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CLINICAL-INSTRUMENTAL EVALUATION OF THE EFFICACY OF A COSMETIC PRODUCT FOR THE EYE CONTOUR

PROFESSIONAL DIETETICS S.P.A.
NUTRAKOS EYE CONTOUR GEL

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com

Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

Sede legale: Via Guido Rossa, 1 20024 Garbagnate M.se (MI) Italy, I P.I./C.F. 11093320155
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L'elenco delle prove accreditate è consultabile su www.accredia.it

KEY PERSONNEL

Customer

PROFESSIONAL DIETETICS S.P.A.
Via Ciro Menotti 1/A
20129 MILANO (MI)
ITALY

Principal Experimenter

Dr. **Enza CESTONE**
Degree in Medicine and Surgery, Specialist in Dermatology and Venereology
Consultant Complife Italia S.r.l.

Study Director

Dr. **Valentina ZANOLETTI**
Chemical and pharmaceutical technologist
Complife Italia S.r.l.

Data analysis and Report

Dr. **Eleonora SPARTA'**
Biologist
Complife Italia S.r.l.

Complife Italia S.r.l

Complife Italia S.r.l
Via Angelini, 21
27028 San Martino Siccomario (PV)
tel. +39-0382 25504 - fax +39-0382 536006
Mail: info@complifegroup.com

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
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GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
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complifegroup.com
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STUDY DESIGN

1.1 Title

Clinical-instrumental evaluation of the efficacy of a cosmetic product for the eye contour.

1.2. Aim of the study

Aim of the study is to assess the efficacy of a cosmetic product for the eye contour in decreasing eye contour wrinkles, dark circles and eyebags visibility, together with its efficacy in improving skin moisturizing, firmness, elasticity and radiance/brightness. In order to reach this goal an instrumental study is carried out on 40 female subjects aged over 40 years old, clinically showing slight to moderate wrinkledness in the eye contour area (50%), bags under eyes (50%) and dark circles (50%). Clinical and instrumental evaluations are performed at baseline (T0) and after 28 days of product use (T28); moreover the study is integrated with the self-assessment questionnaire filled in by the volunteers.

1.3. Tested product

1.3.1. Information provided by the Customer

- ☒ Product name: **NUTRAKOS EYE CONTOUR GEL**
- ☒ The tested cosmetic product conforms to REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30th November 2009 on cosmetic products (recast) (Text with EEA relevance) and to its annexes.
- ☒ Way of use: apply in the eye contour area, gently massaging until complete absorption. Apply twice daily (morning and evening) after cleansing.
- ☒ Qualitative INCI formula: AQUA, GLYCERIN, GLYCINE, SODIUM POLYACRYLATE, PROLINE, ALANINE, PEG-40 HYDROGENATED CASTOR OIL, PHENOXYETHANOL, VALINE, PEG/ PPG-20/6 DIMETHICONE, CITRONELLYL METHYLCROTONATE, PROPYLENE GLYCOL, SODIUM HYALURONATE, PPG-26-BUTETH-26, AMMONIUM ACRYLOYLDIMETHYLTAURATE/VP COPOLYMER, POLYGLYCERYL-10 EICOSANEDIOATE/TETRADECANEDIOATE, CAPRYLYL GLYCOL, 1,2-HEXANEDIOL, LEUCINE, ALBIZIA JULIBRISSIN BARK EXTRACT, LYSINE HCI, DISODIUM EDTA, PARFUM, DARUTOSIDE (SIEGESBECKIA ORIENTALIS EXTRACT).

1.4. Ethical requirements

The study is carried out in compliance with the following ethical requirements:

1. All the subjects participating in the study are healthy volunteers of at least 18 years old.
2. All the subjects participating in the study, were selected with the supervision of a dermatologist according to inclusion/non-inclusion criteria.
3. The subjects' participation in the study was free.
4. All the subjects participating in the study were informed of the aim and the design of the study.
5. All the subjects participating in the study were informed of the possible risk involved in the study execution.
6. All the subjects participating in the study gave their informed consent signed at the beginning of the study.
7. Before the volunteers will be exposed to the product to be tested, all relevant safety information about the product itself and each ingredient are collected and evaluated.
8. All the study procedures are carried out in accordance with the ethical principles for the medical research (Ethical Principles for Medical Research Involving Human Subjects, adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and successive amendments).
9. All the precautions shall be taken in consideration to avoid excessive skin reactions.
10. In case of non-expected/adverse skin reaction occurrence, the medical experimenter will evaluate the severity of the reaction (reporting it in the data collecting sheet) and consequently he will proceed with the appropriate therapy.

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1.5. Subjects selection

The subjects participating in the study are selected under the supervision of a board-certified Dermatologist from a panel of healthy female subjects in accordance with the following inclusion and non-inclusion criteria.

1.5.1. Inclusion criteria

- ☑ Healthy female subjects
- ☑ Aged over 40 years old
- ☑ Caucasian ethnicity
- ☑ 50% of the subjects clinically showing slight to moderate wrinkledness in the eye contour area
- ☑ 50% of the subjects showing bags under eyes
- ☑ 50% of the subjects showing dark circles
- ☑ Subjects who are not recently involved in similar studies
- ☑ Agreement not to use products with the same characteristics as those of the tested product throughout the study duration
- ☑ Agreement not to make any changes to the normal everyday routine
- ☑ Agreement to strictly follow the informative form
- ☑ Absence of skin diseases
- ☑ Subjects aware of the test procedure and having signed an informed consent form.

1.5.2. Non-inclusion criteria

- ✘ Subjects who don't fit the inclusion criteria
- ✘ Pregnant or breastfeeding women
- ✘ Former positive history of allergy or sensitivity to cosmetics, sunscreens and/or topical medications
- ✘ Subjects under both locally and systemically pharmacological treatment (if this condition interferes with the test execution)
- ✘ Subjects with dermatological problems in the test area
- ✘ Positive anamnesis for atopy or hypersensitive skin (if this condition interferes with the test execution)

1.6. Study design

The study is carried out as follow:

- **T0**: enrolment of 40 subjects according to inclusion/non-inclusion criteria.
  Instrumental evaluation of skin moisturization, skin elasticity/firmness, skin radiance/brightness, wrinkle depth, skin smoothness; clinical evaluation of wrinkles visibility → 20 out 40 subjects.
  Instrumental evaluation of eyebags volume; clinical evaluation of eyebags visibility → 20 out 40 subjects.
  Instrumental evaluation of dark circles colour; clinical evaluation of dark circles visibility → 20 out 40 subjects.
- From T1 to T28: daily product application according to provided instructions.
- **T28:** Clinical and instrumental evaluation of the above mentioned parameters after 28 days of product use. Moreover, at the end of the study, all the enrolled subjects (n=40) are asked to express their opinion on tested product by answering to a questionnaire.

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1.7. Materials and methods

Here below the parameters monitored during the study are reported. The instrumental evaluations are carried out in a temperature and humidity-controlled environment (respectively T= 18-26°C and RH= 50±10%).
Subjects, before each visit, observes a 15-20-minute acclimatization period in these conditions.

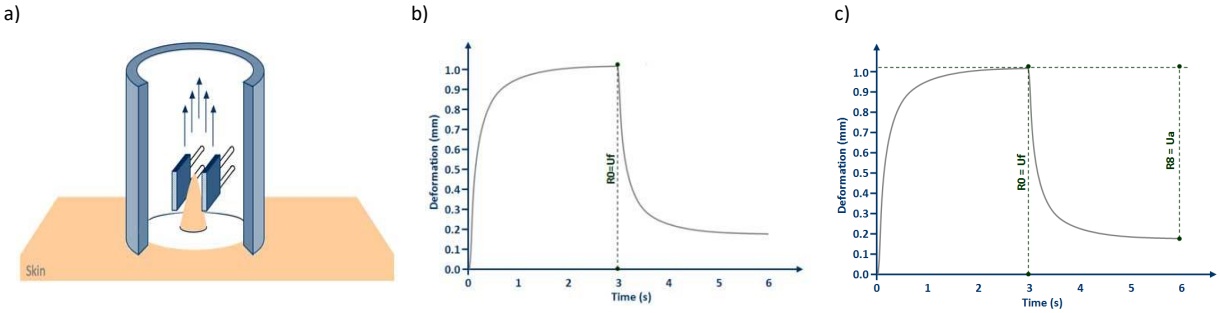
1.7.1. Skin moisturization

Skin moisturizing is evaluated by means of Corneometer® measurement. This measurement is based on the completely different dielectric constant of water (81) and other substances (mostly < 7). The measuring capacitor shows changes of capacitance according to the moisture content of the skin. A metallic lamina separates the metallic tracks (gold) in the probe head from the skin in order to prevent current conduction in the measured area. An electric field between the tracks with alternating attraction develops. One track builds up a surplus of electrons (minus charge) the other a lack of electrons (plus charge). The scatterfield penetrates the very first layer of the skin (10-20 µm) during the measurement and the capacitance is determined.

