

ORIGINAL ARTICLE

A rapid single session in vivo method to quantify blue light protection using immediate pigmentation darkening

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Abstract

Background: Blue light has been implicated in oxidative stress and pigmentary changes, yet no standardized method exists to quantify cosmetic protection in this spectral range.

Objectives: To develop a single session in vivo method that quantifies the protection of topical products against blue light-induced immediate pigmentation darkening (IPD) and to compare its outcomes with a multi-day protocol from the literature.

Methods: IPD was assessed using the Individual Typology Angle (ITA°) measured before (ITA₀) and after exposure (ITA_{imm}) to a 420 nm LED source delivering 80 J cm⁻². Twenty-two cosmetic formulations (mineral and organic filters, nano and non-nano, with or without additional blue light actives) were applied at 2 mg cm⁻² to the backs of volunteers spanning ITA₀ -5 to 45°. For each product, 24 paired (ITA₀, ITA_{imm}) values were modelled by linear regression and compared with a fixed no product control. Protective efficacy was summarized as a Blue Light Protection Index (BLPI), defined as the mean percentage reduction in immediate darkening relative to the control over ITA₀ = 10–40°, where immediate darkening corresponds to the difference between ITA₀ and ITA_{imm}.

Results: BLPI values ranged from 14.6 to 79.2 (median 34.0), demonstrating a wide dynamic range. Mineral filter formulations predominantly occupied the upper BLPI range, whereas organic-only products were mainly in the lower to intermediate range. Non-nano mineral filters generally outperformed their nano equivalents, and mixed TiO₂ + ZnO systems yielded the highest BLPI values within mineral products. A flat increase in BLPI was observed for formulations containing 2%, 4% and 6% benzotriazole, consistent with a concentration effect, although pairwise differences did not reach statistical significance. For four products, rankings obtained with BLPI were concordant with those obtained using a multi-day Blue Light Protection Factor method from the literature.

Conclusions: The proposed single session IPD-based method provides a rapid, reproducible, and biologically relevant assessment of blue light protection. It discriminates effectively between formulations, reflects expected optical behaviour and shows good agreement with a multi-day protocol. This approach is well-suited

for formulation screening and could contribute to future methodological harmonization in the visible light domain.

KEYWORDS

blue light, Blue Light Protection Index, immediate pigmentation darkening, in vivo method, Individual Typology Angle, mineral filters, nano, pigmentation

Résumé

Contexte: La lumière bleue est associée au stress oxydatif et à des modifications pigmentaires, mais il n'existe pas de méthode standardisée permettant de quantifier la protection cosmétique dans cette plage spectrale.

Objectifs: Développer une méthode *in vivo* en une seule session permettant de quantifier la protection des produits topiques contre pigmentation immédiate (Immediate Pigmentation Darkening, IPD) induit par la lumière bleue, et comparer ses résultats à ceux d'un protocole sur plusieurs jours décrit dans la littérature.

Méthodes: L'IPD a été évalué à l'aide de l'Individual Typology Angle (ITA°), mesuré avant (ITA₀) et après exposition (ITA_{imm}) à une source LED de 420 nm délivrant 80 J·cm⁻². Vingt-deux formulations cosmétiques (filtres minéraux et organiques, nano et non nano, avec ou sans actifs supplémentaires contre la lumière bleue) ont été appliquées à raison de 2 mg/cm⁻² sur le dos de volontaires présentant des ITA₀ compris entre -5 et 45°. Pour chaque produit, 24 paires de valeurs (ITA₀, ITA_{imm}) ont été modélisées par régression linéaire et comparées à un contrôle fixe sans produit. L'efficacité protectrice a été synthétisée sous la forme d'un indice de protection contre la lumière bleue (Blue-Light Protection Index, BLPI), défini comme la réduction moyenne en pourcentage de la pigmentation immédiate par rapport au contrôle pour des ITA₀ compris entre 10 et 40°. ITA_{imm}

Résultats: Les valeurs de BLPI variaient de 14,6 à 79,2 (médiane : 34,0), mettant en évidence une large plage de variation. Les formulations à base de filtres minéraux se situaient majoritairement dans la partie supérieure de l'échelle de BLPI, tandis que les produits contenant uniquement des filtres organiques se répartissaient principalement dans les plages basses à intermédiaire. Les filtres minéraux non nano présentaient généralement de meilleures performances que leurs équivalents nano, et les systèmes mixtes TiO₂+ZnO affichaient les valeurs de BLPI les plus élevées parmi les produits minéraux. Une augmentation régulière du BLPI a été observée pour les formulations contenant 2 %, 4 % et 6 % de benzotriazole, ce qui est cohérent avec un effet dose, bien que les différences par paires n'aient pas atteint la significativité statistique. Pour quatre produits, le classement obtenu avec le BLPI concordait avec celui obtenu à l'aide d'une méthode de facteur de protection contre la lumière bleue sur plusieurs jours décrite dans la littérature.

Conclusions: La méthode proposée, qui s'appuie sur l'IPD et se réalise en une seule session, permet une évaluation rapide, reproductible et biologiquement pertinente de la protection contre la lumière bleue. Elle discrimine efficacement les formulations, reflète le comportement optique attendu et présente une bonne concordance avec un protocole sur plusieurs jours. Cette approche est bien adaptée au criblage des formulations et pourrait contribuer à une future harmonisation méthodologique dans le domaine de la lumière visible.

INTRODUCTION

Intentional sun exposure has increased markedly over the last century, leading to greater cumulative UV doses and reinforcing the need for effective photoprotection [1–4]. A robust framework now exists for assessing UV protection, notably in vivo SPF (ISO 24444), in vitro UV spectral transmittance (ISO 23675) and UVA assessments (e.g. PPD and ISO 24442, 24443). These harmonized protocols support reliable product comparison and have reinforced significant advances in sunscreen science [5–8].

Beyond UV, there is growing evidence that visible light, particularly in the blue region (400–470 nm), can induce oxidative stress, inflammation and increased pigmentation, especially in intermediate to darker skin phototypes. However, in contrast to UV, there is currently no standardized method for evaluating protection against blue light. This lack of harmonization limits meaningful comparison between products and complicates substantiation of blue light claims [9–13].

In vitro methods based on spectral transmittance through product films can quantify optical attenuation, but their biological relevance remains uncertain due to an incomplete understanding of the mechanistic thresholds and pathways involved in blue light responses [14–17].

