Issues at the Margins: Validation of Sunscreen in-vitro UVA Test Measurement For FDA, COLIPA and ISO.

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FDA Test

Conducting the in-vitro UVA test for "Broad Spectrum" compliance with the FDA requirement may appear to be simple, but recent experimentation has shown that reproducibility requires attention to detail. Although FDA has moved substantially towards harmonization of this in-vitro method, the state of the art has moved in the interim to an updated version, based on improvements identified in the work of ISO. The in-vitro UVA test method proposed by FDA in the Final Monograph [1] is also light on detail, with potential consequence at the Pass/Fail margins of the test and inter-lab inconsistencies.

The FDA method is based on the COLIPA [2] procedure published in 2007. FDA stated "we have attempted to harmonize the parameters with existing standards, including those of the European Commission and COLIPA ". Subsequent to further validation work that has been conducted as a result of the Sunscreen Working Group of ISO Technical Committee, in which the COLIPA In vitro UV Protection Method Task Force has had heavy commitment, some of the advances in the test methodology proposed for ISO 24443 [3] were incorporated into the recent update to the COLIPA document version published in March 2011 [4]. When released in early 2012, the ISO document should reflect the latest state of the art of this test methodology.

Several Ring Studies conducted during the ISO development process have highlighted the importance of the control of test parameters when performing this test. In particular, to obtain consistency inter-lab, attention needs to be given to product application technique, substrate selection and exposure and test instrument calibration.

Test Method Outline

The ISO, COLIPA and FDA method are all very similar. (Fig 1.)

Fig. 1 Comparison of Test Parameters

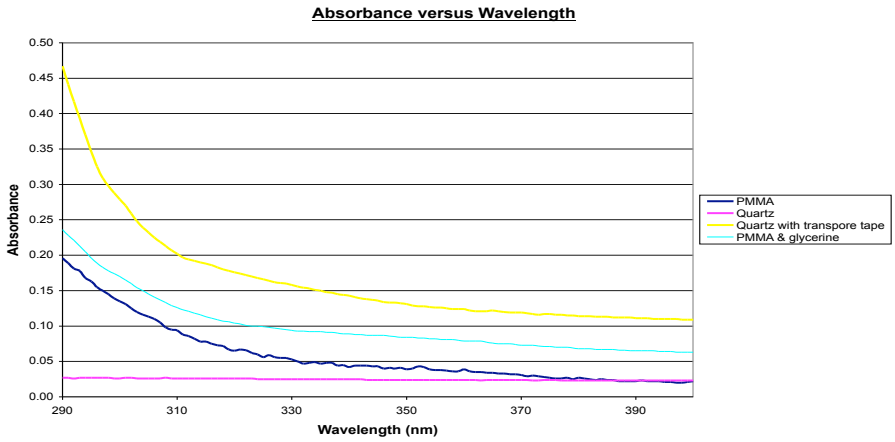
Test Parameter	ISO & Aust Prop March 2012	COLIPA March 2011	FDA FINAL June 2011
Plates	6 um PMMA plates	6 um PMMA plates	2 to 7 um PMMA plates
Plate Surface Characteristics	Moulded	Moulded	Etched or moulded
Application Rate	1.3 mg/sq cm	1.3 mg/sq cm	0.75 mg/sq cm
Drying Time	Temp of exposure min 15 min	min 15 min	15 min
Pre-irradiation Dose	Pre-irradiation 1.2 J x UVAPFo	Pre-irradiation 1.2 J x UVAPFo	Fixed Pre-irradiation 4 MED's
Pre-irradiation Spectrum	UVA Irradiance Spectrum	UVA Irradiance Spectrum	SPF Irradiance Spectrum

SPF used in exposure Calculation	in-vivo SPF	in-vivo SPF	in-vivo SPF
Ratio Calculation (UVAPFDx/label SPF	Ratio minimum 0.33	Ratio minimum 0.33	n/a
Critical Wavelength	TBD	min 370 nm	min 370 nm
Final Expression	Aust “Broad Spectrum” ISO n/a	E.U. interprets Pass/Fail	“Broad Spectrum”
Replicates	4 Measurements on 4 plates	3 Measurements on 4 plates	5 Measurements on 3 plates

The essential and common steps for all are…
1. Apply the sunscreen to a rigid transparent substrate.
2. Dry the film down onto the substrate
3. Measure the absorbance over the wavelength range of 290 to 400 nm.
4. Expose the sample to UV irradiation to imitate in use effect of sunlight.
5. Repeat the measurement post irradiation.
6. Compute the results to arrive at the required value. For ISO and COLIPA, this is UVA Protection Factor Post Irradiation/SPF - a ratio. For FDA and as an additional requirement in the E.U., this is the Critical Wavelength at 370 nm - a different ratio.

