Technical methods

PVP-augmented LISP and DISP AutoAnalyzer haemagglutination channels

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A low-ionic strength-Polybrene (LISP) AutoAnalyzer haemagglutination channel (Lalezari, 1968; Perrault and Högman, 1971) fails to detect a proportion of antibodies, particularly anti-K1, anti-Lewis, and anti-P₁, which can be demonstrated by manual techniques (Moore and Perrault, 1977). Moore *et al.* (1977) found that when dialysis was used instead of dilution to achieve a low ionic-strength reaction environment (dialysed serum-Polybrene or DISP), a greater proportion of anti-K1 and anti-Lewis antibodies was detected.

We have found that the addition of PVP (K-90) to cell suspension medium and Polybrene solution improves the effectiveness of both LISP and DISP channels for antibody detection and identification.

Material and methods

Figures 1 and 2 are modified from Perrault and Högman (1971) and Moore *et al.* (1977). The sampling rate was 60/h with 2:1 sample:wash ratio for both detection and identification of antibodies.

REAGENTS

Red cell diluent

30% BSA	20 ml
AB serum	15 ml
PVP K-90, 50 g/l	8 ml
NaCl 9 g/l	35 ml
K ₂ EDTA·2H ₂ O 1.0 mol/l	2 ml
(For LISP or DISP, use 9 g/l NaCl in place of PVP)	

Cell suspension

Packed red cells	6 ml
Cell diluent	40 ml

<i>BSA-NaCl</i>	
30% BSA	17 ml
10% Tween 20	1 ml
NaCl 9 g/l	982 ml

<i>Citrate (0.2 mol/l)</i>	
Na ₃ citrate 1.0 mol/l	80 ml
10% Tween 20	0.4 ml
Purified water	320 ml

<i>Dialysis solution (DISP)</i>	
Mannitol 50 g/l	400 ml
Citric acid 1.0 mol/l	4 ml

<i>Acidified mannitol (LISP)</i>	
Mannitol 50 g/l	400 ml
Citric acid 1.0 mol/l	1 ml
Verify that pH is 3.2	

<i>Polybrene-PVP</i>	
Polybrene 100 g/l	0.4 ml
PVP K-90 50 g/l	20 ml
NaCl 9 g/l	180 ml

The exact concentration of Polybrene will depend upon the lot used; it may range from 0.08 to 0.8 ml. Polybrene must be stored in plastic bottles.

For LISP or DISP, use 9 g/l NaCl in place of PVP.

<i>Triton (0.5%)</i>	
Triton X-100 5% (v/v)	100 ml
Purified water	900 ml

Manifold

Carry-over between samples was reduced by elimination of the debubbling and resampling step before entry to the flow-cell (Walker, 1977; Walker *et al.*, 1970); this change also lowered the sampling to recording time to ~ 12 minutes. In the LISP channel, acidified mannitol, which is preferred to glucose, was segmented by air to obtain a smoother baseline.

K₂EDTA was added to the cell-suspension medium to reduce 'serum effect' and to smooth the baseline (Moore *et al.*, 1977). Addition of PVP to the cells resulted in slight aggregation but had no deleterious effect.

Results

A total of 90 selected sera with six antibody specificities were tested on LISP and DISP channels and

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Fig. 1 Schematic flow diagram for modified LISP channel.
CR/LA = Curve Regenerator cum Logarithmic Amplifier described by Crockford and Moore (1979).

with the addition of PVP (LIPP and DIPP). The results in Table 1 show that the LIPP and DIPP channels detected a greater proportion of the antibodies tested, and that the mean Δ OD was noticeably increased. A combination of DIPP and LIPP channels detected 88% of the antibodies tested, compared with 81% for the combination of DISP and LISP and 77% for LISP alone. Those antibodies not detected all had manual titres of 4 or <4. The proportion of weak anti-K1 detected was increased from 73% by LISP to 84% by DIPP; six out of eight anti-K1 with an IDAT titre of 1 were detected on DIPP.

