Milan, 30/05/2023

In-vivo DETERMINATION OF THE SUN PROTECTION FACTOR (SPF) ISO 24444:2019/Amd1:2022

METHOD: Ref. E34A rev. 10

CUSTOMER: **THE NUDE ALCHEMIST**
24c Essex St. Phillipstown
Christchurch 8011 (New Zeland)

PRODUCT: **SPF30 The Nude Alchemist**

Ref. LAB: 127/23/01 – 151/23

STARTING DATE OF THE STUDY: 12/05/2023

COMPLETION DATE: 30/05/2023

ETHICAL AND QUALITY CRITERIA

The current study was carried out in compliance with the quality assurance system requirements.

REFERENCES

The data given in this report are exclusively related to the tested sample. This report can be only in full reproduced only with the permission.

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1. SAMPLE DATA SHEET

SAMPLE REF.: **SPF30 The Nude Alchemist**

Ref. LAB: 127/23/01 – 151/23

SAMPLE ARRIVAL DATE: 10/05/2023

PRODUCT:

PHYSICAL FORM: cream

COLOUR: white

QUALITATIVE FORMULA:

- Known / yes /
- Other information / /

OTHER INFORMATION RELATED TO THE PRODUCT SAFETY:
None.

FILE: 1 sample with the code number **Ref. LAB: 127/23/01 - 151/23** and the study findings will be kept filed in our archives for one year and for ten years respectively. After these periods, the sample and the findings report will be discarded, unless otherwise required by the client.

In-vivo DETERMINATION OF THE SUN PROTECTION FACTOR (SPF)
ISO 24444:2019/Amd1:2022
Ref. E34A

2. AIM OF THE STUDY

The effectiveness of a sun protection product is assessed in vivo by determining its sun protection factor (SPF) according to the **ISO 24444:2019/Amd1:2022** norm**.**
This method involves the formation of an erythema by means of an appropriate sun simulator, under controlled conditions, on the back of at least 10 selected subjects.
20 ± 4 hours after exposure, the erythema is visually assessed, and the individual sun protection factor is calculated. This corresponds to the ratio between the lowest UV doses that products first perceptible unambiguous erythema with defined borders appearing over more than 50% of UV exposure subsite (minimal erythemal dose, MED) on protected and on unprotected skin.
The sun protection factor is determined by calculating the arithmetical mean of the individual SPFi values obtained for the subjects participating in the test.

3. SELECTION OF THE VOLUNTEERS

3.a. Criteria for recruitment and admission

The test is carried out according to the Declaration of Helsinki on 10 volunteers, average age 49.2 years.
Subjects are informed about the nature, purpose and risk of the study and give their written consent before participating in the test.
The selection is carried out according to the following criteria:

3.b. Inclusion criteria

- Race: Caucasian.
- Female and male subjects, 18 - 70 years old, in general good health.
- Subjects with ITA° value ≥ 28° (ITA° mean value between 41° and 55°).
- Subjects able to follow all study directions and to commit to all follow-up visits for the duration of the study.
- Subjects who complete the informed consent process.

3.c. Exclusion criteria

- Subjects who are taking topical or systemic drugs that could affect the results of the test (anti-inflammatory agents, etc) or could induce photosensitization reactions.
- Pregnant or nursing females.
- Subjects with a history of unusual skin reactions to skin care toiletry products, cosmetics, or sensitivity to any of the test article components.
- Subjects showing systemic diseases or skin disorders (such as eczema, psoriasis, severe acne, etc.) that may affect the evaluation of the test articles or increase risk to the subject.
- Subjects having marks, tattoo, blemishes or excessive nevi and hair, presenting existing sun damage and/or uneven colour tone in the test area (no ITA° variations > 5° between each test site).
- Subjects with an history of abnormal response to the sun.
- Subjects recently exposed (8 weeks) to intensive doses of UVA+UVB radiation.
- Subjects who have taken part in evaluation tests on sun products during 8 weeks before the test.

