided to test samples from new donors, whose groups were not known, manually and by the machine. In testing 2,013 such samples, 11 discrepancies occurred and are summarized in Table I.

TABLE I
DISCREPANT GROUPINGS DURING COMPARATIVE TESTING
OF 2,013 SAMPLES

	ABO Group	Rh Group	Irregular Antibodies
Machine correct	3	1	5
Manual correct	0	0	2

In the three instances of discrepant ABO grouping, the manual grouping had missed weak anti-A and anti-B agglutinins.

One donor sample was recorded after manual grouping as Rh (D) positive, but by machine had not been agglutinated by either anti-D or anti-C + D + E. Further investigation confirmed that the sample was Rh negative.

In the five instances when irregular agglutinins were detected by the machine but not manually, repeat manual grouping confirmed the presence of weak 'enzyme-only' agglutinins in the plasma. In the two instances in which the manual method is shown as correct, weak irregular agglutinins were detected against the group O enzyme-treated cells in the machine, but repeated manual grouping failed to detect the antibody. It was assumed that these machine results were false positives; it is possible, however, that the results given by the machine may have been the correct ones.

By the end of this phase, 31,585 donor samples had been tested by machine grouping; 12,867 had been grouped in parallel by the manual technique, and in 17,769 the result obtained was checked against the group recorded after manual grouping at a previous blood donation. About 8% of the samples had to be regrouped on the machine, because of clots in the blood or plasma lines, and in 949 instances (approximately 3%) a check had to be made by manual grouping because of weak or missing

agglutinins, agglutination of group O test cells, or failure of the sampling probes to pick up cells or plasma. Since satisfactory ABO and Rh results as well as antibody screening had been obtained by machine in more than 30,000 samples tested, comparative manual grouping was discontinued from this time.

3 During the 15 months of this phase a further 134,214 samples have been grouped by AutoAnalyzer; 116,973 of these were from donors previously grouped and represent therefore a considerable control series. No discrepant ABO results were observed, but one sample recorded as Rh negative was found during manual check grouping, when the donation was being tested for compatibility, to be Rh positive. Reference back to the recorder roll showed that in error an adjacent sample was retested when a 'doubtful positive' result was obtained at the original grouping. This was therefore a human error and not a fault of the machine grouping. It demonstrates the ever-present need for care and the need to adhere to a strict regime for checking; even when a technically accurate mechanized procedure is employed, the system is only as reliable as its weakest link.

To sum up, in some 165,000 samples, allowing for the fact that 3% were manually grouped for the reasons given above, the ABO and Rh groups were correctly determined by machine. One error involving the Rh group was due to the wrong sample having been tested at a repeat grouping.

SUBGROUPS OF A Testing the donor's plasma against A_1 and A_2 cells eliminates the risk of misgrouping A_3 or A_x samples as O. Usually such donor's red cells will be weakly agglutinated by the anti-A of the group O serum, or the result will appear on the recorder roll as ' O_{β} ' and the sample will be selected for further investigation. Plasma from the occasional sample which contains an irregular anti- A_1 agglutinin, and which might be misinterpreted as ' $O_{\alpha\beta}$ ', will agglutinate only the A_1 cells and, again, investigation by the manual technique will be indicated.

RH GROUPING To conserve high titre anti-D serum, antisera too weak for a manual technique are used. All samples found to be Rh (D) negative are then retested in the machine against a more potent anti-D. Approximately 0.2% were shown to be Rh positive by the second test. With experience in reading, D^u can be differentiated from D-positive or D-negative by variation in the intensity of the reaction recorded on the paper.

IRREGULAR ANTIBODIES The machine has proved

highly sensitive in detecting Rh antibodies, anti-Lewis, and anti- P_1 . Initially an occasional anti-Kell and most anti-Fy^a were not detected; since replacing PVP by methyl cellulose, however, these are now detected. Due to the denaturation effect of bromelin on the antigens, anti-M and anti-N fail to react.

