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# The recovery of anaerobic bacteria from swabs

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## SUMMARY

When a standard sample of a simulated exudate containing known numbers of anaerobic bacteria was taken up on a swab and plated on solid medium, the number of colonies subsequently cultured represented a very small proportion of the original sample. Evidence is produced that the apparent loss is not primarily attributable to inactivation on the swab but rather to retention of organisms on the swab. This was demonstrable with *Clostridium welchii* and with *Bacteroides* species that have hitherto been regarded as relatively oxygen-sensitive.

When stock strains of *Bacteroides* species were held for some hours on swabs, some progressive loss of viability was demonstrable. A measure of protection was afforded when these organisms were held aerobically on blood agar medium, but a very exacting anaerobe and some wild strains of faecal anaerobes showed gradual inactivation under these conditions.

These findings may have important implications in relation to currently employed bacteriological sampling procedures with swabs in clinical practice.

## INTRODUCTION

It is generally accepted that, as a routine for the bacteriological sampling of wound exudates and similar material occurring in insufficient amount to be sent as frank pus in a container, serum-coated swabs should be used (Rubbo & Benjamin, 1951; Cruickshank, 1953); steam-sterilized swabs coated with bovine albumin have also been recommended (Bartlett & Hughes, 1969).

The present study was designed to assess the losses that may occur in sampling anaerobic bacteria from an exudate with a swab that is transported under aerobic conditions and is subsequently plated on solid media in the laboratory where the organisms may be subjected to further aerobic exposure pending anaerobic processing. Some of the results seem to be of general relevance to clinical aerobic and anaerobic bacteriology.

## MATERIALS AND METHODS

The test strains included *Bacteroides necrophorus* NCTC 7155 and 10575; *B. fragilis* NCTC 9343; *Clostridium welchii* (*Cl. perfringens*) Lab. nos. L2A and C1; *Cl. tetani* type VI, NCTC 9569; *Cl. oedematiens* type D, Lab. no. GR1D; and

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*Escherichia coli* Lab. no. M15. *Bacteroides*-like organisms isolated in Edinburgh from human wound exudates and faeces were labelled Gram-negative anaerobic bacilli (GNAB) 1–50. The simulated exudate was prepared as follows: A 0·02 ml. drop of a dilution of an overnight cooked meat broth culture of the test organism calculated to give some thousands of bacteria was mixed in a small ovoid area ($2 \times 1$ cm.) with four 0·02 ml. drops of sterile defibrinated horse blood (Wellcome) on a sterile plastic dish surface. The total volume (0·1 ml.) was absorbed on a sterile swab. The actual number of organisms in the diluted broth used for the simulated exudate was subsequently determined by a spread-plate surface viable count procedure.

Commercially available albumen-coated cotton-wool swabs on wooden sticks were used (Exogen Ltd, Dumbarton Road, Glasgow, W.4). These are supplied in plastic tubes; the same product originally labelled 'serum swab' is now labelled 'albu swab'.

Cultures of anaerobes were incubated at 37° C. in anaerobic jars with room-temperature catalysts (Baird and Tatlock Ltd) and a gas mixture of 90% hydrogen and 10% carbon dioxide (Collee, Rutter & Watt, 1971). Blood agar plates contained Columbia Agar Base (Oxoid) with 10% defibrinated horse blood (Wellcome); unless otherwise stated, the plates were used within a day of their preparation to simulate routine laboratory practice. Other media and special technical procedures were as detailed by Watt (1973).

## RESULTS

### *Development of the test model*

A known volume of broth containing a known number of organisms was mixed on a sterile surface with 4 volumes of sterile defibrinated horse blood to make a simulated exudate. The mixture (0·1 ml.) was then completely taken up on a sterile 'Exogen' serum-coated swab. In due course, a swab was plated on to half of the surface of a blood agar plate. The swab was rotated during the plating process to give a generous primary seeding of that area of the plate that bacteriologists call the 'well'.

### *The release of organisms from the swab to solid medium*

The test swabs yielded only a small proportion (3–5%) of their total viable load when they were used to prepare 'wells' in the course of seeding plates of blood agar medium. This was so when *Clostridium welchii* or various *Bacteroides* species or *Escherichia coli* were used and the finding was consistently reproducible. For example, swabs loaded with *ca.* 5000–7000 organisms of various *Bacteroides* species yielded *ca.* 100–300 colony-forming units to the plate, and plates seeded from swabs loaded with *ca.* 1500 *Cl. welchii* organisms yielded *ca.* 50 colonies. In the latter case, there was no doubt that vegetative cells were yielding colonies; there were less than 10 spores in the inoculum on each swab.