3.d. Drop-out

The following reasons are considered sufficient cause for interrupting the subject's participation in the study:

- free choice of the subject;
- reasons not correlated with the treatment (*e.g.*, onset of disease, surgical operation, subjects in quarantine or in fiduciary isolation for Covid-19);
- reasons correlated with the treatment (ex. irritant or allergic reactions).

3.e. Restrictions

For the whole duration of the study, the subjects must not use different products on the tested areas and must avoid the UV exposure.

4. INSTRUMENTS

4.a. Multiport Solar UV Simulator Model 600 - 300 Watt, Solar Light (s/n 15429)

Multiport 601-300 is an arc Xenon lamp with a continuous spectrum ranging from 290 to 400 nm with 6 independent lights. The field of irradiation of each light is of 8 mm in diameter (an area > 0.50 cm^2).
The lamp spectrum is in compliance with the ISO 24444:2019 method.
The light source is placed directly in contact with the skin of the subject's back.

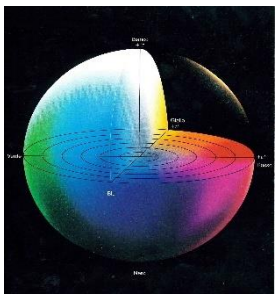
4.b. Chromameter CR 400, Konica Minolta

Chromameter is a portable dual channel, reflecting colorimeter with incorporated microcomputer, liquid crystals display and Xenon light source in the measuring head. The measuring head surface is 8 mm in diameter.

The colour rating system is CIE system L*a*b*:

- ✓ **L*** refers to black - white axis. L* values range from 0 to 100, where 0 corresponds to black colour and 100 to white.
- ✓ **a*** and **b*** refer to two-colours axis: a* represents the red - green colour (-60 green + 60 red) while b* the yellow - blue colour (-60 blue + 60 yellow).

Representation of the colour solid for the colorimetric space L*a*b*.



La comunicazione precisa del colore, Minolta, pp. 19, 1994

L* and b* parameters have been considered in this study as sensible indexes of any changes in the pigmentation intensity. Furthermore **ITA°**, which expresses the melanin index, is calculated from L* and b*, according to the following mathematical expression:

$$\mathbf{ITA° = \{ Arc.tang.\ [(L^*-50) / b^*] \} \times 180 / 3{,}1416}$$

ITA° value is inversely correlated to the pigmentation intensity: the higher the values, the clearer the colour (see graph at page 18).

4.c. Analytical Balance (ME204T/00, Mettler Toledo or Radwag Mod. AS 220.X2 Plus)

An analytical balance is used to determine the amount of product to apply on the skin. It determines up to 4 decimal numbers and has a sensitivity of 0.1 mg.

5. TEST AREAS

The test is carried out on the subject's back, on both sides of the spine, within the region between the scapula line and the waist. Three areas measuring 36 cm^2 each are selected keeping a minimum distance of 1 cm between the borders of adjacent test sites:

- one for the application of the test product (MEDp);
- one for the application of the standard product;
- one for the evaluation of the MEDu (untreated and exposed area).

The position of each area is randomised among the subjects in order to reduce the systematic error due to anatomical differences of the skin.

During the product application, UV exposure and MED reading, the volunteers keep the prone position.

6. REFERENCE SUNSCREEN FORMULATIONS

To guarantee the reliability of the analysis a standard product with a known SPF is tested on the same day as the test product. The average SPF value of the reference sunscreen shall fall within its respective acceptance range (see below). No further statistical requirement is needed.

Any one of the following reference sunscreen formulations shall be used:

P2 = SPF 16.1, between 13.7 and 18.5
P3 = SPF 15.7, between 13.7 and 17.7
P5 = SPF 30.6, between 23.7 and 37.4
P6 = SPF 43.0, between 31.0 and 54.9
P8 = SPF 63.1, between 43.9 and 82.3

The selection of the standard reference depending on the expected SPF of the test sample:

- ✓ **SPF ≤ 24:** any reference standard may be used for each subject.
- ✓ **SPF ≥ 25 but less than 50: P5 or P6** (on at least 5 subjects) **and P2 or P3** on the remaining subjects.
- ✓ **SPF ≥ 50: P8** (on at least 5 subjects) **and P2 or P3** on the remaining subjects.

