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CHAPTER 43

Glove-Fluid Method to Evaluate Acquired Microbial Contamination

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Bacterial contamination of health-worker hands by the emptying of microbially laden medical suction canisters was measured by the glove-fluid sampling method (GFM). Counts recoverable in glove-fluid were estimated by serial dilution and droplet surface plating. The GFM showed that hand contamination increased proportionally to canister bacteria load. The GFM using buffered Tween-80 solution was superior to a single bland soap as a means to elute skin bacteria. The authors found GFM to be convenient and reproducible and suggest its application to the monitoring of hand contamination acquired during the handling of contaminated materials or surfaces.

INTRODUCTION

This report describes application of the glove-fluid method (GFM) as a means to measure bacterial-population changes on the hands of a volunteer laboratory subject engaged in experimental pour-out of medical suction-canister contents. Suction is used in medicine, surgery, and dentistry to remove unwanted materials, including fluids, pus, blood, and occasionally feces from the human body. Depending upon handling techniques and precautions, the suctioning process and subsequent disposal of material aspirated into the suction receiver can comprise a microbiological risk to the health-care worker and a nosocomial hazard to the patient. Microorganisms borne away upon the unwashed hands of personnel who have performed suction procedures, emptied canisters, or who have engaged in other microbially contaminating occupations constitute a substantial portion of the hazard.

The investigation was undertaken to determine the extent to which worker hands became contaminated while emptying microbially populated suction canisters. Differentiation was difficult between hand-population increases which resulted from contact contamination or from aerosols generated above the dumping site as canisters were poured out. Therefore, the need seemed apparent for a sampling method which could remove bacteria from all portions of the hand, nonselectively, rather than from only certain areas as would be the case with contact culture methods. Also, since bacteria acquired on the hands as a result of some occupational procedure will be carried mainly as skin-surface transients, need would not exist to use a scrub-removal sampling method.

Williamson and Kligman (1965) studied a variety of detergents as stripping agents to enhance bacteria removal from the skin. They found these compounds to be effective removal agents. Peterson (1973) developed a GFM which utilized 0.1% Triton X-100 poured into a sterile surgical or examination glove. The method afforded the advantage of total-hand immersion in the stripping liquid, thereby minimizing the variable in attempting to sample only a select portion of the hand surface. He found that 85% of skin bacteria were removed by a 1-min glove wash in Triton, corroborating the findings of Williamson and Kligman (1965). Michaud et al. (1972, 1976) used the gloved-hand method to evaluate hand-skin degerming agents under a variety of conditions and found it to be a satisfactory experimental model which allowed treatment efficacies to be compared with a high level of statistical confidence.

The method used to estimate numbers of cells is an essential factor in any investigation to quantitate bacterial populations. The procedure used in this work was reported initially by Miles and Misra (1938) as the drop-plate method to quantitate numbers of viable bacteria. Although its use has been reported since by several investigators (Mallmann and Broitman 1956; Neblett 1976; Reed and Reed 1948), the method has not been adopted widely despite the reported ability to provide precision and consistently higher numbers than agar embedding procedures. Droplet-plating was selected for use in this investigation because of its advantages (Neblett 1976).

Materials and Methods

Hand Contamination During Pour-Out of Patient Canisters

Test specimens to be evaluated as sources of acquired microbial contamination consisted of 48 disposable, medical suction canisters. Thirty canisters were supplied after having been removed from patients at a nearby large, long-term and acute-care medical center. The canisters were incubated in the laboratory at room temperature to resemble the situation of prolonged patient application in a hospital setting. They were sampled at periodic intervals to measure their bacterial population change.

At the end of 48-h incubation, the canisters were ready to be used in the pour-out, hand-contamination studies. Patient canisters were received in the laboratory in six groups, consisting of four to seven canisters each. A positive control canister containing an overnight mixed culture of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Bacillus subtilis* plus two negative control canisters were tested in parallel with each patient canister group. One negative control contained sterile brain-heart infusion (BHI) broth, while the other was composed of sterilized patient material. All canisters used in the pouring experiments were adjusted to 500 ml with sterile water in an effort to equalize splash aerosol potential. Canister populations were determined by droplet plating immediately prior to beginning the pouring experiments.

