Final Report

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

Study N°.	**KH041/14-01**
Study protocol code	**REL/0477/2014/CLI/SAB**
Customer	**LABORATOIRE DR PAUL ET KARIN HERZOG SA Route de Taillepied, 1 1095 Lutry - SWITZERLAND**
Product/test substance	**VITA A KOMBI 1 – Ref. E1C 100 ml Batch: 0110715**

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Date: **31/03/2014**

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Dott.ssa Mariana Tritapepe - Biologist

Dott.ssa Giulia Caccia – Biologist

AUTHENTICITY OF RESULTS

I hereby declare that the study concerned by this report was carried out under my responsibility, according to the experimental protocol and the quality plan of the Abich S.r.l.. I also state that, whenever applicable, all procedures were compliant with the principles of Good Clinical Practice.
All relevant observations and data recorded during the test are reported in this study report.
I certify the re-reading of this report and a do agree with its content.

Dr. Samuele Burastero

Data **31/03/2014**

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1. SUMMARY

By assignment from the Company **LABORATOIRE DR PAUL ET KARIN HERZOG SA**, on the test substance **VITA A KOMBI 1 – Ref. E1C 100 ml Batch: 0110715** 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 30 days by 20 female subjects aged from 35 to 65.
During this period the participants was asked to avoid the use of any other anti-wrinkle product.

The wrinkles and fine-lines evaluation was made on the crow's feet area before and after 30 days of product application.
At the same experimental times macrophotographies of the crow's feet treated area were taken with digital reflex camera equipped with macro objective.

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 chatacteristics, of the perception of efficacy and to a general satisfaction of the product and its performances.

The study was performed at the Abich Clinical and Cosmetological Trials Center in Via Bruno Buozzi, 4 – 20090 – Vimodrone (Milan), Italy.

The experimentation started the 18th February, 2014 and ended the 27th March, 2014.

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 64th WMA General Assembly, Fortaleza, Brazil, October 2013) and according to the Colipa Guidelines for the evaluation of the efficacy of cosmetic products (May 2008).

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 Clinical and Cosmetological Trials Center 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 a light yellow cream.

Name:	**VITA A KOMBI 1 – Ref. E1C 100 ml**
Batch/ Formule code:	**0110715**
Sample Code Abich:	1164/14-01
INCI composition:	see annex
Pao / Expiration date:	5 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 36 to 60, who were identified from the database of volunteers of the Abich Clinical and Cosmetological Trials Center, 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 Clinical and Cosmetological Trials Center. All patients signed a consent allowing to treat personal data according to the Italian law (Testo unico sulla privacy. D.Lgs 196/2003).

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.

Table 1: Volunteers partecipant to the study.

N°VOLUNTEER	CODE	AGE
1	MATR175	37
2	ROCA405	45
3	PAMU518	50
4	DABE206	47
5	TIRA309	48
6	CACA55	58
7	PAVI307	59
8	FEAD421	57
9	SAPO213	55
10	SACA38	36
11	LUTE520	60
12	DEBO349	58
13	ROIA359	57
14	DAFER17	51
15	GIGA455	51
16	LUFIU18	59
17	LOTU144	57
18	DOGI445	44
19	ANGR385	50
20	MOFO237	50
MEAN		**51.5**

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 pisitionning 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.

- **Canon EOS350D MACRO®:** digital reflex camera equipped with macro objective (Canon Italia,Milano).

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 30 days (time 30 days=T30) 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 nigth) for a period of 30 days on the entire face using a special brush provided by the sponsor.
The volunteers was asked to come in laboratory with clean face skin, without any other cosmetic product or any make-up which could interfer with the measurements.

7.4 Evaluated skin areas

The profilometric evaluation of the Rz and Ra parameters has been made on the crows's feet area; the analyzed areas at T0 and T30 days were as much as possible superimposable.

8. ESSAY METHODOLOGY

8.1 Study duration

The study lasted 30 days for each volunteer.

8.2 Preparation of the volunteer

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

8.3 Wrinkles measurement

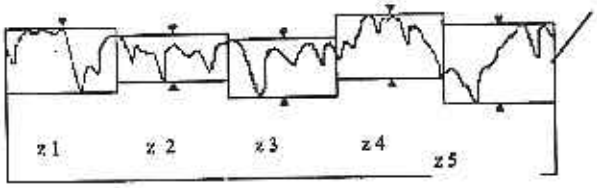
The analysis of the skin surface topography by the means of **Visio3D dermaTOP BLUE system (EOTECH,France)** is an innovative technique of image analysis without the need of replicas.

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.