Several in vivo protocols for blue light protection have been described, most relying on persistent pigmentation following repeated exposures over several days, conceptually similar to UVA PPD methodologies. While biologically meaningful, these protocols are resource-intensive, require multiple visits and precise site repositioning, and are therefore not well-suited to high-throughput formulation screening [14, 18, 19].

The aim of the present work was to develop a simplified in vivo method that causes measurable immediate pigmentation darkening (IPD) after a single blue light exposure, and to derive a quantitative protection index, the Blue Light Protection Index (BLPI), based on ITA° changes. Secondary objectives were to (i) evaluate the method's discriminative power across a diverse set of formulations, and (ii) compare its outcomes with those of a multi-day protocol from the literature, in order to assess concordance in product ranking [18].

MATERIALS AND METHODS

Study objective and design

The study was designed to establish a single-session in vivo protocol for quantifying the protective efficacy of topically applied products against blue light-induced IPD. The primary endpoint was the change in ITA° between baseline (ITA_0) and immediately after exposure (ITA_{imm}) for both treated and untreated (control) sites.

Volunteers

Healthy adult volunteers (women and men, 18–60 years old) were recruited with a hair free area on the back suitable for repeated colourimetric measurements. Eligibility required a baseline ITA_0 between -5 and 45° , covering the range of phototypes expected to be responsive to blue light stimulation (Figure 1). Exclusion criteria included recent sun exposure, use of photosensitizing medications and pigmentation disorders or acne in the test area.

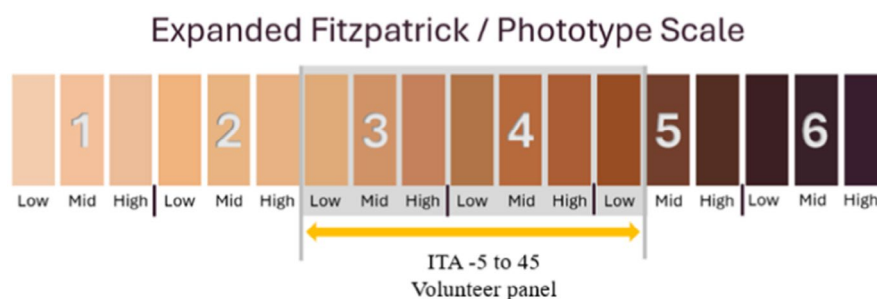
The study was conducted in accordance with the Declaration of Helsinki, all participants provided written informed consent [20].

To ensure adequate statistical power and coverage of the ITA_0 range, 24 paired measurements were targeted per product, with at least 20 valid pairs retained after protocol-related exclusions. The lamp aperture and back area allowed for six distinct exposure sites per volunteer. Two products were assessed per volunteer (three sites per product), so eight volunteers per product yielded the 24 pairs ($8 \times 3 = 24$). Volunteer sets were assembled so that, for each product, the aggregated ITA_0 distribution covered the -5 to 45° interval. In total, 90 volunteers were included across all products.

Products and application

Twenty-two cosmetic formulations were evaluated, including both tinted and untinted products. They covered a variety of UV filters (organic and mineral), particle sizes

FIGURE 1 Expanded Fitzpatrick phototype scale and volunteer selection criteria. Volunteers were selected within the ITA° range shown by the arrow (approximately -5 to 45°), corresponding to the phototypes represented in this panel.



(nano and non-nano) and the presence or absence of specific blue light protective agents.

On each volunteer, six 16 cm² application areas were delineated on the back using a template. Products were applied at 2 mg cm⁻² using a bare finger, following the spreading procedure of ISO 24444 [5]. After a 15-min absorption period, a printed white mask, attached to the exposure device, defined six 4 cm² exposure windows centred within the respective application areas (Figure 2).

Colourimetry and ITA° calculation

Skin colour was measured using a Chroma Meter CR-400 (Konica Minolta) and expressed in CIELAB coordinates (L^* , a^* , b^*). The Individual Typology Angle (ITA°) was calculated as:

$$\text{ITA}(\text{°}) = \arctan\left(\frac{L^* - 50}{b^*}\right) \times \frac{180}{\pi}$$

For each site, ITA₀ was recorded before product application and blue light exposure, and ITA_{imm} was measured immediately after the exposure and a short recovery period (see below). ITA₀ and ITA_{imm} were used throughout as the main descriptive variables of baseline pigmentation and immediate post-exposure pigmentation, respectively.

Blue light exposure

Blue light exposure was delivered using a 420 nm LED device (LED Box 420 nm, BioLambda) controlled by an external unit (BlackBox Smart, BioLambda). A custom 3D

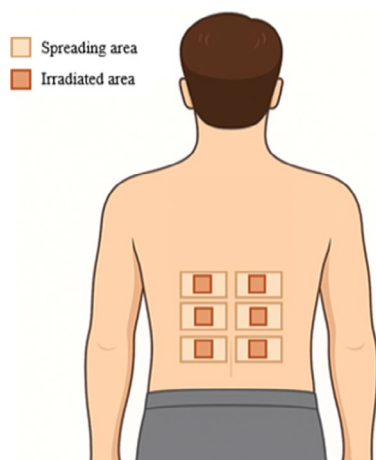


FIGURE 2 Schematic representation of the application and irradiation areas on the volunteer's back. Schematic illustration of the product-application zones (light) and the corresponding irradiation sites (dark) on the volunteer's back.

printed enclosure ensured a fixed distance between the lamp and the skin and secured the white mask. A white mask was chosen to minimize heat and possible erythema. Preliminary tests with dark masks had induced unwanted redness (Figure 3).

Irradiance at each site was measured using a PMA2100 radiometer (Solar Light) with a PMA2132 visible-light sensor. Exposure time was adjusted to deliver a radiant exposure of 80 J cm⁻², selected from preliminary dose-response work as the minimal dose consistently eliciting a clear, reproducible IPD without visible erythema (Figure 4).

After exposure, the skin was gently cleansed with a mild cleanser to remove residual surface product or pigments. ITA° measurements were then performed 15 min after cleansing, allowing short-lived non-pigmentary effects related to mask contact, friction or cleansing-induced skin reactivity to subside. This time point was selected to ensure the stabilization of the immediate pigment darkening response after the resolution of short-lived non-pigmentary effects, while remaining within the immediate pigmentation phase and prior to the influence of later pigmentary processes.

