JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1980, p. 250-255
0095-1137/80/08-0250/06$02.00/0

Vol. 12, No. 2

Membrane Filter Contact Technique for Bacteriological Sampling of Moist Surfaces

JUDY M. CRAYTHORN,[1] ALAN G. BARBOUR,[2] JOHN M. MATSEN,[1,3] MICHAEL R. BRITT,[2] AND RICHARD A. GARIBALDI[2]*

Departments of Medicine,[2] Pathology,[1] and Pediatrics,[3] University of Utah, College of Medicine, Salt Lake City, Utah 84132

We used a membrane filter contact technique to pick up and grow bacteria from artificially contaminated surfaces. We were able to recover individual colony-forming units (CFU) of *Staphylococcus aureus* from a moist agar surface more efficiently with 3- and 5-μm membrane filters than with Rodac plates, velvet pads, velveteen pads, or smaller-pore membrane filters. The effective transfer of bacteria with the 3- and 5-μm membrane filters was 0.96 ± 0.04 (standard error of the mean) and 0.99 ± 0.04, respectively, as compared to 0.49 ± 0.03 for Rodac plates, 0.09 ± 0.01 velvet pad imprints, 0.05 ± 0.01 for velveteen pad imprints, 0.27 ± 0.02 for velvet pad rinses, 0.005 ± 0.001 for velveteen pad rinses, 0.39 ± 0.02 for 0.45-μm filters, and 0.85 ± 0.05 for 1.2-μm filters. In addition, the recovery of *S. aureus* from contaminated bovine muscle surfaces with the 5-μm membrane filter was similar to that of quantitative dilutions of biopsy material and was significantly higher than the recovery from Rodac plates. The 5-μm membrane filters on a paddle recovered 52 ± 5 CFU/cm^2 from artificially contaminated bovine skeletal muscle, the quantitative dilutions of biopsy recovered 69 ± 5 CFU/cm^2, and the Rodac plate recovered 5 ± 3 CFU/cm^2. Sampling of moist surfaces by the membrane filter contact technique is easy to perform and highly efficient; our data suggest that it could be employed for quantitative cultures of clinical surfaces such as surgical wounds or burns.

Quantitative bacteriological methods are now used to evaluate significant bacteriuria (8), predict burn wound sepsis (11), monitor significant bacterial colonization of intravenous catheters (12), and determine skin graft bed receptiveness (9). Quantitative cultures have also been reported to be of value in the early diagnosis of surgical wound infections (17). Raahave (16) used a velvet pad rinse technique to monitor bacterial contamination of routine surgical wounds. Brote et al. (3) used a combination of swabs, biopsies, and Rodac plates to culture surgical wounds and found that the risk of infection was greater if the postdisinfection Rodac plate or the preclosure culture showed any bacterial growth. Jepsen (7) also demonstrated a relationship between positive swab cultures of deep operative sites or subcutaneous tissue and subsequent development of postoperative wound infection. Stone (18) reported that wound infections in gastric surgery patients occurred when the peritoneal or preclosure wound cultures were positive. These studies suggested that colonization of the operative wound predisposed to subsequent clinical infection. Furthermore, they suggested that these infections might be predicted by cultures collected at the time of operation. However, these methods lacked sensitivity and specificity; wounds with negative surveillance cultures sometimes became infected, and positive wounds often did not subsequently develop infection.

We have developed a precise and efficient technique for detecting surface contamination using 5-μm membrane filters. In this report, we compared the sensitivity of the membrane filter contact technique with that of Rodac plates, imprints and rinses of velvet and velveteen pads, and quantitative tissue dilutions of biopsies in bacterial recovery experiments from moist, artificially contaminated agar and tissue surfaces.

MATERIALS AND METHODS

Bacteriological methods. A strain of *Staphylococcus aureus* from a surgical wound and a strain of *Escherichia coli* from an abdominal wound were used in these studies. These fresh, clinical isolates were maintained on 5% sheep blood agar (Columbia Agar base; BBL Microbiology Systems, Cockeysville, Md.) and transferred weekly. As needed, the organisms were grown in 5 ml of Trypticase soy broth (BBL) at 35°C until their turbidity was equivalent to 10^8 to 10^9 colony-forming units (CFU) per ml (standardized broth). Serial 10-fold dilutions of the standardized broth were made in Trypticase soy broth to obtain the desired

inoculum CFU per milliliter.