Two 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 and Ra or arithmetical mean of roughness.**

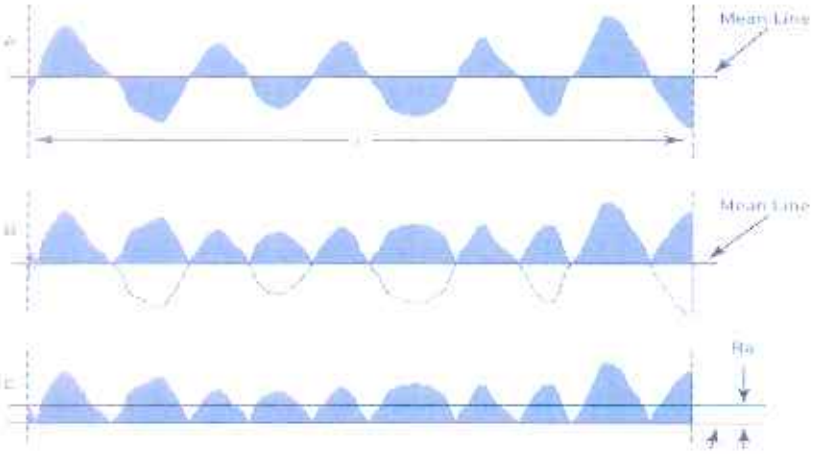
Rz represents the arithmetic average of the different segment roughness calculated from 5 succeeding measurement segments of the same length. In contrast to the other profile roughness parameters Rz is not that much influenced by artifacts due to calculating the average.

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



Ra represents the arithmetical mean of roughness and is the generally used parameter for the evaluation of skin roughness since it is based on the sampling of all the points characterizing the micro profile of the skin.

Ra



Skin surface changes were evaluated by comparing all the described skin parameters values before (T0) and after 30 (T30) 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 analyzed parameters were gathered for each participant and for each measurement time (see annexes).
The average values of each parameter for each measurement time were calculated for the 20 volunteers (Tables 2 and 4, Graphs from 1 to 4).
The % variations of the two parameters were calculated for each volunteer (see annex) and the average % variations were evaluated at T30 VS T0.

The distribution of the values obtained during the measurements at the various experimental times were compared with intra-group analysis (T0 versus T30) using Student's t test. P values<0.05 were considered significant.

11. RESULTS

Under the adopted experimental conditions, the product under examination **VITA A KOMBI 1 – Ref. E1C 100 ml Batch: 0110715** 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 (average maximum profile height difference)** resulted reduced by a mean value equal to 5.59% after 30 days (T30) of bi-daily application of the product respect to the Rz basal value evaluated at T0. This variation resulted statistically significant ($p<0,05$).
The reduction of this parameter indicates an increase in the skin smoothness consequent to a skin roughness decrease.

The tables below report the means of Rz on the panel of 20 volunteers at each observational time (T0 and T30, 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

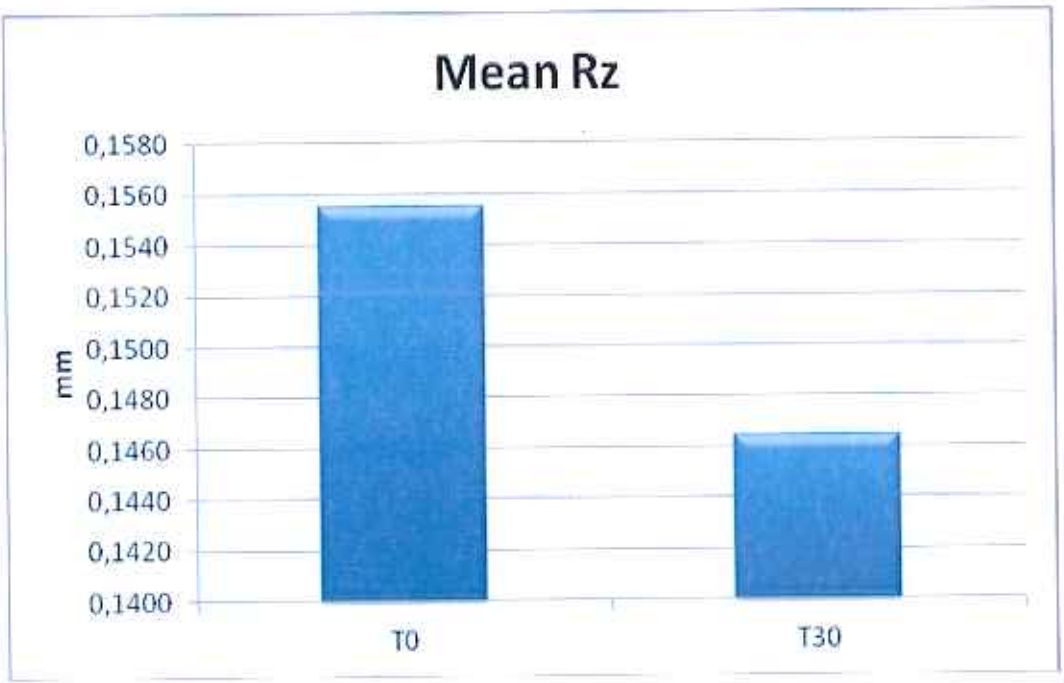
TIME	Mean Rz		Mean Rz L-R
	Left	Right	
T0	0,1527	0,1583	0,1555
T30	0,1430	0,1498	0,1464