Control model

A fixed control model (no product) was built from 58 paired (ITA₀, ITA_{imm}) measurements obtained under

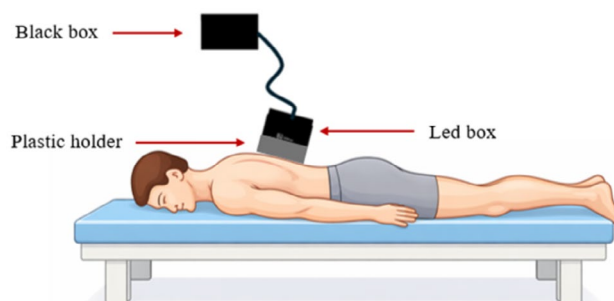


FIGURE 3 Schematic illustration of the measurement configuration. Schematic illustration of the LED light positioned above the back using a plastic holder connected to the control unit.



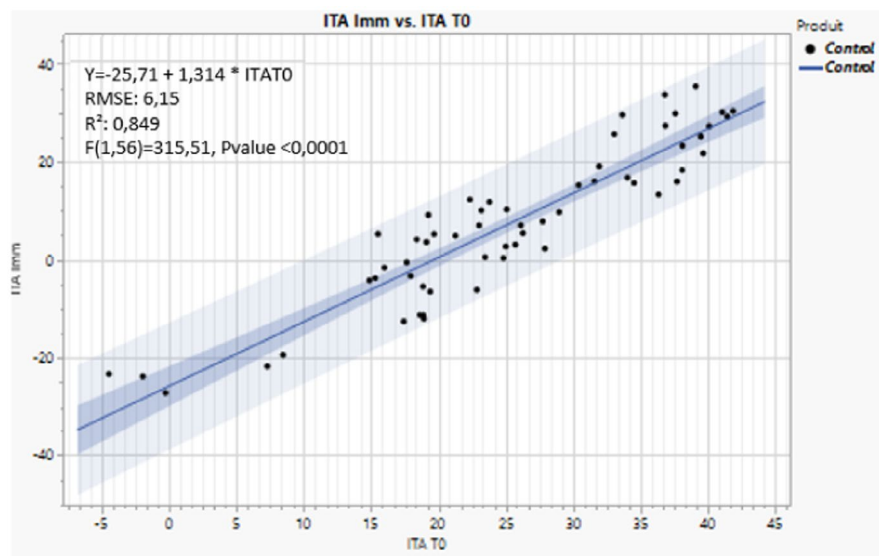
FIGURE 4 Postexposure pigmentation sites. Representative example of the irradiated squares used for IPD assessment. The volunteer is not identifiable.

TABLE 1 Parameter estimates for the fixed control model.

Term	Estimate	SE	t ratio	$p > t $	Lower 95%	Upper 95%
Intercept	-25.70811	2.035443	-12.63	<0.0001*	-29.79	-21.63
ITA T0	1.3143976	0.073998	17.76	<0.0001*	1.1	1.46

Note: Regression coefficients, standard errors and 95% confidence intervals for the linear control model relating baseline ITA₀ to immediate post-exposure ITA_{imm} (no-product condition). * $p < 0.05$.

FIGURE 5 Control model (no-product) relating ITA₀–ITA_{imm}. Scatterplot and linear regression for the fixed control condition (no product) used as the reference model for all analyses.



identical exposure conditions. These data were fitted using simple linear regression:

$$\text{ITA}_{\text{imm,ctrl}}(x) = a_{\text{ctrl}} + b_{\text{ctrl}}$$

where $x = \text{ITA}_0$.

Residuals satisfied normality assumptions (e.g. Shapiro–Wilk, Anderson–Darling), and model performance was high (Table 1) ($R^2=0.85$, $\text{RMSE}=6.15$). Figure 5 shows the scatter, fitted line and confidence/prediction bands. Table 1 reports parameter estimates and 95% CIs. This control model was used as a fixed reference in all subsequent analyses.

Product-specific models and BLPI definition

For each product, a similar linear model was fitted to its 24 paired ITA₀ and ITA_{imm} values (Figures 6 and 7). Although volunteers with baseline ITA₀ values between -5 and 45° were included, BLPI computation was intentionally restricted to the 10 – 40° ITA₀ interval, as this range corresponds to the interval in which the IPD signal was the most stable and reproducible and where model

robustness was optimal. Product and control models were subsequently used to generate predictions of ITA_{imm} over a common ITA₀ grid spanning from 10 to 40° with a 0.5° step ($N=61$ points). This analytical interval represents the densest sampling region of the dataset, encompasses phototypes particularly responsive to blue light-induced pigmentation and is associated with lower predictive variance compared with extreme ITA₀ values.

The Blue Light Protection Index (BLPI) was defined as the mean percentage reduction in IPD provided by the product relative to the control across the grid:

$$\text{BLPI} = \frac{1}{N} \sum_{\text{ITA}_0=10}^{40} \left[100 \times \left(1 - \frac{\text{ITA}_0 - \text{ITA}_{\text{imm,product}}}{\text{ITA}_0 - \text{ITA}_{\text{imm,control}}} \right) \right]$$

where ITA₀ is the baseline Individual Typology Angle value measured before exposure, ITA_{imm,product} is the ITA value estimated immediately after exposure in the presence of the tested product, ITA_{imm,control} is the ITA value estimated immediately after exposure under the reference condition (no product) and ITA₀ values are incremented by 0.5° .

This index reflects the average percentage reduction of blue light-induced immediate darkening across a standardized baseline pigmentation range, thereby

