JOURNAL OF CLINICAL MICROBIOLOGY, June 1976, p. 576-581
Vol. 3, No. 6

Printed in U.S.A.

Differential Quantitation of Surface and Subsurface Bacteria of Normal Skin by the Combined Use of the Cotton Swab and the Scrub Methods

CHARLES A. EVANS* AND ROBERT J. STEVENS

Department of Microbiology and Immunology, University of Washington, Seattle, Washington 98195

Received for publication 8 December 1975

By testing adjacent sites on the hypothenar eminence of the palm, enriched with bacteria by massaging the forehead, we found that the numbers of bacteria recovered from the skin surface by a wet cotton swab in 30 s were not significantly different from the numbers obtained by a brisk scrubbing with a blunted Teflon policeman for 120 s. This was true of aerobes (gram-positive cocci) and anaerobes (propionibacteria). If the same site on the palm was swabbed two times for 15 s each time, 67 to 94% of the total recovered bacteria were obtained on the first swab. Differential localization of bacteria into surface and subsurface populations was accomplished by first swabbing a test skin site to assay the surface flora and then scrubbing the same site to test for subsurface organisms. On the palm the swab yielded more aerobes and anaerobes than did the subsequent scrub. On the forehead the scrub yielded three to eight times as many anaerobes as the preceding swab. In some tests gram-positive cocci were distributed on the forehead like propionibacteria (large excess in scrub specimen); in other tests their numbers were similar in the swab and scrub specimens or there was a large excess in the swab specimen. These results indicate that there was no substantial subsurface flora on the palm. On the forehead propionibacteria were predominantly in deeper locations in all tests; gram-positive cocci were variable: in some test sites they were largely at the surface, whereas at other sites a predominance of cocci was in subsurface locations.

Medical and ecological studies of the microbial flora of normal skin are seriously handicapped by our ignorance of the extent to which surface and subsurface microhabitats are sampled by the various techniques employed for harvesting organisms for culture. There is wide agreement that a well-standardized scrub technique, as introduced by Burtenshaw (3) in 1938, modified by Pachtman et al. (12) in 1954, and further modified to include an improved diluent by Williamson and Kligman (20) in 1965, will yield maximal numbers of bacteria. This method does not permit a separation of organisms occurring in more superficial sites from those growing in subsurface locations. Furthermore, it is cumbersome and involves discomfort and trauma not acceptable in some situations.

Collecting the bacteria from skin on moistened cotton swabs is a most convenient method but is generally viewed as unreliable. Noble and Somerville (11) comment that "only about one-tenth of the organisms on the skin sampled are removed and one-tenth of those removed are deposited on the agar surface." Various authors have expressed concern that toxic substances associated with cotton may kill some of the bacteria (1, 4, 9, 14, 19) removed from skin by swabbing.

Contact cultures (6, 16) and culture of sticky tape (2, 17) or sticky disks (8) are useful in showing the distribution of surface organisms in the area sampled, if the bacteria are not too numerous, but cannot be made quantitative in any but the most approximate sense. Electron microscopy (10, 13) and light microscopy of sections of skin entail additional problems of small sample size and the impossibility of accurate identification of organisms.

In this paper we show that the moistened cotton swab method is as reliable as the scrub method for determining the numbers of bacteria on the surface of skin, whereas the scrub method permits a satisfactory sampling of surface organisms plus those organisms that occur in subsurface locations such as the pilosebaceous units. It is, therefore, possible to use the two methods together to differentiate the microhabitats of different kinds of bacteria inhabiting the same skin area.

