

# Velvet pad surface sampling of anaerobic and aerobic bacteria: an in vitro laboratory model

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**SUMMARY** Velvet pads have been evaluated in an experimental, laboratory model, simulating intraoperative sampling of *Staphylococcus epidermidis*, *Escherichia coli* and *Bacterioides fragilis*. After sampling, the pad was placed in a transport medium and kept in an anaerobic atmosphere, before being shaken and rinsed, followed by anaerobic and aerobic culture. This technique permitted quantitatively high recoveries of the test bacteria. Velvet pad sampling could be a measure to determine the density of aerobic and anaerobic bacteria during operation in an effort to predict the risk of postoperative wound sepsis.

Different types of wounds—for example, the open (traumatic) wound, the burn wound, a graft bed or the operative incision represent different problems in sampling for quantitative bacteriological culture. This laboratory has had an interest in following in particular the course of the operation wound.<sup>1,2</sup> Postoperative wound infections are initiated primarily during operation as a result of contamination with bacteria from different sources.<sup>3-5</sup> However, relatively few investigators have obtained quantitative cultures from the operative incision.<sup>6-10</sup> For this purpose Raahave<sup>2</sup> has used velvet pads, since areas rather than volumes (biopsies) should be sampled, because no deeper penetration by bacteria can have occurred at that time.<sup>11</sup>

It is widely recognised today that anaerobic organisms, especially *Bacteroides fragilis* are implicated in many wound infections.<sup>4,9,10,12</sup> We therefore found it of great interest to investigate whether anaerobic bacteria, in analogy with aerobic, could be sampled quantitatively by velvet pads. An experimental laboratory model was used, mimicking sampling from operation wounds. We studied the influence of the different test bacteria, transport medium, detergent and centrifugation.

## Material and methods

### VELVET PADS

Pale, boiled-out velvet with a close-knit pile was cut into strips of 2.0 × 4.5 cm and the back was glued to

aluminium foil with a water- and heat-resistant contact adhesive.<sup>2,13</sup> The foil extending at both ends allowed the pad to be handled without touching the velvet itself. The pads were heat-sterilised, and were moistened in 0.9% sterile NaCl immediately before use.

### ORGANISMS

Three strains were used: *Staph epidermidis* (CCM 2434), *B fragilis* and *E coli*.

### MEDIA

The test plates contained an agar medium with 5% defibrinated horse blood, 0.35% glucose, 0.001% fatty acids C<sub>12-18</sub>, 0.8% Na<sub>2</sub>HPO<sub>4</sub>·12 H<sub>2</sub>O, 0.67% KCl, 0.25% Nz-amine type B (Sheffield Chemicals) and 0.125 yeast hydrolysate (Orthana). The plates were prerduced before use. The transport media were sterile 0.9% NaCl or peptonewater (peptone Orthana 40 g, NaCl 5 g and distilled H<sub>2</sub>O to 100 ml with 2 g glucose and 10 ml 5% Liquoid Roche added after autoclaving).

### SAMPLING PROCEDURES

Experiments were carried out by a standardised procedure (Fig. 1). Test suspensions were prepared from overnight serum broth cultures (except *B fragilis*: 48 h) by diluting with serum to 1/10<sup>-4</sup>. The blood agar surface was flooded with 1 ml of bacterial suspension and excess liquid was removed by a Pasteur pipette. When visible moisture had disappeared, a sterile velvet pad was applied firmly to

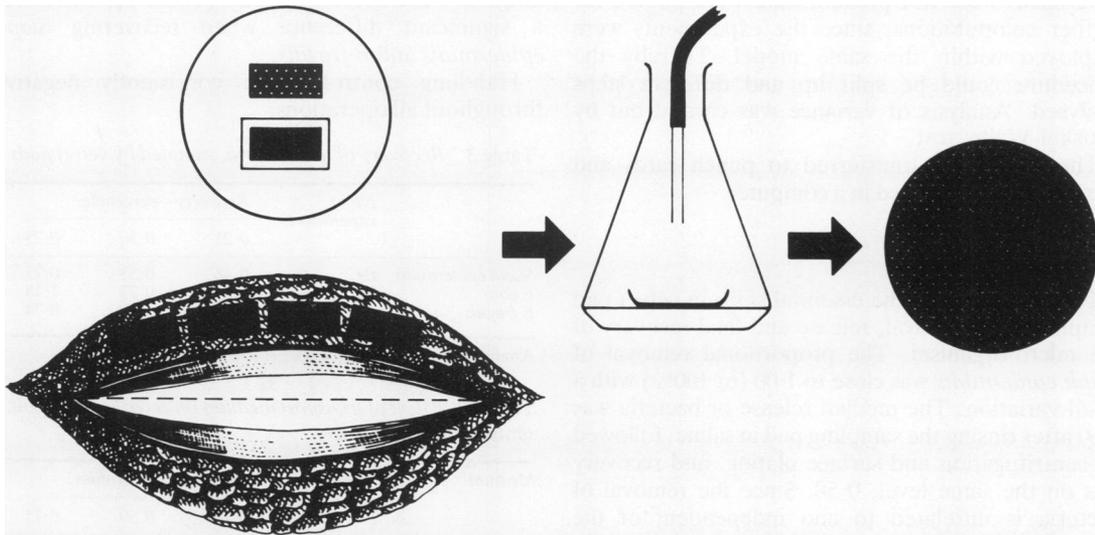


Fig. 1 The experimental laboratory model, mimicking sampling of bacteria from operation wounds by velvet pads.

