Laboratory contamination of blood cultures

R. C. SPENCER AND M. A. SAVAGE

From the Department of Bacteriology, Wright Fleming Institute, St. Mary's Hospital, London W2

SYNOPSIS A prospective study of the use of a laminar flow cabinet, an exhaust-ventilated safety hood, and the open bench for the microbiological examination of blood is described. Blood samples from 1600 patients were subcultured on the open bench, 2700 in a safety hood, and 2607 in a laminar flow cabinet. Use of the laminar flow cabinet produced a significantly greater level of contamination than the other methods, and it is concluded that the exhaust-ventilated safety hood should be used for this procedure.

In the bacteriological examination of blood, the most commonly used culture methods are pour plates (Muir and Ritchie, 1919; Stokes, 1955), broth media (von Haebler and Miles, 1938), and diphasic media (Castaneda, 1947). Improvements in technique have tended to emphasize the advantages and disadvantages of new media (Washington, 1972, 1975; Shanson, 1974; Rosner, 1970, 1974) and little, if any, attention has been paid to the ingress of microbial contaminants which can delay or even lead to an erroneous diagnosis (MacGregor and Beaty, 1972).

Contamination of the blood sample during venesection or on inoculation into the primary culture is beyond the control of laboratory personnel. However, when any cultures are opened for examination or subculture, the possibility of contamination increases. Traditionally, emphasis has been given to 'aseptic technique', the work being done on an open bench, and all operations being carried out close to the bunsen flame. In recent years, safety hoods and laminar flow cabinets have become commonplace, and blood culture work has been undertaken in both. We now report on blood culture contamination in such cabinets.

Material and Methods

BLOOD CULTURES

Each set comprises 50 ml of dextrose broth, 15 ml of Robertson's cooked meat medium, and a modified Castaneda slope, all with added penicillinase (Southern Group Laboratories, Lewisham). Each bottle has a screw cap, and in addition the dextrose broth and Castaneda bottles are fitted with viscaps. These are issued with an instruction sheet which

emphasizes the importance of strict asepsis. The dextrose broth and Castaneda's medium are inoculated with 4.0 ml of blood each, and the Robertson's media with 2.0 ml of blood. Cultures are incubated overnight at 37°C and examined. Using a standard wire loop, samples from the dextrose and Robertson's media are withdrawn for aerobic and anaerobic subculture on blood agar after 1, 5, and 14 days' incubation. At no time is the Castaneda bottle opened after initial inoculation unless obvious growth has occurred and subculture is necessary for identification.

LABORATORY ENVIRONMENTS

The number of blood culture sets examined on the open bench, in an exhaust-ventilated safety hood (LEEC, Colwick, Nottingham), and in a laminar flow cabinet (Microflow, Fleet, Hampshire) were 1600, 2700, and 2607 respectively. Air flow at the entrance to the safety hood was 120 linear ft/min and the laminar flow cabinet had a downwards directed air discharge on to the work area in the region of 90 ft/min. Within the safety hood and laminar flow cabinet it was necessary to use a smaller bunsen flame than that used on the open bench. Air currents in the vicinity of the bunsen flames were followed with titanium tetrachloride 'smoke'.

CONTAMINANTS

A contaminant was defined by MacGregor and Beaty (1972) on the basis of the genus of organism involved, a clinical assessment of the patient, the number of positive cultures, and a comparison of what was grown in a pour plate with that on the routinely employed media. In this study, in addition to their first three criteria, an assessment was based

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on the time the organism was first recorded in subculture. Only those contaminants isolated from the Robertson's media or dextrose broth were considered; organisms grown on the Castaneda slope were either clinically significant or the result of contamination beyond laboratory control.

Results

The total number of bottles examined and contaminated are shown in table I, the contamination rate of each medium in table II, and the relationship between contamination and time of subculture is given in table III. The number of contaminated bottles on the open bench was first compared with that obtained when the work was done under the safety hood, and then with the results from the laminar flow cabinet; with χ^2 values of 65.4 and 98.2 respectively the differences are highly significant ($P < 0.001$). The difference in contamination rates under the safety hood and laminar flow cabinet were similarly compared and with χ^2 4.76 again significant ($0.01 < P < 0.025$).

