Milan, November 23rd 2011

INSTRUMENTAL EVALUATION OF A COSMETIC TREATMENT FOR COUPEROSE SKIN

METHOD: Ref. E16U + E17U

CUSTOMER: **GALENIA BIOTECNOLOGIE srl**
Strada del Casas, 6/3
10090 Rosta (TO) Italy

PRODUCT: **FLEBION FORTE – CREMA SPECIALE VISO COUPE-ROSE**
FLEBION FORTE – ANTI-REDNESS CREAM
Batch 1821 – Code 015

Ref. ISPE: 312/11/01 – 690/11

STARTING DATE OF THE STUDY: 05/10/2011

COMPLETION DATE: 22/11/2011

ETHICAL AND QUALITY CRITERIA

The current study was carried out in compliance with the quality assurance system requirements, according to the principles of good laboratory practice (GLP) and good clinical practice (GCP), as well as the principles established by the World Medical Association in the Declaration of Helsinki.

REFERENCES

The data given in this report are exclusively related to the tested sample.
This report can be only in full reproduced.

ISPE s.r.l.
Director of Laboratory
Dr. Luigi Rigano

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1. SAMPLE DATA SHEET

SAMPLE REF.: **FLEBION FORTE – CREMA SPECIALE VISO COUPE-ROSE**
FLEBION FORTE – ANTI-REDNESS CREAM
Batch 1821 – Code 015

Ref. ISPE: 312/11/01 – 690/11

SAMPLE ARRIVAL DATE: 03/10/2011

PRODUCT:

- Physical Form: cream
- Colour: light green

QUALITATIVE FORMULA:

- Known / yes /
- Other information / - /

OTHER INFORMATION RELATED TO THE PRODUCT SAFETY:
None.

FILE: a sample with code number **Ref. ISPE 312/11/01 – 690/11** and the study findings will be kept filed in our archives for one year and for ten years respectively. After these periods, the sample and the findings report will be discarded, unless otherwise required by the client.

**INSTRUMENTAL EVALUATION OF
A COSMETIC TREATMENT FOR COUPEROSE SKIN
(Ref. E16U + E17U)**

2. AIM OF THE STUDY

The aim of the study is to evaluate the efficacy of a cosmetic treatment in improving skin signs of couperose after repeated applications.

The efficacy of the product is proved by a decrease in skin redness (a* parameter) and in blood micro-flow baseline values in correspondence to the face areas with couperose.

3. SELECTION OF THE VOLUNTEERS

3.a. Criteria for recruitment and admission

The test is carried out according to the Declaration of Helsinki, on 20 females of average age 49.2 years.
Subjects are informed of the nature, purpose and risk of the study and give their written consent before participating in the test.
The selection is carried out according to the following criteria:

3.b. Inclusion criteria

- Race Caucasian.
- Female subjects, 30 – 70 years old, in good health with signs of couperose on the face.
- Subjects able to follow all study directions and to commit to all follow-up visits for the duration of the study.
- Subjects who complete the informed consent process.
- Subjects who avoid the exposure to UV radiation and the use of tanning beds for the duration of the study.

3.c. Exclusion criteria

- Pregnant or nursing females.
-Subjects with a history of unusual skin reactions to skin care toiletry products, cosmetics, or sensitivity to any of the test article components.

- Subjects who are taking topical or systemic drugs that could affect the results of the test (anti-inflammatory agents, corticosteroids, etc).
- Subjects showing systemic diseases or skin disorders (such as eczema, psoriasis, severe acne, etc.) that may affect the evaluation of the test articles or increase risk to the subject.
- Subjects currently involved in another clinical investigation or who have been involved within a period of 30 days prior to admission in this study.

3.d. Drop-out

The following reasons are considered sufficient cause for interrupting the subject's participation in the study:

- o free choice of the subject;
- o reasons not correlated with the treatment (*e.g.*, onset of disease, surgical operation);
- o reasons correlated with the treatment (*e.g.*, irritant or allergic reactions).

Details of any cases of drop-out are anyway included.

3.e. Restrictions

For the whole duration of the test subjects are instructed to wash their skin using their current skin care regimen and should not use different products on the face and should avoid UV and tanning beds exposure.

4. INSTRUMENTS

4.a. Colorimeter Chroma meter CR-400, Minolta

This is a portable dual channel, reflecting colorimeter with incorporated microcomputer, liquid crystals display and Xenon light source in the measuring head. The measuring head surface is 8 mm in diameter.
The colour rating system is CIE SYSTEM L*a*b*:

- **L*** refers to black - white axis. L* values range from 0 to 100, where 0 corresponds to black colour and 100 to white.
- **a*** and **b*** refer to two-colours axis: a* represents the red - green colour while b* the yellow - blue colour (range – 60 + 60).