MATERIALS AND METHODS

Methods of identifying and quantitating bacteria. Specimens were collected in the buffered diluent of Williamson and Kligman (20) that contains Triton X-100 (Rohm and Haas). They were further diluted in half-strength diluent, and four replicate cultures of each dilution were prepared, two for aerobic and two for anaerobic incubation. A 0.05-ml amount of serial 10-fold dilutions was spread with a bent glass rod on the surface of a 2% agar medium (3% Trypticase soy [BBL], 1% yeast extract, and 0.5% Tween 80 [Polysorbate 80, Atlas Chemical, Wilmington, Del.]) in plastic petri dishes partitioned into two or three sectors. An aerobic culture of 0.05 ml of the undiluted specimen was prepared from most specimens on MacConkey medium for enteric organisms and on mannitol salt agar for *Staphylococcus aureus*. Prereduced media were not used; specimen collection and culture manipulations for isolation of anaerobic bacteria were not carried out under anaerobic conditions. The GasPak system (BBL) with methylene blue indicators was used for anaerobic culture; aerobic cultures were incubated in jars or in closed plastic bags to control evaporation.

After incubation for 4 to 7 days at 35 to 36 C, colony types were identified and counted. Gram stains and catalase tests were performed on two colonies of each type. Numbers of bacteria (colony-forming units) were calculated from cultures of those dilutions with the numbers of colonies most suitable for counting, usually between 15 and 150.

Propionibacteria were nearly always identifiable on primary anaerobic cultures as nonsporing, gram-positive, coryneform catalase-positive rods that did not grow on the corresponding aerobic culture.

Test subjects. All subjects were males from 25 to 63 years of age who were known to harbor a rich population of propionibacteria on their foreheads; six of the seven subjects were included in a previously reported study (5). One was of Chicano ancestry; the others were of European ancestry. They used Ivory soap approximately 1 week before the test and were asked to use no other soap for washing their hands and faces or for bathing until the tests were completed. They were also instructed not to wash their hair on the day of the test and not to wash their foreheads within 3 h before the test.

Procedure in the swab method and the scrub method of harvesting bacteria from skin. In the standard swab method we dipped a swab into 2 ml of diluent, squeezed out the excess fluid by pressing on the inside wall of the tube, and gently swabbed the skin surface delineated by a sterile stainless-steel plate with a central hole 22 mm in diameter. After 15 s of swabbing, we agitated the swab in the fluid in the original tube for 15 s to dislodge the bacteria, repeated the 15-s swabbing, and finished with 60 s of agitation of the cotton swab in the fluid. In eight preliminary tests we found that the first 15-s swabbing yielded an average of 85% (range 67 to 94%) of the total propionibacteria obtained in testing the palm this way.

Sterile commercial cotton swabs on wooden applicator sticks (Swubes, Falcon Plastics) were employed. The manufacturer's representative informed us that their quality control includes a leaching test to demonstrate that no inhibition of growth of a *Streptococcus, M. luteus,* or *B. subtilis* occurs in broth exposed for 72 h to the swabs. The only other specification by the supplier is that the cotton shall meet U.S. government standards (18).

In the standard scrub method we placed 1 ml of diluent in a chamber formed by pressing a glass cylinder (22 mm in diameter and 20 mm in height) against the skin. With a blunted Teflon policeman, we scrubbed the skin briskly for 60 s, changed the fluid, and continued the scrubbing for an additional 60 s. We typically recovered 1.8 or 1.9 ml of the fluid.

Numbers of bacteria recovered by swab and by scrub of adjacent sites on the palm. To assess the relative merits of the swab and the scrub methods of sampling the bacterial populations of the skin surface, we carried out tests on the palm, a site where it seemed probable that there would be little subsurface flora. Two adjoining sites on the hypothenar eminence of both palms were tested. They were designated as proximal or distal according to their positions relative to each other. The swab method and the scrub method were each used on the proximal site of one hand and the distal site of the other. Five of the six subjects used in this portion of the study were tested a second time, with the method of harvest from the several sites reversed. Thus, the effects of a difference between the two hands and of proximal versus distal site were balanced.