When the same loaded swab was used to seed a series of plates, there was a fairly reproducible dose given to each plate. Thus, a swab bearing an estimated

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Table 1. *The influence of delay and method of processing on the recovery of* Cl. welchii *from test swabs each bearing* 1500 *organisms*

| At time (hr.) | Viable counts from duplicate platings of test swabs — Plated by procedure* A | B | C |
|---|---|---|---|
| 0 | 36–57 | 44–59 | 252–297 |
| 1 | 27–32 | 14–28 | 225–315 |
| 4 | 46–49 | 26–37 | 324–378 |

* A, Normal swab plating; B, test swab moistened in 1 ml. sterile broth before plating; C, After B, swab was agitated in the remaining broth (0·9 ml.) of which 0·1 ml. was plated in duplicate; the mean number of colonies was then multiplied by 9 to give the total count of organisms released from the swab.

7350 *Bacteroides* organisms (strain GNAB 1) yielded 154, 124, 136, 172, 173 and 152 colony-forming units to 6 successive plates, but on some occasions there was up to a twofold variation in such counts.

*The effect of delay in plating the swab*

Series of swabs were loaded with the test inoculum in a standard manner and plated on blood agar at various times from 0–4 hr. later. Swabs were held in their plastic tubes on the bench (18–20° C) until plated. At each allotted time, a swab was used to seed 2 well areas on a plate; a second swab was moistened by dipping it in 1 ml. of presteamed sterile nutrient broth in a bijou bottle and the moistened swab was used to seed 2 well areas on a second plate; thereafter, the moistened swab was agitated in the remaining bulk of the 1 ml. of broth in the bijou and two 0·1 ml. volumes of that suspension were plated on replicate plates. Seeded plates were immediately incubated anaerobically. The results of these studies (Table 1) indicate that, with *Cl. welchii*, there was the usual low yield of organisms from a swab (loaded with 1500 bacteria) and this was not improved by moistening the swab before plating. The viable counts estimated from broths into which the swabs were expressed indicate that many organisms remained viable and that there was no obvious progressive inactivation in relation to time spent on the swab.

However, when these studies were extended with some *Bacteroides* strains, it was sometimes possible to demonstrate apparently progressive inactivation related to time spent on the swab. This effect varied with different strains and with the same strain at different times. Replicate swabs were loaded with standard inocula of *Bacteroides* species and plated at 0, 1 and 4–5 hr. In this series, we did not investigate the effect of moistening a swab just before plating, as this is not normal laboratory procedure. Immediately after a standard loaded swab had been used to seed four 'well' areas on plates, it was agitated in 1 ml. of sterile broth; on average, this procedure left 0·9 ml. of a broth suspension of organisms shed ('expressed') from the swab. Two 0·1 ml. volumes of that suspension were spread on replicate plates and the mean number of colonies then obtained was multiplied by 9 to give an 'expressed viability' count (EVC); i.e. the colony count for the 0·9 ml. broth

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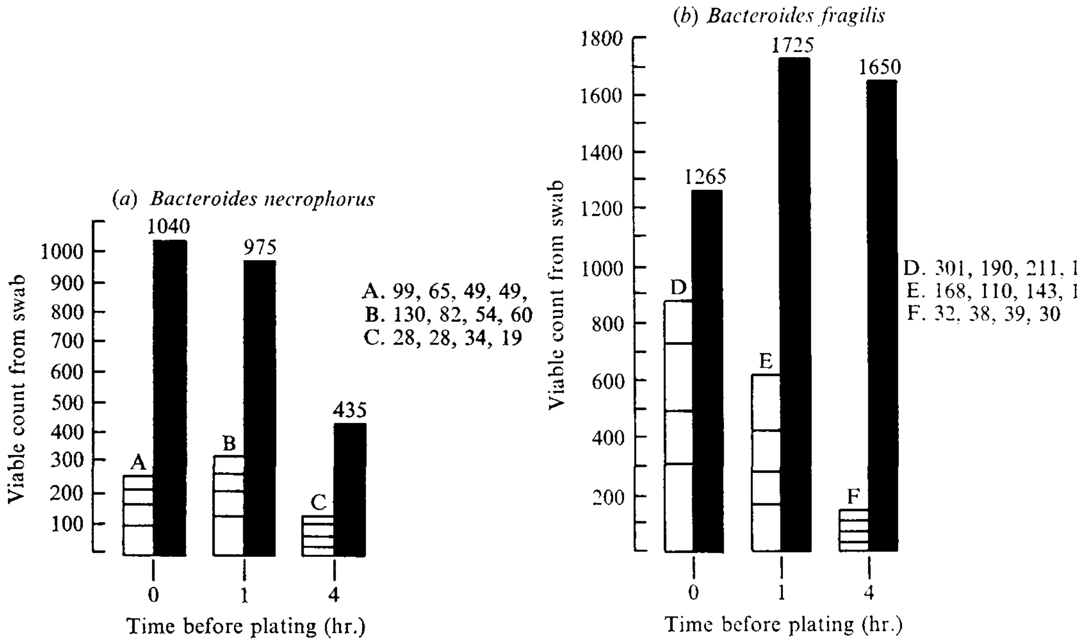


