Technical method

A simple method of concentrating small samples of cerebrospinal fluid for electrophoresis

ARW FORREST* *Department of Biochemistry, Royal Infirmary, Glasgow G4 0SF*

Agarose gel electrophoresis of cerebrospinal fluid (CSF) is a useful test in the investigation of several neurological diseases.¹ However, the sample must be concentrated at least a hundredfold before the electrophoresis can be performed.² If commercially available concentrators are used, sample volumes of several millilitres are required. Clinicians are usually reluctant to withdraw such large amounts of CSF particularly from children.

*Present address: Department of Clinical Chemistry, Royal Hallamshire Hospital, Sheffield, S10 2RX.

Accepted for publication 26 January 1981

There is thus a need for methods of concentrating small samples of CSF that are suitable for use in the routine clinical chemistry laboratory. The method described here is modified from that of Kahn and Thompson³ but requires less technical skill.

Material and methods

The method uses a CSF concentrator (Fig. 1) constructed from two plates of Perspex (ICI) which can be bolted together. Each plate measures 10 cm x 12 cm. The upper plate is drilled with 57 equidistant holes 4 mm in diameter lying within a circle 6.1 cm in diameter centred on the middle of the plate. A Diaflo (Amicon) PM10 ultrafiltration membrane 6.2 cm in diameter is soaked overnight in a wetting agent formulated as follows: glycerol 50 ml; Triton X-100 (British Drug Houses) 2 ml; water to 100 ml; and a few crystals of thiomersal as a preservative. While still wet the membrane is then carefully placed, with the smooth surface towards the Perspex

Fig. 1 The concentrator assembled, ready for use

over the holes drilled in the top plate. Blotting paper (thickness ≥ 0.8 cm) is then laid over the rough surface of the membrane and the upper plate is tightly bolted to the lower plate, sandwiching the membrane and blotting paper.

To use the device 150 μ l of CSF is placed in one of the wells on the top plate, taking care not to damage the membrane. After one hour the sample volume will have decreased by about a third as water is drawn from the well through the membrane into the blotting paper. If the sample is left too long it will be drawn completely through the membrane and the protein precipitated. A further 50 μ l of CSF is added and the device is tilted at an angle of 45° to the bench. After another two hours, only one or two microlitres of concentrated sample will be left in the well from which it may be removed with a micropipette, and either stored in the pipette or

immediately electrophoresed. It is easier to follow the progress of the sample as it concentrates if the device is illuminated from the side. After each well has been used once the membrane and blotting paper should be replaced.

Discussion

This method does not require the constant attention from the operator that the method of Kahn and Thompson demands.³ It is also more economical in its use of the expensive Amicon membranes. With their technique one 62 mm diameter membrane can be used to process 20 samples, whereas 57 samples per membrane can be processed by the technique described here. I have achieved good results (Fig. 2) using this device in conjunction with the Corning agarose cassette electrophoresis

Fig. 2 Electrophoresis in agarose gel at pH 8.4. Amido black stain. Sample 1 is CSF, concentrated $\times 100$, taken from a patient 36 hours after subarachnoid haemorrhage. Bands produced by prealbumin, albumin, α_1 -antitrypsin, haemoglobin, "slow" transferrin, and γ globulins can be seen. Sample 2 is unconcentrated CSF (protein 0.6 g/l) from the same patient. Sample 3 is serum.

equipment. The method can also be used to concentrate urine samples for electrophoresis.

I am grateful to Mr J Millar for constructing the device and to Ms P Price for typing the manuscript.

References

- Laurell CB. Composition and variation of the gel electrophoretic fractions of plasma, cerebrospinal fluid and

- urine. *Scand J Clin Lab Invest* 1972;29:suppl 124:71-81.
- Jeppsson JO, Laurell CB, Franzen B, *et al.* Proposed selected method: agarose gel electrophoresis. *Clin Chem* 1979;25:629-38.
- Kahn SN, Thompson EJ. New ultramicro method for concentration of cerebrospinal fluid. *Lancet* 1976;ii:1275.

