



# SOLAR IN VITRO & IN VIVO TESTING

## Combining Points of view

Hello,

We are glad to share with you the 2nd **FOCUS**, the biannual discussion panel of Skinobs dedicated to the **Solar Testing**. The 1st was about Toxicology & Regulatory. This publication is 12 articles, 12 points of view of the today testing evolution for personal care, actives, ingredients and medical devices.

The sun protection objectivation subject represents a complex issue between in silico, in vitro, in vivo and hybrid methods at least as important as the challenge of the formulation itself. First, it is interesting to consider **what criteria mainly influence** the performance of UV protection products: composition, repartition, photostability, absorbance and distribution of the inorganic and organic filters, galenic (spray, compact powder, oil, cream...), properties to form a stable, homogeneous and resistant film, pleasant to apply. In real use conditions, this performance is impacted by other criteria such as individual wrinkles, skin locations, sweat, hair, application procedures and quantity.

What are the next challenges to optimize these objectivations?

Technically, it seems important to **increase the reproducibility and the accuracy** of the in vitro and in vivo testing by implementing systematic control testing such as BIPEA inter laboratory comparison tests and audit of the global process such as SUNCERT diagnostics. The gap between standardised application versus **real-life conditions of use** may also be deeply studied including anti-salt, anti-sweat or anti-sand claim substantiation.

On the **ethical point of view**, the application of erythema on the subjects which causes skin damages doesn't seem to be a long-term solution for SPF assessment. Fortunately, the HDRS method or in vitro method should propose a **new perspective** within the next years.

Could we open the field of the claim substantiation with the objectivation of all the **various damages that UVB, UVA, Blue Light, Infrared** may cause? Beyond anti-sun spectrum objectivation, and index determination, can we evaluate complementary photo ageing performances such as antioxidants, anti-free radicals, anti-ageing, anti-dark spots...?

Finally, we can expect that both worldwide **industries and regulatory authorities** harmonise the reference methods all over the world and continue with the labelling rules. It will guarantee the appropriate respect of the **human health** (nano, endocrine disruptor...) and **the sustainability for the nature** (ecotoxicity testing, coral protection) while keeping the evolution of the high performance of the sun protection products with all the complementary functionalities the consumers can expect.

### **Easily find the methods and testing labs to substantiate the regulatory framework all over the world**

- For the **in vitro sun tests** : "[Preclinical Testing Platform](#)"
- For the **in vivo tests** SPF and UVA, Infra-red, Blue-light tests: "[Clinical Testing Platform](#)"

### **Here are the subjects we will talk about**

- Future Official In-Vitro SPF Sunscreen Test Method by [Helioscreen](#)
- New developments for SFP measurements complete range of classical skin probes by [C&K](#)
- From topic to oral photoprotection by [Complife](#)
- SPF *in vitro* Testing Options by [Eurofins](#)
- Highest proteomics for a complete understanding of the product efficacy by [Phylogene](#)
- Quantification and stability analysis of UV filters by [Expertox](#)
- Skin responses to UV: a preliminary understanding of microbiome role by [Vitroscreen](#)
- [StratiCELL's](#) solutions for *in vitro* efficacy testing of sun products
- Claim « SPF and water resistance » of cosmetic products by [Intertek](#)
- Evolution of *in vivo* solar standards by [Eurofins](#)
- Advance your solar cosmetics research – customised state of the art *in vitro*, & *ex vivo* models for testing by [Monasterium Laboratory](#)
  - Great challenge of the sunscreen developments by [Grupo Investiga - Allergisa](#)



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In Vitro sunscreen testing solutions

## **Future Official In-Vitro SPF Sunscreen Test Method**

Sébastien MIKSA - General Manager

### **Introduction**

Nowadays, there are different sunscreen testing procedure for claiming the Sun Protection Factor – (SPF), UVA Protection Factor (UVAPF), Critical Wavelength (CW), Water Resistance (WR), etc. Fortunately, mainly according to ISO (International Organization for Standardization), standards are available in order to harmonize these methods worldwide such as:

- In-Vivo SPF according to ISO 24444 (published),
- In-Vivo UVAPF according to ISO 24442 (published),
- In-Vitro UVAPF - CW according to ISO 24443 (published),
- In-Vivo WR procedure according to ISO 16217 (publication in 2020),
- In-Vivo WR percentage calculation according to ISO 18861 (publication in 2020),
- In-Vitro SPF according to ISO 23675 (under development),
- In-Vivo/In-Vitro Hybrid Diffuse Reflectance Spectroscopy SPF – UVAPF according to ISO 23698 (under development).

Among all these methods, the In-Vitro SPF is strongly required by the industry and governmental organizations delivering results equivalent to the In-Vivo SPF according to ISO 24444 method. As evidence, the degree of protection should be measured **using standardized, reproducible testing methods** and take **photo-degradation** into account as recommended by the European Commission [1].

