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# Skin colour, skin redness and melanin biometric measurements: comparison study between Antera® 3D, Mexameter® and Colorimeter®

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**Background:** The actual skin colorimeters analyse reflect values from a limited number of broad spectral bands and consequently present limited reproducibility and specificity when measuring skin colour. Here, Antera 3D®, a new device which uses reflectance mapping of seven different light wavelengths spanning the entire visible spectrum, has been compared with Mexameter® MX-18, an established narrow-band reflectance spectrophotometer and with Colorimeter® CL-400, an established tristimulus colorimetric instrument.

**Methods:** Thirty volunteers were exposed to a controlled ultraviolet B light. Measurements with Antera 3D®, Mexameter® MX-18 and Colorimeter® CL-400 were done before treatment and after 2, 7 and 14 days.

**Results:** Antera 3D® showed to have a better sensitivity and specificity than Mexameter® MX-18 regarding the melanin parameter. A similar sensitivity between Antera 3D® and Mexameter® MX-18 was found for erythema determination and also for the Commission Internationale de l'Eclairage *L**, *a** and *b** parameters between Antera 3D® and Colorimeter® CL-400. Good correlations were observed for all the parameters analysed. Repeatability of Mexameter® MX-18 and Colorimeter® CL-400 values were lower than that of Antera 3D® for all the parameters analysed.

**Conclusion:** Antera 3D®, such as Mexameter® MX-18 and Colorimeter® CL-400, are robust, sensitive and precise equipment for the skin colour analysis.

**Key words:** Antera – Mexameter – Colorimeter – melanin – skin colour – skin redness


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Erythema and pigmentation are the two most important responses to Ultraviolet (UV) irradiation. Because of its good reproducibility and simplicity, the use of UV-induced erythema and pigmentation is an excellent model of inflammation to characterize the skin pathophysiology and to assess the efficacy of various agents (1). The initial inflammatory (erythematous) response in the skin peaks within 2 days and the following increased formation of melanin pigment (melanogenesis) peaks at about 4–7 days and are both readily accessible for assessment by the naked eye or by bioengineering methods (2, 3).

The quantification of experimentally induced colour changes of the skin is a widely used method in dermato-cosmetic research since the colour response can be used as an indicator of skin properties (integrity of the skin barrier and sensitivity), drug properties (concentration, bioavailability), vehicle properties (formulations, enhancers) and skin protection properties (sun screens) (4).

The devices which measure skin colour can be based on scanning reflectance spectrophotometry, others rely on tristimulus colorimetry (such as Colorimeter® CL 400, Courage-Khazaka) and others rely on narrow-band simple reflectance meters based on the difference in absorption between melanin and haemoglobin at well-chosen wavelengths (such as Mexameter® MX 18, Courage-Khazaka) (4–6). The scanning reflectance spectrophotometers are very expensive, cumbersome and not portable enough for routine clinical work. These spectrophotometers are mainly used for fundamental laboratory research. The narrowband reflectance spectrophotometers compute only erythema and melanin indices and are mainly used in dermatological research. The colorimeters represents the colour of materials using the Commission Internationale de l'Eclairage (CIE) *L*a*b**

colour space: lightness is presented as $L^*$, the coordinate $+a^*$ expresses different shades of red and $-a^*$ those of green; $+b^*$ corresponds to yellow and $-b^*$ to blue (7, 8).

Quantification of skin colour evolution appears to be complex, as *in vivo* fluctuations in erythema can affect melanin values and vice versa. Knowing that melanin absorbs light in a large range of wavelengths (including green, red and near-infrared light), the confusion over the discrimination between melanin and erythema (i.e. redness of haemoglobin) can easily occur by colorimetric devices (5). Some commercially available probes used to evaluate erythema and pigmentation rely on measurements of the reflectance at two to four broad spectral bands corresponding to the output of the light-emitting diodes used for illumination. Due to spectral overlap of deoxyhaemoglobin (deoxy-Hb) and melanin, analysis of reflectance values from a limited number of broad spectral bands is often wrought with artefacts (8).

Recently it has been released the Antera 3D® (Miravex Limited, Ireland) which is a camera for image acquisition and analysis of the skin. The chromophores concentration is derived from the spatial and spectral analysis of the acquired image data, obtained by illuminating the skin with LEDs of different wavelengths shining from different directions. The acquired spectral data are used to map the distribution and concentration of melanin and haemoglobin. Unlike traditional imaging techniques, where only three colour channels (red, green, and blue) are used, the Antera 3D® uses reflectance mapping of seven different light wavelengths spanning the entire visible spectrum.

The main goal of this study was to evaluate Antera 3D® potential comparing different biometric properties of the human skin analysed with the equipment Mexameter® MX 18 (Courage-Khazaka, Germany) and the equipment Skin-Colorimeter® CL 400 (Courage-Khazaka, Germany), two of the most used devices in dermatological research. The properties assessed in this study were: melanin content of the skin, skin colour and skin redness. Sensitivity, specificity and reproducibility of the three devices were assessed regarding the referred parameters on human volunteers before and after targeted exposure to different UVB doses.

## Materials and Methods

*Instruments*

*Mexameter® MX 18 (Courage Khazaka, Germany)*

The measurement with this simple reflectance meter is based on absorption/reflection of the light from the skin. In this instrument, 16 light-emitting diodes (LED) arranged circularly emit light at 3 specific light wavelengths: 568, 660 and 870 nm, which respectively correspond to green, red and infrared light. A photo-detector measures the light reflected by the skin. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated. Mexameter provides the measurement of absorbed and reflected light at wavelengths in the green and the red for haemoglobin and wavelengths in the red and the near-infrared for melanin. A melanin index is computed from the intensity of the absorbed and the reflected light at, respectively, 660 and 880 nm. An erythema index is computed from the intensity of the absorbed and the reflected light at, respectively, 568 and 660 nm. The analyses are made in an area of 19.6 $mm^2$, i.e. a disc of 5 mm in diameter. The probe is applied on the skin surface with constant pressure using a spring instrument and is calibrated using a black and white calibration plate. A total of 5 discrete measurements were taken (9) (Fig. 1).

*Colorimeter® CL 400 (Courage Khazaka, Germany)*

This probe sends out white LED, arranged circularly to uniformly illuminate the skin in a range of emitted wavelengths of 440–670 nm. The emitted light is scattered in all directions, some parts travel through the layers and some

*Fig. 1. Mexameter® MX 18 device.*

is scattered out of the skin. The light reflected from the skin is measured in the probe and expressed accordingly. The raw data of the probe are corrected with a special colour matrix to adapt them closely to standard values. The light reflected is collected for a tristimulus colour analysis, using the $L^*a^*b^*$ colour system, as determined by the CIE. $L^*$ gives information about the black-white axis, the brightness and $a^*$ and $b^*$ are the coordinates in the colour space. $a^*$ transfers the values of the erythema to the red-green axis and $b^*$ shows the colour position on the blue–yellow-axis. The probe allows analysing an area of 19.6 $mm^2$, i.e. a disc of 5 mm in diameter. The probe is applied on the skin surface with constant pressure using a spring instrument and is calibrated using a black and white calibration plate (4, 10) (Fig. 2).

*Antera 3D® (Miravex Limited, Ireland)*

The Antera 3D® is a camera for image acquisition and analysis of the skin. The Antera 3D® relies on multi-directional illumination and computer-aided reconstruction of the skin surface, illuminating the surface from different angles and using the differences between these images to reconstruct the surface in three dimensions. The skin topography and the chromophores' concentration are derived from the spatial and spectral analysis of the acquired image data, obtained by illuminating the skin with LEDs of different wavelengths shining from different directions (see Fig. 3) (11).

The acquired spectral data are used to map the distribution and concentration of melanin and haemoglobin. Antera 3D® uses reflectance mapping of seven different light wavelengths spanning the entire visible spectrum. Theoretically this allows for a much more precise analysis of the skin colorimetric properties, which are mostly determined by two dominant chromophores: melanin and haemoglobin. Acquired spectral images are transformed into skin spectral reflectance maps, and the skin surface shape is used to compensate for light intensity variation due to the varying direction of incident illumination. The reflectance data are transformed into skin absorption coefficients and used to quantify melanin and haemoglobin concentrations using mathematical correlation with known spectral absorption data of these chromophores. This equipment measures an area of 3136 $mm^2$ (56 $\times$ 56 mm) (11).

*Measurements*

Thirty volunteers (3 males and 27 females) of Fitzpatrick skin type II and III were recruited with an age range of 19–50 years-old. Exclusion criteria were any sign of diseases or cutaneous alterations (spots, scars, tattoos, etc.) in the tested region that might interfere with the study; currently being under aesthetic or pharmacological treatments or cosmetic usage that might interfere with the study; had been made a prolonged and intense exposure to UV light (solar or artificial) 1 month before the beginning of the study; use of relevant phototoxic and

*Fig. 2. Colorimeter® CL 400 device.*

*Fig. 3. Antera 3D® device.*

photo allergic drugs; being under chronic daily administration of corticosteroids, immunosuppressors and non-steroidal anti-inflammatory agents topically, systemically or by inhalation in the month prior to study beginning; having history of unusual skin reactions to the sun (allergy, photosensitivity); having excessive hair in the tested area, pregnancy and breast-feeding.