Fig. 1. Comparison of colony counts obtained from a test swab loaded with (*a*) 2860 *Bacteroides necrophorus* organisms, or (*b*) 7000 *Bacteroides fragilis* organisms. The yields from identical swabs plated immediately or held aerobically for 1 hr. or 4 hr. are shown thus □ and the actual counts for each plate are given at A, B, C, D, E, F. Each swab was used at the stated time to prepare four 'well' areas on plates. In each case, swabs were finally expressed into broth and viable counts of the resultant suspensions are shown thus ■ and given at the top of the column.

containing the organisms shed from the swab. The results of the experiments are summarised in Fig. 1. In both of these cases, the plated colony yield had decreased considerably when the delay in plating was 4 hr. The counts obtained from the expressed swab series indicate that *B. fragilis* remained essentially viable whereas the colony counts of *B. necrophorus* were much reduced and in each case the results obtained from plating the swab were much less than the maximum possible yield.

Similar experiments were then repeated by four different operators using replicate swabs each loaded with 6700 organisms of a standard strain of *B. necrophorus*. In general, the results (Table 2) show that replicate platings of a test swab yielded 3–5 % of the load to each well area, but counts varied by up to three-fold in some cases; an occasional high yield of up to 9 % was obtained, and low yields were sometimes less than 1 %.

In all cases, after a loaded swab had been used to prepare 4 well areas, the numbers of viable organisms that could be subsequently expressed from it into fluid (the EVC) indicate that significant proportions of the bacterial loads remained viable and were not released by the normal plating procedure. Thus, with swabs examined after immediate plating, 8–50 % of the original inoculum could be recovered by the expression procedure. With swabs plated after 1 hr., the

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Table 2. *The effect of delay in processing on recovery of* B. necrophorus *from test swabs**

| Operator no. | Colony counts in four replicate test well areas and the subsequently expressed viability count (EVC) at time (hr.) 0 | 1 | 4–5 |
|---|---|---|---|
| 1 | 280, 283, 323, 230<br>EVC: **1467** | 277, 242, 239, 214<br>EVC: **1948** | 47, 54, 30, 35<br>EVC: **675** |
| 2 | 184, 109, 74, 60<br>EVC: **500** | 192, 134, 114, 99<br>EVC: **1035** | 80, 60, 56, 46<br>EVC: **1048** |
| 3 | 175, 171, 150, 145<br>EVC: **1174** | 242, 132, 243, 189<br>EVC: **1570** | 166, 147, 87, 92<br>EVC: **882** |
| 4 | 280, 195, 178, 181<br>EVC: **3249** | 563, 375, 290, 242<br>EVC: **2898** | 412, 275, 307, 228<br>EVC: **1278** |

* Replicate colony counts and expressed viability counts are derived from swabs each loaded with a simulated exudate containing *ca.* 6700 organisms of *B. necrophorus* and held for periods of 0–5 hr. before processing.

'residual viability' was within that range (16–45 %), and after 4–5 hr. the expressed viability was diminished but was still about 10–20 % in terms of the original inoculum. When this complete series was repeated with the same strain and the same operators, these findings were generally reproducible. Inconsistently good recoveries occurred on two occasions in the expressed series at 4 hr. and one operator was occasionally able to transfer relatively high numbers of organisms to the plate. When the replicate experiments were done with *B. fragilis*, comparable results were obtained and the same sort of variation occurred. Moreover, it became clear that the sustained viability shown by the expressed count in the figure for *B. fragilis* at 4 hr. was not always demonstrable, and that the loss of viability shown for *B. necrophorus* at 4 hr. was not an invariable finding (see the data for operator 2 in Table 2). The variability is clear; the plate count results were obtained with proper attention to the enumeration of adequately large numbers and the expressed viable counts were derived from duplicate plates in each case. The important findings are that the yield obtained from a swab at any time is very poor with this model and that a considerable proportion of the apparent loss is not due to irreversible inactivation.