Table 3

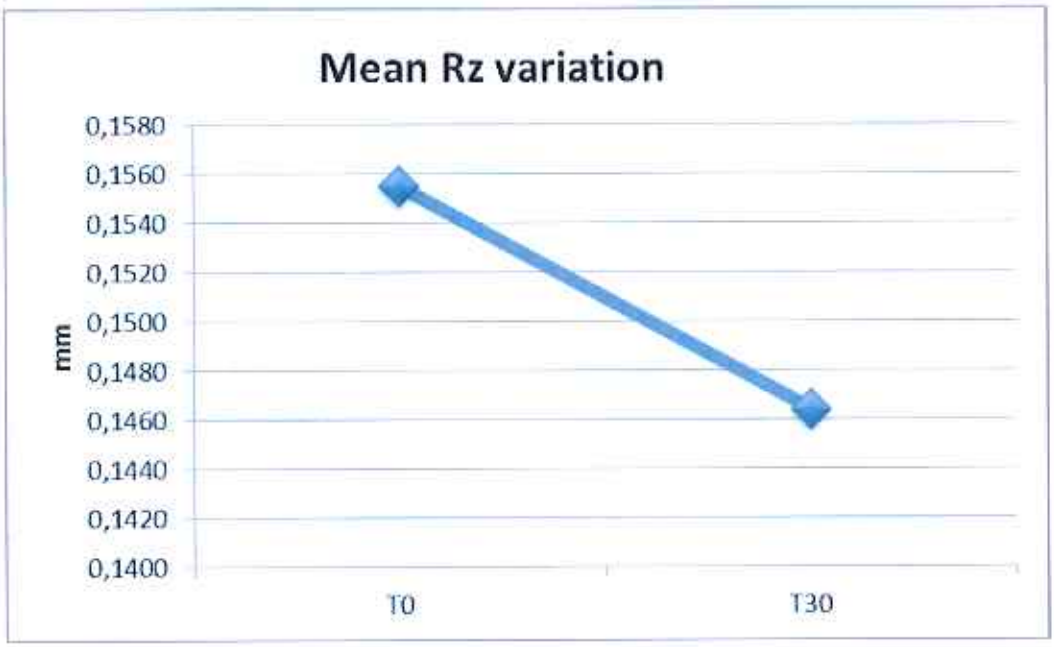
TIME	Mean % variation		Mean % variation L-R	p-value
	Left	Right		
T30 vs T0	-5,72%	-5,46%	-5,59%	0,0012*

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

Graph 1



Graph 2



Ra resulted reduced by a mean value equal to 6.51% after 30 days (T30) of bi-daily application of the product respect to the Ra basal value evaluated at T0.
The variations concerning the product resulted statistically significant vs T0 ($p<0,05$).
The reduction of this parameter indicates an decrease of skin roughness.

The tables below report the means of Ra on the panel of 20 volunteers at each observation times (T0, T30, table 4) and the mean % variation values of the same parameter calculated as arithmetical average of the single % variations of each volunteer (table 5).
The mean Ra value variations are moreover represented in form of graphs (Graph 3-4).

