How to ensure a reliable method deployment? An example with the In Vitro SPF Double Plate method

Pouradier, Florence[1*]; Renoux, Pascale[2]; Mangassi, Modibo[2]; Muller, Benoit[1];

[1] Evaluation Intelligence Department, L'Oréal R&I, Clichy, France; [2] Evaluation Intelligence Department, L'Oréal R&I, Chevilly-Larue, France;

* Florence Pouradier, 9 rue Pierre Dreyfus 92110 Clichy, France, +33149180480, florence.pouradier@rd.loreal.com

Abstract

While the in vivo measurement method of SPF remains the gold standard, industry and the photoprotection expert community continue their efforts to offer a reliable, robust and ethical alternative method. Developed and supported by Cosmetics Europe, the Double Plate method is currently being worked on at the Committee Draft stage by the ISO experts of TC217/WG7 and its ongoing statistical characterization within the ALT-SPF Consortium that could lead to publication as an ISO method in 2025. Previously, some methods published by ISO may have experienced interpretation problems during their implementation in test laboratories. Here we propose a process of appropriation of a new method, to ensure it will be reliably deployed in the industry, based on our experience on the in vitro SPF Double Plate method.

The study involved 3 internal laboratories and 7 voluntary external laboratories. The approach included support and validation in the following points: suitability of equipment, mastery of practical implementation, validation of the results obtained on a set of training formulae.

Each step of this process proved to be crucial for the success of the implementation and the appropriation of the method, highlighting certain pitfalls that could undermine the reliability of the results obtained. With the rigorous implementation of these steps, the Double Plate method for in vitro measurement of the SPF proved to be easy to familiarize with and perform.

The approach implemented constitutes a robust process to ensure a good integration of a new method to obtain reliable and sincere results.

Keywords: Method; Deployment process; SPF; in-vitro; Double Plate

Introduction.

In the cosmetics industry, photoprotection plays a special role. Protecting and maintaining the skin's health against damage caused by the sun's rays is far from being a purely aesthetic issue aimed at avoiding erythema, pigmentation or premature aging. Beyond and even before these aesthetic aspects, sun protection products are part of a public health policy issue aimed at preventing the harmful impact of solar radiation due to repeated or prolonged exposure or for melano-deficient populations, which are linked to the appearance of cutaneous melanomas or carcinomas [1]. The measurement of the level of UV protection provided by a sunscreen product must be robust and reliable, allowing the consumer to choose with confidence a product with adequate protection. The definition of standardized indices to characterize the level of protection against the erythemal effect of UVB, via the Sun Protected Factor (SPF), or against the pigmenting effect of UVA, via the UVA-Protected Factor (UVA-PF), as well as the formalization of international standards specifying the standardized methods of measurement of these indices responds to this public health challenge.

While the in vivo measurement method of SPF (Sun Protection Factor) remains the gold standard and has recently been revised (ISO 24444:2019(Amd1), FDA 2022 ongoing) [2;3], industry and the photoprotection expert community continue their efforts to offer a reliable, robust and ethical alternative method as mandate by the European Commission [4]. Years ago, similar efforts succeeded in developing and standardizing an alternative method to the in vivo measurement method of UVA-PF: considering transmission measurements through thin-layers of product samples on roughened PMMA-plates, then adjusting mathematically the absorption spectrum to the in-vivo SPF made it possible to predict the level of UVA protection with a good correlation with in vivo results [5;6]. This development resulted in the ISO24443 standard, published in 2012 and recently revised (ISO24443:2021).

Developed and supported by Cosmetics Europe, the in-vitro SPF Double Plate method is currently being worked on at the Committee Draft stage by the ISO experts of TC 217/WG7. This method is based on UVR transmittance spectroscopy, whereby spectroradiometric measurement of UVR transmission through appropriate UVR-transparent substrates, allows prediction of in vivo SPF values [7;8]. The test is based on the assessment of UV-

transmittance through a thin film of sunscreen spread on at least three moulded surface PMMA plates and on at least three sandblasted surface PMMA plates, before and after exposure to a controlled dose of radiation from a calibrated solar simulator. Recently, Cosmetics Europe called on the entire industry to start familiarizing themselves with this method [9], the ongoing statistical characterization of which within the ALT-SPF Consortium [10] could lead to publication as an ISO method in 2025.

