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Application of a new microbiological technique to the study of antiperspirant and deodorant soap efficacy

R. F. THEILER, C. L. SCHMIT, and J. R. ROHEIM, *Armour Research Center, New Science and Technology Department, 15101 N. Scottsdale Road, Scottsdale, AZ 85260.*

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Synopsis

A new technique for sampling cutaneous microorganisms has been used to study the effects of deodorant products on aerobic axillary microbial populations. Initially the Thran spray gun was compared to the mechanical scrub method and was found to compare favorably in terms of reproducibility and sensitivity. When a commercial stick antiperspirant was evaluated, both methods demonstrated similar log reductions in axillary bacteria when compared to controls. The Thran spray gun was also used to study the efficacy of a commercial deodorant soap containing 3,4,4′-trichlorocarbanilide. A 55% reduction in aerobic axillary microorganisms was demonstrated 24 hours after washing with the soap.

INTRODUCTION

Many locations on the surface of human skin emit distinctly different odors. However, of all the human scents, those emanating from the axilla are regarded by society as some of the most offensive. In recent years the accumulation of a significant body of scientific evidence has traced the source of axillary odors to the action of bacteria on secretions from the apocrine gland (1-4). In a number of recent publications it has been suggested that a class of Gram positive microorganisms, the diphtheroids, are responsible for the selective generation of the distinctly pungent axillary odors, while the micrococci are responsible for the generation of sweaty, acid odors (2-4). Therefore, numerous manufacturers of deodorant products have marketed formulations with an active ingredient which reduces axillary microbial populations for the purpose of reducing the intensity of axillary odor.

Since bacterial reduction is related to a product's deodorant efficacy, numerous methods have been developed for qualitatively and quantitatively monitoring the distribution and population of skin flora. Among these are mechanical scrub techniques (5-6), swabbing procedures (7-9), tape stripping and contact plates (7-13), as well as basin scrubbing methods (14-15). Among these techniques, a widely employed procedure for quantitating aerobic skin microflora has been the mechanical scrub technique which involves a timed scrub of the skin surface with a blunt teflon scrubber

in the presence of a buffer containing surfactant. Certain problems are encountered with the mechanical scrub procedure, particularly in situations in which a panelist's axillae must be repetitively extracted. In these cases mechanical scrubbing can result in localized edema and irritation of the test site.

In the present investigation we describe the use of a more gentle, pressurized spray technique (Thran bacterial sampler) to quantitatively evaluate total aerobic axillary microbial populations (16). The Thran bacterial sampler was compared to the mechanical scrub procedure, and was subsequently employed to demonstrate the efficacy of a solid stick antiperspirant as well as a deodorant soap containing trichlorocarbanilide (TCC).

EXPERIMENTAL

A. BACTERIAL SAMPLING

Bacterial sampling of the axillary vault was performed utilizing both the mechanical scrub procedure of Williamson and Kligman (5) and the pressurized spray gun technique of Thran (16). In the case of the mechanical scrub method, a glass cylinder which circumscribed a 3.8 cm^2 area was firmly placed onto the exposed skin of the axilla, and 3 ml of 0.075 M phosphate buffer pH 7.9 containing 0.1% Triton X-100 was added. The circumscribed skin surface was rubbed with moderate pressure for one minute with a blunted teflon scrubber; sampling fluid was removed by aspiration, and the procedure repeated. The two samples containing extracted bacteria were pooled and stored on ice prior to serial dilution. Recoveries for the sampling fluid averaged 90%.

The Thran spray gun bacterial sampler was obtained from Dr. Med Vet. Volker Thran, Smetslaan 9, B 1900 Overijse-Maleizen, Brussels, Belgium. In principle, the Thran spray gun technique involves the use of air pressure to aspirate a sampling solution over an isolated region of the skin surface (see Figure 1—a complete description of the apparatus can be found in reference 16.) A jar containing a 100 ml. aliquot of bacterial sampling fluid consisting of 0.075 M phosphate buffer pH 7.9 containing 0.005% Triton X-100 was attached to the spray gun along with an empty sterile collection jar. To extract bacteria the silicon rubber tip of the spray gun was firmly pressed against the axillary vault, and the sampling solution was simultaneously sprayed onto and aspirated from a 1.77 cm^2 area. With a compressed air pressure of 1.8 bars, aspiration times averaged 65 seconds and sampling fluid recovery averaged 98% for the 100 ml sample. Following extraction, bacterial collection jars were removed from the spray gun and stored on ice prior to serial dilution. The Thran spray gun was sterilized between uses by consecutive rinses with sterile water (15 ml), 3A alcohol (15 ml), and one final sterile water rinse (25 ml).