Place of work	Numbers examined		
	Sets	Bottles	Bottles Contaminated
Open bench	1600	3197	96 (3%)
Safety hood	2700	5393	386 (7%)
Laminar flow cabinet	2607	5214	440 (8.5%)

Table I Numbers of culture bottles examined and contaminated

Place of Work	Numbers examined		Numbers contaminated	
	Dextrose	Robertson's	Dextrose	Robertson's
Open bench	1610	1587	56 (3.5%)	40 (2.5%)
Safety hood	2700	2693	224 (8.3%)	162 (6%)
Laminar flow cabinet	2610	2604	242 (9.3%)	198 (7.8%)

Table II Contamination rate in each medium

Place of Work	At 24 hours	At 5 days	At 14 days
Open bench	14 (14.5%)	62 (64.5%)	96 (100%)
Safety hood	36 (9%)	231 (60%)	386 (100%)
Laminar flow cabinet	86 (19.5%)	312 (72%)	440 (100%)

Table III Relation between contamination and time of subculture

After the first subculturing the numbers of contaminants were greatest when the work had been

done under downwards directed laminar flow (table II).

The contaminating organisms encountered were similar at the three working places, and in order of occurrence were *Staphylococcus epidermidis*, 'diphtheroids', micrococcus, aerobic spore-bearing organisms, mixed cultures, and small numbers of streptococcus, *Staphylococcus aureus*, and *Enterobacteriaceae*. More 'diphtheroids' were encountered when subculturing was carried out under the hood and under laminar flow than when transfers were made near a bunsen flame on the open bench. No anaerobes isolated were regarded as contaminants.

Discussion

That the introduction of working in a safety hood and in a laminar flow cabinet should lead to a higher rate of contamination was unexpected. Indeed, laboratory procedures in laminar flow cabinets have been recommended by several authors (Favero and Berquist, 1968; Stokes, 1974), and it is probable that technical care was reduced as a consequence of working in positions where contamination should have been unlikely. Contamination rates after 24 hours' incubation were highest in the laminar flow cabinet. At this stage in the microbiological examination of blood contamination is particularly important since it is the most critical time with respect to clinical diagnosis and can lead to confusion and delay. The surface of the filter in the laminar flow cabinet was examined several times and found to be sterile on each occasion, thus ruling out the filters as a possible source of contamination.

The contamination rate of each medium (table II) showed that the dextrose broth was contaminated more often than the Robertson's medium. However, this was a constant feature in all three working environments. The possibility of a vacuum being present in the bottles when opened was also considered. It was found that this was present on very few occasions with the dextrose broth, a factor that may have contributed to the medium's greater contamination rate, but not enough to affect the overall results.

In microbiological subculture with traditional techniques, wire loops and bottle necks are sterilized by flaming. In the exhaust-ventilated safety hood, the heat from the bunsen had no discernible effect on air flow so that a flow of contaminating bacteria from outside into the bottles is unlikely (fig 1). However, in the laminar flow cabinet the heat generated by even the small flame employed resulted in a replacement of laminar flow by turbulence (fig 2). Therefore, other aseptic techniques would have to be employed, such as the use of sterile disposable

Fig 1 Heat from the lighted bunsen has no discernible effect on air flow in exhaust-ventilated safety hood.

Fig 2 Heat, even from small flame employed, results in turbulence in the laminar flow cabinet.

swabs for culture transfer and inoculation. Another way would be to use 'spirit' to clean the bottle caps and employ syringes to remove samples for sub-culturing.

Bacteria are displaced from the skin surface due to the friction of a sleeve cuff over the wrist (unpublished), and many of the contaminating organ-

isms occur in the normal flora of the skin. In the studies described, gloves were not worn and sleeves were not rolled up. It is most likely that the contamination rates would have been reduced if gloves had not only been worn but had covered the cuffs of the worker's gown or covered bare 'clean' arms.

Although the open bench gave the best results, a return to working close to a bunsen flame in this environment is unacceptable for safety reasons, especially where the isolation of *Brucella* is a possibility (Spencer, 1975), and when dealing with specimens from Australia antigen positive patients.

We therefore recommend the use of the exhaust-ventilated safety hood, the worker wearing gloves covering the cuffs of his gown. The use of a diphasic medium has much to recommend it, since contamination by subculture is avoided, and it may be repeatedly examined for so long as is necessary.

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