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# EVALUATION OF SUN PROTECTION BY INTERNATIONAL STANDARD - ISO 24442 IN VIVO DETERMINATION OF SUNSCREEN UVA PROTECTION (UVAPF)

AMA Ref. No.: MS15.UVA.PPD.N8862.LACL.ISO10

Date: February 12, 2015

Sponsor: LABORATORIOS ARENSBURG SAIC
Los Limoneros 3777
Macul, Santiago
Chile

## 1.0 Objective:

Sunscreen protection against the full solar ultraviolet (UV) spectrum (290 - 400 nm) is measured and expressed as the Sun Protection Factor (SPF). The sun protection Factor (SPF) rating does not provide explicit information on the magnitude of the protection provided specially in the UVA range of the spectrum (320 – 400 nm). Sunscreen protection against the UVA spectrum is measured and expressed as the UVA-Protection Factor (UVA-PF). This panel has been convened to evaluate the Ultraviolet a Protection Factor (UVA-PF) of one formula. All testing will be performed in accordance with the Declaration of Helsinki and national regulations regarding human studies as described by the International Standard ISO 24442 – Cosmetics – Sun protection test methods – In vivo determination of sunscreen UVA protection.

## 2.0 Sample Description:

On January 15, 2015 one test sample labeled RAYTAN PROTECTOR SOLAR SPF 50+ Lot# 1410097 Batch# 1419052 / Formula con N° Reg. ISP : 1218C-2/10 was received from LABORATORIOS ARENSBURG SAIC and assigned AMA Lab No.: N-8862.

Expected UVA: 17

## 3.0 Test Material Handling:

Upon arrival at AMA Laboratories, Inc., the test material is assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and tests requested.

Samples are retained for a period of three months beyond submission of final report unless otherwise specified by the sponsor or if sample is known to be in support of governmental applications, in which case retained samples are kept two years beyond final

report submission. Sample disposition is conducted in compliance with appropriate federal, state and local ordinances.

4.0 Panel Demographics:

| | | |
|---|---|---|
| Number of subjects enrolled | | 10 |
| Number of subjects completing study | | 10 |
| Age Range | | 21 - 59 |
| Sex | Male | 3 |
| | Female | 7 |
| Race | Caucasian | 4 |
| | Hispanic | 6 |
| | Asian | 0 |

4.1 Standards for Inclusion in a Study:

a. Individuals between eighteen and seventy years old.
b. Individuals free of any dermatological or systemic disorder which would interfere with the results, at the discretion of the Investigator.
c. Individuals free of any acute or chronic disease that might interfere with or increase the risk of study participation.
d. Individuals with untanned skin on the test area and an ITA° value within the range of 20° and 41° by colorimetric method or Fitzpatrick phototypes II, III and IV.
e. Individuals with no uneven skin tones, pigmentation, scars, solar lentigo other irregularities or hair in test site areas that would interfere with UVAPF determination.
f. Individuals who complete a preliminary medical history form mandated by AMA Laboratories, Inc. and are in general good health.
g. Individuals who will read, understand and sign an informed consent document relating to the specific type of study they are subscribing. Consent forms are kept on file and are available for examination on the premises of AMA Laboratories, Inc. only.
h. Individuals able to cooperate with the Investigator and research staff, be willing to have test materials applied according to the protocol, and complete the full course of the study.
i. Individuals with excessive hair on their back who are willing to have hair removed by AMA technicians prior to commencement of study.
j. Individuals willing to refrain from using any sunscreen products, sunbathing or tanning bed use, 24 hours prior to study initiation and the entire duration of the study.

