Annex I - Protocol in vitro SPF Double Plate method

STANDARD OPERATIONAL PROCEDURE

DOUBLE PLATE METHOD

ASSESSMENT OF THE IN-VITRO SPF OF SOLAR FORMULAE

INTRODUCTION

It has long been a desire to develop an in vitro SPF test method, recognizing the potential advantages of such methodology, including (i) the use of a non-human model, (ii) the significant improvements in speed and cost, (iii) the improved repeatability and reproducibility, (iv) the elimination of technically challenging procedures (e.g. MED determination) and (v) the use of a method which is significantly more amenable to continuous improvement. Importantly, there is also a current mandate from the European Commission to develop and deploy in vitro sun protection test methods (EC Recommendation 2006/647/EC [1]), which states '...preference should be given to in vitro testing methods delivering equivalent results, as in vivo methods raise ethical concerns. Industry should increase efforts to develop in vitro testing methods for protection against both UVB and UVA radiation'.

Various efforts to develop in vitro SPF test methods have resulted in a variety of potential approaches, all united by the combined use of a UVR-transparent substrate (which acts as a skin analogue and upon which product is spread) and a UVR spectrophotometer (to measure transmitted UVR). In the in vitro sun protection methods groups of COLIPA/Cosmetics Europe, coherent programs to develop such methods have been underway since 2008. Considerable effort, therefore, has been given to the development of, for example, optimal UVR transparent substrates, irradiation protocols/parameters and automated spreading procedures to enable the in vitro prediction of in vivo SPF with accuracy, repeatability and reproducibility [2-7].

All these considerations lead to the development of a full in vitro method, using:
- 2 complementary PPMA plate types to overpass the limitation of difference in affinity of one type of plate for specific sunscreen formulae
- Automated spreading procedures mimicking the in vivo gesture while improving reproducibility of this key step and thus of the SPF assessment
- UV exposure with same spectrum than in vivo ISO 24444 method, to take into account the photostability of the formulae.

This document describes the Double Plate technical procedure, which is under ISO development as the 23675 International Standard, and currently in a validation process developed by ISO sunscreen experts.

[1] European Commission Recommendation 2006/647/EC. Available at: http://eur-lex.e uropa.eu/legal-content/EN/TXT/?uri=CELEX %3A32006H0647

[2] Diffey, B.L. and Robson, J. A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum. J. Soc. Cosmet. Chem. 40, 127–133 (1989).

[3] Fageon, L., Moyal, D., Coutet, J. and Candau, D. Importance of sunscreen products spreading protocol and substrate roughness for in vitro sun protection factor assessment. Int. J. Cosmet. Sci. 31, 405–418 (2009).

[4] Pissavini, M., S. Marguerie and O. Doucet (2016). "SPF tests reveal no ideal in vitro substrate exists." Cosm & Toil.

[5] Miksa, S., D. Lutz and C. Guy (2013). "In vitro UV testing-robot vs. human spreading for repeatable, reproducible results." Cosmt. Toil 128: 742-752.

[6] Pissavini, M., C. Tricaud, G. Wiener, A. Lauer, M. Contier, L. Kolbe, C. Trullas Cabanas, F. Boyer, V. Nollent, E. Meredith, E. Dietrich and P. J. Matts (2018). "Validation of an in vitro sun protection factor (SPF) method in blinded ring-testing." Int J Cosmet Sci.

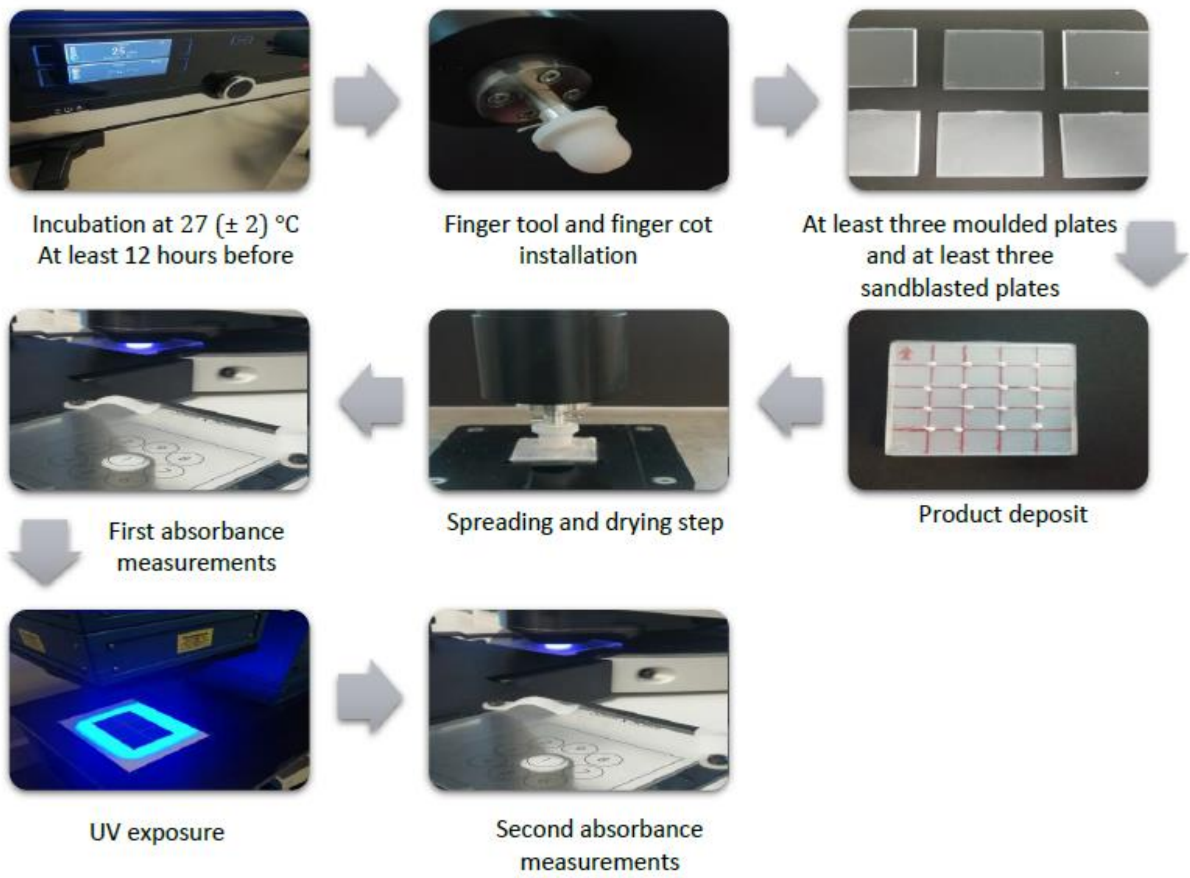
[7] Pissavini, M., C. Tricaud, G. Wiener, A. Lauer, M. Contier, L. Kolbe, C. Trullas Cabanas, F. Boyer, E. Meredith, J. de Lapuente, E. Dietrich and P. J. Matts (2020). "Validation of a new in vitro Sun Protection Factor method to include a wide range of sunscreen product emulsion types." Int J Cosmet Sci 42(5): 421-428.

