Modified Scrub Technique for Sampling Infant Skin Microflora

BRUCE H. KESWICK* AND D. FRANK
The Procter & Gamble Co., Cincinnati, Ohio 45224

Received 2 July 1987/Accepted 3 September 1987

Two techniques for quantitatively recovering normal-flora microorganisms from the skin of infants were compared. A technique using a swab to dislodge microorganisms from the skin compared favorably to a technique using a rubber policeman. The swab was easier to use and is suitable for use on infant skin.

Skin is inhabited by a wide variety of microorganisms, but the factors which control normal skin flora are not well understood. To understand how outside influences affect changes in normal skin flora, it is necessary to evaluate the skin flora and to monitor changes in these organisms.

The study of the microbiology of skin was facilitated by the development of the skin scrub technique (2, 7, 10). However, in some respects the technique is difficult to apply and produces unacceptable discomfort and trauma (3). Since we planned to study small children with a sensitive skin condition, atopic dermatitis, a more suitable technique was required. Also, since we wanted to detect large shifts in flora on the surface of the skin (major surface flora types) and not every organism present, a modification of the scrub technique using a cotton swab that had been tested on adults (8) was thought suitable. A wetted swab is softer than a rubber or teflon policeman and eliminates the loss of sample buffer due to movement of the child. We compared the two techniques for the recovery from infants' skin of major groups of organisms which have an established etiological role in skin or urinary tract disease.

Informed parental permission was obtained for all participants. Twenty children aged 2 months to 2 years were studied. On three separate occasions, four samples were collected from each child. Two samples were collected from sites inside the area covered by the diaper on the suprapubic region of the abdomen, and two were from the suprapubic region, about 5 cm outside of the area covered by the diaper. Two of the samples were collected with a rubber policeman (modified scrub technique), and two were taken by the swab technique. For the scrub technique, a sterile glass cylinder (2.4-cm diameter) was pressed against the skin of the abdomen and 2.0 ml of 0.075 M phosphate buffer (pH 7.9) containing Triton X-100 (0.1%) (10) was added. The skin was scrubbed with a rubber policeman for 30 s, and the buffer was removed with a transfer pipette. For the swab technique, a Dacron swab (American Scientific Products catalog no. A5005-1) was dipped in a tube containing 2 ml of the buffer and used to swab the skin in the area delineated by a sterile glass cylinder for 30 s. The swab was then placed back in the tube containing buffer and mixed on a vortex mixer for 1 min. Fluid was expressed from the swab, and the swab was discarded. Within about 1 h after collection, the samples were plated at an independent laboratory by personnel unaware of the sample identities.

The samples were plated on Trypticase (BBL Microbiology Systems) soy agar containing 5% sheep erythrocytes (blood agar), mannitol salt agar, MacConkey agar, and Sabouraud dextrose agar with chloramphenicol and cycloheximide (0.5 g/l). Dilutions to 10−2 were plated. Strep- tococci were identified by hemolysis reaction and Strep-tek identification. Staphylococcus aureus was confirmed by the Staphylox test. Escherichia coli was confirmed by the Minitest indole test on lactose-fermenting colonies. Candida albicans was confirmed by germ tube formation. The mean log counts recovered by each technique were compared by a paired t test.

The swabs used did not possess antibacterial activity as has been reported previously (1, 9). We tested the survival of E. coli, Staphylococcus epidermidis, and representative organisms from infant skin in the buffer system and found no significant reduction in numbers after 0, 1, 2, or 4 h at room (25°C) or refrigerator (4°C) temperature or in an ice bath (0°C).

The recovery of test organisms from inside the diaper area ranged from undetectability (the limit of detection was 5 CFU/cm²) to 10⁵ CFU/cm². This agrees with the ranges reported in similar studies (5, 6). With the exception of the undifferentiated total plate count organisms, coagulase-negative staphylococci and S. aureus were the most frequent isolates. The log mean recovery of each organism was similar by both methods and higher inside than outside the diaper area (Fig. 1). No significant differences (P < 0.05) between the methods were detected when they were compared at each sample period (Table 1). Although the number of organisms on the skin of individual subjects varied considerably over time, the two methods remained comparable for the recovery of the test organisms.

The methods compared favorably for the frequency of isolation of each organism from skin from both inside and outside the diaper area (Table 2). No significant differences were detected between the methods when they were compared.

**TABLE 1.** Total microorganism count from infant skin by two methods in three sampling periods

<table>
<thead>
<tr>
<th>Type of value</th>
<th>Log₁₀ total plate count/cm² at sample (wk)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Scrub</td>
</tr>
<tr>
<td>Mean</td>
<td>4.8</td>
</tr>
<tr>
<td>SD</td>
<td>2.1</td>
</tr>
<tr>
<td>Maximum</td>
<td>7.7</td>
</tr>
</tbody>
</table>

* Twenty children's skin was sampled on three separate occasions. No significant difference between the two sampling techniques was detected when they were compared at each of the three sampling periods. The P values were 0.80, 0.96, and 0.41 for week-1, -4, and -10 samples, respectively.
pared at each sample period. Values are presented in summary form as the means of the means from each sample period.

On the basis of the results of this study, we calculated that the technique has sufficient sensitivity to detect 1 log CFU/ml difference between groups of eight subjects (alpha, 0.05; beta, 0.50).

This study demonstrated that the technique using a swab for sampling infant skin was equivalent to the technique using a rubber policeman in terms of the numbers and types of organisms recovered and was easier to use. The swab technique also was visibly less irritating to infant skin.

The reported major limitations of the swab technique are inability to recover subsurface organisms (3) and inability to detect very small numbers of organisms. Since we were interested in major shifts in flora commonly found on the skin surface, these limitations were not encountered. The swab technique has been used successfully to study the skin flora of children with normal skin and children with a sensitive skin condition, atopic dermatitis (4).

LITERATURE CITED