



Final Report

EVALUATION OF THE ANTI-WRINKLE EFFICACY OF A COSMETIC PRODUCT ON 20 VOLUNTEERS THROUGH PROFILOMETRIC ANALISYS (LONG TERM TEST)

<u>Study N°.</u>	KH392/12-01
<u>Study protocol code</u>	REL/0666/2013/CLI/SAB
<u>Customer</u>	LABORATOIRE DR PAUL ET KARIN HERZOG SA Route de Taillepied, 1 1095 Lutry - SWITZERLAND
<u>Product/test substance</u>	Oxygen Hyalu' Lift (Face Cream) Day&Night Cream-Crema Mattina e Sera Batch: n.a.

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AUTHENTICITY OF RESULTS

I hereby declare that the study concerned by this report was carried out under my responsibility and all observation or data recorded during this test are reported in this study report.

I certify the re-reading of this report and I do agree with its content and I confirm its correspondence with the raw data.

Dr Samuele Burastero

Date

30/04/2013



QA STATEMENT

The volunteers recruitment is done according to specific internal procedures, according to GCP directive and according to Helsinki Declaration, 2008 requests. The volunteers signed the personal informal consent, they were informed about the complete study plan under development and they were visited by the Medical during their recruitment.

The collected data derived from this study are managed according to internal procedures following the GLP directive and they are verified by the QA manager who check the different parts of this study (comparison between raw data and recorded data, laboratory books and files, protocol and report) according to the quality plan of the ABICH Cosmetic Lab (internal audits, periodical calibration status of the instruments if they are involved in the test).

Faithfully

Quality Assurance – Valentina Celada

Date **30/04/2013**



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1. SUMMARY

By assignment from the Company **LABORATOIRE DR PAUL ET KARIN HERZOG SA**, on the test substance **Oxygen Hyalu' Lift (Face Cream) Day&Night Cream-Crema Mattina e Sera** an *in vivo* test has been carried out in order to evaluate its anti-wrinkle efficacy on healthy volunteers by means of an *in-vivo*-3D-Scanner **dermaTOP-blue (Eotech, France)** dedicated to non contact local measurement of the skin surface topography.

For this purpose the product under examination, was applied twice a day for a period of 90 days by 20 female subjects aged from 35 to 65.

The wrinkles evaluation was made on the crow's feet area before and after 45 and 90 days of product application .

During this period the participants was asked to avoid the use of any other anti-wrinkle product.

The described *in vivo* long-term test allows to evaluate instrumentally the capacity of the product to reduce the appearance of the periorbital wrinkles through the analysis of the parameter "**Rz- mean depth of roughness**" that represents the arithmetical mean of the roughness depths of five adjacent identical distances of the digitally filtered profile and the parameter "**Ra- arithmetical mean of roughness**" that represents the arithmetical mean of roughness and is the generally used parameter for the evaluation of skin roughness.

Moreover at the end of this period the participants to the study, filled in a questionnaire relative to a subjective evaluation of the cosmetic pleasantness, of the organoleptic characteristics, of the perception of efficacy and to a general satisfaction of the product and its performances.

The study was performed at the Abich Cosmetic Lab. in Via Bruno Buozzi, 4 – 20090 – Vimodrone (Milan), Italy.

The experimentation started the 22nd January, 2013 and ended the 23rd April, 2013.

This study has been carried out in compliance with the most recent recommendations of the World Medical Association Declaration of Helsinki- ethical principles for medical research involving human subjects (Helsinki Declaration 59th WMA General Assembly, Seoul, October 2008) and according to the Colipa Guidelines for the evaluation of the efficacy of cosmetic products (May 2008).

On the basis of the results obtained with the adopted experimental procedure, it can be concluded that, the substance under examination

Oxygen Hyalu' Lift (Face Cream) Day&Night Cream-Crema Mattina e Sera

on the subjects that had undergone the test, determines a statistically significant decrease of the skin roughness at the level of the analyzed skin area since caused a decrease of the Rz and Ra analyzed parameters.



2. INTRODUCTION

The surface of the skin is intersected by primary and secondary lines like a topographical map with plateaus and valleys. The micro-relief is a good indicator of the aging process of the skin. The primary lines are characteristic of each single individual, at every age and part of the body. They are influenced by external factors such as temperature, humidity, nutrition and pharmaceuticals. Modifications at the level of the micro-relief occur because of the loss of elastic fibers in the dermis and are typical of the aging process (Baumann, 2007; Callaghan and Wilhelm, 2008; Uitto, 2008). Image analysis consents to study in a quantitative way the skin roughness with scientifically validated methodologies, largely used in controlled clinical trials (Kim et al., 2009; Koh et al.). On the basis of these preliminary considerations, the following parameters can be accurately monitored and maintained constant in the execution of assays that quantify the roughness (Dobrev, 2002):

- a) the area of the analyzed skin, that may differ significantly by its roughness in topographical areas even only slightly different between each other;
- b) ambient humidity and temperature (to higher environmental humidity and temperature correspond higher skin hydration and lower skin roughness, respectively).

The ideal measurement conditions are approximately 20°C and 50% relative humidity.

3. DISCLAIMER

According to COLIPA guidelines, the test was performed with the assumption that the Sponsor under its responsibility provided to the personnel of Abich Cosmetic Lab. truthful information on any ingredient of the test product endowed with potential toxicological relevance.

On the basis of such information, a general assessment of the toxicological information concerning the product was preliminarily carried out and ethical implications as to its use during the present study have been considered.

4. TEST SUBSTANCE

The test substance consists of an emulsion of white color.

Name: Oxygen Hyalu' Lift (Face Cream) Day&Night Cream-Crema
Mattina e Sera

Batch/ Formule code: n.a.