1.7.2. Skin elasticity

The measurement of skin elasticity is based on the suction method using a negative pressure mechanically deforming the skin (Cutometer® method). A Negative pressure (450 mbar) is created in the device and the skin was drawn into the aperture of the probe for 2 seconds and after a defined time (2 seconds) released again. Inside the probe, the penetration depth is determined by a non-contact optical measuring system. The optical measuring system consists of a light source and a light receptor, as well as two prisms facing each other, which project the light from transmitter to receptor. The light intensity varies due to the penetration depth of the skin. The resistance of the skin to the negative pressure and its ability to return into its original position are displayed as curves (penetration depth in mm/time) in real time during the measurement. The used device is the Cutometer® MPA 580 (Courage+Khazaka, electronic GmbH). In this study, R0 (skin firmness) and R2 (overall skin elasticity) parameters (Box 1) are measured.

Box 1 - Skin elasticity measurement. a) Schematic representation of the measurement process. (b) R0 (skin distensibility) represents the passive behaviour of the skin to force (i.e. gravity). Conceptually R0 parameter is correlated to skin firmness. (c) R2 (Ua/Uf, gross elasticity or overall elasticity) represents the ability of the skin to return to its basal state.



1.7.3. Skin radiance/brightness

The skin radiance (or skin brightness), is the ability of the skin to reflect the light and it is measured using the gloss parameter taken using the spectrophotometer/colorimeter CM-700D (Konica-Minolta). The instrument emits diffuse light that reaches the skin through an opening located at the extreme of the lighting sphere. A sensor located at 8 ° compared to the vertical axis of the opening detects then the reflected light and calculates a parameter known as "gloss". The gloss value is used in the management of the brilliance of the colour.
For further information on the principle of the measurement and data analysis see box 2.

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complifeitalia@legalmail.it
complifegroup.com
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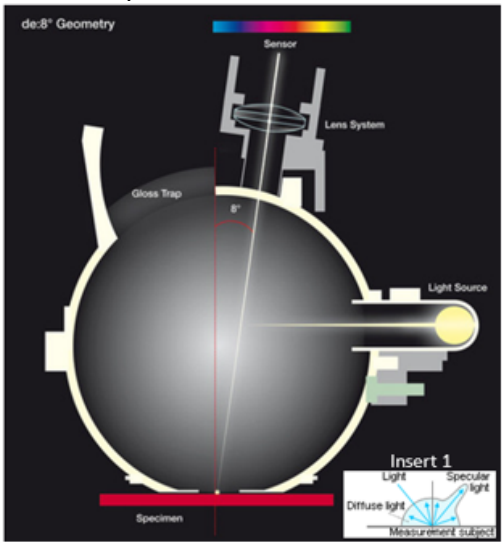
Box 2 – Gloss parameter

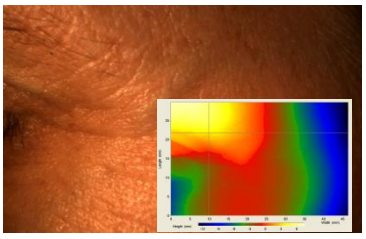


When light reach a surface it is reflected at the equal but opposite angle from the light source; this is called specularly reflected light. This specular component is reflected as if reflected by a mirror. The light that is not specularly reflected, but scattered in many directions, is called diffuse reflectance (insert 1). The sum of the specular reflectance plus the diffuse reflectance is called the total reflectance. For objects with shiny surfaces, the specularly reflected light is relatively strong and the diffused light is weaker. On rough surfaces with a low gloss, the specular component is weak and the diffused light is stronger. The measuring geometry d: 8° features an optical device which provides diffuse illumination (Ulbricht sphere). The light (Xenon lamp) is projected into a sphere. The interior of the sphere is coated with a white highly reflecting substance (barium sulphate, ceramic, special plastic) which reflects the light manifold. A shutter, an optical element inside the sphere, prevents the directional rays from reaching the measuring sample directly. The sample is positioned at an opening of the sphere and is illuminated from all directions with a close to perfect diffuse light. Through an opening at the top of the sphere the sensor is viewing the surface being measured with an angle of 8° to the vertical. In order to prevent reflection of specular light from the sample surface, the instrument feature a gloss trap. When the trap which is arranged with an angle of -8° to the viewing opening, is open, the light which would otherwise be reflected from the interior wall of the sphere, will be eliminated and can therefore not Illuminate the sample. The relation between directional and diffuse reflection allows calculating the gloss component. The measuring system including gloss is named di: 8° whilst the measuring system excluding gloss is described as de: 8°.

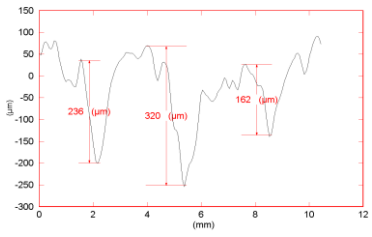
1.7.4. Skin profilometry

Skin surface is quantitatively assessed by Primos 3D (GFMesstechnik GmbH). Primos 3D is a non-contact in vivo skin measurement device based on structured light projection. In conjunction with a comprehensive 3-D measurement and evaluation software, the sensor allows to evaluate skin surface properties (i.e. wrinkle depth, volume, skin roughness, etc.). In this study periocular wrinkle depth, periocular skin smoothness (Sa parameter) and eye bags volume are evaluated. For further information see box 3.

Box 3. Skin profilometry by means of Primos 3D analysis



The technique. Primos 3D is a 3D scanner that create a point cloud (set of vertices in a three-dimensional coordinate system) of geometric samples on the surface of the object to be measured. These points are then used to extrapolate the shape of the object (a process called reconstruction). Like cameras, 3D-scanner has a cone-like field of view, and like cameras, they can only collect information about surfaces that are not obscured. While a camera collects color information about surfaces within its field of view, 3D scanners collect distance information about surfaces within its field of view. The "picture" produced by a 3D scanner describes the distance of a surface at each point in the picture (see the image in the insert).



Calculation of wrinkle depth. It is calculated the height of wrinkles in the sampling length. This calculation is done on the sectional picture (wrinkle depth vs. section).

$$Sa = \frac{1}{nx * ny} * \sum_{i=1}^{nx} \sum_{j=1}^{ny} R(x_i, y_j)$$

Calculation of skin roughness. The surface roughness is calculated through the arithmetic roughness (Sa parameter). Sa is the arithmetic mean of the surface roughness and it is a vertical parameter. This means it describes the roughness in vertical direction. The parameter is used to evaluate the surface smoothness.

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
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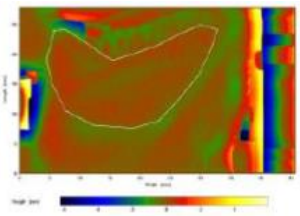
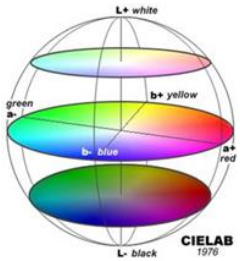
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504



Calculation of eye bags volume. The technique allows to: a) take a high resolution image of the skin, b) take a 3 dimensional image and c) analyze by means of image analysis the profilometrical information of the 3D image.

1.7.5. Dark circles colour

The colorimetric evaluation of dark circles colour is performed by means of image analysis carried out on Visia®-CR (Canfield Scientific) digital images.



Red (Δa) and blue (Δb) components of the skin are measured using a software dedicated for colorimetric image analysis, in the chromatic CIELAB space (parameters a* and b*). The measurements are performed in a defined region of interest (ROI). All the measurements are acquired in the same ROI, under standards light conditions.

1.7.6. Clinical evaluations

The decrease of skin wrinkledness, eyebags puffiness and dark circles colour is clinically evaluated by the dermatologist according to the clinical scores reported respectively in box 4, 5 and 6.