In the present study the standard **P8 (subjects 1-5) and P5 (subjects 6-10)** were used.

7. METHOD OF EVALUATION

Product application, UV exposures and MED assessment are carried out in an air-conditioned room maintaining room temperature between 20 and 26 °C.

7.a. Evaluation of the MED on untreated skin (MEDu)

The visually determined **MEDu** corresponds to the lowest UV dose that produces the first perceptible unambiguous erythema with defined borders appearing over more than 50% of the field of UV exposure on unprotected skin, 20 ± 4 hours after UV exposure.
For each subject the unprotected MEDu is made on the same day as the MEDp. A provisional individual MEDu can be made before the test.

The intensities of the radiations emitted by the 6 independent lights are increased with a geometric incremental progression of 1.15 (15%). The exposition time is constant.
The minimal erythemal dose of the unprotected skin (MEDu) is visually assessed in blind 20 ± 4 hours after UV exposure in sufficient and uniform illumination (450 lux and colour temperature 6500°K).

7.b. Evaluation of the MED on treated skin (MEDp)

The visually determined **MEDp** corresponds to the lowest UV dose that produces the first perceptible unambiguous erythema with defined borders appearing over mere than 50% of the field of UV exposure on protected skin, 20 ± 4 hours after UV exposure.

The product is weighed with a syringe and applied in doses of 2.00 ± 0.05 mg/cm^2.
About 15 droplets/30 cm^2 are deposited within the test site and then the product is spread over the whole test site using light pressure for 20-50 seconds using a finger cot. A method of weighing by loss is used.
Exposure of the test site is made 15-30 minutes after the application of the product. The intensities of the radiations emitted by the 6 independent lights are increased with a geometric incremental progression of 1.15 (15%).
The time of exposure is kept constant and it is equal to the time of exposure of the MEDu multiplied by the expected SPF.
The minimal erythemal dose of the protected skin (MEDp) is visually assessed in blind 20 ± 4 hours after UV exposure in sufficient and uniform illumination (450 lux and colour temperature 6500°K).

The same procedure is used for the standard reference.

8. SPF CALCULATION

The individual Sun protection factor (SPFi) corresponds to the ratio between the minimal erythemal dose on product protected skin (MEDp) to the minimal erythemal dose on unprotected skin (MEDu).

For each product is calculated:

- **Individual sun protection factor (SPFi)**:

$$\textbf{SPFi} = \frac{\text{MEDpi (J/m}^2\text{)}}{\text{MEDui (J/m}^2\text{)}}$$

- **Product sun protection factor (SPF)**, corresponding to the arithmetical mean of the individual SPFi obtained from the total number of subjects used:

$$\textbf{SPF} = (\Sigma\ \textbf{SPFi} / \textbf{n})$$

- **Standard error of the mean (SEM)**, calculated as follows:

$$\textbf{SEM} = \textbf{s} / \sqrt{\textbf{n}}$$

where:

n= total number of subjects used
s= standard deviation

- the **95% confidence interval (95% CI)** of the mean value of SPF, indicating the SPF range within which the real value of SPF has the 95% probability to fall, calculated as follows:

$$\textbf{95\% CI} = \textbf{from (SPF - c) to (SPF + c)}$$

where:

c = "SEM" **x** "*t*" = **s x *t* /** $\sqrt{\textbf{n}}$

CI % = 100 x **c** / SPF

t = is the *t* value from the 'two-sided' Student-*t* distribution at a probability level *p* = 0.05 and with degrees of freedom ν= (n° of subjects **-** 1).