### In-Vitro SPF Method Principle

The In-Vitro SPF method in progress at the ISO level (project ISO 23675) includes **new requirements and appliances** to ensure the reliability of the results. The method was validated by Cosmetics Europe (CE) in a recent publication [2] according to the different simplified steps:

1. **Topographic parameters** of the substrates shall be controlled and respected a control chart for both PMMA molded HD6 (1.3 mg/cm<sup>2</sup>) and PMMA sandblasted SB6 plates (1.2 mg/cm<sup>2</sup>).
2. **The temperature** of the interface substrate/sample shall be controlled.
3. To ensure reproducibility, automated spreading is the only way by using a robotic arm with specific characteristics such as the HD-SPREADMASTER.
4. After a **drying step** and prior to any UV irradiation, the acquisition of the initial UV absorbance spectrum by a spectrophotometer (including specific calibrations).
5. From the initial UV absorbance spectrum using **correction factors**, a single UV exposure dose D (MED/h) is applied by using a solar simulator (including specific calibrations).
6. Finally, after UV exposure, a **second In-Vitro absorbance** measurement is required in order to calculate the final In-Vitro SPF using mathematical adjustment.

### Acceptance of In-Vitro SPF Method

One of the problems raised for the In-Vitro SPF method validation was that no acceptance criteria existed among the ISO TC217 (Technical Committee for Cosmetics) WG7 (Working Group for Sun Protection Test Methods) consensus.

To respond to this issue, after a huge work from different parties (ISO TC217 WG7 Ad Hoc group, CE, statisticians, experts, etc.) during several years, an **international consensus** was proposed and accepted years ago by the ISO/TC217/WG7 including a balance between the statistical requirements, the cost efficiency and the realistic feasibility by checking:

- The ascertain minimal method bias (matrix effect, overall bias, etc.).

- At least 95% of the paired SPF values for 24 products, derived from the 3 in-vivo test institutes (at least 5 test subjects per laboratory) and the 3 in-vitro testing labs (both in a blinded fashion), fit within the upper and lower limits of a funnel across the full range of labelled SPF categories (SPF 6, 10, 15, 20, 25, 30, 50 and 50+).

## Conclusion

Today, all barriers are solved for the alternative In-Vitro SPF method according to the ISO 23675 projects, including:

- The technical limits (the method is reproducible and correlated to the In-Vivo SPF values),
- The fulfilment of the ISO acceptance criteria (as explained in a publication about the CE method [2]),
- The established international majority consensus.

To summarize, this most advanced In-Vitro SPF method is based on the UV transmittance measurement process using a multi-substrates approach (molded and sandblasted PMMA plates) with correction factors, a robotic spreading and a UV exposure step.

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## References

[1] Commission Recommendation of 22 September 2006 on the efficacy of sunscreen products and the claims made relating thereto (notified under document number C(2006) 4089)

[2] M. Pissavini et al., Validation of an In Vitro Sun Protection Factor (SPF) method in blinded ring-testing, IJCS, April 2018.





## New developments for SPF measurements complete range of classical skin probes

Diana KHAZAKA - General Manager

The most important property of sun protection products is certainly, that they can absorb and reflect large amounts of the solar radiation- especially UV-, which is described quantitatively by the sun protection factor (SPF). Up to now, the SPF value given on the bottle of each sun protection product has to be determined by the generation of sunburns at the back of at least 10 volunteers. This is not only invasive but also time consuming, since volunteers have to come back after 24h for evaluation of the minimal dose which caused sunburn.

Since there is an obvious need for **alternative methods**, spectroscopic in vitro methods have been used for many years. However, they could not replace the invasive in vivo method so far because skin properties could not yet be completely simulated by the plastic plates used.

A **new hybrid measurement principle**, which combines spectroscopic in vivo and in vitro data (HDRS - hybrid diffuse reflectance spectroscopy), has been introduced by Ruvolo et al. and is currently applied by different groups. The ISO technical committee TC 217 (Cosmetics) is simultaneously working on a new ISO norm for the HDRS method.

Together with researchers of a clinic in Berlin, C+K currently develops a new system to measure **UV reflectance in vivo directly on the skin**, which uses state of the art UV-LED technology instead of traditional solar simulators and a sensitive spectroscopic system\*. The UV-dose applied does not cause sunburn and the SPF is determined based on the measured light attenuation of the sunscreen. Since there is no need to wait for the sunburn formation, the method is also much faster. The system has been shown to successfully implement hybrid SPF measurements using the HDRS principle.

Protection against solar radiation is not the only task of sun protection products. They have to offer much more than that. This is where the "classic" C+K probes come into play. When skin is exposed to sun, it dries out quickly. An SPF product should therefore provide moisture, which is measured by the Corneometer®. Sunscreen products must also preserve the skin barrier (keywords: anti-irritating, anti-allergic, anti-comedogenic). Tewameter®, Skin-pH-meter, Sebumeter®, Mexameter®, Visiopor® are used for these assessments. Additionally to TEWL, the new Tewameter® TM Hex is able to determine the local heat balance of the skin separately for diffusion and evaporation cooling, offering interesting research approaches in the development of sun protection products. Last but not least, UV radiation is one of skin's major aging factors:

Therefore, the renowned Cutometer® device (standard measuring principle for determining skin elasticity) and the flexible Visioscan® characterizing skin topography have been used for decades in the field of sun protection development.