Total aerobic bacterial count was determined by serially diluting 1 ml aliquots from each sample in 10 fold steps with 0.0375 M phosphate buffer, pH 7.9 containing 0.05% Triton X-100. In deodorant soap and antiperspirant experiments 0.5% lecithin and 5.0% Tween-80 were added to this dilution buffer to neutralize any antimicrobial activity associated with the active ingredients. Diluted samples were plated in duplicate in trypticase soy agar supplemented with 0.25% dextrose, 0.1% yeast extract, and 0.2% Tween-80 to facilitate growth of diphtheroids (17). Following a 48 hour aerobic

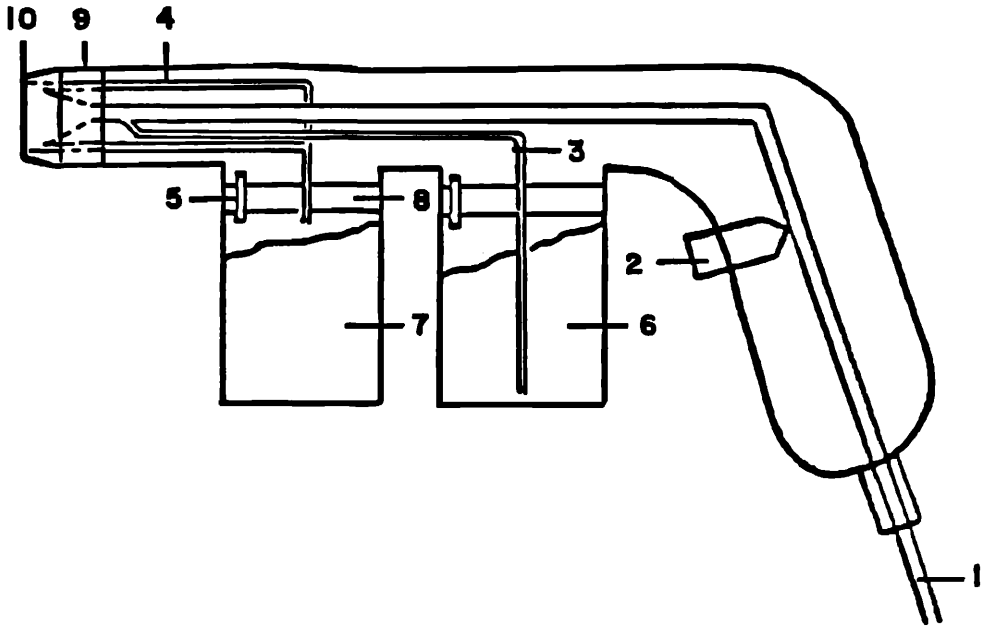

Figure 1. Schematic drawing of the Thran spray gun bacterial sampler. 1. Compressed air entry; 2. Valve; 3. Entry to spray head; 4. Exit from spray head; 5. Air vent; 6. Sampling fluid reservoir (detachable); 7. Collecting reservoir (detachable); 8. Special fastening; 9. Spray head; 10. Sealing band.

incubation at 37°C, plates were counted under a dissecting microscope and colony forming units (CFU) per plate converted to log CFU per cm^2 of axilla sampled. Average log CFU's per axilla were calculated for the duplicate plates and paired t-tests conducted when appropriate as a test of statistical significance of differences in axillary aerobic bacterial populations.

B. CLINICAL TESTING PROCEDURES

Male and female subjects over 18 years of age were recruited for clinical studies. All subjects were in excellent health and free of visible dermatitis. Prior to laboratory testing all subjects participated in a one week washout period with placebo soap (containing no germicide, fragrance, or color) during which time they were restricted from the use of all deodorant or medicinal soaps, antiperspirants or deodorants, and antibiotics. In addition, subjects were restricted from swimming in chlorinated water within three days of the test period. Female subjects were instructed to stop underarm shaving three days before bacterial extraction.