### *Non-toxicity of components of the test model*

It could be argued that some of the observed inactivation of bacteria on the test swab is attributable to a direct toxic effect of one of the components of the swab or the simulated exudate. Accordingly, identical test inocula of a sensitive *B. necrophorus* strain (NCTC 10575) were exposed for periods of up to 4 hr. at 18–20° C to broth extracts of swab materials, or to broth containing disintegrated swabs, with and without 80 % horse blood. The viable counts obtained were compared with those from control inocula that had been held under similar conditions in nutrient broth. In all cases, there was no demonstrable toxic effect when the test bacteria had been in contact with any of the swab materials, or



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extracts of the materials, or horse blood, or with any mixtures of these components. Indeed, the swab materials seemed to have some protective effect for the test bacteria in liquid suspensions.

*The effect of aerobic exposure of strict anaerobes on blood agar pending anaerobic incubation*

Quantitative studies with *Cl. oedematiens* type D indicate that inactivation of a seeded inoculum on blood agar may proceed quite rapidly (97—98 % loss within 90 min.) if the plates are held aerobically on the bench. For example, a series of standard inocula from an overnight cooked meat broth culture of *Cl. oedematiens* type D were spread on 20 plates of a freshly prepared special solid medium (see Watt, 1973) and immediately transferred to two anaerobic jars that were promptly processed and incubated. A parallel series of plates spread with identical inocula were held aerobically on the bench for 90 min. before being processed anaerobically. The first series gave mean colony counts of $239{\cdot}0 \pm 11{\cdot}1$ and $246{\cdot}6 \pm 9{\cdot}8$. The other series gave a mean count of $5{\cdot}2 \pm 2{\cdot}4$ for one jar and virtually no growth was obtained (2 colonies observed on a total of 10 plates) with the other jar; control plates excluded the possibility that 'jar failure' might account for these differences.

Results of similar studies with *Cl. tetani* and *B. fragilis* show that these organisms are significantly less sensitive than *Cl. oedematiens* under these conditions and there was quite consistent evidence of 'protection on the plate' for these test organisms. For example, replicate blood agar plates spread with essentially vegetative (non-sporing) standard inocula containing *ca.* 250 cells yielded *ca.* 160 colonies of *Cl. tetani* when they were promptly processed; after exposure for 24 hr. before anaerobic incubation, similarly seeded plates subsequently yielded *ca.* 100 colonies. Clearly, vegetative cells of *Cl. tetani* are able to survive aerobic exposure on seeded plates.

Similar studies showed that different *Bacteroides* strains seeded on plates of solid medium varied in their sensitivity to aerobic conditions. With all the test organisms, however, the seeded plates that had been exposed for up to 24 hr. before anaerobic incubation showed reductions of less than 50 % in the colony counts; with some, e.g. *Bacteroides* strains GNAB 7 or 21, there was no reduction in the numbers of colonies obtained on seeded plates held aerobically for 24 hr. before anaerobic incubation.

In studies of total anaerobes in dilutions of human faeces, there was again a considerable degree of survival during aerobic exposure of the seeded plates. In this special case, sterile blood agar plates were held anaerobically for up to 20 hr. so that they were 'reduced' before receiving the inocula. Such a procedure might help to minimize losses attributable to transient aerobic exposure. Despite this precaution in these latter studies without swabs, it is clear that a gradual process of inactivation of wild strains of bacteria taken from a natural environment may start as soon as sampling on plates is begun (Table 3) and there is therefore a case for prompt processing. However, the losses that take place at this stage of the processing are very slight compared with those that we have associated with the use of the swab.

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Table 3. *The effect of aerobic exposure of seeded plates bearing standard inocula of diluted faecal suspension on the colony counts obtained after subsequent anaerobic incubation*

| Time of exposure of seeded plates (hr.) | Mean colony counts ± S.E.* |
|---|---|
| 0 | 513·5 ± 36·9 |
| ½ | 503·5 ± 33·6 |
| 1 | 455·8 ± 22·3 |
| 2 | 432·2 ± 19·0 |
| 4 | 387·4 ± 12·1 |
| 7 | 401·8 ± 12·4 |
| 24 | 249·1 ± 12·4 |

* Each colony count is expressed as the mean ± standard error (S.E.) of the mean derived from 10 replicate plates of prereduced blood agar, each seeded with 0·02 ml. inocula of a ($8{\cdot}0 \times 10^5$) dilution of human faeces.