- **SKIN WRINKLEDNESS**

Box 4a. Classification of skin wrinkledness at T0	Score	Box 4b. Variation at T28 vs T0	Score
No wrinkle. No visible wrinkles; continuous skin line.	**0**	No variation.	**1**
Very shallow yet visible wrinkles.	**0.5**	Slight improvement.	**2**
Fine wrinkle. Visible wrinkles and slight indentation.	**1**	Moderate improvement.	**3**
Visible wrinkles and clear indentation	**1.5**	Remarkable improvement.	**4**
Moderate wrinkles. Clearly visible wrinkles.	**2**		
Prominent and visible wrinkles.	**2.5**		
Deep wrinkles. Deep wrinkles and furrows.	**3**		

- **EYE BAGS PUFFINESS**

Box 5a. Classification of eye bags puffiness at T0	Score	Box 5b. Variation at T28 vs T0	Score
Eyebags are very swollen	**3**	No variation.	**1**
Eyebags are slightly swollen	**2**	Slight improvement.	**2**
Eyebags are not swollen	**1**	Moderate improvement.	**3**
		Remarkable improvement.	**4**

- **DARK CIRCLES COLOUR**

Box 6a. Classification of dark circles colour at T0	Score	Box 6b. Variation at T28 vs T0	Score
Under the eye circles are very dark	**3**	No variation.	**1**
Under the eye circles are slightly dark	**2**	Slight improvement.	**2**
The palpebral skin colour is normal	**1**	Moderate improvement.	**3**
		Remarkable improvement.	**4**

1.7.8. Self-assessment

At the end of the study subjects are asked to express their opinion on tested product by answering to a self-assessment questionnaire.

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1.8. Results and statistics

1.8.1. Results

The results are reported in their respective unit in tables:

1) The mean values are calculated as:

$$m = \frac{\sum_{1}^{n} p}{n}$$

where:
n is volunteers' number and p is the value of the parameter under analysis.

2) The percentage variations obtained for each volunteer are calculated as:

$$\text{var.}(\%)_i = \frac{T_{xi} - T_{0i}}{T_{0i}}$$

where:
T_{xi} is the individual value of the parameter at the x time
T_{oi} is the individual value of the parameter at baseline

3) The mean percentage variations are calculated as:

$$\text{var.}(\%) = \sum_{1}^{n} \text{var}(\%)_i$$

4) The mean standard error of data is calculated as:

$$SEM. = \frac{\sqrt{\sum_{1}^{n}(p_i^2) - \frac{\sum_{1}^{n} p_i^2}{n}}}{\sqrt{n}}$$

All the calculations are done using a Microsoft® Excel worksheet.

The results of self-assessment questionnaire are calculated as percentage (%) of subjects who assigned a determined judgment (among those proposed). For each question, the number of subjects related to each judgment is counted → (number of subjects) and this number is then divided by the total number of subjects → % of answers.

1.8.2. Statistical analysis

The instrumental data are submitted to the 2-way Student's test t for paired data. The variations are considered statistically significant when the p value is <0.05. Statistical analysis is carried out using a Microsoft® Excel 2013 (vers. 15.0.4815.1001; Microsoft, USA) worksheet running on Microsoft® Windows 10 Professional (Microsoft, USA).

1.8.3. Interpretation of results

The study here above reported was designed to demonstrate the test product claim(s) in the current framework proposed by Commission Regulation (EU) No 655/2013. Endpoints are measured using techniques currently accepted in the cosmetic field while biases are minimized by procedure(s) standardization according to ISO 9001 Quality Management System. Data are analysed and interpreted by skilled technician according to both descriptive and inferential statistical analysis procedures. Due to the lack of reference values in the cosmetic field, statistical significance (for instrumental analysis) and percentage of subjects showing an effect (for clinical/sensorial endpoints) are the primary criterion to evaluate the correspondence between the proposed claim(s) and the study output(s). In particular, intragroup (vs. T0) or intergroup (eg. active vs. placebo, treated vs not treated) statistical analysis criterion to reject the null hypothesis (no product effect) is set at p<0.05. For clinical evaluations, the positive effect of the product on the measured parameter is confirmed if more than 50% of the subjects register an improvement. Finally, for the self-assessment questionnaires, the performance and the pleasantness of the product must be perceived by at least 60% of the subjects. Whenever reference values or threshold values exists those values are used to validate product claim(s).

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
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1.9. Start/end date of study

The table here below shows date of beginning and end of the study.

Start date	End date
09/06/2021	17/11/2021

1.10. Report change record

The table here below reports the change log of all approved changes made to the document that make up the course after initial approval.

Rev. no	Date	Description
00	05/08/2021	First release
01	10/08/2021	Modified product commercial name. Digital pictures added.
02	17/11/2021	20 more subjects added.
02	29/11/2021	Version A: efficacy study

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
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RESULTS AND GRAPHS
PANEL DEMOGRAPHY

TABLE 0: The table below summarize the evaluated parameters for each subject participating in the study. Self-assessment questionnaire has been submitted to 40 out 40 subjects.

Volunteer no		AGE	EVALUATED PARAMETER
01	F3656S	63	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND DARK CIRCLES**.
02	R1927M	59	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND DARK CIRCLES**.
03	P2126A	62	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND DARK CIRCLES**.
04	P1492V	58	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND DARK CIRCLES**.
05	C0074M	56	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND DARK CIRCLES**.
06	D0097E	57	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND DARK CIRCLES**.
07	F2070C	60	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND DARK CIRCLES**.
08	A4003S	52	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND DARK CIRCLES**.
09	F4631M	47	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND DARK CIRCLES**.
10	V3024C	58	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND DARK CIRCLES**.
11	M3661M	64	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND EYE BAGS***.
12	R1784M	61	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND EYE BAGS***.
13	P2181M	59	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND EYE BAGS***.
14	G0587G	61	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND EYE BAGS***.
15	M3265G	59	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND EYE BAGS***.
16	D2925G	53	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND EYE BAGS***.
17	V2130R	55	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND EYE BAGS***.
18	V2827L	65	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND EYE BAGS***.
19	T4522I	65	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND EYE BAGS***.
20	T4004E	54	MOISTURIZATION, ELASTICITY, RADIANCE, WRINKLES* AND EYE BAGS***.
21	F5051I	49	DARK CIRCLES**
22	M5087A	50	DARK CIRCLES**
23	C5263N	57	DARK CIRCLES**
24	N5078M	47	DARK CIRCLES**
25	M5118V	46	DARK CIRCLES**
26	M5083S	55	DARK CIRCLES**
27	M5126M	51	DARK CIRCLES**
28	B5101F	51	DARK CIRCLES**
29	E5122A	49	DARK CIRCLES**
30	B5580A	51	DARK CIRCLES**
31	C5071M	45	EYE BAGS***
32	L4924M	55	EYE BAGS***
33	T5202F	60	EYE BAGS***
34	F5119F	52	EYE BAGS***
35	L5748M	53	EYE BAGS***
36	G5674V	43	EYE BAGS***
37	R5121M	54	EYE BAGS***
38	B5102L	42	EYE BAGS***
39	P5166A	54	EYE BAGS***
40	M5326D	64	EYE BAGS***
	Mean	**54,9**	**50% WRINKLES**
	Min	**42**	**50% DARK CIRCLES**
	Max	**65**	**50% EYEBAGS**

*WRINLES: intrumental analysis of skin profilometry, clinical analysis of wrinkles visibility
**DARK CIRCLES: instrumental analysis of dark circles colour, clinical analysis of dark circles visibility
*** EYE BAGS: instrumental analysis of eye bags volume, clinical analysis of eye bags visibility