Requests for reprints to: Dr ARW Forrest, Department of Clinical Chemistry, Royal Hallamshire Hospital, Sheffield S10 2RX.

Letters to the Editor

Growth of *Neisseria gonorrhoeae* in a simple medium at 28°C

Although it is generally documented that *Neisseria gonorrhoeae* does not grow below 30°C there seems no more precise information on the minimum of its growth temperature range.¹ During some studies of simple media for the cultivation of this organism, it was observed that growth of all of a few strains tested did in fact occur at 28°C but not at 24°C. It was decided to examine a larger batch of isolates with a view to obtaining more data on the minimum growth temperature for this species.

The test medium was a semi-solid one which consisted of brain heart infusion broth (Difco) to which was added agar powder (0.1%) and soluble starch (0.1%). It was dispensed in 4 ml volumes in plastic tubes (100 × 12 mm) and autoclaved at 121°C for 15 minutes.

The organisms tested were a batch of 65 freshly collected clinical isolates. After ensuring purity of the isolates, growth was emulsified in nutrient broth and 2 drops of dense suspension were inoculated on to the surface of the test medium. One tube was incubated in a cabinet at 36°C and the other in an agitated water bath at a temperature of 27.7 ± 0.2°C (hereafter referred to as 28°C). The bath thermometer was calibrated against a standard instrument. Two chocolate agar plates were also inoculated; one was incubated at 36°C in 10% carbon dioxide and the other in air at room temperature (20-22°C).

The control plates at 36°C all yielded growth and those at room temperature

none. All tubes of the test medium showed growth after varying periods. Those at 36°C showed distinct turbidity within 24 hours whereas those at 28°C did so within 24 to 48 hours. Growth first appeared as a shallow surface sludge. When this was disturbed by gentle shaking and distributed into the deeper regions of the medium, growth continued and produced an evenly turbid suspension to a depth of about 15 mm. The final density of this growth varied widely among the isolates and in general correlated with the colony size of the isolates on chocolate agar. No critical survival study was made on the cultures but many, though not all of those tested, yielded growth when plated after about 30 days.

The semi-solid agar is proving a useful medium for growth and short term maintenance of gonococcal strains. Other semi-solid media such as that devised by Vera² and marketed as Cystine Trypticase Agar (Baltimore Biological Laboratories) have been used for many years for this purpose. Serum enrichment as advocated by some workers³ would appear to be unnecessary in media designed for the maintenance of the gonococcus.

It should be of interest to extend the investigations on low temperature growth of the gonococcus particularly to include observations on agar surfaces. Meanwhile it is now established that this organism does, under some cultural conditions, grow at a temperature of as low as, at least, 28°C.

DI ANNEAR

Department of Microbiology,
Royal Perth Hospital,
Box X 2213, GPO,
Perth, Western Australia

I should like to thank Mr M Blums, State Health Laboratory Services, Perth, Western Australia for kindly identifying and supplying the strains tested.

References

- Wilson GS, Miles AA. *Topley and Wilson's Principles of Bacteriology, Virology and Immunity*. 6th edn. London: Edward Arnold, 1975:688.
- Vera HD. A simple medium for identification and maintenance of the gonococcus and other bacteria. *J Bacteriol* 1948;55:531-6.
- Public Health Laboratory Service Publications. Monograph Series Number 1. *Laboratory Diagnosis of Venereal Disease*. London: HMSO, 1972.

IgM mesangial deposits in nephrotic syndrome

We have studied 14 patients (aged 8-39 years) with an idiopathic nephrotic syndrome. Seven patients were male and seven female. On light microscopy, moderate increase in cells and mesangial matrix were observed in all cases. The most characteristic findings by immunofluorescence microscopy were generalized and diffuse mesangial granular deposits of IgM in all the biopsies. There was C3 deposition in 12 cases, and C1q and C4