4.2 Standards for Exclusion from a Study:

a. Individuals who are under doctor's care.
b. Individuals who are currently taking any medication (topical or systemic) that may mask or interfere with the test results.
c. Subjects with a history of any form of skin cancer, melanoma, lupus, psoriasis, connective tissue disease, diabetes or any disease that would increase the risk associated with study participation.
d. Subjects receiving chemotherapy or radiotherapy.
e. Subjects using anti-inflammatory medication.
f. Individuals diagnosed with chronic skin allergies.
g. Individuals with a history of adverse effects upon sun exposure.
h. Subjects accustomed to using tanning beds.
i. Female volunteers who indicate that they are pregnant or nursing an infant.
j. Individuals with blemishes, nevi, sunburn, suntan, scars, moles, active dermal lesions or uneven pigmentation in the test sites.
k. Individuals with known hypersensitivity to any sunscreen products.
l. Subjects who have had UV exposure on the back area in the four weeks prior to UVAPF testing.
m. Children and persons below the age of consent or older than 70 years.
n. Subjects using medication with photo-sensitizing potential.
o. Subjects that are immunosuppressed, such as HIV-positive patients or transplant patients.
p. Subjects with a family history of skin cancer.
q. Subjects with dermatological conditions.

4.3 Informed Consent And Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms are available for inspection on the premises of AMA Laboratories, Inc. only. Reference 21 CFR Ch. 1 Part 50, Subpart B.

A trained technician performed a physical examination of the panelist's back to determine if study eligibility criteria were satisfied.

4.4 Panel Composition:

The panel consists of fair-skin individuals with untanned skin on the test area and an ITA° value within the range of 20° and 41° by colorimetric method or of subjects with Fitzpatrick phototypes II, III, IV defined as follows (Federal Register 43:38260, 1978)*:

Type II - Always burn easily, tans minimally;
Type III - Burns moderately, tans gradually;
Type IV - Burns minimally, always tans well;

* Based on first 30 to 45 minutes sun exposure after a winter season of no sun exposure.

Skin color categories ITA° values ranges:
Intermediate: >28° to 41°
Tan (or matte): >20° to 28°

4.5 Institutional Review Board:

Reference: CFR Title 21 Part 56, Subparts A, B, C, and D. The IRB of AMA Laboratories, Inc., consists of five or more individuals, chosen from within the company for technical expertise and from the local community for lay interaction. The list of IRB members is kept on file at AMA Laboratories, Inc. and is available for inspection during the hours of operation.

5.0 Artificial Light Source:

The light source employed is a 150 watt Xenon Arc Solar Simulator (Solar Light Co., Philadelphia, Pennsylvania, Model 14S, Model 15S or Model 16S) equipped with an Ultraviolet (UV) reflecting dichroic mirror (which reflects all radiation below 400nm), 3mm thick Schott WG-335 filter together with a 1mm thick Schott UG-11 filter was used to produce simulation of the UVA solar spectrum.

Xenon arc is selected on the basis of its black body radiation temperature of 6000° K which produces continuous UV spectra (all wavelengths) substantially equivalent to that of natural sunlight1.

UVA radiation was monitored continuously during exposure using a Model DCS-1 Sunburn UV Meter/Dose Controller System (Solar Light Co.) formerly known as the Robertson-Berger Sunburn meter (R-B meter). Measurements were taken at a position within 8 mm from the surface of the skin. The size of the exposure site will be ≥ 1cm$^2$. The solar simulator will be allowed a warm up time of at least 15 minutes before use and power supply output will be recorded.

Realignment of the Light Sources and calibration of the sunburn meters are conducted semi-annually by independent certification facilities and more often as necessary at the discretion of the operating technician or Investigator. The spectral analysis of the solar simulators used in this study is in compliance with the proposed International harmonization standard using the International Standard ISO 24442 – Cosmetics – Sun protection test methods – In Vivo Determination of the Sunscreen UVA Protection. A certificate, for each solar simulator, of the %RCEE compliance is on file at AMA Laboratories, Inc. The spectroradiometric measurements are performed at least annually.

