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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-

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Table 1 Daily calibration of CR/LA

Instrument settings		Action
CR	LA	
'Normal'	'Direct'	Place blank aperture in front of flowcell; set recorder pen to zero with Zero Control Knob on Colorimeter
'Normal'	'Logarithmic' Scale factor 000 Scale factor 999	(i) Pump all reagents except Citrate. Adjust Baseline Position Knob on LA until pen records 99. It is usually necessary to <i>reduce</i> the gain control inside the recorder (ii) Reset pen to 99 using 100% Transmission Knob on colorimeter. <i>Colorimeter controls must not be altered again</i>
'Normal'	'Logarithmic'	Pump Citrate. Adjust Scale Factor on LA to make the recorder pen read between 1 and 2; the setting is usually around 500. <i>This is the baseline</i>
'Regenerated'	'Logarithmic'	Place several cups of dilute anti-D in the Sampler and sample at 30/h. Adjust Time Constant on CR to produce a square peak on the recorder. It is easiest to accomplish this by 'over-regeneration' and then reducing the time constant. See Fig. 1 for examples. An average reading (in seconds) would be 07.0. Adjustment of the time constant is needed only at infrequent intervals for a particular machine and a particular channel

tor, as this instrument generates a signal proportional to linear absorbance. ΔOD may be read directly from the chart as peak height; subtraction of OD 'peak' from OD baseline is unnecessary. A peak height of 5 or > 5 graph units is considered as positive and comparable to ΔOD of 0.05 or > 0.05.

Results

ANTIBODY DETECTION

Since a curve regenerator alters only the shape of the recorded peaks, an increase in sampling rates from 60/h to 90/h should have little or no effect upon the proportion of antibodies detected. Table 2 shows that such is the case.

Figure 2 illustrates some of the benefits of curve regeneration. Although both sets of recordings were made at 90/h, the relative heights of the peaks are not comparable because one set was on linear and

Table 2 Results obtained at sampling rates of 60/h and 90/h on a LISP channel

Specificity	No. of examples	Sampling rate			
		60/h		90/h with curve regenerator	
		No. detected	Mean ΔOD	No. detected	Mean ΔOD
Anti-K1	22	19	0.36	19	35
Anti-Fy*	6	6	0.51	6	50
Anti-M	6	6	0.45	6	46
Anti-Lewis	22	19	0.31	19	32

the other on logarithmic paper. 'Shoulder peaks' and 'carry-over' (Walker *et al.*, 1970) were minimal when curve regeneration was used. Thus samples 4, 11, and 12 were unequivocally detectable, and the peak produced by sample 8 was clearly defined, only with the aid of curve regeneration. Sample 9 was a control of serologically inert AB serum.

ANTIBODY IDENTIFICATION

Identification of antibody specificity at a sampling rate of 90/h with curve regeneration poses no problems. It saves time and conserves serum. No more than 0.7 ml of serum is needed to test a 10-cell panel at 90/h compared with 2.0 ml at 30/h.

ANTIBODY QUANTIFICATION

Figure 3 shows the regression lines in the 'zone of maximum accuracy' (Moore and Fernandez, 1972), that is, from 1 to 2.5 ng/ml anti-D at two sampling speeds with and without curve regeneration.

When quantifying anti-D, regenerated peaks reach a maximum height or 'plateau' when the sampling time is 40 s (60 samples/h; 2:1 sample:wash ratio), whereas unregenerated peaks show a reduction in

Fig. 1 Diagrammatic examples of under-regeneration, over-regeneration, and correct setting.

Fig. 2 Recordings at 90 samples/h of 16 different antisera and one inert AB serum with and without curve regeneration.

height compared to that obtained after 80 s sampling (30 samples/h; 2:1 sample:wash ratio). The lower values for ΔOD on line B compared with line A in Fig. 3 and the absence of any substantial difference in the ΔOD values for lines C and D support this conclusion. Use of a sampling time of 40 s may, therefore, be used for the quantification of antibody. The slope of line B (60 samples/h, no curve regeneration) differs from the others; the best fit is given by line D (60 samples/h with curve regeneration).

Table 3 supplements Fig. 3 by supplying, for lines A and D, the standard deviations of ΔOD for each dilution tested. Reproducibility is substantially the same at both sampling speeds, and better than that produced at 60/h without curve regeneration.

Table 3 Results of replicate tests ($n = 10$) on one anti-D serum by a LISP technique at two sampling speeds (sample:wash ratio, 2:1)

Serum dilution (reciprocal)	Sampling speed			
	30/h		60/h†	
	Mean ΔOD^*	SD	Mean ΔOD	SD
4 000	32.7	1.64	34.2	1.34
6 000	20.8	0.92	22.5	1.20
8 000	14.2	0.63	16.3	1.42
10 000	10.3	0.82	12.5	0.82
20 000	4.6	0.52	6.6	0.68

* $\Delta OD \times 10^3$

† plus curve regeneration.

Fig. 3 Quantification of anti-D on a LISP channel: comparison of effects of two sampling rates and presence or absence of CR/LA on regression lines.

Discussion

Sampling rates of 120/h have been used for antibody detection when the single-channel instrument was coupled to a 15-channel AutoAnalyzer (Cotton and Ray, 1976), but the power of detection of the apparatus was reduced. Curve regeneration, on the other hand, enables sampling rates of at least 90/h to be used for antibody detection and identification, and 60/h for quantification, with no loss in sensitivity. The apparatus may be used with either ordinary LISP or DISP channels or when PVP is added (LIPP and DIPP). Its use with bromelain-PVP channels is not so rewarding, as problems in producing a steady baseline are difficult to solve.

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