The same volunteer subject performed all pouring trials in order to reduce possible variables in handling canisters. The particular volunteer was experienced in hospital-floor unit procedures and mimicked pouring as in actual practice. The subject who had neither been exposed to germicidal soaps nor had received antibiotics prior to the experiments washed hands twice for 1 min each time, lathering thoroughly using bland soap (Ivory). Hands were air-dried. Subject's preferred hand (right) was inserted into a sterile examination glove (Pharmaseal #8822), and 75 ml of 0.1% Tween-80 solution in pH 7.9, 0.075 *M* phosphate buffer were poured into the glove. A sterile catheter (approximately 100-mm long) attached to a sterile, disposable plastic syringe was inserted into the glove's palmar region, and the glove wrist was sealed off by a doubled rubber band. The hand was moved and massaged vigorously for 1 min, and 10 ml of the stripping fluid were removed via syringe.

Subject removed the glove, and the hand was allowed to air-dry. Subject then gripped the canister with left hand, removed the canister's lid with the right hand, and poured its contents into a deep laboratory sink chosen because of its resemblance to a hospital utility-room disposal hopper. The right hand was sampled once again by GFM, followed by a single 1 min soap wash, air drying, and a final sampling by GFM.

Serial 10-fold dilutions were made in half-strength buffered Tween. Columbia base blood and MacConkey agar plates were inoculated with duplicate 0.01-ml samples by droplet plating. Inoculated plates were allowed to imbibe the surface inoculum prior to handling. Incubation was under ambient atmosphere at 37 C. Only total colony-forming units (cfu) were tallied in this study. No species differentiations were made, and the medium giving the highest count was tabulated. Airborne bacteria above the outpouring site were measured by use of a battery-powered, portable, centrifugal air sampler (Biotest Reuter Centrifugal Sampler, Folex-Biotest-Schleussner, Inc., Moonachie, NJ) clamped 18 inches above the top rim of the receiving sink. The capacity of the device was 40 liters/min. Agar strips were incubated under ambient atmosphere at 37 C, and counts were tabulated as cfu/ft^3 of air (Gröschel 1980).

Comparison of Bacteria Removed by GFM and Soap Wash

Since the GFM is an effective way to strip skin bacteria, special procedures need to be designed when using it experimentally to measure the effectiveness of other bacteria removal agents and methods. Twenty-two canisters containing the contrived polymicrobic load equivalent to the one used in positive controls, as previously described, were poured in order to compare individual rates of hand-bacteria removal by the bland soap and by GFM. Populations of these canisters were determined, and the canisters were divided into two experimental groups as follows:

Group A (N = 11)
1. Hands received 2× wash
2. Hand sampled by GFM
3. Subject poured canister
4. Hand sampled by GFM
5. Hands *did not* receive a single wash
6. Hand sampled by GFM

To estimate how many bacteria are removed by GFM following pouring of the canister.

Group B (N = 11)
1. Hands received 2× wash
2. Hand sampled by GFM
3. Subject poured canister
4. Hand *not sampled* by GFM
5. Hands washed in bland soap a single time
6. Hand sampled by GFM

To estimate how many bacteria are removed by a single, bland soap wash following pouring of the canister.

Canisters belonging within Groups A and B containing the contrived populations were processed alternately. Thereby, Group A contained odd-numbered canisters, while Group B contained the even-numbered specimens. All canisters received identical preparation and handling. Thus, no direct pairings were structured other than those obviated by the order of handling. Estimates of bacterial populations were derived by droplet plating as described. For ease and convenience of plotting, exponential cfu values were converted to log 10. Means of individual raw values within groups were calculated prior to converting to log 10. Graphically represented data are means of canister groups specified.

RESULTS

The mean hand population recoverable by GFM following an initial 2X soap wash for all experimental trials (N = 70) was 3.8×10^3 cfu/ml glove fluid, or 2.8×10^5 cfu for the entire glove contents.

Patient Canisters

The mean data for patient suction-canister trials plus their controls are presented in Fig. 1. For the patient canister group (N = 30), mean prewash (2W) glove-fluid population was 5.6×10^3 cfu/ml. Following canister pour-out (P), the population recoverable rose to 5.3×10^4 cfu/ml; and following the second single wash (W), the mean population returned to 4.2×10^3 cfu/ml. The mean canister population for this group (represented by solid diamonds) was 3.4×10^8 cfu/ml at the time of pouring, while the mean air-sample population (represented by open squares) was 37 cfu/ft^3.