	EQUIPMENT
SUBSTRACT	1) Moulded PMMA plates 2) Sandblasted PMMA plates *Cf specifications in Annex A*
PLATES SURFACE TEMPERATURE CONTROLLER	To store plates and product, in the dark, at 27 (± 2) °C for at least twelve hours before the start of the test, then for 30-60min after the spreading
ANALYTICAL BALANCE	Laboratory balance with at least 10^{-4} g precision
AUTOMATIC POSITIVE-DISPLACEMENT PIPETTE	Positive displacement pipettes, micro-pipettes, automatic pipettes or any similar device should use piston-driven displacement and shall be capable of delivering accurate and repeatable aliquots of approximately 1.6 mg to 1.8 mg of a sunscreen product
FINGERCOT	Latex, untextured, unpowdered, Medium size
AUTOMATIC SPREADING ROBOT	The robot shall have (i) positional repeatability of at least ± 0,1 mm in x, y and z axes, (ii) degrees of freedom equal to at least 6 rotating joints, (iii) a payload of at least 0,5 kg, (iv) a vertical force (z axis), measured in the centre of the plate (with the finger tool and finger cot, without x and y axis movement), of 6,0 ± 0,5 N *Cf specifications in Annex B*
UV TRANSMITTANCE SPECTROPHOTOMETER	As described in ISO 24443 /!\ The substrate should remain positioned in a horizontal plane as : - Vertical position could create product migration. To limit migration, be sure the full measurement would take less than 2 minutes. - In vertical spectrophotometer, the plates need to be pinched in a support which damages the film and removes partly the deposited product. Adapted support could be considered. The spectrophotometer shall allow to perform 1-9 measurements on the plate (covering 16cm²) in a very reproducible position of the measurement areas for measurements before/after exposure. *Refer to Annex A of ISO 24443*
SOLAR SIMULATOR	A xenon arc solar simulator with appropriate filters should be used. It shall be able to maintain a stable, sample-level temperature of (27 ± 2) °C and to irradiate at least 2 plates at the same time. Pay special attention to ensure having: - SPF vivo spectrum - No flux of air on the plate - Good temperature stability - Good homogeneity of UV irradiation - Possibility to place the plates without interrupting the UV flux *Cf specifications in Annex C*
RADIOMETER / SPECTRORADIOMETER	Use a radiometer able to provide flux measurement in MED/Hr Or use a spectroradiometer and perform the right calculation (e.g. 1 MED = 210 J/cm²).
REFERENCE SUNSCREEN	P2 (SPF range 2-24) P5 or P6 (SPF range 25-49) P8 (SPF range ≥50)
PRODUCT FOR BLANK	White petroleum

+ SPREADSHEET FOR CALCULATION

KEY STEPS OF THE METHOD

1. Preparation of reagents and materials
2. Product application on substrates and robot automatic spreading
3. Measurement of initial absorbance using two plate types (290 nm to 400 nm).
4. Calculation of initial in vitro SPF.
5. Calculation of irradiation dose (based on initial in vitro SPF).
6. Irradiation with calculated dose.
7. Measurement of final post-irradiation absorbance using two plate types (290 nm to 400 nm).
8. Calculation of final in vitro SPF.



1) PREPARATION OF REAGENTS AND MATERIALS

1.1. Plate preparation and handling

- Pick at least 3 moulded PMMA plates from the same batch, and at least 3 sand-blasted plates from the same batch.

NB : Pick additional plates for saturation of the finger cot (1 moulded or sandblasted plate) and for blank measurement (1 moulded plate and 1 sansdblasted plate)
/!\ Some spectrophotometer allows you to save calibration data so that you do not have to do it for every product. Calibration for moulded and sandblasted plates should be performed at least once a week, and at each batch change.
/!\ 3 pairs of plates is a minimum but depending on the product, additional pair(s) of plates may be need to meet the statistical criterion CI95% ≥ 17% of the mean SPF.

- Store the plates (opened or removed plastic bag) and product, in the dark, at 27 (± 2) °C for at least twelve hours before the start of the test. The time for the plate being in different temperature condition (such as at room temperature) should be limited at the minimum. To do so, proceed the product deposition and spreading then the absorbance measurement plate by plate while you let the other plates in the dark at 27(± 2) °C.

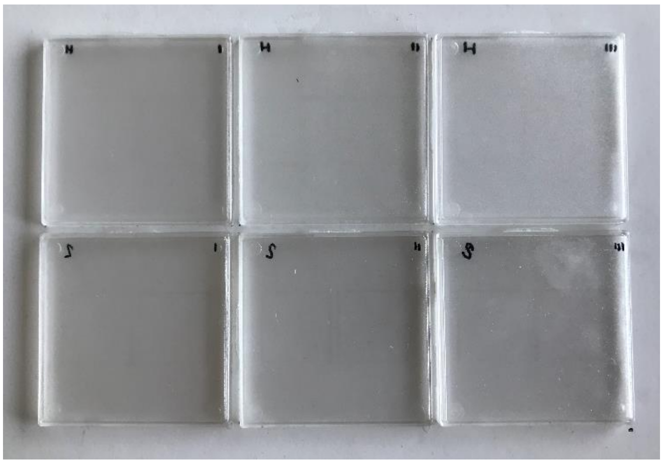
- Clean the surface of sand-blasted plates with a dry, antistatic microfibre cloth right before the product deposition.

- Always be cared to handle the plates carefully by holding them by the edges, avoiding finger contact with the surface.

- The plates shall be used without additional treatment on surface (chemical and/or physical).

- The plates shall be used only once.

- Make some form of reference mark on each plate to ensure the whole measurement process proceeds in the same order and direction each time. Better is to handle all the plates in the same direction. See examples below:



1.2 Finger cot

- Place a latex finger-cot on the robot finger probe.

/!\ It is important to verify that there are no creases in the finger cot after fitting and to verify that the finger cot remains firmly in place and does not break during the spreading procedure. If creases should appear on the finger-cot or if it breaks during the spreading procedure, it shall be replaced, and the plate currently being spread shall be discarded.

- Ensure saturation of the finger cot, by performing at first a standard spreading procedure with a moulded PMMA plate, using the same protocol as described in 2. This plate shall be discarded and is not part of the final calculation.

/!\ Each time that there is a change in finger cot, this procedure shall be repeated. There is no need to repeat this procedure before performing spreading procedure on sandblasted PMMA plate if the finger cot is unchanged.

2) PRODUCT APPLICATION ON SUBSTRATES AND ROBOT AUTOMATIC SPREADING

2.1 Weighing of product and deposit on plates

/!\ Liquid sunscreen products shall be shaken well before application to the plates.
/!\ to achieve an uniform droplet application, it is advice to use a support where the diagram of droplet application is drawn.

- Place a plate onto the weighing pan of an analytical balance (with the roughened side uppermost), to check the weight of the plate before and after application of product.

- Using an automatic positive-displacement pipette capable of dispensing repeated identical aliquots of product:
  - moulded plate: 28,7 mg ± 0,5 mg (corresponding to 1,3 mg/cm² (± 1,6 %)) of product is applied to each plate
  - sandblasted plate: 26,5 mg ± 0,5 mg (corresponding to 1,2 mg/cm² (± 1,5 %)) of the same product is applied to each plate

- Achieve the coverage of the whole area of the plate with 16 uniform equally-spaced droplets of approximately 1.6 mg to 1.8 mg each (corresponding to approximately 1,6 µL to 1,8 µL each; as described in the diagram here after for example).

/!\ Deposit and weighing shall not take more than 30s.

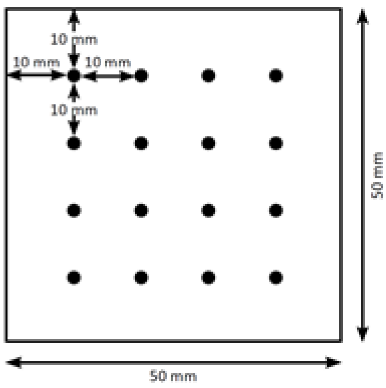


Figure 1 — Diagram of droplet application

/!\ If the applied weight is too low, up to four more equal droplets can be added to the plate in a uniform manner, to achieve the desired target final weight. If the applied weight is too high, product can be removed carefully using the pipette tip, until the desired weight is achieved.

2.2 Automatic spreading

- Once the product is deposited on plate, immediately place it on the plate holder of the robot and start the automated spreading sequence (see Annex D).

  /!\ For each plate, the time between the start of droplet application and placing the plate on the robot plate holder shall be no more than 30s.

- Immediately after the robot application sequence stops, place the plate in a dark environment for at least 30min (up to a maximum of 60min) at 27 (± 2) °C.

Repeat steps 2.1 and 2.2 for each of the 3 moulded PMMA plates then each of the 3 sandblasted PMMA plates.