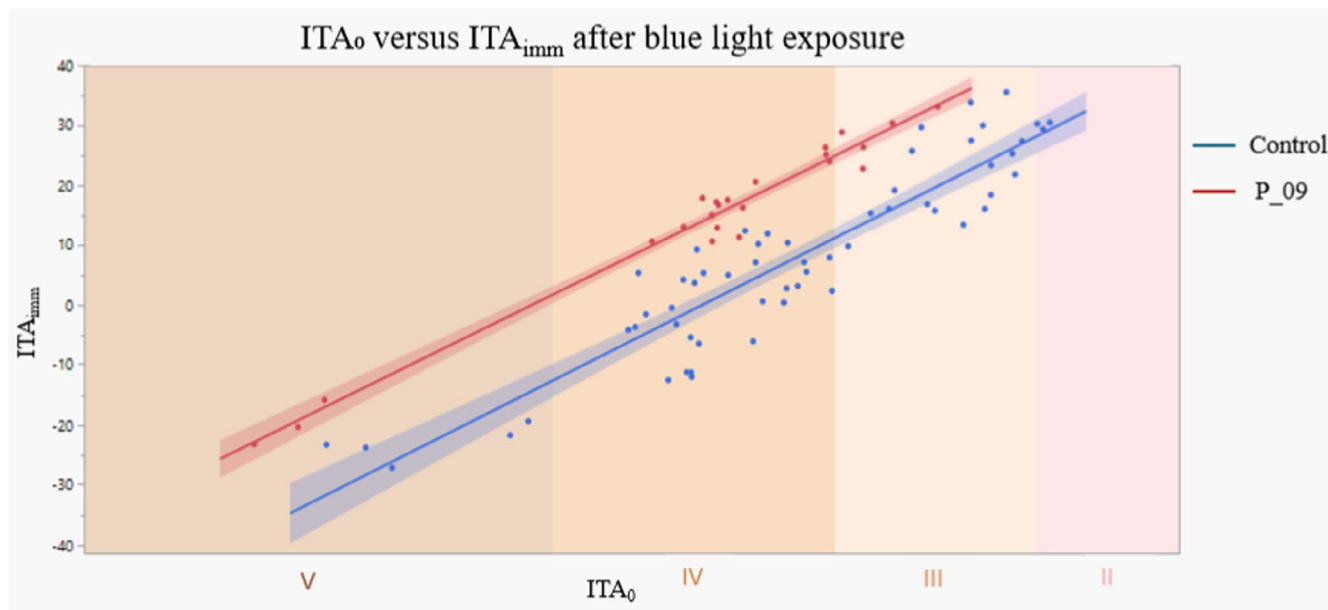


FIGURE 6 ITA_0 – ITA_{imm} relationship for a well-protecting product. Comparison between the fixed control model (blue) and product P_09 (red). The upward shift of the product regression line indicates reduced immediate pigmentation and therefore good blue light protection.

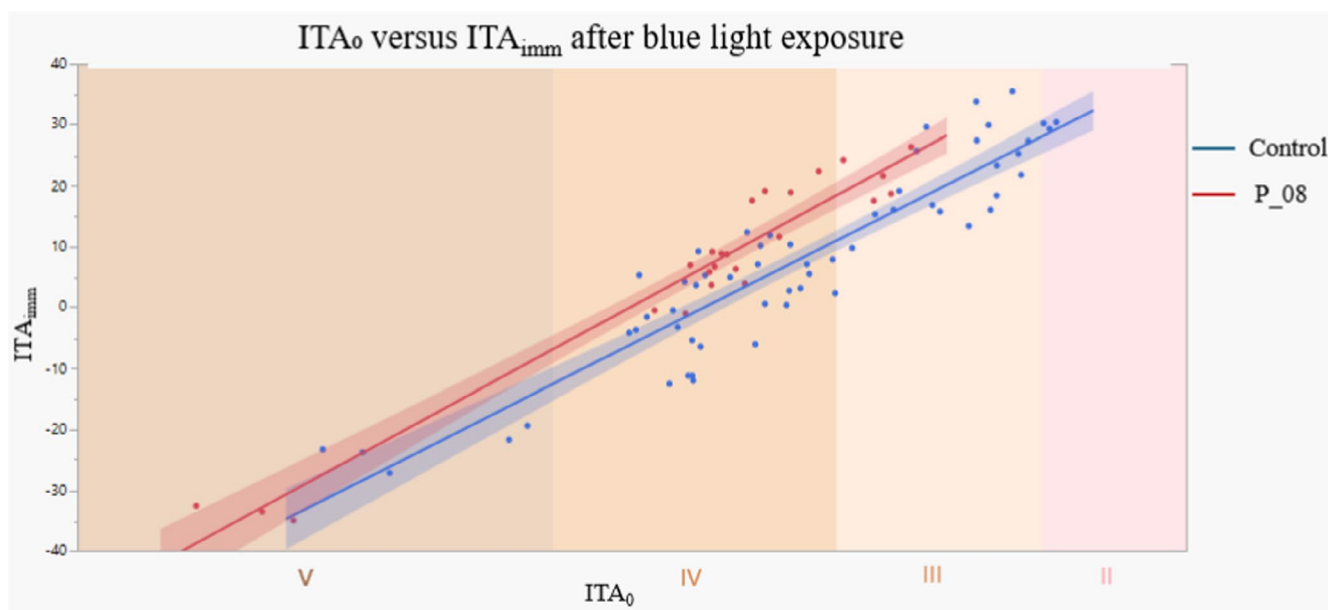


FIGURE 7 ITA_0 – ITA_{imm} relationship for a less protective product. Comparison between the fixed control model (blue) and product P_08 (red). The limited upward shift of the product regression line indicates weaker protection against blue light-induced pigmentation.

minimizing inter-individual variability and enabling direct comparison of products.

Uncertainty estimation and statistical analyses

Uncertainty combines (i) parameter and residual variability of the fixed control model (58 pairs) and (ii) variability of each product-specific model.

Step 1—Prediction standard error at a given $ITA_0(x_0)$

For each model, the standard error (SE) of the mean prediction at x_0 follows the usual linear-regression formula:

$$SE_{\hat{y}} = s \sqrt{\frac{1}{n} + \frac{(x_0 - \bar{x})^2}{\sum_{i=1}^n (x_i - \bar{x})^2}}$$

where s is the residual standard deviation, n the number of observations, x_0 , the baseline value for which the prediction is computed, $\bar{x}$ the mean of ITA₀ values and $\sum (x_i - \bar{x})^2$ the centred sum of squares.

Step 2—SE of the ratio at a grid point

Using a delta-method approximation:

$$\sigma_{\text{Ratio}} = \text{Ratio} \sqrt{\frac{\sigma_{\text{Num}}^2}{\text{Num}^2} + \frac{\sigma_{\text{Den}}^2}{\text{Den}^2} - 2\rho \frac{\sigma_{\text{Num}}}{\text{Num}} * \frac{\sigma_{\text{Den}}}{\text{Den}}}$$

With:

$$\text{Num} = x - \widehat{\text{ITA}}_{\text{imm,product}}(x)$$

$$\text{Den} = x - \widehat{\text{ITA}}_{\text{imm,control}}(x).$$

And where ρ is the Pearson correlation between Num and Den at that x . Because control and product models are fitted on independent datasets, ρ was taken as = 0.