To assure a countable population of bacteria on the palmar surface, the subject massaged his forehead for 15 s with one hand and then for 15 s with the other hand, thus transferring bacteria and the associated fatty secretions from the forehead to the palm. He then rubbed his two hands together for 60 s, using a pattern of motion aimed at distributing the bacteria as uniformly as possible over the palmar areas to be cultured. In preliminary tests of various sites on the palm, we found the least quantitative variability in cultures from the hypothenar eminence (the smooth fleshy part of the palm on the side opposite the thumb). The latter area was, therefore, used in all subsequent tests.

All four specimens, proximal and distal from right and left hands, were collected before further processing was carried out. We arranged the time sequence of harvests from the several sites to compensate for any possible decline in numbers of viable organisms on the palm from the start to completion of the harvesting process, about 20 min. No such decline could be detected in control tests over a much longer time.

Tests of the same site by both methods: swab for surface and scrub for subsurface flora. Having validated the wet swab procedure as a satisfactory method for sampling the surface flora, we proceeded to test the palm and the forehead by using both methods on the same site. The hypothenar eminence of the palm and the center of the forehead were cultured first by a single 15-s swabbing of an area 31 mm in diameter and immediately thereafter by one 60-s scrub with a slightly smaller area (diameter, 25 mm) to be sure that no new skin was included.

Tests for toxicity of cotton. In two experiments we collected bacteria from the forehead by swabbing

and dispersed them in 10 ml of diluent. A 0.2-ml amount was removed for quantitative cultures, and five fresh cotton swabs were then immersed in the original tube of diluent and agitated for 1 min. A 0.2-ml amount was again removed for culture, and 10 fresh swabs were now placed in the original fluid and agitated for 1 min. A final set of cultures was then prepared.

RESULTS

The predominant organism in aerobic culture was in all cases a gram-positive, catalase-positive coccus. Well-isolated colonies were flat or slightly convex, gray to white, and 3 to 6 mm in diameter, an appearance consistent with *S. epidermidis*. With a single exception, all anaerobic cultures yielded a great predominance of propionibacteria with the colonial appearance of *Propionibacterium acnes*. One site on JC yielded 70% of colonies with the appearance of *P. granulosum*. In other specimens such colonies usually were less than 1% of the total and never exceeded 3%.

Tests for toxicity of cotton. The tests for inhibition of bacterial growth by agitation of 5 or 10 cotton swabs in a suspension of bacteria yielded entirely negative results. There was no significant decrease after the exposure to 5 swabs nor after the final exposure to 10 swabs.

Comparison of numbers of bacteria from scrub and swab of palm. Tables 1 and 2 show the results of our comparative tests of the scrub and swab methods of harvesting bacteria from the palm. There was no clear superiority of one

TABLE 1. *Comparison of numbers of propionibacteria and gram-positive cocci recovered from adjacent sites on the palm by the wet cotton swab and the scrub methods*[a]

Subject	Test	Hand	Propionibacteria				Gram-positive cocci			
			Proximal		Distal		Proximal		Distal	
			Scrub	Swab	Scrub	Swab	Scrub	Swab	Scrub	Swab
JC	1	R		2,100	9,600			<50	170	
		L	1,200			6,700	95			95
JC	2	R	4,100			5,000	<50			<50
		L		1,700	4,300			<50	100	
CE	1	R		14,000	31,000			450	480	
		L	11,000			5,900	270			290
CE	2	R	57,000			47,000	230			150
		L		12,000	6,300			190	160	
NG	1	R		12,000	2,700			130	100	
		L	29,000			5,000	460			130
NG	2	R	14,000			3,400	240			120
		L		11,000	2,600			100	<50	
WL	1	R	7,300			1,300	360			130
		L		5,300	3,200			200	150	
JL	1	R		7,100	8,200			120	89	
		L	1,300			2,200	<50			63
JL	2	R	11,000			13,000	100			190
		L		1,400	1,400			63	<50	
RS	1	R		3,000	1,500			79	37	
		L	8,900			10,000	140			190
RS	2	R	7,500			8,300	170			220
		L		17,000	20,000			340	190	
RS	3	R		13,000	16,000			<50	<50	
		L	4,900			6,700	79			74
Geometric mean			3.8859	3.7868	3.7499	3.7928	2.1702	2.0626	2.0154	2.0992
Standard error			0.140	0.114	0.127	0.113	0.092	0.094	0.092	0.066