3) MEASUREMENT OF INITIAL ABSORBANCE USING TWO PLATE TYPES (290 NM TO 400 NM)

3.1 Blank measurement

- Prepare a “blank” plate for both moulded and sandblasted plates by spreading a few microliters of white petroleum (approximately 15 µl for a 50x50 mm ± 4% plate) on the roughened side of the plate.

  /!\ The amount of white petroleum should be such that the entire surface is completely covered without excess

- Spread (manually or by robot) using a finger cot.

- Measure UV transmission through plates spread in this way using the spectrophotometer and used as the “blank” for subsequent spectrophotometric measurement

3.2 Initial absorbance measurement

/!\ An equal number of moulded and sandblasted plates shall to be used. A minimum of three plates of each type is required, and a maximum of 10 plates of each type shall be used.

- Measure the absorption spectrum (290 nm to 400 nm) of product films spread on either moulded or sandblasted plates using a calibrated spectrophotometer. Measurements should be taken at one center location within the plate if a large beam of at least 16 cm² is used or, if a small beam is used, then at least 9 separate locations shall be used, equally-spaced, with a total area of at least 16 cm² (as described in the diagram here after for example (Figure 2).

  /!\ Initial absorbance (290 nm to 400 nm) shall be measured after at least 30min and no more than 60min after product spreading, using the spectrophotometric procedure described above. Care shall be taken to measure absorbance on the same plate locations (using a standard template) for each plate and before / after UV exposure.

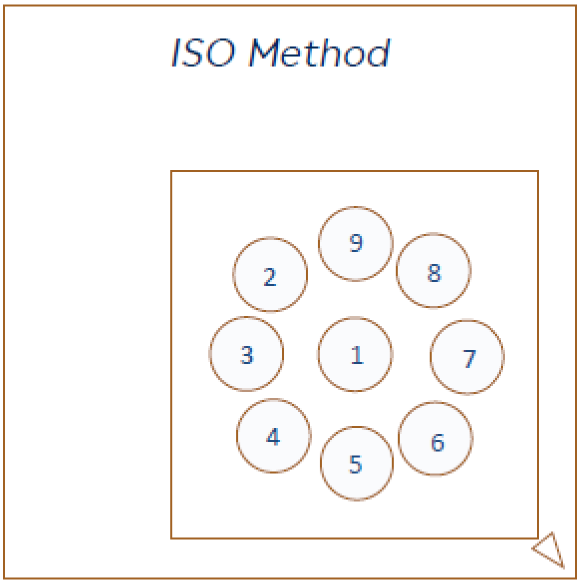


Figure 2 – Example of diagram of the 9 measurements locations in the case when a small beam is used. (cf Annex G for construction guide)

- Initial absorbance data from both plates are then combined in the spreadsheet provided to yield a single term describing initial absorbance, using the following Equation 1.
Pairing of plate shall be performed by pairing the 1st moulded plate with the 1st sandblasted plate, the 2nd moulded plate with the 2nd sandblasted plate and the 3rd moulded plate with the 3rd sandblasted plate. i.e. at least 3 combinations:

$A_{Initial}(\lambda) = C_{Moulded} * A_{Moulded\text{-}pre\text{-}irradiation}(\lambda) + C_{Sandblasted} * A_{Sandblasted\text{-}pre\text{-}irradiation}(\lambda)$ (Eq. 1)
where:

$A_{Moulded\text{-}pre\text{-}irradiation}$ = absorbance of the moulded plate before UV exposure;

$A_{Sandblasted\text{-}pre\text{-}irradiation}$ = absorbance of the sandblasted plate before UV exposure;

With $C_{Moulded}$ and $C_{Sandblasted}$ = Correction factors defined in Table here after (according to product type);

Type of product	**$C_{moulded}$**	**$C_{sandblasted}$**
Emulsions	0.225	0.800
Alcohol	0	0.800

4) CALCULATION OF INITIAL IN VITRO SPF

- Calculate the initial in vitro SPF_i for each pair of plates by using the following Equation 2:

$$Initial\ in\ vitro\ SPF_i = \frac{\int_{290}^{400} E(\lambda)\, I(\lambda)\, d\lambda}{\int_{290}^{400} E(\lambda)\, I(\lambda)\, 10^{-A_{initial}(\lambda)}\, d\lambda} \qquad \text{(Eq. 2)}$$

where:

E(λ) = CIE erythema action spectrum;

I(λ) = Midday mid-summer global irradiance at 40°N;

$A_{Initial}$(λ) = Mean monochromatic absorbance of the test product layer before UV exposure for each pair of plates from Eq. 1

dλ = Wavelength step (1 nm).

Note : initial in vitro SPF could be calculated directly in the spreadsheet provided (see for details on how the calculation sheet works on Annex G) or using the spectra described in Annex E.

/!\ be sure that, whatever the unit of the data you import from the Spectrophometer (T, A or mAF), the SPF calculation is based on Absorbance.

- Use the spreadsheet to check the homogeneity of absorbance measurements between the plates: if COV > 20% then it is advisable to make a new set of 3 moulded and 3 sandblasted plates.

5) CALCULATION OF IRRADIATION DOSE (BASED ON INITIAL IN VITRO SPF)

- Calculate the irradiation dose Dx (of full-spectrum solar-simulated radiation) using the following Equation 3 (for each pair of plates):

$$Dx\ (MED) = 0{,}25 \times Initial\ in\ vitro\ SPF_i \qquad \text{(Eq. 3)}$$

- Measure the irradiation flux of your solar simulator with radiometer

- Enter the measured value in the spreadsheet, using MED/Hr unit.

- Update the correction factor to adapt it to your own equipment: if the correction factor is already included into your radiometer, then enter "1" as correction factor in the spreadsheet.

- Calculate the time for exposure for each pair of sunscreen treated plates using the spreadsheet or with the following equation T *(Hr)* = Dx *(MED)* / Flux *(MED / Hr)*

6) IRRADIATION WITH CALCULATED DOSE

- Irradiate each pair of sunscreen treated plates with the relevant calculated dose (Dx) of full-spectrum solar-simulated radiation using a solar simulator (see annex C and annex E), at a stable, sample-level temperature of 27 (± 2) °C.

7) MEASUREMENT OF FINAL POST-IRRADIATION ABSORBANCE USING TWO PLATE TYPES (290 NM TO 400 NM)

- Measure the post-irradiation absorbance (290 nm to 400 nm) using the spectrophotometric procedure described above.

  /!\ Care should be taken to measure absorbance on the same plate locations used in 3.2 (using the same standard plate template).

- Final absorbance data from both plates are then combined in the spreadsheet provided to yield a single term describing final absorbance, using the following Equation 4.

Pairing of plate shall be performed by pairing the 1st moulded plate with the 1st sandblasted plate, the 2nd moulded plate with the 2nd sandblasted plate and the 3rd moulded plate with the 3rd sandblasted plate. i.e. at least 3 combinations:

$$\text{Final A}(\lambda) = C_{\text{Moulded}} * A_{\text{Moulded-post-irradiation}}(\lambda) + C_{\text{Sandblasted}} * A_{\text{Sandblasted-post-irradiation}}(\lambda) \qquad \text{(Eq. 4)}$$

where:

C_{Moulded} and $C_{\text{Sandblasted}}$ = Correction factors defined in Equation 1;

$A_{\text{Moulded-post-irradiation}}$ = absorbance of the exposed moulded plate;

$A_{\text{Sandblasted-post-irradiation}}$ = absorbance of the exposed sandblasted plate;

8) CALCULATION OF FINAL IN VITRO SPF

- Calculate the final in vitro SPF_i value of each pair using the calculation sheet provided or the Equation 5 below. Pairing of plate shall be performed by pairing the 1st moulded plate with the 1st sandblasted plate, the 2nd moulded plate with the 2nd sandblasted plate and the 3rd moulded plate with the 3rd sandblasted plate. i.e. at least 3 combinations.:

$$Final\ in\ vitro\ SPF_i = \frac{\int_{290}^{400} E(\lambda)\, I(\lambda)\, d\lambda}{\int_{290}^{400} E(\lambda)\, I(\lambda)\, 10^{-Final\ A(\lambda)}\, d\lambda} \qquad \text{(Eq 5)}$$

where,

$E(\lambda)$, $I(\lambda)$ and $d\lambda$ are defined in Equation 2;

