Results and discussion

After evaluation and comparison of many different methods, Sörnäs developed and recommended the sedimentation method for cytology of CSF (Sörnäs, 1967; Aronson *et al.*, 1974). The cytocentrifuge method has also been recommended recently by a few investigators (Drewinko *et al.*, 1973; Evans *et al.*, 1974). We have found much cell distortion with the cytocentrifuge method and very poor yields, as has been reported by others (Addiego and Woodruff, 1973). The distortion of cell morphology, especially the spreading of the cytoplasm, makes interpretation difficult (Fig. 1). Reduced distortion of cell morphology was well demonstrated in CSF from a patient with Chediak-Higashi's syndrome. Giant granules in the cytoplasm of lymphocytes were seldom seen in cytocentrifuge preparations but were well demonstrated in the preparations by our sedimentation method (Fig. 2).

The method described here is also simpler and more practical. It is difficult to scrape off completely the petroleum jelly in the method of Sörnäs (1967), and this method has also been criticised as time-consuming (Castleberry *et al.*, 1975). Our method not only eliminates the procedure of applying and scraping off the petroleum jelly but also shortens the time considerably. Since the height of fluid within the circle was no more than 3 mm, the time required for cells to settle at the rate of 0.1 mm/min will be

less than 30 minutes. The cells, of course, are less concentrated in our method than in that of Sörnäs. However, the same amount of CSF will be adequate to make more slides. By using low-power lens for scanning the suspicious cells and oil lens for detailed morphology, we probably examine as many cells as in Sörnäs' method. In a few patients whose CSF contained few cells per microlitre the smear revealed a few definitely leukaemic cells (Fig. 3).

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A new method of quality control for the Coulter Model S Counter

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Conventional methods of quality control depend on the use of standards for machine calibration and the use of control samples to detect drift. The methods applied to haematology require the use of expensive whole blood reference preparations. Control samples, although they can be prepared daily or slightly less frequently (Hamilton and Davidson, 1973), must be interposed at frequent intervals between test samples. The control value can be affected by carry-over from the previous specimen (Brittin

et al., 1969) and therefore must be used twice and the second value taken as the more accurate. Control specimens may thus occupy a significant proportion of machine time. The use of a method independent of whole blood standards and controls offers a considerable saving in expense and time.

The method described depends on the stability of the modal values of the population passing through the laboratory. The mode is chosen because—unlike the mean value (Cavill, 1971)—it is not affected by moderate numbers of highly abnormal results which inevitably form an unrepresentatively large proportion of the workload.

Method

PREPARATION OF THE MACHINE

Recalibration of the machine is rarely necessary provided that routine cleaning is carried out. Overnight the counting orifices and diluting chambers are

soaked in Isoterge¹. Each morning the machine is washed through with Isoton¹ and primed with blood at least 10 times to allow distribution of pressures within the valves and flow lines (Overton, 1971).

CHECKING THE HAEMOGLOBIN VALUE

The haemoglobin of fresh blood anticoagulated with EDTA (1.8 mg/ml) is measured on a flow through EEL spectrophotometer². This machine is calibrated against three commercial cyanmethaemoglobin standards³ conforming to British Standard 3985 (1966). The mean of three results on the spectrophotometer is compared with the mean of three readings performed on the Coulter S. Agreement should be within ± 0.1 g/dl.

PRECISION

A blood sample from a single donor anticoagulated with citrate phosphate dextrose is counted 10 times. The 10 readings taken are used to check the precision of the seven measured and computed values. In our opinion, the maximum acceptable range of results is as shown in Table 1. A similar

Table 1 *Maximum range of values of 10 repeated measurements on a single specimen*

White cell count	(WCC)	$0.3 \times 10^9/l$	(300/mm ³)
Red cell count	(RCC)	$0.10 \times 10^{12}/l$	($0.1 \times 10^6/mm^3$)
Haemoglobin	(Hb)	0.2 g/dl	(0.2 g/100 ml)
Packed cell volume	(PCV)	0.01	(1%)
Mean cell volume	(MCV)	1.0 fl	(1.0 cμ)
Mean cell haemoglobin	(MCH)	0.6 pg	(0.6 pg)
Mean cell haemoglobin concentration	(MCHC)	1.0 g/dl	(1%)

range is quoted by Cavill and Ricketts (1974). If these results are unacceptable corrective action is taken as detailed below—items 1 to 4.

PREPARING THE FREQUENCY DISTRIBUTION HISTOGRAM

In routine use the machine is operated by two technicians: the first feeds the machine with specimens and the printer with cards; the second marries the printed cards with the request forms and records the results on the frequency histogram chart (Fig. 1). This is done by marking a single stroke in the appropriate column. Only WCC, MCV, MCH, and

MCHC are recorded in this way. The values are truncated to the whole number below. The results of all specimens are included. Individual values for MCV or WCC may fall outside the range plotted on the histogram but these values do not affect the mode.

After 12 results it is usually possible to read the modal value for MCH and MCHC. The WCC and MCV require a greater number of results but an estimate of where the mode will settle can often be made by drawing a 'best fit' line.

The machine is in control if the modal values are within the ranges shown in Table 2. An example of the histograms obtained is shown (Fig. 1).