The total irradiance of UVA source shall not exceed 1 600 W/m2 (reciprocity has been tested over the range of 370 W/m2 to 1 440 W/m2). The maximum level of visible and infrared (IR) radiation in the source beam shall be less than 5 % of the total source output. The amount of UVA I radiation shall be between 80 % and 92 % of the total UVA output (UVA I/UVA = 80 % to 92 %), and the amount of UVA II (320 nm to 340 nm) shall be between 8 % and 20 % of the total UVA irradiance (UVA II/UVA = 8 % to 20 %). There shall be less than 0.1 % of UVB contained in the source beam. The intensity of the beam for each subsite shall be as uniform as possible.

1/Berger, D.S.: Specification and Design of Solar Ultraviolet Simulators. J. Invest Dermatol. 53:192-199, 1969.

6.0 Procedure:

**TREATMENT AND EXPOSURE AREAS**

The number of sites was restricted to no more than six. The infrascapular area of the back was delineated within the region between the scapula line and the waist. Within this area, a 30 $cm^2$ test sites were delineated with a gentian violet surgical skin marker. Sites were observed to ensure uniform pigmentation, skin tone and texture, and absence of warts, moles, nevi, scars, solar lentigo and blemishes. Any areas that might be expected to produce erratic results were excluded from UV exposures. A minimum distance of one cm was maintained between the borders of adjacent test site application areas. The unprotected test site used to determine MPPDDu were randomized as one of the test sites across the test area and across subjects.

Test site that has been exposed to UV were free of any sign of previous pigmentation marks.

## MPPD DETERMINATION

The threshold dose for PPD in unprotected skin was determined over the mid to lower back by administering a series of exposures in 25% dose increments of UVA radiation in geometric progression. The minimal persistent pigment darkening dose (MPPDD) corresponds to the lowest Ultraviolet A (UVA) dose that produces the first perceptible unambiguous persistent pigment darkening response with defined borders appearing over most of the field of UVA exposure, observed between 2 h and 24 h after the end of the UVA exposure. A minimum of 5 exposures were administered within this site. The threshold response was taken as an unequivocal pigment darkening with distinct borders which persist for at least 2 to 4 hours.

Persistent pigmentation on each subsite was graded according to the following 4 point ordinal scale:

0 = No discernible pigment darkening
+/- = Barely perceptible pigment
1 = Unequivocal pigment darkening, distinct borders, lasting more than two to four hours
2 = Pronounced pigment darkening, lasting more than two to four hours

Observations of protected (product) and unprotected test sites was conducted at the same relative time point after the end of the exposures. All observations were made on the same day.

Visual evaluation were performed in a blinded manner by a qualified observer under standardized, sufficient and uniform illumination conditions (white lamps, industry type, delivering at least 450 lx over the examination plane), with the subject in the same position that was used for the product application and UVA exposures.

## TEST MATERIAL APPLICATION

The room temperature was between 18°C and 26°C when test product and the S1 or S2 control were applied. The test product was shaken and/or swirled with a glass rod before use. Test product and the S1 or S2 control were applied through plastic volumetric syringes to rectangular areas measuring 30 $cm^2$ in the amount of 2.0 $mg/cm^2$ ± 0.05 $mg/cm^2$. Dry products such as powders, pastes or ointments that cannot be drawn into a syringe are weighed and applied by spreading on the test site. Purified water is applied on the skin before the powder application to help

the sample adhere to the application site. Evenness of application was verified by observation with a Woods Lamp and the product was allowed to dry at least 15 minutes prior to UV exposure.

The product was gently spread with a finger cot using circular and then linear movements (up and down), without excessive pressure. The time used to spread the product on the test site was from 20 seconds to not more than 50 seconds. New finger cot was used for each new application of product and was not pre-saturated with the test product.

Product was applied to subjects in the same position as it was utilized for the irradiation procedure.

**UVA EXPOSURE**

Fifteen to thirty minutes after test material and S1 or S2 standard application, the test sites received a series of five UVA exposures calculated at 25% increments based upon previously determined MPPDDu and the expected UVA-PF of the test product. An adjacent unprotected site received a series of five UV exposures based upon previously determined MPPDDu.