Sampling techniques. (i) Rodac plates. The slightly convex agar surface (17 ml of Columbia agar base) of a 50-mm Rodac plate (1, 5, 13) was placed without pressure in direct contact with a contaminated surface for 5 s and incubated overnight at 35°C to determine the number of bacterial colonies transferred.

(ii) Pad imprint. White velvet (100% rayon) and white velveteen (100% cotton) pads (20 by 45 mm) were prepared for replica plating by the methods of Holt (6) and Raahave (14). Pads were moistened by immersing them in 0.85% NaCl and then shaken to remove the excess fluid. Premoistened pads were applied to the artificially contaminated surface for 5 s and then imprinted on Columbia agar plates for 5 s. The imprinted agar was incubated overnight at 35°C.

(iii) Pad rinse. Premoistened velvet or velveteen pads were applied to the test surface for 5 s and then transferred to a 150-ml flask containing 10 ml of sterile 0.85% NaCl as previously described by Raahave (15). The flask was shaken at 300 rpm for 10 min. The suspension was centrifuged at 1,200 × *g* for 30 min, and the supernatant was decanted. All of the sediment was spread over a Columbia agar plate and incubated overnight at 35°C.

(iv) Membrane filter contact. Sterile membrane filters of 0.45-, 1.2-, 3-, and 5-μm pore sizes (Millipore Corp., Bedford, Mass.) were placed without pressure in direct contact with the artificially contaminated surfaces for 5 s and then placed on Columbia agar with the contact side up. Recovered bacteria produced identifiable colonies on the agar surface after overnight incubation.

(v) Membrane filter paddles. The commercially available Millipore sampler (4) for untreated water was adapted by the manufacturer to our specifications: a 5-μm membrane filter replaced the 0.45-μm membrane filter in the plastic paddle applicator (Fig. 1). An absorbent pad containing dehydrated culture media (tryptone glucose yeast extract) was placed behind the filter in the paddle. After application of the paddle to the contaminated surface for 5 s, 1 ml of sterile water was allowed to diffuse over the filter surface to rehydrate the culture medium. Recovered organisms grew as colonies on the filter surface.

(vi) Quantitative tissue dilution. A sample of bovine skeletal muscle tissue was excised and inoculated with a known quantity of *S. aureus*. The contaminated surface area of the sample was measured to the nearest 1 cm^2. The specimen was minced with scissors, ground in a mortar and pestle, and homogenized with 5 ml of Trypticase soy broth; serial dilutions were plated on Columbia agar plates to determine the CFU recovered per square centimeter of surface area (CFU/cm^2) (4, 9, 11).

Artificial contamination of test surfaces. (i) Agar. Dilutions of the standardized bacterial broth suspensions were prepared in cold Trypticase soy broth to prevent bacterial multiplication. Surfaces were contaminated and sampling procedures were performed in a refrigerated room to keep the bacterial population static. An even distribution of bacteria with minimal running or pool formation was obtained by spreading 2 ml of broth culture over the Columbia agar test surface in 140-mm petri dishes. The plate was tipped for 1 min to allow the excess fluid to pool; the fluid was removed with a Pasteur pipette. A 5×10^{-4} dilution of standardized *S. aureus* solution contained 5×10^4 CFU/ml and deposited 10^2 CFU/cm^2 by this contamination procedure.

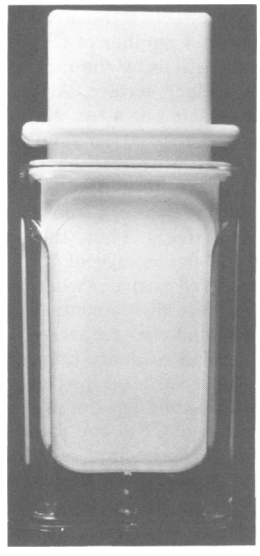

FIG. 1. *The membrane filter paddle (an adaptation of the Millipore sampler).*

Sampling with Rodac plates, velvet pads, velveteen pads, or membrane filter contact was performed within 5 min by direct contact for 5 s. After overnight 35°C incubation, the clones transferred by the sampling method and the clones remaining at the sample site were counted on a dark-field Quebec colony counter with an electronic register (American Optical Corp., Buffalo, N.Y.). The inoculum level was determined by counting the CFU/cm^2 growing on five unsampled control plates.