the inoculated test surface with an even pressure for 2–3 s. Sterile gloves were worn. After sampling, the velvet pad was transferred to a 250 ml Erlenmeyer flask containing 10 ml sterile transport medium. In the experiments with *B fragilis* carbon dioxide was led into the flask through a Pasteur pipette, after which the flask was capped with a cotton plug and aluminium foil. The flask with its contents was agitated in a Gallenkamp shaker for 10 min, after which the fluid was centrifuged at 3000 rpm for 30 min (Sorvall). The supernatant was removed and the deposit was spread on the plates and incubated aerobically and anaerobic. In some experiments centrifugation was not done, but aliquots of 1 ml were spread directly after shaking.

The detergents tested were Triton X-100, 0.1% and Tween-80, 0.5%.

In all experiments, control of sterilisation and contamination was carried out by taking velvet pads through all procedures except for the sampling step.

#### COUNTING AND CALCULATIONS

The following counts (Abacus counter) were made (i) control colony forming units (CFU),  $N_0$ , through a template with an aperture the size of one pad (Fig. 1) (ii) residual CFU,  $R_1$ , at the site of velvet pad sampling (Fig. 2) and (iii) final CFU,  $I$ , from the final spread. Removal, release and recovery efficiencies were calculated by using the following equations:

$$\text{Removal} = \frac{N_0 - R_1}{N_0}$$

$$\text{Release} = \frac{I}{N_0 - R_1}$$

$$\text{Recovery} = \frac{N_0 - R_1}{N_0} \times \frac{I}{N_0 - R_1} = \frac{I}{N_0}$$

Distribution-free statistical terms were used. Calculations were carried out at first within the experiments. Wilcoxon's test (paired data) and Mann-Whitney's test (unpaired data) were used<sup>14</sup> to examine the significance of differences. Where

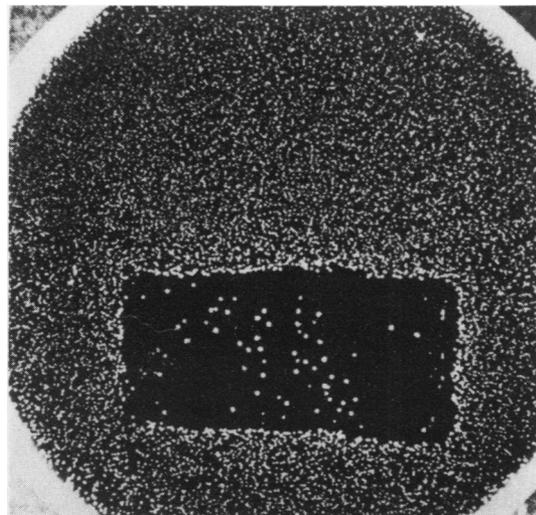


Fig. 2 Residual colonies of *B fragilis* after velvet pad sampling and subsequent anaerobic incubation.

differences were not proven, data were pooled for further computations, since the experiments were employed within the same model. Thereby the procedure could be split up and different steps analysed. Analysis of variance was carried out by Kruskal-Wallis' test.

The data were transferred to punch cards and subsequently processed in a computer.

## Results

Table 1 summarises the essential steps in velvet pad sampling: the removal, release and final recovery of the micro-organism. The proportional removal of *Staph epidermidis* was close to 1.00 (or 100%) with a small variation. The median release of bacteria was 0.60 after rinsing the sampling pad in saline, followed by centrifugation and surface plating, and recovery was on the same level, 0.58. Since the removal of bacteria is unrelated to and independent of the procedures following removal (as also seen from the formula), median removals (0.91 to 0.96) have been compared for different bacteria in Table 2. The pooled value was 0.95 and no significant differences were found. In contrast, significant differences were seen in final recoveries of the test bacteria (Table 3).

The recovery of *E coli* after rinsing the pad in saline or in peptonewater is compared in Table 4; no significant differences were found.

The effect of adding a detergent to the transport medium is shown in Table 5. There was an equal and significant rise in recovery of *Staph epidermidis* when Tween-80 and Triton X-100 were added to saline.

Whereas the recovery of *E coli* did not differ significantly when centrifugation took place or 1 ml

aliquots were spread directly (Table 6), there was a significant difference when recovering *Staph epidermidis* and *B fragilis*.

Handling controls were consistently negative throughout all operations.

Table 3 Recovery of test bacteria, sampled by velvet pads

	No of experiments	Recovery—percentiles		
		0.25	0.50	0.75
<i>Staph epidermidis</i>	89	0.48	0.58	0.73
<i>E coli</i>	30	0.39	0.73	1.15
<i>B fragilis</i>	20	0.30	0.48	0.74

Analysis of variance:  $p < 0.05$ .