Table 2 *Range of acceptable modal values*

WCC	7.5	$\pm 1.0 \times 10^9/l$	($\pm 1000/mm^3$)
MCV	87	± 1.0 fl ¹	(± 1.0 cμ)
MCH	29.5	± 0.5 pg	(± 0.5 pg)
MCHC	33.5	± 0.5 g/dl	($\pm 0.5\%$)

¹Mode of best fit line.

CORRECTIVE ACTION

If the machine is not in control by the stated criteria action is taken in the following order:

- 1 The running of the machine, functioning of valves and other components is checked.
- 2 The presence and cleanliness of the reagents are checked.
- 3 The machine-performed calculations are checked.
- 4 The diluting and counting orifices are cleaned and bleached.
- 5 The value of the haemoglobin is checked against the cyanmethaemoglobin standards as described.
- 6 Only if these checks fail to reveal the cause of error is the machine recalibrated. In practice, this is necessary only very occasionally—once every few months.

With experience it is often possible to predict the type of fault by the changes in the mode values.

SENSITIVITY

The minimum changes in mode values that are easily detectable are shown in Table 3. The method is especially sensitive to small changes in haemoglobin or red cell count.

A change of MCH or MCHC by 0.25 unit reflects

Table 3 *Minimum changes in mode value detectable from the histograms*

WCC	$0.5 \times 10^9/l$	(500/mm ³)
MCV	1.0 fl	(1.0 cμ)
MCH	0.25 pg	(0.25 pg)
MCHC	0.25 g/dl	(0.25%)

¹Coulter Electronics Ltd

²EEL Flowthrough spectra, Evan Electroselenium Ltd

³(1) Acuglobin; (2) BDH cyanmet haemoglobin standard; (3) Diagen cyanmet haemoglobin standard.

Fig. 1 The frequency histogram chart.

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Letters to the Editor

Effect of growth at 43°C on phage typing of staphylococci

During 1975 we noted that a number of initially untypable strains of *Staphylococcus aureus* had become susceptible to lysis by one or more of the basic set of typing phages after varying periods of storage at room temperature on slopes of nutrient agar. During an investigation of this phenomenon it was shown that loss of resistance to phage lysis could also be greatly accelerated by growing the cultures of staphylococci in broth at 43°C before they were typed. The results of testing 110 random isolates in parallel after growth in broth culture at 37°C and 43°C are shown in Tables 1 and 2. There was a 10% increase in the number of typable strains at routine test dilution (RTD), and a further 30% increase in typability in strains that required examination at 100 RTD, with no apparent loss in discrimination.

Although Ma and Mandle (1961) reported a similar observation 15 years ago the effect of growth at elevated temperature on typability does not appear to be widely known. I draw attention to it because it may be useful in routine typing.

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Table 1 Results of typing *Staphylococcus aureus* strains at RTD and 100 RTD after growth in broth at 37°C and 43°C

	RTD		100 RTD	
	37°C	43°C	37°C	43°C
Number of strains typed	68	73	16	23
Number of strains NOT typed	42	37	16	9
Total	110	110	32	32

Table 2 Different typing patterns of *Staphylococcus aureus* obtained after growth at 37°C and 43°C

Strain No.	RTD	
	37°C	43°C
1	NT	80
2	NT	52/52A/80
3	NT	54
4	NT	94/96
5	NT	95
6	NT	52/52A/80
7	77	NT
8	83A/85	42E/54/83A/85
9	80/95	29/52/52A/80/95
10	80/95	52/52A/80/95
11	95	52A/80/95/53
100 RTD		
	37°C	43°C
12	NT	29/52/52A/79/53/54
13	NT	52/80/85
14	NT	47/53
15	NT	84/85
16	NT	52/80
17	NT	52A/79
18	NT	85
19	80	52/52A/80
20	47	6/47/54/75/84/85
21	52	29/52/52A
22	77	42E/47/53/54/75/77

Outbreaks of food poisoning attributed to lecithinase-negative *Clostridium welchii*

The ability of *Clostridium welchii* type A to produce a lecithinase which splits the insoluble fatty material in human serum was first described by Nagler (1939). Since then this reaction, and its specific inhibition, has been recommended and widely used as a rapid diagnostic and confirmatory test for *Cl. welchii* (Hayward, 1943; McClung and Toabe, 1947).

In the investigation of food poisoning outbreaks where *Cl. welchii* type A is suspected as the causative agent, typical colonies from the primary isolation medium, after anaerobic incubation, are streaked across a plate of egg yolk medium. Half of this has previously been spread with 2 or 3 drops of standard *Cl. welchii* type A antitoxin and allowed to dry. After anaerobic incubation growth of *Cl. welchii* is surrounded by a zone of opalescence (Nagler reaction). This is inhibited on the other half of the plate by the specific antitoxin. *Cl. bifermentans*, *Cl. sordellii*, and *Cl. centrosporogenes* also produce lecithinase partially inhibited by *Cl. welchii* type A antitoxin. In the past two years we have encountered three outbreaks of food poisoning attributed to lecithinase-negative strains of *Cl. welchii*.

Outbreak 1

In October 1974, 105 of the patients and staff in a hospital suffered symptoms of food poisoning. *Cl. welchii* was isolated from 16 of 20 faecal specimens. Roast lamb and gravy were suspected as the cause of the outbreak but samples were not available for examination.

Outbreak 2

In August 1975, 18 of 36 people attending