New Technique for Quantitative Bacteriological Sampling of Wounds by Velvet Pads: Clinical Sampling Trial

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A new surface sampling method, the velvet pad rinse technique, was compared with the velvet pad imprint method, in a randomized bacteriological sampling trial, using surgical wounds to evaluate quantitative results. The efficiency of bacterial recovery was increased 20-fold by the new technique, and the improvement was ascribed in particular to the introduction of a procedure of rinsing the velvet pad after sampling.

The importance of bacterial quantitation for the clinical approach to treatment has been emphasized during the last decade. There undoubtedly still remains a need for quantitative surface sampling methods, even though there is the gauze capillarity method of Brentano and Gravens (3) and a moist swab culture method, described by Georgiade and co-workers (5, 6), both methods being capable of providing quantitative values for the recovery of bacteria in burn wounds. Velvet pads were used clinically for the first time by Gorrill and Penikett (7), who sampled the bacterial flora of infected wounds, but the study was not quantitative. The normal skin has since been sampled quantitatively by velvet pads, followed by imprinting onto culture plates (8, 9), and the method has also been employed for the quantitation of bacteria in surgical wounds (13).

A new technique, the so-called velvet pad rinse (VPR) technique, was recently evaluated in laboratory experiments to yield a constant recovery rate of bacteria of 61% (D. Raahave, *Acta Pathol. Microbiol. Scand. Sect. B*, in press), and the present paper reports a clinical sampling trial initiated to compare this improved technique with the conventional velvet pad imprint (VPI) method.

MATERIALS AND METHODS

Velvet pad sampling and culture techniques. Two groups of patients were selected at random and examined peroperatively, 24 patients by the VPI method and 27 patients by the VPR technique. The sampling pads were sterile velvet pads, 2.0 by 4.5 cm (Fig. 1), backed by aluminium foil with a water- and heat-resistant contact adhesive, UHU; the foil was extended at both ends, allowing the pad to be handled without touching the velvet itself. After the incisional wound was completed surgically and prior

to entering bacteria-containing viscera, I sampled the two wound surfaces by imprinting the sterile pads, moistened in sterile saline before use. As high a proportion of the entire wound area as possible was sampled in every instance, the unsampled area being less than one velvet pad on each side. After sampling, the velvet pad was either imprinted onto a blood agar plate (VPI method) or transferred to a 300-ml Erlenmeyer flask with 0.9% sterile sodium chloride (VPR technique). The flask with its contents was agitated mechanically in the laboratory for 10 min, after which the fluid was centrifuged at 3,000 rpm for 30 min. The supernatant was removed, and the deposit was resuspended and finally spread by a bent glass rod over a blood agar plate (blood agar base [Oxoid], 4% and glucose, 0.1%, in demineralized water, pH 7.5 after autoclaving, containing defibrinated horse blood, 5%, and yeast extract [Difco], 0.75%, dispensed into 14-cm plastic petri dishes). The exact viable count of each plate was then determined after aerobic incubation at 37 C for 48 h.

All patients were sampled in the same operation ward, and no changes were made in the aseptic or antiseptic policy, which I observed and recorded. The preparation of the operation site was carried out in the operating theater immediately prior to surgery. The skin was first cleansed with an aqueous solution of cetrimide (0.15%, wt/vol and chlorhexidine 0.015%, wt/vol [Savlon]) for 3 min and then disinfected with 0.5% (wt/vol) alcoholic solution of chlorhexidine (Hibitane), applied twice (12; M. L. Nielsen, D. Raahave, J. G. Stage, and T. Justesen, *J. Clin. Pathol.*, in press). All room personnel followed the established procedures for use of the sterile gowns, caps, masks, and gloves, and the entire surgical team, including myself, scrubbed with a disposable brush and sponge containing a detergent solution and 3% hexachlorophane (E-Z-scrub).

All wound samples were taken under strictly aseptic precautions, and velvet pads were included for control of sterilization and artificial contamination during sampling and laboratory handling of the

FIG. 1. Sterile velvet pads, 2.0 by 4.5 cm, backed by aluminium foil, ready for use.

pads. These were cleansed and sterilized for re-use if not damaged. No disinfectant inactivator was used, since no suitable antagonists exist (12). None of the patients received antibiotics prior to or at the time of sampling.

Biostatistics. The viable count of each plate was the yield of one velvet pad, whether the imprint method or the rinse technique was followed. The degree of bacterial contamination for the single operation wound was expressed at the bacterial density: viable count per wound area sampled (square centimeters), denoted as viable counts $\times 10^{-1}$ per square centimeter. The sample distributions of bacterial densities were characterized by the median, and comparisons were made by distribution-free statistical tests (2), as these do not involve any assumption that the distributions from which the samples are drawn at random are normal.