All these measurement methods ensure that an SPF product becomes what it is – a true and complex multi-talent in protecting and nourishing the skin.

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## From topic to oral photoprotection

Vincenzo NOBILE - R&D Manager and Cosmetics Market Manager

Sunlight is an essential prerequisite for life; however too much sun may be extremely dangerous to the human health. The harmful effects of an excessive exposure to the sun consists of both acute and chronic reactions affecting primarily the skin and the eyes. That is why when we speak about sun exposure, we need to speak at the same time about photoprotection.

Photoprotection aims to reduce the deleterious effects of an excessive sun exposure and its critical for a correct exposure to the sunlight. But what does photoprotection mean in real world? The answer to this question is not trivial and depends on the public awareness on sun **exposure deleterious effects** and on the efficacy of public health campaign. Sunscreens for sure are the most known form of topic photoprotection and represent the first line in protecting the skin by absorbing or reflecting the UV radiation. The performance of these products is provided by sunscreen testing of both their effective in protecting the skin from UVB (Sun Protection Factor testing) and UVA (UVA Protection Factor testing) radiations according to standard methods that can vary based on the product marketplace and the regulatory status of the product in that marketplace. But photoprotection is not the only protection and is not only topic.

In recent years products fighting the deleterious effects of sun exposure and improving the response of the skin to the sun are available on the marketplace. This is how the oral photoprotection was born. Conversely to topic photoprotectors, oral photoprotectors do not directly protect the skin against the damage induced by UV radiation. These **oral photoprotective** products usually contain one or more ingredients that **activate(s) biological mechanisms of photoprotection, especially the ones related to antioxidants**.

Based on a consolidated experience in the topic photoprotection testing field, in the last years we have focused our attention in developing study protocols to evaluate the performance of oral photoprotective products. These study protocols include the assessment of both systemic and skin benefits of using oral photoprotectors. A standardized skin stripping procedure combined to biochemical assays was developed to assess the performance of products in decreasing the oxidative stress, in decreasing the **oxidative effects of UV on skin lipids**, and in replenishing the skin with antioxidants. **Bioengineering and image analysis techniques** were also implemented to specifically target the mechanism related to oral photoprotections as well as in vitro testing was developed to demonstrate the mechanism of actions.

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## SPF *in vitro* Testing Options

John Staton Scientific Director: Solar Eurofins - Cosmetics & Personal Care

In the European Union, there are recommendations that require that alternatives to use of human volunteers be pursued for the testing of sunscreens. These *in vitro* tests are also of particular use for the screening of products for R&D purposes. A number of methods have been in use from 1990's<sup>(1)</sup>, based on the use of thin films of products being applied to a rigid substrate and now measured with special purpose spectrophotometers such as a LabSphere® or Solar Light SPF 290®.

Although useful for formulation comparative purposes, the *in vitro* test method has suffered from not fully correlating with *in vivo* SPF. In order to advance this methodology, two *in vitro* SPF methods, ISO 23675<sup>(2)</sup> and ISO 23698<sup>(3)</sup> are currently under development as Work Items.

Both *in vitro* methods have been supported by publications and an outline of the methodology can be seen there<sup>(4,5)</sup>, even though the ISO drafting of the two *in-vitro* methods is not complete and formalised. Below is a comparison of the *in vitro* test methods available.

### Comparison of 3 *in vitro* SPF Test Methods:

Parameter of test method	Modified Diffey Method LabSphere SPF 290	ISO 23675 In Vitro SPF Test Method <sup>(2)</sup>	ISO 23698 DRS SPF Test Method <sup>(3)</sup>
Invasive Test	No	No	No
Application of Test Sample	By syringe or equivalent	By syringe or equivalent	By syringe or equivalent
Spreading of Sample	By finger	using robotics	by finger
Substrate for test	PMMA or Quartz Plates	PMMA Plates - 2 Types	Human back
Protection measured by	Calculation from UV Spectra	Calculation from UV Spectra	Calculation from UV Spectra
Products Tested to date	>1000	>148	>200
Types of Products Tested	Broad Range	w/o, o/w emulsions Mineral sunscreens	Broad range
Correlation with In vivo SPF	poor	R <sub>2</sub> = 0.88	R = 0.978
Measures SPF	yes	yes	Extrapolation
Water Resistance Test Capability	no	no	yes
Use of SPF number to perform ISO 24443 Broad Spectrum	no	no	yes

**As active participants** in international technical review committees, solar experts from Eurofins Cosmetics & Personal Care laboratories participate in validation cycles of various standards relating to solar tests (whether *in-vivo* or *in-vitro*) and facilitate their implementation in Eurofins sites. Consistent **inter- and intra-laboratory harmonisation** guarantees homogeneous test methods for all solar products and optimal quality across all our sites. This optimises the performance of multicenter solar studies.

