Improving the performance of anaerobic bacteriology in a hospital laboratory

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SUMMARY   A comparison was made of the performance of a newly established anaerobic section of a clinical laboratory and the routine diagnostic section in terms of isolation and identification of anaerobic bacteria. Both sections attempted to isolate obligate anaerobes from the same clinical specimens which were not transported in anaerobic containers. Anaerobic and diagnostic sections isolated anaerobes from 35% and 6% respectively of clinical specimens. The use of antibiograms greatly improved the identification of anaerobic organisms.

Improved isolation of anaerobic bacteria from clinical specimens has been related to improved methods of specimen transport, special techniques employed during the setting up of specimens in the laboratory, and improved methods of cultivation of anaerobic bacteria. There are a number of published reports which compare different protocols for the isolation of anaerobes from clinical specimens (McMinn and Crawford, 1970; Dowell, 1972; Killgore *et al.*, 1973; Rosenblatt *et al.*, 1973). In summary, these studies have examined the improvement in anaerobic isolation resulting from the use of anaerobic glove boxes and roll-tube culture methods. A consistent feature of the studies quoted is that clinical specimens were transported in anaerobic containers.

Thus, the clinical microbiologist, in order to upgrade the quality of anaerobic technology, is faced with the problem of selecting from a variety of complex and expensive procedures.

This is a report of our experience in anaerobic microbiology after the introduction of a number of simple procedures and an assessment of the resulting improvement in the capacity to isolate anaerobes. All innovations were confined to the laboratory and no attempt was made to change existing methods of specimen-taking or transport. All specimens were received in 'non-anaerobic' containers.

Material and methods

All clinical specimens from a 400-bed teaching hospital, except sputum, urine, throat, stool and

vaginal swabs, were submitted for anaerobic bacteriology. Swabs used in the hospital were Handiswabs (Fisher Scientific). Specimens were received and processed by the new anaerobic section of the laboratory as well as by the existing procedures in the routine diagnostic section which had, hitherto, performed both aerobic and anaerobic bacteriology on relevant clinical specimens. A comparison between the diagnostic and anaerobic sections of the results obtained provided an assessment of performance of the two sections.

ANAEROBIC SECTION

A portion of the laboratory was entirely devoted to anaerobic bacteriology and was staffed by a full-time qualified technologist.

The media used in the anaerobic section were 5% sheep blood agar (BA); menadione blood agar (MBA), which consisted of 5% sheep blood Columbia agar supplemented with vitamin K_1 (10 $\mu g/ml$) and haemin (5 $\mu g/ml$); neomycin-vancomycin blood agar (NVBA) which was menadione blood agar supplemented with 100 $\mu g/ml$ of neomycin and 7·5 $\mu g/ml$ of vancomycin; thioglycollate broth (Difco) supplemented with vitamin K_1 (10 $\mu g/ml$) and haemin (5 $\mu g/ml$). Prereduced agar media were not used.

A Gram stain was performed on every specimen. Upon arrival in the laboratory each specimen was immediately inoculated onto MBA, NVBA, and thioglycollate broth for anaerobic incubation and onto BA for aerobic incubation in 10% CO_2. Anaerobic cultures were incubated in anaerobic jars with Gas Paks (BBL) for two days. Palladium catalyst was reactivated daily by dry heating to 160°C

for two hours and, when necessary, stored over silica gel. Bacteria that grew in both aerobic and anaerobic conditions were disregarded. Colonies suspected as being obligate anaerobes were sub-cultured onto MBA and incubated anaerobically, and also onto BA for aerobic incubation in 10% CO_2. All subcultures were incubated overnight and read the following day. Further identification of obligate anaerobes was by Gram stain reaction colonial morphology and antibiotic sensitivity patterns, as described by Finegold *et al.* (1967, 1972). Sensitivity profiles were performed on 5% sheep blood Columbia agar containing 5 mg/ml vitamin K_1.

DIAGNOSTIC SECTION

In this section, specimens were investigated for the presence of aerobes as well as anaerobic organisms when appropriate. Media for the isolation of anaerobes included prereduced 5% sheep Columbia BA and thioglycollate broth (Difco). Inoculated media were incubated in gas jars with Gas Paks (BBL) for two days at 37°C. An obligate anaerobe was defined by its capacity to grow on the anaerobic blood agar plate and concomitant inability to grow on the aerobic blood agar plate. The thiogly-collate broth was examined daily for five days for visible evidence of growth and, when appropriate, subcultured onto prereduced BA for anaerobic incubation, and onto BA for aerobic incubation.

Identification of major genera rested on Gram stain reaction and colonial morphology.

Results

During the period of evaluation, 347 specimens were received for which anaerobic bacteriology was required. The regular diagnostic section of the laboratory isolated anaerobic bacteria from 21 of the specimens while the section devoted entirely to anaerobic work isolated anaerobes from 123 specimens of a total of 347 (Table 1). The regular

diagnostic section isolated anaerobic bacteria from 6/163 (3·6%) specimens of pus and from 15/148 (10%) cervical swabs, while the anaerobic section isolated them from 40/163 (24·5%) specimens of pus and from 82/148 (55·4%) cervical swabs. Furthermore, on no occasion did the diagnostic section isolate more than one species of anaerobe from a single specimen while the anaerobic section found 42 of 347 specimens to be polymicrobic (Table 1). A larger variety of organisms was identified by the anaerobic section (Table 1). Table 2 illustrates the distribution of the anaerobic species isolated. The time taken for a report to be issued was also analysed and, as shown in the Figure, there appeared to be no obvious difference between the two sections.