Step 3—SE of BLPI

The BLPI SE follows from averaging the per-grid contributions (scaling by 100/ N). BLPI values are reported as mean $\pm$ SE, and 95% confidence intervals (CIs) were derived using the same delta-method framework. Bootstrap confidence intervals, obtained by resampling residuals and refitting models, can be used as a robustness check.

Group comparisons (e.g. mineral vs. organic filters, nano vs. non-nano, concentration levels) were performed using appropriate tests (e.g. Welch's t -tests or non-parametric Mann–Whitney U , with correction for multiple testing such as Benjamini–Hochberg when relevant).

RESULTS

BLPI distribution across products

Across the 22 formulations assessed, BLPI values ranged from 14.6 to 79.2, with a median of 34.0 and an interquartile range (IQR) of 24.3–47.6 (Table 2 and Figure 8). The distribution was continuous, with no clustering at the extremes, indicating a broad dynamic range and good discriminative capacity of the method. The highest BLPI values were obtained for P_09, P_18, P_06, P_14 and P_16 (BLPI $\geq$ 53), whereas P_01, P_02 and P_21 exhibited the lowest protection (BLPI $\leq$ 17.9).

TABLE 2 Ranked BLPI values, standard deviation (σ) and coefficient of variation (CV%) for the 22 tested products.

Rank	Product	BLPI	σ	CV%
1	P_09	79.2	3.53	4.5
2	P_18	75.6	4.58	6.1
3	P_06	63.2	6.61	10.5
4	P_14	54.0	5.52	10.2
5	P_16	53.4	4.70	8.8
6	P_19	48.2	4.23	8.8
7	P_04	45.7	4.09	9.0
8	P_05	42.1	6.72	16.0
9	P_08	41.8	4.34	10.4
10	P_13	36.5	6.83	18.7
11	P_17	34.4	3.69	10.7
12	P_20	33.6	2.67	8.0
13	P_12	30.3	4.58	15.1
14	P_03	29.8	6.60	22.2
15	P_07	29.0	3.19	11.0
16	P_11	26.4	5.44	20.6
17	P_22	23.6	8.53	36.1
18	P_15	23.5	1.71	7.3
19	P_10	19.2	1.46	7.6
20	P_01	17.9	4.00	22.3
21	P_02	14.9	4.46	29.8
22	P_21	14.6	4.53	31.0

Repeatability, summarized by within-product standard deviation and coefficient of variation (CV%), showed a median CV of 10.6% (IQR 8.8–20.1%). Eight products exhibited CV $\leq$ 10%, indicating high precision, while six had CV $>$ 20%. Lower-performing products tended to show higher variability (e.g. P_03, P_01, P_02, P_21, P_22), consistent with small effect sizes closer to the control response. Several high-ranking products combined high BLPI with relatively low variability (e.g. P_09, P_18, P_16), supporting the precision of the method in the range of effective protection.

Influence of filter type

Products were categorized as 'mineral', 'organic' or 'BL reflector' (formulations without filters, using blue light reflective/dispersion agents) (Figure 10). Mineral filters consistently populated the upper segment of the BLPI ranking (41.8–79.2), with several among the top performers (P_09, P_18, P_06, P_14, P_16; all BLPI $>$ 50). Organic-only formulations generally appeared in the lower half of the distribution (14.6–42.1), and none occupied the highest BLPI tier. 'BL reflector' products showed intermediate,

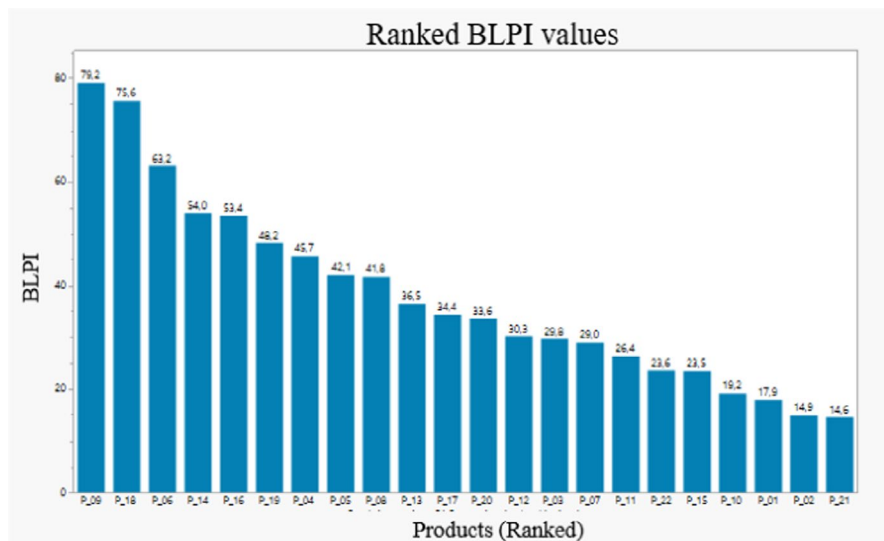


FIGURE 8 Ranked BLPI values of the 22 tested products (highest to lowest).

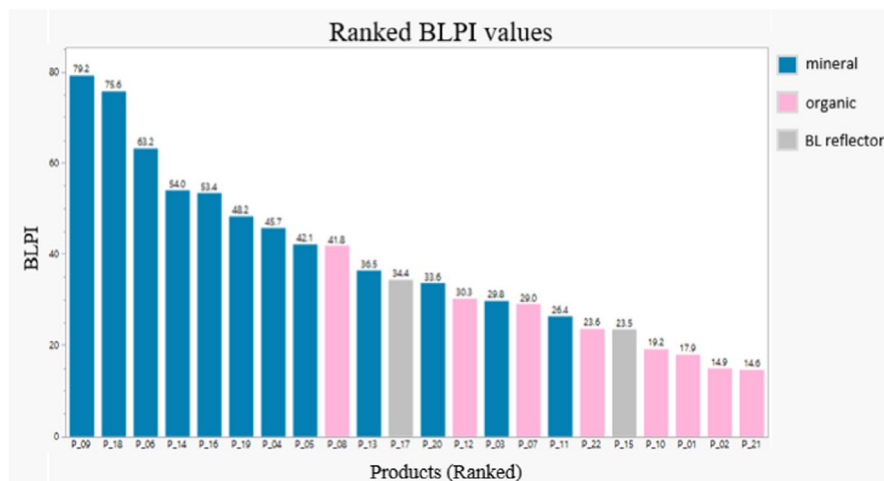


FIGURE 9 Ranked BLPI values of the 22 tested products by filter category (mineral, organic, BL reflector).

heterogeneous outcomes (23.6–36.5) (Figure 9). Overall, mineral-based products provided greater attenuation of blue light-induced IPD than organic-only systems under the test conditions.