Our regulatory expertise, coupled with our years of experience in toxicology, physico-chemical analyses, ecotoxicity, biodegradability, and OTC product support make Eurofins Cosmetics & Personal Care THE

solar center of excellence!

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1. Diffey B, Robson J. A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum. *J Soc Cosm Chem.* 1989;40(5):127-133.
2. ISO 23675 Cosmetics - Sun protection test methods - *In Vitro* Determination of Sun Protection Factor (SPF).
3. ISO 23698 Cosmetics - Sun protection test methods -Measurement of the Sunscreen Efficacy by Diffuse Reflectance Spectroscopy.4. Pissavini M, Tricaud C, Wiener G, Lauer A, Contier M, Kolbe L, Trullás Cabanas C, Boyer F, Nollent V, Meredith E, Dietrich E, Matts PJ. Validation of an *in vitro* sun protection factor (SPF) method in blinded ring-testing. *Int J Cosm Sci.* 2018;40(3):263-268.
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## High Resolution Proteomics for a Complete Understanding of the Product Efficacy

Gilbert SKORSKI - CEO

With the "omics" and bioinformatics analysis, PHYLOGENE offers a broad and unproven evaluation of the effects of cosmetics on the skin and / or its microbiota.

The UVs, among other stresses, induces a response from the exposed skin with a number of protective and recovery mechanisms. These mechanisms are diverse and may be differentially activated by a protection or an ingredient. Anyway protection products have their own specific signature which is very informative. Using high resolution proteomics with nanoLC-MS/MS, these mechanisms may be uncovered as proteomics tells us **which protein is there**, and **relative quantitation between a UV exposed sample** and a protected UV exposed sample gives access to activated mechanisms.

For example, we had the opportunity to study the UV effects on various models of skin cells and tissues by LC-MS/MS proteomics followed by CORAVALID™ analysis. It showed metabolic changes on different scales and degrees, in effectors and regulators.

We were able to unravel the mechanisms underlying changes in interactions in the extracellular matrix, by examining interactors and protein domains. We could **link part of the activities and variations to specific promoters, catalytic or biological functions** (inflammation, immune cells interactions, vesicular transport, DNA repair), metabolic (anti-oxidant mechanisms, vitamin biosynthesis, energetic components, ubiquitination) and signaling pathways (apoptosis, NFkB, Ras/Jun, PP2A, PKC, calcineurin, cell cycle and proliferation regulations), and even specific compartments involved in the matter at work. It was even

possible to correlate the changes with pathological mechanisms like atopic dermatitis, Cantú syndrome, or photosensitivity, and to accurately identify various changes in cellular equipment like ribosomal proteins, various structural proteins (collagens, actins, integrins, dynamins, desmosomes components, fibronectin, keratins, ankyrins), transporters (ATPases mitochondrial channels, RCPG or VOC subunits).

**The exhaustivity of the method** allowed interpreting the results to objectively explain the phenomena in progress depending on the parameters of the experimental context, discriminating the changes between apoptosis, cornification or senescence, with the advantage of taking post-translational modifications and signalling pathway interplay into account.

Results allow quick integration of these into current researches, multiple metabolic pathways displays allowing additionally to replace the intervention level in the whole picture of the assessed as pertinent mechanisms. Furthermore now, the whole answer of skin is not only at the human level but includes the **microbiome** which is very exposed and reactive. This may be integrated using our metaproteomics approach followed with HolXplore™ analysis.

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## Quantification and stability analysis of UV filters

Ségolène DE VAUGELADE - Fonctions dans l'entreprise Responsable R&D

UV filters are chemical compounds used in variety of sunscreen products, as creams, lotions ; but also in some anti-aging or day care products. These active compounds are used to prevent or minimise the harmful effects of UV radiation on the skin.

However, it has been demonstrated that some skin reactions due to photoallergic contact are caused by using many **commonly organic sunscreens** as 4-methylbenzylidene camphor, Drometrizole trisiloxane, benzophenone-3, or ethylhexyl dimethyl (PABA) [1] [2].

In this context, the maximum content of UV filter in products has been limited. **A list approved UV filters** and their allowed maximum concentration in cosmetic products has been established by the European Commission in Annex VI of the cosmetics Regulation 1223/2009 [3].

Different instrumental techniques have been used to analyze the UV filters:

- NMR spectroscopy,
- Raman spectroscopy [4],
- gas chromatography/mass spectrometry (GS/MS) [5],
- high-performance liquid chromatography/UV detector (HPLC/UV-Vis) [6], [7], [8].

EXPERTOX has been set up and validated a method for **separation and quantification of 16 UV filters**,

by liquid chromatography (LC/UV-Vis).

Moreover, UV filters can lead to **a partial or complete loss of their effectiveness** or even to a **possible transformation into a hazard substance**. These reactions can lead to a decrease in concentrations of UV filters and the formation of sometimes undesirable by-products.