[a] Letters used to identify subjects in this table correspond to the letters used for the same subjects in Table 3. Numbers refer to bacteria per square centimeter of skin of the hypothenar eminence and are rounded to the closest two digits. Fewer than 5 colonies/aerobic culture are recorded as <50 (5 colonies = 50/cm^2).

method over the other. For example, comparison of numbers of propionibacteria recovered by the swab and the scrub tests of adjoining sites of the same hand shows that 12 times the swab yielded a higher number, 11 times the scrub yielded a higher number, and in one test the results were identical. The geometric mean values in the various combinations showed 10 to 25% more organisms recovered by the scrub method in five of the six combinations, but this difference was not significant. To take into account individual variations, we analyzed the data by the paired *t* test (Table 2). In every case the observed differences are likely to have been due to chance alone (P = 0.2 to 0.8). This was true when we compared the two adjacent sites of the same hand or the proximal or distal sites of the two hands, using either anaerobic or aerobic cultures.

TABLE 2. *Probabilities based on paired t test*

Comparison of scrub and swab:	Propionibacteria		Gram-positive cocci	
	t	*P*	*t*	*P*
On the same hand	−0.33	0.7	0.57	0.6
On proximal sites of right and left hands	−0.71	0.5	−1.32	0.2
On distal sites of right and left hands	0.25	0.8	0.89	0.4

Similar analysis of numbers of gram-positive cocci recovered from adjacent sites of the same hand (Table 1) showed that a larger number was obtained by the swab method 13 times and by the scrub method 8 times, and there was no difference in three tests.

Two successive tests of the same site, first by swab for surface flora and second by scrub for subsurface flora. When two successive harvests, the first by swabbing and the second by scrubbing, were collected from the same site, there was a striking difference between results of tests of the palm and those of the forehead (Table 3). On the palm we recovered propionibacteria by the swab in numbers that ranged from essentially the same as the scrub to 14 times as many as from the scrub. In seven of eight tests the swab yielded more than two times as many as the scrub. On the forehead we consistently obtained more propionibacteria from the scrub than from the preceding swab. The ratio ranged from 3 to 8, with a median value of 5.

Our data with respect to gram-positive cocci growing aerobically show that several tests of the palm and one of the forehead were unsatisfactory because the numbers of organisms were too few to give reliable results. In all six in-

TABLE 3. *Numbers of bacteria recovered by successive harvests of the same site first by swab and second by scrub method*[a]

Subject	Side	No. of bacteria/cm²					
		Propionibacteria			Gram-positive cocci		
		Swab	Scrub	Swab/scrub ratio	Swab	Scrub	Swab/scrub ratio
Palm							
CE	L	7,600	600	13:1			
	R	23,000	11,000	2:1	200	26	8:1
RS	L	630	800	1:1	<8	<20	
	R	2,400	1,100	2:1	300	64	5:1
JS	L	4,000	400	10:1	800	70	11:1
	R	2,600	1,200	2:1	500	220	2:1
JL	L	1,850	130	14:1	97	52	2:1
	R	1,750	<300	>6:1	67	<20	≥3:1
Forehead							
CE	L	1,200,000	10,000,000	1:8	40,000	36,000	1:1
	R	1,200,000	6,000,000	1:5	9,800	39,000	1:4
RS	L	560,000	1,600,000	1:3	<80	200	
	R	480,000	1,900,000	1:4	29,000	78,000	1:3
JS	L	500,000	3,900,000	1:8	240,000	34,000	7:1
	R	1,500,000	10,000,000	1:7	27,000	5,200	5:1
JL	L	2,000,000	10,000,000	1:5	26,000	28,000	1:1
	R	3,000,000	8,000,000	1:3	43,000	34,000	1:1

[a] The swab/scrub ratio is rounded to the nearest whole number. The symbol < means that the average number of colonies on duplicate cultures of the specimen was fewer than 5.

stances in which countable numbers of bacteria were recovered from at least one test site, the number of aerobic cocci recovered from the palm by the scrub method was smaller than the number obtained from the swab.