RESULTS

The VPR technique yielded bacterial growth in 125 out of 163 samples (76.7%), whereas this was the case in 47 out of 192 samples (24.5%) for the VPI method. Twenty-three samples were included as indicators of sterilization and artificial contamination of the velvet pads during handling in the operating theater and laboratory (VPI method); none of them showed any growth, whereas 15 out of 27 samples (55.6%) showed no growth, 11 samples (40.7%) yielded 1 to 2 viable counts, and one sample yielded 3 viable counts by the VPR technique.

Figure 2 shows the bacterial densities of surgical incisions sampled by the two methods. After investigation by the VPI method, 6 out of 24 surgical wounds had zero bacterial density, but this was never the case after sampling 27 wounds by the VPR technique. The median bacterial density increased from 3.0×10^{-2} viable counts/cm² (VPI method) to 5.9×10^{-1} viable counts/cm² (VPR technique), a highly significant difference at $P < 0.001$ (Mann-Whitney test) and a 20-fold increase.

DISCUSSION

The surgical incision required for any main surgical intervention has been shown to be contaminated initially with bacteria from exogenous (immediate environmental) sources, irrespective of the type of operation to be performed or of the patient in general (13). It is therefore a reasonable assumption in the present study that no bacteriological differences existed between the wounds other than those accounted for by chance at the moment of sampling, whether by the VPI method or the VPR technique. The latter improved the bacterial recovery by as much as a factor of 20, the sampling step itself remaining unchanged, being derived

FIG. 2. The quantitative yield (viable counts/square centimeter) from surgical wounds examined peroperatively after incision by the VPI method (left) and the VPR technique (right). The ascending line shows the improvement in median bacterial density.

from the replica plating technique of Lederberg and Lederberg (10). Recent quantitative laboratory experiments (D. Raahave, in press) showed an increase in the bacterial recovery rate of *Staphylococcus epidermidis* from 2% (VPI method) to 61% (VPR technique), a factor of 30.5. This improvement was due to an enhanced release of bacteria from the velvet pads by the shake and rinse procedure, and not to breaking up clusters of bacteria. The clinical and experimental figures thus agree well, since in clinical sampling, by comparison with the experimental use of bacteria in pure cultures, a loss in efficiency would be expected due to less viable or damaged bacteria and to the presence of different species.

Only a few previous investigators have indicated the magnitude of bacterial contamination of surgical wounds. Taylor (15) found that an average of 103 bacteria was removed by a first-wound washing with saline during clean operations, but no definite wound sizes were reported. Also, by irrigation, Lilly et al. (11) found a mean viable count of 25.3 in surgical incisions at the commencement of femoral nail-plate insertions, and on the assumption of wound sizes equal to those in the present series the degree of contamination can be calculated as 3.5×10^{-1} viable counts/cm², agreeing well with the bacterial densities estimated by the VPR technique. Likewise, where only 18 to 25% of the surface of the surgical wound was sampled by Rodac plates in an air flow study (1) (total hip replacements), the density of bacteria can be calculated as an average of 2.3×10^{-1} viable counts/cm².

The quantitative comparison of the VPI method and the VPR technique was carried out by counting aerobic bacteria. The anaerobic flora could probably also have been sampled by means of velvet pads, if immediately after sampling these had been transferred to a tube with a prereduced medium in an oxygen-free, carbon dioxide atmosphere, for transportation to the final anaerobic cultivation in a "glove box." Anaerobic skin bacteria were recently sampled in this way (M. L. Nielsen, D. Raahave, J. G. Stage, and T. Justesen, *J. Clin. Pathol.*, in press).

Favero et al. (4) have recently pointed out the difficulties connected with the determination of "naturally" occurring microbial contamination and the interpretation of the numerical results, where the true numbers of contaminants are not known. Consequently, the aim was to sample the total surgical incision area. The swab-rinse method continues to be widely used in clinical work, although quantitative results are difficult to achieve because a definite area can-

not be sampled uniformly. Brentano and Gravens (3) and Georgiade and co-workers (6), in their clinical research on burn patients, have emphasized the importance of being able to express the results as the numbers of bacteria recovered per unit area (in square centimeters). This principle was followed in the present, which is the first, clinical assessment of the VPR technique, which linked together the ease of a contact method and the high efficiency of bacterial recovery of a rinse technique. The preparation of the velvet pads demands no special effort, and they are conveniently packed and sterilized in plastics bags (Medi-Plast), ready for immediate use.

Open wounds, including burns, have been studied quantitatively by means of biopsies (14), but as far as surgical wounds are concerned quantitative sampling from the surface peroperatively seems to be efficient, since bacteria do not invade the tissues as soon after incision.

Efficient enumeration of bacteria is important, as it permits evaluation of prophylactic precautions to prevent contamination of wounds and provides guidance for antibiotic treatment, grafting, or reclosure of infected wounds. Whether the VPR technique can be used for these purposes remains to be demonstrated in a larger number of patients.

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