EXPERTOX laboratory works on R&D projects for new protocols to anticipate physical and chemical degradation of raw materials based on photodegradation and new criteria (chemical and toxicological) to help industries and to ensure the safety of their products [9-10].

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## Skin responses to UV: a preliminary understanding of microbiome role

Marisa Meloni - Founder and CEO

The skin and its **microbiome have a strong symbiotic relationship** in a continuous crosstalk, which influences both counterparts' behavior. Within exogenous factor influencing skin conditions, UV light plays

a major role interacting with the physical and functional skin barrier unit through effects on both host cells and the skin microbiota. **UV rays can alter integrity, metabolic and immune-mediated responses of the skin** causing important modifications on skin appearance (hyper-pigmentation and photo-ageing) but also on skin health as inflammatory and immunological status.

Today, in the microbiome “era”, it is recognized that biologically relevant UVA and UVB rays interact not only with skin’s cells but also with bacteria and yeasts that inhabit skin and its appendages: it seems important to have a different approach to **UV-mediated responses** taking into account the contribution of microbiota and its evolutionary modifications. Considering the complexity of the individual components of skin microbiota and the biological diversity, a pre-clinical approach on 3D human skin models appears relevant to gain basic and mechanistic knowledge on this triple and new biological interaction.

Within this framework, in our laboratory we are currently investigating the effects of UV light on viable and **fully differentiated skin models colonized with *S. aureus*, *S. epidermidis* and *C. Acnes*** (either virulent and commensal strain) as relevant components of the microbiota community and their effects on the host by a multiple endpoint approach after UV-light induced damages. We are using two different skin models (RHE-epidermis including Type III melanocytes and full thickness models).

As expected, colonized tissues respond differently to UV exposure compared to not colonized and irradiated models: a delayed NFkB nuclear translocation, reduced oxidative stress, reduction of sunburn cells and melanin production, modulation of b-defensin 2 expression levels have been reported in presence of *S.epidermidis*. These results underline a **higher skin sensitivity** (stronger inflammatory response, deeper pigmentation and oxidative stress) to the UV in absence of associated microbiota thus confirming a fundamental but **almost unknown protective role of microbiome** towards UV exposure. Furthermore, the results derived after the topical application of reference probiotic strains, such as *L. rhamnosus*, on colonized tissue models have contributed to understand which mechanisms of skin innate response are activated by this type of actives to protect the host by UV exposure, demonstrating the applicability of the colonized 3D models for basic research and efficacy studies. So far we have been able:

- To identify a **role of bacteria** representative of microbiota community (the most abundant and supported by clinical findings) against UV light induced damages.
- To understand basic mechanisms by which they could play a **protective, rebalancing, detrimental role** on skin under UV.
- To develop a simplified but biologically **relevant pre-clinical model** for photo-biology investigations for R&D.
- To validate **in Vitro biological responses** by confirming available *in Vivo* literature data.

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## StratiCELL's solutions for *in vitro* efficacy testing of sun products

Michel SALMON - PhD MBA - Founder & CEO of StratiCELL

The sun light produces multiple types of radiations, which are ultra-violet (UVA, UVB and UVC), visible light and infra-red. Solar radiations penetrate the skin and their energy is absorbed by constitutive molecules, with consecutive damages like oxidation and inflammation. Evolution brought effective defences such as pigmentation to quench radiations, anti-oxidation and clearance processes. However, an excess of sun exposure, in conjunction with other environmental pressures, disrupts those self-defences, leading to uncontrolled reactive oxygen species (ROS) production, DNA damages, proinflammatory response and microbial dysbiosis.

Cellular pathways controlling responses to stress have been long investigated using *in vitro* assays on skin explants. Nowadays, *in vitro* skin models based on reconstructed 3D epidermis are also available, offering **more standardization** and less subjectivity related to the history and the characteristic of the donor. To highlight the efficacy of cosmetics to protect or repair light damages, both skin models can be challenged **with controlled radiations after topical application of ingredients**. Innovation relies on choosing the key skin biological markers of stress to be followed by functional and transcriptional assays.

UV radiations, as well as infra-red and blue light, are potent cause of **oxidative stress**, mainly resulting in an **overproduction of deleterious ROS**. *In vitro*, the amount of ROS is easily evaluated with fluorescent probes. Multiple solutions have been developed to reduce the amount of ROS, which can be addressed *in vitro* too. For instance, regulation of cytoprotective genes by key transcription factors like Nrf-2 activation or downstream heme-oxygenase (HO-1) expression, are relevant biomarkers to demonstrate a sun protection effect.

