Letters to the Editor

Detection of bacteraemia by an automated blood culture system

Many clinical microbiology laboratories in the UK and the USA are currently detecting bacteraemia using an automated C¹⁴ labelled substrates blood culture system (Bactec, Johnston Labs, Inc).

Positive blood cultures are initially detected by release of radioactive carbon dioxide into the gaseous space which exceeds a printout value of 40 cpm. We report two cases of endocarditis which, respectively, provide a cautionary reminder and a reassurance to clinicians and microbiologists who rely upon this blood culture system.

CASE 1

A 39-year-old woman was admitted with a ten-day history of malaise, joint pains, sweating attacks and rigors. On examination she was pyrexial and the tip of the spleen was palpable and an Osler's node was seen on a finger. She had a mitral systolic murmur. Six sets of blood cultures were taken over 24 h and antibiotics were commenced using penicillin and gentamicin, a clinical diagnosis of infective endocarditis having been made. The temperature returned to normal within 24 hours of starting treatment and apart from a transient ischaemic attack on day 6 of treatment, no new symptoms or signs were noted.

Blood cultures were examined using the Bactec 460. On the fifth day of incubation one aerobic bottle gave a positive reading. Nothing was seen in the Gram film but *Haemophilus parainfluenzae* was grown in subculture the following day. All other blood culture bottles remained negative. All bottles were then subcultured onto conventional media and *Haemophilus parainfluenzae* was grown from a further two bottles from different sets on day 7. These two bottles persistently gave negative readings up to 21 days of incubation at which time one of the two bottles gave a low positive reading of 59 cpm. *Haemophilus parainfluenzae* was subcultured from both these bottles at 21 days of incubation.

CASE 2

A 28-year-old man with known mixed aortic valve disease presented with a three-

month history of a 'flu-like illness with a sore throat, malaise and generalised aches and pains in his limbs. On examination he had a temperature of 37.7°C, hepatosplenomegaly and murmurs of aortic regurgitation and stenosis. A clinical diagnosis of infective endocarditis was made.

Seven sets of blood cultures were taken over three days. These were all processed using the Bactec 460 system: All sets were positive in the aerobic bottles on the first day of reading showing Gram positive cocci on Gram film.

Culture of the blood on standard media failed to produce growth at 24 h but a very scanty growth was visible at 48 h. The organism was alpha-haemolytic on sheep blood agar and optochin resistant and was subsequently identified as *Streptococcus mitior*. The patient was started on intravenous benzylpenicillin and netilmicin.

Minimal inhibitory concentration of penicillin was not assayable because the organism would not produce turbid growth in either glucose broth or thioglycollate broth. It was then tested and found to be 0.1% cystein dependent due to a pyridoxine deficiency.

The recent departure from traditional methods to automated technology in medical microbiology has inevitably led to an assumption of the veracity of the machine. This was illustrated by the first case described in which all except one blood culture bottle were believed to be culture negative. This might have led to a failure to appreciate the significance of *H. parainfluenzae* in this case but a strong clinical suspicion overcame the inclination to believe the machine print-out.

The growing number of reports¹⁻⁴ of nutritionally fastidious organisms causing disease creates concern among microbiologists about the laboratory's capacity to detect routinely their presence in clinical specimens.

The second case provides reassurance about the performance of an automated blood culture system in this context. The inclusion of either pyridoxine or L-cystein blood culture in the medium supported growth of the organism while traditional sheep blood agar failed to support growth in subculture.

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Quantifying the microbial flora of the cervix

Many studies have been undertaken to define the indigenous bacterial flora of the female genital tract. The majority of these have been qualitative, based on the culture of material obtained from swabs of vagina or cervix. Quantitative studies are few because of difficulties in sampling and also because cervical mucus is both sparse and extremely viscous. It is not, therefore, readily amenable to accurate bacterial enumeration. The few quantitative investigations have assessed specimens obtained with sterile calibrated loops,¹⁻⁴ pipettes, swabs which are weighed before and after sampling.^{2,4,5} Difficulties have been encountered with all of these methods. We describe here a further technique for obtaining cervical secretions and quantifying the microbial flora at this site.

Cervical mucus is aspirated using a sterile disposable sampling syringe and aspirator (IMV—Aspiglaire, Ref UA311) which can be passed easily through the speculum without touching the walls of the vagina. The syringe is calibrated and a freshly prepared 10% solution of dithiothreitol (Sputolysin, Calbiochem/Behring Corp, La Jolla, California) is aspirated into it diluting the secretions in a ratio which may vary from 1 to 3:1, depending on the viscosity of the mucus. The syringe is then placed in a sterile test tube and the mixture alternately expelled and aspirated several times until it is liquefied and homogenous. Six serial tenfold dilutions are made in pre-reduced glucose broth and 10 µl samples of the undiluted specimen and each dilution are deposited in the form of drops or plates out, in duplicate, on appropriate media. After incubation colony types are described, counted, and isolated for subsequent identification. From the colony

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counts it is possible to calculate the number of each species present in the undiluted specimen.