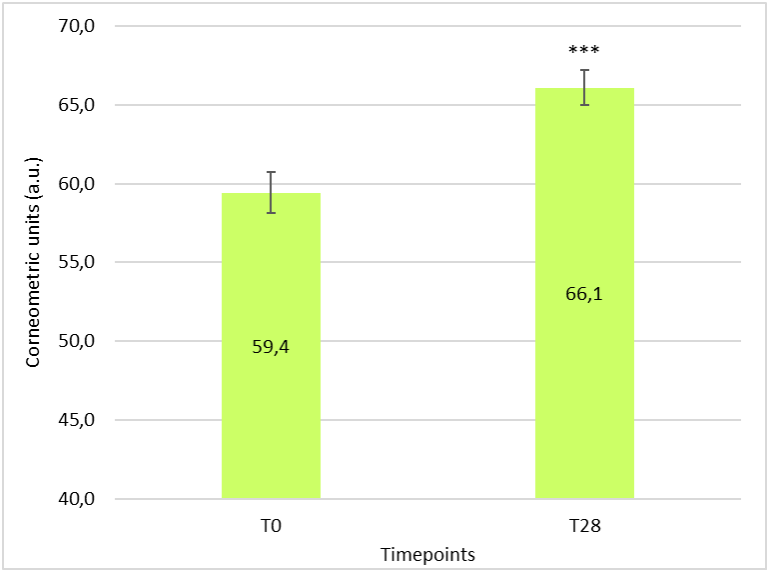
COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

SKIN MOISTURIZATION

TABLE 1: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed in corneometric units (a.u.).

n	Vol ID	T0	T28	% VARIATION VS. T0 T28
01	F3656S	54,4	64,7	18,9%
02	R1927M	60,3	74,5	23,5%
03	P2126A	52,6	66,3	26,0%
04	P1492V	57,7	64,7	12,1%
05	C0074M	66,3	65,6	-1,1%
06	D0097E	51,4	64,6	25,7%
07	F2070C	67,2	73,9	10,0%
08	A4003S	65,1	67,4	3,5%
09	F4631M	58,4	69,6	19,2%
10	V3024C	64,3	68,3	6,2%
11	M3661M	57,8	62,4	8,0%
12	R1784M	51,5	61,4	19,2%
13	P2181M	63,6	67,5	6,1%
14	G0587G	52,5	61,4	17,0%
15	M3265G	50,3	56,4	12,1%
16	D2925G	59,7	57,3	-4,0%
17	V2130R	69,4	71,7	3,3%
18	V2827L	59,5	67,5	13,4%
19	T4522I	65,8	72,9	10,8%
20	T4004E	60,7	63,5	4,6%
	Mean	59,4	66,1	11,7%
	SEM	1,3	1,1	Min -4,0%
	t-test vs. T0	--	0,000	Max 26,0%

GRAPH 1. The graph shows the mean data obtained at each monitored check for the parameter under analysis. Data are reported as mean ± SEM. Above the error bar the intragroup statistical analysis is reported as follows: *p<0.05; ** p<0.01; ***p<0.001.



COMMENT:
as it is possible to notice, tested product determines a statistically significant increase of skin moisturization by +11.7% after 28 days of use.

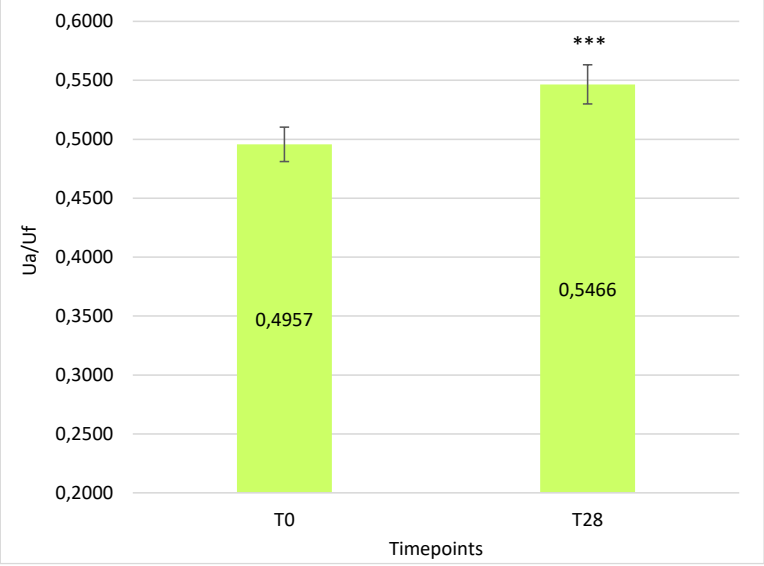
COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

SKIN ELASTICITY – R2 parameter

TABLE 2: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed as Ua/Uf.

n	Vol ID	T0	T28	% VARIATION VS. T0 T28
01	F3656S	0,6065	0,6455	6,4%
02	R1927M	0,4229	0,4632	9,5%
03	P2126A	0,6264	0,6435	2,7%
04	P1492V	0,4537	0,4900	8,0%
05	C0074M	0,5765	0,6436	11,6%
06	D0097E	0,4824	0,5094	5,6%
07	F2070C	0,5385	0,6047	12,3%
08	A4003S	0,5151	0,5925	15,0%
09	F4631M	0,4695	0,4900	4,4%
10	V3024C	0,4882	0,5760	18,0%
11	M3661M	0,4645	0,5354	15,3%
12	R1784M	0,4228	0,5222	23,5%
13	P2181M	0,3959	0,3952	-0,2%
14	G0587G	0,5528	0,5890	6,5%
15	M3265G	0,5228	0,5775	10,5%
16	D2925G	0,3864	0,4206	8,9%
17	V2130R	0,5160	0,5046	-2,2%
18	V2827L	0,4545	0,5164	13,6%
19	T4522I	0,4941	0,6398	29,5%
20	T4004E	0,5245	0,5721	9,1%
	Mean	0,4957	0,5466	10,4%
	SEM	0,0146	0,0165	Min -2,2%
	t-test vs. T0	--	0,000	Max 29,5%

GRAPH 2. The graph shows the mean data obtained at each monitored check for the parameter under analysis. Data are reported as mean ± SEM. Above the error bar the intragroup statistical analysis is reported as follows: *p<0.05; ** p<0.01; ***p<0.001.



COMMENT:
as it is possible to notice, tested product determines a statistically significant increase of skin elasticity (R2 parameter) by +10.4% after 28 days of use.

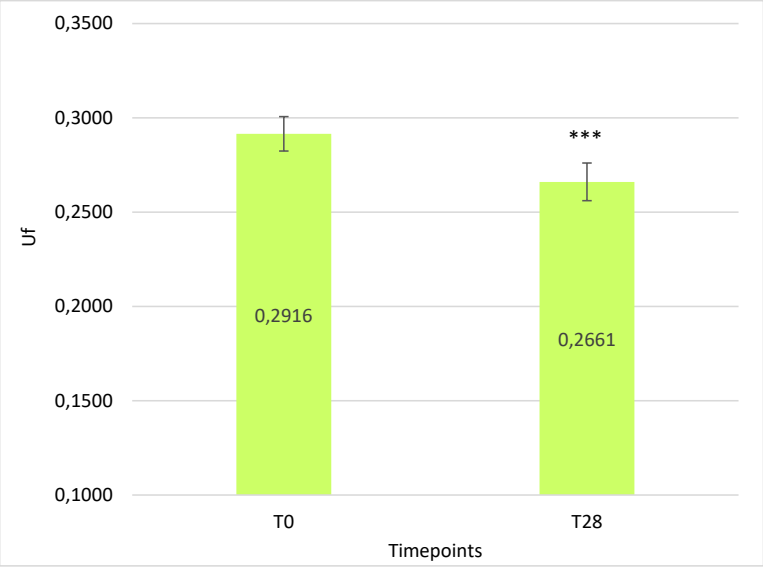
COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

SKIN ELASTICITY – R0 parameter

TABLE 3: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed as Uf.

n	Vol ID	T0	T28	% VARIATION VS. T0 T28
01	F3656S	0,3100	0,2860	-7,7%
02	R1927M	0,2940	0,2640	-10,2%
03	P2126A	0,2650	0,2480	-6,4%
04	P1492V	0,2830	0,2510	-11,3%
05	C0074M	0,2550	0,2020	-20,8%
06	D0097E	0,3400	0,3210	-5,6%
07	F2070C	0,2990	0,2540	-15,1%
08	A4003S	0,3650	0,3530	-3,3%
09	F4631M	0,2790	0,2510	-10,0%
10	V3024C	0,3380	0,3110	-8,0%
11	M3661M	0,2820	0,2590	-8,2%
12	R1784M	0,2720	0,2010	-26,1%
13	P2181M	0,2450	0,2430	-0,8%
14	G0587G	0,2840	0,2650	-6,7%
15	M3265G	0,1970	0,1820	-7,6%
16	D2925G	0,3640	0,3210	-11,8%
17	V2130R	0,3120	0,3290	5,4%
18	V2827L	0,3080	0,2810	-8,8%
19	T4522I	0,2530	0,2360	-6,7%
20	T4004E	0,2860	0,2630	-8,0%
	Mean	0,2916	0,2661	-8,9%
	SEM	0,0091	0,0100	
	t-test vs. T0	--	0,000	
			Min	-26,1%
			Max	5,4%

GRAPH 3. The graph shows the mean data obtained at each monitored check for the parameter under analysis. Data are reported as mean ± SEM. Above the error bar the intragroup statistical analysis is reported as follows: *p<0.05; ** p<0.01; ***p<0.001.