The result is valid for the first 10 subjects if the resulting range of the 95% confidence interval (95%CI) of the mean SPF is within ± 17% of the mean SPF. If it is not within ± 17% of the mean SPF, the number of the subjects shall be increased until the 95%CI statistical criterion is met (up to a maximum of 20 valid results from a maximum of 25 subjects tested). If the statistical criterion has not been met after 20 valid results from the maximum 25 subjects, then the test shall be rejected.

9. RESULTS

TABLE 1: *general information about the subjects, the technicians and intensity of the solar simulator highest intensity port.*

Subject	ID Number	Age	Sex	Applied/ Exposed by	Exposure Date	Read by	Sim EE (highest) W/m²	ITA°	Rejected?
1	591	41	F	C.C.	16/05/2023	A.B.	10.00	49.2°	**NO**
2	1344	49	F	C.C.	16/05/2023	A.B.	10.00	33.6°	**NO**
3	3011	39	M	C.C.	18/05/2023	A.B.	10.00	51.4°	**NO**
4	626	58	F	C.C.	23/05/2023	A.B.	10.00	43.5°	**NO**
5	2047	26	M	C.C.	23/05/2023	A.B.	10.00	46.2°	**NO**
6	153	63	F	C.C.	24/05/2023	A.B.	10.00	55.8°	**NO**
7	594	69	F	C.C.	24/05/2023	A.B.	10.00	40.6°	**NO**
8	222	38	F	C.C.	24/05/2023	A.B.	10.00	54.5°	**NO**
9	939	54	F	C.C.	25/05/2023	A.B.	10.00	44.7°	**NO**
10	1108	55	F	C.C.	29/05/2023	A.B.	10.00	46.7°	**NO**
							ITA° mean value	**46.6°**	

TABLE 2: SPF30 The Nude Alchemist

Ref. LAB: 127/23/01 – 151/23

	MEDu		MEDp		
Subject	**sec**	**J/m²**	**sec**	**J/m²**	**SPFi**
1	35	307	1050	9135	**29.7**
2	50	438	1600	16000	**36.5**
3	31	271	930	9300	**34.3**
4	40	303	1200	10440	**34.5**
5	35	267	1050	7938	**29.7**
6	27	237	810	7047	**29.7**
7	35	267	1050	10500	**39.3**
8	31	178	930	7031	**39.4**
9	35	353	1050	10500	**29.7**
10	35	267	1050	9135	**34.2**
				SPF	**33.7**
				s	**3.9**
				95% CI	**from 30.9 to 36.5**
				c	**2.8**
				CI%	**8.3%**
				17%SPF	**5.7**

The test study complies with the statistical validations (CI% ≤ 17%).

TABLE 3: Standard reference P8

	MEDu		MEDp		
Soggetti *Subjects*	**sec**	**J/m²**	**sec**	**J/m²**	**SPFi**
1	35	307	2209	22085	**71.9**
2	50	438	3155	27449	**62.6**
3	31	271	1956	19561	**72.1**
4	40	303	2524	19081	**63.1**
5	35	267	2209	14510	**54.4**
				SPF	**64.8**
				s	**7.4**

TABLE 3: Standard reference P5

	MEDu		MEDp		
Soggetti *Subjects*	**sec**	**J/m²**	**sec**	**J/m²**	**SPFi**
6	27	237	826	8262	**34.9**
7	35	267	1071	8097	**30.3**
8	31	178	949	6232	**34.9**
9	35	353	1071	9318	**26.4**
10	35	267	1071	9318	**34.9**
				SPF	**32.3**
				s	**3.8**

10. CONCLUSIONS

The Sun Protection Factor evaluation of **SPF30 The Nude Alchemist, Ref. LAB: 127/23/01 - 151/23**, was carried out on 10 subjects under the experimental conditions described in detail in the present report. Below the result:

Sun Protection Factor: SPF 33.7 (CI% = 8.3%)