Initial experiments were designed to compare the reproducibility of bacterial extraction using the Thran spray gun or the mechanical scrub procedure. Twenty-four hours after the final placebo soap wash, two adjacent sites on a subject's left and right axillae were extracted—the first site via mechanical scrubbing, the second site using the Thran spray gun. In antiperspirant experiments, following a one week washout period and a placebo soap wash, a commercial antiperspirant stick based on an active ingredient aluminum zirconium tetrachlorohydrex glycine, cyclomethicone, stearyl alcohol, water, glyceryl monostearate, quaternium-18 hectorite, talc, fragrance and SD alcohol 40, was applied

(0.5 gm per male/0.3 gm per female) to either the left or right axilla based on a computer randomized assignment. The corresponding placebo stick, in which the active ingredient was removed and replaced with talc and cyclomethicone, was applied to the opposite axilla. Twenty-four hours later adjacent sites of antiperspirant and placebo treated axillae were extracted, the first site with mechanical scrubbing and the second site with the Thran spray gun.

In clinical experiments designed to evaluate the *in situ* antibacterial efficacy of a commercial deodorant soap based on an active ingredient (3,4,4',Trichlorocarbanilide), sodium tallowate, sodium cocoate, water, glycerin, fragrance, sodium chloride, preservatives, and colors, only the Thran spray gun procedure was employed. Panelists participated in a one week washout with placebo soap (with the same restrictions listed previously) prior to reporting to the laboratory. Placebo and deodorant soaps were assigned to opposite axillae using a computer generated randomization schedule, and panelists participated in four supervised washes as described below. Panelists were instructed to wet their right axilla with a clean washcloth (15 seconds), after which the assigned soap bar was wet under running water (15 seconds) and applied directly to the axilla (20 seconds). Following a 15 second hand rinse, panelists wet a clean washcloth and removed all remaining soap lather from their axillae (45 seconds). Panelists' axillae were patted dry with a clean towel, and the procedure was repeated for the opposite axilla with the other test soap using a fresh supply of linen. This process was repeated each morning for four mornings. Twenty-four hours following the last wash each axilla was extracted using the Thran spray gun, and samples were serially diluted for plating and analysis of total aerobic bacterial count as previously described.

RESULTS AND DISCUSSION

The Thran spray gun (TSG) and mechanical scrub (MS) techniques represent distinctly different methods of bacterial extraction. The TSG delivers a fine mist of buffer and surfactant which we believe removes only surface bacteria. For TSG sampling, the surfactant concentration (Triton X-100) was reduced to 1/20th of the level used with the MS procedure to reduce foaming during extraction. The MS procedure involves extensive agitation of the skin surface resulting in the removal of greater amounts of the desquamating stratum corneum. In contrast to the MS method where microorganisms present in lower levels of the stratum corneum might also be removed, the tendency of the TSG is to only remove surface microorganisms. For this reason there was concern about the percentage of total axillary skin bacteria extracted utilizing either method.

To answer this question, initial experiments focused on evaluating the *in situ* axillary bacterial levels on human subjects 24 hours following a supervised wash with placebo soap. For purposes of comparison, adjacent areas in the axillary vault of each panelist were extracted, with the first site using the MS method and the second site using the TSG. This process enabled a comparison of total aerobic bacteria extracted and an examination of the reproducibility of both methods. In addition, total aerobic bacteria could be compared between left and right axillae. Experimental results for five panelists are illustrated in Table I with bacteria levels for each subject expressed in log units per cm^2. Based on the MS method, total axillary bacterial count was found to range from 10^5 to 10^7 per cm^2 which is in agreement with previously published information (18-19).

Table I
A Comparison of Mechanical Scrub (MS) and Thran Spray Gun (TSG) Methods of Bacterial Extraction on Placebo Soap Washed Axillae

	Mechanical Scrub[a] log CFU/cm^2			Thran Spray Gun[a] log CFU/cm^2		
Panelist	Left Axilla	Right Axilla	Difference	Left Axilla	Right Axilla	Difference
1	5.72	7.03	−1.31	5.42	5.16	0.26
2	6.26	6.14	0.12	5.61	5.07	0.54
3	6.32	6.11	0.21	5.59	4.66	0.93
4	6.87	6.68	0.19	5.53	5.67	−0.14
5	6.67	6.89	−0.22	5.98	6.25	−0.27
Average			−0.20			0.26
Std. Dev.			0.64			0.49
P			≤0.60			<0.30

[a]Comparison of MS to TSG for total bacteria extracted, as follows: ave. Σ_{n-1}^{n-5} (log MS_{left} − log TSG_{left}) = (log MS_{right} − log TSG_{right})/2 resulting in $\overline{X}$ = 0.98, std. dev. = 0.21, P < 0.001.