COMMENT:
as it is possible to notice, tested product determines a statistically significant decrease of R0 parameter by -8.9% after 28 days of use. A decrease of R0 parameter is related to an improvement of skin firmness and for marketing purposes this variation can be expressed in absolute value as an improvement of skin firmness by 8.9%.

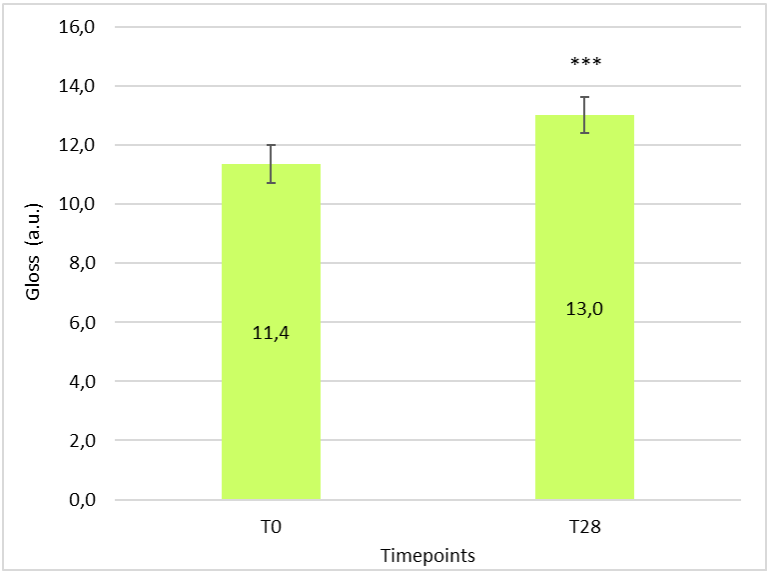
COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

SKIN RADIANCE/BRIGHNTESS - GLOSS PARAMETER

TABLE 4: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed as arbitrary units.

n	Vol ID	T0	T28	% VARIATION VS. T0 T28
01	F3656S	6,8	9,3	36,5%
02	R1927M	9,4	12,1	29,1%
03	P2126A	11,6	13,3	15,0%
04	P1492V	12,4	14,4	16,0%
05	C0074M	10,8	14,2	31,4%
06	D0097E	6,4	8,0	24,8%
07	F2070C	14,4	16,4	14,1%
08	A4003S	9,3	12,3	32,3%
09	F4631M	10,3	9,3	-10,1%
10	V3024C	10,3	14,8	44,1%
11	M3661M	8,2	8,7	5,8%
12	R1784M	10,1	11,8	16,6%
13	P2181M	14,6	13,4	-8,0%
14	G0587G	11,4	13,2	15,6%
15	M3265G	14,1	14,8	5,2%
16	D2925G	18,0	17,2	-4,5%
17	V2130R	14,2	15,4	8,4%
18	V2827L	13,3	15,6	17,5%
19	T4522I	9,3	10,2	9,1%
20	T4004E	12,7	16,0	26,4%
	Mean	11,4	13,0	16,3%
	SEM	0,6	0,6	Min -10,1%
	t-test vs. T0	--	0,000	Max 44,1%

GRAPH 4. The graph shows the mean data obtained at each monitored check for the parameter under analysis. Data are reported as mean ± SEM. Above the error bar the intragroup statistical analysis is reported as follows: *p<0.05; ** p<0.01; ***p<0.001.



COMMENT:
as it is possible to notice, tested product determines a statistically significant increase of skin radiance/brightness (gloss parameter) by +16.3% after 28 days of use.

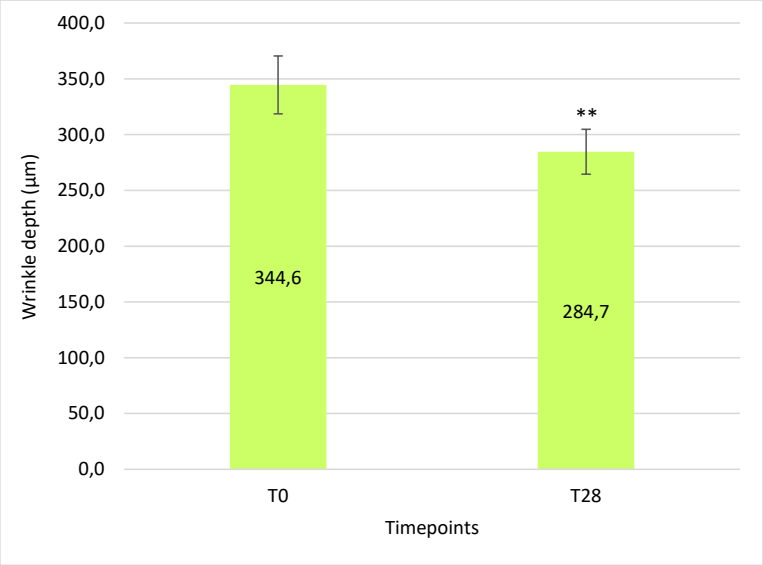
COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

SKIN PROFILOMETRY – PERIOCULAR WRINKLE DEPTH PARAMETER

TABLE 5: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed as µm.

n	Vol ID	T0	T28	% VARIATION VS. T0 T28
01	F3656S	331,5	295,3	-10,9%
02	R1927M	366,5	337,6	-7,9%
03	P2126A	329,2	247,6	-24,8%
04	P1492V	348,3	376,5	8,1%
05	C0074M	369,0	350,8	-4,9%
06	D0097E	342,8	278,6	-18,7%
07	F2070C	179,3	183,0	2,1%
08	A4003S	266,6	224,8	-15,7%
09	F4631M	150,8	123,0	-18,4%
10	V3024C	357,1	295,9	-17,1%
11	M3661M	355,9	347,1	-2,5%
12	R1784M	431,3	318,8	-26,1%
13	P2181M	441,0	269,3	-38,9%
14	G0587G	345,2	302,8	-12,3%
15	M3265G	434,8	468,6	7,8%
16	D2925G	231,3	169,4	-26,8%
17	V2130R	161,0	148,0	-8,1%
18	V2827L	654,0	422,0	-35,5%
19	T4522I	466,5	320,0	-31,4%
20	T4004E	329,5	214,5	-34,9%
	Mean	344,6	284,7	-15,8%
	SEM	25,9	20,1	Min -38,9%
	t-test vs. T0	--	0,001	Max 8,1%

GRAPH 5. The graph shows the mean data obtained at each monitored check for the parameter under analysis. Data are reported as mean ± SEM. Above the error bar the intragroup statistical analysis is reported as follows: *p<0.05; ** p<0.01; ***p<0.001.



COMMENT:
as it is possible to notice, tested product determines a statistically significant decrease of periocular wrinkle depth by -15.8% after 28 days of use.