(ii) Bovine muscle. A sterile, 16-mm cork borer was used to cut tissue cores from bovine skeletal muscle obtained from fresh cadavers. The cores were placed in a freezer until they became firm but not hard. Uniform, 4-mm slices (1.11 g) of the rigid cores were prepared by using a sterile razor blade and slicing block. The surface area of the 16-mm-diameter circle was 2 cm^2, and the total surface area of the slice was 6 cm^2. A tissue slice was immersed in *S. aureus* suspension (9×10^3 CFU/ml) and immediately sampled by either Rodac plate (2-cm^2 area), membrane filter paddle (2-cm^2 area), or quantitative tissue dilution (6-cm^2 area). After overnight incubation, the CFU/cm^2 for each method was calculated. Uncontaminated slices were sampled by membrane filter paddle to detect presence of background contamination.

Mathematical and statistical methods. The effective uptake rates were calculated by dividing the number of CFU/cm^2 remaining at the sampled agar site by the number of CFU/cm^2 growing on an unsampled agar surface (inoculum level) and subtracting that

value from 1. The effective transfer rates were calculated by dividing the number of CFU/cm^2 recovered by the sampling method by the number of CFU/cm^2 on an unsampled agar surface. Artificially contaminated agar was selected as a test surface because the actual number of bacteria remaining after sampling could be counted after overnight incubation. In addition, the number of bacteria originally present on the test surface could be deduced from control counts of unsampled agar surfaces. Thus, the effective uptake rate describes the percentage of bacteria picked up from a contaminated surface, whereas the effective transfer rate describes the percentage of bacteria originally on a surface that were recovered by the sampling technique. The mean recovery rates from artificially contaminated tissue surfaces were expressed both as CFU/cm^2 and as percentages of the technique with the highest number of CFU/cm^2 recovered. Comparisons between the experimental results were made by both Student's t test and distribution-free statistical test (Kruskal-Wallis) (19).

RESULTS

Recovery of bacteria from contaminated agar surfaces. We compared the efficiency of bacterial uptake and transfer of Rodac plate, pad imprint, pad rinse, and membrane filter techniques by artificially contaminating an agar surface with approximately 10^2 CFU of *S. aureus* per cm^2. The Rodac plate mean uptake of *S. aureus* from the agar was 48%; however, all of the bacteria which were picked up from the sample site were transferred effectively (Table 1). As a sampling method, the single-phase Rodac plate technique was insensitive but efficient. Velvet pads, on the other hand, picked up a greater percentage of organisms than did the Rodac plate technique but transferred organisms very inefficiently. The introduction of a second phase in the velvet pad techniques resulted in a considerable difference between the effective uptake and transfer rates. The effective transfer by velvet pad rinse was significantly better than that by velvet pad imprint ($P < 0.001$, Student's t test). The uptake and transfer of *S. aureus* was significantly better with velvet pads than with velveteen pads by both the pad imprint and the pad rinse techniques ($P < 0.01$, Student's t test).

Recovery of *S. aureus* was significantly better with 3- and 5-μm membrane filters than with Rodac plate, pad imprint, or pad rinse techniques ($P < 0.001$, Student's t test) (Table 1). The effective uptake and transfer of *S. aureus* from a contaminated agar surface by the membrane filter contact technique was better with 3- and 5-μm membrane filters than with 0.45- and 1.2-μm filters (Table 1). Similar results demonstrating efficient bacterial uptake and transfer were obtained with 5-μm filters placed on a paddle containing nutrient medium. Very few CFU remained on the agar surface after sampling with a 5-μm membrane filter (Fig. 2). The effective uptake and transfer of *S. aureus* by 5-μm membrane filter was efficient and reliable at both low (10^0 CFU/cm^2) and high (10^2 CFU/cm^2) inocula (Table 2). The uptake and transfer of *E. coli* by 5-μm membrane filter was also precise and efficient.