Table 4

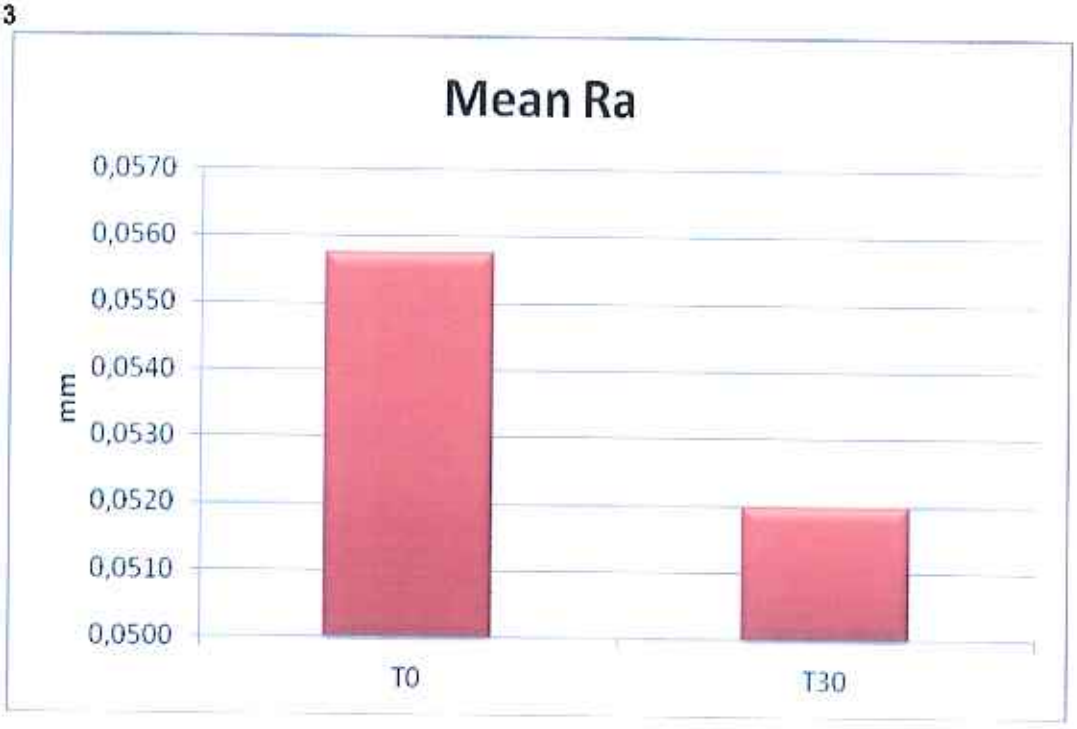
TIME	Mean Ra		Mean Ra L-R
	Left	Right	
T0	0,0534	0,0581	0,0558
T30	0,0498	0,0541	0,0520

Table 5

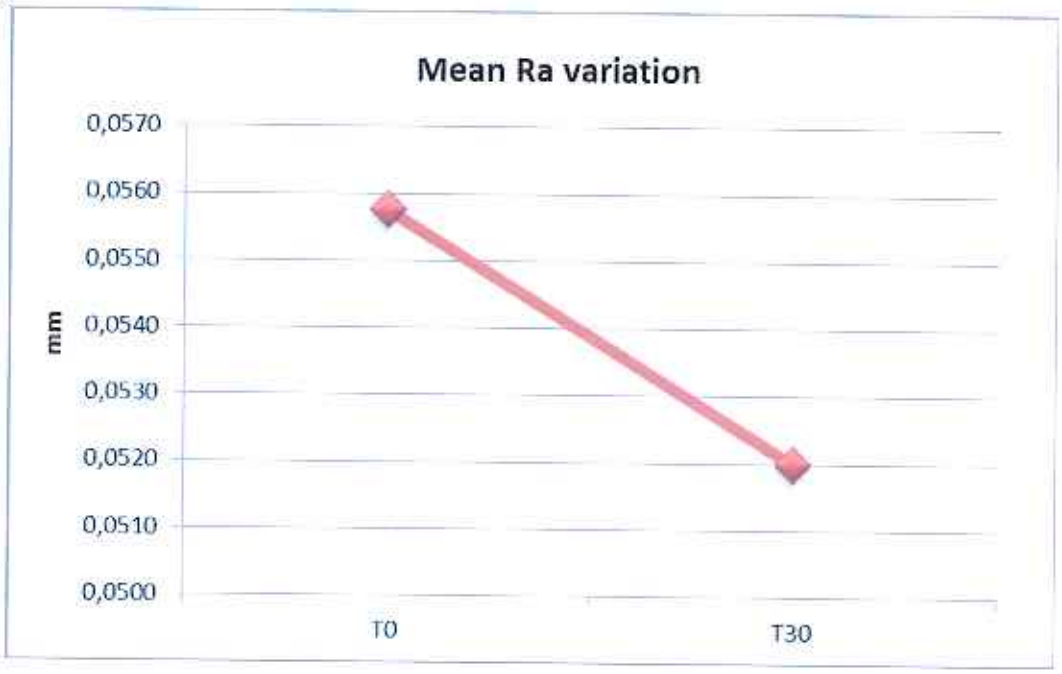
TIME	Mean % variation		Mean % variation L-R	p-value
	Left	Right		
T30 vs T0	-5,99%	-7,02%	-6,51%	P<0,0001*

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

Graph 3



Graph 4



MACROPHOTOGRAPHY

Representative images of the decrease of the skin roughness in the treated area made with digital reflex camera equipped with macro objective.