Final $A(\lambda)$ = Mean monochromatic absorbance of the test product layer after UV exposure for each pair of plates from Eq. 4

The SPF of the product is the arithmetical mean of the individual plate pair. In Vitro SPF value are calculated using the total number (n, with $n \geq 3$) of pairs used, expressed as a whole number (truncation):

$$\text{Final In Vitro SPF} = \left(\Sigma \ \ \text{Final } \textit{In Vitro } \text{SPF}_i \right) / \ n \qquad \text{(Eq. 6)}$$

- Calculate the standard deviation (s) defined as:

$$s = \sqrt{[(\Sigma (\text{Final } \textit{In Vitro} \text{ SPF}_i)^2 - ((\Sigma \text{ Final } \textit{In Vitro} \text{ SPF}_i)^2 / n)) / (n - 1)]} \qquad \text{(Eq. 7)}$$

8.1 Validation of in vitro SPF

At least three pairs of treated plates should be used to establish the in vitro SPF value of the test sample.
If the 95 % Confidence Interval (CI) of the calculated in vitro SPF value is greater than 17 % of the mean, additional pair(s) of treated plates can be added until the 95 % CI is reduced to below 17 % of the mean. If this criterion is not fulfilled after ten valid pairs, then the entire test shall be rejected and repeated.
The 95 % confidence interval (95 % CI) of the mean in vitro SPF is expressed as:

$$95\ \%\ \text{CI} = (\text{Final In Vitro SPF} - c) \text{ to } (\text{Final In Vitro SPF} + c) \qquad \text{(Eq. 8)}$$

c is calculated as:

$$c = (\text{t value}) \times \text{SEM} = (\text{t value}) \times s / \sqrt{n} \qquad \text{(Eq. 9)}$$

The percentage half-range confidence interval is calculated as:

$$\text{CI}[\%] = 100 \times c / \text{Final In Vitro SPF} \qquad \text{(Eq.10)}$$

where

SEM = the standard error of the mean;
n = total number of plates used;
t = t value from the “two-sided” Student-t distribution table at a probability level p = 0,05 and with degrees of freedom v = (n - 1).

8.2 Validation of the test

Check at least once a month that the mean SPF of the reference sunscreens fall within the acceptance limits shown in Annex D.

9) CALCULATION OF FINAL IN VITRO UVA-PF

The Final *In Vitro* UVA-PF_i shall be calculated according to Equation 11 for each pair of plates, using the single observation value or the mean of multiple observations on that pair of plates.

$$Final\ in\ vitro\ UVA - PF_i = \frac{\int_{\lambda=320}^{\lambda=400} P(\lambda)\ I(\lambda) d\lambda}{\int_{\lambda=320}^{\lambda=400} P(\lambda) I(\lambda) 10^{-Final\ A(\lambda)} d\lambda} \quad \text{(Eq 11)}$$

where

P(λ)	is the PPD action spectrum (see Annex E);
I(λ)	is the spectral irradiance received from the UV source (UVA 320 nm to 400 nm for PPD testing) (see Annex F);
Final A(λ)	Mean monochromatic absorbance of the test product layer after UV exposure for each pair of plates from Eq. 4
dλ	is the wavelength increment (in step of 1 nm)

The UVA-PF of the product is the arithmetical mean of the individual plate pairs. In Vitro UVA-PF value is calculated using the total number (n with n ≥ 3) of pairs used, expressed as a whole number (truncation):

$$\text{Final In Vitro UVA-PF} = \left(\Sigma\ \text{Final } \textit{In Vitro}\ \text{UVA-PF}_i\right) / n \quad \text{(Eq. 12)}$$

- Calculate the standard deviation (s) defined as:

$$s = \sqrt{\left[\left(\Sigma\left(\text{Final } \textit{In Vitro}\ \text{UVA-PF}_i\right)^2 - \left(\left(\Sigma\ \text{Final } \textit{In Vitro}\ \text{UVA-PF}_i\right)^2 / n\right)\right) / (n-1)\right]} \quad \text{(Eq. 13)}$$

9.1 Validation of in vitro UVA-PF

At least three pairs of treated plates should be used to establish the in vitro UVA-PF value of the test sample.
If the 95 % Confidence Interval (CI) of the calculated in vitro UVA-PF value is greater than 17 % of the mean, additional pairs of treated plates can be added until the 95 % CI is reduced to below 17 % of the mean. If this criterion is not fulfilled after ten valid pairs, then the entire test shall be rejected and repeated.
The 95 % confidence interval (95 % CI) of the mean in vitro UVA-PF is expressed as:

$$95\ \%\ CI = (\text{Final In Vitro UVA-PF} - c) \text{ to } (\text{Final In Vitro UVA-PF} + c) \quad \text{(Eq. 14)}$$

c is calculated as:

$$c = (t\ value) \times SEM = (t\ value) \times s / \sqrt{n} \quad \text{(Eq. 15)}$$

The percentage half-range confidence interval is calculated as:

CI[%] = 100 × c / Final In Vitro UVA-PF (Eq. 16)

where

SEM = the standard error of the mean;
n = total number of plates used;
t = t value from the "two-sided" Student-t distribution table at a probability level p = 0,05 and with degrees of freedom v = (n - 1).

10) CALCULATION OF CRITICAL WAVELENGTH

The critical wavelength " λ_c " is the wavelength at which the area under the absorbance curve represents 90 % of the total area under the curve in the UV region. This is expressed mathematically as Formula (17):

$$\text{Final In Vitro } \lambda c_i = \int_{\lambda=290}^{\lambda_c} Final\ A(\lambda) \times d\lambda = 0.9 \times \int_{\lambda=290}^{\lambda=400} Final\ A(\lambda) \times d\lambda \quad \text{(Eq. 17)}$$

where

Final A(λ) monochromatic absorbance of the test product layer after UV exposure for each pair of plates from Eq. 4

dλ is the wavelength interval between measurements.

The critical wavelength is expressed as a whole number (truncation) and with nanometer (nm) unit.

The Critical Wavelength of the product is the arithmetical mean of the individual plate pairs. In Vitro Critical Wavelength value is calculated using the total number (n with n ≥ 3) of pairs used, expressed to one decimal point:

$$\text{Final In Vitro } \lambda c = \left(\Sigma \ \text{Final } In\ Vitro\ \lambda c_i \right) / n \quad \text{(Eq. 12)}$$

ANNEX A

Sample substrate specifications

A. 1 GENERAL

Moulded and sandblasted PMMA plates shall be used as the substrate for sunscreen application according to this method. Samples substrates shall be UVR-transparent, non-fluorescent, (i.e., no detectable fluorescence when exposed to UVR and measured with a spectrophotometer), photostable and inert to all ingredients in the preparations to be tested. Furthermore, to enable the application of stable, thin films of different types in a skin-like manner, the substrate shall have a standard, textured upper surface. The substrate shall allow an optimal, homogeneous film to be formed after the product has been spread.

A. 2 SIZE

The size of the substrate shall be chosen such that the application area is not less than 16 cm^2. It is important to verify the exact dimension of the substrate before performing this method and / or to check the specification in the accompanying Certificate of Analysis from the manufacturer.

A. 3 SPECIFICATION

Correct substrate / plate specification is of primary importance to the correct deployment of this method . UVR-transparent PMMA is the preferred material for test substrates. Specifications for plate-to-plate reproducibility and surface roughness parameters are defined here after.