Recovery of bacteria from artificially contaminated tissue surfaces. The recovery rates from contaminated bovine tissue surfaces

TABLE 1. *S. aureus effective uptake and transfer rates from contaminated agar surfaces by sampling method*

Sampling method	n	Inoculum (CFU/cm^2)	Effective uptake[a]	Effective transfer[a]	Significance[b]
Rodac plate	11	134	0.48 ± 0.04	0.49 ± 0.03	$P < 0.001$
Velvet pad					
Imprint	19	99	0.63 ± 0.02	0.09 ± 0.01	$P < 0.001$
Rinse	18	84	0.53 ± 0.04	0.27 ± 0.02	$P < 0.001$
Velveteen pad					
Imprint	11	99	0.07 ± 0.04	0.05 ± 0.01	$P < 0.001$
Rinse	19	84	0.15 ± 0.04	0.005 ± 0.001	$P < 0.001$
Membrane filter, pore size:					
0.45 μm	19	70	0.17 ± 0.08	0.39 ± 0.07	$P < 0.001$
1.2 μm	19	70	0.76 ± 0.05	0.85 ± 0.05	$P < 0.05$
3 μm	19	70	0.84 ± 0.02	0.96 ± 0.04	NS
5 μm	19	70	0.83 ± 0.03	0.99 ± 0.04	

[a] Results expressed as mean ± standard error of the mean.

[b] The effective transfer of each sampling method was compared to that of the 5-μm membrane filter by Student's t test. NS, Not significant.

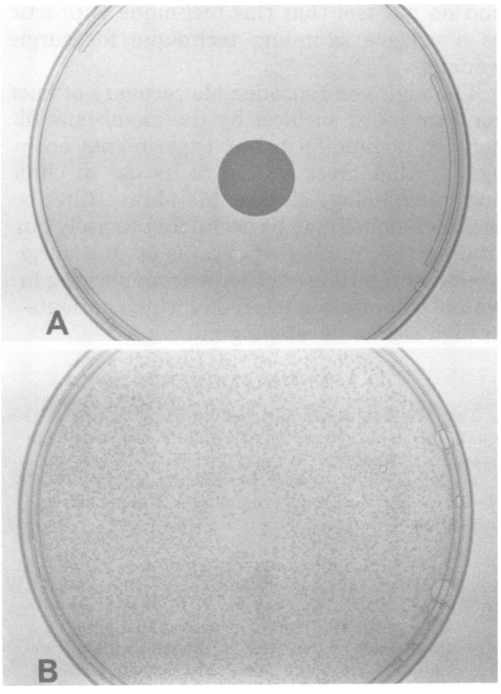

FIG. 2. *(A) The membrane filter was applied to the agar surface for 5 s and then removed. (B) The CFU remaining at the sample site grew as macroscopic colonies after incubation.*

TABLE 2. *Effective uptake and transfer rates from contaminated agar surfaces of the 5-μm membrane filter at various inoculum levels*

Organism	*n*	Inoculum (CFU/cm²)	Effective uptake[a]	Effective transfer[a]
S. aureus	18	4	0.93 ± 0.01	0.93 ± 0.04
	19	70	0.83 ± 0.03	0.99 ± 0.04
	20	136	0.97 ± 0.003	0.85 ± 0.04
E. coli	20	100	0.95 ± 0.005	1.00 ± 0.02

[a] Results expressed as mean ± standard error of the mean.

with the Rodac plate technique were significantly lower than rates with either the membrane filter paddle or the quantitative tissue dilution techniques ($P < 0.001$, Kruskal-Wallis test) (Table 3). Although the membrane filter paddle mean recovery rate was lower than the quantitative tissue dilution technique rate, there was no statistical difference ($P < 0.05$, Kruskal-Wallis test). Background bacterial contamination had no significant effect on the number of CFU recovered by the sampling methods (e.g., 1 CFU/30 cm² was recovered from uncontaminated bovine muscle by membrane filter paddle). We obtained similar results when we artificially contaminated abdominal tissue of live anesthetized rabbits with *S. aureus* and then sampled the tissue surface by Rodac plate, membrane filter paddle, and quantitative tissue dilution techniques.