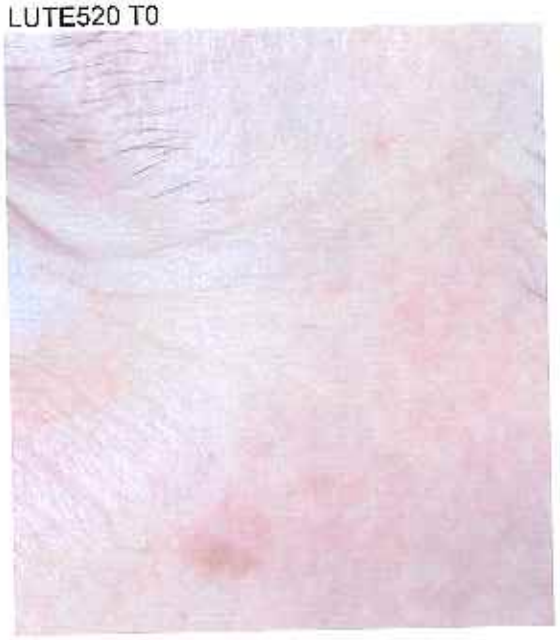
DABE206 T0

DABE206 T30

LOTU144 T0

LOTU144 T30

LUTE520 T0



LUTE520 T30

ROIA359 T0

ROIA359 T30

AZIENDA CERTIFICATA
UNI EN ISO 9001:2008
Certificato N. 501004992

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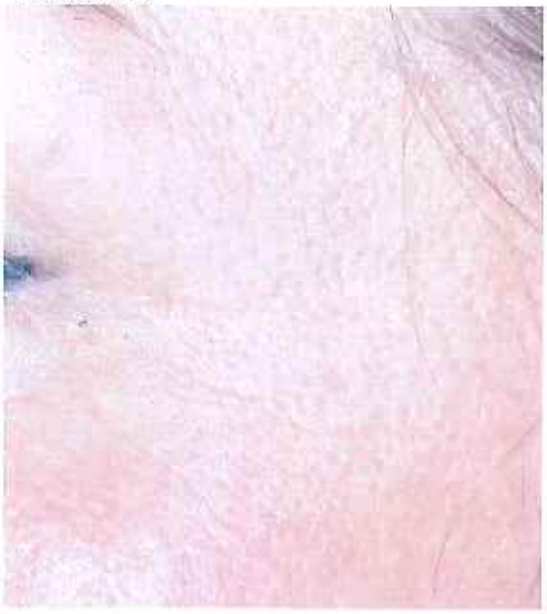
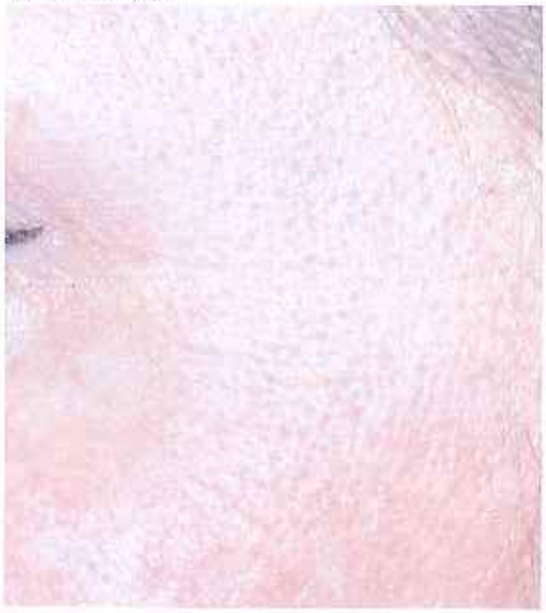
R.E.A.: 189901
Cap. Soc. € 16.000,00 i.v.

TIRA309 T0



TIRA309 T30



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

VITA A KOMBI 1 – Ref. E1C 100 ml Batch: 0110715

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 30 days of bi-daily application of the product.
In particular Rz resulted reduced by a mean value equal to 5,59% while Ra resulted reduced by a mean value equal to 6,51%.
These results are 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 Clinical and Cosmetological Trials Center, 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.