Table 2   *Distribution of 123 anaerobes isolated*

Organism	%
Bacteroides fragilis	33
Bacteroides melaninogenicus	11
Fusobacterium	6·4
Clostridium welchii	4·6
Veillonella	5·8
Peptococcus	21
Peptostreptococcus	10
Eikenella corrodens	1·7
Anaerobic nonsporing Gram-positive rods	4

Discussion

The purpose of this project was to achieve a better performance in the isolation of anaerobes from appropriate clinical specimens and to compare the efficiency of the newly introduced techniques with those already existing in the laboratory. The two salient findings were that a 6% anaerobic isolation rate by the traditional methods was unacceptably low and that the new methods produced an anaerobic isolation rate (37%) that was much improved and reached the proportions quoted in other publications (Zabransky, 1970; Martin, 1974). It was interesting to observe that the improvement was achieved without improving the methods of specimen collection

Table 1   *A comparison between the diagnostic and anaerobic sections of the anaerobic isolation rate and quality of identification*

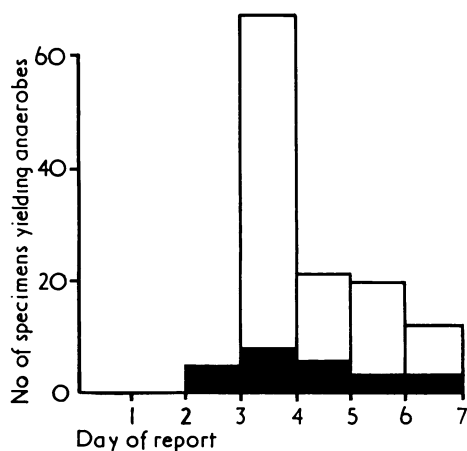|	Diagnostic section	Anaerobic section
No. of specimens yielding anaerobes	21/347 (6%)	123/347 (35%)
No. of specimens yielding two anaerobes	0	32/347 (9%)
No. of specimens yielding three anaerobes	0	10/347 (2·8%)
Organisms reported	*Bacteroides* sp.	*Bacteroides fragilis*
	Peptococcus	*Bacteroides melaninogenicus*
	Clostridium welchii (type A)	Peptococcus
		Peptostreptococcus
		Fusobacterium nucleatum
		Fusobacterium mortiferum
		Clostridium welchii (type A)
		Eikenella corrodens
		Veillonella

Figure *A comparison of reporting time by the diagnostic and anaerobic sections of the laboratory: open columns—anaerobic section; hatched columns—diagnostic section.*

and transport that are claimed to be essential for subsequent anaerobic isolation (Killgore *et al.*, 1973; Rosenblatt *et al.*, 1973).

The low isolation rate by the diagnostic section could in part have been the result of a reduction in the number of organisms on swab specimens after inoculation onto several media in the anaerobic section. This was not investigated, but, in future, all specimens taken in the hospital which will require aerobic and anaerobic culture procedures will be duplicated to avoid this possible dilution phenomenon. The anaerobic section was the first to receive specimens which were inoculated and immediately passed on to the diagnostic section. The transit time was 1 to 3 minutes between the two sections which is unlikely to have resulted in the death of a significant number of anaerobic organisms.

It was considered that an important factor which led to the improvement was the establishment of a section of the laboratory devoted entirely to anaerobic bacteriology. Furthermore, the use of menadione and haemin supplements in the MBA media together with the addition of neomycin and vancomycin in the NVBA medium was considered to have enhanced anaerobic isolation. It was theoretically possible that the anaerobic section's better performance was due entirely to the use of a selection medium (NVBAP) which inhibits bowel aerobic flora, thus allowing anaerobes to flourish. However, heavy contamination with faecal flora is unlikely with properly obtained cervical swabs and from these specimens the anaerobic section achieved higher isolation rates than the diagnostic section.

The comparison of anaerobic identification by the two sections was unfair since the methods used by the diagnostic section were rudimentary. However, the comparison was made to illustrate how much better identification may be achieved using the simple antibiograms of Finegold *et al.* (1967, 1972).

At the onset of the study, we were concerned that the new protocol for anaerobic isolation and identification would significantly delay the time taken to issue a positive report. However, it was found that, in the majority of specimens, a report with identification was available by the fourth day of receipt of the specimen and was a day earlier than in the case of the majority of specimens reported by the diagnostic section.

The information from this study suggests that the introduction of a specialised anaerobic section using media supplemented with menadione and haemin and identifying anaerobic isolates with the aid of antibiograms significantly improves the quality of anaerobic microbiology in a hospital laboratory. Gas chromatography and anaerobic glove boxes appear not to be essential at the onset for significantly improving the performance of anaerobic bacteriology in a routine clinical microbiology laboratory although we are now using the former as an important adjunct to the accurate identification of anaerobic bacteria.

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