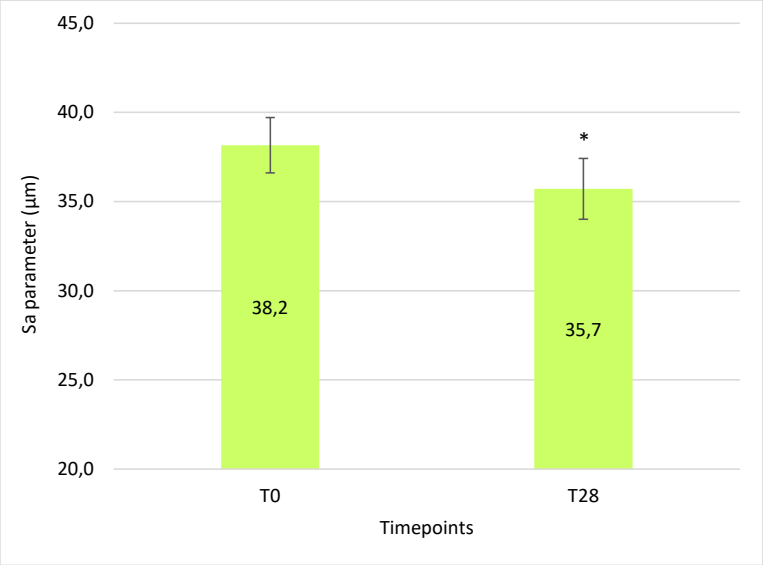
COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

SKIN PROFILOMETRY – Sa PARAMETER (related to skin smoothness)

TABLE 6: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed as µm.

n	Vol ID	T0	T28	% VARIATION VS. T0 T28
01	F3656S	38,7	38,6	-0,3%
02	R1927M	39,8	37,6	-5,5%
03	P2126A	27,5	23,7	-13,8%
04	P1492V	54,1	57,6	6,5%
05	C0074M	37,9	36,5	-3,7%
06	D0097E	41,7	39,4	-5,5%
07	F2070C	26,1	29,1	11,5%
08	A4003S	34,7	33,6	-3,2%
09	F4631M	36,3	27,0	-25,6%
10	V3024C	39,8	35,1	-11,8%
11	M3661M	34,5	34,3	-0,6%
12	R1784M	48,5	37,7	-22,3%
13	P2181M	36,3	29,6	-18,5%
14	G0587G	46,3	44,9	-3,0%
15	M3265G	34,3	32,8	-4,4%
16	D2925G	28,6	25,4	-11,2%
17	V2130R	39,1	43,5	11,3%
18	V2827L	39,8	37,0	-7,0%
19	T4522I	45,3	38,6	-14,8%
20	T4004E	34,0	32,3	-5,0%
	Mean	38,2	35,7	-6,3%
	SEM	1,6	1,7	
	t-test vs. T0	--	0,011	
			Min	-25,6%
			Max	11,5%

GRAPH 6. The graph shows the mean data obtained at each monitored check for the parameter under analysis. Data are reported as mean ± SEM. Above the error bar the intragroup statistical analysis is reported as follows: *p<0.05; ** p<0.01; ***p<0.001.



COMMENT:
as it is possible to notice, tested product determines a statistically significant decrease of Sa parameter by -6.3% after 28 days of use. A decrease of Sa parameter is related to an improvement of skin smoothness and for marketing purposes this variation can be expressed in absolute value as an improvement of skin smoothness by 6.3%.

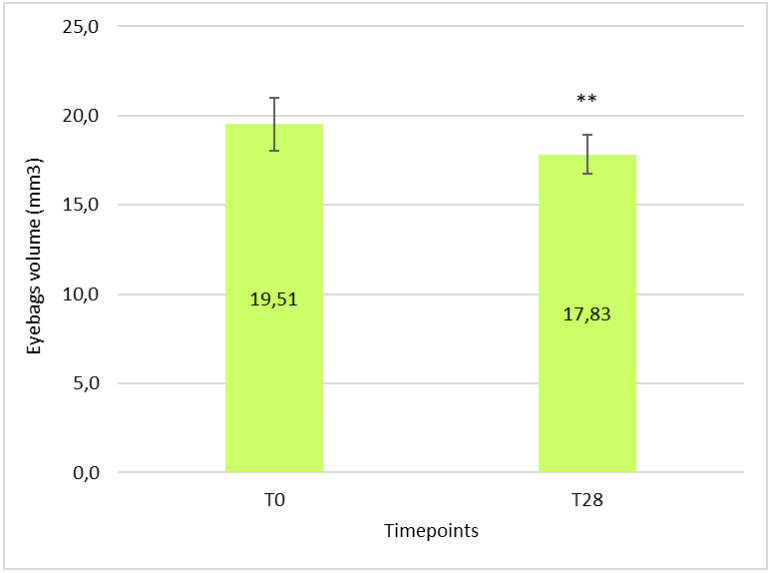
COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

SKIN PROFILOMETRY – EYEBAGS VOLUME

TABLE 7: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed as mm^3.

n	Vol ID	T0	T28	% VARIATION VS. T0 T28
11	M3661M	22,73	20,54	-9,6%
12	R1784M	23,54	21,28	-9,6%
13	P2181M	12,93	13,08	1,2%
14	G0587G	17,12	17,26	0,8%
15	M3265G	18,98	19,03	0,3%
16	D2925G	21,97	19,46	-11,4%
17	V2130R	20,27	18,65	-8,0%
18	V2827L	15,22	15,97	4,9%
19	T4522I	30,20	25,84	-14,4%
20	T4004E	10,85	10,80	-0,5%
31	C5071M	21,18	19,09	-9,9%
32	L4924M	36,66	27,15	-25,9%
33	T5202F	12,69	11,11	-12,5%
34	F5119F	14,55	14,02	-3,6%
35	L5748M	23,22	24,34	4,8%
36	G5674V	23,21	20,89	-10,0%
37	R5121M	21,13	17,02	-19,5%
38	B5102L	8,51	9,03	6,1%
39	P5166A	14,45	12,95	-10,4%
40	M5326D	20,82	19,05	-8,5%
	Mean	19,51	17,83	-6,8%
	SEM	1,48	1,11	Min -25,9%
	t-test vs. T0	--	0,005	Max 6,1%

GRAPH 7. The graph shows the mean data obtained at each monitored check for the parameter under analysis. Data are reported as mean ± SEM. Above the error bar the intragroup statistical analysis is reported as follows: *p<0.05; ** p<0.01; ***p<0.001.



COMMENT:
as it is possible to notice, tested product determines a statistically significant decrease of eyebags volume by -6.8% after 28 days of use.

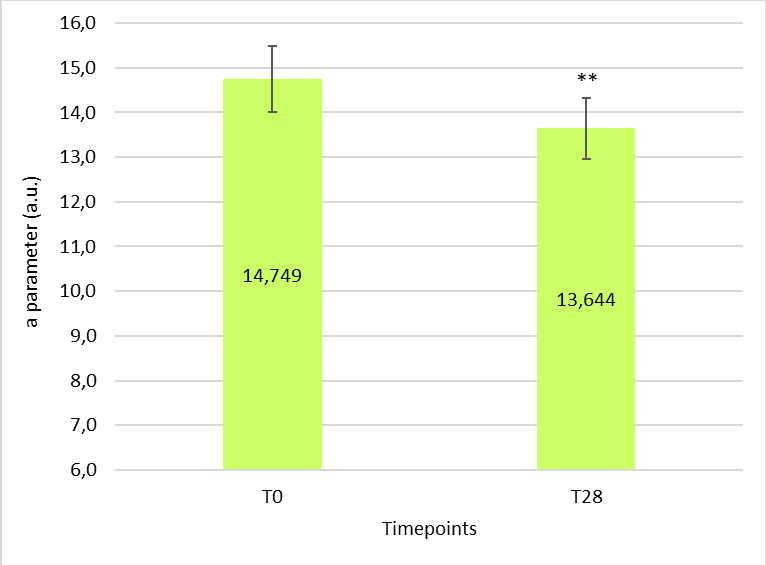
COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

DARK CIRCLES COLOUR – RED COMPONENT

TABLE 8: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed in arbitrary units (a.u.).

n	Vol ID	T0	T28	% VARIATION VS. T0 T28
01	F3656S	16,212	14,558	-10,2%
02	R1927M	13,967	11,370	-18,6%
03	P2126A	9,697	10,206	5,2%
04	P1492V	12,788	11,289	-11,7%
05	C0074M	12,067	11,170	-7,4%
06	D0097E	19,000	18,359	-3,4%
07	F2070C	10,626	10,407	-2,1%
08	A4003S	12,319	10,835	-12,0%
09	F4631M	14,014	15,098	7,7%
10	V3024C	19,670	15,607	-20,7%
21	F5051I	16,628	15,986	-3,9%
22	M5087A	17,377	15,626	-10,1%
23	C5263N	15,277	13,225	-13,4%
24	N5078M	11,277	10,141	-10,1%
25	M5118V	14,179	14,205	0,2%
26	M5083S	22,259	21,823	-2,0%
27	M5126M	14,466	11,513	-20,4%
28	B5101F	18,232	16,359	-10,3%
29	E5122A	12,043	12,654	5,1%
30	B5580A	12,878	12,440	-3,4%
	Mean	14,749	13,644	-7,1%
	SEM	0,739	0,687	Min -20,7%
	t-test vs. T0	--	0,001	Max 7,7%

GRAPH 8. The graph shows the mean data obtained at each monitored check for the parameter under analysis. Data are reported as mean ± SEM. Above the error bar the intragroup statistical analysis is reported as follows: *p<0.05; ** p<0.01; ***p<0.001.