Sample Code Abich: 2759/12-01

INCI composition: see annex

Pao / Expiration date: 4 M

Storage conditions: room temperature

The characterization of the test substance is under responsibility of the Sponsor.



5. PANEL RECRUITMENT

5.1 Characteristics of the panel

The study was performed on 20 female volunteers, aged from 35 to 65, who were identified from the database of volunteers of the Abich Test Centre, and who were evaluated as appropriate for participation in the study and not suffering from diseases to the skin areas to treat.

Before the beginning of the study each volunteer has read and signed an informative form (informed consent form, C.I.). Each volunteers has had the opportunity to ask any kind of questions regarding the study to which was given an exhaustive answer. The volunteer was explained the aim of the test, the procedure and the possible risks related.

Only after signature of the informed consent the participation in the study was permitted.

Only volunteers in good general health conditions were included in the study.

The originals of these informed consent forms were archived at the Abich Cosmetic Lab. All patients signed a consent allowing to treat personal data according to the Italian law (Testo unico sulla privacy. D.Lgs 196/2003).

Table 1: Volunteers participant to the study.

NVOLUNTEER	CODE	AGE
1	DABE206	46
2	CACA55	58
3	ROCA405	45
4	ROTE181	62
5	LOTU144	56
6	TIRA309	47
7	MACAT1	60
8	ESA8	46
9	ROVA262	50
10	LOPA162	40
11	LUBEL22	55
12	FRMA177	59
13	MACA64	45
14	SILBO37	54
15	LUFIU18	53
16	FEAD421	57
17	PAVI307	58
18	ANCON12	48
19	MAMU403	39
20	LUPR276	43
MEAN		

5.2 Exclusion criteria

The following criteria of exclusion were applied:

- Pregnancy or nursing condition.
- Medication (local and/or systemic) which might interfere with the test evaluation.
- Subjects with signs of irritation at the application site.
- Subjects with dermatological problems which that might interfere with the study.
- Simultaneous participation to other studies, which that might interfere with the test evaluation.
- Subjects otherwise evaluated as not suitable by the Doctor.



5.3 Criteria for study withdrawal

After study start, the following withdrawal criteria were applied:

- volunteers who did not longer wish to participate in the study;
- volunteers who during the study suffered any illness or accident or developed any condition which could affect the outcome of the study;
- volunteers who did not follow the conditions as described in the Study Protocol.

6. INSTRUMENTATION AND MATERIALS

The following instrumentations and materials were used:

- **derma TOP-blue di Eotech**: in-vivo 3D scanner optimized to provide precise and reproducible measurements of human skin for dermatological and cosmetic applications without the need of using facial replicas. To ensure the reliable and accurate reproducibility of the proband's positioning for several skin scanning session over a period of several minutes, hours or weeks, a professional measuring station was developed and optimized for the specific requirements of this application. Together with the alignment possibilities of a sophisticated 3D software, it is made sure that in each measuring session the same area of examination is being analyzed.

Data acquisition, visualization and analysis are performed by dermaTOP software, based on Breuckmann's program OPTOCAT. Intelligent data post-processing functions provide high-quality 3D results and powerful evaluation tools are proposed to compute different parameters that are representative of the efficacy of the product or treatment.

The software compares the results obtained evaluating the volunteers at time 0 and after the treatment with the product to be tested. The instrument measures very precisely the performances of cosmetic products formulated to reduce skin imperfections such as wrinkles.



- **Thermohygrometer**: Taylor Precision, model Lp, to monitored temperature and humidity in the room.



7. EXPERIMENTAL DESIGN

7.1 Structure of the study

The study has been executed with an open observational modality.

7.1 Aim of the study

This study is finalized to evaluate the effectiveness of the product in reducing the appearance of wrinkles and fine-lines and in improving the skin aspect.

The evaluation implied the comparison of the analyzed parameters:

- **Rz- mean depth of roughness**
- **Ra- arithmetical mean of roughness**

of the area of interest prior to the product application (time 0=T₀) with the same parameter detected in the same area after 45 days (time 45 days=T₄₅) and after 90 days (time 90 days=T₉₀) of product application.

7.2 Environmental conditions

The study was carried out under standard environmental conditions for each reading time, monitoring and maintaining constant temperature and humidity.

7.3 Method of application

Each subject applied the test product twice a day (morning and night) for a period of 90 days on the face massaging until absorption and insisting on the periorbital area using a special brush provided by the sponsor.

The volunteers were asked to come in laboratory with clean face skin, without any other cosmetic product or any make-up which could interfere with the measurements.

7.4 Evaluated skin areas

The profilometric evaluation of the Rz and Ra parameters has been made on the crow's feet area; the analyzed areas at T₀, T₄₅ and T₉₀ days were as much as possible superimposable.

8. ESSAY METHODOLOGY

8.1 Study duration

The study lasted 90 days for each volunteer.

8.2 Preparation of the volunteer

Before each measurement with the dermaTOP-blue, each volunteer was allowed to relax in an air conditioned room to avoid anomalous sampling due to excessive sweating or stress.

8.3 Wrinkles measurement

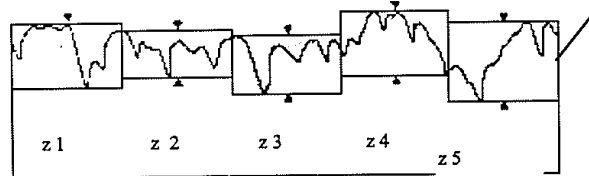
The analysis of the skin surface topography by the means of **Visio3D dermaTOP BLUE system (EOTECH,France)** is an innovative technique of image analysis without the need of replicas.

The accuracy and the reproducibility of the measurements is guaranteed thanks to a special system studied for the volunteer positioning which allows the measurement of the same areas at different measurement times for the same subject and thanks to the optoCAT software utilized for the visualisation, acquisition and for the results analysis.