DEVELOPMENTAL WORK

HEN EGG ALBUMEN Suspension of the test cells, A_1 , A_2 etc, in a solution containing bovine albumin was recommended by Rosenfield and Haber (1965) both to maintain stability during the machine run and to act as an additional rouleaux-inducing agent. Initially we used up to 150 ml of 20% bovine albumin per day for this purpose, but now dilute this successfully with two parts of hen egg albumen to effect a significant saving in cost.

SCREENING FOR COMPATIBLE BLOODS A water jacket fitted round one of the delay coils has enabled the temperature of the coil to be maintained at any selected temperature between 0°C and 37°C. This line has been used to screen donor bloods at 12°C for a patient whose serum contained anti- P_1 , and at 37°C for other patients whose sera contained anti-c or anti-E.

HIGH TITRE GROUP O DONORS Methods investigated for screening these donors have included testing the plasma, diluted within the machine to 1 in 50, against A_1B enzyme-treated cells, and partially neutralizing the plasma with group AB specific substance. Neither procedure has proved sufficiently absolute to be recommended for routine use.

We have previously established the reliability of an A^p haemolysin test for the manual detection of high titre group O donors (Lockyer and Tovey, 1960). Currently therefore we are attempting to automate this technique by adding an excess of guinea-pig complement to each donor plasma before adding enzyme-treated A^p cells. Because of the anti-species agglutinins, agglutination occurs in all samples with the exception of those containing significant A^p haemolysins. These haemolyse the A^p cells and show themselves on the recorder roll as a negative (no agglutination) reaction. It is too early yet to state whether this technique will prove consistently reliable.

THE SINGLE CHANNEL MACHINE

The value of the single-channel AutoAnalyzer in the automated detection of blood group antibodies (Marsh *et al*, 1968), Rh antibody titration (Tovey and Lockyer, 1968), and quantitation in terms of

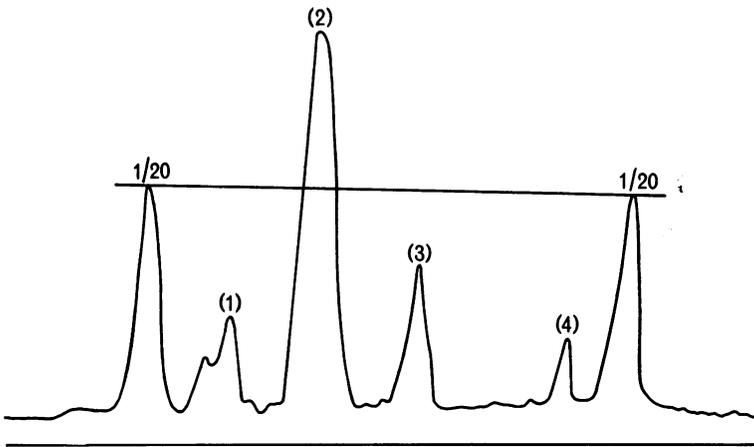


FIG. 2. Recorder graph tracing for automated antibody titration. A line is drawn between the peaks of the control tests (1/20 dilution). Sera (1), (3), and (4), from Rh-negative expectant mothers, contain anti-D, but at a titre lower than 1/20. Serum (2) contains anti-D at a titre greater than 1/20; this mother will be advised to have amniotic fluid examination.

micrograms of antibody nitrogen per millimetre (Rosenfield *et al*, 1968) has been described in detail elsewhere. Only a brief general summary will therefore be given here.

The principle of the machine is essentially that of the multichannel AutoAnalyzer, except that following interaction of cells and serum in a delay coil and removal of the agglutinates at T-junction decanters, the residual red blood cells are lysed and the haemoglobin level is measured colorimetrically and recorded.