Lamp irradiance was monitored continuously throughout the duration of the UV exposures.

7.0 Evaluation of Responses:

The threshold PPD within each site was determined according to the 4 point ordinal scale stated in section 6.0.

In order to ensure observer blinding, the observer was not the same individual that applied the product or administered the UVA exposures. Observation documents are coded so that the identity of the location and identity of MPPDDu, the UVA treatment doses, the test products, and the control product are not revealed.

The UVAPFi was calculated for each the test product for each volunteer as the ratio of the minimal UVA dose necessary to induce the defined pigmentation response on the MPPDDp and the minimal UVA dose necessary to induce the MPPDDu:

$$UVAPFi = \frac{MPPDDp\ (Seconds)\text{-}Protected\ skin}{MPPDDu\ (Seconds)\text{-}Unprotected\ skin}$$

where MPPDD(u,p) is the minimal persistent pigment darkening dose.

7.1 All technical employees of AMA Laboratories, Inc. are required to take and pass a visual discrimination examination conducted by a Board Certified Ophthalmologist using the Farnsworth-Munsell 100 Hue Test as published which determines a person's ability to discern color against a black background. This test was additionally modified to include a flesh tone background more nearly approaching actual use conditions, wherein pigment darkening of the skin is graded according to intensity.

8.0 Rejection and Study Discontinuation Criteria:

Test results would not be accepted if:

a. There is no pigmenting response on any UVA exposure subsites;
b. All subsites have a pigmented response;
c. There are random pigmenting responses that do not follow the logical sequence of the test (randomly absent responses);
d. The test subject is non-compliant or becomes ill, or does not shield the test area from sunlight after exposures;
e. A technical error occurs during UVA exposure.

If invalid data (whether MPPDDu or MPPDDp) have to be rejected for any one product on more than five subjects, then the whole test for that product is invalid and shall be rejected.If invalid data have to be rejected for the reference sunscreen on more than five subjects, then the whole test is invalid and shall be rejected.

Criteria for the discontinuation of a subject during the study include the following:

a. Significant protocol violation.
b. Serious adverse experience.
c. At the request of the subject.
d. At the Investigator's discretion where any unmanageable factor may interfere significantly with the protocol or interpretation of results.

9.0 Results:

Please see attached Table.

10.0 Observations:

No adverse effects or unexpected reactions of any kind were observed on any of the subjects.

11.0 Archiving:

All original samples, raw data sheets, technician's notebooks, correspondence files, copies of final reports and remaining specimens are maintained on the premises of AMA Laboratories, Inc. in limited access marked storage files. A duplicate DVD copy of final reports is separately archived in a bank safe deposit vault.

12.0 Security Label Disclosure:

To prevent loss of and protect intellectual property, original, certified documents issued by AMA Laboratories Inc. can be identified by a proprietary, tamper evident security hologram affixed to all Conclusion/Signature pages on final reports. Any attempt to remove the hologram will irreversibly damage the label and leave an immediate trace, thus invalidating the document.

Only reports containing the AMA LABS, INC. hologram intact will be recognized by AMA Laboratories Inc. as a certified original.

13.0 Conclusions:

The mean UVA Protection Factor (UVA-PF) of the above test material (AMA Lab No.: N-8862; Client No.: RAYTAN PROTECTOR SOLAR SPF 50+ Lot# 1410097 Batch# 1419052 / Formula con N° Reg. ISP : 1218C-2/10) when tested on ten subjects with a WG-335 filter under static conditions according to the reference described herein was 19.2. The mean UVA Protection Factor of S2 standard on the same panel was 13.5.