A. 3. 1 Moulded PMMA plate

Ra (µm) = 4,853 ± 0,501
Rv (µm) = 13,042 ± 0,989
Rdq (°) = 11,122 ± 2,032
A1 ($µm^2$ mm-1) = 239,750 ± 70,165
Ssc (1/µm) = 0.033 ± 0.021
Vvv (mm3/mm^2) = 1.044.10-6 ± 9.76.10-7

A. 3. 2 Sandblasted PMMA plate

Ra (µm) = 4,188 ± 0,514
Rv (µm) = 11,402 ± 2,499
Rdq (°) = 11,004 ± 1,938
A1 ($µm^2$ mm-1) = 238,252 ± 72,663
Ssc (1/µm) = 0.032 ± 0.012
Vvv (mm3/mm^2) = 8.701.10-7 ± 2.325.10-7

ANNEX B

Automatic spreading robot specifications

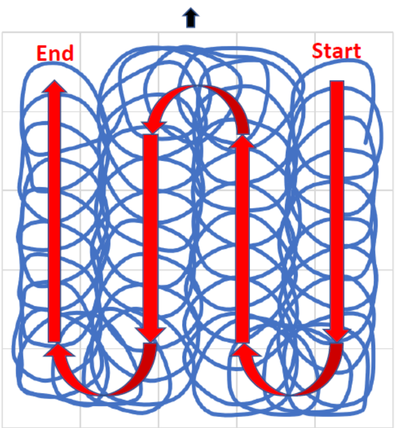
The robot shall have (i) positional repeatability of at least ± 0,1 mm in x, y and z axes, (ii) degrees of freedom equal to at least 6 rotating joints, (iii) a payload of at least 0,5 kg, (iv) a vertical force (z axis), measured in the centre of the plate (with the finger tool and finger cot, without x and y axis movement), of 6,0 ± 0,5 N

B. 1 SPREADING

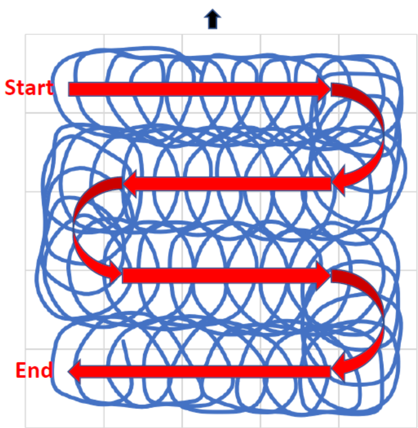
The complete spreading routine should be performed in two passes, over one minute, with a tool maximum speed of 1 m/sec:

B.1.1 STEP 1

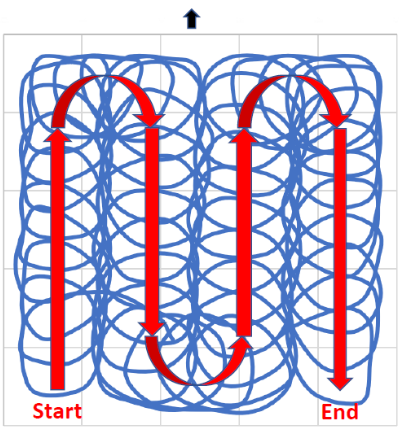
Three circular cycles (one circular cycle equals to about eight circles per linear arrow including continuous circles movement for the hemispherical arrows between the four linear arrows covering the whole area) with 90° rotation between each circular cycle



First cycle
(11.0 ± 0.5 sec)



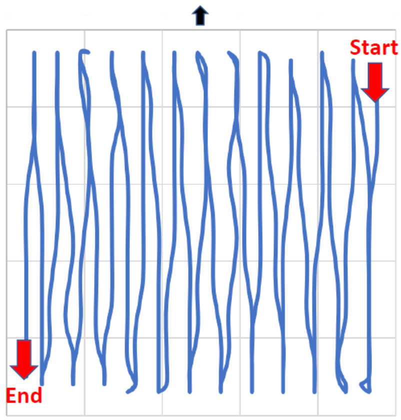
Second cycle
(11.0 ± 0.5 sec)



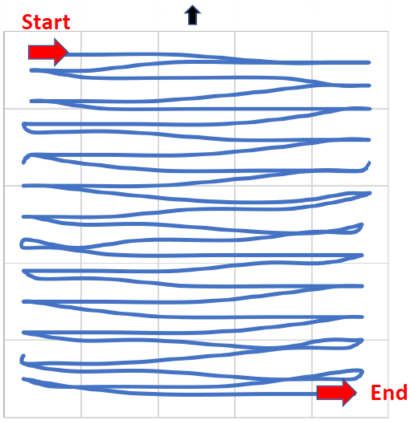
Third cycle
(11.0 ± 0.5 sec)

B.1.2 STEP 2

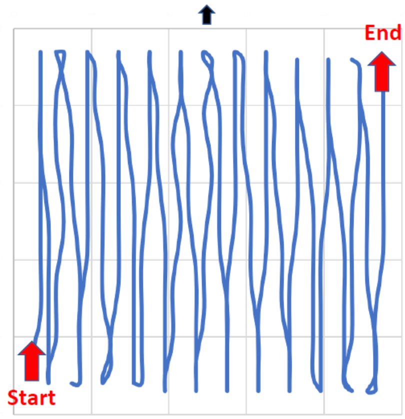
Three linear cycles (one linear cycle equals to about thirteen back and forth movements covering the whole area), with 90° rotation between each linear cycle.



Fourth cycle
(7.5 ± 0.5 sec)



Fifth cycle
(6.0 ± 0.5 sec)



Sixth cycle
(7.5 ± 0.5 sec)

B.2 Finger tool

The robot should use a hemi-spherical finger tool, (i) made of a silicon material (ii) with a hardness of between 20-25 shores (iii) and a diameter of (20,0 ± 1,0) mm and a 90° radius of curvature.

B.3 Checking robot parameters

The robotic appliance shall be checked by a suitably qualified expert at regular intervals (at least every twelve months) to ensure compliance to the mechanical and spreading specifications above.

The finger tool shall be replaced after every cycle of 400 spreading operations or when damaged.

ANNEX C

UV exposure and erythema action spectra and solar simulator UV spectrum

A xenon arc solar simulator with appropriate filters should be used and shall comply with the spectral specifications described in Annex E.

It shall be able to maintain a stable, sample-level temperature of (27 ± 2) °C and to irradiate at least 2 plates at the same time.

1.C.1 Quality of UVR

The output from the solar UVR simulator shall be continuous, uniform, stable, with no gaps or extreme peaks of emission in the UVR region and suitably filtered to create a spectral quality that complies with the required acceptance limits (see Table A in Annex E).

To ensure that appropriate amounts of UVA radiation are included in the spectrum of the solar UV simulator, the total radiometric proportion of UVA-II irradiance of the simulator (320 nm to 340 nm) shall be ≥ 17.5 % of total UVR irradiance (290 nm to 400 nm). Additionally, UVA-I region (340 nm to 400 nm) irradiance shall be ≥ 60 % of total UVR irradiance. The source spectral specification is described in terms of cumulative erythemal effective irradiance by successive wavelength bands, 290nm to 400 nm. The erythemal effective irradiance of each wavelength band is expressed as a percentage of total erythemal effective irradiance, 290 nm to 400 nm, or as percentage relative cumulative erythemal effectiveness (%RCEE). The definition and calculation of %RCEE values is described in Annex A and acceptance limits are shown in Table A.

1. C.2 Total irradiance (UVR, visible and near infrared rays)

Total irradiance shall not exceed 1 600 W/m2. The output of the solar simulator shall be measured with a broad spectrum sensor (capable of measuring between 280 nm and 1 600 nm) calibrated against a standard reference source over the range of 280 nm to 1 600 nm. Alternatively, the source may be measured with a calibrated spectroradiometer over this same wavelength range to determine the total irradiance.

1.C.3 Maintenance and monitoring the solar simulator

The emission of the UV exposure source used for exposure shall be checked for compliance with the given acceptance limits by a suitably qualified expert (at least) every 12 months, or 2500 hours of lamp running time. The inspection should be conducted with a spectroradiometer that has been calibrated against a standard lamp that is traceable to a national or an international calibration standard. In addition to the spectroradiometric inspection, the intensity of the UV exposure source used for exposure shall be checked prior to each use.

This can be done using either a spectroradiometer or a radiometer with sensitivity in the UVA, calibrated for the same UV exposure source spectrum used for the exposure step of the procedure, applying the coefficient of calibration to adjust for variance between the UVA radiometer and the reference spectroradiometer.