Responsible for the laboratory
Dr. Adriana Bonfigli

Responsible for the evaluation
Dr. Claudia Cartigliani

Dermatologist
Dr. Enzo Berardesca

11. BIBLIOGRAPHY

International Standard ISO 24444: 2019. Cosmetics – Sun Protection Test Methods - In vivo determination of the sun protection factor (SPF)

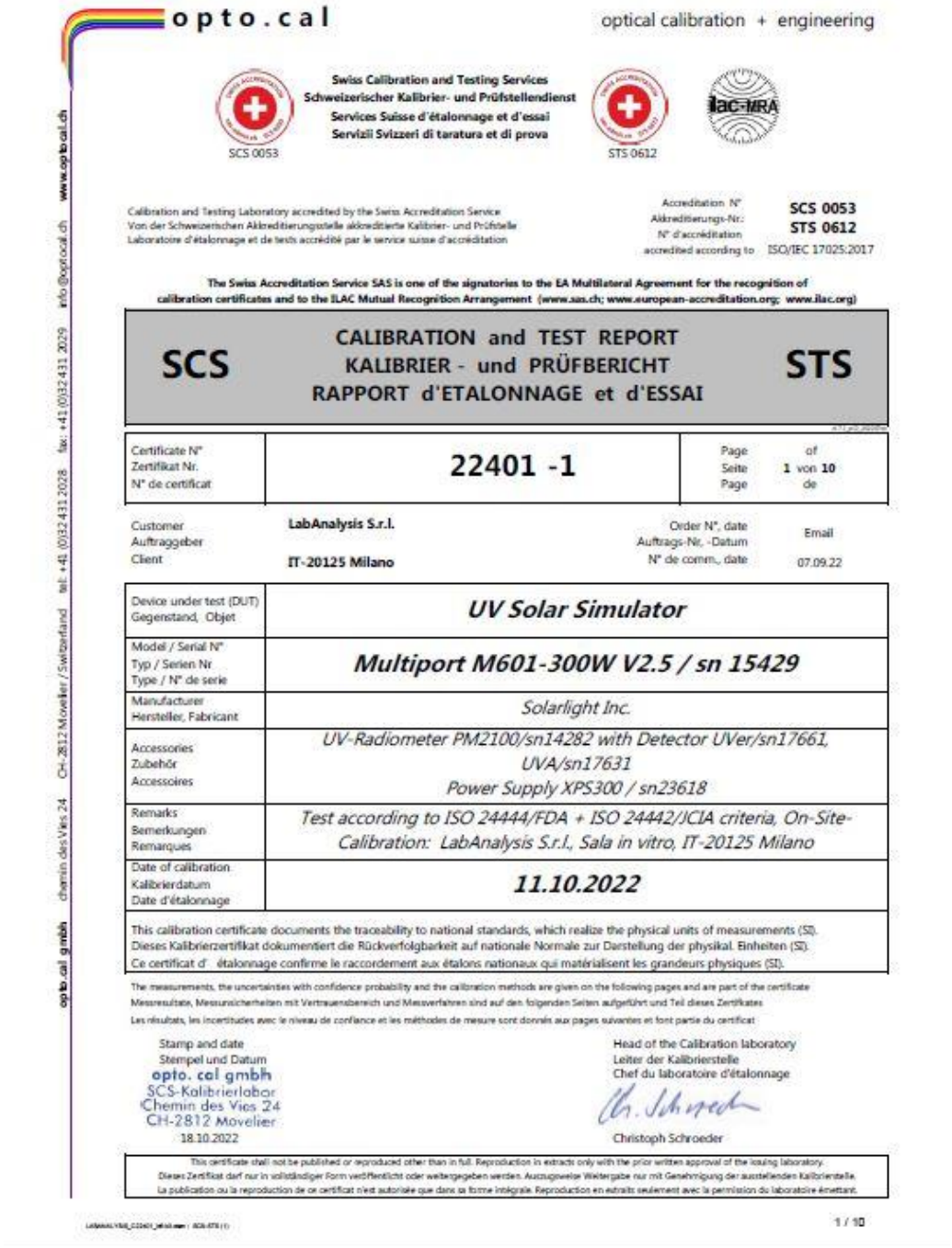
International Standard ISO 24444: 2019. Cosmetics – Sun Protection Test Methods - In vivo determination of the sun protection factor (SPF) – Amd1:2022.