Donna Muratschew, M.D.
Study Director

Kaitlyn Callaghan, B.S. (Candidate)
Technician

Jaime M. Reidy, A.A.
Technician

Erica Petrillo, B.S.
Technician

Tara Grube, B.S.
Technician

David R. Winne, B.S.
Technical Director

2/12/15
Date

## EVALUATION OF UVA PROTECTION FACTOR (ISO 24442)

Table

Sponsor: LABORATORIOS ARENSBURG SAIC
AMA Lab No.: N-8862
Client No.: RAYTAN PROTECTOR SOLAR SPF 50+ Lot# 1410097 Batch# 1419052 / Formula con N° Reg. ISP : 1218C-2/10
Expected UVA-PF: 17

| No. | Date | Subject ID # | Sex | Race | Age | ITA° | Skin Type | Lamp No. | MW/cm² Amps I | MPPDDu I (sec) | MW/cm² Amps II | MPPDDu II (sec) | MW/cm² Amps S2 | MPPDDp S2 Std (sec) | MW/cm² Amps | MPPDDp Product (sec) | UVA-PFi S2 Std | UVA-PFi Product | Tech. Initials |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1/20/2015 | 31 9721 | F | H | 51 | 26.3° | II | 16614 | 65.6/6.0 | 52 | 65.7/6.0 | 52 | 65.2/6.0 | 624 | 65.3/6.0 | 884 | 12.0 | 17.0 | TG |
| 2 | 1/20/2015 | 78 6702 | F | C | 26 | 28.8° | II | 16614 | 66.9/6.0 | 65 | 66.8/6.0 | 65 | 67.4/6.0 | 975 | 67.3/6.0 | 1105 | 15.0 | 17.0 | TG |
| 3 | 1/20/2015 | 78 4366 | M | H | 24 | 32.1° | II | 4553 | 67.2/6.1 | 65 | 67.6/6.1 | 65 | 67.4/6.1 | 780 | 67.5/6.1 | 1385 | 12.0 | 21.3 | |
| 4 | 1/21/2015 | 76 1025 | F | C | 37 | 25.2° | II | 16613 | 65.7/6.0 | 65 | 65.9/6.0 | 65 | 67.3/6.0 | 975 | 68.0/6.0 | 1105 | 15.0 | 17.0 | |
| 5 | 1/23/2015 | 48 4004 | F | H | 45 | 27.4° | II | 1695 | 67.1/5.1 | 52 | 67.4/5.1 | 52 | 67.5/5.1 | 780 | 67.7/5.1 | 1108 | 15.0 | 21.3 | KC |
| 6 | 2/03/2015 | 46 0672 | F | H | 59 | 29.4° | II | 16613 | 67.3/6.0 | 52 | 67.7/6.0 | 52 | 67.8/6.0 | 624 | 67.6/6.0 | 1108 | 12.0 | 21.3 | EP |
| 7 | 2/03/2015 | 18 8404 | F | H | 40 | 26.9° | III | 1695 | 68.6/6.9 | 101 | 68.5/6.9 | 101 | 68.0/6.9 | 1515 | 68.4/6.0 | 2151 | 15.0 | 21.3 | TG |
| 8 | 2/03/2015 | 82 3905 | M | H | 21 | 29.1° | III | 4553 | 68.5/6.2 | 81 | 68.4/6.2 | 81 | 68.4/6.2 | 1215 | 68.8/6.2 | 1377 | 15.0 | 17.0 | |
| 9 | 2/03/2015 | 11 8412 | F | C | 45 | 29.5° | II | 4553 | 68.9/6.1 | 65 | 68.6/6.1 | 65 | 68.2/6.1 | 780 | 68.5/6.1 | 1105 | 12.0 | 17.0 | |
| 10 | 2/03/2015 | 74 1676 | M | C | 26 | 28.6° | II | 16613 | 65.8/6.0 | 65 | 65.9/6.0 | 65 | 66.1/6.0 | 780 | 66.4/6.0 | 1385 | 12.0 | 21.3 | EP |
| **MEAN UVA-PF** | | | | | | | | | | | | | | | | | **13.5** | **19.2** | |
| **STANDARD DEV (s)** | | | | | | | | | | | | | | | | | **1.6** | **2.3** | |
| **STD. ERROR** | | | | | | | | | | | | | | | | | **0.5** | **0.7** | |
| **S.E. % OF MEAN** | | | | | | | | | | | | | | | | | **3.7%** | **3.8%** | |
| **t** | | | | | | | | | | | | | | | | | **2.262** | **2.262** | |
| **95% CONFIDENCE INTERVAL** | | | | | | | | | | | | | | | | | **12.4 - 14.6** | **17.5 - 20.8** | |
| **N OF CASES** | | | | | | | | | | | | | | | | | **10** | **10** | |