Other biomarkers can be studied *in vitro*, such as the typically modified DNA bases emerging after UVA and/or UVB radiations. Today, histologic immunolabelling targeting 8-oxo-2'-deoxyguanosine (8-oxoG) or cyclobutane pyrimidine dimers (CPD) allows *in vitro* evaluation of the anti-oxidative or UV-filter effect of sunscreen formulations. Moreover, uncontrolled amount of mismatching 8-oxoG or CPD directly activates the p53 pathway, that can be quantified by immunolabeling and image analysis through p53 phosphorylation. This tumour-repressor pathway stimulates the cellular DNA repair system and triggers cell apoptosis when eradication of potentially tumorigenic cells is required. Alternatively, the **activation of the apoptotic pathways** in UV-damaged cells can be addressed through the monitoring of the proteolytic activation of the executioner caspase-3, as well as the appearance of sunburn cells presenting a pyknotic nucleus and an eosinophilic cytoplasm.

Moreover, the inflammatory status is also linked to the skin microbiota, with direct and indirect UV-induced microbial dysbiosis having potential consequences on the development of pathogenic strains. Given recent advances on **adding microbiota components to 3D reconstructed epidermis**, this *in vitro* model is **becoming the model of choice** to confirm the protective effect of cosmetic ingredients on sunburned skin flora.

In conclusion, the sun, alone but also in combination with the rest of the exposome, can be deleterious for the skin, even though epidermal and dermal natural defences do exist. **Multiple and interconnected cell signalling pathways** are triggered to counteract the sun-driven deteriorations and maintain cellular homeostasis. Cosmetic ingredients play an important role in restoring those natural self-defences. This is why it is important to define and understand the mechanisms of action that can be modulated by dermo-cosmetic ingredients, at the cellular and molecular level. Such information is commonly provided by *in vitro* studies. As an expert on *in vitro* dermo-cosmetic assays, StratiCELL can support you to reveal the mechanism of action of your innovative ingredient.

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## **Claim « SPF and water resistance » of cosmetic products**

Céline Orechenkoff - Solar department Manager

The SPF (Sun Protection Factor) allows the measurement of the level of protection that the product offers in the UVB part of the spectrum. In addition to SPF, a product can also claim water resistance properties. Like the SPF determination, there are also several methods for assessing the water resistance of products.

Depending on the marketing area, the method differs.

2 main methods for testing the water resistance of a product containing sun protection:

- **Food and Drug Administration** 21 CFR Parts 201 and 310 (June 2011) Labelling and Effectiveness Testing; Sunscreen Drug Products for Over-the-Counter Human Use
- **GUIDELINES COLIPA 2005** associated with the standard NF EN ISO 24444: 2020 Cosmetics - Sun protection test methods - In vivo determination of the sun protection factor (SPF)

#### • Which method to choose?

The method to be applied is defined according to the marketing area.

Marketing area	GUIDELINES COLIPA 2005 associated with the standard NF EN ISO 24444: 2020	Food and Drug Administration 21 CFR Parts 201 and 310 (June 2011)
EUROPE	YES	NO
USA	NO	YES
ASEAN*	YES	NO
CHINE	NO	YES
CANADA	YES	YES
MERCOSUR**	YES	YES

\* Brunei, Cambodia, Indonesia, Laos, Malaysia, Burma, Philippines, Singapore, Thailand and Vietnam

\*\* Argentina, Brazil, Paraguay, Uruguay, Venezuela, Bolivia, Chile, Colombia, Ecuador, Peru, Guyana, Suriname

South Africa requires the water resistance assessment according to the SANS 1557: 2014 method and Australia according to the AS/NZ S2604: 2012 method.

#### The main technical characteristics of the 2 methods

Characteristics	GUIDELINES COLIPA 2005 associated with the standard NF EN ISO 24444: 2020	Food and Drug Administration 21 CFR Parts 201 and 310 (June 2011)
<b>Volunteers</b>	10 subjects minimum / 25 subjects maximum including 5 invalid cases maximum  Average ITA° between 41° and 55°	10 subjects minimum / 13 subjects maximum including 3 invalid cases maximum  Phototype I, II and III
<b>Finger stall</b>	Optional	Obligatory
<b>UV dose progression</b>	Identical progression for all exposed areas  SPF≥25: geometric progression 1.25 SPF <25: geometric progression 1.25, 1.15 or 1.12	Different progressions between the control zone and the product zones  Witness: progress 1.25 SPF <8: progression 1.25 8≤SPF≤15: progression 1.20 SPF > 15 : progression 1.15
<b>Standard products</b>	To be chosen according to the index of products tested SPFs24: P2 or P3 25≤SPF <50: P5 or P6 SPF≥50 : P8	P2
<b>Quantity of product applied</b>	2.0mg/cm <sup>2</sup> ± 2.5%	2mg/cm <sup>2</sup>
<b>Bath temperature</b>	Between 27°C and 31°C	Between 23°C and 32°C
<b>Statistics</b>	95% CI included in ± 17% of the average SPF Unilateral lower confidence limit 90% ≥ 50% Average SPF of the standard product in the expected range *	Average SPF of the standard product P2 in the range 16.3 ± 3.43
<b>Specific aspects</b>	Determination of the product index before immersion then after immersion in order to be able to determine the % water resistance.	Determination of the product index after immersion only. No calculation of % water resistance.