Be care that the solar simulator UV shall :

- Provide the same spectrum than the one used in SPF vivo exposure (SSR – cf table)
- Ensure that there is no flux of air on the plates

- Ensure a good temperature stability (+/- 2°C)
- Ensure a good homogeneity of UV irradiation of the whole irradiated area
- Ensure the possibility to place the plates without interrupting the UV flux

ANNEX D

SPF reference sunscreen products

The formulation and preparation procedure for product reference sunscreen P2, P5, P6 and P8 are the same than the one described in ISO 24444:2019.

D.1 Mean SPF and acceptance limits for reference sunscreen formulations

Reference sunscreen formulation	Mean SPF	Acceptance limits	
		Lower limit	Upper limit
P2	11,3	9,9	12,6
P3	TBD	TBD	TBD
P5	31,3	26,3	36,3
P6	43,0	38,3	47,7
P8	104,4		

ANNEX E

UV exposure and erythema action spectra and solar simulator UV spectrum

Table E.1 — UV exposure and erythema action spectra and solar simulator UV spectrum

	UV calculation	Erythema	Solar Simulator UV spectrum	Sol. Sim.	RCEE accept. range	
	Midday midsummer global irradiance at 40°N (W·m-2·nm-1)	Action Spectrum	SSR (W·m-2·nm-1)	%RCEE	Lower	Upper
Nm	I(l)	E(l)	S(l)	Sum{E*S}/T	limit	limit
290	**3.680E-06**	**1.00E+00**	**4.41E-05**	**0.00 %**	**-**	**< 0.2%**
291	1.300E-05	1.00E+00	5.50E-05			
292	4.500E-05	1.00E+00	8.28E-05			
293	1.300E-04	1.00E+00	2.38E-04			
294	3.400E-04	1.00E+00	8.22E-04			
295	7.970E-04	1.00E+00	2.69E-03			
296	1.650E-03	1.00E+00	8.03E-03			
297	3.000E-03	1.00E+00	2.10E-02			
298	5.500E-03	1.00E+00	5.03E-02			
299	8.500E-03	8.05E-01	1.04E-01			
300	**1.280E-02**	**6.49E-01**	**1.89E-01**	**4.00 %**	**1.00 %**	**16.00 %**
301	2.100E-02	5.22E-01	3.35E-01			
302	3.000E-02	4.21E-01	5.36E-01			
303	4.100E-02	3.39E-01	8.05E-01			
304	5.300E-02	2.73E-01	1.13E+00			
305	6.510E-02	2.20E-01	1.56E+00			
306	8.400E-02	1.77E-01	2.01E+00			
307	9.700E-02	1.43E-01	2.58E+00			
308	1.200E-01	1.15E-01	3.08E+00			
309	1.450E-01	9.25E-02	3.70E+00			
310	**1.710E-01**	**7.45E-02**	**4.25E+00**	**55.70 %**	**49.00 %**	**65.00 %**
311	1.950E-01	6.00E-02	4.77E+00			
312	2.150E-01	4.83E-02	5.38E+00			
313	2.450E-01	3.89E-02	5.98E+00			

314	2.700E-01	3.13E-02	6.40E+00			
315	2.950E-01	2.52E-02	6.90E+00			
316	3.100E-01	2.03E-02	7.25E+00			
317	3.300E-01	1.64E-02	7.73E+00			
318	3.500E-01	1.32E-02	8.06E+00			
319	3.700E-01	1.06E-02	8.34E+00			
320	**3.980E-01**	**8.55E-03**	**8.70E+00**	**87.40 %**	**85.00 %**	**90.00 %**
321	4.050E-01	6.89E-03	8.99E+00			
322	4.300E-01	5.55E-03	9.32E+00			
323	4.600E-01	4.47E-03	9.55E+00			
324	5.000E-01	3.60E-03	9.76E+00			
325	5.360E-01	2.90E-03	9.91E+00			
326	5.450E-01	2.33E-03	1.02E+01			
327	5.650E-01	1.88E-03	1.03E+01			
328	6.000E-01	1.51E-03	1.04E+01			
329	6.150E-01	1.41E-03	1.06E+01			
330	**6.300E-01**	**1.36E-03**	**1.07E+01**	**93.30 %**	**91.50 %**	**95.50 %**
331	6.350E-01	1.32E-03	1.09E+01			
332	6.370E-01	1.27E-03	1.10E+01			
333	6.420E-01	1.23E-03	1.11E+01			
334	6.470E-01	1.19E-03	1.12E+01			
335	6.500E-01	1.15E-03	1.13E+01			
336	6.520E-01	1.11E-03	1.14E+01			
337	6.600E-01	1.07E-03	1.14E+01			
338	6.700E-01	1.04E-03	1.15E+01			
339	6.750E-01	1.00E-03	1.16E+01			
340	**6.800E-01**	**9.66E-04**	**1.17E+01**	**95.50 %**	**94.00 %**	**97.00 %**
	UV calculation	Erythema	Solar Simulator UV spectrum	Sol. Sim.	RCEE accept. range	
	Midday midsummer global irradiance at 40°N (W·m-2·nm-1)	Action Spectrum	SSR	%RCEE	Lower	Upper
nm	I(l)	E(l)	S(l)	Sum{E*S}/T	limit	limit
341	6.820E-01	9.33E-04	1.18E+01			
342	6.840E-01	9.02E-04	1.19E+01			
343	6.860E-01	8.71E-04	1.19E+01			

344	6.880E-01	8.41E-04	1.19E+01			
345	6.900E-01	8.13E-04	1.20E+01			
346	6.920E-01	7.85E-04	1.20E+01			
347	6.940E-01	7.59E-04	1.20E+01			
348	6.960E-01	7.33E-04	1.21E+01			
349	6.980E-01	7.08E-04	1.22E+01			
350	**7.000E-01**	**6.84E-04**	**1.22E+01**	**97.20 %**		
351	7.020E-01	6.61E-04	1.22E+01			
352	7.040E-01	6.38E-04	1.23E+01			
353	7.060E-01	6.17E-04	1.23E+01			
354	7.080E-01	5.96E-04	1.23E+01			
355	7.100E-01	5.75E-04	1.23E+01			
356	7.140E-01	5.56E-04	1.23E+01			
357	7.180E-01	5.37E-04	1.23E+01			
358	7.220E-01	5.19E-04	1.23E+01			
359	7.260E-01	5.01E-04	1.23E+01			
360	**7.300E-01**	**4.84E-04**	**1.23E+01**	**98.50 %**		
361	7.340E-01	4.68E-04	1.23E+01			
362	7.380E-01	4.52E-04	1.23E+01			
363	7.420E-01	4.37E-04	1.22E+01			
364	7.460E-01	4.22E-04	1.21E+01			
365	7.500E-01	4.07E-04	1.20E+01			
366	7.530E-01	3.94E-04	1.18E+01			
367	7.600E-01	3.80E-04	1.17E+01			
368	7.700E-01	3.67E-04	1.15E+01			
369	7.750E-01	3.55E-04	1.13E+01			
370	**7.800E-01**	**3.43E-04**	**1.10E+01**	**99.30 %**		
371	7.840E-01	3.31E-04	1.07E+01			
372	7.880E-01	3.20E-04	1.04E+01			
373	7.920E-01	3.09E-04	1.01E+01			
374	7.960E-01	2.99E-04	9.65E+00			
375	8.000E-01	2.88E-04	9.37E+00			
376	8.060E-01	2.79E-04	8.98E+00			
377	8.120E-01	2.69E-04	8.60E+00			