This software is able to extract from the acquired images the same area of interest at the different times of analysis and to overlap it aligning to the previous one.



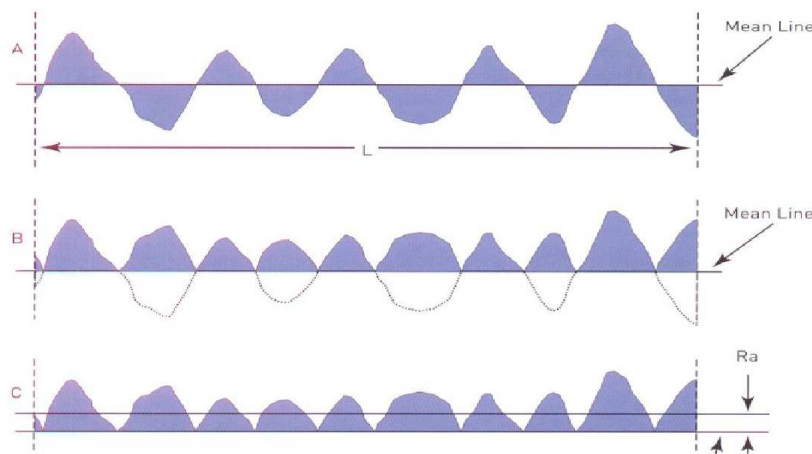
One of the most representative parameters for the anti-wrinkle efficacy evaluation, taken into account in this study, is **Rz or average maximum profile height difference** that represents the arithmetic average of the different segment roughness calculated from 5 succeeding measurement segments of the same length. In contrast to the other profile roughness parameters Rz is not that much influenced by artifacts due to calculating the average.



$$Rz = \frac{z1 + z2 + z3 + z4 + z5}{5}$$

Ra represents the arithmetical mean of roughness and is the generally used parameter for the evaluation of skin roughness

Ra



Skin surface changes were evaluated by comparing all the described skin parameters values before (T0), after 45 (T45) and 90 (T90) days of product application.

9. TOLERABILITY

None of the 20 volunteers enrolled in this study during the product use showed signs of intolerance or allergic reactions to the product.



10. DATA EVALUATION AND STATISTICAL ANALYSIS

All the values of the parameters analyzed were gathered for each participant and for each measurement time (see annex 1).

The average values of the 20 volunteers for each parameter for each time were calculated (Tables 2 e 4, Graphs from 1 to 4).

Then the % variation of all parameters values from T0, T45 and T90 was calculated for each volunteer (see annex 1) and the average % variations were evaluated (Tables 3 e 5).

The distribution of the values obtained during the measurements at the various experimental times were compared with intra-group analysis (T0 versus T45, T45 versus T90 and T0 versus T90) using Student's t test. P values < 0.05 were considered significant.

11. RESULTS

In the adopted experimental conditions, the product under examination **Oxygen Hyalu' Lift (Face Cream) Day&Night Cream-Crema Mattina e Sera** has demonstrated efficacy in reducing the skin roughness at the level of the analyzed skin area since caused:

- a decrease of the Rz analyzed parameter;
- a decrease of the Ra analyzed parameter;

In particular **Rz** resulted reduced by a mean value equal to 13,18% after 45 days (T45) and 23,18% after 90 days (T90) of bi-daily application of the product respect to the Rz basal value evaluated at T0. The reduction of this parameter indicates an increase in the skin smoothness consequent to a skin roughness decrease.

The variations concerning the product resulted statistically significant vs T0 ($p < 0,05$).

The tables below report the means of Rz on the panel of 20 volunteers at the three observation times (T0, T45 and T90, table 2) and the mean % variation values of the same parameter calculated as arithmetical average of the single % variations of each volunteer (table 3).

The mean Rz value variations are moreover represented in form of graphs (Graph 1-2).

Table 2

TIME	Rz (mm)
T0	0,161
T45	0,140
T90	0,123

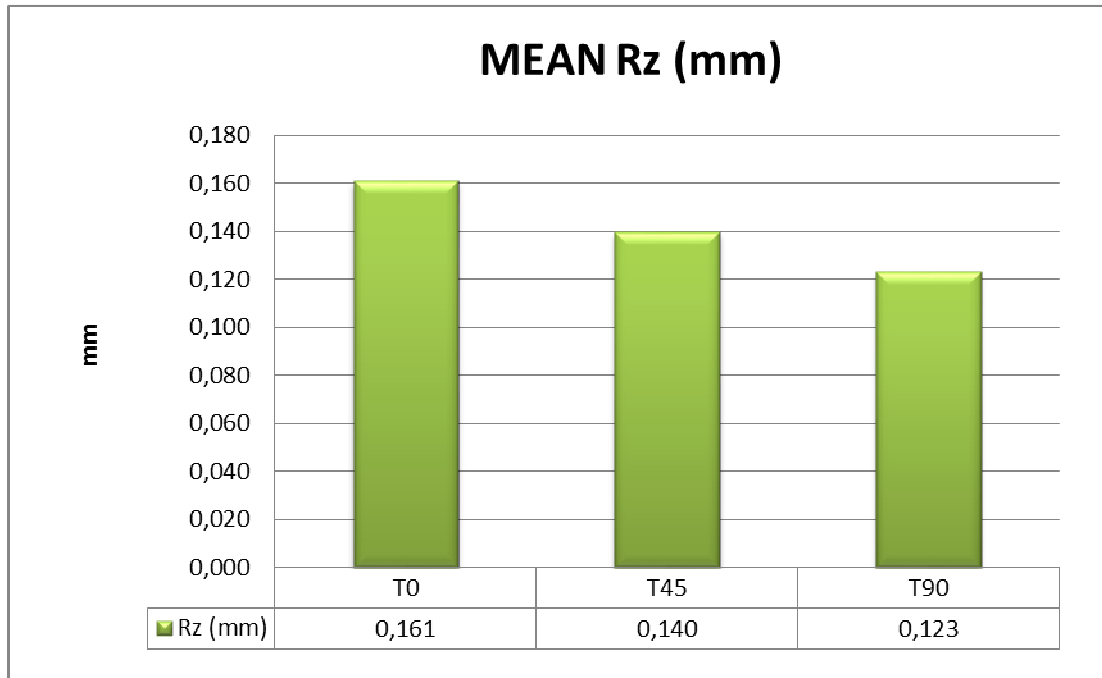
Table 3

TIME	Rz Mean % variation	p-value
T45-T0	-13,18%	0,0000000*
T90-T0	-23,18%	0,0000000*
T90-T45	-11,91%	0,0000000*