14. BIBLIOGRAPHY

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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
64th WMA General Assembly, Fortaleza, Brazil, October 2013

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)

Rz (mm)	T0		T30		T0	T30
Vol. Code	Left	Rigth	Left	Rigth	mean L-R	mean L-R
angr385	0,1456	0,1167	0,1429	0,1152	0,1312	0,1291
caca55	0,1659	0,1373	0,1464	0,1022	0,1516	0,1243
dabe206	0,2461	0,3031	0,2463	0,2861	0,2746	0,2662
dafer17	0,1219	0,1963	0,1321	0,2106	0,1591	0,1713
debo349	0,1700	0,1882	0,1591	0,1876	0,1791	0,1734
dogi445	0,1426	0,1132	0,1397	0,1085	0,1279	0,1241
fead421	0,1156	0,1017	0,1044	0,0837	0,1086	0,0940
giga455	0,0992	0,1464	0,0998	0,1388	0,1228	0,1193
lotu144	0,2241	0,2142	0,1872	0,1991	0,2192	0,1931
lufiu18	0,1303	0,1120	0,0918	0,1135	0,1212	0,1027
lute520	0,1460	0,1813	0,1336	0,1480	0,1636	0,1408
matr175	0,0918	0,0900	0,0919	0,0924	0,0909	0,0922
mofo237	0,1359	0,1285	0,1307	0,1221	0,1322	0,1264
pamu518	0,1138	0,1636	0,1136	0,1526	0,1387	0,1331
pavi307	0,2200	0,1623	0,1842	0,1489	0,1911	0,1666
roca405	0,2376	0,2106	0,2321	0,2087	0,2241	0,2204
roia359	0,1429	0,2075	0,1431	0,2028	0,1752	0,1729
saca38	0,0798	0,0758	0,0786	0,0747	0,0778	0,0766
sapo213	0,1616	0,1580	0,1670	0,1502	0,1598	0,1586
tira309	0,1638	0,1591	0,1363	0,1501	0,1614	0,1432
Mean	**0,1527**	**0,1583**	**0,1430**	**0,1498**	**0,1555**	**0,1464**

Raw data of Ra parameter (in mm)

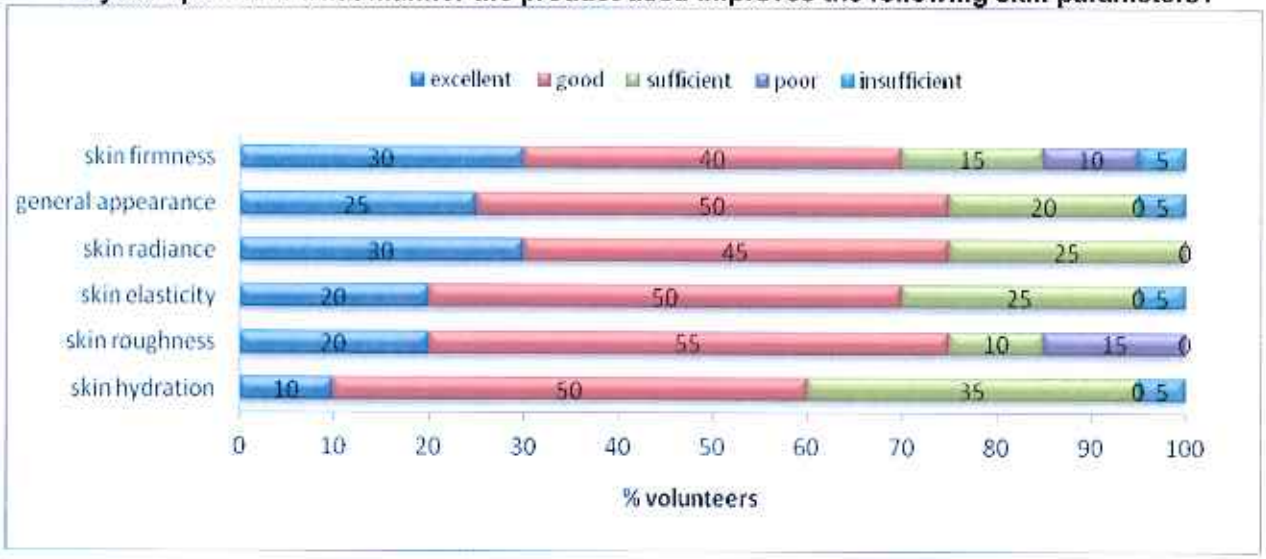
Ra (mm)	T0		T30		T0	T30
Vol. Code	Left	Rigth	Left	Rigth	mean L-R	mean L-R
angr385	0,0642	0,0714	0,0578	0,0684	0,0678	0,0631
caca55	0,0514	0,0453	0,0456	0,0364	0,0484	0,0410
dabe206	0,1072	0,0953	0,1035	0,0975	0,1013	0,1005
dafer17	0,0461	0,0841	0,0438	0,0831	0,0651	0,0635
debo349	0,0528	0,0733	0,0504	0,0688	0,0630	0,0596
dogi445	0,0516	0,0467	0,0485	0,0426	0,0492	0,0456
fead421	0,0401	0,0299	0,0366	0,0283	0,0350	0,0324
giga455	0,0312	0,0607	0,0301	0,0583	0,0460	0,0442
lotu144	0,0673	0,0713	0,0595	0,0642	0,0693	0,0618
lufiu18	0,0473	0,0518	0,0421	0,0420	0,0496	0,0420
lute520	0,0509	0,0652	0,0455	0,0533	0,0580	0,0494
matr175	0,0313	0,0282	0,0309	0,0276	0,0298	0,0292
mofo237	0,0458	0,0589	0,0421	0,0542	0,0524	0,0482
pamu518	0,0417	0,0619	0,0418	0,0548	0,0518	0,0483
pavi307	0,0686	0,0499	0,0587	0,0461	0,0592	0,0524
roca405	0,0876	0,0729	0,0819	0,0675	0,0803	0,0747
roia359	0,0516	0,0693	0,0508	0,0694	0,0605	0,0601
saca38	0,0232	0,0231	0,0252	0,0242	0,0232	0,0247
sapo213	0,0592	0,0546	0,0564	0,0532	0,0569	0,0548
tira309	0,0489	0,0483	0,0458	0,0427	0,0486	0,0442
Mean	**0,0534**	**0,0581**	**0,0498**	**0,0541**	**0,0558**	**0,0520**