Table 2 shows that addition of PVP increases the power of detection of LISP and DISP channels for the examples of the specificities shown. It also illustrates how sensitivity of these two channels differs with antibody specificity, and how one channel complements the other. The example of

Table 1 Comparison of four Auto Analyzer channels for detection of selected blood group antibodies

Antibody specificity	No. of examples tested	No. of examples detected (mean Δ OD)			
		LISP	LIPP*	DISP	DIPP†
Anti-Rh	12	12 (60)	12 (62)	12 (42)	12 (64)
Anti-K1	51	37 (28)	39 (39)	41 (45)	43 (68)
Anti-Jk ^a	6	5 (31)	5 (31)	2 (15)	3 (23)
Anti-Fy ^a	10	9 (59)	10 (72)	8 (47)	8 (59)
Anti-Lewis	6	3 (16)	3 (23)	4 (32)	5 (50)
Anti-M	5	3 (37)	4 (52)	3 (43)	4 (64)

*Low ionic strength-Polybrene-PVP.

†Dialysed-serum-Polybrene-PVP.

anti-S was not detected by manual methods; it was a 'machine-only' antibody.

Titration of a standard anti-D showed that 0.7 ng/ml could be detected (Δ OD 0.05 or >0.05) on LISP or LIPP and 1.7 ng/ml on DISP or DIPP.

Fig. 2 Schematic flow diagram for modified DISP channel.

Discussion

No single AutoAnalyzer haemagglutination channel described to date will detect every example of each known antibody specificity; certain deficiencies in

Table 2 Reciprocals of highest doubling dilutions of sera producing ΔOD of > 0.05 on different AutoAnalyzer channels

Channel	Antibody specificity				
	Anti-E	Anti-K1	Anti-Fy ^a	Anti-S	Anti-Jk ^a
LISP	64	64	256	4	32
LIPP	128	128	512	8	64
DISP	8	128	32	0	4
DIPP	32	1024	64	1	8
Manual IDAT titre	2	2048	128	0	32

IDAT = indirect antiglobulin test.

each type of channel are generally accepted (see Moore and Perrault (1977) for a review). A number of different approaches must be used in combination. What combination is best for a particular purpose is still a matter of opinion.

The weaknesses of LISP can be decreased by running DISP in parallel. One noteworthy advantage of DISP (unpublished observations) is that, perhaps owing to the higher pH and ionic strength compared to LISP, a number of 'machine-only' panagglutinins reacting by LISP fail to be demonstrable on DISP; such antibodies we disregard.

Addition of PVP renders both LISP and DISP channels more effective for antibody detection and identification; its use does not increase the proportion of unidentifiable 'machine-only' antibodies. For anti-D quantification we continue to use the modified LISP channel as it is adequate for the purpose.

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Curve regeneration in modified LISP and DISP AutoAnalyzer haemagglutination channels

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In order to obtain clear, reproducible results and an adequate power of detection, sampling rates in single-channel AutoAnalyzer haemagglutination systems are usually restricted to 60/h for antibody detection and to 30/h for antibody identification or quantification.

We describe here the use of curve regeneration on single-channel low ionic strength-Polybrene (LISP) (Lalezari, 1968; Perrault and Högman, 1971) and dialysed serum-Polybrene (DISP) (Moore *et al.*, 1977) systems which permits significantly higher sampling rates to be used for all types of test with no loss, and in some instances a gain, in both precision and the power of detection of the instrument.

Material and methods

The flow diagrams and reagents currently used have been described by Crockford and Moore (1979).

CURVE REGENERATOR/LOGARITHMIC AMPLIFIER (CR/LA)

A Curve Regenerator Type 201 and Logarithmic Amplifier Type 202 were obtained from Fisons-MSE, Crawley, Sussex, UK. They are connected between the colorimeter output and the recorder input; no modifications are needed.

The logarithmic amplifier linearises the output of the Technicon Colorimeter I; the Technicon Colorimeter II already has a linear output. The curve regenerator is an analogue computer, which must be fed a linear absorbance signal. It continuously computes peak height from the rate of change of the electronic signal from the colorimeter and passes a corrected signal to the recorder.

When sampling time is too short, or there is too much carry-over between samples, Δ OD is not proportional to the strength of the antigen:antibody reaction being measured. Curve regeneration largely eliminates the effect of short sampling times on Δ OD (Walker *et al.*, 1972; Carlyle *et al.*, 1973; Dueck, 1975).

Table 1 shows the method of daily calibration; Fig. 1 illustrates correct and incorrect settings.

ELECTRONIC TIMER

A model F1 CR/T Electronic Timer (obtained from the same source as the CR/LA) was connected to a Technicon Sampler II or IV in place of the usual cam. Sampling and wash times could be individually set from 0 to 99 s; readout was digital.

RECORDER CHART PAPER

Linear paper must be used with the curve regenera-