All measurements were performed in the same room with no daylight under controlled ambient conditions (22°C ± 2°C of temperature and 50 ± 15% relative humidity). Each volunteer was acclimatized for 10 min prior testing. For each time-point and condition, measurements with Mexameter, Colorimeter and Antera were collected five times on each specific skin area of interest. In parallel, high resolution photographs of the tested area were taken in standardized lightening and volunteers positioning conditions with the equipment Visioface® (Courage Khazaka, Germany) for visual assessment before UVB exposure, after 2 days, 7 days and 14 days of UVB exposure.

In order to reduce the effects caused by desquamation (which interferes with the measurements) and so that the volunteers won't use any other topical product in the tested area, a hydrophilic emollient (Nivea® Body Lotion Moisturizing Express for dry skin) was provided and was applied twice a day on the irradiated skin during the study except in the measurement days.

Previously to the experiments, all volunteers received oral and written information about the objectives and protocols of the study, and signed an informed consent. The study was performed according to the principles of the Declaration of Helsinki of World Medical Association.

*UV exposure*

Four skin sites (A, B, C and D) – squares with a side of 3 cm (3 × 3 cm) – were drawn in a homogeneous skin colour site of the back of the volunteer. One of the sites was the control where no treatment with UVB light was performed, latter referred as treatment 1. On each of the other three sites the volunteers were exposed once to a controlled UVB broad band light (MEDlight-PSOR-Comb, MEDlight, Germany equipped with Philips® lamps) of three different intensities each: 89 mJ/cm$^2$, 129 mJ/cm$^2$ and 169 mJ/cm$^2$, respectively at a fixed distance between the UVB lamp and the skin. These three different intensities, 89 mJ/cm$^2$, 129 mJ/cm$^2$ and 169 mJ/cm$^2$ will be latter referred as treatment 2, 3 and 4, respectively. Measurements with Antera, Mexameter as well as Colorimeter were done before treatment (i.e. day 0) as well as after 2, 7 and 14 days.

*Calculations and statistics*

The median and interquartile range of the variation coefficient of 5 replicate measurements obtained with all time-points and in all skin sites was used to evaluate the equipment repeatability ($n$ = 480) for each analysed parameter.

Statistical analysis was performed to evaluate if the different skin sites were in equal conditions at time 0. Statistical analysis was also performed to evaluate if, at each time-point, there were statistically significant differences between the different doses tested so that the sensitivity of the equipment was evaluated for each parameter. This analysis was done using the differences between the values obtained at each time-point and the value obtained at time = 0 (Dif. t2-t0; Dif. t7-t0; Dif. t14-t0) for all parameters. Normality tests (Shapiro–Wilk test) were performed in order to assure normal distribution of the data obtained. Parametric tests, namely analyses of variances (ANOVA tests), were used whenever the normality of the results could be assumed, and Tukey test was used as post hoc comparison test.

To determine the correlations between the different parameters measured with Antera and with Mexameter and Colorimeter, the Pearson correlation factors and respective level of significance were calculated for each skin site, for time 0 and for the differences to time 0 obtained at time-points 2, 7 and 14 days. This analysis was also performed using the differences between the values obtained at each time-point and the value obtained at time = 0 (dift2-t0; dift7-t0; dift14-t0) for all parameters.

The level of significance was set at $\alpha = 0.05$ and the statistical analysis was performed with IBM SPSS 22.0 for Windows software (IBM SPSS Inc., Chicago, USA).

The following parameters were analysed (Table 1):

## Results and Discussion

*Visual perception*

According to visual perception observed by the researcher during the study and confirmed in the macrophotographies, skin erythema reached its maximum at day 2 (Fig. 4) following the UVB exposure on the volunteer's back, and then gradually decreased. Similarly to what occurred in the Baquié, M. and Kasraee, B. study (5), the intensity of the erythema and pigmentation (melanin) seemed to be proportional to the applied UVB dose. Visual assessment considered an increase in dark pigmentation (tan) up to day 7, which remained until day 14. Approximately half of the volunteers presented desquamation at time-point 14 days in the tested sites were the two highest UVB doses were applied.

*Sensitivity*

Previously, it was verified that there were no statistical significant differences between the time 0 values obtained in the 4 different skin sites tested (for all parameters measured with the three equipment), so the skin was in equal conditions in the beginning of the study.

*TABLE 1. Skin properties parameters to be analysed with Antera 3D, Mexameter and Colorimeter equipment*

| Skin property | Parameters analysed with Antera | Comparison equipment | Parameters analysed with the comparison equipment |
|---|---|---|---|
| Melanin content of the skin | Melanin average level | Mexameter® MX 18 (Courage Khazaka) | Melanin value |
| Skin colour | *L** parameter | Colorimeter® CL 400 (Courage Khazaka) | *L** parameter |
| | *a** parameter | | *a** parameter |
| | *b** parameter | | *b** parameter |
| Skin redness | Haemoglobin average level | Mexameter® MX 18 (Courage Khazaka) | Erythema value |
| | Haemoglobin average level | Colorimeter® CL 400 (Courage Khazaka) | *a** parameter |

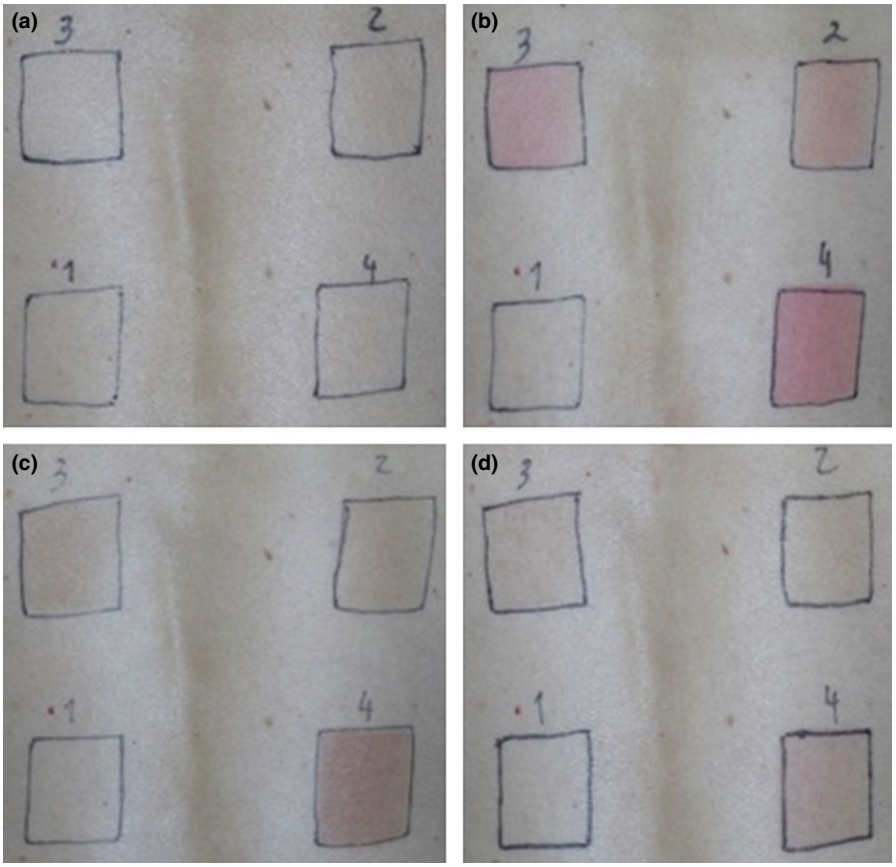


*Fig. 4. Photos obtained at all time-points.*

*Melanin: Mexameter vs. Antera*

In Table 2 it is presented the mean, variation coefficient and differences in the melanin parameter measured with Antera and Mexameter equipment for all the volunteers.