\*P2 : 16.1 ± 2.4 ; P3 : 15.7 ± 2.0 ; P5 : 30.6 ± 6.9 ; P6 : 43.0 ± 12.0 ; P8 : 63.1 ± 19.2

Internationally, ISO is currently working on a standard to assess the water resistance of solar products. On French territory, AFNOR is actively participating in the drafting of this **new standard** which could end up replacing the 2005 Colipa Guidelines. Intertek is integrated into this working group in order to bring, among other actors, its expertise on this topic. Intertek Clinical Studies, in Paris, confirmed expert for over 25 years in the field of clinical cosmetic studies and medical devices.

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## Evolution of *in vivo* solar standards

Anne Sirvent - R&D Manager Eurofins - Cosmetics & Personal Care

The efficacy of sunscreen products in protecting consumers from the undesirable effects of the sun on the skin (sunburn, photoaging, skin cancer) is well established.

In terms of evaluation, sunscreens are unlike other cosmetic products as they must conform to standardised and internationally recognised methods for efficacy testing. At the end of 2019, the ISO/TC 217 Cosmetics committee published a technical revision of the **ISO 24444** - *In vivo* determination of the sun protection factor (SPF).

The main changes are as follows:

- **Revision of the definition** of the minimal erythema response (MED) criteria;
- Modification of **lighting conditions** for visual determination of DEM;
- Choice of eligible test subjects now based solely on individual typology angle (ITA°);
- **ITA°** used to define the range of unprotected MED doses;
- Validation of **three new references standard sunscreens** for products with SPF equal to 25 or higher (P5, P6 and P8);
- Description of new test methods to determine the **uniformity of the beam** of solar simulators;
- Description of **sunscreen application procedures** in greater detail;
- Addition of an informative annexe, with photographic examples of erythema responses and guidelines for grading;
- Modification of the reporting tables and requirements to provide more complete information on results of testing;
- Bibliography update;

These modifications aim to reduce the remaining variability between test laboratories, in order to obtain similar FPS regardless of the laboratory that carried out the evaluation.

Revisions to standards **ISO 24442** - *In vivo* determination of UVA protection, **ISO 16217** - *Water immersion procedure for determining water resistance* as well as **ISO / FDIS 18861** - *Percentage of resistance to water* are also expected this year.

As active participants in international technical review committees, solar experts from Eurofins Cosmetics & Personal Care laboratories participate in validation cycles of various standards relating to solar tests (whether *in-vivo* or *in-vitro*) and facilitate their implementation in Eurofins sites. Consistent **inter- and intra-laboratory harmonisation** guarantees homogeneous test methods for all solar products and optimal quality across all our sites. This optimises the performance of multicenter solar studies.

Our regulatory expertise, coupled with our years of experience in toxicology, physico-chemical analyses, ecotoxicity, biodegradability, and OTC product support make Eurofins Cosmetics & Personal Care THE solar center of excellence!

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**Advance your solar cosmetics research  
customized state of the art *in vitro*, & *ex vivo* models for testing**

Marta Bertolini - Principal Scientist

Monasterium Laboratory Skin & Hair Research Solutions GmbH is one of the most innovative CROs in the field of hair and skin research. Based on our strong roots in academic excellence, we have the world-class expertise in hair & skin research, pre-clinical and clinical services, and innovative methodologies for dermatological, therapeutic, and cosmeceutical applications.

We offer state of the art validated *ex vivo* models (human hair follicle organ culture & human skin organ culture) for testing the effect of compounds and, complex formulations used in solar cosmetics on hair and skin physiology. We employ advanced **microdissection techniques** to isolate hair follicles from human scalp skin or follicular units and culture them (amputated/full-length hair follicles or entire follicular units) in a serum-free defined media. Our human hair follicle organ culture *ex vivo* models can be applied in various customized assays to answer specific questions related to sun care testing on human hair. Our well established in-house experimental readouts for hair follicle responses include hair cycle, pigmentation, cytotoxicity, aging, immunological phenotype, etc.

We also offer *ex vivo* cutting edge assays by applying culture of human skin samples in serum-free media. Our *ex vivo* models can be customized for the application of test agents either added directly onto the epidermis at the liquid -air interphase (topical application), or to the medium (systemic application) or injected intradermally. The main advantages of our ***ex vivo* human hair follicle & skin organ culture model** are cost-effectiveness, animal-free, and clinically relevant test systems that can be efficiently used for pre-clinical evaluation of various active agents, SPF testing and we can test the full chemical formulations used in the development of solar cosmeceuticals.

In addition, our *ex vivo* models are very well suited to examine the effect of environmental factors such as **ultraviolet radiation in skin and hair biology**. We have recently developed an **innovative *ex vivo* assay** (published research) to investigate the **impact on hair physiology when solar UV radiation** hits the human scalp skin surface. Our advanced models can be applied to test agents/compounds or to identify novel sun photo-protectants for hair and skin against UV radiation.