Previously, some methods published by ISO may have experienced interpretation problems during their implementation in test laboratories, so how can you be sure that this new method will be reliably deployed in the industry? The work of standardization consists in framing sufficiently the formalization to ensure the good realization of the method, but ambiguities, misunderstandings or implementation gaps may remain. All the equipment suppliers offer support in getting started with their tools, but this support can be insufficient when a method requires several steps with different devices from different suppliers. The ISO24444 standard or FDA OTC sunscreen monography considered sunscreen reference samples which are good indicators for quality monitoring, but they may be not sufficient for method appropriation. The continuous work of standardization shows that the observation of deviations feeds future revisions, in a process of continuous improvement. It is important to make a distinction between the intrinsic variability of the method and the proficiency of laboratories to apply it reliably.

ISO/IEC17025, standard providing “General requirements for the competence of testing and calibration laboratories” [11], details a review of requirements relating to resources (installation, equipment), then relating to processes, simply requesting (clause 7.2.1.5) "The laboratory shall verify that it can correctly apply methods before implementing them by ensuring that it can achieve the required performance." In some domains, additional initiatives can be observed:

- In nondestructive testing and nuclear, the ENIQ published a qualification method [12] which provides for a technical justification of capability as well as practical tests on deliberately defective parts (controlled defects)
- In sensory evaluation, the process of training and qualification of panelists, and a panel, is widely described (in terms of sensitivity, reproducibility, accuracy, consensually) [13]

Post-deployment, it is possible and encourage to voluntarily participate in interlaboratory proficiency tests such as those organized by BIPEA, according to ISO/IEC 17043 [14]. But it requires a minimum of 12 laboratories so it may not be so adapted to the deployment of new methods which may be integrated in only a few laboratories at the start.
The purpose of this communication is to highlight the key steps in the process of appropriation of a new method, based on our collaborative experience on the In Vitro SPF Double Plate method.

Materials and Methods.

The study involved 3 internal laboratories (France (coordinator), US, Japan) as well as 7 voluntary external laboratories (France, Poland, Germany, Ireland). Each of the laboratories has previous experience in in vitro photoprotection measurement (ISO24443) and has expressed its willingness to be accompanied in the implementation of the in vitro SPF Double Plate method as well as its agreement on the course of each of the proposed steps. Our laboratory (internal – France) was coordinated and supported all the participants. Two other laboratories (external – France), which were in the core group of this method development for years, were identified for additional support. Each of these 3 “reference laboratories” followed nevertheless the global process.

The approach that was deployed included support and validation by one or more of the reference laboratories in each of the following points:

A- Suitability of equipment

This first step consisted in defining and sharing the requirements in terms of installation and equipment, considering the completeness of the necessary equipment. The list and technical specifications of the equipment available in each of the test laboratories was collected in a declarative way and used to verify the conformity of the resources to the specifications. Table 1 provides an extract of such requirements.

B- Mastery of practical implementation

The second step consisted in a video monitoring with 1-2 referent auditors who observed the practice of an operator in real conditions. After auditing the general installation and each of the required equipment, the auditors observed the practice supported by a monitoring guide containing together the key steps of the method. Those steps are 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.

C- Validation of the results obtained on a set of 10 training formulae

The third step consisted in an inter-laboratory testing on 10 sunscreen formulas. The 10 formulas were W/O emulsion, contained variable UV filters systems, and covered a large range of SPF (previously assessed in ISO24444 testing). One of the ISO24444 / FDA 2011 sunscreen reference formula (P2) was included in this training set. The 10 formulas were blinded, coded from P1 to P10 and simultaneously sent to the participating laboratories. Each laboratory measured the in vitro SPF of these samples by autonomously performing the in vitro SPF Double Plate method. The data were collected in a predefined template. The results were analyzed in Excel through general descriptive statistics and Pearson's correlation to a "reference laboratory". Such "reference laboratory" was defined considering the median results of the 3 reference laboratories. If we would have more reference values, it would have been better to consider the assigned value as estimated in the ISO13528 standard [15]. To not consider the in vivo SPF of the formulas as the assigned value was on purpose since the aim of this approach is to assess the proficiency of the laboratories to perform the method and not to characterize the method in its correlation to the in vivo, which was already investigated and published [7;8].

When necessary, the step B-C were repeated to understand and improve inconsistent results.

Results.

Each of the steps of this process proved to be crucial for the success of the implementation and the appropriation of the method. The approach has highlighted certain pitfalls or errors of interpretation which would be likely to undermine the reliability of the results obtained.