378	8.180E-01	2.60E-04	8.20E+00			
379	8.250E-01	2.51E-04	7.71E+00			
380	**8.300E-01**	**2.43E-04**	**7.18E+00**	**99.80%**		
381	8.350E-01	2.34E-04	6.70E+00			
382	8.400E-01	2.26E-04	6.15E+00			
383	8.480E-01	2.19E-04	5.58E+00			
384	8.550E-01	2.11E-04	4.99E+00			
385	8.600E-01	2.04E-04	4.42E+00			
386	8.650E-01	1.97E-04	3.86E+00			
387	8.750E-01	1.91E-04	3.35E+00			
388	8.820E-01	1.84E-04	2.85E+00			
389	8.860E-01	1.78E-04	2.39E+00			
390	**9.000E-01**	**1.72E-04**	**2.00E+00**	**100.00%**		
391	9.060E-01	1.66E-04	1.63E+00			
392	9.120E-01	1.60E-04	1.30E+00			
393	9.180E-01	1.55E-04	1.02E+00			
394	9.250E-01	1.50E-04	7.81E-01			
395	9.300E-01	1.45E-04	5.92E-01			
396	9.350E-01	1.40E-04	4.44E-01			
397	9.420E-01	1.35E-04	3.25E-01			
398	9.540E-01	1.30E-04	2.31E-01			
399	9.620E-01	1.26E-04	1.59E-01			
400	**9.700E-01**	**1.22E-04**	**1.07E-01**	**100.00 %**	**99.90 %**	**100.00 %**

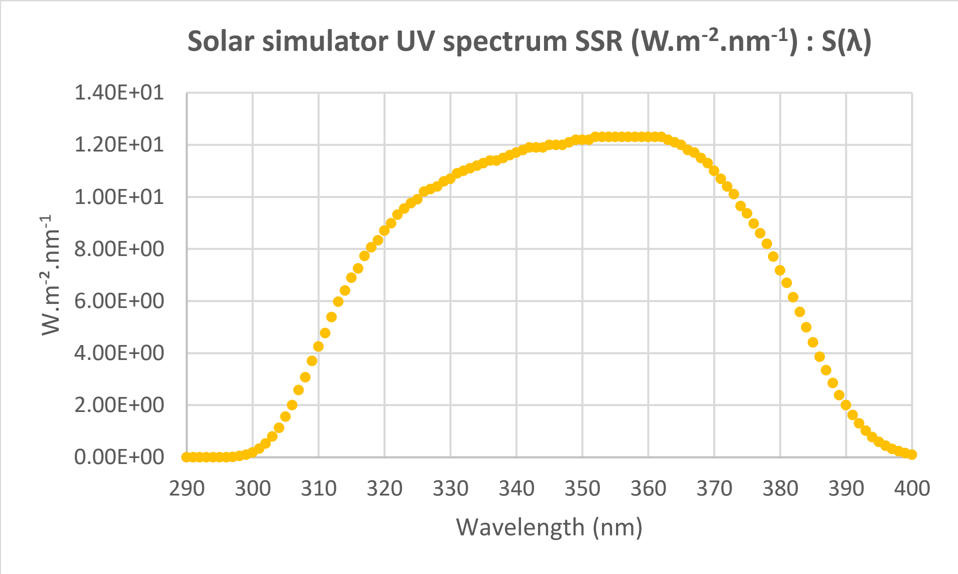


Figure E.1 – Solar simulator UV spectrum

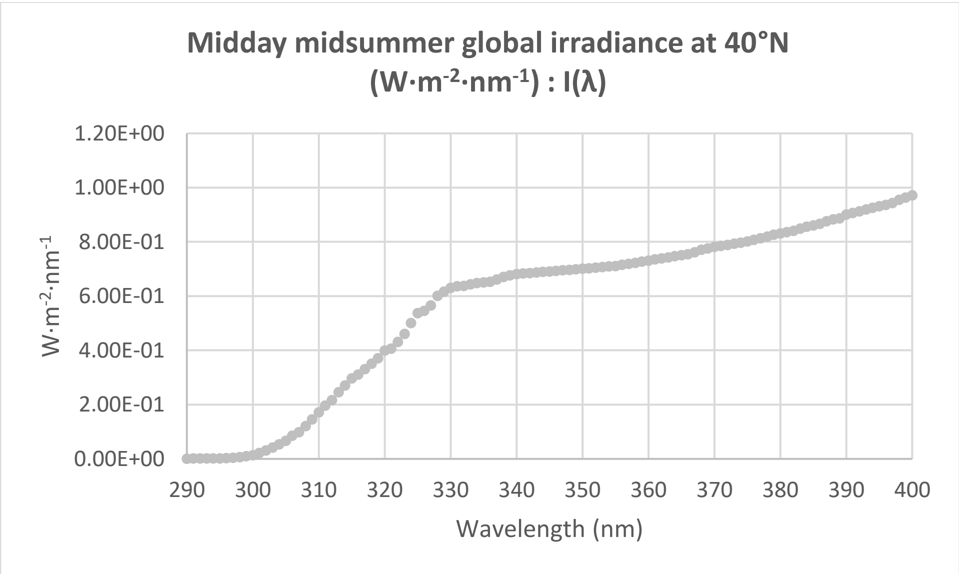


Figure E.2 – Midday midsummer global irradiance at 40°N – "Sun" spectrum

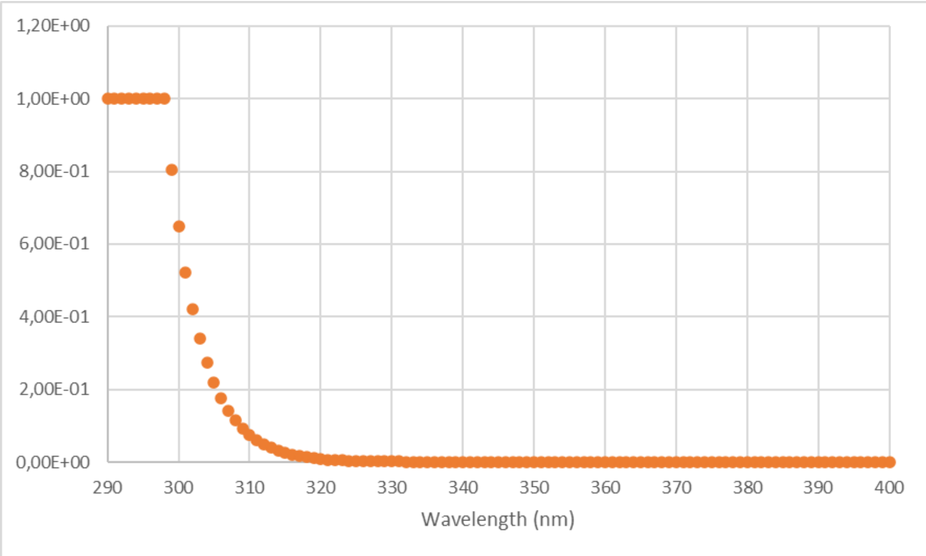


Figure E.3 – Erythema Action Spectrum

ANNEX F

Computation values: PPD and erythema action spectra and UV SSR spectral irradiances

Table F.1 — PPD and UVA spectral irradiances

Wavelength nm	**PPD** **action spectrum**	**UVA radiation source** W/m2/nm
290	—	—
291	—	—
292	—	—
293	—	—
294	—	—
295	—	—
296	—	-
297	—	—
298	—	—
299	—	—
300	—	—
301	—	—
302	—	—
303	—	—
304	—	—
305	—	—
306	—	—
307	—	—
308	—	—
309	—	—
310	—	—
311	—	—
312	—	—
313	—	—
314	—	—
315	—	—
316	—	—
317	—	—
318	—	—

319	—	—
320	1,000E+00	4,843E-06
321	9,750E-01	8,466E-06
322	9,500E-01	1,356E-05
323	9,250E-01	2,074E-05

Wavelength nm	PPD action spectrum	UVA radiation source W/m2/nm
324	9,000E-01	3,032E-05
325	8,750E-01	4,294E-05
326	8,500E-01	5,738E-05
327	8,250E-01	7,601E-05
328	8,000E-01	9,845E-05
329	7,750E-01	1,215E-04
330	7,500E-01	1,506E-04
331	7,250E-01	1,811E-04
332	7,000E-01	2,132E-04
333	6,750E-01	2,444E-04
334	6,500E-01	2,833E-04
335	6,250E-01	3,186E-04
336	6,000E-01	3,589E-04
337	5,750E-01	3,980E-04
338	5,500E-01	4,387E-04
339	5,250E-01	4,778E-04
340	5,000E-01	5,198E-04
341	4,938E-01	5,608E-04
342	4,876E-01	5,998E-04
343	4,814E-01	6,384E-04
344	4,752E-01	6,739E-04
345	4,690E-01	7,123E-04
346	4,628E-01	7,468E-04
347	4,566E-01	7,784E-04
348	4,504E-01	8,180E-04
349	4,442E-01	8,427E-04
350	4,380E-01	8,754E-04