For the bacterially seeded control group (N = 6), 2W was 6.5×10^3, P was 3.3×10^5, and W was 8.0×10^3 cfu/ml glove fluid, respectively. The mean seeded canister population was 1.6×10^9 cfu/ml at time of pouring, while the air population was 91 cfu/ft^3.

Within the BHI broth control group (N = 6), 2W was 7.6×10^3, P was 1.4×10^4, and W returned to 4.1×10^3 cfu/ml glove fluid each. Because these controls consisted of sterile broth poured into a new but nonsterile canister whose surface sometimes contained rare numbers of *Bacillus*, low-level growth occurred but was negligible (<100) with respect to populations usually in excess of 10^8/ml within the seeded canisters. Air population which occurred while pouring out these controls averaged 47 cfu/ft^3.

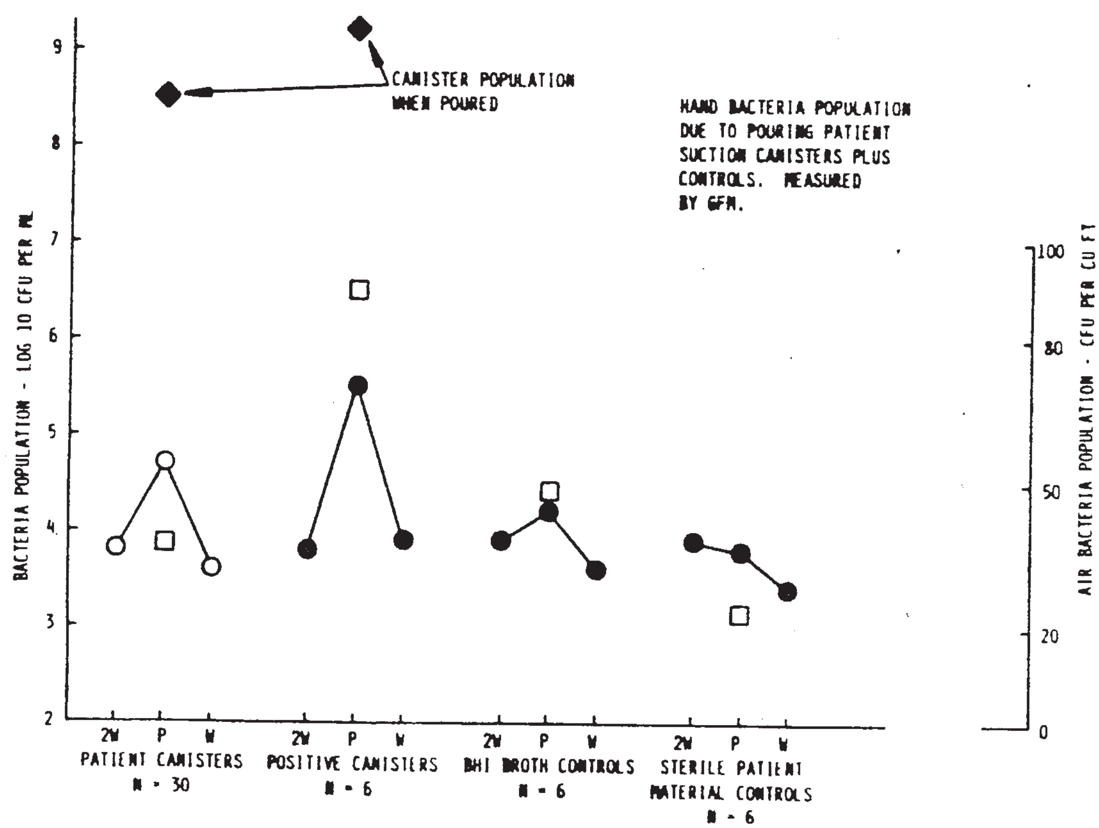

FIG. 1. Hand-bacteria population due to pouring patient suction canisters plus controls. Measured by GFM.

The 2W hand population for sterile, patient material controls ($N = 6$) averaged 7.3×10^3 cfu/ml glove fluid. Following pour-out procedure, the P value became 5.6×10^3/ml, and the W value dropped to an average 2.3×10^3 cfu/ml glove content. Airborne bacteria during these six pourings averaged 23 cfu/ft^3.

