Technical methods

Flexible electrophoresis applicator for use with the AutoAnalyzer in routine protein analysis

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The 'multiapplication' principle originated by Kohn (1967, 1968) for the simultaneous electrophoresis of batches of serum samples greatly improved reproducibility and comparability in this procedure. However, there was not a great saving in time as the samples had to be separately spread on wells in an applicator plate before being transferred to the grooved castellations of the sample applicator. There was also the possibility of errors in transferring and in drying of the samples on the applicator plate. A cheap and simple flexible applicator which overcomes these problems by the direct transfer of serum from the AutoAnalyzer cups to the sheet of cellulose acetate paper has been developed.

The Applicator

The applicator consists of a 12 in. $\times$ $\frac{1}{2}$ in. stainless steel engineer's rule, with eight 'feet'. Each foot consists of a tapestry needle bent to a right angle in a bunsen flame at the junction of the eye with the shaft. For general purposes Milward Gold Seal size 24 was found to be suitable, having an eye 8 mm long, narrowed to 0.2 mm with a feeler gauge, and delivering 1.7 μ l of serum. Larger and smaller sizes were also available for applying different amounts, as when larger sample volumes were required for lipoprotein electrophoresis. Optimal application was achieved when the gold plating on the eyes of the needles was preserved. The needles were fastened with Araldite onto filed notches on the rule at 16 mm intervals to fit the cup intervals on the mark I Technicon sampler, or at 11 mm intervals for the mark II sampler. For easy identification, the first 'foot' is 26 mm from the second and anteverted, like the cox of a rowing eight. The Shandon Multi-Microband electrophoresis tank was modified to take this applicator by cutting two pairs of vertical notches 1 cm deep in the tank sides, 6.5 cm from both ends.

Method

After the AutoAnalyzer sample plate has been made

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up for the day's run, relevant cup numbers are marked along the sheet of cellulose acetate 150 $\times$ 85 mm (Celogram) with a ball point pen. The sheet is soaked with buffer and laid across the bridge of the Shandon Multi-Microband electrophoresis tank. The applicator is then bent to fit the curve of the sampler plate and its 'feet' are simultaneously immersed in the samples (Fig. 1). As long as the first, anteverted, foot is in the correct cup, no transfer errors can occur. As an additional precaution every eighth sample is labelled with one drop of bromophenol blue dye, which does not interfere with subsequent total protein and albumin estimation. After checking that there are no obvious excess droplets of serum, the straightened applicator is lowered into the applicator guides at the edges of the tank (Fig. 2). The applicator is allowed to rest under its own weight on the sheet of cellulose acetate for 20 seconds and the lid of the tank is replaced. Special care should be taken that the weight of the applicator does not depress the sheet to the extent of touching the central partition in the tank. The applicator is immediately washed in detergent and

Fig. 1 The flexed applicator about to be immersed in the sample cups.

Fig. 2 The straightened applicator in position in the electrophoresis tank.

Fig. 3 The cleared cellulose acetate sheet after staining showing four normal and four abnormal patterns.

dried. Using 0.05 Molar barbitone buffer, pH 8.6, and a current of 10 m Amp, good separation is achieved within 45 min as can be seen by the migration of the albumin in the sample, stained with bromophenol blue. The sheet is then removed from the tank and stained with Ponceau S, or other more specialized stains if required. After drying, the sheet can be rendered completely clear by being painted with Whitmore oil (Ondina oil 17, Shell), or any other suitable oil, on a white tile (Fig. 3). Alternatively the sheet can be made transparently dry and clear by the other methods recommended by the manufacturers. At this stage the row of electrophoretic patterns can be accurately compared for abnormalities by visual inspection or by scanning in a densitometer.

The sheet is then cut into its individual patterns which can be mounted with Sellotape on the request forms, when they again become opaque. In the case of strips stained with Ponceau S, the brilliance of colouring of the bands is better retained after treatment with oil than if they are mounted on the forms directly after drying.

Results

With consecutive running of batches of eight sera,

which can be sampled while the AutoAnalyzer is carrying out the total protein and albumin analyses, the electrophoretic patterns are available as soon as these other results can be read off the chart. Applications are reproducible, and the method is suitable for screening both proteins and lipoproteins. Though electrophoresis cannot be performed on plasma samples, the increasing use of serum in screening studies, as with the Technicon SMA 12/60, and at the Hammersmith Hospital, should provide in the applicator a valuable additional means of investigation in this field.

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References

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