Substrate
Polymethylmethacrylate (PMMA) is the substrate of choice. This has been arrived at when cost, convenience and reproducibility have been taken into account. FDA came to a similar conclusion, after originally proposing Quartz. The PMMA plates available provide a maximum Abs value 0.2 and this is manageable without impacting too much on instrument sensitivity. The plates are required to be roughened so as to imitate skin roughness and to assist in "binding" of the applied thin film of test sample. FDA simplifies this requirement to a specification of mean roughness (Sa) of 2 to 7 um whilst COLIPA 2011 and ISO specify a quality of this with a mean roughness (Ra) equivalent to 6 um, but further detailed in terms of quality [5.]

FIG 2 – Relative Substrate Performance.



According to the studies conducted by the ISO GROUP, the combination of substrate roughness and sample application rate is important. A nominal roughness of 2 micron is more relevant for a sample application rate of 0.75 mg/ sq cm, whereas 6 micron was found to be most reproducible for organic sunscreens when 1.3 mg/sq cm is applied, but less for inorganics. Additionally, the more detailed specification given in the ISO 24443 document is as a result of the recognition by the working group that the surface profile is far more complex than only specifying an Sa value. Annex D - "PMMA Test Plate Surface Specifications" (of Ref 3) details a total of 6 parameters (Ra, Rv, Rdq, A1, SSc and Vw). These were honed down from an original list of 10 reviewed by the ISO committee experts!

Fig 3. Substrate Roughness for 0.75 mg/sq cm

Sample ID	SPF (Estimated)	PMMA plate Roughness	Application rate (mg/cm2)	UV Exposure	λc (pre)	λc (post)	E.U. UVAPFDx/SPF
ZnO Sunscreen Containing 25% dispersed ZnO	30	6μm	0.75	Std protocol	369.0	367.6	0.330
ZnO Sunscreen Containing 25% dispersed ZnO	30	2μm	0.75	Std protocol	368.8	367.0	0.304

Application Technique

According to ISO and COLIPA, this test parameter is the most critical for providing reproducibility. COLIPA has produced a short training video with the purpose of demonstrating a standardized technique found to provide consistency. This can be downloaded from their website [6]. Thorough practice is needed in order to achieve the same answer on the replicate films and to achieve consistent results between technicians within the same laboratory.

Film Thickness

Originally, FDA proposed as film thickness of 2 mg/sq cm in line with the in-vivo test amount. The final proposal is for 0.75 mg/sq cm. The impact of film thickness on ratio and critical wavelength can be seen in Fig 3 above.

Fig. 4 Measured parameters vis Film Thickness

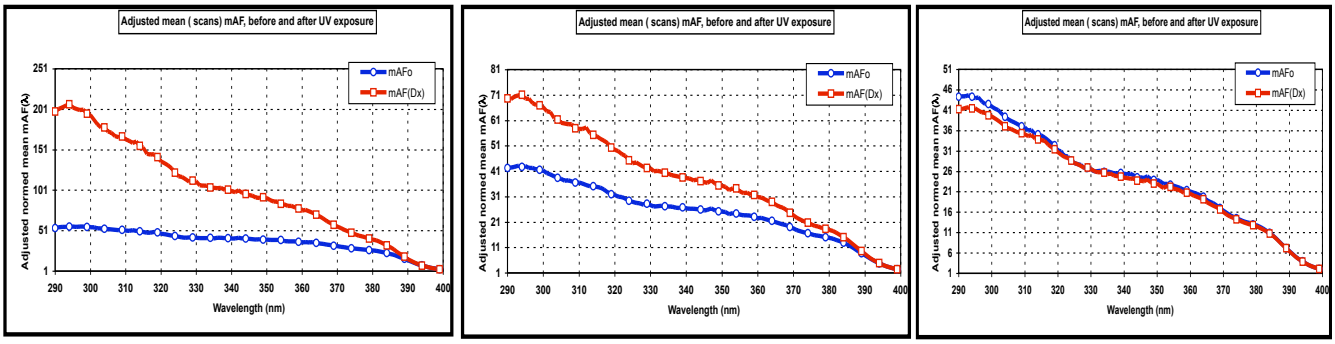
Film Thickness Sample ID	SPF (Estimated)	PMMA plate Roughness	Application rate (mg/cm2)	UV Exposure (J/sq.cm)	λc (pre)	λc (post)	E.U. UVAPFDx/SPF
1) ZnO Sunscreen Containing 25% dispersed ZnO	30	6μm	0.75	17	369.0	367.6	0.330
2) ZnO Sunscreen Containing 25% dispersed ZnO	30	6μm	1.3	17	369.9	368.3	0.337
3) ZnO Sunscreen Containing 25% dispersed ZnO	30	6μm	2.0	17	370.7	368.8	0.346

