fluorescein (Marshall *et al*, 1975b). This is not removed by the desalting procedure, although it may be removed chromatographically (Graichen and Molitor, 1959). However, we have found that this is unnecessary for the present staining procedure. Tribromofluorescein-free eosin and eosin deliberately contaminated with a much larger proportion of tribromofluorescein than is present in commercial samples gave indistinguishable results.

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Modifications to improve the reliability of the Coulter 'S'

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The Coulter Counter Model 'S' System (Coulter Electronics Ltd) measures automatically the haematological parameters haemoglobin concentration, white cell count, red cell count, and mean cell volume and also computes from them the haematocrit, mean cell haemoglobin, and mean cell haemoglobin concentration. The instrument can handle one sample every 20 seconds and can be both precise and reliable (Barnard *et al*, 1969). However, in our laboratory the model 'S' is used to process up to 800 samples per day, and we have found that these conditions of virtually continuous operation expose limitations in the design of the instrument with a concomitant fall off in its reliability. We now describe some of these limitations and suggest ways of modifying the machine to overcome them.

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The Rinse and Drain Mechanisms

The most troublesome limitation exposed when the instrument was run continuously was the failure of the aperture baths to drain completely. (This happened several times within a period of three months.) In the original version of the instrument, during each cycle waste fluid was drawn under vacuum from the aperture baths into the waste chamber and then discharged to waste under 0·35 kgf/cm^2 positive pressure. Owing to the low surface tension of the effluent, caused by the lysing agent, and the turbulence created by the vacuum, there was a tendency for excess foam to accumulate in the waste chamber which then overflowed into the trap bottle and from there into the switching valve (SV) No. 6, which controls the vacuum in the pneumatic card (L2). This caused the switching valve to become progressively less efficient with the result that the aperture baths were sometimes only partially drained, and carry-over between successive samples was observed. The valve eventually failed completely. The manufacturers then modified the instrument by fitting an anti-foam device (Coulter Alteration Number ECO-0367) which gave only partial improvement; however the excess foam still over-

flowed into the trap bottle (see below). In addition, the design of the device resulted in a residual pressure being held in the waste chamber. When vacuum was applied to this chamber, the back pressure which tended to build up with continuous operation of the instrument impaired the efficiency of the drainage system due to the shortening of the time interval between emptying and refilling of the baths. Eventually the baths were only partially emptied before they began to refill. We have corrected this flaw by fitting a Norgren one-way bleed valve No. 4 LD-010 (supplied by Coulter Electronics Ltd) to the port of the SV No. 6 which is normally blanked off. This allows the residual pressure to be discharged just before the vacuum is applied. Our modification also permitted the volume of the rinse in each aperture bath to be increased to more than 9 cm^3. Since we previously suggested that this volume was not large enough we have now had 12 cm^3 rinse dispense units fitted.

Recently the manufacturers have begun to replace switching valves with pinch valves (either 'normally closed' or 'normally closed and normally open'). SV No. 6 must therefore be replaced with a double-acting pinch valve rather than with the single acting one which is being fitted by the manufacturers. The input pressure line is fed through the 'normally closed' part of the pinch valve and is then divided by a Y-piece. One branch continues to the waste chamber and the other is passed back through the 'normally open' port and then to the bleed valve.

It was stated above that even after Coulter's modification excess foam still overflowed into the trap bottle, continuously increasing the volume of liquid in it; the foam also tended to find its way into the components of the anti-foam device. We partially resolved this problem (*a*) by raising the 0·35 kgf/cm^2 pressure to 0·42 kgf/cm^2 (also increasing the pressure of the orifice clear system); and (*b*) by replacing the T-piece junction between the waste lines from the sample aspirator and the waste chamber with a wider bore transparent plastic Y-piece (5 mm internal diameter). We completely resolved the problem by extending the line from the waste chamber to within 3 mm of the bottom of the trap bottle; foam is then drawn back into the waste chamber when the pressure is applied.

It is possible to prevent foam formation entirely, for instance by injecting an anti-foam solution (1% aqueous Silicone MS Antifoam Emulsion RD, Hopkin and Williams Ltd) into the waste chamber simultaneously with the effluent from the vacuum isolator chamber. However, in practice this has not been found to be necessary.

When the instrument operated under a heavy workload poor drainage of the waste chamber also occurred as a result of an accumulation of proteinaceous material in the main waste line near the waste chamber. In the original instrument this part of the line was out of sight, which made routine inspection difficult. We have therefore re-routed this line along the front of the instrument above the drip tray, thus sacrificing good looks for easy maintenance. In addition, the two one-way check valves, which are connected to the side arms of the waste chamber and were behind the left front panel, have been repositioned at the front of it; the operator can now see when they become faulty.