In the case of melanin quantification, Antera detected an increase in melanin values already at day 2 (Fig. 5) which was expected once the UV light stimulates the melanin production in the skin (3). This increase was proportional with the increased UVB applied doses. On the contrary, Mexameter showed a false but significant decrease in melanin values at the same time-point for all the UVB conditions showing that melanin values were significantly affected by the increased erythema that was verified at that time-point (Fig. 6). The incorrect diminution in pigmentation was inversely proportional to the degree of erythema. This result was not expected once, like already mentioned, the UV light stimulates the melanin production, so it should not decrease. Park et al. (1) also confirm that after UV irradiation the melanin index increase slowly until day 7, when maximum pigmentation is verified. Baquié and Kasraee (5) performed a similar comparison study between the equipment Mexameter and Dermacatch® (Scientis Pharma, Switzerland) and confirm our results as they concluded that Mexameter had falsely measured a significant decrease in

*TABLE 2. Mean, variation coefficient and differences obtained with the melanin parameter measured with Antera and Mexameter equipment*

| | Treatment | Mean T0 | VC T0 | Mean T2 | VC T2 | Mean T7 | VC T7 | Mean T14 | VC T14 | Dif. T2-T0 | Dif. T7-T0 | Dif.T14-T0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antera | 1 | 0.56 | 14.46% | 0.56 | 14.50% | 0.56 | 14.59% | 0.55 | 15.13% | 0.00 | 0.00 | −0.01 |
| | 2 | 0.56 | 14.31% | 0.60 | 12.78% | 0.59 | 13.02% | 0.58 | 13.44% | 0.03 | 0.02 | 0.02 |
| | 3 | 0.56 | 14.67% | 0.65 | 12.42% | 0.65 | 12.05% | 0.63 | 12.39% | 0.09 | 0.08 | 0.06 |
| | 4 | 0.57 | 14.15% | 0.69 | 11.67% | 0.69 | 11.59% | 0.64 | 12.41% | 0.12 | 0.12 | 0.07 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |
| Mexameter | 1 | 171.65 | 33.55% | 167.25 | 32.15% | 171.02 | 32.23% | 183.67 | 29.83% | −4.41 | −0.63 | 12.02 |
| | 2 | 169.79 | 33.35% | 162.93 | 37.13% | 184.52 | 30.34% | 197.67 | 28.73% | −6.86 | 14.73 | 27.88 |
| | 3 | 168.85 | 33.86% | 150.82 | 41.27% | 214.07 | 29.77% | 224.00 | 28.35% | −21.68 | 45.21 | 55.15 |
| | 4 | 174.53 | 33.23% | 130.44 | 53.01% | 239.71 | 28.83% | 224.59 | 25.94% | −44.09 | 65.19 | 50.06 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |

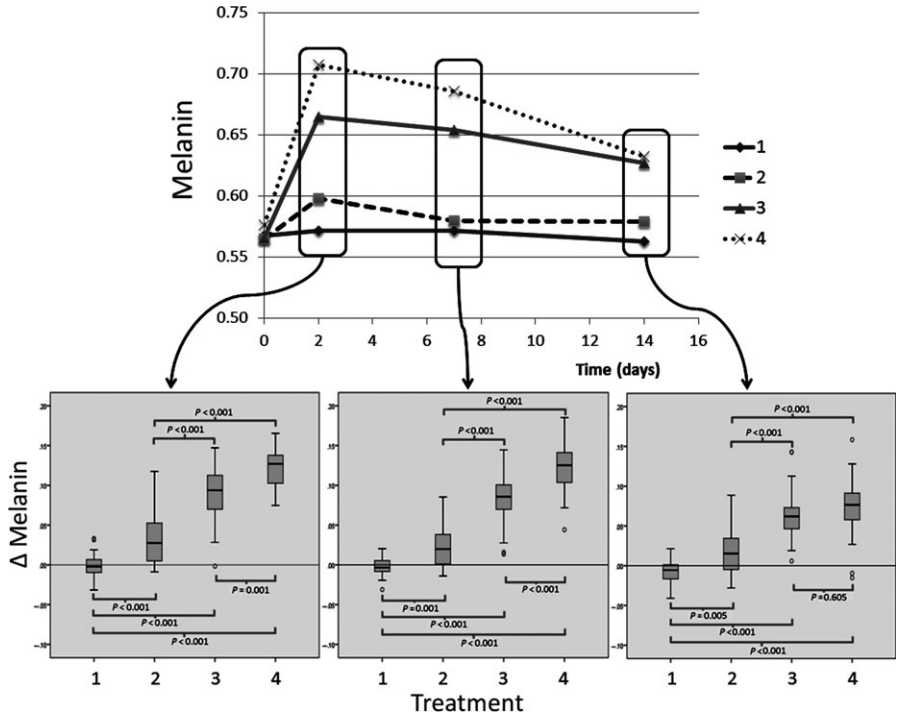


*Fig. 5. Median melanin values at each time-point for the different UVB applied doses obtained with the equipment Antera. Box-plots of the variation in melanin in relation to time = 0. The P-values were obtained from the post hoc comparison tests.*

pigmentation at day 2 which was inversely proportional to the increase in cutaneous redness. Thus, the melanin measurement with Mexameter appeared to be biased by the induced erythema. The reason associated with this phenomenon is due to a partial overlap in wavelengths, which leads to erroneous measurements of melanin which can be falsely biased by a fluctuation in skin erythema and vice versa (5, 8). Antera showed specificity for the melanin quantification once its melanin values were not affected by the increase in skin erythema.

Both Antera and Mexameter showed that at time-point = 7 days the melanin values were high and were proportional to the different UVB doses applied which was consistent with the visual perception. At this time-point the melanin production reached its maximum level. For Antera and Mexameter, at time-point = 14 days, the melanin values of the two highest UVB doses applied became more close, especially in the case of Mexameter. This is probably due to the fact that the two highest UVB doses applied originated desquamation between day 7 and day 14 and consequently the skin became lighter and with a more similar colour.

The post hoc comparison tests demonstrated that Antera presented a better sensitivity for the determination of melanin obtained through UVB light stimulation with different doses at the different time-points evaluated.

*Erythema: Mexameter vs. Antera*

The mean, variation coefficient and differences in the haemoglobin parameter measured with Antera and for erythema parameter measured with Mexameter equipment for all the volunteers can be seen in Table 3.

Interestingly, both devices showed the same pattern for erythema values which was proportional to the applied dose of UVB (Figs 7 and 8). The results of the haemoglobin parameter obtained with Antera at the time-point = 2 days showed a considerable increase in this parameter which was consistent with the visual perception of erythema at this time-point. This increase was proportional with the increased UVB applied doses. Like Antera, the erythema values obtained with the equipment Mexameter showed an increase in these values proportional to the UVB doses applied. At time-point = 7 days the haemoglobin value measured with Antera and the erythema measured with Mexameter decreased (in comparison to

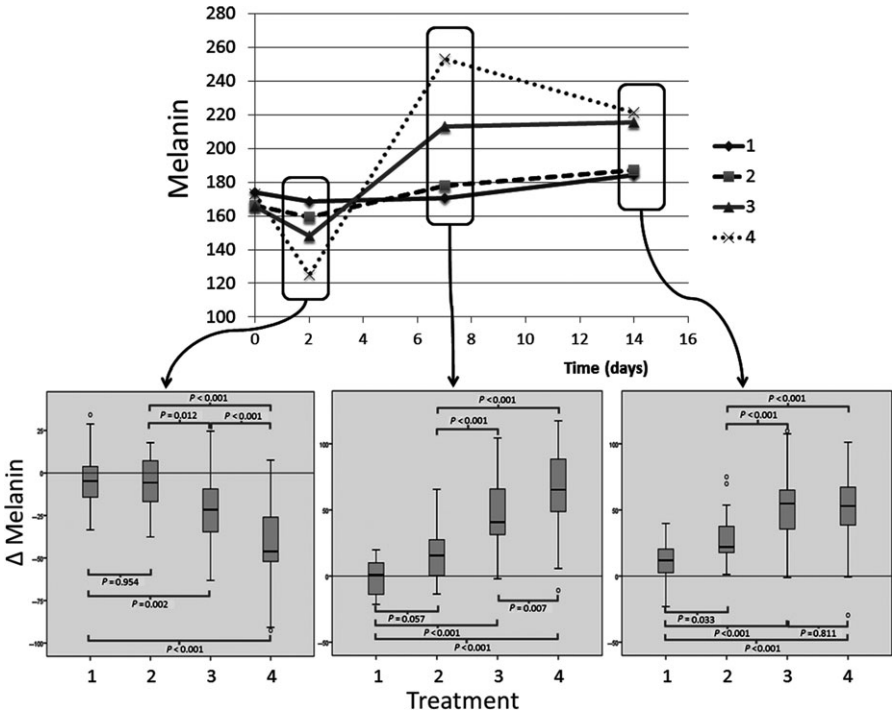


*Fig. 6. Median melanin values at each time-point for the different UVB applied doses obtained with the equipment Mexameter. Box-plots of the variation in melanin in relation to time = 0. The P-values were obtained from the post hoc comparison tests.*

day 2 which was the erythema peak) but still in a proportional relation to the different UVB doses applied. At time-point = 14 days, the haemoglobin and erythema behaviour was similar to day 7, but with lower values. At this time-point the skin has recovered from the inflammatory response provoked by the UVB light, so the erythema decreased. A similar behaviour was observed in Baquié, M. and Kasraee study (5), in which the erythema reached its peak at day 2 in a proportional relation to the UVB applied dose and then decreased over the time as skin recovered from the inflammatory process.