TABLE 3. *S. aureus recovery rates from contaminated bovine skeletal muscle by sampling method (n = 15)*

Sampling method	Recovery rate[a]	% of quantitative tissue dilution	Significance[b]
Rodac plate	5 ± 3	36	$P < 0.001$
5-μm membrane filter	52 ± 5	75	NS
Quantitative tissue dilution	69 ± 5	100	NS

[a] Results are expressed as mean recovered CFU/cm² ± standard error of the mean.

[b] Statistical comparisons of all sampling methods by Kruskal-Wallis variance analysis. NS, Not significant.

DISCUSSION

Our study reports a new method for quantitating the number of bacteria on a moist surface by using a membrane filter contact technique. Traditionally, membrane filters are used for bacteriological analysis of water and wastewater by filtration. Our technique does not rely on passing fluid through the membrane filter. Rather, the filter is applied directly to the moist surface to be sampled. Bacteria are presumably absorbed into the filter and trapped in its interstitial spaces. Bacterial adherence to the filter surface through electrostatic forces may also be of importance. The trapped bacteria are then placed on a nutrient medium for culture and quantitation. The 5-μm membrane filter recovered significantly more bacteria from artificially contaminated surfaces than did Rodac plates, velvet pads, velveteen pads, or smaller-pore-size membrane filters. Over 90% of the individual *S. aureus* and *E. coli* CFU on contaminated agar surface were consistently picked up by the 5-μm membrane filter. In sampling contaminated tissue surfaces, the bacterial recovery of the 5-μm membrane filter was similar to that of the quantitative dilutions of tissue biopsies, and the 5-μm membrane filter was considerably easier to process in the laboratory. In these experiments, we found that a filter paddle containing dehydrated medium was less cumbersome than individual filter membranes. In our hands, the membrane filter contact technique produced easy and accurate quantitative surface culture results.

Our Rodac plate recovery rates of approximately 50% of the bacteria from contaminated agar surfaces were similar to those of Angelotti et al. (2). However, our Rodac plate recovery rates from contaminated tissue and wound surfaces were significantly lower than membrane filter contact or quantitative tissue dilution recovery rates. Apparently, the bacteria tested attach to tissue surfaces and membrane filter surfaces more avidly than to agar surfaces.

The recovery rates of velvet and velveteen pads have been studied by other investigators (6, 10, 13). Velvet has a longer nap than does velveteen. The velveteen pads used in our study had consistently lower uptake and transfer rates by both the pad imprint and pad rinse techniques than did the velvet pads. Raahave (14) reported that the velvet pad rinse technique resulted in a 30-fold improvement in transfer rates, from 2 to 61%, over the velvet pad imprint technique. In our hands, there was only threefold improvement, from 9 to 27%. The rinse technique was awkward and laborious to perform. Both velvet and velveteen pad techniques had substantially lower transfer rates as compared with Rodac plate and membrane filter contact methods. Because of the differences in fiber content, threads per square inch, length of nap, presence or absence of fabric sizing and dye, and general lot-to-lot variability, it appears that velvet or velveteen surface sampling cannot be standardized to yield precise and consistent results from laboratory to laboratory.

Traditionally, the quantitative tissue dilution technique is used to determine the tissue level of bacterial colonization rather than the degree of surface contamination (9). Because colonization of a surgical or burn wound is a surface phenomenon, a knowledge of the surface contamination level was of interest in our study. Use of the quantitative tissue dilution technique as a surface sampling method has three inherent problems: (i) accidental cross-contamination of clean tissue surfaces while collecting specimens, (ii) inaccuracies in measuring the exact area of the contaminated surface excised, and (iii) difficulties in processing samples with contaminated surfaces of more than 2 cm^2. We avoided these problems in our experiments with bovine skeletal muscle by first preparing uniform tissue specimens and then artificially contaminating their surfaces before sampling them by any method. However, in experiments in which we artificially contaminated the abdominal tissue of live, anesthetized rabbits before sampling, we obtained highly variable CFU/cm^2 counts with the quantitative tissue dilution technique. We attribute this variance to the problems mentioned above and do not feel that this technique is practic as a surface sampling technique for surgic wounds.