Drying Down Time

The ISO and COLIPA monographs require that the sample is held for a period of time in order to allow the film to dry down. This is in line with what is applied for in-vivo SPF testing and reflects what happens in use. COLIPA 2007 and 2011 require a minimum period of 15 minutes and the current ISO wording is " During the exposure the samples should be maintained at between 25°C and 35°C at the same temperature used for the drying period". Our experience is that some sunscreen products will continue to dry out for up to several hours after the film has been applied (Fig 5). In some instances, this is apparent as

a change in the spectral appearance pre and post irradiation – as an effect of light and heat during the UV exposure – and this can be misinterpreted as sample degradation. If the temperature of the plates during exposure exceeds the dry down temperature, further dry out will occur for those samples containing volatiles. This is the reason for the ISO approach.

Fig 5 – Example of Dry Down effect during irradiation.



Room Temp 15 min **35oC 15 min** **35oc 75 min**

Sample Orientation

Even when the COLIPA application method has been mastered, there will often be some inconsistency in dried down films according to how the substrate is oriented in the reading instrument. For this reason, it is good practice to rotate the sample for repetitive readings from the same plate, or to ensure that replicate pates are oriented in the same direction and position for repeat readings.

UV Light Exposure Device

This is the source of the irradiation required for the test and in effect, is a challenge for photostability. Critical parameters are the light intensity, the quality of the spectrum and the control limit on heat build up in the device. Proposed ISO 24443 has addressed this in detail. Set out below is a comparison of these requirements for ISO, very little of which is reflected in the FDA requirements (Fig 6).

Fig. 6 Comparison of Pre-irradiation Light Source Parameters.

Specification	ISO	FDA
Spectrum	290-400 nm	290-400 nm (Solar Sim)
Intensity	40 to 200 W/m2	Not specified
UVA Irradiance Ratio	UVA/UVB 8 - 22	Solar Sim
Run Temperature	20oC to 35oC	Not specified
Recalibrate	18mths or 3,000 hrs	(annual (solar sim)
NIST Traceable	yes	

Reference Sunscreen.

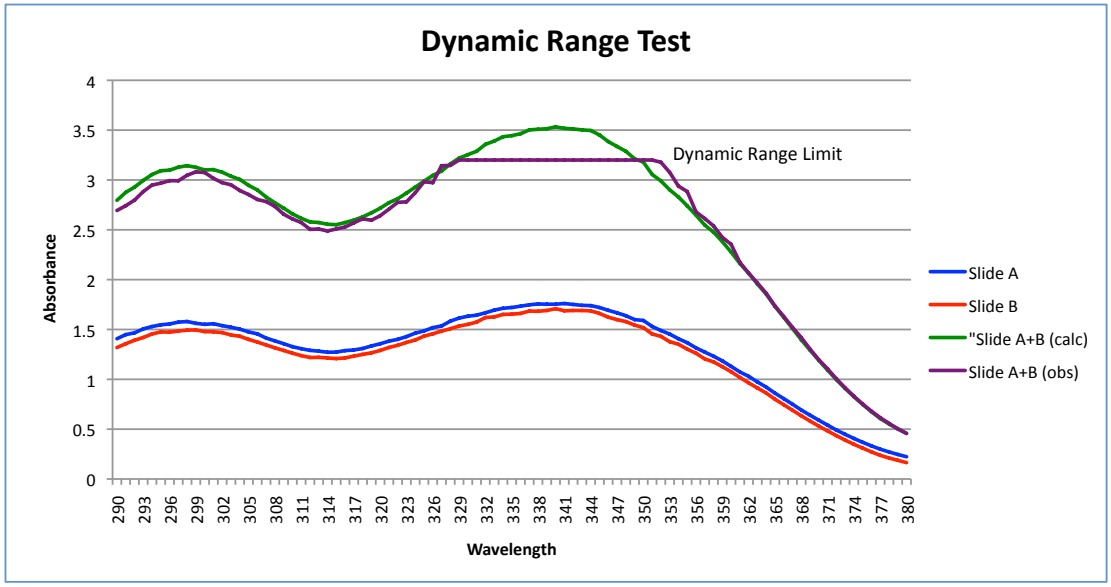
Both ISO 24443 and COLIPA 2011 include the requirement to utilize a reference standard sunscreen. This is a useful procedure, both for the revalidation of technique and as a training tool. The Reference sunscreen required is “S2” with a Mean and Range specified.

