Fig. 1 U device comprising disposable 1.0 ml glass pipette.

Fig. 2 Isolated fibril of collagen from human dermis with phosphotungstic acid negative staining. $\times 24\ 000$.

were collected in a coherent fraction immediately before the nuclear fraction. Figure 2 shows a negatively stained fibril of collagen displaying clear and dark bands. A neat fraction of collagen fibril was obtained despite the reduced volume: no special device was required to collect it on to the grids of the electron microscope.⁴ The slow freezing of the homogenate did not cause distortion of the ultrastructures of the collagen fibrils but produced an undisturbed and distinct layer. Undoubtedly, this device will be useful for isolating cellular fractions whenever the volumes are reduced, and great precision is not required. The main advantage is the cost of the device because it can be made in the laboratory with standard pipettes or plastic tubing without using any special equipment.

HAROL NUNEZ-DURAN*
ULISES GELOS†

*Physiological Sciences Department,
Instituto de Ciencias, UAP,
A Postal 406,
Puebla,
Mexico

†Department of Biophysics,
Facultad de Medicina,
Montevideo, Uruguay.

References

- 1 Anderson NG. Techniques for the mass isolation of cellular components. In: Oster G, Pollister AN, eds. *Physical techniques in biological research*. Vol 3. New York: Academic Press, 1956:300-89.
- 2 Neuhoff V. Capillary centrifugation. *Mol Biol Biochem Biophys* 1973;14:205-13.
- 3 Tan KB. A simple device for fractionating density gradients. *Anal Biochem* 1972;45:306-8.
- 4 Richard RD. Adaptation of a screw-top vial for use in centrifugation of tissue onto EM grids. *Stain Technol* 1972;47:272-4.

Rapid detection of bacteraemia

We read with interest the paper on the rapid detection of bacteraemia by Mr Corkill.¹ His extremely low detection rate of 9.5% at 24 hours using a manual system differs considerably from the average of 71% reported by us,² and this rate has subsequently risen to 78% using the same technique. This striking difference may be

ascribed to three main aspects of our methods: the consistent use of a biphasic medium containing Columbia agar in parallel with fastidious anaerobe broth; incubation without delay of blood cultures taken after laboratory hours; and blind subculture of both media at 10 pm on the day the specimen was received.

Mr Corkill's detection rate rose from 9.5% to 76.0% with a semiautomated radiometric system (Bactec 460). Our overall rate of 83% for the first 24 hours with Bactec is not significantly higher than our manual rate. With the manual system, however, the isolates were available on solid media 24 hours earlier than with the radiometric system. The radiometric system has some important drawbacks; a further study showed negative radiometric growth indices in the first 24 hours when blind subcultures were positive. The cost is also considerably greater than that of a manual system.³

LA GANGULI
MGL KEANEY
Department of Microbiology,
Hope Hospital,
University of Manchester School of
Medicine,
Eccles Old Road,
Salford M6 8HD.

References

- 1 Corkill JE. Effects of media, working practice, and automation on the rapid detection of bacteraemia. *J Clin Pathol* 1985;38:336-40.
- 2 Ganguli LA, O'Hare W, Hyde WA. Rapid detection of bacteraemia by early subculture. *J Med Microbiol* 1984;17:311-5.
- 3 Ganguli LA, Keaney MGL, Hyde WA, Fraser SB. More rapid detection of bacteraemia by manual than radiometric method. *J Clin Pathol* (in press).

Mr Corkill replies as follows:

Our detection rate of bacteraemia at 9.5% with traditional broth systems (preincubated for up to 24 hours and blindly subcultured once a day) highlights the delay occasioned by standard techniques for blood culture.¹ Routine application of a biphasic system over 12 months more than doubled our detection rate at 24 hours, even with a single daily inspection. We decided to discontinue this system, however, because of recurrent contamination related to the manufacturer and a lack of support for pneumococci. If we had inspected the biphasic bottles more frequently in an extended working day our detection rate could probably have reached the level subsequently obtained using Bac-

Letters

tec, method 1. In their previous work Ganguli *et al*² were unable to study the performance of more than one blood culture system in parallel; nor could we. Without such comparative data the value of retrospective conclusion is limited.

The single most important factor in reducing our reporting time was the revised work schedule. This comprised an initial examination at < 16 hours after collection, followed by a further two at 24 hours. It is the interval between these two later samplings that influences the rate of detection.

JE CORKILL

Department of Medical Microbiology,
7th Floor,
Duncan Building,
Royal Liverpool Hospital,
Prescot Street, Liverpool L7 8XP.

157 (93%) gave no growth or gave contaminants only using either method.

As regards the positive results (judged clinically important), on two occasions *Escherichia coli* was isolated from both systems at one day, and in two other instances a mixture of *E coli* with *Klebsiella* sp was isolated from Bactec after three days and by the Isolator after one day. Additionally, one isolate of *Alkaligenes* sp and one of *Candida parapsilosis* were recovered by the Isolator only. Two isolates of *Staphylococcus aureus* and one each of *Geotrichum* sp, *Streptococcus pneumoniae*, and a group C streptococcus were recovered in the Bactec only. Failure of the Isolator to detect these organisms is disappointing but probably not conclusive, given the small total number of isolates and the performance in other studies.