The high and reproducible recovery of bact ria from moist surfaces by the membrane filt contact technique in our experiments enco ages further investigation of its use in clini and microbiology studies. Membrane filter cc tact techniques may be useful for precisely qu titating the number of organisms in a surgi wound at the time of closure or monitoring b wound colonization to predict which patients at high risk of developing infection.

ACKNOWLEDGMENTS

This research was supported by Public Health Se grants AI-14533 and 1T32 AI-07011-03 from the Nati Institutes of Health.

Membrane filter paddles were kindly provided by F Ruderman and Christopher Simmons of Millipore Corp., ford, Mass.

LITERATURE CITED

1. **Aglietti, P., E. A. Salvati, P. D. Wilson, and Kutner.** 1974. Effect of a surgical horizontal unic tional filter air flow unit on wound bacterial con nation and wound healing. Clin. Orthop. **1**:99–104
2. **Angelotti, R., J. L. Wilson, W. Litsky, and W Walter.** 1964. A comparative evaluation of the c swab and rodac methods for the recovery of *Ba subtilis* spore contamination from stainless steel faces. Health Lab. Sci. **1**:289–295.
3. **Brote, L., J. Gillquist, and A. Tarnvik.** 1976. W infections in general surgery. Wound contamin rates of infection, and some consequences. Acta Scand. **142**:99–106.
4. **Cotton, R. A., K. J. Sladek, and B. I. Sohn.** Evaluation of a single-step bacterial pollution mo J. Am. Water Works Assoc. **67**:449–451.
5. **Hall, L. B., and M. J. Hartnett.** 1964. Measureme the bacterial contamination on surfaces in hosp Public Health Rep. **79**:1021–1024.
6. **Holt, R. J.** 1966. Pad culture studies on skin surfac Appl. Bacteriol. **29**:625–630.
7. **Jepsen, O. B.** 1973. Contamination of the wound d operation and post-operative wound infection. Surg. **177**:178–180.
8. **Kass, E. H.** 1957. Bacteriuria and diagnosis of infecti the urinary tract. Arch. Int. Med. **100**:709–714.
9. **Krizek, T. J., and M. C. Robson.** 1975. Evolutic quantitative bacteriology in wound management. J. Surg. **130**:579–584.
10. **Lederberg, J., and E. M. Lederberg.** 1952. Re plating and indirect selection of bacterial mutan Bacteriol. **63**:399–406.
11. **Loebl, E. C., J. A. Marvin, E. L. Heck, P. W. Cur and C. R. Baxter.** 1974. The method of quantit burn wound biopsy cultures and its routine use in care of the burned patient. Am. J. Clin. Pathol. **61** 24.
12. **Maki, D. G., C. E. Weise, and H. W. Sarafin.** 19 semiquantitative culture method for identifying i venous catheter-related infection. N. Engl. J. Med. 1305–1309.
13. **Raahave, D.** 1973. Agar contact plates in evaluatic skin-disinfection. Dan. Med. Bull. **20**:204–208.
14. **Raahave, D.** 1973. Bacterial density in operation wo Acta Chir. Scand. **140**:585–593.

15. **Raahave, D.** 1975. Experimental evaluation of the velvet pad rinse technique as a microbiological sampling method. Acta Pathol. Microbiol. Scand. **83**:416–424.
16. **Raahave, D.** 1975. New technique for quantitative bacteriological sampling of wounds by velvet pads: clinical sampling trial. J. Clin. Microbiol. **2**:277–280.
17. **Robson, M. C., and J. P. Heggers.** 1970. Delayed wound closures based on bacterial counts. J. Surg. Oncol. **2**: 379–383.
18. **Stone, H. H.** 1977. Gastric surgery. Biliary tract surgery. South. Med. J. Suppl. **1**:35–42.
19. **Swinscow, T. D. V.** 1978. Statistics at square one, 4th ed. Dawson & Goodall Ltd., The Mendip Press, Bath, England.