ANNEX 2

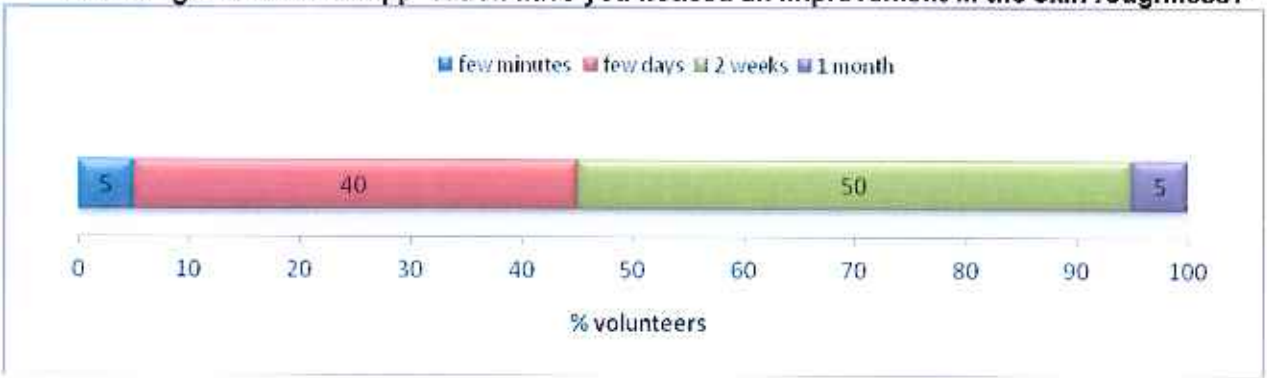
Questionnaire concerning a sensorial / psichorheological assessment relative to the tested product