The post hoc comparison tests demonstrated that Antera presented a similar sensitivity for the detection of different grades of erythema provoked by the different UVB doses applied at the different time-points evaluated in comparison to Mexameter. Antera also demonstrated to have specificity to detect an erythema when the skin is highly pigmented.

*L** parameter: Antera vs. Colorimeter

In Table 4 it is presented the mean, variation coefficient and differences in the *L** parameter measured with Antera and with Colorimeter equipment for all the volunteers.

The results of the *L** parameter obtained with Antera at the time-point = 2 days showed a considerable decrease in this parameter at all UVB doses applied (Fig. 9). This decrease was

*TABLE 3. Mean, variation coefficient and differences obtained with the haemoglobin parameter (Antera) and with erythema parameter (Mexameter)*

| | Treatment | Mean T0 | VC T0 | Mean T2 | VC T2 | Mean T7 | VC T7 | Mean T14 | VC T14 | Dif. T2-T0 | Dif. T7-T0 | Dif. T14-T0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antera | 1 | 0.88 | 13.81% | 0.88 | 16.89% | 0.85 | 16.76% | 0.82 | 16.63% | 0.00 | −0.03 | −0.05 |
| | 2 | 0.89 | 16.32% | 1.03 | 18.59% | 0.89 | 16.35% | 0.86 | 16.61% | 0.14 | 0.00 | −0.03 |
| | 3 | 0.88 | 14.59% | 1.35 | 16.11% | 0.97 | 14.35% | 0.92 | 14.22% | 0.47 | 0.09 | 0.04 |
| | 4 | 0.88 | 13.51% | 1.63 | 15.69% | 1.10 | 14.13% | 0.97 | 13.94% | 0.75 | 0.22 | 0.08 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |
| Mexameter | 1 | 214.75 | 24.09% | 214.62 | 26.73% | 194.21 | 26.38% | 186.16 | 29.83% | −0.13 | −20.55 | −28.59 |
| | 2 | 220.52 | 25.60% | 279.33 | 23.95% | 214.43 | 24.69% | 205.77 | 26.83% | 58.82 | −6.09 | −14.75 |
| | 3 | 222.05 | 22.34% | 378.21 | 15.93% | 263.51 | 18.82% | 248.51 | 18.79% | 156.16 | 41.46 | 26.46 |
| | 4 | 221.84 | 25.29% | 434.82 | 13.46% | 309.87 | 14.58% | 274.27 | 16.17% | 212.98 | 88.03 | 52.43 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |

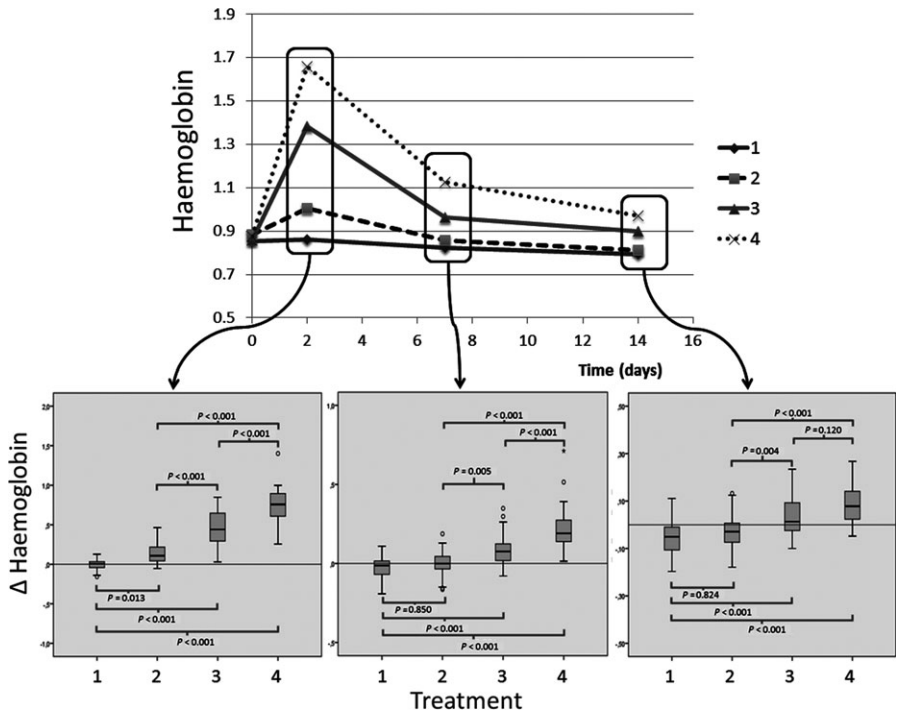


*Fig. 7. Median haemoglobin values at each time-point for the different UVB applied doses obtained with the equipment Antera. Box-plots of the variation in haemoglobin in relation to time = 0. The P-values were obtained from the post hoc comparison tests.*

inversely proportional with the increased UVB applied doses as expected. Like Antera, the *L** values obtained with the equipment Colorimeter showed a decrease in these values for all the UVB doses applied in an inverse relation (Fig. 10). *L** is the value of the lightness of an object, regardless of its lightness. *L** value is mainly influenced by the green light which is considerably absorbed not only by melanin but also by haemoglobin, and is likely to be interfered with by both pigmentation and erythema (1). The results published by Park et al. (12) are in accordance with our results in which the *L** value decreased significantly in erythematous skin. According to Shriver et al. (13) *L** is a good indicator of UV tanning: when *L** decreases indicates less lightness, less reflectance which happens when the skin is tanned. Both Antera and Colorimeter showed that at time-point = 7 days the *L** values remained lower than the basal level. However, between the time-point = 2 days and 14 days the *L** values increased in a proportional relation to the different UVB doses applied, meaning that the skin started to become lighter (closer to the initial values). Our observations were in accordance with the results obtained in the study of Park et al. (1) in which after one day of UV

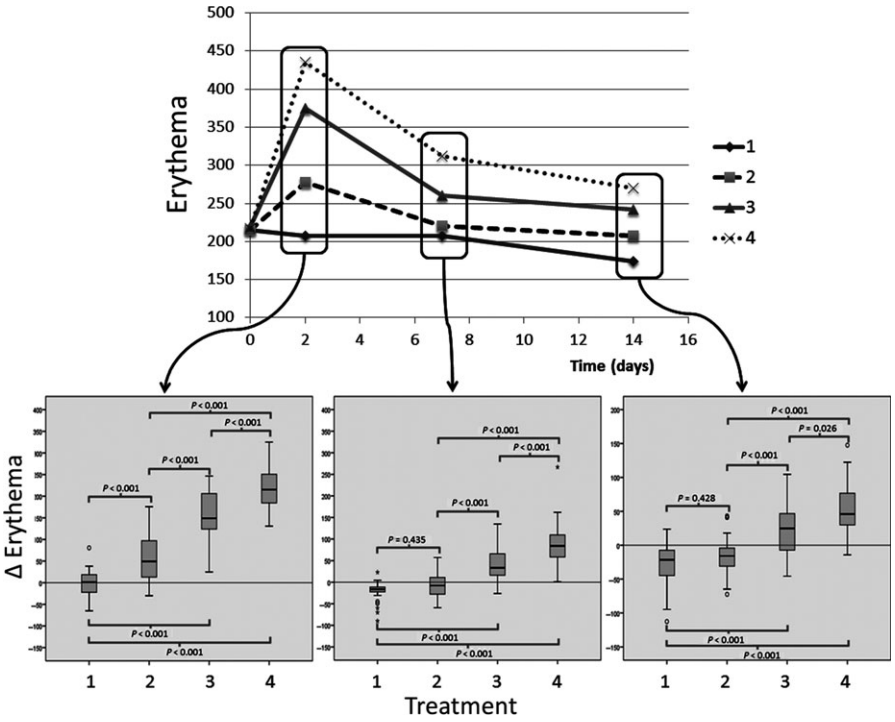


*Fig. 8. Median erythema values at each time-point for the different UVB applied doses obtained with the equipment Mexameter. Box-plots of the variation in erythema in relation to time = 0. The P-values were obtained from the post hoc comparison tests.*

*TABLE 4. Mean, variation coefficient and differences obtained with the* L* *parameter measured with Antera and Colorimeter equipment*

| | Treatment | Mean T0 | VC T0 | Mean T2 | VC T2 | Mean T7 | VC T7 | Mean T14 | VC T14 | Dif. T2-T0 | Dif. T7-T0 | Dif. T14-T0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antera | 1 | 69.37 | 5.26% | 69.13 | 5.56% | 69.69 | 5.88% | 69.90 | 5.84% | −0.24 | 0.32 | 0.54 |
| | 2 | 69.23 | 5.26% | 67.68 | 5.55% | 68.58 | 5.24% | 68.72 | 5.35% | −1.55 | −0.66 | −0.51 |
| | 3 | 69.37 | 5.08% | 65.09 | 5.60% | 65.87 | 5.39% | 66.48 | 5.59% | −4.28 | −3.50 | −2.89 |
| | 4 | 69.09 | 4.95% | 62.48 | 6.28% | 62.74 | 5.75% | 65.35 | 5.64% | −6.61 | −6.35 | −3.74 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |
| Colorimeter | 1 | 66.42 | 5.47% | 66.63 | 5.79% | 67.47 | 5.88% | 68.18 | 5.85% | 0.21 | 1.04 | 1.76 |
| | 2 | 66.46 | 5.30% | 64.40 | 5.77% | 66.13 | 5.50% | 66.95 | 5.30% | −2.07 | −0.34 | 0.49 |
| | 3 | 66.41 | 5.24% | 60.77 | 5.33% | 62.62 | 5.74% | 64.32 | 5.93% | −5.64 | −3.80 | −2.10 |
| | 4 | 66.29 | 5.63% | 58.91 | 5.90% | 59.38 | 5.72% | 63.32 | 5.42% | −7.39 | −6.91 | −2.97 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |

exposure the *L** parameter significantly decreased and then increased up to a week after UV exposure.