COMMENT:
as it is possible to notice, tested product determines a statistically significant decrease of the red component of dark circles by -7.1%.

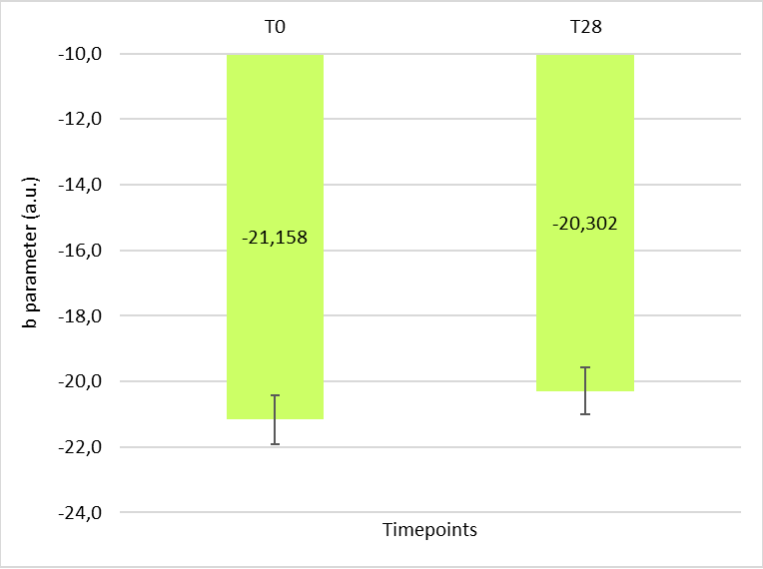
COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

DARK CIRCLES COLOUR – BLUE COMPONENT

TABLE 9: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed in arbitrary units (a.u.).

n	Vol ID	T0	T28	% VARIATION VS. T0 T28
01	F3656S	-21,382	-20,503	-4,1%
02	R1927M	-22,250	-20,960	-5,8%
03	P2126A	-20,268	-21,261	4,9%
04	P1492V	-23,022	-22,264	-3,3%
05	C0074M	-18,695	-17,642	-5,6%
06	D0097E	-24,675	-23,905	-3,1%
07	F2070C	-16,144	-15,848	-1,8%
08	A4003S	-23,912	-22,567	-5,6%
09	F4631M	-22,929	-23,462	2,3%
10	V3024C	-22,226	-21,199	-4,6%
21	F5051I	-19,286	-18,296	-5,1%
22	M5087A	-22,866	-20,070	-12,2%
23	C5263N	-24,131	-22,297	-7,6%
24	N5078M	-19,678	-18,366	-6,7%
25	M5118V	-21,724	-22,173	2,1%
26	M5083S	-29,870	-28,338	-5,1%
27	M5126M	-18,131	-16,163	-10,9%
28	B5101F	-19,063	-18,069	-5,2%
29	E5122A	-16,315	-16,601	1,8%
30	B5580A	-16,586	-16,056	-3,2%
	Mean	-21,158	-20,302	-4,0%
	SEM	0,748	0,716	Min -12,2%
	t-test vs. T0	--	0,001	Max 4,9%

GRAPH 9. The graph shows the mean data obtained at each monitored check for the parameter under analysis. Data are reported as mean ± SEM. Above the error bar the intragroup statistical analysis is reported as follows: *p<0.05; ** p<0.01; ***p<0.001.



COMMENT:
as it is possible to notice, tested product determines a statistically significant decrease of the blue component of dark circles by -4.0%.

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

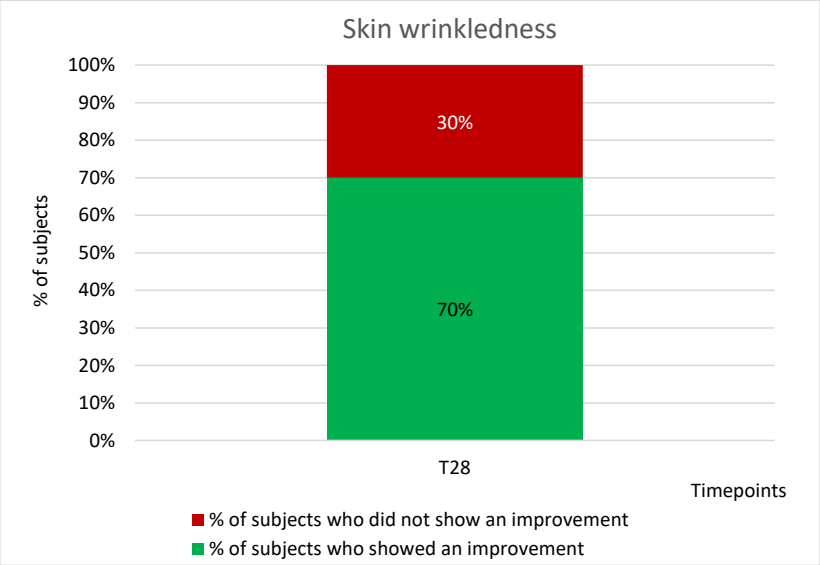
CLINICAL EVALUATION – SKIN WRINKLEDNESS

TABLE 10: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed according to the clinical scores reported in box 4a/4b.

n	Vol ID	T0	T28
01	F3656S	2,0	2
02	R1927M	2,0	2
03	P2126A	2,5	3
04	P1492V	2,5	1
05	C0074M	2,0	1
06	D0097E	1,5	2
07	F2070C	1,5	1
08	A4003S	1,5	2
09	F4631M	1,5	1
10	V3024C	1,5	2
11	M3661M	2,5	1
12	R1784M	2,5	2
13	P2181M	1,5	3
14	G0587G	2,5	2
15	M3265G	2,5	1
16	D2925G	2,0	2
17	V2130R	1,5	2
18	V2827L	2,5	2
19	T4522I	2,5	2
20	T4004E	2,0	2
	Mean	2,0	1,8
	SEM	0,1	0,1

Box 4a. Classification of skin wrinkledness at T0	Score
No wrinkle. No visible wrinkles; continuous skin line.	**0**
Very shallow yet visible wrinkles.	**0.5**
Fine wrinkle. Visible wrinkles and slight indentation.	**1**
Visible wrinkles and clear indentation	**1.5**
Moderate wrinkles. Clearly visible wrinkles.	**2**
Prominent and visible wrinkles.	**2.5**
Deep wrinkles. Deep wrinkles and furrows.	**3**
Box 4b. Variation at T28 vs T0	**Score**
No variation.	**1**
Slight improvement.	**2**
Moderate improvement.	**3**
Remarkable improvement.	**4**

GRAPH 10. The graph reports the percentage of subjects related to the effect.



COMMENT: as it is possible to notice, a clinical decrease of skin wrinkledness is recorded (in the eye contour area) in 70% of the enrolled subjects.

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

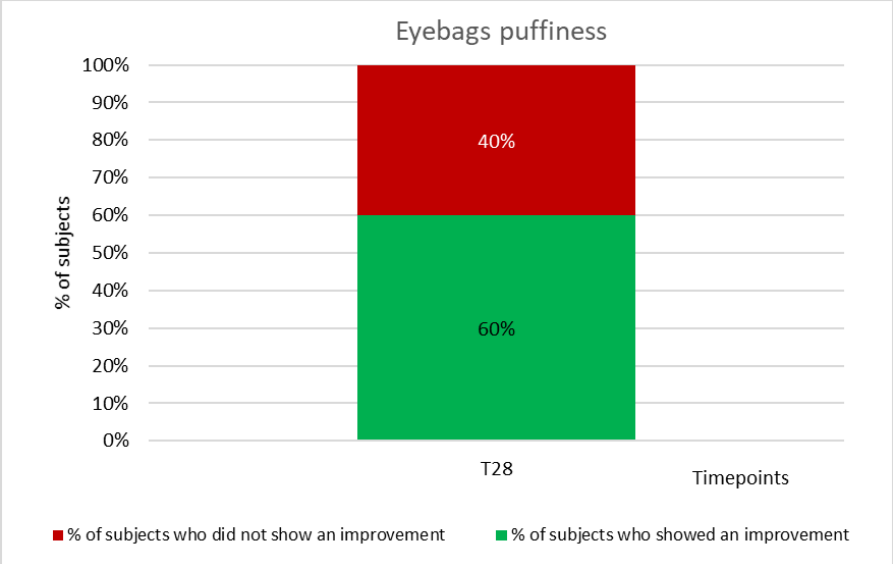
CLINICAL EVALUATION – EYEBAGS PUFFINESS

TABLE 11: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed according to the clinical scores reported in box 5a/5b.