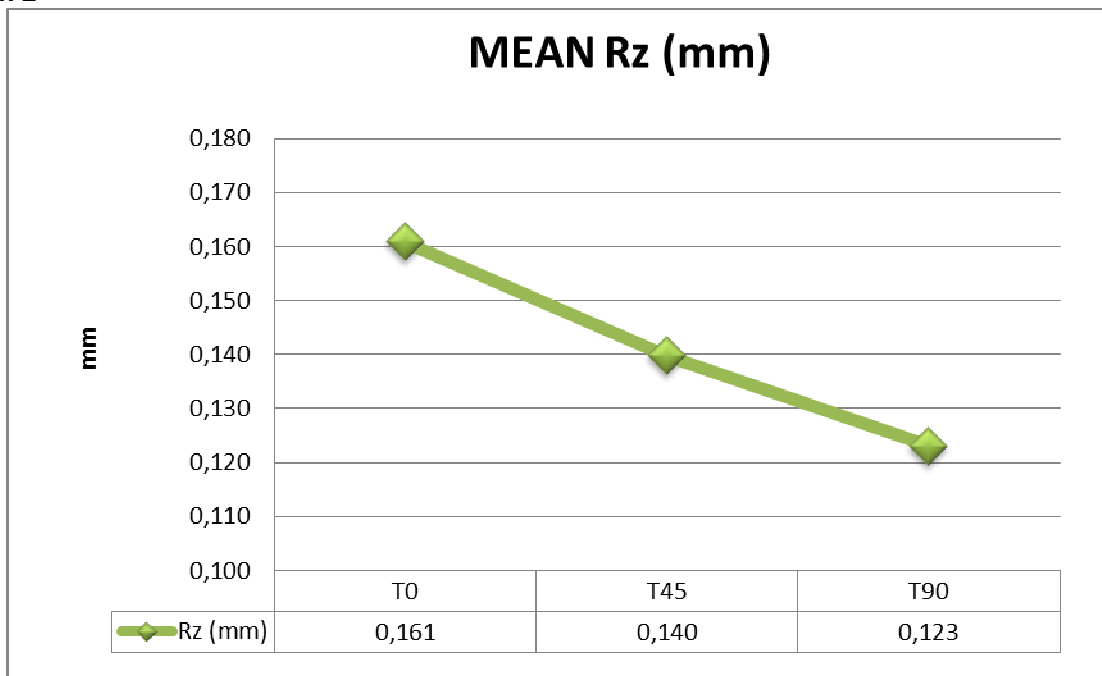
* P-values relative to statistically significative variations ($p < 0,05$).



Graph 1



Graph 2





Ra resulted reduced by a mean value equal to 14,19% after 45 days (T45) and 30,49% after 90 days (T90) of bi-daily application of the product respect to the Ra basal value evaluated at T0. The reduction of this parameter indicates an decrease of skin roughness. The variations concerning the product resulted statistically significant vs T0 ($p < 0,05$).

The tables below report the means of Ra on the panel of 20 volunteers at the three observation times (T0, T45 and T90, table 2) and the mean % variation values of the same parameter calculated as arithmetical average of the single % variations of each volunteer (table 4). The mean Ra value variations are moreover represented in form of graphs (Graph 3-4).

Table 4

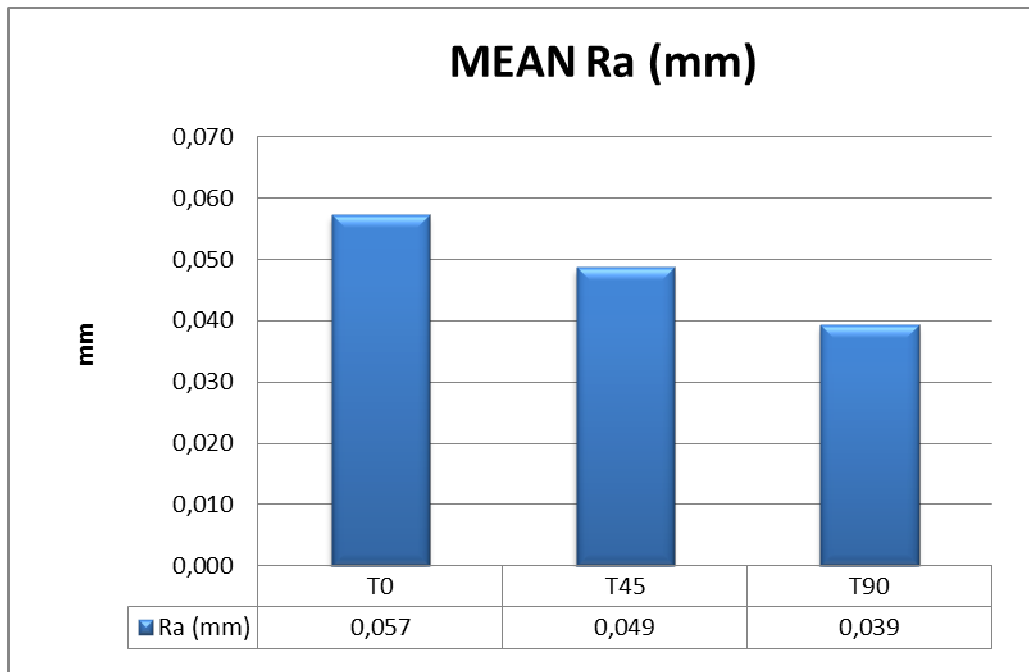
TIME	Ra (mm)
T0	0,057
T45	0,049
T90	0,039