In order to ensure that no organisms were being lost with this technique, endocervical swabs of five subjects were taken through a sterile Cusco's speculum at the same time as the aspirate. Each swab was broken into a bijou containing 2 ml of glucose broth and mixed vigorously by vortex mixer. The resultant suspension was diluted and plated out in an identical manner to the aspirate. After 48 h incubation the plates were examined and the isolates recovered by the two techniques were compared. In all five cases there was no qualitative difference in the organisms isolated. However, while the cervical flora obtained by aspiration can be accurately enumerated, that recovered from the swabs cannot since the volume of mucus on each swab is unknown.

Dithiothreitol is widely used in the liquefaction and homogenisation of sputum and has not been shown as inhibitory to the microbial flora of this secretion. Similarly, no antimicrobial activity was detectable following the treatment of cervical mucus. However, to exclude this possibility, a suspension of *Neisseria gonorrhoeae*, probably the most labile organism potentially isolated from the cervix, was prepared and diluted 1:1 with either dithiothreitol or glucose broth. The number of viable organisms in each suspension was estimated according to the method of Miles and Misra.⁶ No difference was noted. It is likely that since dithiothreitol does not inhibit the growth of the gonococcus it should not do so to any other more rigorous constituent of the cervical flora.

The method described is rapid, technically easy and precise. We recommend its use when quantitative assessment of the microbial flora of the cervix is required.

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Ring sideroblasts and myelodysplastic syndromes

In the May 1983 issue, Juneja *et al*¹ conclude that ring sideroblasts are not a distinctive feature of sideroblastic anaemia but "also seem to occur frequently in refractory anaemia with excess of blasts." Moreover, they state that their findings are in marked contrast to those reported recently by myself and colleagues,² since we found that none of the cases of refractory anaemia with excess of blasts had more than 5% ring sideroblasts. I believe that the conclusions by Juneja *et al*¹ are confusing. For example, faced with a patient having anaemia and 40% ring sideroblasts with 10% myeloblasts in the bone marrow, which diagnosis should one make? Refractory anaemia with excess of blasts and ring sideroblasts, or primary acquired sideroblastic anaemia? I will try to clarify this point.

In counting sideroblasts, it is of fundamental importance to distinguish between ferritin (or intermediate) sideroblasts and ring sideroblasts. High numbers of ferritin sideroblasts are usually found in patients with iron loading anaemias. Such patients have high transferrin saturation, increased proportion of diferric transferrin, and an iron supply to the erythroid marrow which is in excess of that required for haemoglobin synthesis. This excess iron is stored as ferritin in the cytoplasm of developing red cells, which therefore may contain two to 10 iron-staining particles.³ Many patients with myelodysplastic syndromes have iron overload and increased numbers of ferritin sideroblasts. On the other hand, ring sideroblasts are found in patients with impaired porphyrin synthesis, in whom the mitochondria become entrapped with iron. Precise criteria for the distinction between ferritin sideroblasts and ring sideroblasts have been defined by Hillman and Finch.³ The term sideroblastic anaemia should be reserved for anaemia with a significant

number of ring sideroblasts in the bone marrow,⁴ usually over 30%. Nevertheless, some haematologists call sideroblastic anaemia cases of refractory anaemia with ferritin sideroblasts. Apparently, Juneja *et al*¹ and we² adopted the same criteria for defining ring sideroblasts. Thus, the discrepancy between the two studies cannot be explained by different criteria in counting ring sideroblasts. Such discrepancy is due to different criteria in defining myelodysplastic syndromes.

In the Department of Internal Medicine, University of Pavia, Pavia, Italy, we have studied more than 70 cases of myelodysplastic syndromes. In 20 such patients we found more than 30% ring sideroblasts, and therefore we made a diagnosis of primary acquired sideroblastic anaemia. Three of these patients had also an excess of blasts in the bone marrow, and two of them eventually developed acute leukaemia. Juneja *et al*¹ would have called these cases refractory anaemia with excess blasts and ring sideroblasts.

In the other patients studied in Pavia, ring sideroblasts were almost absent. Due to difficulties in distinguishing between ferritin sideroblasts and ring sideroblasts in subjects with marked iron overload, in some cases 1 to 4% ring sideroblasts were recorded. Since these subjects had increased numbers of ferritin sideroblasts, I believe that also those recorded as ring forms were indeed of the ferritin type. Anyhow, all patients with refractory anaemia with excess of blasts had less than 5% ring sideroblasts. It should be considered that Dreyfus,⁵ revising 29 cases of the disorder he defined, found that morphological abnormalities in erythroblasts were not conspicuous, and ring sideroblasts could only sometimes be seen.

The recent effort by the FAB Group⁶ to formulate new diagnostic criteria for the myelodysplastic syndromes should be useful to remove at least part of the confusion presently existing in this field. I suggest that, as a further refinement, the category of primary acquired sideroblastic anaemia should be divided into two subcategories: sideroblastic anaemia with only erythroid abnormalities, and sideroblastic anaemia with abnormalities in other cell lines.

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