On the forehead the relative numbers of cocci in the two harvests were variable: in three tests they were essentially the same, in two tests the swab recovered a great excess (ratios of 7 and 5), and in two tests the scrub recovered three and four times as many as the swab.

DISCUSSION

Use of wet cotton swabs for harvesting bacteria from the surface of normal skin has fallen into disrepute for reasons that we think are largely not justified. Our data show that the cotton swab was essentially as good as the scrub method for quantitation of both aerobes and anaerobes on the palm. There was no indication that cotton was toxic or failed to pick up organisms or to release them into the diluent.

Concern about the toxicity of cotton is derived largely from studies of comparatively delicate pathogens, such as *Neisseria gonorrhoeae* or streptococci (1, 4, 9, 14, 19), held on a swab for hours or even 1 or 2 days before culture. Such evidence can hardly apply a priori to indigenous organisms of skin transferred in a matter of seconds to diluent in which these bacteria are known to survive well. The "toxicity" of cotton has been attributed to a lipid constituent of cotton (4) or to an acid pH (1). Bacteria indigenous to normal skin inhabit a lipid-rich environment that is usually acid. The alkaline pH of the Williamson-Kligman diluent might help counter a possible bactericidal effect of some acid cotton swabs; the Triton X-100 undoubtedly is important in achieving good dispersion of the bacteria.

In our opinion wet cotton swabs can be used with confidence in quantitative studies of the flora of the surface of normal skin, provided organisms are dispersed promptly into a suitable diluent.

The great variability in the numbers of bacteria recovered from adjacent skin sites (Table 1) is a troublesome but unavoidable feature in any assay of skin flora. With well-standardized methods of harvesting, this variability reflects the conditions on skin where bacteria grow in microcolonies and are distributed unevenly, not as a diffuse, evenly mixed film.

The results of our tests with the swab method and the scrub method applied in succession to the same site on the palm are consistent with the interpretation that the populations of both anaerobes (propionibacteria) and aerobes (gram-positive cocci) on the hypothenar eminence are largely surface populations and can be assayed as reliably with a simple wet cotton swab as by the scrub method. The scrub method recovered no more or a negligibly greater number of bacteria than did the swab method. On the forehead, the scrub method yielded substantially larger numbers of propionibacteria than did the swab, presumably because scrubbing recovered a larger proportion of those organisms located beneath the skin surface, chiefly in the pilosebaceous units. From Table 3 it is clear that on the forehead the cocci were more superficially located than were the propionibacteria in most of the skin sites sampled. However, in one test of CE and one of RS the cocci, like the propionibacteria, were predominantly in a subsurface location, as shown by the higher numbers recovered by the scrub method. Apparently, on the foreheads of our subjects the cocci occurred predominantly in a more superficial location than propionibacteria, with only an occasional microcolony extending so deeply that it was not well sampled by the wet cotton swab.

In comparative tests of methods of quantitating aerobic organisms from the back, Shaw et al. (15) found that scrubbing with a Teflon spatula yielded numbers of bacteria not significantly different from those obtained with a moistened rayon swab. Cotton swabs were not tested. Their data support the concept that cocci occur at a relatively superficial site on the back. They did not compare the recovery of anaerobes by the several methods. Holland et al. (7) have described a method of identification and quantitation of skin bacteria of deep sites based on removing the contents of pilosebaceous units with cyanoacrylate gel and dispersing surviving organisms for culture by mechanical agitation with glass beads. In future studies it will be interesting to compare the subsurface flora as determined by this technique with that based on successive swab and scrub tests of a single site.