Table 5

TIME	Ra Mean % variation	p-value
T45 vs T0	-14,19%	0,0000000*
T90 vs T0	-30,49%	0,0000000*
T90 vs T45	-18,77%	0,0000000*

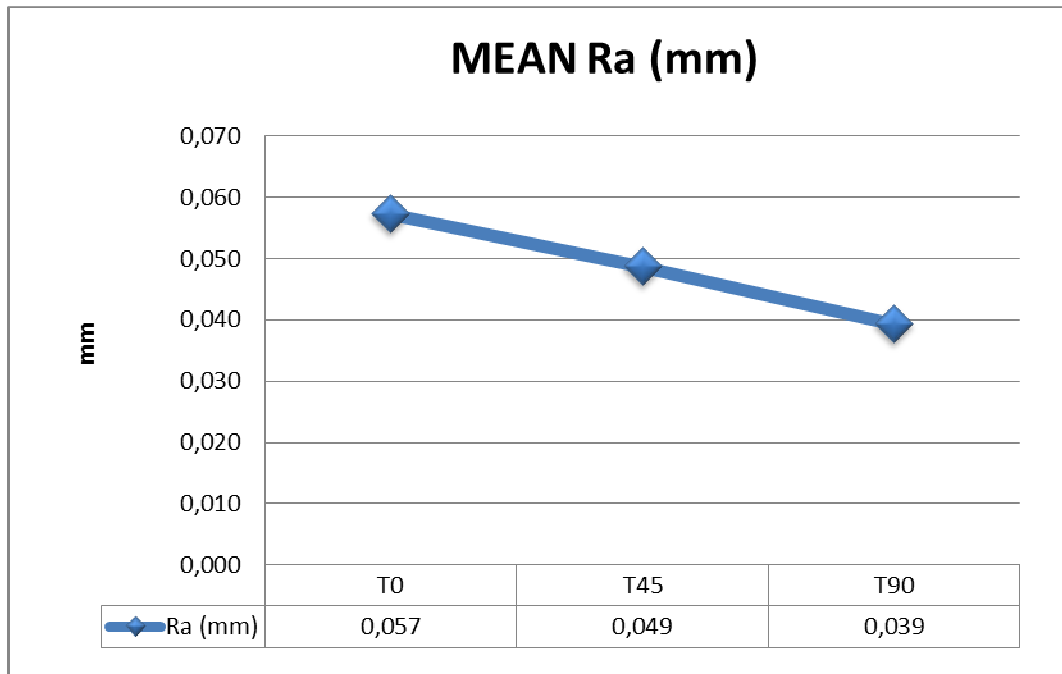
* P-values relative to statistically significative variations ($p < 0,05$).

Graph 3





Graph 4



12. DISCUSSION AND CONCLUSIONS

On the basis of the results obtained with the adopted experimental procedure, it can be concluded that the substance under examination

Oxygen Hyalu' Lift (Face Cream)Day&Night Cream-Crema Mattina e Sera

on the subjects that had undergone the test, determines a statistically significant reduction of the two most accredited parameters in the profilometric evaluation, Rz and Ra (which indicate respectively the mean depth of roughness and the arithmetical mean of roughness) evaluated after 90 days of bi-daily application of the product. These results correlate to an improvement in skin roughness, and hence, the treatment has been proved to be significantly effective in reducing wrinkle appearance and smoothing the skin's surface.

13. ARCHIVING

The clinical study protocol, the corresponding raw data and the final report are kept in the archives of Abich Testing Centre, in Via Buozzi, 4, 20090-Vimodrone (MI), both in electronic format and in reduced paper format for a period of 10 years from the issue of the final report. The control samples of the test substance and eventual specific reference material will be kept for 3 month, unless a specific request is made by the customer.



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Linee guida EEMCO. Valutazione della topografia cutanea. JL Lèvêque. Centro Charles Zviak- Clichy Cedex-France

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Koh, B.K., Lee, C.K., and Chae, K. Photorejuvenation with Submillisecond Neodymium-Doped Yttrium Aluminum Garnet (1,064 nm) Laser: A 24-Week Follow-Up. *Dermatol Surg*

Saunders, J., and Wainwright, P. (2003). Risk, Helsinki 2000 and the use of placebo in medical research. *Clin Med* 3, 435-439

COLIPA guidelines for the evaluation of the efficacy of cosmetic products, May 2008.

Declaration WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects
Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

Consensus documents Number 4.
OECD SERIES ON PRINCIPALES OF GLP AND COMPLIANCE MONITORING
"Quality assurance and GLP" 26 Oct. 1999.

Consensus documents Number 5.
OECD SERIES ON PRINCIPALES OF GLP AND COMPLIANCE MONITORING
"Compliance of laboratory suppliers with GLP principles" 28 Sept. 2000.

Consensus documents Number 7.
OECD SERIES ON PRINCIPALES OF GLP AND COMPLIANCE MONITORING
"The application of to GLP principles to short term studies" 15 Sept. 1999.

Consensus documents Number 8.
OECD SERIES ON PRINCIPALES OF GLP AND COMPLIANCE MONITORING
"The role and responsibility of the Study Director in the GLP studies" 15 Sept. 1999.



