

A Noninvasive Objective Measure of Sunscreen Use and Reapplication

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Abstract

Objectives: To study whether a noninvasive swabbing technique can detect sunscreen use for up to 6 hours, and whether the technique can detect reapplication of sunscreen. **Methods:** Thirty volunteer office workers were randomly assigned to have one of a variety of sunscreens applied using recommended application techniques, and half were randomly assigned to have sunscreen reapplied after 3 hours. Alcohol-based swabs were used to obtain a sample from participants' arm at 20 minutes, and hourly from 1 to 6 hours post-application. Absorption readings were analyzed using an UV-visible spectrophotometer.

Results: The swabbing technique was consistently able to distinguish the sunscreen from control swabs for up to

6 hours. The absorption readings between 20 minutes and 6 hours were significantly higher than control swabs. There were no differences between the group that had sunscreen reapplied and the group that did not.

Conclusion: The sunscreen swabbing technique is an effective noninvasive method for detecting a variety of sunscreen products in adults over a 6-hour period. No differences in absorption readings were found with sunscreen reapplication. This procedure will be a useful adjunct to other objective measures of sun protection and UV radiation exposure, resulting in a more accurate picture of the sun protection habits of individuals. (*Cancer Epidemiol Biomarkers Prev* 2005;14(3):722-6)

Introduction

Skin cancer, the most common form of cancer in the United States, is increasing (1, 2). Most skin cancers can be prevented by reducing sun exposure and covering up: seeking shade, using sunscreen properly, and wearing protective hats and clothing (1). When used properly, sunscreen can prevent sunburn (3, 4), nonmelanocytic (5), and melanocytic (6-8) skin cancers. Sunscreens have become very popular for the prevention of sun damage, and are often the method of choice for people who are trying to reduce their UV radiation exposure and its effects (9, 10).

Most sunscreens combine organic UV-absorbing and inorganic UV-reflecting chemicals to provide broad-spectrum protection (11). The sun protection afforded by a sunscreen is defined by its sun protection factor (SPF), which is assessed by an internationally agreed application thickness of 2 mg/cm² (11). The SPF protection afforded may be reduced due to inadequate application (<2 mg/cm²), water resistance, and abrasion from clothing and/or sand (12, 13). Several studies have found that consumers apply much less than this recommendation (14-16), providing a level of protection 20% to 50% less than that stated by the SPF (17).

Our understanding of the use of sunscreens relies on self-report data, which introduces a major limitation due to possible social desirability bias (18, 19). Observational measures are useful for assessing body coverage from hats and clothing, but observation is a weak measure of sunscreen use since it is only possible to observe when a person is applying sunscreen and not whether it is on the skin. Indirect procedures such as returning bottles for weighing the remaining sunscreen have been used in trials where

sunscreen was provided to study subjects (5, 20), but these methods cannot be applied to most population-based studies. Fluorescence spectroscopy is a recently developed technique to measure the thickness of sunscreen application (21, 22), but it also seems to have limited utility in large-scale community studies as it requires expensive equipment to be taken into the field.

Sunscreen swabbing has been proposed as a quick and reliable method for determining whether sunscreen has been applied to the skin (23). This procedure is based on the fact that organic compounds make up the majority of sunscreens and absorb radiation in the UV spectrum (23). Sunscreen swabbing has proven successful within a laboratory setting (21 office workers) and on a small sample of 12 children 2 to 4 years of age (23). The procedure is portable, rapid, and easy to perform, and swabs can be stored and analyzed in batches days or weeks later. Therefore, it is promising for use as an objective measure of sun prevention behaviors and should be tested in the United States using commonly sold sunscreens.

Reapplication is generally considered an important element of the effective use of sunscreen (11). Compared to the first application, the second sunscreen application is believed to provide approximately two times more protection against sunburn (24). As reapplication is an important aspect of effective sunscreen use, the purpose of this study is to ascertain if a noninvasive swabbing technique can determine both sunscreen use and reapplication in adults.

Materials and Methods

Participants. Approval for this study was obtained from the Committee on Human Studies at the University of Hawaii at Manoa. An e-mail invitation to participate in the trial was circulated to all staff at the Cancer Research Center and two academic departments at the University of Hawaii. The invitation provided a brief overview of the study, and described the amount of involvement required. Interested staff were provided a packet containing more detailed information about the project and a consent form to be signed and returned

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to researchers. A total of 30 volunteer office workers were recruited for this study and remained indoors throughout their involvement in the study. Participants were predominantly female (70%), had a mean age of 34 years (range 19-59), and represented a range of ethnic backgrounds (Caucasian 70%, Asian 23%, Latino 7%). All participants received a small incentive for their involvement in the study.