Comparative Efficacies of GFM and Soap Wash

Comparison of bacterial populations eluted from hands by GFM and bland soap wash, respectively, is presented in Fig. 2. As a general observation, the hand washed with soap had higher numbers of residual bacteria than the hand which had been subjected to GFM. The mean recoverable bacteria population for Group A, GFM-treated hands (open circles), was 9.8×10^3 cfu/ml glove fluid. The Group A range was 1.2×10^3 to 3.4×10^4 cfu/ml. Group B, soap-washed hands, produced a mean population of 1.8×10^4 cfu/ml. The Group B range was 2.1×10^3 to 4.6×10^4 cfu/ml. The difference between population means of the two groups was 8.2×10^3 cfu/ml greater in favor of Group B trials.

DISCUSSION

The experimental design used here, which included only a single subject in the glove-hand procedures, seemed satisfactory because of the consistent hand-skin populations obtained over several days. Wide variation may exist between the skin populations of individuals. However, Michaud et al. (1972) reported that by the gloved-hand procedure, quite consistent counts were obtained from the same individual. Our pre-pouring counts following the 2× bland soap wash were in close agreement and tended

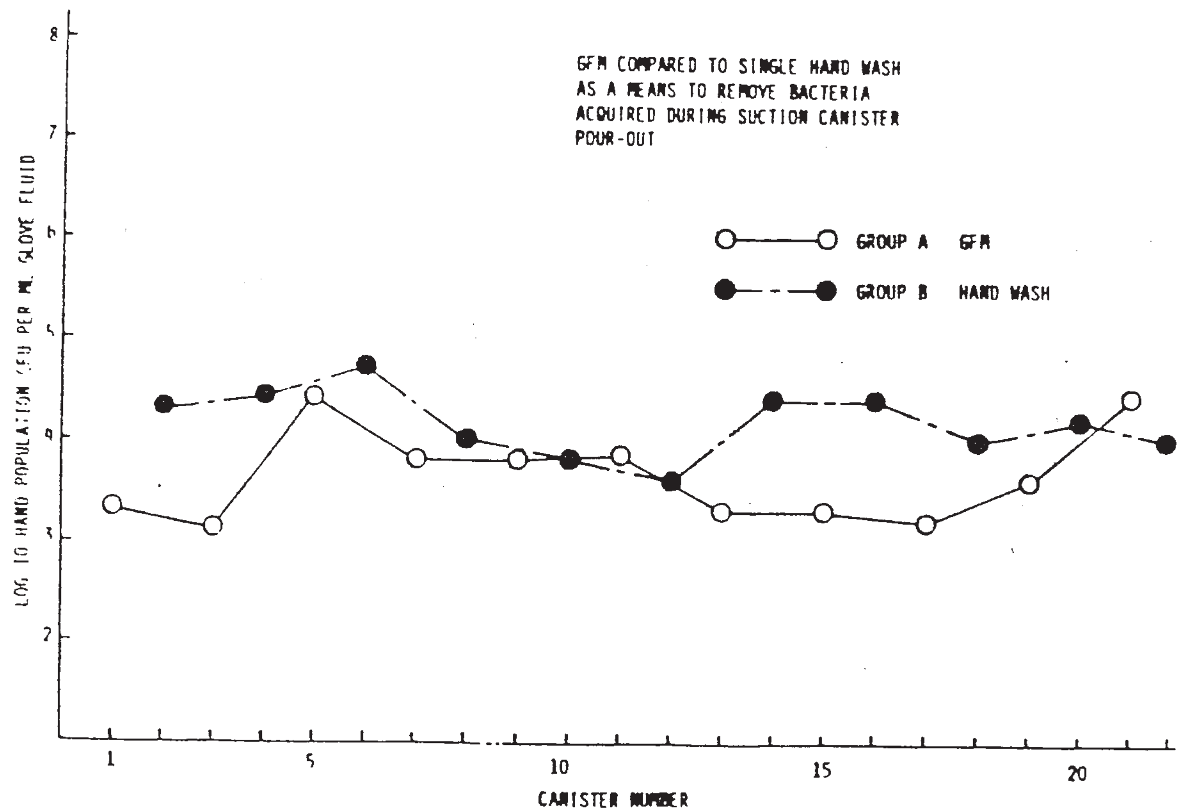

FIG. 2 GFM compared to single hand wash as a means to remove bacteria acquired during suction canister pour-out

to support the premise. Our effort was to determine how extensively the hands became contaminated by a microbiologically hazardous procedure, and a consistent pre-pouring count facilitated that objective.