In the present study, only the values relating to the skin redness (**a*** parameter) were taken into consideration.

4.b. Flowmeter Periflux PF4001, Perimed

The blood micro-flow is measured by means of a computerized laser Doppler device named Periflux PF4001.
A laser light, carried by an optic fibres probe, is partially reflected and partially absorbed by the examined tissue.
The light, hitting the moving haematic cells, is subjected to wavelength variation (Doppler effect) while the light hitting static bodies does not change its wavelength.
The power and frequency distribution of the wavelength variations is correlated to the number and the speed of the haematic cells, but not to their direction.
The relative information are picked up by a return optic fibre, turned into an electronic signal and analyzed.
The perfusion is expressed in Perfusion Units (P.U.), arbitrary units of the laser Doppler-device.

4.c. Digital images, Fotofinder Dermoscope Ver. 2.0

Fotofinder Dermoscope is a powerful and versatile system that allows carrying out and memorizing recorded images of any skin surface.
The system consists in a high-definition colour video camera which is able to magnify any surface on which it is placed by means of a series of magnifying lenses.
The digital images are shown on the screen in their real colours. This allows the observer to view the smallest details.
The software can also compare the recorded images to references recorded by the user.

5. METHOD

5.a. Method of evaluation

The instrumental measurements were carried out in a temperature and humidity-controlled room (24 ± 2 °C; 50 ± 10 % rh) after a 30 minutes acclimatization.
Volunteers were asked not to wash their face for 3 hours before the measurements and not to apply any product for 12 hours before each visit.

The basal measurements of skin colour and blood microcirculation were performed on the skin area of the face with couperose signs.

Then the product was given to the volunteers, who applied it on the whole face twice a day, in the morning and in the evening, for 4 weeks.

After 4 weeks of treatment the subjects returned to the laboratory for the final instrumental measurements.

5.b. Mathematical elaboration

Mean values, standard deviations, variations and percentage variations were calculated for each set of values.

Following the results of normality tests (Kolmogorov-Smirnov test, Lilliefors test and Shapiro-Wilk test) the instrumental data (T_0, T_{4wks}) were statistically compared by means of **paired samples t-test** for parametric and dependent data.

The groups of data were considered statistically significant for a probability value **$p \leq 0.05$**.

6. RESULTS, TABLES and GRAPHS

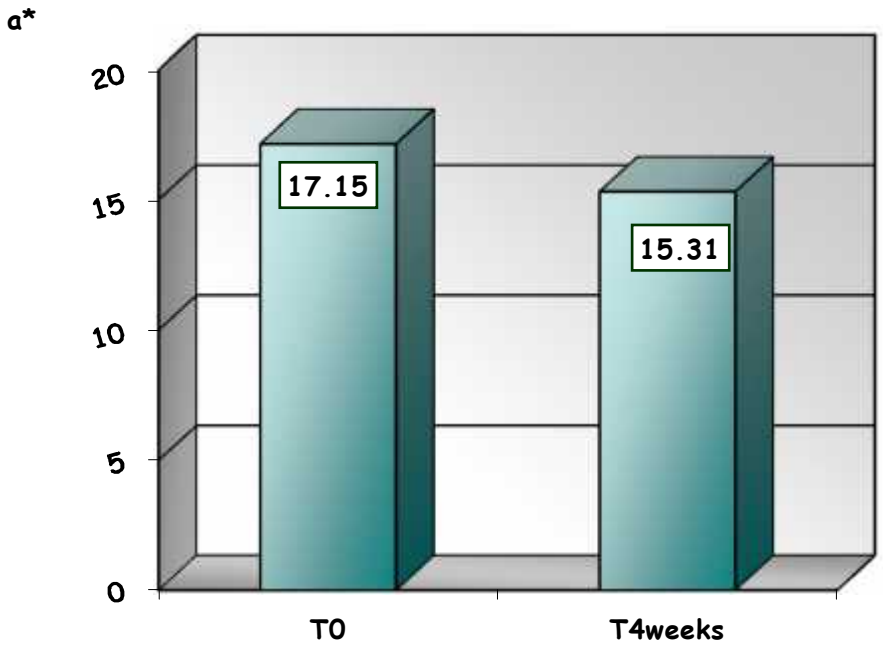
6.a. SKIN REDNESS (a* parameter)

A significant decrease in the baseline mean value of skin redness was detected after 4 weeks of treatment in correspondence of the face areas with couperose.