Spectrophotometer Calibration

Dynamic Range

The importance of this becomes evident when high SPF sunscreens are measured. At an application film thickness of 1.3 mg/sq cm., an SPF 30 sunscreen typically has an Absorbance max around Abs 1.5 and an SPF 60 around Abs 2.0 As both the UVAPF and critical wavelength end points essentially involve the measurement of areas under a curve, it is obvious that the areas need to be fully plottable on the spectrophotometer used for this measurement (see Fig 7). COLIPA/ISO approach this by requiring a dynamic range of not less than 2.2. FDA has specified that this “should be sufficient to measure transmittance accurately”. As FDA specifies a film thickness of 0.75 mg/sq cm, this range should be more than adequate for most sunscreens up to SPF 60.

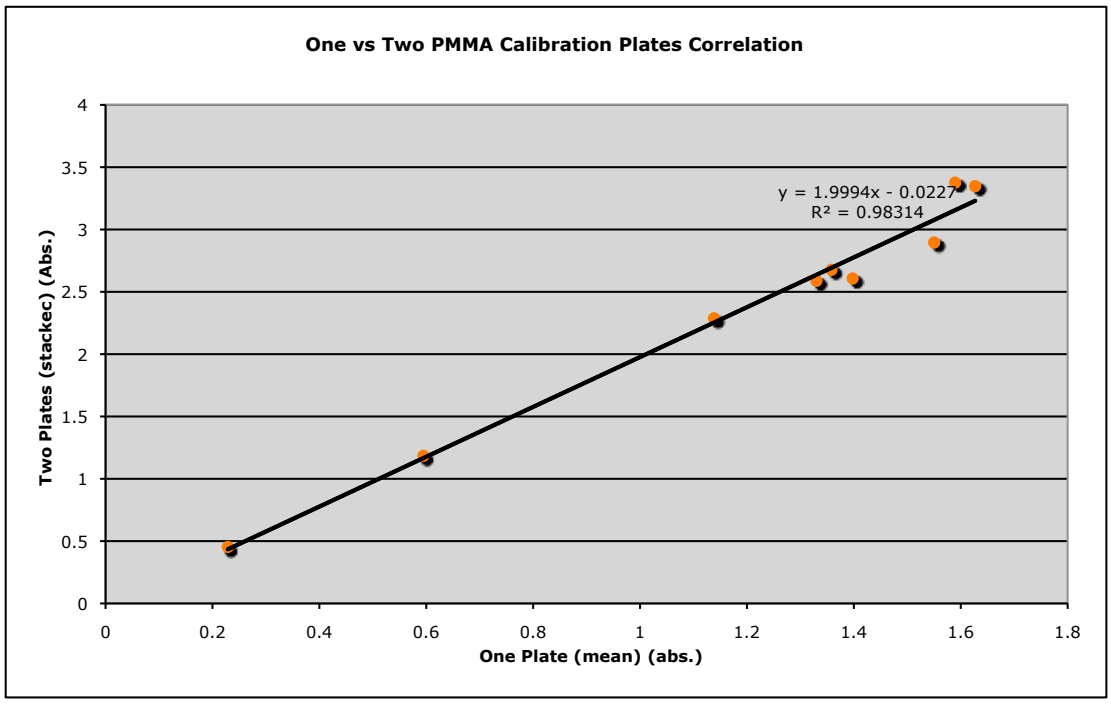
Fig 7. Showing the flat line effect when the instrumental limit is exceeded.