Mineral products: particle size and filter composition

Within mineral formulations, we examined the impact of particle size (nano vs. non-nano) and filter composition (TiO_2 , ZnO or $\text{TiO}_2 + \text{ZnO}$ mixtures at the same concentrations) (Tables 3 and 4, Figure 10). Non-nano formulations tended to show higher BLPI than their nano counterparts. This trend reached statistical significance for ZnO ($z = 2.02$, $p = 0.0433$) and $\text{TiO}_2 + \text{ZnO}$ mixes ($z = 2.98$, $p = 0.0029$), but not for TiO_2 alone ($z = 1.17$, $p = 0.2400$).

Regarding composition, ZnO-based products achieved higher BLPI than TiO_2 based products; concentration

differences may partly explain this contrast. In both nano and non-nano subsets, $\text{TiO}_2 + \text{ZnO}$ mixtures provided the highest BLPI values, with all pairwise comparisons significant in the non-nano category.

Interestingly, although nano mineral filters are often highlighted for their UV attenuation performance, our data suggest the opposite trend under blue light exposure, with non-nano mineral formulations generally delivering higher BLPI than their nano equivalents.

Influence of tinting on BLPI

Across products, tinted formulations tended to rank among the highest BLPI values (Figure 11), indicating a positive, yet secondary, contribution of colouration compared with the predominant effect of mineral filters. Within mineral products, tinted variants clustered in the upper BLPI range. Among non-mineral formulations (organics and BL reflectors), the tinted product

TABLE 3 BLPI (mean $\pm$ SD) of nano and non-nano mineral filters and nano versus non-nano comparison.

Filter	Nano (BLPI $\pm$ SD)	Non-nano (BLPI $\pm$ SD)	<i>z</i>	<i>p</i> -Value	Significance
TiO ₂	26.4 $\pm$ 5.44	33.6 $\pm$ 2.67	1.19	0.2348	ns
ZnO	36.5 $\pm$ 6.83	53.4 $\pm$ 4.70	2.04	0.0415	*
Mix (TiO ₂ + ZnO)	54.0 $\pm$ 5.52	75.6 $\pm$ 4.58	3.01	0.0026	**

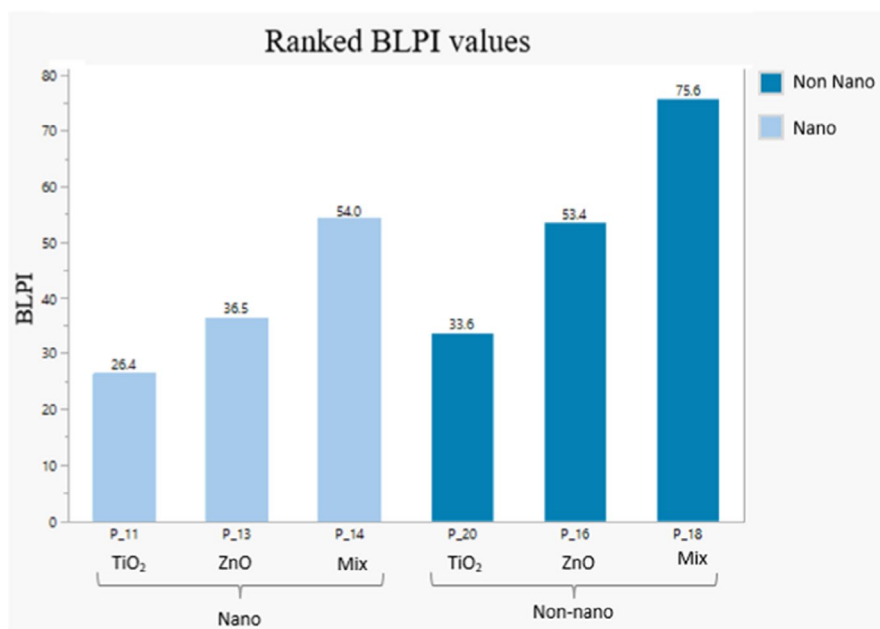
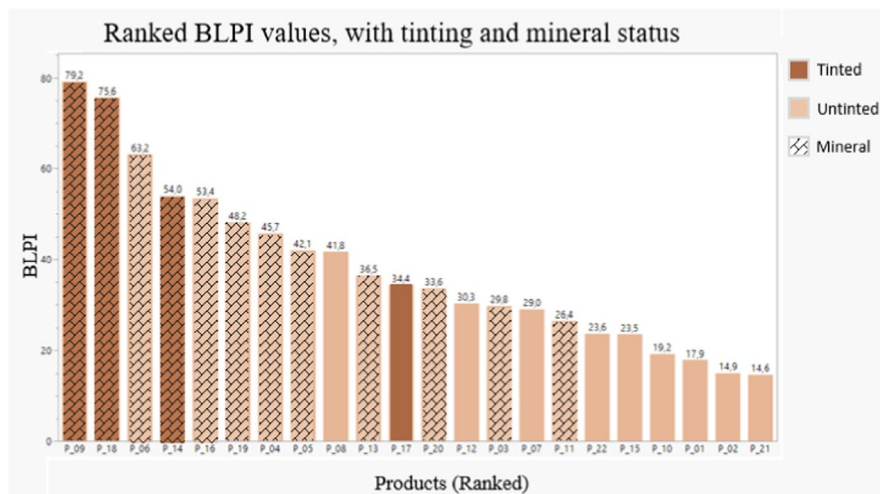
Note: * $p < 0.05$; ** $p < 0.01$.

TABLE 4 Intragroup comparisons of BLPI among mineral filters.

Nano formulations				Non-nano formulations			
Comparison	<i>z</i>	<i>p</i> -Value	Significance	Comparison	<i>z</i>	<i>p</i> -Value	Significance
TiO ₂ versus ZnO	1.16	0.2474	ns	TiO ₂ versus ZnO	3.66	0.0002	***
TiO ₂ versus Mix	3.56	0.0004	***	TiO ₂ versus Mix	7.92	<0.0001	***
ZnO versus Mix	1.99	0.0463	*	ZnO versus Mix	3.38	0.0007	***

Note: * $p < 0.05$; *** $p < 0.001$.