***Table 1**: Mean values, standard deviation, variation, percentage variation and statistical comparison.*

T_0	T_{4weeks}	Variation T_{4weeks} - T_0 (%)	t-test T_0 vs T_{4weeks}
mean 17.15 *std. dev. 2.63*	mean 15.31 *std. dev. 2.36*	**-1.84** **(-10.7%)**	**p < 0.001**

***Graph 1**: Mean values of a* parameter.*



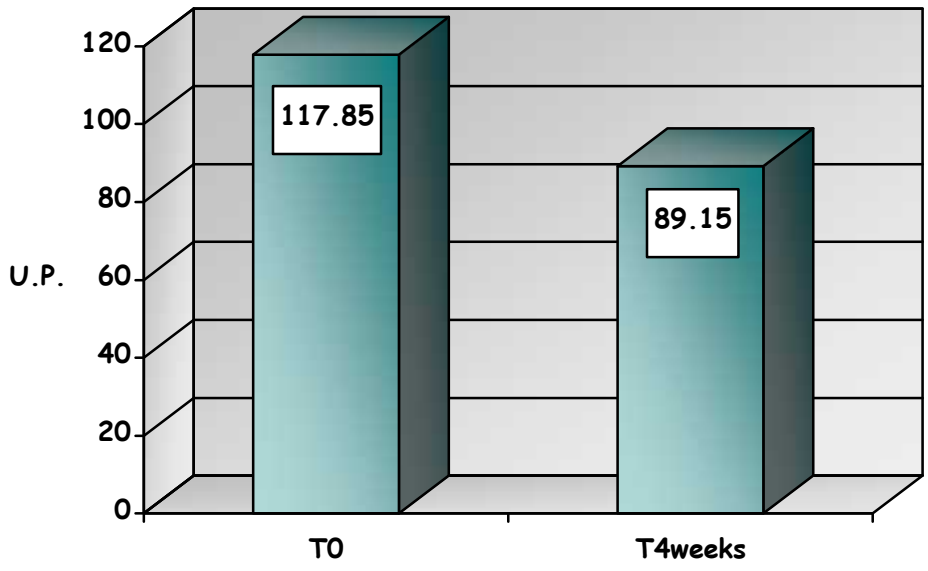
6.b. SKIN BLOOD MICROFLOW

A significant decrease in the baseline mean value of skin blood microflow was detected after 4 weeks of treatment in correspondence of the face areas with couperose.

Table 2: *Mean values, standard deviation, variation, percentage variation and statistical comparison.*

T_0	T_{4weeks}	Variation T_{4weeks} - T_0 (%)	t-test T_0 vs T_{4weeks}
mean 117.85 *std. dev. 44.73*	mean 89.15 *std. dev. 34.25*	-28.70 (-24.3%)	p < 0.001

Graph 2*: Mean values of skin blood microflow.*



8. CONCLUSIONS

In order to evaluate the efficacy of the treatment **FLEBION FORTE – CREMA SPECIALE VISO COUPE-ROSE, FLEBION FORTE – ANTI-REDNESS CREAM, Batch 1821 – Code 015, Ref. ISPE: 312/11/01 – 690/11**, 20 volunteers with couperose skin applied it on the face for 4 weeks.

The instrumental measurements performed at the beginning and after the application period showed a significant decrease in the mean value of skin redness and skin blood microflow in correspondence of the areas with couperose signs.

These findings evidence the application of the product **FLEBION FORTE – CREMA SPECIALE VISO COUPE-ROSE, FLEBION FORTE – ANTI-REDNESS CREAM** improved the strong redness and the high blood microflow values of the skin with couperose signs.

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Dr. Monica Prigioni

Dermatologist
Dr. Fernanda Distante

9. BIBLIOGRAPHY

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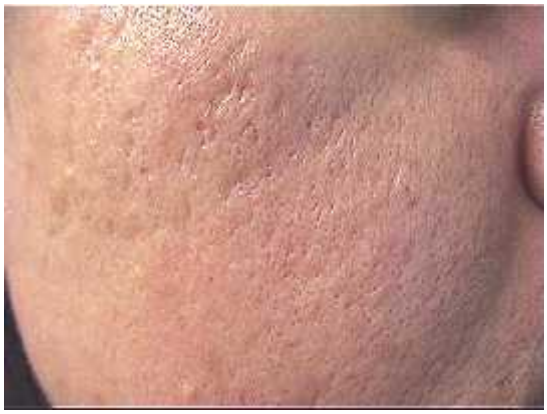
E. Berardesca, J.L. Lévêque, P. Masson, EEMCO group: "EEMCO GUIDANCE FOR THE MEASUREMENT OF SKIN MICROCIRCULATION", Skin Pharmacol Appl Skin Physiol.; 15(6): 442-456 (Nov-Dec. 2002);

J. Wilkin, M. Dahl, M. Detmar, L. Drake, M.H. Liang, R. Odom, F. Powell "STANDARD GRADING SYSTEM FOR ROSACEA: REPORT OF THE NATIONAL ROSACEA SOCIETY EXPERT COMMITTEE ON THE CLASSIFICATION AND STAGING OF ROSACEA"; J. Am. Acad. Dermatol. 2004;50:907-912.