Sunscreens. The sunscreens included a range of popular brands of various SPF strengths sold in the United States. They included: Coppertone (SPF 30), Coppertone Sport (SPF 30), Neutrogena (SPF 30), Banana Boat Sport (SPF 30), Banana Boat Faces (SPF 23), and Hawaiian Tropic (SPF 15+). We used a variety of brand name products to avoid the risk of drawing conclusions that would be based on only one type of sunscreen. An overview of the sunscreens that shows the manufacturer, SPF, and active ingredients is provided in Table 1.

Swabbing Procedure. Each participant was assigned a number from a random number list, corresponding to the different sunscreen products. The participant's right and left arm were cleaned with an alcohol swab prior to having sunscreen applied. Data collectors were responsible for sunscreen application and reapplication in addition to swabbing participants. Sunscreen was applied to a 50 cm² area on the surface of the right and left forearms at the beginning of the day using a standard dose of 0.1 ml over the delineated area (dose rate, 2 mg/cm²). Sunscreen was then lightly and evenly rubbed into the skin by the researcher using a disposable, nonabsorbent glove, and participants were asked to leave their forearms free of clothing for the duration of the trial. Each 50 cm² test area was divided into a grid of five equal sections (2.5 × 4 cm). The grid was drawn onto the skin using an indelible marking pen that could easily be removed at the end of data collection. We used BD Alcohol Swabs (70% isopropyl alcohol) for swabbing the skin as they were individually wrapped and small in size (~2.5 cm²). The most distal section of the right arm was swabbed before applying any sunscreen (baseline control), and each successively more proximal grid

on the right arm was swabbed after intervals of 20 minutes, 1, 2, and 3 hours after initial application, taking care to only wipe within the specified test section.

Sunscreen reapplication was undertaken at hour 3. Half of the participants were randomly selected to have their assigned sunscreen reapplied to the designated area on the left forearm by the researcher, using the same procedure described above. The most distal section of the left arm was swabbed prior to reapplying any sunscreen (reapplication control), and each successively more proximal grid on the left arm of all participants was swabbed at intervals of 20 minutes, 1, 2, and 3 hours, respectively, after reapplication. Thus, swabbing continued for 6 hours after the initial application.

Sunscreen Absorption Readings and Analysis. Eluted washings (0.5 mL) were transferred to a UV-rated cuvette (BrandTech UVB ultra micro, 70-880 μL) and absorbance was determined using a UV-Visible Spectrophotometer (Beckman DU-530) at 5 nm intervals over the wavelength 280 to 400 nm (the UVA and UVB spectrum). Absorbance is defined as the logarithm to the base 10 of the ratio of the spectral radiant power of light transmitted through a reference sample to that of the light transmitted through a control sample. A swab placed directly into ethanol was used as a reference standard (control swab) for all other swabs, thereby limiting potential light-absorbing properties of the swab itself. In previous research, absorbance readings at 320 nm have been found to be the most reliable indicators (23). We were able to ascertain the presence of sunscreen by comparing absorbance readings at 320 nm with a baseline swab wiped on the skin before sunscreen was applied. Due to coding of samples, laboratory staff were blinded to specific information relating to individual swabs.

Absorbance readings from the spectrophotometer were imported to a database for statistical analysis using SPSS (version 12; ref. 25). Descriptive and graphical analyses were undertaken on all absorbance readings. ANOVA was used to determine if there was any statistical difference between application and reapplication, and repeated measures

Table 1. Details of sunscreens used

Sunscreen Name	Manufacturer	SPF	Active Ingredients	
			Organic	Inorganic
(1) Coppertone	Schering-Plough	30	<ul style="list-style-type: none"> • Avobenzine (Parsol 1789) • Homosalate • Octocrylene • Octisalate • Oxybenzone 	• No inorganic ingredients
(2) Coppertone Sport	Schering-Plough	30	<ul style="list-style-type: none"> • Homosalate • Octyl methoxycinnamate • Octyl salicylate • Oxybenzone 	• No inorganic ingredients
(3) Neutrogena	Neutrogena Corp.	30	<ul style="list-style-type: none"> • Avobenzone (2%) • Homosalate (12%) • Octinoxate (7.5%) • Octisalate (5%) • Oxtbenzone (6%) 	• No inorganic ingredients
(4) Banana Boat Sport	Sun Pharmaceuticals	30	<ul style="list-style-type: none"> • Octinoxate (7.5%) • Octisalate (4.75%) • Oxybenzone (4.75%) 	• Titanium dioxide (1.2%)
(5) Banana Boat Faces	Sun Pharmaceuticals	23	<ul style="list-style-type: none"> • Octyl methoxycinnamate • Oxybenzone • Octyl salicylate 	• No inorganic ingredients
(6) Hawaiian Tropic	Tanning Research Labs, Inc.	15+	<ul style="list-style-type: none"> • Octinoxate • Octisalate 	• Titanium dioxide