Raising the pressure to 0·42 kgf/cm^2 and fitting the Y-piece increased the flow rate from the waste chamber from the typical 11 cm^3/s to above 19 cm^3/s, and consequently the amount of residual foam in the chamber has been markedly reduced.

The Aperture Baths

Drainage of the aperture baths was further improved by replacing the T-piece which accepts the effluent from them with a transparent Y-piece (4 mm internal diameter). On the original instrument the aperture baths were not easily observed, so making it difficult to detect changes in the rate of flow into and out of them. The mirror assembly shown in fig 1 was therefore fitted, and it was so positioned that visual detection of drainage faults was possible since the operator could observe the flow rates continuously. (This modification did not interfere with the haemoglobin estimation.)

Contamination of the Photocell

When the aperture baths are overfilled reagent is forced out, drenching the orifice assembly and photocell housing. Although the photocell is well protected it eventually becomes contaminated and loses

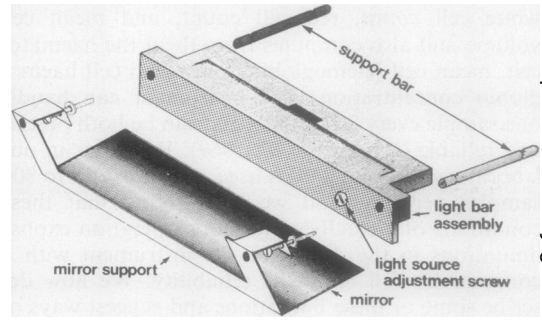

Fig 1 *Diagram of mirror assembly showing relation of mirror to light bar assembly.*

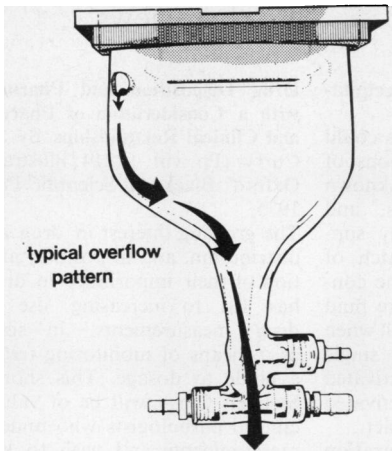

Fig 2 *White cell aperture bath showing position of overflow outlets and typical overflow pattern.*

sensitivity, while the orifice assembly becomes encrusted. This was rectified by persuading the manufacturers to drill a hole 9·5 mm (⅜ inch) in diameter in each bath to act as an overflow outlet. The exact positioning of the holes is shown (fig 2).

Calibrating Flow Rate Control Valves

The diluter unit houses six pneumatic cards which form the major part of the mechanisms controlling its operation. Each card has specific functions so that when a fault is detected it is relatively easy to decide in which individual component it is contained. Four of the cards are fitted with air-flow control valves and we have used these to help in pin-pointing the faulty component and to determine whether it was inside or outside the card. The method depends on measuring the fraction of a revolution the valve has to be turned from some base line to give a satisfactory flow rate. This angle is easily determined by etching a horizontal line on the knurled head of the valve and supporting a plastic disc, calibrated like a protractor, behind the locking nut securing the valve to the card. Even when the machine is working normally, the angle will vary slightly—in our experience by about ± 4°. If the valve has to be adjusted by more than this, a fault is suspected in one of the components it supplies. If the angle is routinely recorded, slowly developing faults can be corrected before a major breakdown occurs. It should be noted that this system requires the air flow rates and hence the compressor to be stable; in our experience, this requirement is met. It also assumes that the needle valves themselves are operating satisfactorily.

Two typical faults in the detection of which this calibration has proved useful are:
(1) where a blocked nipple may cause the transfer rate from the mixing chambers to the aperture baths to become too slow. It is usually countered by adjusting the needle valve without, of course, correcting the underlying fault.
(2) where a leak or other failure develops in one or both of the two SVs which are involved in the transfer of fluid from the lyse chamber to the white cell aperture bath. This is usually countered by increasing the operating pressure by adjustment of the appropriate needle valve. If the valve has been calibrated, the location of the underlying fault is simplified.

An alternative way of calibrating the valves is to replace the existing needle valves with a calibrated (Vernier scale) Fine Metering Valve (Techmation Ltd).

Conclusion

The Model 'S' is a complex instrument of great potential, the design of which is still evolving. Clearly, it is when the instrument is being used continuously that any faults in design are most likely to become apparent. In our experience, the modifications described considerably improve reliability in that the major failures are reduced in frequency. In addition, some incipient failures can be detected before they significantly affect the operation of the instrument.

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