Chardon A, Cretois I, Hourseau C. Comparative colorimetric follow-up on humans of the sun tannings induced by cumulative exposure to UVB, UVA and UVB+A radiations. 16th IFSCC Congress, NY, Preprint 1990: **1**:51-70 & Skin colour typology and suntanning pathways. *Int. Cosm.Scien.* 1991: **13**:191-208.

Piérard G E. EEMCO guidance for the assessment of skin colour. *J. Eur. Acad. Dermatol. Venereol*. Jan.1998: **10(1)**: 1-11.

Guidelines for the colorimetric determination of skin colour typing and prediction of the minimal erythemal dose (MED) without UV exposure, proposed by COLIPA 2007.

ANNEX I



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Certificate N° Zertifikat Nr. N° de certificat	22401 -1	Page Seite Page	of 1 von 10 de

Customer Auftraggeber Client	LabAnalysis S.r.l. IT-20125 Milano	Order N°, date Auftrags-Nr, -Datum N° de comm., date	Email 07.09.22

Device under test (DUT) Gegenstand, Objet	*UV Solar Simulator*
Model / Serial N° Typ / Serien Nr Type / N° de serie	*Multiport M601-300W V2.5 / sn 15429*
Manufacturer Hersteller, Fabricant	*Solarlight Inc.*
Accessories Zubehör Accessoires	*UV-Radiometer PM2100/sn14282 with Detector UVer/sn17661, UVA/sn17631 Power Supply XPS300 / sn23618*
Remarks Bemerkungen Remarques	*Test according to ISO 24444/FDA + ISO 24442/ICIA criteria, On-Site-Calibration: LabAnalysis S.r.l., Sala in vitro, IT-20125 Milano*
Date of calibration Kalibrierdatum Date d'étalonnage	***11.10.2022***

This calibration certificate documents the traceability to national standards, which realize the physical units of measurements (SI).
Dieses Kalibrierzertifikat dokumentiert die Rückverfolgbarkeit auf nationale Normale zur Darstellung der physikal. Einheiten (SI).
Ce certificat d'étalonnage confirme le raccordement aux étalons nationaux qui matérialisent les grandeurs physiques (SI).

The measurements, the uncertainties with confidence probability and the calibration methods are given on the following pages and are part of the certificate
Messresultate, Messunsicherheiten mit Vertrauensbereich und Messverfahren sind auf den folgenden Seiten aufgeführt und Teil dieses Zertifikates
Les résultats, les incertitudes avec le niveau de confiance et les méthodes de mesure sont donnés aux pages suivantes et font partie du certificat

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opto. cal gmbh
SCS-Kalibrierlabor
Chemin des Vies 24
CH-2812 Movelier
18.10.2022

Head of the Calibration laboratory
Leiter der Kalibrierstelle
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ANNEX II

Quality of ultraviolet radiation

To ensure that appropriate amounts of UVA radiation are included in the spectrum of the solar simulator throughout the entire UVA range, the total radiometric proportion of the UVA II (320-340 nm) irradiance of the simulator must be equal or exceed 20% of the total UV (290-400 nm) irradiance. Additionally, the UVA I region (340-400 nm) irradiance must equal or exceed 60% of the total UV irradiance.