Table 4 Effect of transport medium on recovery of *E coli*, sampled by velvet pads

Medium	No of experiments	Recovery—percentiles		
		0.25	0.50	0.75
Saline	30	0.39	0.73	1.15
Peptonewater	20	0.23	0.75	1.41

Mann-Whitney test:  $p > 0.05$ .

Table 5 Effect of detergent on recovery of *Staph epidermidis*, sampled by velvet pads and subsequently rinsed

	No of experiments	Recovery—percentiles		
		0.25	0.50	0.75
No detergent	15	0.34	0.47	0.52
Triton X-100	15	0.52	0.79	0.98* †
Tween-80	15	0.71	0.80	0.99* †

\*  $p < 0.01$ , as compared to no detergent (Wilcoxon test).

†  $p > 0.05$  (Wilcoxon test).

Table 6 Effect of centrifugation on recovery of *Staph epidermidis*, *E coli* and *B fragilis*, sampled by velvet pads

	No of experiments	Recovery—percentiles		
		0.25	0.50	0.75
Centrifugation				
<i>Staph epidermidis</i>	15	0.68	0.80	1.04*
<i>E coli</i>	20	0.23	0.75	1.41†
<i>B fragilis</i>	20	0.30	0.48	0.74‡
No centrifugation				
<i>Staph epidermidis</i>	15	1.12	1.27	1.65
<i>E coli</i>	19	0.46	0.85	1.54
<i>B fragilis</i>	20	1.06	1.65	2.20

Centrifugation v no centrifugation.

\*  $p < 0.01$ , as compared to no centrifugation (Wilcoxon test).

†  $p > 0.05$ , as compared to no centrifugation (Mann-Whitney test).

‡  $p < 0.01$ , as compared to no centrifugation (Mann-Whitney test).

Table 1 Removal, release and recovery of *Staph epidermidis* after velvet pad sampling

	No of experiments	Percentiles		
		0.25	0.50	0.75
Removal	89	0.93	0.96	0.98
Release	89	0.50	0.60	0.78
Recovery	89	0.48	0.58	0.73

Table 2 Proportional removal of test bacteria after sampling with velvet pads

	No of experiments	Proportional removal—percentiles		
		0.25	0.50	0.75
<i>Staph epidermidis</i>	133	0.94	0.96	0.97
<i>E coli</i>	70	0.91	0.96	1.00
<i>B fragilis</i>	40	0.86	0.91	0.93
Total	255	0.91	0.95	0.97

Analysis of variance:  $p > 0.05$  (Kruskal-Wallis test).

## Discussion

Blood agar plates were freshly seeded with aerobic and/or anaerobic bacterial cells, mimicking an

operation wound contaminated with bacteria. *Escherichia coli*, *B fragilis* and a staphylococcus species were chosen as test bacteria, since these organisms are important pathogens in postoperative wound infections.<sup>1 3 4 9 12</sup> A uniform finding was the very effective removal of bacteria, from 91 to 96%. This is in contrast to the results by Lederberg and Lederberg,<sup>15</sup> who found that only 10 to 30% of the initial cells were taken up by the velvet. It seems that the type of velvet used here greatly facilitates the removal of bacteria, irrespective of species, probably because of adhesive forces. In order to release the bacteria the velvet pad was shaken and rinsed in the medium. In experiments with cotton swabs, rinse techniques have similarly been shown release enhancing.<sup>16 17</sup>

Several studies have shown that avoiding desiccation of bacteria after sampling seems much more important than the type of transport medium used, if bacteria are to survive.<sup>18-20</sup> As the aim was to extend the method to clinical use only two media were compared, sodium chloride and peptonewater, and no difference was found between the high recoveries. It was also shown that a detergent could further improve the recovery rate, probably due to an enhanced release of bacteria from the sampling velvet pad.

When we started the experiments anaerobiosis was not produced during the initial sampling step, except for rendering the flask oxygen-free by flushing with carbon dioxide.<sup>21 22</sup> However, the aerotolerance of— for example *B fragilis*, seems relatively high, as shown by equal recovery rates, even after a six-hour exposure to oxidised fluid.<sup>23</sup> Finally, centrifugation and resuspension of the sediment followed by surface plating were compared to a more simple manoeuvre of spreading 1 ml aliquots directly onto the agar plates. The recovery rates of *E coli* did not differ significantly. However, incorporating centrifugation seems to lower the recovery of *B fragilis*, perhaps because of aggregation of bacterial cells. On the other hand, plating 1 ml aliquots, the recoveries exceed the optimal 1:00.

There are no elaborate methods to determine adequately the true number of bacteria in the operation wound. Swabs have been used widely, but are inadequate to indicate the true degree of contamination.<sup>5 24-26</sup> While burn and traumatic wounds have been sampled by impression gauze<sup>27</sup> or by homogenised tissue samples<sup>28</sup> these methods have apparently not been used for operation wounds. However, quantitative sampling of such wounds has until recently been done also by irrigation procedures.<sup>6 9 10</sup> Clearly, there is a need for quantitative methods to measure the degree of contamination, not only manifest infection. We

think, that velvet pad sampling could probably contribute to fill this gap.

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