Patient canisters and seeded controls produced approximately 1-log and 2-log increases on the subject's hand, respectively, following pour-out procedures, attributable to the microbial population contained within the canister and to any aerosol generated during the activity. The purpose of the second single soap wash was to simulate an attempt in a patient-care environment to break the canister-to-hands contamination cycle. Since GFM performed to measure bacteria acquired on the hands during the disposal procedure removed bacteria to achieve its purpose, the numbers of bacteria removed by GFM and the numbers removed by soap wash could not be distinguished readily from one another. In the absence of distinct data, no claim could be made to support the second hand wash as a reasonable means to interrupt any hand-contaminating cycle. Bacteria acquired on the hands during the discard procedure would be transient, hence likely would be easily removed by GFM sampling. The comparison experiments between Groups A and B were performed to estimate the contribution by each elution method to the removal of hand contamination.

The data determined for Groups A and B, microbially contrived canisters, represent bacteria remaining on the hand following GFM or soap wash as estimated by the final GFM sampling of each. Cautiously interpreted, the results seem to indicate that more bacteria remained on the hand following soap wash than following GFM. Because no true pairings resulted from the alternate A-B processing of canisters, the samples needed to be compared as similar populations which differed from one another by the treatment used to remove bacteria from the hand. As shown graphically in Fig. 2, Group B results appeared consistently higher, individually and as a group, than those of Group A. The difference between means of the two sets of data, 8.2×10^3, does not exceed 1 $\log_{10}$ order of magnitude, however.

Conclusions

The glove-fluid sampling method has been used as a convenient and reproducible means to estimate bacteria acquired on the hand of a worker experimentally pour-discarding the contents of microbially contaminated medical suction canisters. The GFM is to be recommended as a highly satisfactory procedure to monitor hands suspected of having been microbially contaminated by occupational practices.

Pour-discard of medical suction canisters having a mean bacterial population in the 8th log produced hand populations averaging in the 4th log, and positive control canisters having a mean bacterial population in the 9th log produced hand populations averaging in the 5th log. Baseline hand populations determined after a 2 × bland soap wash and prior to emptying canisters averaged in the 3rd log. Hands subjected to single soap wash, GFM alone, or GFM plus soap wash following emptying procedures returned to a mean bacterial population in the 3rd log. The GFM as an elution method was found to be somewhat superior to a single wash in bland soap.

Literature Cited

Groschel, D. H. M. 1980. Air sampling in hospitals. *Ann. N.Y. Acad. Sci.* 353:230–240.

Mallmann, W. L., and S. W. Broitman. 1956. A surface plating technic for determining bacterial population of milk. *Am. J. Public Health* 46:1018–1020.

Michaud, R. N., M. G. McGrath, and W. A. Goss. 1972. Improved experimental model for measuring skin degerming activity on the human hand. *Antimicrob. Agents Chemother.* 2:8–15.

————. 1976. Application of a gloved-hand model for multiparameter measurements of skin degerming activity. *J. Clin. Microbiol.* 3:406–413.

Miles, A. A., and S. S. Misra. 1938. The estimation of the bactericidal power of the blood. *J. Hyg.* (Cambridge) 38:732–749.

Neblett, T. R. 1976. Use of droplet plating method and cystine-lactose electrolyte-deficient medium in routine quantitative urine culturing procedure. *J. Clin. Microbiol.* 4:296–305.

Peterson, A. F. 1973. The microbiology of the hands: Evaluating the effects of surgical scrubs. *Dev. Ind. Microbiol.* 14:125–130.

Reed, R. W.; and G. B. Reed. 1948. "Drop Plate" method of counting viable bacteria. *Can. J. Res. Sect. E* 26:317–326.

Williamson, P., and A. M. Kligman. 1965. A new method for the quantitative investigation of cutaneous bacteria. *J. Invest. Dermatol.* 45:498–503.