The post hoc comparison tests demonstrated that Antera presents specificity to determinate the *L** parameter and presented a similar sensitivity compared to the Colorimeter for the determination of *L** values when different UVB doses were applied in the different time-points evaluated.

*a** parameter: Antera vs. Colorimeter. The mean, variation coefficient and differences in the *a** parameter measured with Antera and Colorimeter equipment for all the volunteers are represented in Table 5.

The results of the *a** parameter obtained with Antera (Fig. 11) show a considerable increase in this parameter after 2 days of UVB exposure which is expected once the UVB light triggers an inflammatory reaction of the skin which causes redness (2). The *a** parameter value reached its peak at this time-point, as expected. This increase was proportional with the increased UVB applied doses. Like Antera, the *a** parameter values obtained with the equipment Colorimeter showed an increase in these values for all the UVB doses applied in a proportional relation for time-point = 2 days (Fig. 12). According to Park et al. (2002) (1) the index of *a** increased significantly on days 1 and 2 after UV exposure in the direction of the mean colour of haemoglobin. Both Antera and Colorimeter showed that between the time-point = 2 days and time-point = 7 days the *a** values decreased but remained higher than the basal level and were still proportional to the different UVB doses applied. These results were expected as the skin becomes darker and the redness disappears, however, the erythematous reactions had not completely ceased. For Antera and Colorimeter, at time-point = 14 days, the *a** parameter values decreased as expected once the inflammatory reaction of the skin becomes resolved.

The post hoc comparison tests demonstrated that Antera presents a good sensitivity (similar to Colorimeter) for the determination of the *a** parameter obtained through UVB light stimulation with different doses at the different time-points evaluated. Additionally, the ability to distinguish and measure the *a** parameter value of skin when the skin is highly pigmented showed that Antera presents specificity when measuring the *a** parameter of the skin.

*b** parameter: Antera vs. Colorimeter. The mean, variation coefficient and differences in

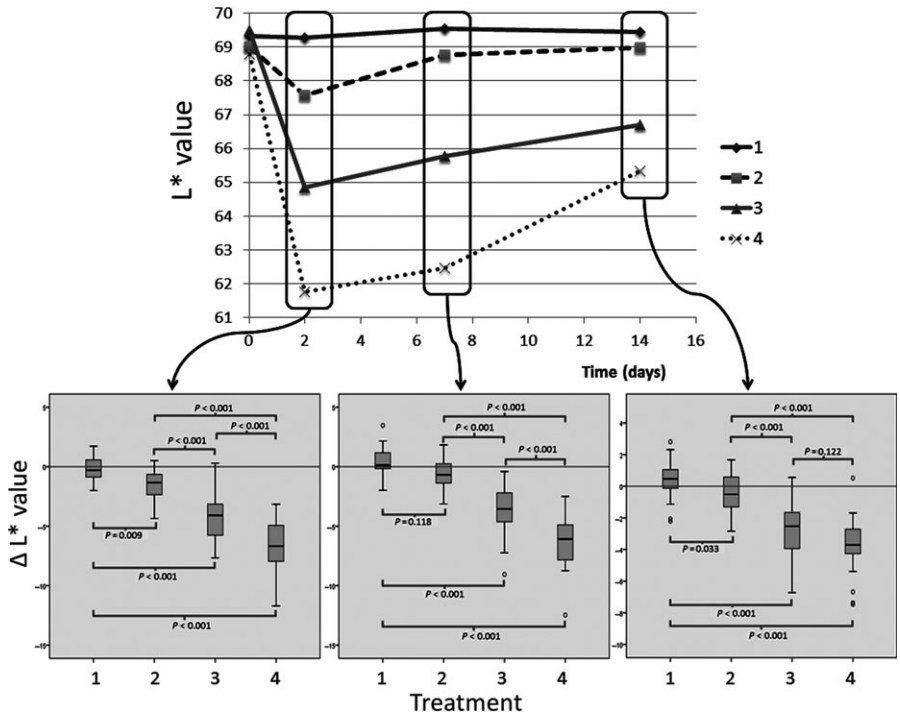


*Fig. 9. Median L* values at each time-point for the different UVB applied doses obtained with the equipment Antera. Box-plots of the variation in L* values in relation to time = 0. The P-values were obtained from the post hoc comparison tests.*

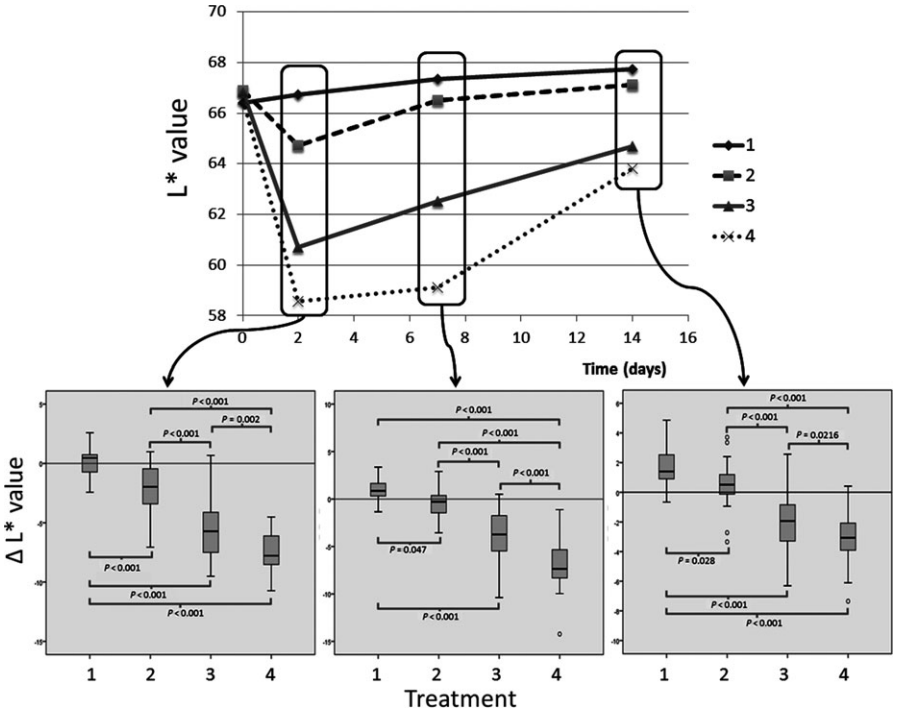


*Fig. 10. Median L* values at each time-point for the different UVB applied doses obtained with the equipment Colorimeter. Box-plots of the variation in L* values in relation to time = 0. The P-values were obtained from the post hoc comparison tests.*

*TABLE 5. Mean, variation coefficient and differences obtained with the* a* *parameter measured with Antera and Colorimeter equipment*

| | Treatment | Mean T0 | VC T0 | Mean T2 | VC T2 | Mean T7 | VC T7 | Mean T14 | VC T14 | Dif. T2–T0 | Dif. T7–T0 | Dif. T14–T0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antera | 1 | 12.30 | 12.24% | 12.28 | 15.40% | 12.01 | 14.66% | 11.67 | 14.77% | −0.02 | −0.29 | −0.63 |
| | 2 | 12.52 | 14.31% | 14.32 | 16.72% | 12.61 | 14.24% | 12.18 | 14.20% | 1.80 | 0.09 | −0.34 |
| | 3 | 12.39 | 12.86% | 18.19 | 13.61% | 13.69 | 11.93% | 12.96 | 12.20% | 5.80 | 1.29 | 0.57 |
| | 4 | 12.38 | 11.92% | 21.26 | 12.78% | 15.03 | 10.95% | 13.48 | 11.55% | 8.88 | 2.64 | 1.09 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |
| Colorimeter | 1 | 10.68 | 13.74% | 10.16 | 15.74% | 9.96 | 13.73% | 9.38 | 14.17% | −0.52 | −0.72 | −1.30 |
| | 2 | 10.74 | 13.20% | 12.76 | 18.76% | 10.44 | 12.88% | 9.85 | 12.64% | 2.02 | −0.30 | −0.88 |
| | 3 | 10.81 | 13.26% | 16.92 | 13.48% | 11.83 | 12.54% | 10.78 | 10.25% | 6.11 | 1.02 | −0.03 |
| | 4 | 10.68 | 14.60% | 18.77 | 9.90% | 12.96 | 10.77% | 11.19 | 9.05% | 8.09 | 2.28 | 0.52 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |

the *b** parameter measured with Antera and Colorimeter equipment can be seen in Table 6.