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?**

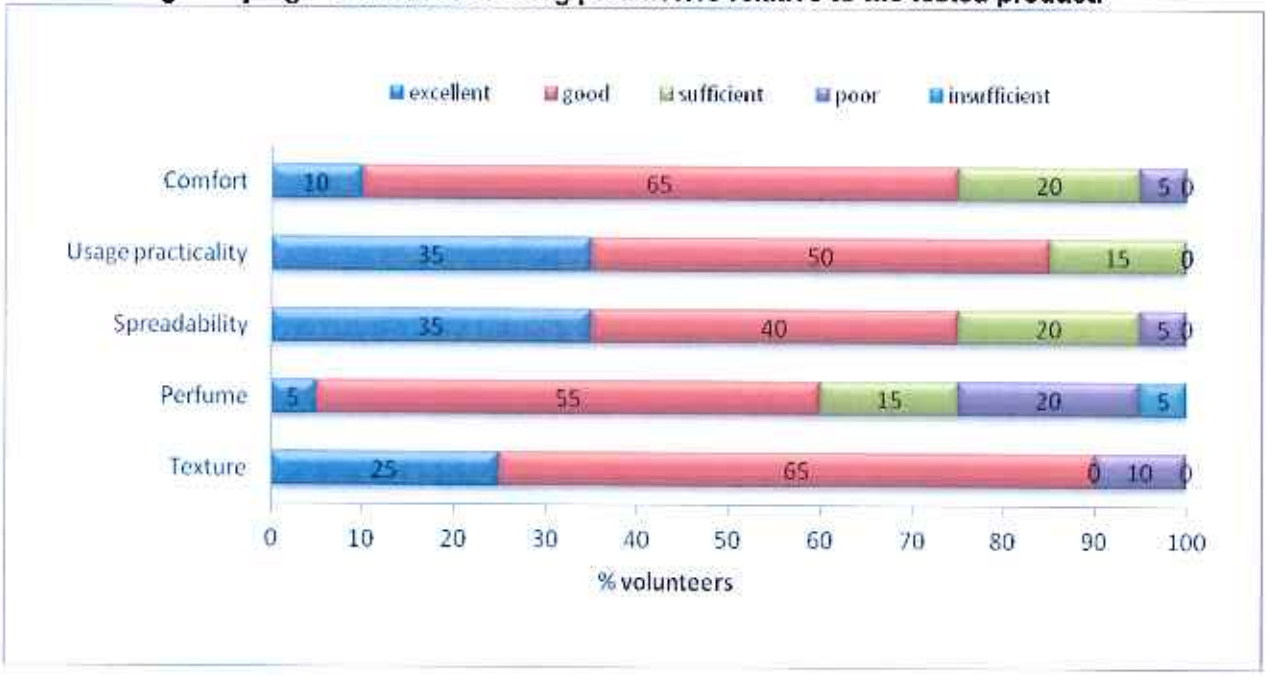


➢ **How long after the first application have you noticed an improvement in the skin roughness?**

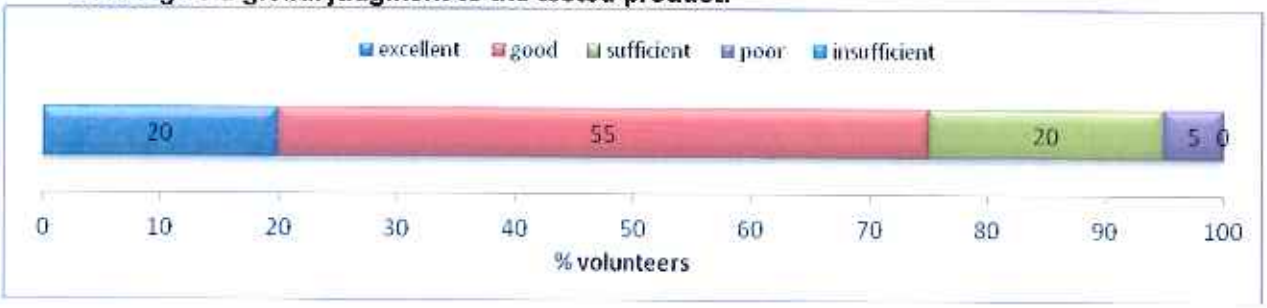


➢ **How long after the first application have you noticed an improvement in the skin hydration?**

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



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

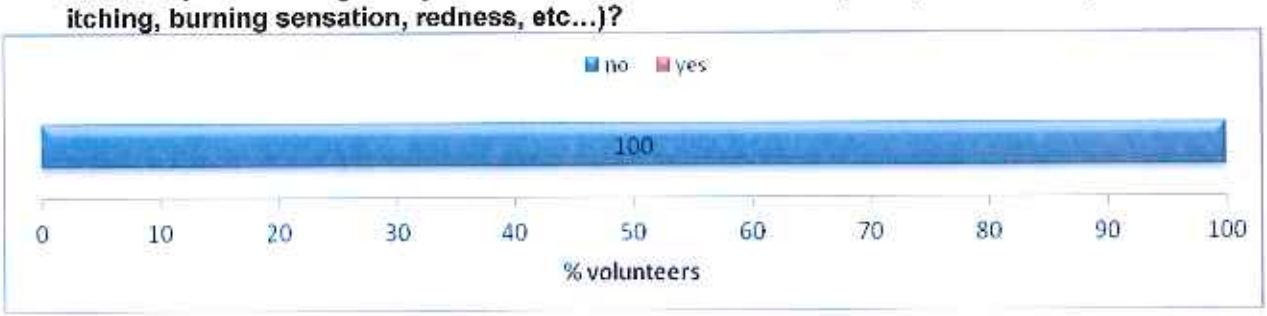


➢ **Would you recommend to someone the product purchase?***

*The subjects who answered "NO" to this question was asked to indicate the motivation.
The answers were:

- The product has an unpleasant odour.

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



ANNEX 3:

Inci list

Aqua, Petrolatum, Glyceryl stearate, Paraffinum liquidum, Tocopheryl acetate, Polysorbate 80, Stearyl alcohol, Cetyl alcohol, Hydrogen peroxide, Cetearyl ethylhexanoate, Isopropyl myristate, Salicylic acid, Fructose, Retinol, Parfum.