n	Vol ID	T0	T28
11	M3661M	3,0	2
12	R1784M	3,0	2
13	P2181M	2,0	1
14	G0587G	2,0	1
15	M3265G	3,0	1
16	D2925G	2,0	2
17	V2130R	2,0	2
18	V2827L	3,0	1
19	T4522I	3,0	3
20	T4004E	2,0	1
31	C5071M	2,0	2
32	L4924M	3,0	3
33	T5202F	2,0	2
34	F5119F	2,0	1
35	L5748M	2,0	1
36	G5674V	2,0	2
37	R5121M	3,0	2
38	B5102L	2,0	1
39	P5166A	2,0	2
40	M5326D	2,0	2
	Mean	2,4	1,7
	SEM	0,1	0,1

Box 5a. Classification of eye bags puffiness at T0	Score
Eyebags are very swollen	3
Eyebags are slightly swollen	2
Eyebags are not swollen	1
Box 5b. Variation at T28 vs T0	**Score**
No variation.	1
Slight improvement.	2
Moderate improvement.	3
Remarkable improvement.	4

GRAPH 11. The graph reports the percentage of subjects related to the effect.



COMMENT: as it is possible to notice, a clinical decrease of eyebags puffiness is recorded in 60% of the enrolled subjects.

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

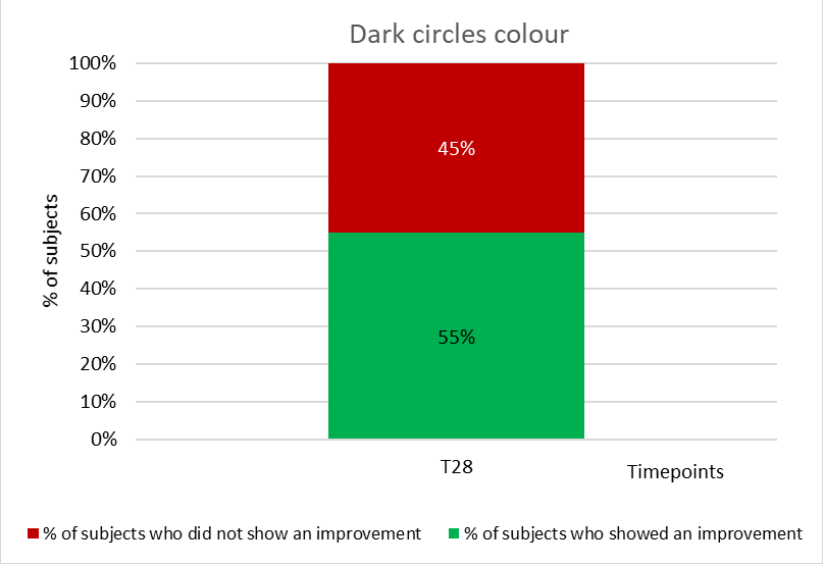
CLINICAL EVALUATION – DARK CIRCLES COLOUR

TABLE 12: The table below reports the raw data obtained for each subject participating in the study for the analyzed skin parameter. Data refer to 20 out 40 subjects and are expressed according to the clinical scores reported in box 6a/6b.

n	Vol ID	T0	T28
01	F3656S	2,0	2
02	R1927M	2,0	2
03	P2126A	2,0	1
04	P1492V	3,0	2
05	C0074M	2,0	2
06	D0097E	2,0	1
07	F2070C	2,0	1
08	A4003S	3,0	2
09	F4631M	2,0	1
10	V3024C	3,0	3
21	F5051I	2,0	1
22	M5087A	2,0	2
23	C5263N	2,0	2
24	N5078M	3,0	2
25	M5118V	2,0	1
26	M5083S	3,0	1
27	M5126M	3,0	2
28	B5101F	3,0	3
29	E5122A	2,0	1
30	B5580A	3,0	1
	Mean	2,4	1,7
	SEM	0,1	0,2

Box 6a. Classification of dark circles colour at T0	Score
Under the eye circles are very dark	3
Under the eye circles are slightly dark	2
The palpebral skin colour is normal	1
Box 6b. Variation at T28 vs T0	**Score**
No variation.	1
Slight improvement.	2
Moderate improvement.	3
Remarkable improvement.	4

GRAPH 12. The graph reports the percentage of subjects related to the effect.



COMMENT: as it is possible to notice, a clinical decrease of dark circles colour is recorded in 55% of the enrolled subjects.

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

SELF-ASSESSMENT QUESTIONNAIRE

TABLE 14: The table below summarizes the results of the self-assessment questionnaire. Data refer to 40 out 40 subjects.

no.		Completely agree	Agree	Disagree	Completely disagree	Positive answers
01	The product reduces eyebags puffiness*	15%	75%	10%	0%	90%
02	The prodcut reduces dark circles colour*	25%	70%	5%	0%	95%
03	The product reduces fine lines/wrinkles visibility	20%	70%	10%	0%	90%
04	The product improves skin brightness	35%	65%	0%	0%	100%
05	The product improves skin firmness	40%	58%	3%	0%	98%
06	The product improves skin moisturization	35%	65%	0%	0%	100%
07	The product has a smoothing effect	35%	60%	5%	0%	95%
08	The product reduces signs of fatigue	20%	73%	8%	0%	93%
09	The product has a pleasant texture	63%	35%	3%	0%	98%
10	The product is quickly absorbed	48%	50%	3%	0%	98%
11	The product is easy to apply	63%	38%	0%	0%	100%
no.		Yes	No	--	--	Positive answers
12	Are you satisfied with the product?	100%	0%	--	--	100%
13	Would you buy the product?	98%	3%	--	--	98%
14	Is the product well tolerated?	100%	0%	--	--	100%

*questions 1 and 2 refer to 20 subjects, respectively showing eyebags puffiness or dark circles.

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

CONCLUSIONS

According to the obtained results we can conclude that the product

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NUTRAKOS EYE CONTOUR GEL

after 28 days of use, determines:

- an increase of skin moisturization by +11.7%;
- an increase of skin elasticity (R2 parameter) by +10.4%;
- an improvement of skin firmness (R0 parameter) by 8.9%;
- an increase of skin radiance/brightness (gloss parameter) by +16.3%;
- a decrease of wrinkle depth (in the periocular area) by -15.8%;
- an improvement of skin smoothness (Sa parameter) by 6.3%;
- a decrease of eyebags volume by -6.8%;
- a decrease of both red and blue component of dark circles colour, respectively by -7.1% and by -4.0%.

Obtained variations are statistically significant ($p<0.05$) compared to baseline values (T0).

Instrumental results are also confirmed by the clinical evaluation carried out by the dermatologist, that highlighted a decrease of wrinkles visibility (in the periocular area) in 70% of the enrolled subjects, a decrease of eyebags puffiness in 60% of them and a decrease of dark circles colour in 55% of them.
No skin reactions or adverse events related to product use were reported during the study. The product is “well tolerated” by 100% of included subjects.

Moreover, tested product is positively judged by most of the enrolled subjects for all the investigated aspects.

Principal Investigator

Dr Enza CESTONE

DOTT.SSA ENZA CESTONE
MEDICO CHIRURGO
SPECIALISTA IN DERMATOLOGIA E VENEREOLOGIA
Via Mortara n. 27 - 100 - PAVIA
Partita IVA 0 9 5 4 1 1 1 0 1 8 3
C.F. CST NZE 80G43M109Z
Parere O.d.M. PV n. 32 del 03/11/2009

Digitally signed by Enza Cestone
Date: 2021.12.15 14:30:01 +01'00'

Study Director

Dr Valentina ZANOLETTI

Digitally signed by Valentina Zanoletti
Date: 2021.12.16 09:27:22 +01'00'

Data analysis & Report

Dr Eleonora SPARTA’

Digitally signed by Eleonora Spartà
Date: 2021.12.15 19:26:54 +01'00'

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
Sedi principali:
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504