Linearity

This is a measurement of the ability of the spectrophotometer to produce the same sensitivity response over its dynamic range. COLIPA and ISO protocols address this by utilising 2 matching PMMA plates which have been impregnated with UV absorber. The shape of the spectral absorbance curve should be the same when two plates are stacked in the light path, vis one plate. The absorbance value of two at all wavelengths should be double the value obtained by one. A similar effect can be achieved by use of Neutral Density filters, utilizing the same principle of 2 vis 1 in order to determine linearity.

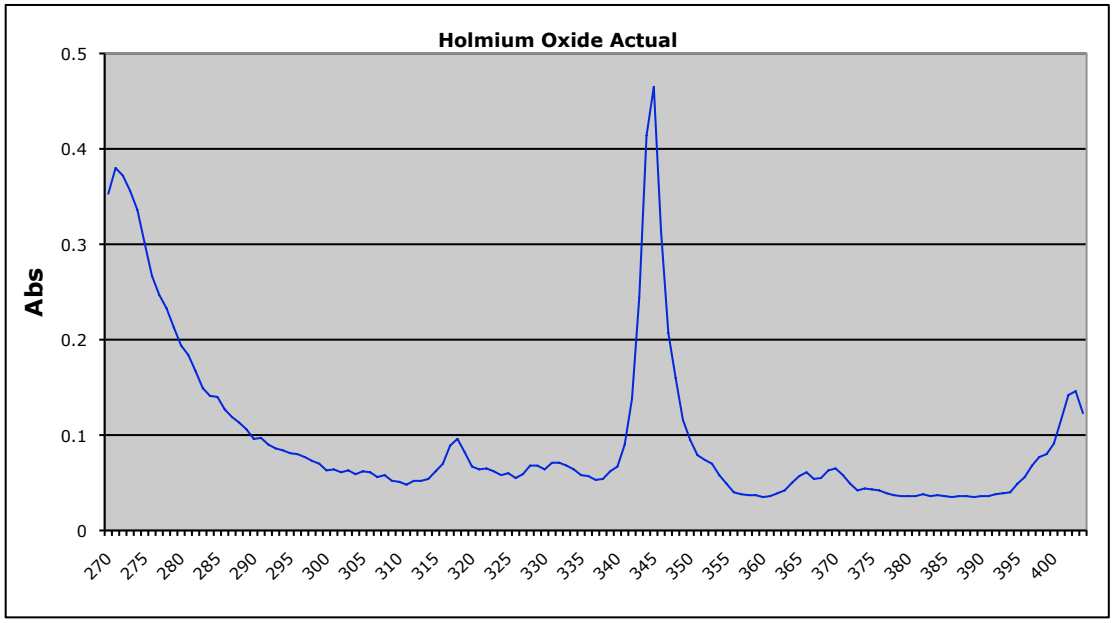
Fig 8. One vis Two Calibration Plate Correlation



Wavelength Calibration

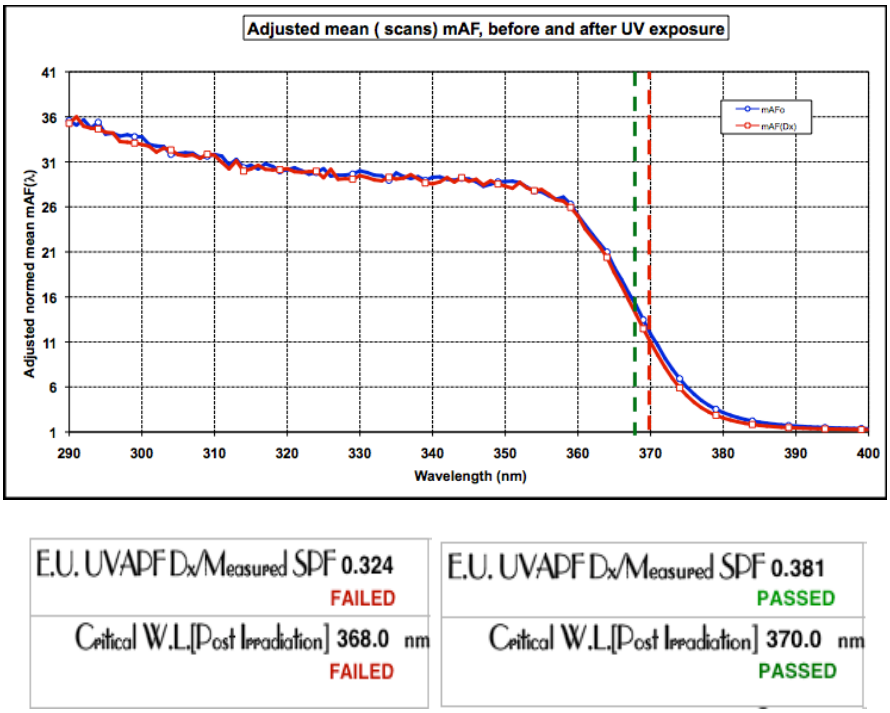
A reference spectra is utilized for this purpose. Holmium is most commonly used and COLIPA 2011 recommends Holmium perchlorate solution, whilst ISO and instrument supplier utilise a Holmium Oxide filter.