With either method when bacterial concentrations from left versus right axilla were compared, no significant differences were observed. This suggests that the random assignment of two test products represents an appropriate testing model. However, significant differences were observed between numbers of total bacteria extracted using the two procedures. The MS method removed more than five times the number of microorganisms as compared to extraction with the TSG. This difference was highly significant ($p < 0.001$), and was apparently due to more vigorous scrubbing and hence the removal of greater numbers of microorganisms. In these experiments a significant number of subjects developed erythema as a result of the pressure applied during scrubbing (approximately 100–120 g. pressure). In subsequent bacterial extraction on one axilla of 20 panelists the physical pressure exerted during scrubbing was reduced to approximately 60–70 g. pressure, resulting in a concomitant reduction in the incidence of erythema and a reduction in the total number of bacteria extracted (Table II).

Table II
A Comparison of Gentle Mechanical Scrub (MS) and Thran Spray Gun (TSG) Methods of Bacterial Extraction on Placebo Soap Washed Axillae

Panelist	Log CFU/cm^2 MS minus TSG	Panelist	Log CFU/cm^2 MS minus TSG
1	0.52	11	0.08
2	−1.38	12	−0.45
3	0.86	13	−0.68
4	−1.60	14	−0.71
5	−0.18	15	1.37
6	0.04	16	0.31
7	0.12	17	0.21
8	0.91	18	−0.81
9	0.59	19	−0.68
10	−0.53	20	3.34
		Average	0.07
		Std. Dev.	1.08
		P	<0.8

Experimental results indicated that if very gentle mechanical scrubbing is performed nearly equivalent numbers of bacteria are extracted with the two methods.

A number of experiments were conducted which involved repetitive extraction of the same axillary skin site. As illustrated in Table III, regardless of the number of extractions, the MS method removed more total bacteria than did the TSG. In addition, the first extraction with the MS method tended to remove a greater percentage of the total *in situ* axillary bacteria than did the TSG. While the TSG tended

Table III
Quantitation of Aerobic Axillary Bacterial Populations as a Function of the Number of Extractions

Extraction #	log CFU/cm² Panelist # 1	2	3	4	Ave. log CFU/cm²	CFU/cm²	% of Total Bacteria Per Extraction
Mechanical Scrub[a]							
1	6.66	7.01	6.36	5.87	6.48	3.02×10^6	79.68
2	5.98	6.44	5.85	4.52	5.70	5.01×10^5	13.22
3	5.77	5.89	5.50	3.86	5.26	1.82×10^5	4.80
4	5.40	5.78	5.00	3.64	4.96	9.12×10^4	2.41
Total						3.79×10^6	
Thran Spray Gun							
1	6.86	6.42	5.32	4.97	5.89	7.76×10^5	44.34
2	6.46	6.18	5.27	4.42	5.72	5.25×10^5	30.00
3	6.30	5.86	4.93	4.48	5.39	2.45×10^5	14.00
4	6.05	5.90	4.96	4.31	5.31	2.04×10^5	11.66
Total						1.75×10^6	

[a]Each extraction consisted of a single 60 second scrub with 3 ml. of buffer.

to extract fewer bacteria, this does not suggest the procedure is any less sensitive or statistically less reliable in detecting reductions in a given microbial population. As a test of reproducibility and sensitivity, both test methods were used to evaluate the germicidal efficacy of a stick antiperspirant versus a placebo stick (no antimicrobial agent). Immediately following a placebo soap wash, a placebo stick or an active containing stick antiperspirant was applied to the axillae of seven subjects. Using both bacterial extraction methods, adjacent locations in each axilla were extracted 24 hours after treatment with the antiperspirant sticks. As illustrated in Table IV, a significant reduction in bacteria was observed for axillae treated with the active containing antiperspirant 24 hours following product application. Both methods detected log reductions in CFU's. The TSG demonstrated a 1.82 log decrease in axillary bacteria or 98.49% reduction in the geometric mean bacteria per cm², compared to a 1.02 log reduction for the MS or 90.45% reduction in geometric mean bacteria per cm². Despite the fact that the MS harvested three times the number of bacteria per cm², both methods demonstrated log reductions in axillary bacteria as well as similar percent errors and statistical probabilities. These results suggest that either method represents a satisfactory technique for measuring *in situ* reductions in axillary microorganisms.