		Known Sunscreen	
		YES	NO
Above Cut-off	YES	a	b
	NO	c	d

Figure 1. A 2 × 2 table demonstrating how sensitivity and specificity were calculated. Sensitivity was defined as $a / (a + c)$, whereas specificity was defined as $d / (b + d)$.

ANOVA were used to test for differences between swabs with no sunscreen and swabs taken at various lengths of time after application (20 minutes, 1 hour, 2 hours, etc.). To determine sensitivity and specificity, we categorized absorbance readings for all samples taken with sunscreen of the 6 hours including those with reapplication ($n = 240$) as sunscreen-positive, and those taken before sunscreen was applied as sunscreen-negative ($n = 30$). These known results were then compared with absorbance readings at 320 nm. The mean absorbance and Standard Deviation (SD) of each group was taken and the maximum distance of 5.4 SDs from each mean was an absorbance of 0.147. Therefore, this measurement was used as the cutoff, and sensitivity and specificity were calculated according to Fig. 1.

Results

When compared with the control swab, all sunscreens showed considerable absorbance throughout the UVB (280-320 nm) and UVA (320-400 nm) wavelengths. The mean absorbance readings for the six sunscreens are shown in Fig. 2. This figure illustrates considerable variation in the absorbance of sunscreen at shorter (280-300 nm) and longer wavelengths (340-400 nm) with the most stable indicator of sunscreen being around 320 nm for all sunscreens tested.

Figure 3 illustrates mean absorbance readings of all sunscreens at 320 nm at time points throughout the 6-hour

study. Repeated measures ANOVA revealed that there was a significant difference between the baseline swab and all timeframes after sunscreen was applied. For example, the absorbance readings at 320 nm for the baseline swab was 0.02 [95% confidence interval (CI), 0.01-0.03] compared with absorbance readings at 20 minutes (0.25; 95% CI, 0.23-0.26; $P < 0.000$) and at 6 hours (0.25; 95% CI, 0.23-0.26). Repeated measures ANOVA identified that there were no significant changes in the absorbance readings over time between any of the sites that had sunscreen applied. Interestingly, there was a significant difference ($P < 0.02$) between the absorbance readings for the control swab placed directly into ethanol (-0.005; 95% CI, -0.02-0.01) and the baseline swab (0.02; 95% CI, 0.01-0.03), indicating that the swab may remove some UV-absorbing compounds from the skin. The sensitivity of the swabbing technique was 99.6%, and specificity was 96.7%, providing a valid and reliable measure of sunscreen application. Thus, the swabbing technique could accurately validate 99.6% of samples with sunscreen and 96.7% of samples without.

Sunscreen was reapplied to a random sample of participants 3 hours after the initial application. Figure 4 illustrates the mean absorbance readings of participants who had sunscreen reapplied and those who had no sunscreen reapplied. ANOVA's at each time point revealed that there were no between-group differences in absorbance readings between participants who had sunscreen reapplied at 3 hours and those who had no reapplication. For example, there were no significant variation ($F = 1.47$; $P < 0.24$) in absorbance 20 minutes after reapplication between participants that did not reapply sunscreen (0.25; 95% CI, 0.23-0.26) compared with those who did have sunscreen reapplied (0.26; 95% CI, 0.25-0.26). Similar readings were recorded 3 hours after reapplication (not reapplied: 0.25; 95% CI, 0.23-0.26; reapplied: 0.25; 95% CI, 0.25-0.26).