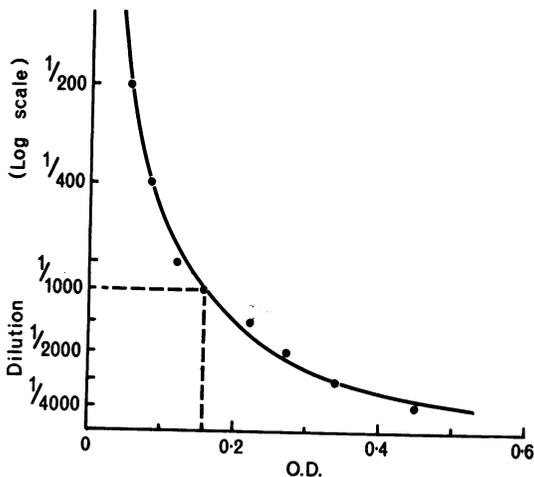


FIG. 3. Curve prepared after determining optical density (O.D.) of a standard serum containing 10 µg of Rh antibody nitrogen/ml. Tested at a dilution of 1/100, the 'unknown' serum shows an optical density of 0.16. Reference to the standard curve shows that this corresponds to a 1/1,000 dilution of the standard serum. The unknown serum contains therefore 1/10th the antibody nitrogen of the standard, that is, 1.0 µg/ml.

The greater the number of red cells removed by agglutination the fewer will remain for haemolysis, and the magnitude of the light transmission in the colorimeter will thus be directly proportional to the strength of the original antigen-antibody reaction.

It is now generally agreed that examination of amniotic fluid is not indicated in a first Rh incompatible pregnancy unless the Rh antibody titre reaches a certain critical level (Tovey, 1966; Fairweather, Tacchi, Coxon, Hughes, Murray, and Walker, 1967). Using control sera at this titre Tovey and Lockyer (1968) have automated the procedure for selecting cases for amniocentesis (Fig. 2). Correlation with manual anti-globulin and enzyme techniques is good, and, with a sampling rate of 60 per hour, a considerable amount of technicians' time is saved.

By reference to a curve prepared from analysis of an accurately diluted standard (Fig. 3), the Rh antibody content of a serum may be determined in terms of antibody nitrogen. It is too soon to say whether this technique will advance the more accurate prediction of severity in Rh haemolytic disease, but intercontinental exchanges of test sera have shown that an encouraging consistency of quantitation can be obtained (Moore, 1969).

Automated antibody screening has been considerably advanced by the thoroughness which Marsh and his colleagues (1968) have applied to this problem. Suspending the reagent red cells in albumin and AB serum, replacing PVP by methyl cellulose, and incubation in one cell at 37°C followed by another at 4°C, allows both warm and cold antibodies to be detected. The remaining, but critical, problem is maintaining an adequate supply of fresh red cells, containing all appropriate antigens.

For this reason, the procedure is as yet only suitable for a large reference laboratory. As Marsh *et al* conclude, 'the apparatus requires skill, patience and constant supervision for its successful operation. Like all complex equipment it is subject to occasional process failure'.

The same may be said of all the automated blood grouping procedures described in this paper, but the past six years have indeed laid a firm foundation for rapid progress in this field.

SUMMARY

The routine grouping of donor blood samples has been automated at the South West Regional Transfusion Centre since mid-1967. Details are given of the multichannel machine used, which has grouped correctly more than 165,000 samples during this time. Automation of blood grouping results in a saving of technician time and eliminates a considerable volume of dull repetitive work. Snail anti-A is used in the machine in place of human anti-A, and economies have been effected in the use of anti-D and bovine albumin.

Single-channel AutoAnalyzers are used for Rh antibody titration, quantitation in terms of micrograms of antibody nitrogen per millilitre, and the

screening of sera for irregular blood group antibodies.

Much of the detail concerning the multichannel machine has been taken from a report prepared for the Department of Health and Social Security to which I am indebted for permission to publish. I am grateful also to Mr W. J. Lockyer, BSc, principal scientific officer, for his assistance in the preparation of this paper, and to Mr Christopher Darke for the illustrations.

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