FIGURE 10 BLPI values for mineral formulations. BLPI values for mineral products grouped by particle size (nano vs. non-nano) and filter type (TiO₂, ZnO or TiO₂ + ZnO). Non-nano formulations show higher BLPI than nano equivalents, and the TiO₂ + ZnO mix displays the highest protection.

**FIGURE 11** Ranked BLPI by product; colours indicate tinted versus non-tinted; hatching denotes mineral filters.

also ranked among the most effective. These findings are descriptive and should be interpreted as exploratory rather than inferential.

Concentration dependence: benzotriazole

Three formulations containing methylene bis-benzotriazolyl tetramethylbutylphenol (MBBT; Bisotriazole) in nano form, prepared on the same base and containing no additional UV filters, were compared at 2%, 4% and 6% (Table 5 and Figure 12). BLPI values increased monotonically with concentration ($14.6 \rightarrow 19.2 \rightarrow 23.5$); however, pairwise comparisons (2% vs. 4%, 2% vs. 6%, 4% vs. 6%) did not reach statistical significance ($z=0.51-0.96$; $p=0.3386-0.6105$). Given the limited sample size and relatively high variability in two of the formulations, this observation should be considered descriptive and non-significant. The directional consistency of the trend is merely suggestive and indicates that, under appropriate conditions, the method may be sensitive to concentration-dependent differences. To improve robustness, additional testing with larger sample sizes and replicate batches would be required before drawing any dose-response conclusions.

Comparison with a published method

Four products were evaluated using a multi-day Blue Light Protection Factor (BPF) method described by Zhang et al. [18], which employs a broad band blue light source (400–500 nm, peak = 416 nm) and four consecutive daily exposures at 3/4 Minimal Persistent Pigmentary Dose (MPPD). Protection is derived from the slope of daily ITA° changes versus an untreated control.

Despite differences in exposure regimen and read-out strategy (Table 6), BLPI rankings were qualitatively concordant with BPF outcomes for the four products tested (Table 7 and Figure 13). The magnitude of protection and relative ordering were equivalent, indicating that the single-session IPD-based BLPI captures biologically meaningful differences that are also reflected in a more complex, cumulative protocol.

DISCUSSION

This study introduces a simplified in vivo approach to quantify the protection provided by cosmetic products against blue light-induced immediate pigmentation. The method yielded a broad dynamic range of BLPI values across 22 formulations and revealed formulation-dependent trends that are biologically coherent. These results demonstrate that a single-session IPD-based protocol can serve as a robust and discriminative tool for evaluating blue light protection.

A first key observation is the amplitude of BLPI values, which reflects meaningful performance differences between formulations. Repeatability was satisfactory for most products, with median CV values around 10%. As expected, the highest variability was observed among low-performing products, whose pigmentation responses lie close to the untreated condition. In this region, small fluctuations in ITA readings or skin response translate proportionally into higher uncertainty. In contrast, high-performing formulations combined elevated BLPI values with narrower dispersion, supporting the sensitivity of the method for identifying effective blue light protection.

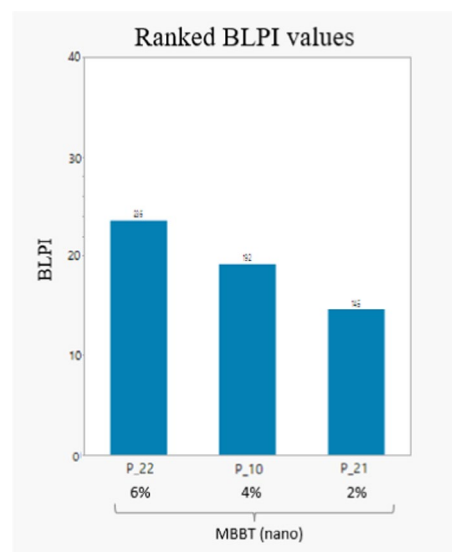


FIGURE 12 BLPI values across increasing benzotriazole concentrations. Products containing higher benzotriazole levels show higher BLPI values, indicating a concentration-dependent increase in blue light protection.

TABLE 5 Dose-response effect of filter concentration on BLPI.

Filter	Concentration	BLPI $\pm$ SD	Comparison	z	p -Value	Significance
MBBT (nano)	2%	14.6 ± 4.53	2% versus 4%	0.96	0.3386	ns
MBBT (nano)	4%	19.2 ± 1.46	2% versus 6%	0.93	0.3532	ns
MBBT (nano)	6%	23.6 ± 8.53	4% versus 8%	0.51	0.6105	ns

TABLE 6 Comparison of the BLPI protocol and the BPF published multi-day method.

Item	Studied protocol (BLPI)	Reference protocol (BPF)
Protection factor	BLPI (mean % attenuation of immediate pigmentation over $ITA_0 = 10-40^\circ$)	BPF (angle-based factor from daily pigmentation change versus control) [europmc.org]
Light source (λ)	LED 420 nm (monochromatic)	Blue LED 400–500 nm, peak = 416 nm [europmc.org]
Dose/Pattern	1 exposure, 80 J cm^2 (IPD in the absence of erythema)	4 exposures on 4 consecutive days, each at 3/4 MPPD (individual dose) [europmc.org]
Measured response	Immediate pigmentation (IPD)	Cumulative pigmentation over days (PPD-like)
Read-out-metric	Percent reduction of ($ITA_0 - ITA_{imm}$) versus control, averaged across ITA_0 grid	Angle (θ) of linear fit of daily ΔL^* , ΔM , ΔITA ; $BPF = \theta_{\text{control}}$ to θ_{sample}
Data model	Linear regressions $ITA_{imm} = a + b - ITA_0$ (product and control)	Linear fit of daily pigmentation deltas (day 0 $\rightarrow$ 4) per site
Normalization/range	Integration on $ITA_0 = 10-40^\circ$ (step 0.5°)	Baseline-to-day deltas; comparison to mean control angle
No. of visits/volunteer	1	4 days (+MPPD determination at baseline)
No. of exposures/volunteer	1	4 (1/day) at 3/4 MPPD [europmc.org]
Panel (reported)	$ITA_0 - 5 \rightarrow 45^\circ$ (phototype III–IV)	30 adults, Fitzpatrick III–IV

Note: Summary of the main methodological differences between the present BLPI protocol and the published BPF method, including exposure conditions, read-out metrics and modelling approaches.