The results of the *b** parameter obtained with Antera showed an increase in this parameter after 2 days of UVB exposure (Fig. 13). This increase was not proportional with the increased UVB applied doses. Unlike Antera, the *b** parameter values obtained with the equipment Colorimeter show a decrease in these values for the highest UVB doses applied and an increase for the two lowest UVB doses applied at time-point = 2 days (Fig. 14). According to Park et al. (2002) (1) after UV exposure the *b** parameter decreases significantly (detection of bluish shade of UV-induced melanin oxidation) and then starts to increase up to the first week after UV exposure (yellow component of newly generated melanin). Antera results at time-point = 2 days were not in accordance with the observations of this study. Antera results show that at time-point = 7 days the *b** parameter values increased for the two highest UVB doses applied and slightly increased for the lowest dose and control in a non-proportional relation. At this time-point the *b** parameter measured with Colorimeter increased for all the UVB doses applied as expected. For Antera and Colorimeter at time-point = 14 days, the *b** parameter values remained stabilized for the lowest UVB dose

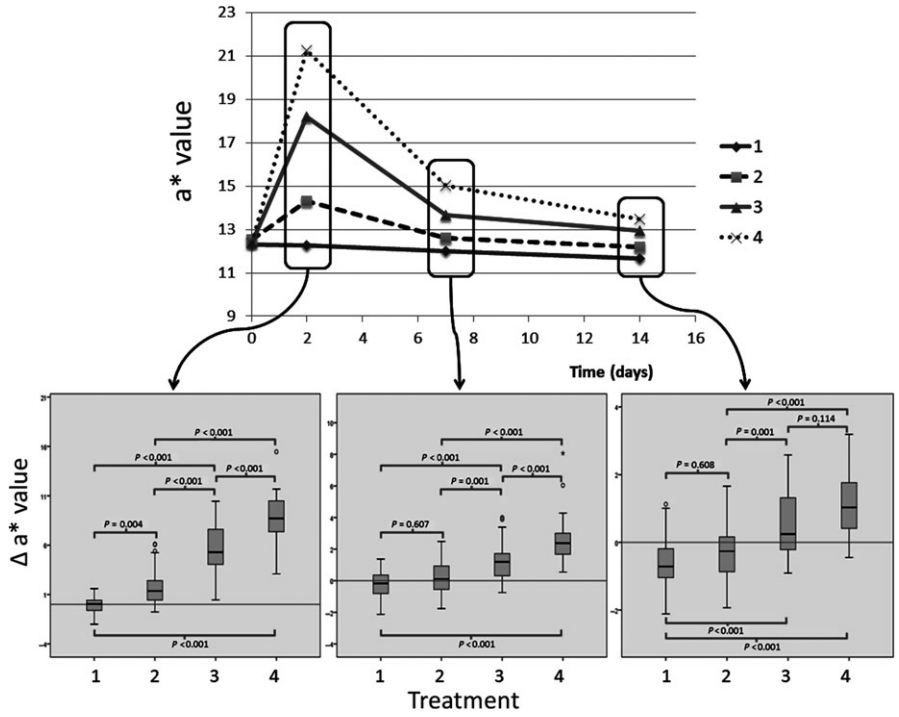


*Fig. 11. Median a* values at each time-point for the different UVB applied doses obtained with the equipment Antera. Box-plots of the variation in a* values in relation to time = 0. The P-values were obtained from the post hoc comparison tests.*

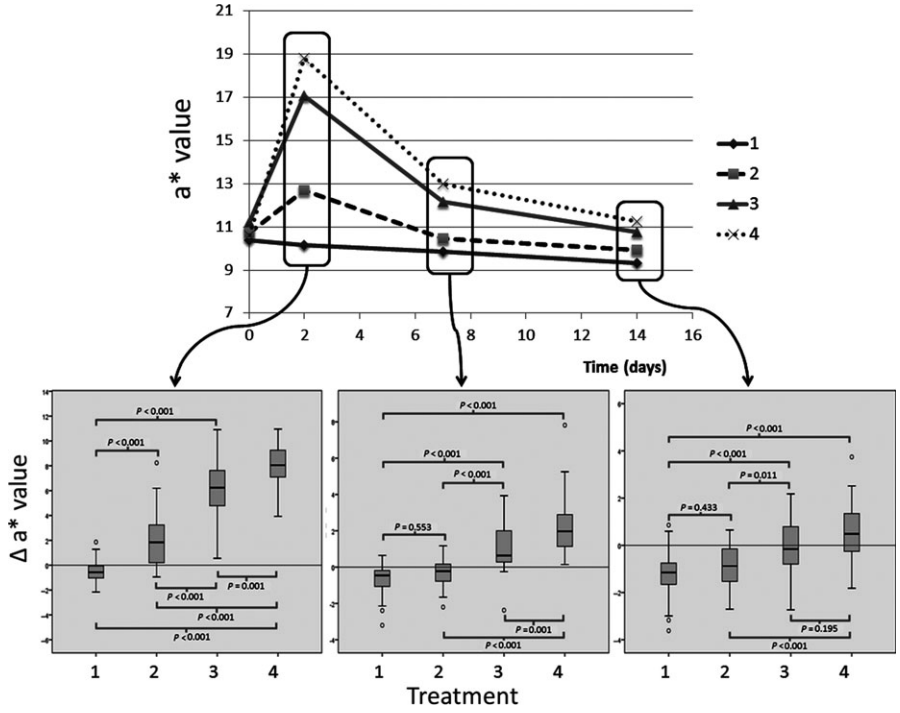


*Fig. 12. Median a* values at each time-point for the different UVB applied doses obtained with the equipment Colorimeter. Box-plots of the variation in a* values in relation to time = 0. The P-values were obtained from the post hoc comparison tests.*

applied and because the skin was desquamated and consequently less tanned, for the two highest UVB doses applied the *b** parameter decreased. The post hoc comparison tests demonstrated that Antera presents a similar sensitivity in comparison to Colorimeter for the

determination of the parameter *b** in the different UVB doses applied at the different time-points except for time-point = 14 days. Although in the Seitz et al. (14) study it is suggested that the *b** value would be a good indicator of tanning, in this case it should be noted that this parameter does not seem to be highly related with skin colour variations caused by UVB exposure.

*Haemoglobin and a** parameter: Antera vs. Colorimeter

The mean, variation coefficient and differences in the haemoglobin measured with Antera and *a** parameter measured with Colorimeter equipment can be seen in Table 7.

The *a** parameter values obtained with the equipment Colorimeter showed an increase for all the UVB doses applied in a proportional relation at time-point = 2 days (Fig. 12). The increase in this parameter immediately after 2 days of UVB exposure is expected once the UVB light triggers an inflammatory reaction of the skin which causes redness (2). The *a** parameter value reaches its peak at this time-point, as expected. The results of the haemoglobin parameter obtained with Antera at this time-point showed a considerable increase which is consistent with the visual perception of erythema (Fig. 7). This increase was proportional with the increased UVB applied doses. Colorimeter showed that between the time-point = 2 days

*TABLE 6. Mean, variation coefficient and differences obtained with the* b* *parameter*

| | Treatment | Mean T0 | VC T0 | Mean T2 | VC T2 | Mean T7 | VC T7 | Mean T14 | VC T14 | Dif. T2-T0 | Dif. T7-T0 | Dif. T14-T0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antera | 1 | 20.22 | 11.16% | 19.97 | 10.63% | 20.19 | 9.85% | 20.18 | 10.87% | −0.25 | −0.03 | −0.04 |
| | 2 | 20.16 | 11.07% | 20.67 | 8.57% | 20.81 | 8.82% | 20.74 | 9.48% | 0.50 | 0.65 | 0.58 |
| | 3 | 20.27 | 11.78% | 21.43 | 9.48% | 21.99 | 8.11% | 21.55 | 8.41% | 1.16 | 1.72 | 1.28 |
| | 4 | 20.27 | 11.03% | 21.17 | 8.76% | 22.17 | 7.54% | 21.42 | 8.65% | 0.90 | 1.90 | 1.14 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |
| Colorimeter | 1 | 14.72 | 17.15% | 14.89 | 15.77% | 15.24 | 15.48% | 15.47 | 16.02% | 0.17 | 0.52 | 0.75 |
| | 2 | 14.75 | 16.43% | 15.16 | 13.39% | 16.00 | 12.73% | 16.02 | 13.56% | 0.40 | 1.24 | 1.27 |
| | 3 | 14.62 | 16.92% | 14.84 | 15.78% | 16.66 | 12.43% | 16.56 | 12.76% | 0.23 | 2.05 | 1.94 |
| | 4 | 14.89 | 16.50% | 14.15 | 15.57% | 16.85 | 11.30% | 16.36 | 12.00% | −0.74 | 1.96 | 1.46 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | 0.001 |