ACKNOWLEDGMENTS

This study was aided by Public Health Service research grants AI 10938 and AI 11915 from the National Institute of Allergy and Infectious Diseases.

We greatly appreciate the advice of Patricia Wahl on the statistical aspects of this study.

LITERATURE CITED

1. **Anderson, K. F.** 1965. Antibacterial bacteriological swabs. Br. Med. J. 2:1123–1124.
2. **Beetz, H.-M.** 1972. Zur Tiefenverteilung der Hautbakterien im Stratum corneum. Arch. Dermatol. Forsch. 244:76–80.
3. **Burtenshaw, J. M. L.** 1938. The mortality of the haemolytic *Streptococcus* on the skin and on other surfaces. J. Hyg. 38:575–586.

4. **Ellner, P. D., and C. J. Ellner.** 1966. Survival of bacteria on swabs. J. Bacteriol. 91:905-906.
5. **Evans, C. A.** 1975. Persistent individual differences in the bacterial flora of the skin of the forehead: numbers of propionibacteria. J. Invest. Dermatol. 64:42-46.
6. **Evans, C. A., W. M. Smith, E. A. Johnston, and E. R. Giblett.** 1950. Bacterial flora of the normal human skin. J. Invest. Dermatol. 15:305-324.
7. **Holland, K. T., C. D. Roberts, W. J. Cunliffe, and M. Williams.** 1974. A technique for sampling microorganisms from the pilo-sebaceous ducts. J. Appl. Bacteriol. 37:289-296.
8. **Kooyman, D. J., and R. W. Simons.** 1965. "Sticky-disc" sampling of skin microflora. Arch. Dermatol. 92:581-584.
9. **Mair, N. S., and D. A. McSwiggan.** 1965. Antibacterial bacteriological swab. Br. Med. J. 2:230.
10. **Montes, L. F., and W. H. Wilborn.** 1970. Anatomical location of normal skin flora. Arch. Dermatol. 101:145-159.
11. **Noble, W. C., and D. A. Somerville.** 1974. Microbiology of human skin. W. B. Saunders Co. Ltd., London.
12. **Pachtman, E. A., E. E. Vicher, and M. J. Brunner.** 1954. The bacteriologic flora in seborrheic dermatitis. J. Invest. Dermatol. 23:389-396.
13. **Papa, C. M., and B. Farber.** 1971. Direct scanning electron microscopy of human skin. Arch. Dermatol. 104:262-270.
14. **Rubbo, S. D., and M. Benjamin.** 1951. Some observations on survival of pathogenic bacteria on cotton-wool swabs. Development of a new type of swab. Br. Med. J. 1:983-987.
15. **Shaw, C. M., J. A. Smith, M. E. McBride, and W. C. Duncan.** 1970. An evaluation of techniques for sampling skin flora. J. Invest. Dermatol. 54:160-163.
16. **Somerville, D. A., and W. C. Noble.** 1973. Microcolony size of microbes on human skin. J. Med. Microbiol. 6:323-328.
17. **Updegraff, D. M.** 1964. A cultural method of quantitatively studying the microorganisms in the skin. J. Invest. Dermatol. 43:129-137.
18. **U. S. General Services Administration.** Federal Supply Service, Commissioner. 1960. Federal specification: cotton, sterile (purified) and nonsterile. JJJ-C-561d. Government Printing Office, Washington, D.C.
19. **White, W. D.** 1965. Antibacterial bacteriological swab. Br. Med. J. 2:229-230.
20. **Williamson, P., and A. M. Kligman.** 1965. A new method for the quantitative investigation of cutaneous bacteria. J. Invest. Dermatol. 45:498-503.