Our contamination rate for the Isolator was 18%, in contrast with 2.4% for the Bactec. Although this could probably be improved with experience, even the use of special precautions may not reduce contamination of the Isolator to the level encountered in other systems.⁹

The advantage of the Isolator system is that, at the first indication of a positive culture, a colony on solid media is available rather than simply a Gram stained film of culture fluid. Identification can thus be much closer at this stage, and tests of antibiotic susceptibility can be set up with greater confidence and ease than from blood culture fluid. Furthermore, the amount of growth in four of the six positive cultures suggested that it would have been possible to set up direct sensitivities by the Stokes technique at the same time as the original cultures. The possibility of direct microscopy of the original extract, using acridine orange staining, has also been raised.^{10,11} The quantitative expression of positive results may be another useful feature.¹² There suggestions await further investigation.

The main disadvantage of the new system is that it requires more labour time than the Bactec system to process each specimen. Furthermore, although some delay may be tolerable,^{10,13} manufacturers recommend that the initial processing and culture procedure lasting 40 minutes should be performed as soon as possible after collection of the specimen. This would be an additional demand on those laboratories that do not usually devote "out of hours" technical time to blood cultures. The cost of consumable materials in the United Kingdom also seems likely to be greater than that of Bactec bottles, but the

capital equipment is cheaper; the Isolator uses more agar plates, however.

The financial and logistical considerations and the possible remaining disadvantage in recovering some streptococci^{2,4} and anaerobes^{3,7} suggest that the Isolator may not rapidly replace other systems as a mainstay method for all blood cultures. On the other hand, the possibility of earlier results and the improved recovery of staphylococci^{1,2,5} and yeasts^{1-3,5,11,14,15} reported by other workers may well indicate selective usage of the system as an additional procedure in investigating patients in whom venous lines or other immunocompromising factors are present. We thank Dr Pont for providing the Isolator tubes used.

SR MARPER

E ROBBINS

MS AHMED

P NOONE

Department of Microbiology,
The Royal Free Hospital,
Pond Street,
Hampstead,
London NW3 2QG.

References

- Shanson DC. Blood culture techniques. In: Williams JD, ed. *Modern topics in infection*. London: Heinemann, 1978:14-30.
- Ganguli LA, O'Hare W, Hyde WA. Rapid detection of bacteraemia by early subculture. *J Med Microbiol* 1984;17:311-5.

New system for blood culture

Recent American reports have favourably compared the performance of a new system for blood culture, the Isolator (Du Pont Co, Wilmington, Delaware, United States), with various existing methods in a routine setting.¹⁻⁷ We recently had an opportunity to use the system in this laboratory, alongside our existing Bactec radiometric procedure.

The new method, described by Dorn and Smith,⁸ differs in principle from the basis of most other systems—that is, enrichment with broth—in that organisms are harvested directly from the patient's blood sample in a centrifuged tube containing fluids of different densities and plated directly on to several solid media. Any bacterial cells are thus separated from the different factors in the blood that inhibit growth, and the system is claimed to be sufficiently sensitive to allow recovery of organisms present at less than 1 colony forming unit/ml of sample.

For this assessment an Isolator tube and explanatory note were issued with each of 300 routine blood culture sets comprising a Bactec 6B aerobic and 7D anaerobic bottle. The same range of solid media was used in both systems. Of 168 sets returned with a properly filled Isolator tube,

References

- Dorn GL, Land GA, Wilson GE. Improved blood culture technique based on centrifugation: clinical evaluation. *J Clin Microbiol* 1979;9:391-6.
- Henry NK, McLimans CA, Wright AJ, Thompson RL, Wilson WR, Washington JA. Microbiological and clinical evaluation of the isolator lysis-centrifugation blood culture tube. *J Clin Microbiol* 1983;17:864-9.
- Kiehn TE, Wong B, Edwards FF, Armstrong D. Comparative recovery of bacteria and yeasts from lysis-centrifugation and a conventional blood culture system. *J Clin Microbiol* 1983;18:300-4.
- Kelly MT, Buck GE, Fojtasek MF. Evaluation of a lysis-centrifugation and biphasic bottle blood culture system during routine use. *J Clin Microbiol* 1983;18:554-7.
- McLaughlin JC, Hamilton P, Scholes JV, Bartlett RC. Clinical laboratory comparison of lysis-centrifugation and Bactec radiometric blood culture techniques. *J Clin Microbiol* 1983;18:1027-31.
- Gill VJ, Zierdt CH, Wu TC, Stock F, Pizzo PA, MacLowry JD. Comparison of lysis-centrifugation with lysis-filtration and a conventional unvented bottle for blood cultures. *J Clin Microbiol* 1984;20:927-32.
- Kellogg JA, Manzella JP, McConville JH. Clinical laboratory comparison of the 10-ml isolator blood culture system with Bactec radiometric blood culture media. *J Clin Microbiol* 1984;20:618-23.
- Dorn GL, Smith K. New centrifugation blood culture device. *J Clin Microbiol* 1978;7:52-4.