Discussion

The findings of this trial confirm reports by Whiteman et al. (23) that this objective method could detect sunscreens and has an extremely high specificity and sensitivity for detecting a range of sunscreens. All the swabs on the skin treated with sunscreen showed much greater levels of absorption than the control swabs. Our sunscreen assays were effective in detecting absorbance of sunscreen in the

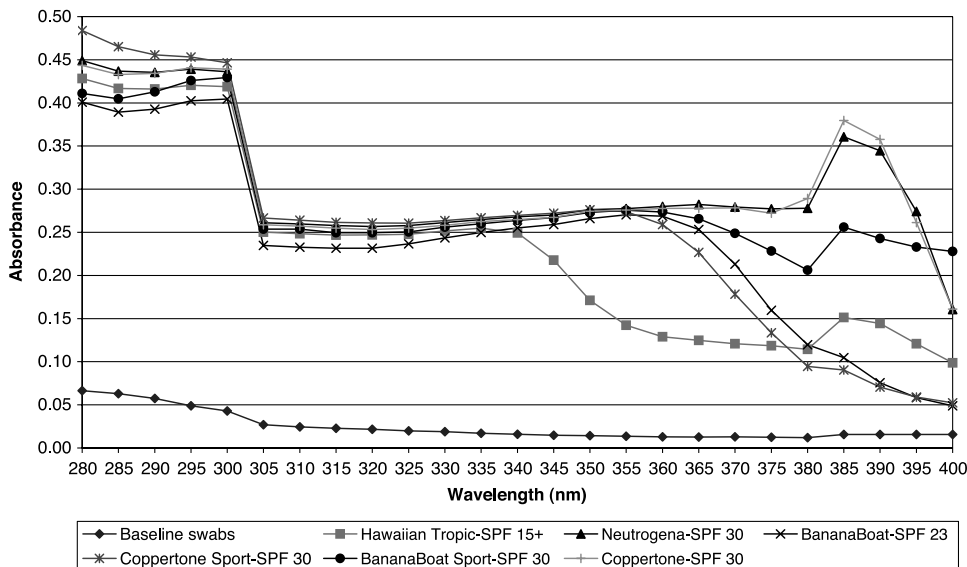


Figure 2. Mean absorbance reading of each sunscreen throughout the UVA (280-320 nm) and UVB (320-400 nm) wavelength.

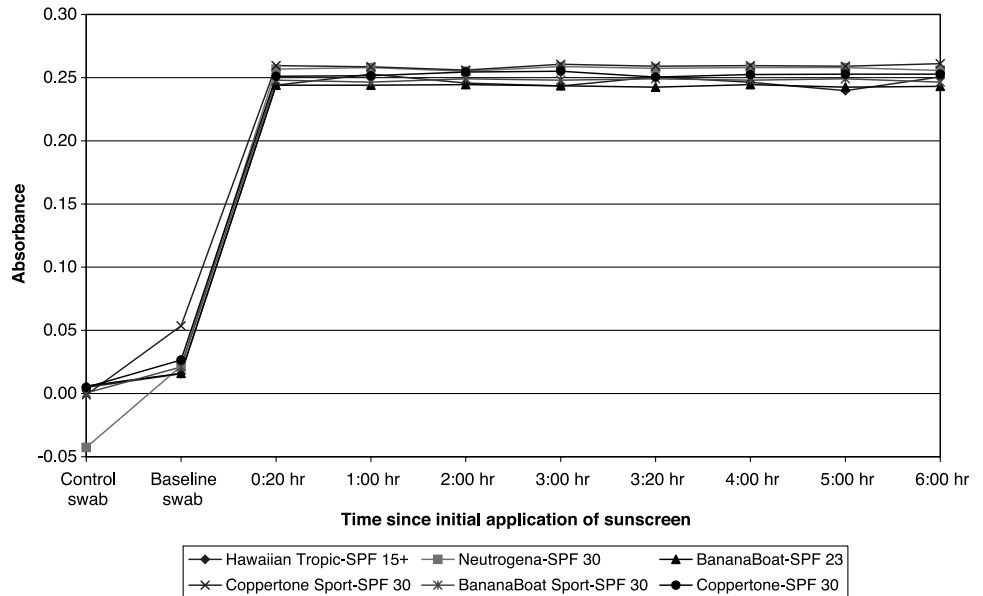


Figure 3. Mean absorbance readings at 320 nm of all sunscreens over time.

UVA range (320-400 nm) and within the UVB range specifically between 305 and 320 nm. High absorbance readings over shorter wavelengths (280-300 nm) seemed less reliable with various sunscreens. This may indicate that the swab is picking up other substances present on the skin that absorb at shorter wavelengths or the swab itself may have some absorbance properties in that range.

The sensitivity and specificity of this technique was very high. In fact, only 2 out of 300 samples were incorrectly categorized using the swabbing technique. It is important to note that in settings that are less controlled (e.g., self-application of sunscreen, water, and sand abrasion) the cutoff may need to be adjusted, or the accuracy might be lower. Our test of sensitivity and specificity may have been strengthened by including a lotion with no SPF in the study.