- **Suitability of equipment**

At this stage we realized that it is difficult for laboratories to have access to all the necessary equipment and their precise specification before the publication of a standard. The descriptions in publications are often succinct and omit details that are considered as

technical expertise, which may be lacking in new adopters. Table 1 summarizes the general observed adequacy of the equipment regarding specifications and highlights the critical points encountered.

This first step resulted in the formal withdrawal of 5 laboratories out of the initial 10, revealing this step as the most limiting.

- **Mastery of practical implementation**

In this second step, the 5 laboratories that have passed successfully the first step were included. Additionally, 4 laboratories which were withdrawn during the first step requested to be involved in similar parallel process to get feedback on their partial practice (often stopped before UV irradiation at the stage of calculating the initial in vitro SPF because missing adequate solar simulator was the critical points for each of them).

This stage allowed us to observe certain misunderstandings or misappropriations linked to the installation constraints. It appeared to be an important phase in the transmission of expertise, allowing the exchange of good practices and tips. Nevertheless, it was sometimes difficult at this stage to distinguish habits from requirements. Indeed, to quantify the impact of the different points of the procedure was done previously concerning the main ones (substrates, quantity, robotic application, spectrum and irradiation conditions... [16-19]) but not for each of them (wiping of the sandblasted plate before the product deposition, time spent by the plate at room temperature during the phases of deposition and spreading of the product, position and time spent by the plate in the UV transmittance spectrophotometer...).

Here appeared also the importance of considering a controlled and harmonized results processing file. Indeed, in the Double Plate method, the in vitro SPF is calculated as:

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

where
E(λ) = CIE erythema action spectrum;
I(λ) = Midday mid-summer global irradiance at 40°N;
dλ = Wavelength step (1 nm);
Final A (λ) = Mean monochromatic absorbance of the test product layer after UV exposure for each pair of plates calculated as:

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

where:
$A_{Moulded\text{-}post\text{-}irradiation}$ = absorbance of the moulded plate after UV exposure;
$A_{Sandblasted\text{-}post\text{-}irradiation}$ = absorbance of the sandblasted plate after UV exposure;
$C_{Moulded}$ and $C_{Sandblasted}$ = Correction factors defined according to product type (for emulsion $C_{Moulded}$ = 0.225 and $C_{Sandblasted}$ = 0.800).

Some softwares offer to calculate an in vitro SPF based on similar equation but considering the absorbance of each type of plate separately. An encountered error of interpretation was to consider the in vitro SPF Double Plate calculated as a weighted average of the independent in vitro SPF calculated for each of the plate, instead of applying the coefficient $C_{moulded}$ and $C_{sandblasted}$ on the absorbance as required.

No laboratory was withdrawn at this stage since this step allowed the observation but also the correction of wrong practices.

- **Validation of the results obtained on a set of 10 training formulas**

In this third step, the results were analyzed considering 2 sets of data:

- After irradiation for the 5 laboratories that have passed successfully the first and second steps
- Before irradiation for the 5 laboratories that have passed successfully the first and second steps, plus the 4 laboratories that have been withdrawn at step A because of equipment missing but were voluntary to continue the process in a partial procedure.

For both “after-” and “before- irradiation” datasets, we considered a “reference laboratory” which result for each product was calculated as the median of the 3 reference laboratories SPF values.

Unsurprisingly, the 3 reference laboratories (Lab1*, Lab2*, Lab3*) provided consistent results and presented a very high correlation between them and to the arbitrary created “reference laboratory” (R^2 between 0.98 and 0.99). The variability observed within these 3 laboratories allowed us to estimate for each product the uncertainty on the mean, which goes from 1.7 for a SPF mean at 10.1 (P2) to 11.6 for a SPF mean at 92.7 (P5). For the 2 other laboratories (Lab4, Lab5), a trend to underestimate the SPF value for the high levels of protection was observed (Figure1). We observed a good correlation coefficient (R^2=0.96 for these two laboratories) but a slop around 0.7 so we judged necessary to have action to improve the individual SPF results. For Lab4, we evidenced an equipment issue in the UV transmittance spectrophotometer. Once fixed, the underestimated values tend to get closer

from the expected ones. For Lab5, we supposed an impact of the fingercot, but we miss evidence at this time to confirm this hypothesis.