In a final series of experiments the TSG was utilized to evaluate the effect of a germicide containing soap bar on the levels of *in situ* axillary microflora. Following a one week washout period, 20 subjects participated in four days of supervised washes with placebo soap and an antimicrobial soap containing 3,4,4′-Trichlorocarbanilide.

Table IV
Reduction of Aerobic Axillary Bacterial Populations by a Solid Antiperspirant as Measured by Mechanical Scrub and Thran Spray Gun Methods of Bacterial Extraction

	Mechanical Scrub log CFU/cm^2			Thran Spray Gun log CFU/cm^2		
Panelist	Placebo Treated Axilla[a]	Antiperspirant Treated Axilla	Difference	Placebo Treated Axilla[a]	Antiperspirant Treated Axilla	Difference
1	5.73	4.96	0.77	5.39	3.92	1.47
2	5.27	4.18	1.09	5.23	3.44	1.79
3	6.71	5.88	0.83	6.29	4.85	1.44
4	6.59	4.16	2.43	6.21	3.20	3.01
5	6.17	5.47	0.70	5.73	3.38	2.35
6	6.06	5.37	0.69	4.73	3.23	1.50
7	6.24	5.60	0.64	5.85	4.68	1.17
Average			1.02			1.82
Std. Dev.			0.64			0.64
P			<0.005			<0.0005
% Reduction			90.45			98.49

[a]Comparison of MS to TSG total bacteria extracted on placebo treated axillae yields $\overline{X} = 0.48$, Std. Dev. = 0.40, $P < 0.01$.

Table V
Reduction of Aerobic Axillary Bacterial Populations by a Deodorant Soap as Measured by the Thran Spray Gun Method of Bacterial Extraction

Panelist	Placebo Soap Treated Axilla log CFU/cm^2	Deodorant Soap Treated Axilla log CFU/cm^2	Difference
1	4.50	4.53	−0.03
2	7.01	5.96	1.05
3	5.14	4.35	0.79
4	5.71	6.39	−0.68
5	5.66	5.85	−0.19
6	5.76	4.72	1.04
7	5.29	5.42	−0.13
8	4.33	5.71	−1.38
9	5.51	4.63	0.88
10	5.83	5.24	0.59
11	3.83	3.26	0.57
12	5.37	4.91	0.46
13	4.85	3.79	1.06
14	5.80	6.05	−0.25
15	5.14	4.82	0.32
16	5.97	5.29	0.68
17	4.96	4.64	0.32
18	6.23	4.85	1.38
19	4.88	4.88	0.00
20	3.12	2.55	0.57
Average			0.35
Std. Dev.			0.67
P			<0.025
% Reduction			55.36

Twenty-four hours following the last supervised wash, placebo and antimicrobial soap washed axillae were extracted with the TSG and total aerobic bacteria counted. Experimental results revealed a significant reduction in recoverable aerobic axillary microbial population by the antimicrobial soap when compared to the placebo soap (Table V). The antimicrobial soap was shown to reduce bacterial populations an average 0.35 log units ($p < 0.03$), representing a 55% overall reduction in recoverable aerobic axillary bacteria.

In conclusion, these results demonstrate that the Thran spray gun can be employed to quantitate the *in vivo* antimicrobial efficacy of antiperspirant and bar soap products. This technique compares favorably with the mechanical scrub method in terms of both reproducibility and in its ability to detect changes in the population levels of cutaneous microorganisms. The mechanical scrub method, however, extracts larger numbers of bacteria per cm^2 in a smaller sampling volume and is recommended for studies requiring concentrated bacterial samples such as those involving quantifying pathogens or other low level ($<1 \times 10^3$ CFU/cm^2) bacterial populations. The Thran spray gun offers several advantages over the mechanical scrub including ease of sampling, increased sampling volume with recoveries of 98%, and the reproducibility of sampling pressure which allows collection of several samples on the same site without inducing localized erythema. This method should have broad application in assessing the antimicrobial efficacy of topically applied products.

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