15. ANNEXES

ANNEX 1

Raw data of Rz parameter (in mm)

Cod. vol	T0		T45		T90		T0	T45	T90
	L	R	L	R	L	R	mean L-R	mean L-R	mean L-R
ancon12	0,154	0,135	0,132	0,117	0,124	0,106	0,145	0,125	0,115
caca55_	0,087	0,091	0,072	0,081	0,060	0,079	0,089	0,077	0,070
dabe206	0,311	0,184	0,309	0,160	0,279	0,140	0,247	0,235	0,209
esa8__	0,150	0,125	0,090	0,112	0,067	0,105	0,137	0,101	0,086
fead421	0,150	0,114	0,118	0,100	0,110	0,093	0,132	0,109	0,101
frma177	0,165	0,146	0,132	0,127	0,112	0,117	0,155	0,130	0,114
lopa162	0,104	0,103	0,090	0,091	0,078	0,078	0,104	0,090	0,078
lotu144	0,288	0,180	0,262	0,173	0,195	0,160	0,234	0,218	0,177
lubel22	0,150	0,149	0,132	0,130	0,118	0,121	0,149	0,131	0,120
lufiu18	0,250	0,237	0,194	0,181	0,167	0,158	0,243	0,187	0,163
maca64_	0,158	0,155	0,134	0,127	0,128	0,113	0,157	0,131	0,120
macat1_	0,206	0,192	0,181	0,143	0,177	0,123	0,199	0,162	0,150
pavi307	0,201	0,145	0,162	0,124	0,147	0,113	0,173	0,143	0,130
roca405	0,176	0,170	0,168	0,154	0,146	0,132	0,173	0,161	0,139
rote181	0,182	0,177	0,157	0,167	0,129	0,142	0,179	0,162	0,135
rova262	0,122	0,092	0,107	0,087	0,091	0,079	0,107	0,097	0,085
silbo37	0,133	0,121	0,127	0,112	0,117	0,102	0,127	0,120	0,110
tira309	0,238	0,187	0,210	0,169	0,166	0,149	0,213	0,189	0,157
mamu403	0,091	0,102	0,074	0,099	0,066	0,092	0,097	0,087	0,079
lupr276	0,188	0,127	0,164	0,121	0,137	0,111	0,158	0,142	0,124
means	0,175	0,147	0,151	0,129	0,131	0,116	0,161	0,140	0,123



Raw data of Ra parameter (in mm)

Cod. vol	T0		T45		T90		T0	T45	T90
	L	R	L	R	L	R	mean L-R	mean L-R	mean L-R
ancon12	0,053	0,050	0,046	0,041	0,039	0,032	0,052	0,043	0,036
caca55_	0,034	0,029	0,030	0,029	0,027	0,022	0,031	0,030	0,025
dabe206	0,105	0,095	0,111	0,054	0,068	0,054	0,100	0,083	0,061
esa8_	0,050	0,043	0,038	0,042	0,028	0,039	0,047	0,040	0,034
fead421	0,061	0,056	0,035	0,044	0,028	0,040	0,059	0,040	0,034
frma177	0,057	0,054	0,043	0,053	0,039	0,039	0,056	0,048	0,039
lopa162	0,041	0,032	0,033	0,029	0,029	0,022	0,036	0,031	0,026
lotu144	0,076	0,061	0,084	0,057	0,067	0,046	0,068	0,070	0,056
lubel22	0,051	0,053	0,040	0,051	0,036	0,037	0,052	0,046	0,037
lufiu18	0,072	0,058	0,068	0,044	0,044	0,036	0,065	0,056	0,040
maca64_	0,040	0,044	0,034	0,040	0,025	0,035	0,042	0,037	0,030
macat1_	0,079	0,066	0,061	0,041	0,053	0,031	0,073	0,051	0,042
pavi307	0,066	0,060	0,049	0,041	0,039	0,040	0,063	0,045	0,039
roca405	0,053	0,054	0,042	0,047	0,036	0,039	0,054	0,044	0,038
rote181	0,074	0,071	0,061	0,059	0,059	0,052	0,055	0,073	0,055
rova262	0,046	0,042	0,035	0,029	0,028	0,023	0,044	0,032	0,025
silbo37	0,041	0,042	0,030	0,032	0,021	0,028	0,041	0,031	0,025
tira309	0,084	0,057	0,073	0,040	0,056	0,035	0,070	0,057	0,046
mamu403	0,071	0,063	0,118	0,032	0,092	0,030	0,067	0,075	0,061
lupr276	0,079	0,060	0,054	0,042	0,041	0,040	0,069	0,048	0,041
means	0,062	0,055	0,054	0,042	0,043	0,036	0,057	0,049	0,039



ANNEX 2

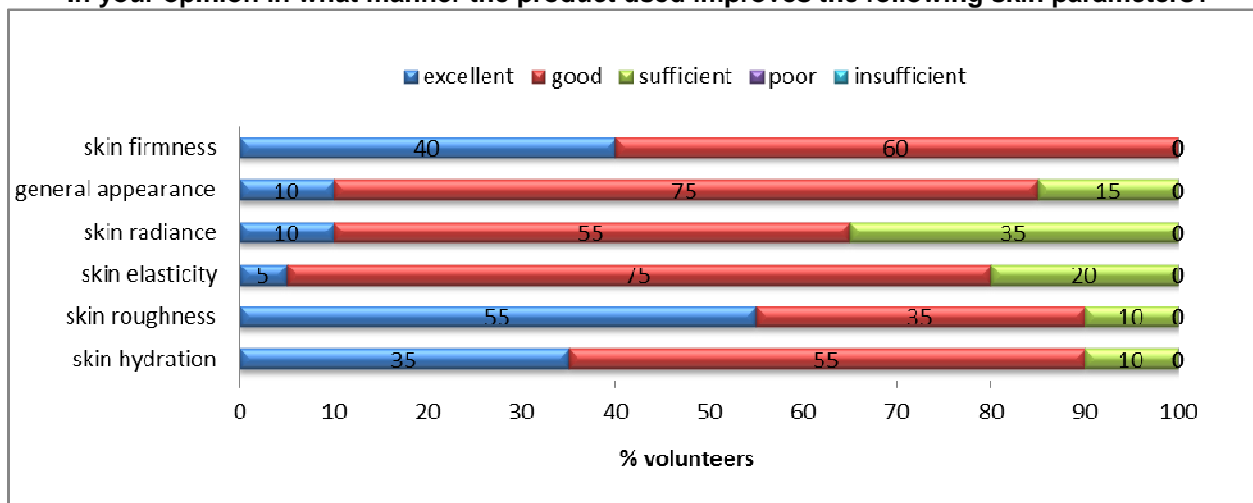
Questionnaire concerning a sensorial / psichorheological assessment relative to the tested product