Analysis of the “before exposure dataset” showed consistent results for the 3 reference laboratories and the same tendency to underestimate the SPF value for high levels of protection for Lab4 and Lab5 (Figure2). These observations are consistent with what was observed on the “after exposure dataset” for the same 5 laboratories. Thus, we assumed that the observations “before exposure” are a good indicator of what would be the final results for the laboratories which were not in position to realize the full method. The Lab6, Lab7 and Lab8 showed good consistency with the “reference laboratory”, unless on one product (P6) which is underestimated, which could suggest an issue in sample dispatching as this same product was also underestimated by Lab4 and Lab5. For Lab9, a strong overestimation is observed for almost all the products, suggesting an issue in the product application (e.g. linked to the robotic arm pressure settings) or in the UV transmittance measurements (e.g. saturation of signal due to accumulation of product on the measurement probe) but this last hypothesis was tested and rejected.

Discussion.

This work made it possible to identify some critical points, the understanding and mastery of which were not as obvious as expected. Thus, it highlighted points of improvement in the formalization of the method. The timing of this approach was in good phase to integrate the exhaustive equipment aspects and detailed experimental protocol in the appendix of the Cosmetics Europe’s 26th recommendation [9]. Based on our experiment with the laboratories included in the present approach, we expect this appendix to be a key helper for the industry to familiarize with the method, in complement of the previous publications [7-8;20-21].

Identifying reference laboratories from which new adopters can seek support, advice and reference for their measured values has been a key point in the approach deployed. While such a choice may be unusual when a method is developed by a service provider, for the Double Plate method, the context is different as it is developed and supported by a group of members within Cosmetics Europe.

Discussion with new adopters sometimes led to challenges to minor points of practice. Future studies could aim to investigate the impact of these "details" of the procedure on the final value of the SPF (type of fingercot...).

To not have “true value” for in vitro SPF forced us to create this arbitrary “reference laboratory”, as there was no justification to consider one of the 3 historically trained laboratories (Lab1*, Lab2*, Lab3*) as measuring the true value versus the others. To go further with the statistical analysis, it will be interesting to consider the statistical testing suggested by the ISO5725 (“Accuracy (trueness and precision) of measurement methods and results”) and ISO13528 (“Statistical methods used in proficiency tests by interlaboratory comparison”) standards.

For the 4 laboratories which were withdrawn in the first step (because of equipment missing) but voluntary continue with the second and third steps, it should be noticed that obtaining good results “before exposure” on the training formulas set encouraged 2 laboratories to invest in the missing equipment. The approach will then be repeated to confirm the previous observations on the full procedure and final in vitro SPF Double Plate values.

Conclusion.

To obtain reliable measurement of the level of protection offered by a sunscreen is essential, as part of an efficient public health policy to help consumers to be adequately protected against the damaging impact of solar exposure. Efforts were deployed here to help voluntary laboratories to familiarize themselves with and appropriate the in vitro SPF Double Plate method, as it could be published as an ISO method in 2025. The key elements of the approach were: identification of referent laboratories, detailed resources checking, monitoring of the practice, validation of the results on a set of training samples. Such approach is based on generic steps which can be implemented for the deployment of any new instrumental method. It had the dual advantage of identifying possible ambiguities, thus indicating where it is relevant to strengthen the formalization of the procedure and ensuring easy and reliable implementation in laboratories new to the SPF in vitro Double Plate method.

Acknowledgments. The authors salute the 7 external laboratories without which this approach would not have existed: Beiersdorf AG, Coty Lancaster, Eurofins Dermscan

Poland, HelioScreen Cosmetic Science SAS, Naos les Laboratoires SAS, Public Analyst's Laboratory Cork, SP Equation, as well as the L'Oreal R&I hubs US and Japan. Formula training sets were provided by L'Oréal. Processing file was funded by Cosmetics Europe.

Conflict of Interest Statement. NONE.

References.

1. Iannacone, M. R., et al. (2014). "Effects of sunscreen on skin cancer and photoaging." Photodermatol Photoimmunol Photomed 30(2-3): 55-61.

2. ISO 24444:2019/AMD 1:2022 Cosmetics — Sun protection test methods — In vivo determination of the sun protection factor (SPF) — Amendment 1

3. U.S. Food and Drug Administration Over-the-Counter Monograph M020: Sunscreen Drug Products for Over-the-Counter Human Use

4. European Commission Recommendation 2006/647/EC. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX %3A32006H0647

5. Matts, P. J., et al. (2010). "The COLIPA in vitro UVA method: a standard and reproducible measure of sunscreen UVA protection." Int J Cosmet Sci 32(1): 35-46.