MPPDD: Minimal Persistent Pigment Darkening Dose
I: Intensity of light source

**Technicians:**

KC = Kaitlyn Callaghan, B.S. (Candidate)
EP = Erica Petrillo, B.S.
JR = Jaime Reidy, A.A.
TG = Tara Grube, B.S.

Evaluation Period: This study was conducted from January 15, 2015 through February 3, 2015.

14.0 Quality Assurance Statement:

This study was inspected in accordance with the Standard Operating Procedures of AMA Laboratories, Inc. To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the study records and report.

Report reviewed by:

Tasmiya Masud, B.A.
Quality Assurance Supervisor

2/12/15
Date

## 15.0 Appendix I - Certificates of Spectral Measurement – SUMMARY

| Certificate of Spectral Measurement - SUMMARY (December 2014) | | | | | | |
|---|---|---|---|---|---|---|
| *Characterization of the UV source with intensity in units of energy/unit area* | | | | | | |
| **Solar Simulator SN:** | **1695** | **4553** | **16613** | **16614** | **11471** | |
| Total Irradiance (250-1500 nm) | 4.89E-02 | 6.46E-02 | 5.54E-02 | 5.30E-02 | 6.60E-02 | $W/cm^2$ |
| Total UV Irradiance (250-400 nm) | 4.81E-02 | 6.36E-02 | 5.43E-02 | 5.20E-02 | 6.52E-02 | $W/cm^2$ |
| UVC Irradiance (250-290 nm) | 3.37E-08 | 1.16E-08 | 5.22E-08 | 3.43E-08 | 3.68E-08 | $W/cm^2$ |
| UVB Irradiance (290-320 nm) | 1.09E-05 | 2.58E-05 | 1.97E-05 | 1.84E-05 | 2.30E-05 | $W/cm^2$ |
| UVA Irradiance (320-400 nm) | 4.81E-02 | 6.36E-02 | 5.43E-02 | 5.19E-02 | 6.52E-02 | $W/cm^2$ |
| UVA2 Irradiance (320-340 nm) | 6.22E-03 | 8.99E-03 | 7.51E-03 | 7.33E-03 | 9.18E-03 | $W/cm^2$ |
| UVA1 Irradiance (340-400 nm) | 4.19E-02 | 5.46E-02 | 4.68E-02 | 4.46E-02 | 5.60E-02 | $W/cm^2$ |
| % UVC of Total UV | 0.00007% | 0.00002% | 0.0001% | 0.0001% | 0.0001% | |
| % UVB of Total UV | 0.02% | 0.04% | 0.04% | 0.04% | 0.04% | |
| % UVA of Total UV | 99.98% | 99.96% | 99.96% | 99.96% | 99.96% | |
| ISO 24442 Spectral Characteristics | | | | | | |
| <320nm (<0.1% of UV) | 0.02% | 0.04% | 0.04% | 0.04% | 0.04% | ***Passed*** |
| UVA II (8-20% of UVA) | 12.9% | 14.1% | 13.8% | 14.1% | 14.1% | ***Passed*** |
| UVA1 (80-92% of UVA) | 87.1% | 85.9% | 86.2% | 85.9% | 85.9% | ***Passed*** |
| Visible & NIR 400-1500 nm (<5%) | 1.56% | 1.53% | 1.92% | 2.04% | 1.18% | ***Passed*** |