Even in a controlled setting, sunscreen application thickness and associated SPF was shown to decrease over a 2-hour period (12), resulting in the need to reapply sunscreen. Whiteman et al. (23) also showed that there is a decrease in absorbance over a 4-hour period with most substantive decreases occurring in the first 2 hours after application. Our

study did not show a decrease in absorbance over a 6-hour period. This result may be in part due to the lack of alcohol-based sunscreens or sunscreens with benzophenone-3 in this study, so there may have been less decrease due to absorption into the skin, which has been reported to be higher in sunscreens with these ingredients (26). Another factor could be methodologic differences such as cuvette type (UVB-sensitive plastic versus quartz) and swab type (ethanol versus baby wipe).

We were surprised to find that no significant variation in the level of absorption between participants who had sunscreen reapplied compared with those that did not reapply sunscreen. Reapplication of sunscreen is thought to aid the maintenance of an adequate application thickness and subsequently an adequate UV radiation protection resulting in better protection than a single application of sunscreen (27). Yet, we found no increase in absorbance between application and reapplication of sunscreen. This result may be an artifact of our sampling methods: maximum swab uptake of sunscreen, supersaturated solution in the vial, or cuvette type (disposable UV-rated). Alternatively, this may indicate that reapplication does not

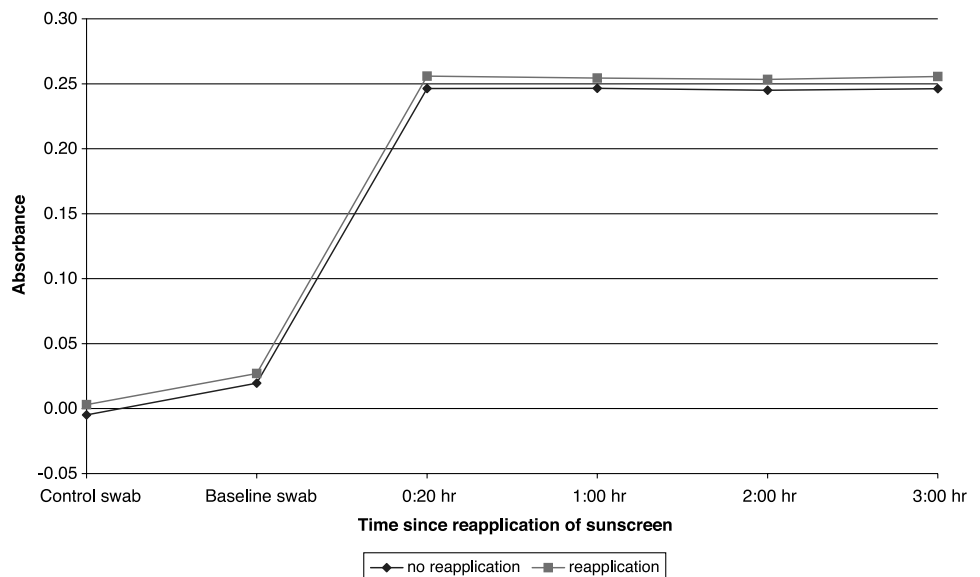


Figure 4. Mean absorbance at 320 nm comparing application and reapplication.

afford greater protection to the wearer from UV radiation, but rather provides maintenance of an adequate layer of protection that might normally be reduced by the rubbing of clothing, perspiration, or immersion in water.

The swabbing procedure is effective in a controlled setting and can effectively detect whether someone is wearing sunscreen across a range of sunscreens. The next step is to test the swabbing procedure among adults in outdoor settings, where they are exposed to UV radiation and self-apply sunscreen. We have begun to incorporate this methodology as one of a range of measures (objective and self-reported) to assess sun protection practices among beach-goers, and to see if it can distinguish between sunscreens with low and high SPF.

This procedure will be most useful when combined with other objective measures of sun protection and UV radiation exposure, resulting in a more accurate picture of sun protection practice of populations. There is a further need to compare this objective measure with self-report in outdoor settings to help us learn more about the biases introduced by self-report of sunscreen use.

Recent evidence reviews have continued to examine whether sunscreen can prevent melanoma, the deadliest form of skin cancer, or whether sunscreen is associated with increased risk of melanoma, as has been argued in the past (6, 8). Even so, as Bigby (7) points out, it may take many years to detect a protective effect of newer formulations of sunscreens on melanoma. The methodology reported in this study will be a useful tool in such research as well as in studies of skin cancer prevention.

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