6. Moyal, D., et al. (2013). "The revised COLIPA in vitro UVA method." Int J Cosmet Sci 35(1): 35-40.

7. Pissavini, M., et al. (2018). "Validation of an in vitro sun protection factor (SPF) method in blinded ring-testing." Int J Cosmet Sci 40: 263–268.

8. Pissavini, M., et al. (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.

9. Cosmetics Europe Recommendation On the Use of Alternative Methods to ISO24444:2019. Available at: https://cosmeticseurope.eu/files/5216/4846/8648/No_26_Use_of_Alternative_Methods_to_ISO24444_-_2019.pdf

10. ALT-SPF Consortium position paper available at https://www.alt-spf.com

11. ISO/IEC 17025:2005 “General requirements for the competence of testing and calibration laboratories”

12. European Network for Inspection and Qualification, 2007, European Methodology for Qualification of Non-Destructive Testing - Third Issue - 3rd edition; ENIQ Report nr. 31 EUR 22906 EN.

13. Tabary, C., et al. (2021). "A tool to help the panel leader to best monitor a sensory panel performance." Journal of Sensory Studies 36(1): e12613.

14. ISO/IEC 17043:2010 Conformity assessment — General requirements for proficiency testing

15. ISO 13528:2015 Statistical methods for use in proficiency testing by interlaboratory comparison

16. 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).

17. 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).

18. Pissavini, M., S. Marguerie and O. Doucet (2016). "SPF tests reveal no ideal in vitro

substrate exists." Cosm & Toil.

19. 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.

20. Miksa, S., et al. (2015). "New approach for a reliable in vitro sun protection factor method Part I: Principle and mathematical

21. Miksa, S., et al. (2016). "New approach for a reliable in vitro sun protection factor method - Part II: Practical aspects and implementations." Int J Cosmet Sci 38(5): 504-511.

Tables and figures

	GENERAL ADEQUACY TO SPECIFICATIONS	SPECIFICATIONS ENCOUNTERED AS BEING LIMITING DURING THE METHOD DEPLOYMENT
SUBSTRACT	★★★	1. Moulded PMMA plates 2. Sandblasted PMMA plates
PLATES SURFACE TEMPERATURE CONTROLLER	★★☆	To store plates and product at 27 (± 2) °C in the dark
ANALYTICAL BALANCE	★★★	with at least 10^{-4} g precision
AUTOMATIC POSITIVE-DISPLACEMENT PIPETTE	★★☆	capable of delivering accurate and repeatable aliquots of approximately 1.6 mg to 1.8 mg of a sunscreen product
AUTOMATIC SPREADING ROBOT	★★☆	The robot spreading is defined in gesture and time to reproduce the in-vivo gesture. Vertical force (z axis), measured in the centre of the plate (with the finger tool and finger cot, without x and y axis movement), shall be of 6,0 ± 0,5 N
UV TRANSMITTANCE SPECTROPHOTOMETER	★☆☆	As described in ISO 24443. Precise positioning of the plate, which should remain positioned in a horizontal plane.
SOLAR SIMULATOR	★☆☆	A xenon arc solar simulator with appropriate filters. 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 with: - 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
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).

Table 1: General observed adequacy of the equipment regarding specifications and the most critical points encountered for the Double Plate method to be deployed in the participating laboratories. To be noticed that some important specifications which were meet in all the participant laboratories are not re-detailed here but are available in the Cosmetics Europe's 26th recommendation appendix.