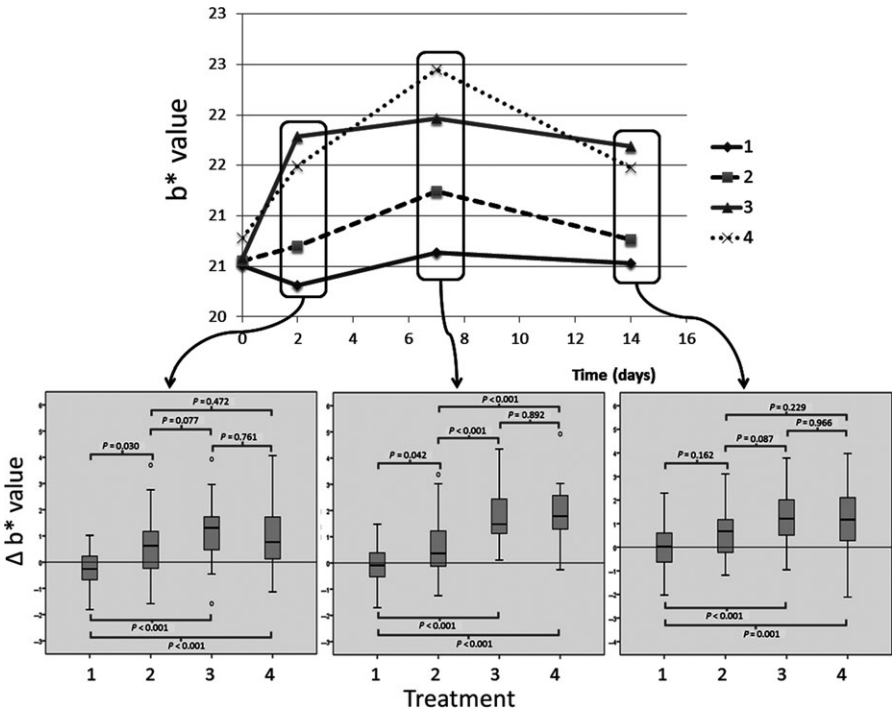


*Fig. 13. Median b* values at each time-point for the different UVB applied doses obtained with the equipment Antera. Box-plots of the variation in b* values in relation to time = 0. The P-values were obtained from the post hoc comparison tests.*

and time-point = 7 days the *a** values decreased but remained higher than the basal level and were still proportional to different UVB doses applied. At time-point = 7 days the haemoglobin value measured with Antera decreased (in comparison to day 2) but still in a proportional relation to different UVB doses applied. These results are expected as the skin becomes darker and the redness disappears. For Colorimeter, at time-point = 14 days, the *a** parameter values decreased as expected once the inflammatory reaction of the skin becomes resolved. At time-point = 14 days, the haemoglobin measured with Antera had a similar behaviour to day 7, but with lower values. At this time-point, the skin had recovered from the inflammatory response provoked by the UVB light, so the erythema decreased.

*Repeatability*

Table 8 shows the values of the variation coefficient (VC) median and inter-quartile range for all the parameters analysed with Antera, Mexameter and Colorimeter.

The VC varied as a function of the parameter evaluated, but generally repeatability of Mexameter and Colorimeter values was found to be

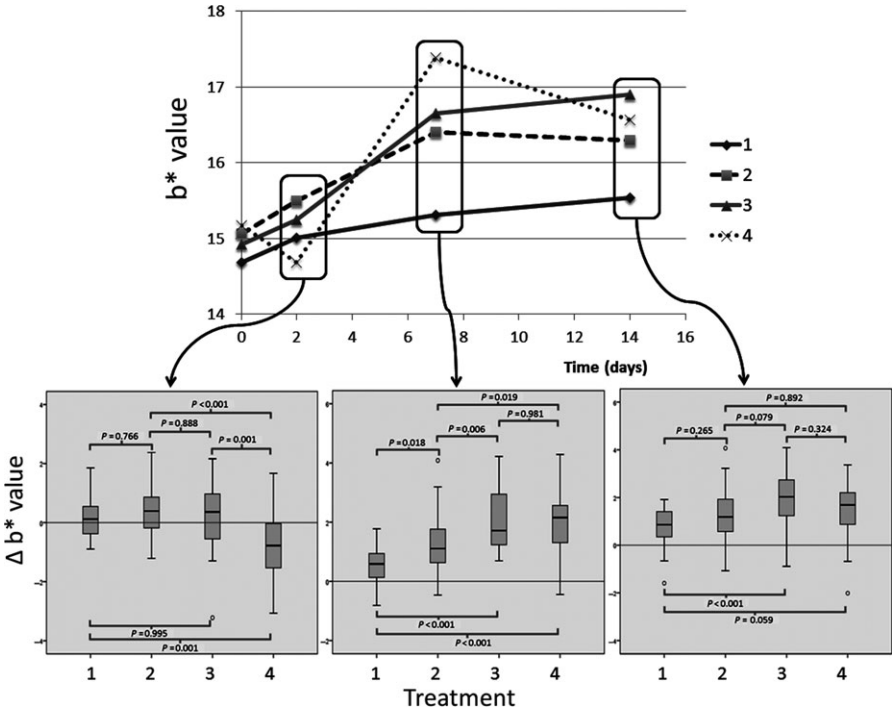


*Fig. 14. Median b* values at each time-point for the different UVB applied doses obtained with the equipment Colorimeter. Box-plots of the variation in b* values in relation to time = 0. The P-values were obtained from the post hoc comparison tests.*

*TABLE 7. Mean, variation coefficient and differences obtained with the Haemoglobin measured with Antera and* a* *parameter measured with Colorimeter*

| | Treatment | Mean T0 | VC T0 | Mean T2 | VC T2 | Mean T7 | VC T7 | Mean T14 | VC T14 | Dif. T2-T0 | Dif. T7-T0 | Dif. T14-T0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antera | 1 | 0.88 | 13.81% | 0.88 | 16.89% | 0.85 | 16.76% | 0.82 | 16.63% | 0.00 | −0.03 | −0.05 |
| | 2 | 0.89 | 16.32% | 1.03 | 18.59% | 0.89 | 16.35% | 0.86 | 16.61% | 0.14 | 0.00 | −0.03 |
| | 3 | 0.88 | 14.59% | 1.35 | 16.11% | 0.97 | 14.35% | 0.92 | 14.22% | 0.47 | 0.09 | 0.04 |
| | 4 | 0.88 | 13.51% | 1.63 | 15.69% | 1.10 | 14.13% | 0.97 | 13.94% | 0.75 | 0.22 | 0.08 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |
| Colorimeter | 1 | 10.68 | 13.74% | 10.16 | 15.74% | 9.96 | 13.73% | 9.38 | 14.17% | −0.52 | −0.72 | −1.30 |
| | 2 | 10.74 | 13.20% | 12.76 | 18.76% | 10.44 | 12.88% | 9.85 | 12.64% | 2.02 | −0.30 | −0.88 |
| | 3 | 10.81 | 13.26% | 16.92 | 13.48% | 11.83 | 12.54% | 10.78 | 10.25% | 6.11 | 1.02 | −0.03 |
| | 4 | 10.68 | 14.60% | 18.77 | 9.90% | 12.96 | 10.77% | 11.19 | 9.05% | 8.09 | 2.28 | 0.52 |
| *P*-value | | | | | | | | | | <0.001 | <0.001 | <0.001 |

*TABLE 8. Median and inter-quartile range of the variation coefficient (VC) for all the parameters analysed with Antera, Mexameter and Colorimeter*

| | VC (%) Median | VC (%) Inter-quartile range |
|---|---|---|
| Melanin Antera | 0.46 | 0.33 |
| Melanin Mexameter | 3.52 | 2.45 |
| Haemoglobin Antera | 1.58 | 1.43 |
| Erythema Mexameter | 5.98 | 4.52 |
| *L** Antera | 0.36 | 0.27 |
| *L** Colorimeter | 0.66 | 0.43 |
| *a** Antera | 1.56 | 1.11 |
| *a** Colorimeter | 3.06 | 2.11 |
| *b** Antera | 0.58 | 0.46 |
| *b** Colorimeter | 2.01 | 1.18 |

lower than that of Antera for all the parameters analysed as can be seen by higher median and Inter-quartile range VC values.