To obtain a judgement from potential customers on product performances, the 20 subjects who took part to the study answered to a questionnaire on a subjective evaluation of the tested product.

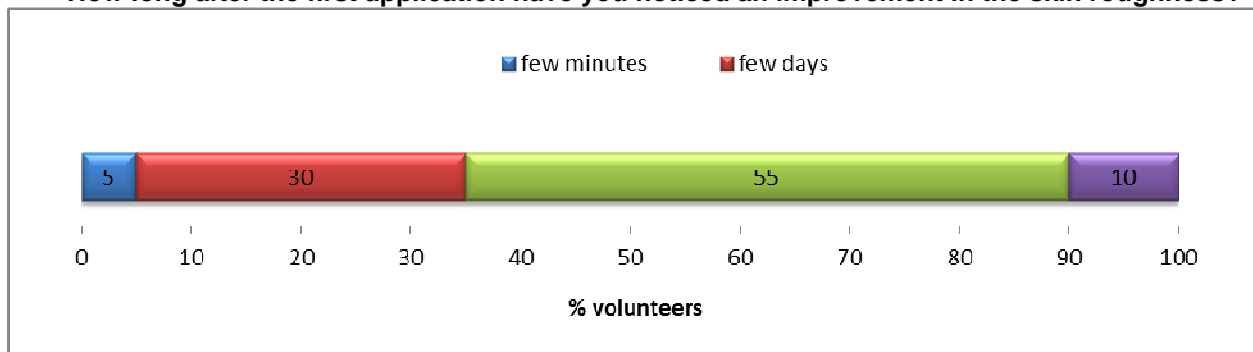
Here below are reported all questions of the questionnaire and their answers are represented in the form of graphs.

For the graphical representation of the multiple choice answers of each question, the percentage of volunteers who expressed the same opinion was calculated.

➤ **In your opinion in what manner the product used improves the following skin parameters?**

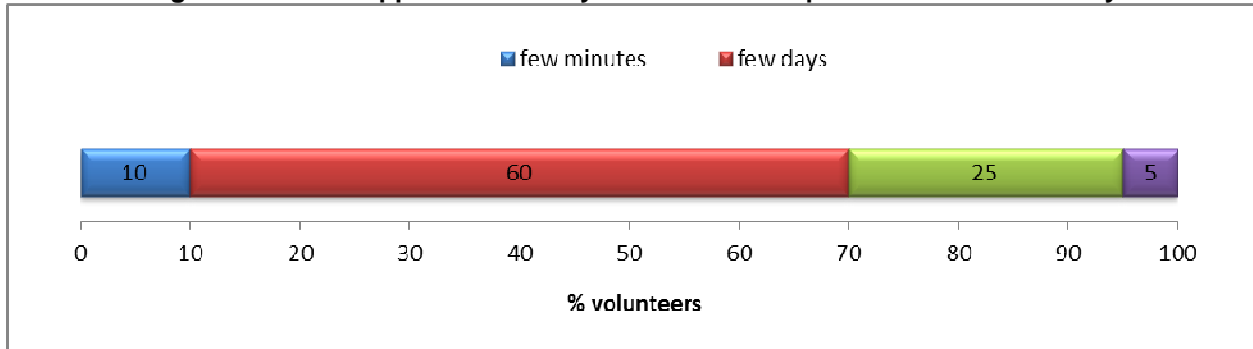


➤ **How long after the first application have you noticed an improvement in the skin roughness?**