351	4,318E-01	9,044E-04
352	4,256E-01	9,288E-04
353	4,194E-01	9,486E-04
354	4,132E-01	9,733E-04
355	4,070E-01	9,863E-04
356	4,008E-01	1,009E-03
357	3,946E-01	1,028E-03
358	3,884E-01	1,045E-03
359	3,822E-01	1,062E-03
360	3,760E-01	1,078E-03
361	3,698E-01	1,086E-03
362	3,636E-01	1,098E-03
363	3,574E-01	1,095E-03
364	3,512E-01	1,100E-03
365	3,450E-01	1,100E-03
366	3,388E-01	1,093E-03

Wavelength nm	**PPD action spectrum**	**UVA radiation source** W/m2/nm
367	3,326E-01	1,087E-03
368	3,264E-01	1,082E-03
369	3,202E-01	1,071E-03
370	3,140E-01	1,048E-03
371	3,078E-01	1,026E-03
372	3,016E-01	9,953E-04
373	2,954E-01	9,703E-04
374	2,892E-01	9,367E-04
375	2,830E-01	9,057E-04
376	2,768E-01	8,757E-04
377	2,706E-01	8,428E-04
378	2,644E-01	8,058E-04
379	2,582E-01	7,613E-04
380	2,520E-01	7,105E-04
381	2,458E-01	6,655E-04
382	2,396E-01	6,115E-04
383	2,334E-01	5,561E-04

384	2,272E-01	4,990E-04
385	2,210E-01	4,434E-04
386	2,148E-01	3,876E-04
387	2,086E-01	3,363E-04
388	2,024E-01	2,868E-04
389	1,962E-01	2,408E-04
390	1,900E-01	2,012E-04
391	1,838E-01	1,640E-04
392	1,776E-01	1,311E-04
393	1,714E-01	1,028E-04
394	1,652E-01	7,897E-05
395	1,590E-01	5,975E-05
396	1,528E-01	4,455E-05
397	1,466E-01	3,259E-05
398	1,404E-01	2,302E-05
399	1,342E-01	1,581E-05
400	1,280E-01	1,045E-05

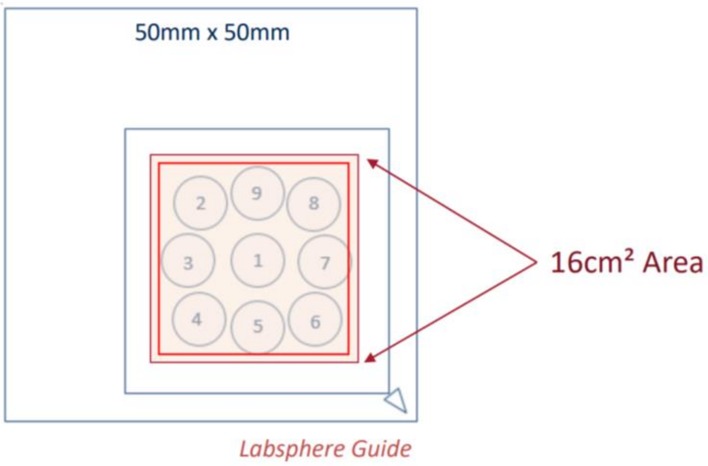
The reference sun has a total UV irradiance of 51,4 to 63,7 W/m^2 and a UVA to UVB irradiance ratio of 16,9 to 17,5.

Annex G

16cm² 9 scans measurement guide

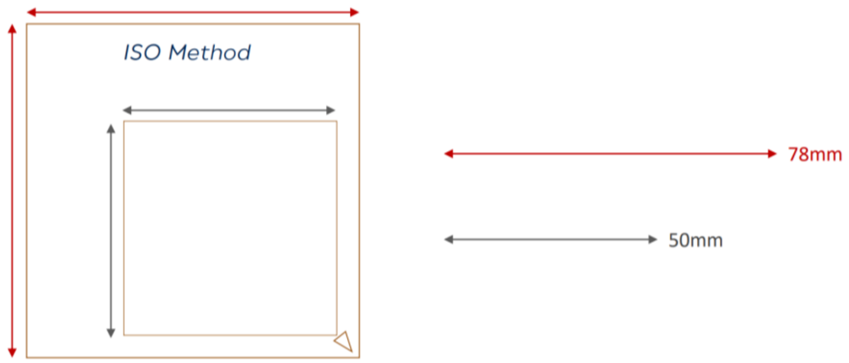
16CM² 9 SCANS MEASURMENT GUIDE

PROPOSITION & CONSTRUCTION

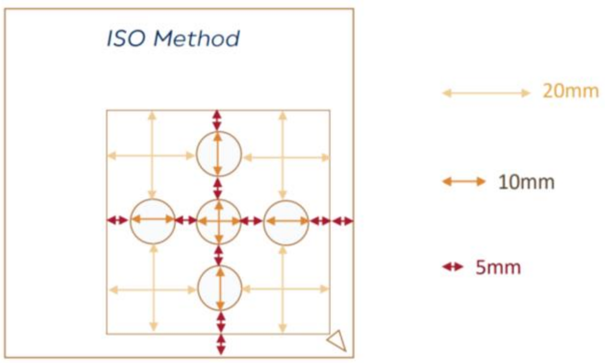


The 9 scans measurment guide proposed by labsphere doesn't respect the 16cm² Area recommanded by ISO

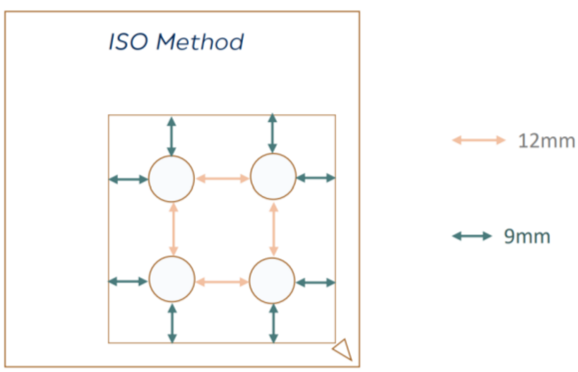
CONSTRUCTION



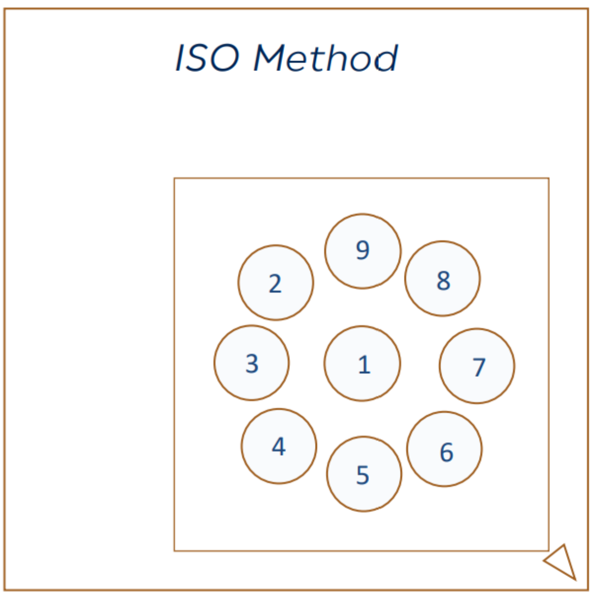
New Marks for Iso method



New Marks for Iso method



New Marks for Iso method



WARNING : Figure not to scale

Annex H

ALT-SPF ISO 23675 - UVB Calculation

ALT-SPF%20ISO%202
3675%20-%20UVB%2