*Correlations*

In Table 9 are represented the Pearson correlation coefficients and respective level of significance obtained for the correlation between the Antera and Mexameter values and between Antera and Colorimeter values at time-point = 0 days and for the difference values obtained for each time-point (dift2-t0; dift7-t0 and dift14-t0) for each UVB dose applied for all parameters.

Antera and Mexameter presented high melanin correlations for normal skin ($R$ = 0.862–0.907) and for skin with high pigmentation level ($R$ = 0.726–0.787), as seen 14 days after UV exposure.

Antera and Mexameter showed good haemoglobin and erythema correlations for normal skin ($R$ = 0.773–0.836) and for skin with high level of erythema, as seen 2 days after UV exposure ($R$ = 0.726–0.839).

Regarding the $L^*$ parameter, Antera and Colorimeter showed good correlation for normal skin ($R$ = 0.934–0.958) and for highly pigmented skin ($R$ = 0.727–0.884), as seen in day 7.

Antera and Colorimeter also showed good correlation regarding the $a^*$ parameter for normal skin ($R$ = 0.762–0.861) and for skin with high erythema ($R$ = 0.769–0.928), as seen 2 days after UV exposure.

Antera and Colorimeter showed good correlation as well regarding the $b^*$ parameter for normal skin ($R$ = 0.931–0.953) and not so good correlation when the skin was highly pigmented ($R$ = 0.590–0.723) or with an erythema ($R$ = 0.586–0.805).

Haemoglobin measured with Antera and $a^*$ parameter measured with Colorimeter showed good correlations for normal skin ($R$ = 0.754–0.856) and for skin with high erythema level ($R$ = 0.676–0.905) as seen 2 days after UV exposure. Takiwaki et al. (15) measured the colour of normal skin with a reflectance spectrometer and tri-stimulus colorimeter and reported that the $a^*$ value (measured with the tri-stimulus colorimeter) and E-index (measured with a reflectance spectrometer) showed a strong linear correlation. The $a^*$ value is known to reveal a significant linear correlation with the dermatologist's perception of skin redness and erythema. In the referred study, this value as well as the E-index reached a peak towards the mean colour of haemoglobin on the first 2 days after irradiation, and then gradually returned to its original value. These findings are in accordance to our results.

## Conclusion

The aim of this study was to compare different biometric properties of the human skin, namely melanin content of the skin, skin colour and skin redness, assessed with Antera 3D®, with Mexameter® MX 18 and Colorimeter® CL 400.

Concerning sensitivity, for the melanin content analysis Antera presented a better sensitivity in comparison to Mexameter. On the contrary to Mexameter, Antera could reliably discriminate between skin erythema and melanin. Regarding the erythema parameter, Antera presented a similar sensitivity for the detection of different grades of erythema (through the haemoglobin parameter) at the different time-points evaluated in comparison to Mexameter. Regarding the $a^*$, $b^*$ and $L^*$ parameters, Antera presented a similar sensitivity in comparison to Colorimeter.

It is also relevant to mention that Antera presented a better specificity than Mexameter when measuring melanin in skin with erythema.

The correlations performed between the parameters analysed by the different parameters confirm these findings:

- Antera and Mexameter showed good haemoglobin and erythema correlations for normal skin and for skin with high level of erythema;

*TABLE 9. R coefficients and respective level of significance obtained between Antera and Mexameter values for the melanin and erythema parameters; between* L* a* b* *values measured with Antera and Colorimeter and between haemoglobin values measured with Antera and* a* *values measured with Colorimeter*

| | | | 0 mJ/cm$^2$ | 89 mJ/cm$^2$ | 129 mJ/cm$^2$ | 169 mJ/cm$^2$ |
|---|---|---|---|---|---|---|
| Melanin: Antera vs. Mexameter | Day 0 | *R* | 0.901 | 0.862 | 0.907 | 0.894 |
| | | *P*-value | <0.001 | <0.001 | <0.001 | <0.001 |
| | Day 2 | *R* | | −0.695 | −0.364 | −0.313 |
| | | *P*-value | | <0.001 | 0.052 | 0.092 |
| | Day 7 | *R* | | 0.693 | 0.572 | 0.356 |
| | | *P*-value | | <0.001 | 0.001 | 0.053 |
| | Day 14 | *R* | | 0.729 | 0.787 | 0.726 |
| | | *P*-value | | <0.001 | <0.001 | <0.001 |
| Haemoglobin and Erythema: Antera vs. Mexameter | Day 0 | *R* | 0.807 | 0.836 | 0.773 | 0.833 |
| | | *P*-value | <0.001 | <0.001 | <0.001 | <0.001 |
| | Day 2 | *R* | | 0.834 | 0.839 | 0.726 |
| | | *P*-value | | <0.001 | <0.001 | <0.001 |
| | Day 7 | *R* | | 0.523 | 0.619 | 0.771 |
| | | *P*-value | | 0.003 | <0.001 | <0.001 |
| | Day 14 | *R* | | 0.399 | 0.644 | 0.561 |
| | | *P*-value | | 0.029 | <0.001 | 0.001 |
| *L** parameter: Antera vs. Colorimeter | Day 0 | *R* | 0.949 | 0.949 | 0.934 | 0.958 |
| | | *P*-value | <0.001 | <0.001 | <0.001 | <0.001 |
| | Day 2 | *R* | | 0.584 | 0.871 | 0.713 |
| | | *P*-value | | 0.001 | <0.001 | <0.001 |
| | Day 7 | *R* | | 0.727 | 0.882 | 0.884 |
| | | *P*-value | | <0.001 | <0.001 | <0.001 |
| | Day 14 | *R* | | 0.648 | 0.838 | 0.664 |
| | | *P*-value | | <0.001 | <0.001 | <0.001 |
| *a** parameter: Antera vs. Colorimeter | Day 0 | *R* | 0.773 | 0.861 | 0.762 | 0.833 |
| | | *P*-value | <0.001 | <0.001 | <0.001 | <0.001 |
| | Day 2 | *R* | | 0.928 | 0.878 | 0.769 |
| | | *P*-value | | <0.001 | <0.001 | <0.001 |
| | Day 7 | *R* | | 0.675 | 0.767 | 0.846 |
| | | *P*-value | | <0.001 | <0.001 | <0.001 |
| | Day 14 | *R* | | 0.744 | 0.772 | 0.591 |
| | | *P*-value | | <0.001 | <0.001 | 0.001 |
| *b** Antera vs. Colorimeter | Day 0 | *R* | 0.931 | 0.932 | 0.953 | 0.945 |
| | | *P*-value | <0.001 | <0.001 | <0.001 | <0.001 |
| | Day 2 | *R* | | 0.676 | 0.586 | 0.805 |
| | | *P*-value | | <0.001 | 0.001 | <0.001 |
| | Day 7 | *R* | | 0.590 | 0.731 | 0.625 |
| | | *P*-value | | 0.001 | <0.001 | <0.001 |
| | Day 14 | *R* | | 0.617 | 0.601 | 0.723 |
| | | *P*-value | | <0.001 | <0.001 | <0.001 |
| Haemoglobin and *a** Antera vs. Colorimeter | Day 0 | *R* | 0.769 | 0.856 | 0.754 | 0.838 |
| | | *P*-value | <0.001 | <0.001 | <0.001 | <0.001 |
| | Day 2 | *R* | | 0.905 | 0.826 | 0.676 |
| | | *P*-value | | <0.001 | <0.001 | <0.001 |
| | Day 7 | *R* | | 0.615 | 0.717 | 0.805 |
| | | *P*-value | | <0.001 | <0.001 | <0.001 |
| | Day 14 | *R* | | 0.683 | 0.735 | 0.457 |
| | | *P*-value | | <0.001 | <0.001 | 0.011 |

- A good correlation was observed between Antera and Colorimeter regarding the *a** parameter when the skin was without erythema or pigmentation and when it presented a pronounced erythema;
- The haemoglobin measured with Antera and *a** parameter measured with Colorimeter showed good correlations for normal skin and for skin with higher erythema level;
- Antera and Mexameter presented high melanin correlations for normal skin and for skin with high pigmentation;
- Antera and Colorimeter showed good correlation regarding *b** and *L** parameters for normal skin.

Antera also presented better repeatability than Mexameter and Colorimeter for the parameters analysed.

In conclusion, Antera is a robust, a sensitive and a precise equipment for the skin colour analysis, which can be useful for skin colour assessment studies. Moreover, this equipment has the advantage to be portable, user friendly and allows the analysis of a larger skin area in comparison to other equipment such as Mexameter and Colorimeter.

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