## DISCUSSION

Many workers have considered possible mechanisms of inactivation of organisms sampled on swabs and most have taken account of potentially toxic primary or secondary factors in the swab material or in the stick (Pollock, 1947, 1948; Rubbo & Benjamin, 1951; Beakley, 1957; Rowatt, 1957; Anderson, 1965; Mair & McSwiggan, 1965; White, 1965; Dadd, Dagnall, Everall & Jones, 1970).

Deleterious influences such as desiccation and overgrowth and other aspects of loss or inactivation in transit have been studied (Cooper, 1957; Crookes & Stuart, 1959; Huet & Bonnefous, 1960; Bartlett & Hughes, 1969; Dadd *et al.* 1970) and various transport media have been developed for delicate organisms (e.g. those of Venkatraman & Ramakrishnan, 1941; Stuart, 1946, 1956; Cary & Blair, 1964). When especially delicate organisms are involved, the culture medium is sometimes seeded directly from the patient and promptly incubated, as is the practice with some cases of gonococcal infection for example. It is surprising that analogous precautions are not routinely associated with the submission of specimens that may contain strictly anaerobic pathogens, particularly when there is a degree of clinical urgency.

Anaerobic infections occur fairly commonly in hospital practice; as laboratory help with these cases is often slow or inadequate, our traditional bacteriological procedures should be critically re-appraised. The laboratory model for the swab studies that we have developed might be challenged on several grounds. Relatively small numbers of organisms from a stationary-phase pure culture were held on a non-toxic swab in a simulated exudate, whereas there might be physiological, immunological and chemotherapeutic factors operating separately or in combination in a 'natural' exudate; moreover there may be bacterial competition within the mixed flora that often occurs naturally. Such important reservations indicate a real requirement for relevant clinical data, though quantitative studies with clinical material along the lines of the present work will be difficult. Meanwhile, several important points emerge from the present study.



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The general finding is that, when a simulated exudate containing some thousands of anaerobic organisms was taken up on a swab and plated on solid medium, the yield in terms of the numbers of colonies that we could grow was only about 3–5 % of the sample. This is not an index of toxicity of the swab for the delicate anaerobes that we used. The test swab was non-toxic and the finding was reproducible with an unexacting aerobic organism included in our tests. When a similar test swab was successively plated on separate plates, it continued to release about 3–5 % of its load in terms of colony-forming units per plate for several plates. Thus, the swab appears to be an inefficient sampling device if the aim is to get as many organisms as possible on to the first plate and this may be of importance for the success of a primary culture. In turn, a primary culture is essential if a really prompt diagnosis is to be made in many cases. However, if our aim is merely to get a sample dose on several plates, the swab has some merit – provided that 3–5 % of its load provides acceptable numbers of colonies. It seems that the usual laboratory practice of finally seeding the swab into broth, or breaking it off into the broth, is well based – though this procedure does not allow prompt bacteriological diagnosis and usually leads to the complications that attend the selection of likely pathogens from mixed cultures.

When the loaded swab was agitated in 1 ml. of sterile broth, we could actually express considerably better numbers of organisms from it and we could show that these were viable. This finding is of immediate importance as it illustrated that at least a proportion of the losses in transit that might be attributed to death of delicate organisms is really attributable to non-release of organisms onto plates (i.e. retention on the swab). In addition, our results suggest that some species progressively die on the swab exposed to aerobic conditions. It may be that such 'death in transit' is due primarily to desiccation rather than to aerobic inactivation and we are investigating the various factors involved in such losses. As the swab yielded relatively few colonies to the solid medium in our studies and as its load loses viability in transit, the traditional procedure of plating a swab on a series of primary plates seems to have little chance of prompt success unless large numbers of organisms present in an exudate are plated promptly. There might be a good case for prompt subculture from a very young anaerobic broth culture after about 4–8 hr. incubation, so that the swab's potential is fully exploited before overgrowth with a mixed flora obscures the important evidence.

The fact that some strictly anaerobic bacteria can survive aerobic exposure when seeded on plates pending anaerobic incubation is of considerable interest, but we report this with caution lest it is taken as a general excuse for further delay in the laboratory. Although the results of our current studies (Watt, Collee & Brown, to be published) indicate that ultraspecialized equipment such as an anaerobic cabinet does not seem to be necessary for the laboratory processing of strict anaerobes, we stress the importance of prompt and careful attention; the possible adverse effects of delays in conventional processing are clearly evident in the present work.

It is generally accepted that work with anaerobic bacteria is difficult. Our present evidence suggests that we may start at a disadvantage when we use a

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swab; we may greatly add to the disadvantage of accepting a 'normal' delay in transit while the inoculum is not protected on the swab; and we may further increase the disadvantage when we process the swab on primary plates in the traditional manner.

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