Digital images of some volunteers of the study:

Subject 12



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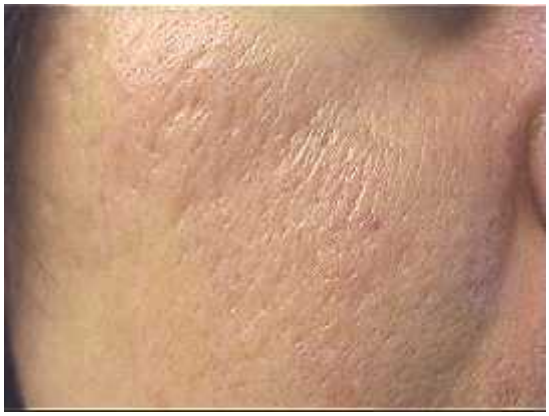

T4weeks

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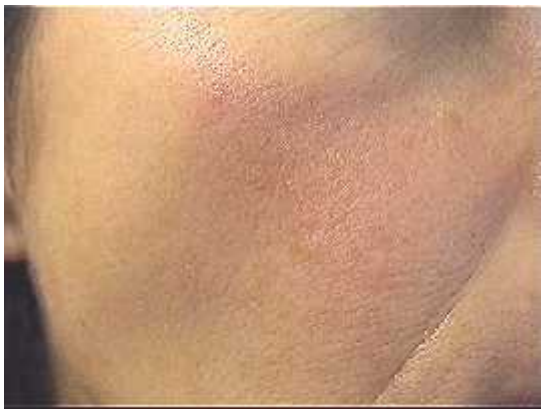

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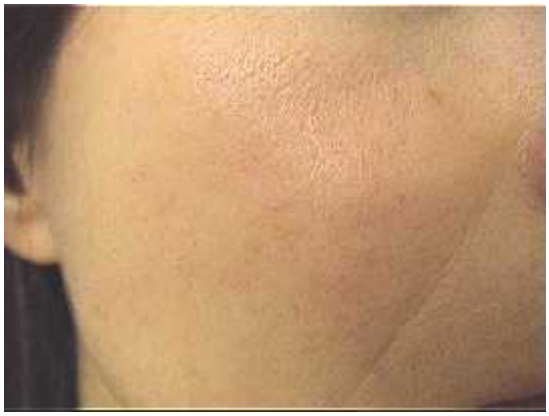

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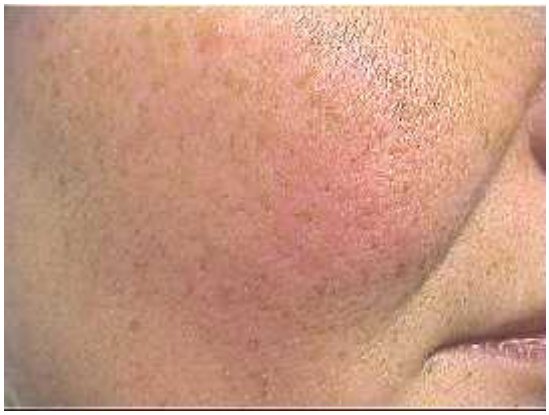

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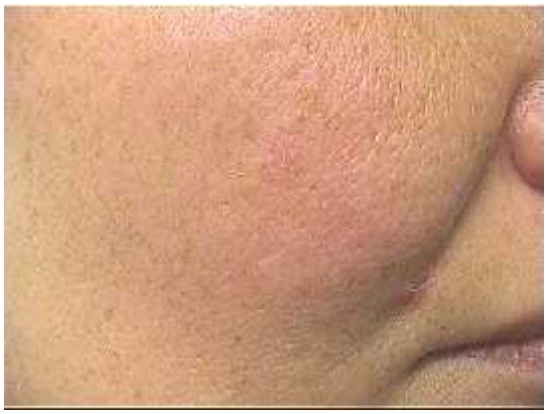

T4weeks