TABLE 7 Comparison of BLPI and BPF (ΔITA°) values for the four products tested in parallel.

Product	BLPI	BPF (ΔITA)
P_04	45.7	47.9
P_07	29.0	32.6
P_03	29.8	31.9
P_12	30.3	31.1

Note: Side-by-side comparison of BLPI and BPF (ΔITA°) values for the four products using the two methods.

Clear and consistent formulation dependent trends emerged. Mineral-based systems systematically outperformed organic-only formulations. Within mineral filters, non-nano materials tended to produce higher BLPI values than nano-sized equivalents, an effect opposite to what is typically observed under UV, but fully plausible in the visible range where scattering by larger particles is stronger. Differences between TiO_2 and ZnO-based products must be interpreted cautiously, as they were tested at different concentrations. Moreover, the performance of inorganic filters showed an additional cumulative effect: formulations combining TiO_2 and ZnO consistently produced higher BLPI values than

those containing either mineral alone. ZnO-rich and $TiO_2 + ZnO$ mixed formulations frequently produced the highest BLPI values.

Across the tested panel, tinted formulations tended to rank higher in BLPI; however, this observation is descriptive and secondary to the stronger effect of mineral filters and should be interpreted as exploratory rather than inferential.

The comparison with the multi-day protocol of Zhang et al. provides important external validation. Despite major differences in exposure design, single session versus cumulative four-day regimen, narrowband versus broadband source and immediate versus delayed pigmentation endpoints, both methods produced BLPI/BPF values of similar magnitude and identical ranking for the four formulations tested. This convergence strongly suggests that the simplified approach captures a biologically relevant component of the skin's visible light response and can differentiate formulations in a manner consistent with longer, more demanding protocols.

Several methodological considerations merit discussion. The BLPI was calculated over the $ITA_0 = 10-40^\circ$ interval, which represents the zone where blue light-induced pigmentation is most responsive and most reproducible. At higher ITA_0 (lighter skin tones), pigmentary changes tend to saturate early, while very low ITA_0 values (darker tones)

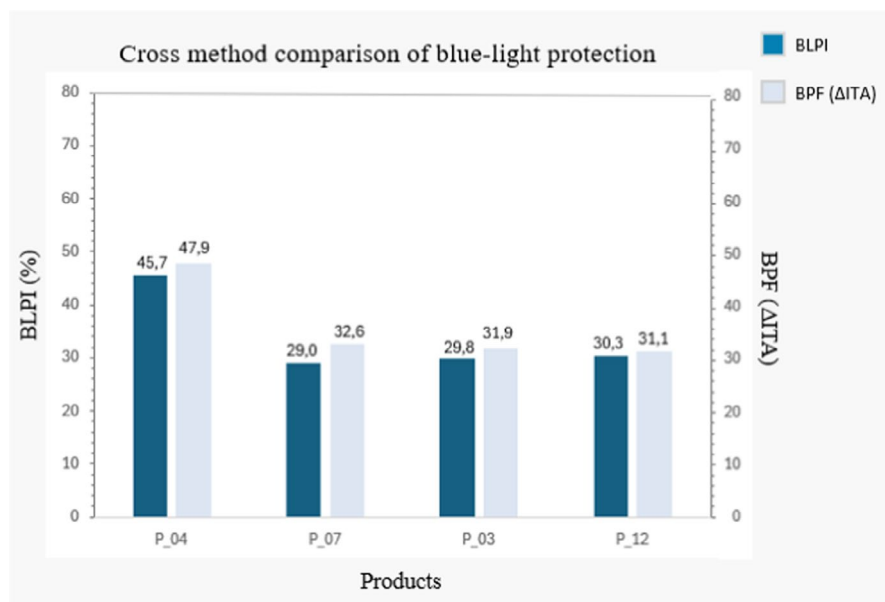


FIGURE 13 Cross-method comparison of bluelight protection. BLPI and BPF(Δ ITA $^\circ$) values for four products are shown side-by-side for visual comparison. The two metrics reflect different methods and are not expressed in the same unit.

produce smaller incremental IPD responses, both situations increasing model uncertainty. Importantly, this interval also corresponds to the phototypes predominantly assessed in the multi-day published protocol (III–IV), supporting methodological coherence between the two approaches and facilitating comparison of protection levels across methods. Focusing the calculation on the 10–40° interval maximizes biological discriminability while limiting prediction variance. Standardizing this interval also enhances future comparability of BLPI results across studies or laboratories.

Focusing on immediate pigmentation darkening refines the biological scope toward early visible light responses, while intentionally avoiding the variability introduced by multi-day designs. However, this choice was deliberate: a single-session design eliminates multi-day variability related to repositioning, skin condition changes or volunteer compliance, while substantially reducing operational time and cost. Concentrating all measurements within one controlled session also minimizes geometric drift and strengthens the internal consistency of ITA measurements. The strong agreement with the multi-day protocol indicates that this operational simplification does not compromise the method's capacity to detect meaningful differences between formulations.

Future work may explore larger formulation panels, increased sampling for low-performing products or statistical approaches better accommodating heteroscedasticity. It would also be relevant to investigate the link between IPD and longer-term biological pathways, including melanogenesis markers or oxidative stress signatures, to clarify how immediate responses relate to sustained pigmentation under visible light.

Taken together, these results demonstrate that the proposed single-session IPD-based method offers a fast,

reproducible and discriminative way to assess blue light protection, while maintaining strong biological coherence with published multi day protocols.

CONCLUSION

This work presents a rapid and simplified in vivo method that quantifies blue light protection through ITA-based immediate pigmentation darkening. Applied to a diverse set of 22 formulations, the method generated a wide discriminative range, captured expected formulation-dependent trends and showed outcomes consistent with a published multi-day protocol. By condensing the assessment into a single exposure session, the approach offers clear operational advantages without compromising biological relevance.

The method provides a practical and efficient screening tool for early-stage formulation development and represents a promising alternative when rapid evaluation is required. With further validation across additional products, phototypes and laboratories, this simplified approach could contribute to future methodological alignment in the assessment of blue light protection.

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The authors have nothing to report.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The

data are not publicly available due to privacy or ethical restrictions.

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