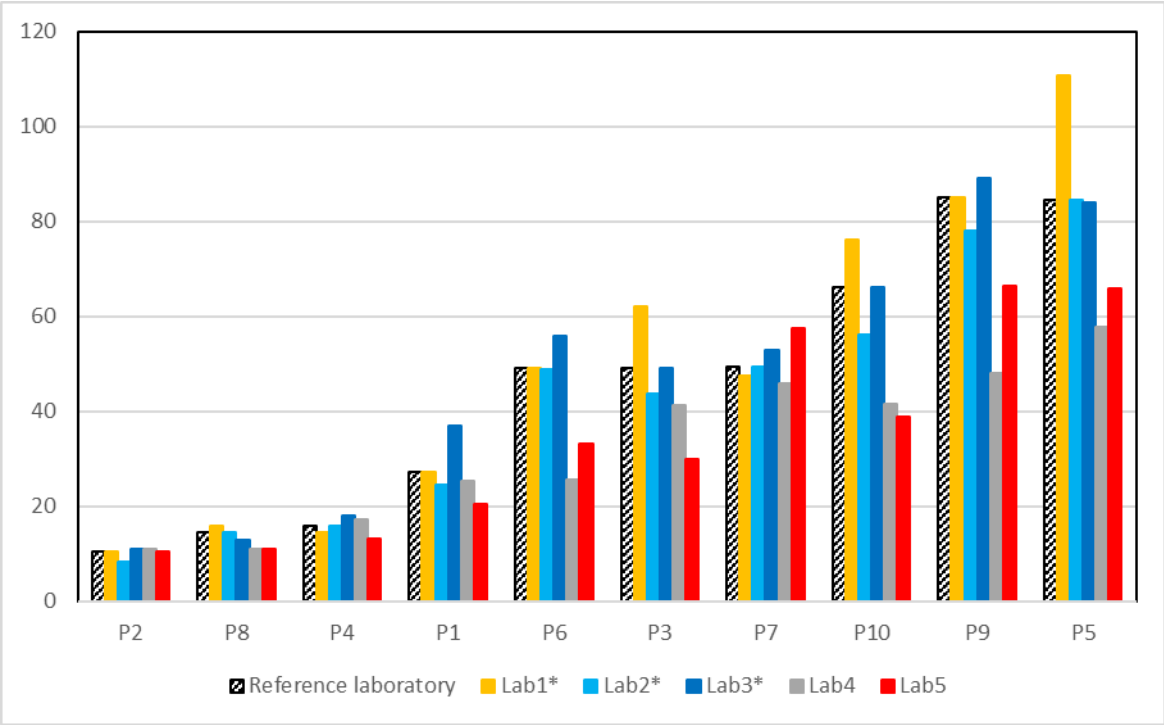

Figure 1 : Bar graph comparing the individual final in vitro SPF results for the 5 laboratories which pass the steps 1 and 2. The reference laboratory is created artificially by considering the median value from the 3 reference laboratories (Lab1*, Lab2* and Lab3*) which are mastering the method for long time, while Lab4 and Lab5 are new adopters. Such comparison allowed us to detect underestimation for Lab4 and Lab5, to identify the cause and to suggest corrective actions.

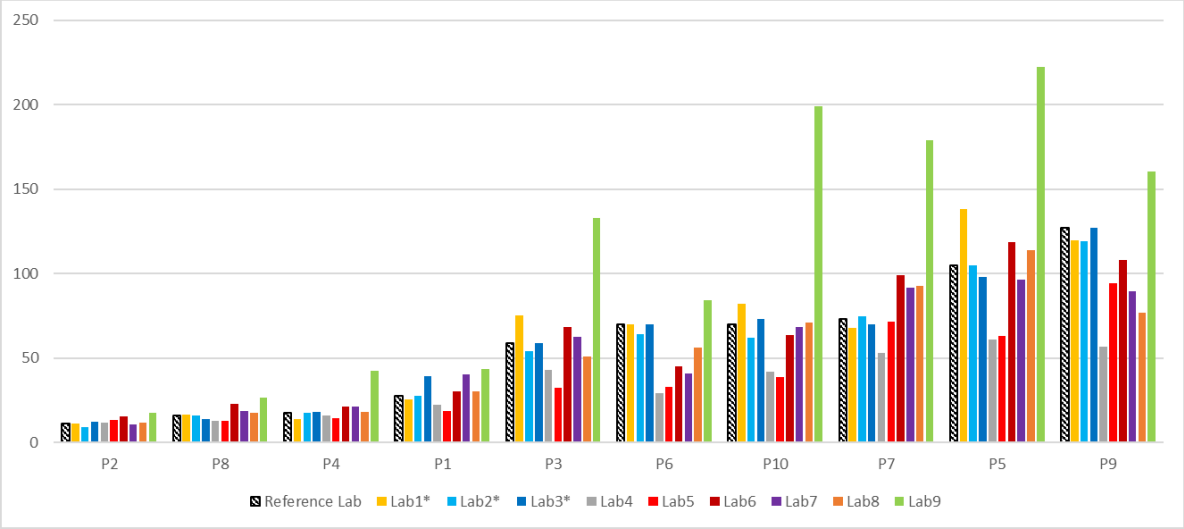

Figure 2: Bar graph comparing the individual initial in vitro SPF results (before exposure) for the 5 laboratories which pass the steps 1 and 2 (Lab1 to Lab5), plus the 4 laboratories which missed key equipment but were voluntary to participate on the “before exposure” stage only (Lab6 to Lab9).