Last but not the least, we also have the expertise in applying our *in vitro* models (human cell lines, human primary cells, 2D/3D culture models) to investigate early-stage compounds/agents used for developing solar cosmetics for efficiency, toxicity and dose-ranging studies. Our vast worldwide network of scientific advisors, consultants, dermatologists from academia, and industry exemplify our strong expertise and knowledge in the field of skin and hair research. We promise to provide our clients with the highest quality research – from the basic research questions to industry-relevant translational research. Please do not hesitate to (<https://www.monasteriumlab.com/contact/>) if you have any queries, we are looking forward to

supporting you to advance your solar cosmetic research.

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## Great challenge of the sunscreen development

Juliana CRISTINA - Coordinator of the photobiology laboratory  
Guerra LUCAS - Research Director

In the complex universe of the development of sunscreens, there is a great challenge to formulate effective products due to many factors that influence on the SPF value. Small alterations on formulas may have great impacts on the product's SPF.

Currently, there are many international guidelines to standardize the methods of evaluation of photoprotection to have a reliable result there are several quality controls necessary that may assure a more precise evaluation. Even so, there is a considerable variability, especially among laboratories, that may get as high as 30%.

The objective of this article is to share the **state of the art of the main methods** that are being developed, led by the ISO group which has been working with the aim of turning this method less variable, with more liability among different laboratories and also to develop alternative methods that are less invasive and faster.

- **Static SPF (ISO 24444 / FDA 2011) – Gold Standards in vivo**

The study consists on the determination of the minimal erythemal dose (MED, minimum radiation dose necessary to produce the first visible erythemal reaction with clearly defined borders) on the unprotected skin (MED<sub>u</sub>) and the protected skin (MED<sub>p</sub>). The ratio between MED<sub>p</sub> / MED<sub>u</sub> represents the SPF value of the product. After the products' application and drying, the irradiation is performed with six progressive intensities sequential doses of energy, controlled with radiometers.

This method presents **some factors of imprecision**, for instance the biological response of the skin, which varies among subjects, the product application, which requires a precise technique, the evaluation of the erythema, which is visual and subjective and the equipment use, which may present great variability.

- **Single Exposure Method – in vivo**

This is a Pass/Fail method and it does not require the appearance of erythema, reducing, thus, the impact on the subjects. In this method, the subjects undergo one single ultraviolet radiation exposure with a dose

proportional to the product's expected SPF and one single endpoint is evaluated: the formation or not of erythema, which makes it possible to conclude if the product is effective or not for that SPF value. It is still an in vivo method, that stimulates erythema formation, even if minimal, and it is very imprecise.

- **Hybrid Method of Spectroscopy and Diffuse Reflectance (in vivo/in vitro)**

This method consists on the product application on the subjects and, after drying, a measurement of diffuse reflectance is performed, using radiation skin reflection measurement while emitting a minimal UV dose insufficient to cause erythema, which is the main advantage of this method. The ratio between the reflected and the incident radiation corresponds to the reflectance, from which it is possible to calculate the absorbance.

Only the UVA absorbance spectrum is obtained on this stage, since the skin absorbs most part of the UVB radiation. Thus, the method is complemented with an in vitro measurement, (application on PMMA plates). The complete absorbance spectrum (UVB/UVA) is obtained through a spectrophotometer.

The UVA absorbance spectrum (spectrophotometry) is compared to the one obtained by in vivo reflectance and a correction factor is generated to extrapolate the curve for the UVB. With complete absorbance curve (UVB+UVA) the SPF is calculated, based on the ISO 24443 calculations.

- **Transmission Method (in vitro)**

It consists of the use of plates, without the need of subjects, accelerating the study and reducing costs. However, the absence of the product application on the skin creates an extra challenge of the behavior interaction of the product with the plates.

The product is applied by a robot on two types of plates (sandblasted and molded) and the absorbance is measured using a spectrophotometer. The product is exposed to the ultraviolet radiation to also assess photostability.

The main challenge of this method is the viability of this high cost application robot, which is also difficult to calibrate, and, also, the development of substrates or calculations that reproduce better results on the skin.

- **Calculation of the SPF with the use of Softwares (in silico)**

The softwares developed for this purpose are based on the same principles of the transmission method, but instead of using the film absorbance measurement of the product applied over a substrate, they use the characteristic absorbance of the composition of filters used in the formulation.

In this method, the **photodegradation of the filters** is not taken into account, neither the formula characteristics, as for the quality of the emulsion and molecular excitation of the filters, which may create divergences between the SPF calculated and the one obtained through the in vivo method.

## **Conclusion**

For the publication of a new alternative method, systematic errors inherent of each proposed method is being evaluated. Also, intercomparisons studies are being performed considering several types of models to evaluate the repeatability, the reproducibility and the accuracy regarding the in vivo method.

The need for reduced variability in in vivo studies, as well as the validation of alternative methods that reduce harm to subjects while presenting greater accuracy and precision, are still under consideration and are the greatest challenge of the scientific community.

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We remain at your disposal for any specific information

Good reading !

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Ceo

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