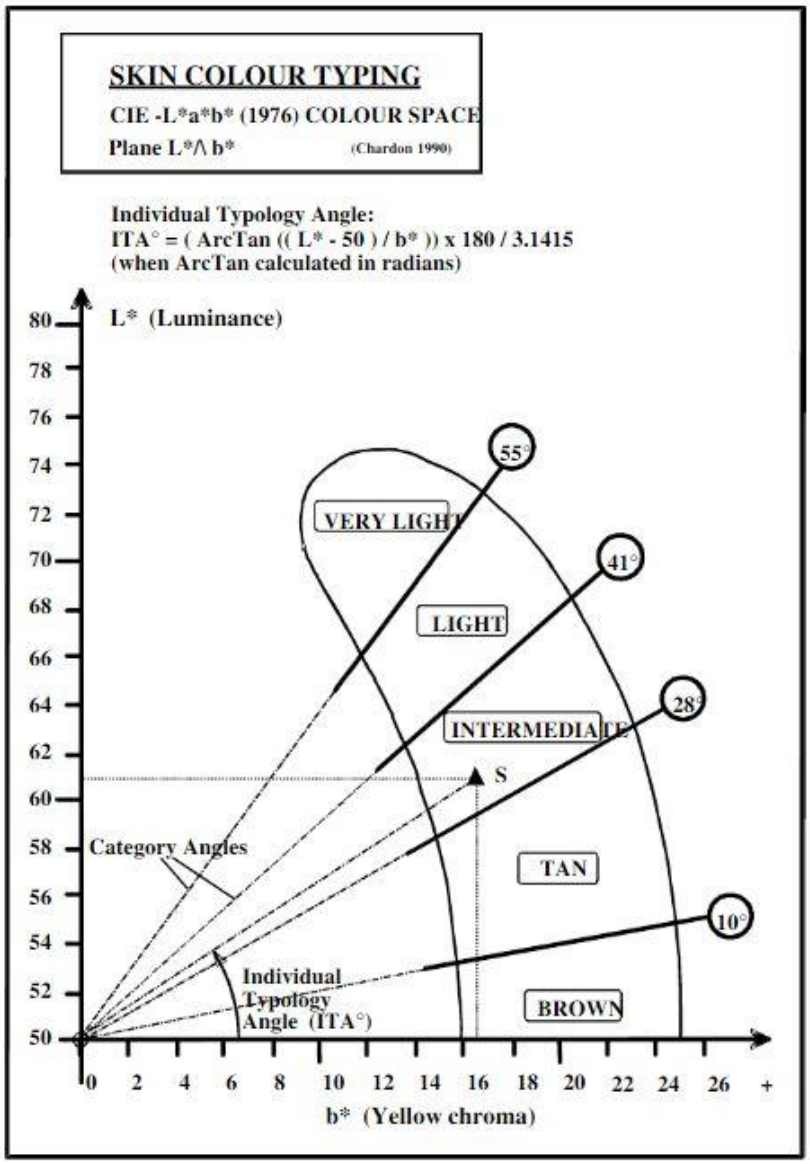
Table1: %RCEE acceptance limits for the UV solar simulator output

Spectral Range (nm)	%RCEE Accepted Limits		Measured %RCEE
	Lower limit	Upper limit	
<290		<0.1%	0.001%
290-300	1.0%	8.0%	5.9%
290-310	49.0%	65.0%	59.2%
290-320	85.0%	90.0%	88.3%
290-330	91.5%	95.5%	93.7%
290-340	94.0%	97.0%	95.9%
290-400	≥ 99.9%		100.0%

Table2: Uniformity of the beam (≥ 90%)

Port 1	Port 2	Port 3	Port 4	Port 5	Port 6	**Mean**
95%	96%	98%	92%	92%	92%	**94%**

ANNEX III Individual Typology Angle (**ITA°**)



Chardon A ., Cretois I ., Hourseau C., Skin colour typology and suntanning pathways. *Int. J. Cosmet. Sci.*, 13, pp. 191–208, 1991

Skin colour type: the graph enables the classification the subjects' skin according to its colour by referring to the **ITA°** value. This is obtained from L* and b* colorimetric parameters. The graph shows a correlation between the changes in **ITA°** value and the changes in skin colour.