females of adolescent or adult age with a local injection history, but there has been only one report on childhood LIL[2]. Thus, we think that this case is very interesting in terms of age of onset, gender, and unidentified triggering factor.

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Stamp-Form Contact Plate: A Simple and Useful Culture Method for Microorganisms of the Skin

Jung Won Shin, Hye Ryung Choi, Kyoung Chan Park

Department of Dermatology, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea

Dear Editor:

The distribution, type and density of cutaneous microflora are variable depending on the anatomical regions involved, and this diversity may affect the pathogenesis and progress of skin disorders. In particular, atopic dermatitis (AD) has been known to be closely related with *Staphylococcus aureus* colonization. *S. aureus* affects AD by producing exotoxins with superantigenic properties[1]. Therefore, the dynamics of cutaneous microbial population is important in AD. Several methods have been developed to quantify the microflora of the skin, including the scrub method, swab method, tape method, and contact plate method. Apart from the contact plate method, all of these techniques require two steps: sampling and inoculation. If the contact plate method is used, sampling and inoculation can be performed in one step by inoculating bacteria directly from skin to the agar plate. Furthermore, distribution of bacteria in the tested area can be observed by direct contact. The purpose of this study was to quantitatively analyze *S. aureus* in AD and a normal control using a self manufactured stamp-form contact-plate.

Nine AD patients (two male, seven female, mean age of 12.3 years old) and ten normal controls (three male, seven

Table 1. Demographics of atopic dermatitis patients

	Age (yr)	Sex	The most severe area	SCORAD
1	24	Female	Left wrist	9.56
2	26	Female	Right popliteal fossa	9.81
3	23	Female	Left cubital fossa	42.26
4	6	Female	Neck	60.41
5	11	Male	Right popliteal fossa	18.16
6	8	Female	Left popliteal fossa	52.10
7	10	Female	Left cubital fossa	12.74
8	8	Male	Left popliteal fossa	24.97
9	6	Female	Right popliteal fossa	18.26

SCORAD: SCORing atopic dermatitis.

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Corresponding author: Kyoung Chan Park, Department of Dermatology, Seoul National University Bundang Hospital, 82 Gumi-ro 173 beon-gil, Bundang-gu, Seongnam 463-707, Korea. Tel: 82-31-787-7311, Fax: 82-31-787-4058, E-mail: gcpark@snu.ac.kr

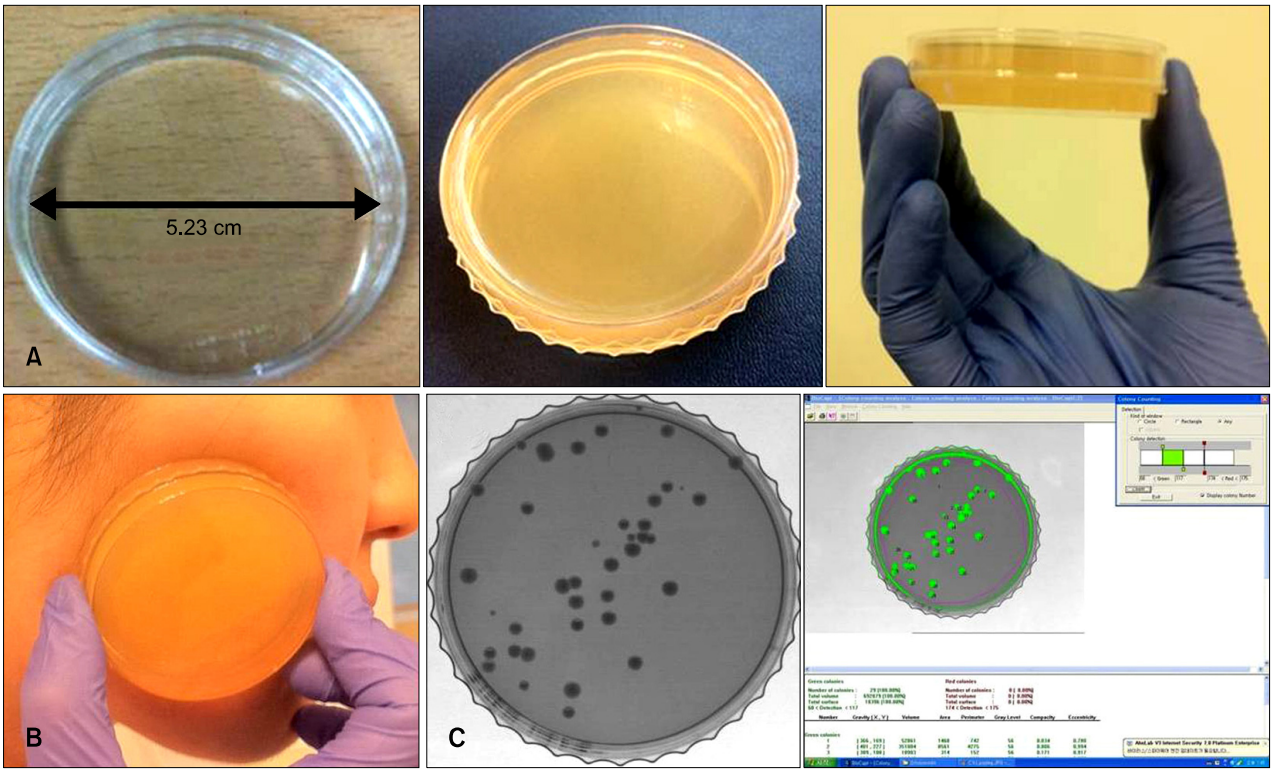

Fig. 1. Stamp-form contact-plate. (A) Self manufactured stamp plate-like contact-plate, (B) sampling technique, (C) colony forming units (CFU) counting using image analysis (Biocapat, Vilber Lourmet, France).