Fig 9. Holmium Spectrum



It is important that the measurement instrument be accurate to within one nanometer as any greater variation could mean that one lab reports a pass and another a failure.

Fig 10. Effect of 2 nm Wavelength Shift on Pass/Fail



The ISO standard provides a standard format spreadsheet in which the data can be computed and documented.

Recalibration Interval

Both the pre-irradiation device and the measurement instrument need to be re-calibrated at intervals. These are tabulated below.

Fig 11. Recalibration Interval Comparison.

	ISO	COLIPA (2011)	FDA
Pre-irradiation Device	18 mths or 3,000 hrs	annually	At least annually
Measurement Device	monthly	monthly	Not specified

Compilation of Results

How Method Sensitive are Sunscreens

Based in a sample of several hundred, one in ten sunscreen has a critical wavelength which falls between 368 and 372 nm. Subtle changes in test technique will mean the difference between pass and fail for these candidates. From the author's experience, Zinc Oxide sunscreens are the most likely examples of the effect of the pass/fail margin for both UVAPF and

Critical wavelength. This is because the absorption curve for ZnO sits very much on the limits. Additionally, variation in grade can change the shape of the curve and product thin film tends to be more thickness dependent than organic sunscreens. See Fig 5 for this effect.

Suitable Spectrophotometers ?

The measurement of the spectral performance requires the use of a spectrophotometer incorporating an integrating sphere device. Several instruments, which were originally designed for the measurement of in-vitro SPF are suited to the purpose as well as standard UV-Vis spectrophotometers fitted with the same device.

The instrument suppliers have recently updated software to provide the data in COLIPA/ISO compatible format. ISO 24443 does provide a pre-formatted spreadsheet with the intention that this be used for standardized management and harmonization of data.

At the time of preparation of this review, COLIPA 2011 and FDA compatible software was yet to be offered by the two main suppliers. No doubt, this will appear shortly.

1. Labeling and Effectiveness Testing; Sunscreen Drug Products for Over-the-Counter Human Use Federal Register / Vol. 76, No. 117 / Friday, June 17, 2011 / Rules and Regulations

2. The European Cosmetic Toiletry and Perfumery Association, COLIPA (2007): Method for the *in vitro* determination of UVA protection provided by sunscreen products.

3. *FDIS ISO 24443 :2011 (E)* Cosmetics — Sun protection test method - Determination of sunscreen UVA photoprotection in vitro

4. The European Cosmetic Toiletry and Perfumery Association, In Vitro Method for the Determination of the UVA Protection Factor and "Critical Wavelength" Values of Sunscreen Products *Guideline March 2011*

5. M. Pissavini, S. Marguerie, A. Dehais, L. Ferrero and L. Zastrow, Characterizing Roughness: A New Substrate to Measure SPF Cosmet. Toiletries, (2009) 9:56-62

6. http://www.colipa.eu/publications-colipa-the-european-cosmetic-cosmetics-association/guidelines.html?view=item&id=33&catid=46

Author Background

John Staton is the Australian Lead Delegate to the ISO Working Group on sunscreens (WG 7) and a collaborator on the calibration methods for the instrumentation and analytical methods for reference sunscreens. He is currently also the Convener of a new ISO project on a harmonized method for water resistance testing.

Craig Dennyson has been active in the development of the instrumental validation processes for ISO 24443 and ISO 24444, as well as the analytical method used for certification of the ISO reference sunscreens.