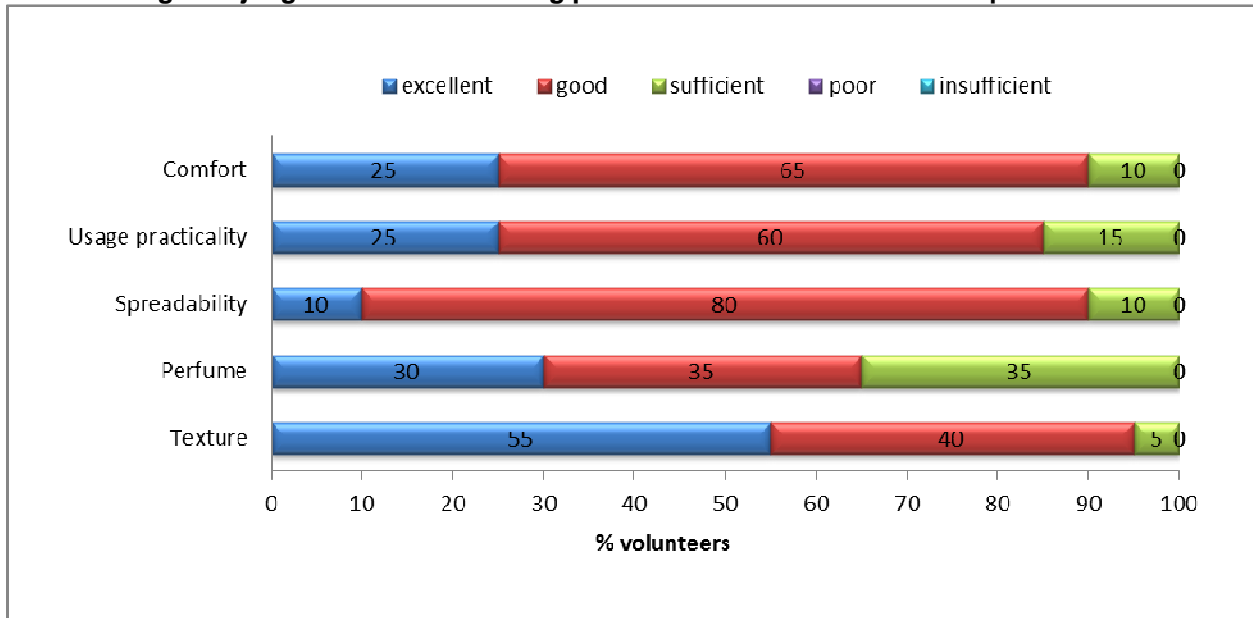




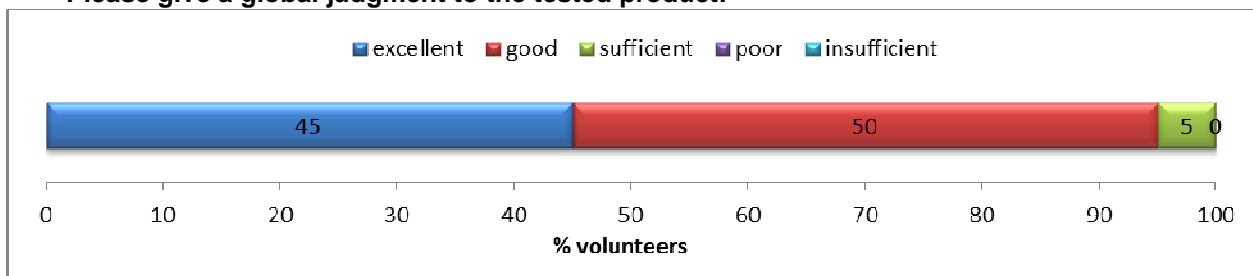
➤ **How long after the first application have you noticed an improvement in the skin hydration?**



➤ **Please give a judgment to the following parameters relative to the tested product:**

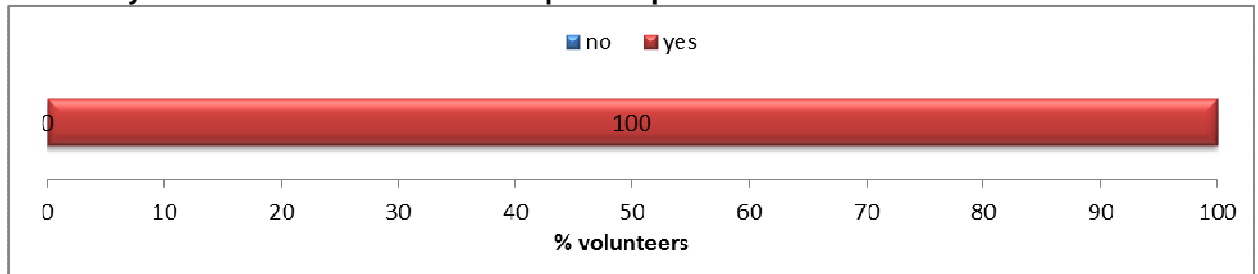


➤ **Please give a global judgment to the tested product:**

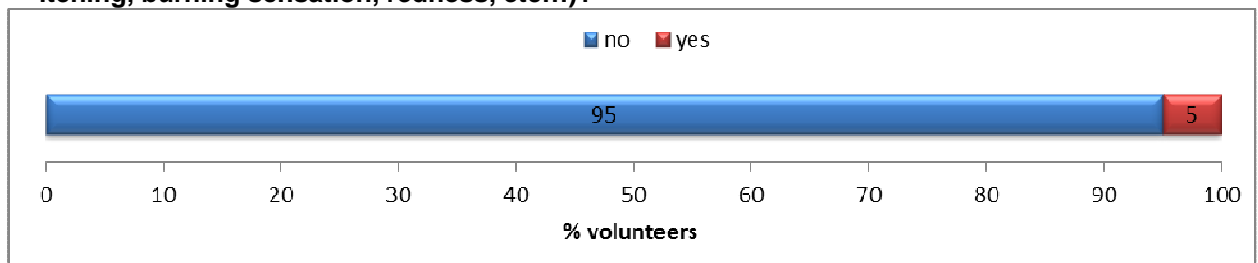




➤ **Would you recommend to someone the product purchase?**



➤ **After the product usage did you note adverse effect caused by the product itself (irritation, itching, burning sensation, redness, etc...)?***



*The subjects who answered "YES" to this question was asked to indicate the motivation.

The answers were:

- After its use, the product leaves the skin dry.

➤ **Others general comments:**

- The product is too moisturizing
- In my opinion this product is not suitable as a base for makeup



ANNEX 3:

Inci list

AQUA,
PETROLATUM,
GLYCERYL STEARATE,
PARAFFINUM LIQUIDUM,
POLYSORBATE 80,
STEARYL ALCOHOL,
CETYL ALCOHOL,
CETEARYL ETHYLHEXANOATE,
ISOPROPYL MYRISTATE,
1% HYDROGEN PEROXIDE,
TOCOPHERYL ACETATE,
PERSEA GRATISSIMA OIL,
PARFUM,
SODIUM HYALURONATE,
ASCORBIC ACID,
RETINOL,
DAUCUS CAROTA SATIVA SEED OIL,
BIOTIN,
BETA-CAROTENE.