Table 2. Frequency and density of *S. aureus* colonization in atopic dermatitis (AD) patients and the normal control

	Frequency of colonization (%)		CFU/cm^2	
	AD patients	Normal control	AD patients	Normal control
Cheek	88.9*	40	3.55±3.55	1.91±3.42
Neck	100*	60	13.81±32.30†	0.65±1.09
Forearm	88.9	50	2.91±4.92*	0.80±1.53
Cubital fossa	100*	60	14.31±20.21	1.87±3.34
Abdomen	88.9	50	1.07±1.55	1.15±1.11
Leg	77.8	60	1.70±1.93	1.97±2.72
Popliteal fossa	88.9*	40	25.58±34.8†	0.77±1.13

*$p<0.05$, $^{\dagger}p<0.01$ measured by chi-square test. CPU; colony forming units.

female, mean age of 29.6 years old) were enrolled in the present study. Our study was approved by the institutional review board of Seoul National University Bundang Hospital. Clinical information for the AD patients is summarized in Table 1. Stamp-like contact-plates were prepared in our laboratory. A petri dish with a diameter of 5.23 cm was completely filled with sterilized liquid blood heart infusion agar and coagulated on a clean bench to create a solid agar (Fig. 1A). The surface of the agar became a little concave after coagulation but was in even contact with the skin when the agar plate was attached to the skin. Samples were obtained from seven sites (cheek, neck, forearm, cubital fossa, abdomen, lower leg, and popliteal fossa) by contacting the plate to the skin for ten seconds (Fig. 1B). These were then incubated at a temperature of 36°C for 24 hours. Colony forming units (CFU)/cm^2 were calculated using an image analyzer BioCapt (Vilber Lourmat, Marne la Vallée, France) and confirmed with the unaided eye (Fig. 1C). *S. aureus* was identified through secondary culture using Gram staining and coagulase tests. In AD patients, the SCORing atopic dermatitis (SCORAD) index was calculated to evaluate disease severity.

The positive rates of *S. aureus* colonization were signi-

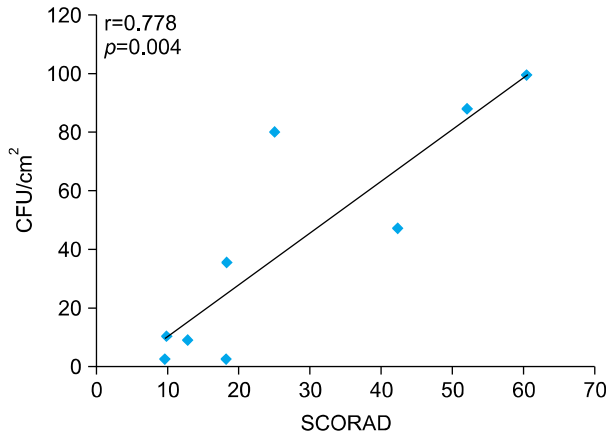

Fig. 2. Relationship between SCORing atopic dermatitis (SCORAD) index and colony forming units (CFU) in the most severe lesion in atopic dermatitis patients.

ficantly higher in the AD group than in the control group, and a similar result was observed for the mean CFU/cm^2 (Table 2). The mean SCORAD index measured in AD patients was 27.59 (9.56～60.41) and there was a significant positive correlation between the SCORAD index and CFU/cm^2 measured at the most severe lesions ($r=0.778$, $p<0.01$, Fig. 2).

Several culture methods have been previously developed for quantitative analysis of microorganisms, including the swab method, scrub method, tape method, and contact plate method. The swab method is one of the most commonly-used ways of collecting bacteria and involves the use of a cotton swab and inoculation on agar[2]. It is simple and convenient but generally unreliable for quantitative analysis. The scrub method also allows the collection of bacteria and involves scrubbing with a sterilized instrument, followed by inoculation on agar. Although this process is time consuming and requires skillful techniques, it is known to be precise[3]. However, the distribution of bacteria cannot be observed by this method. The tape method is relatively cheap and simple but can cause suppression of bacterial growth due to the adhesive material of the tape[4]. By contrast, the contact plate method is practical and very simple. Unlike the other techniques, it does not require inoculation onto agar. Furthermore, this approach can be used to determine the distribution of bacteria in tested areas. However, the contact plate method has not been routinely used for this purpose.Previously, Sung et al.[5] designed a 'stamp-like' contact agar plate, which had a convex surface and the convex agar plate was acquired as a commercial custom order. However, it is not commercially available. Thus, we prepared a modified 'stamp-form' contact plate using a small petri dish, as an item generally used in the laboratory. Although the surface of the agar plate was slightly concave, we found that it could contact the skin well when it was applied. In this study, *S. aureus* colonization was clearly observed in AD by using the stamp-form contact plate. In addition, our results showed that colonization severity was highly correlated with the SCORAD index. Thus, this contact plate method is a useful means of studying bacterial colonization on the skin.

In conclusion, our modified stamp-form contact-plate sampling technique is a simple, inexpensive and reliable method to culture skin *S. aureus* quantitatively. Further studies using this method need to be conducted with increased numbers of subjects and age matched controls to study the microorganisms of many skin diseases, including AD.

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