EVALUATION OF THE ANTI-AGING EFFICACY
OF A COSMETIC PRODUCT
CHECKING ITS ACCEPTABILITY AFTER APPLICATION
UNDER NORMAL CONDITIONS OF USE
OBJECTIVE ASSESSMENT OF ITS QUALITIES AND EFFICACY

In use test with clinical control by a dermatologist
and with instrumental evaluation

Short Report
This report is a summary of the report 790613.A, and reflects all data concerning
the study developed with the product.

I. OBJECTIVE

Skin aging is a combination of multiple aspects that consistently modify the appearance of skin. The most characteristic signs are the stratum corneum dryness, the loss of skin elasticity and firmness, due to a decrease in the fibroblast number and a decrease in the levels of collagen and elastin, the loss of architectural regularity, with the consequent appearance of wrinkles and fine lines, a change in the integrity of the skin barrier function, the appearance of hyperpigmented spots and a yellow type aspect.

The aim of the present study is to assess the anti-wrinkle efficacy and cutaneous recovery of elasticity, as well as to check the acceptability and assess the qualities and efficacy of the cosmetic product CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655 after application under the normal conditions of use planned by the Sponsor.

The anti-aging efficacy and cutaneous recovery of elasticity of the product was assessed:

- **objectively and quantitatively**, by instrumental measurements of the wrinkles with a VISIA-CA system, before and after treatment,
- **objectively and quantitatively**, by instrumental measurements of the wrinkle, skin roughness and topography with a Fringe projection system (Primos 3D°), before and after treatment,
- **objectively and quantitatively**, by instrumental measurements of the skin firmness and elasticity with a Cutometer™ (Dual-Cutometer MPA 580°), before and after treatment.

The acceptability was:

- controlled after visual examination of the experimental area, by the dermatologist or the technician, under his authority, and after questioning of the subjects.
- checked every day, by the subjects themselves at home,
The subjective qualities and efficacy of the products are assessed, at the end of the study, using a target questionnaire.

This study allows to justify the claim "anti-aging".

**II. DATES OF STUDY**

Beginning: June 26\(^{th}\), 2013  
End: July 24\(^{th}\), 2013

**III. SUBJECTS**

Fifteen (15) subjects were included in the study. There were neither withdrawals nor exclusions. The efficacy and compatibility of the test product was, therefore, assessed in fifteen (15) subjects, corresponding to these specific inclusion criteria:

a) Age: 35 to 65 years,

b) Gender: female,

c) Phototype (Fitzpatrick): I to V,

d) with visible signs of ageing (expression wrinkles in ocular area),

e) Type of skin: all types of skin.

**IV. METHODOLOGY**

**IV.1. Assessment of the firming effect and skin elasticity recovery**

**IV.1.1 Principle**

Skin biomechanical evaluation was performed by a Cutometer® dual MPA 580 using a 2mm probe. This system was used to measure elasticity of the upper skin layers using negative pressure which deforms the skin mechanically. The measuring principle is based on the suction method. Negative pressure is created in the device (450 mbar) and the skin is drawn into the aperture of the probe (2s) and after a defined time released again (2s). Inside the probe, the penetration depth is determined by a non-contact optical measuring system which 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 changes due to the penetration depth of the skin. The resistance of the skin to the negative pressure (firmness) and its ability to return into its original position (elasticity) are displayed as curves (penetration depth in mm/time) in real time during the measurement. This measurement principle allows getting information about the elastic and mechanical properties of skin surface.

**IV.1.2 Equipment**

The measurements were performed with a Cutometer dual MPA 580 (Courage & Khazaka, Germany) equipped with a 2 mm diameter probe and which generates 450 mbars negative pressure, allowing
stretching vertically the upper layers of the skin. The probe was located on an adhesive ring to perfectly immobilize the skin site. Each left and right ocular, malar and labial area was evaluated.

**IV.1.3 Frequency of measurements**

The measurements were performed on **D0 and D28**.

**IV.1.4 Expression and interpretation of the results**

At each experimental time, the biomechanical properties of skin were represented by a curve of deformation according to time.

![Figure 1 – Graphic representation of the Cutometer curves](image)

From this recording, the following parameters are retained:

**R0 (Uf) (in mm):** Highest point of the first curve. This parameter represents the passive behavior of the skin to force and is a measurement of firmness. A lower result is related with a higher firming effect.

**R2 (Ua/Uf) (in %):** Ratio between the ability of returning to the original position and the maximum amplitude. It is a standard parameter of elasticity. The closer the value is to 1 (100 %) the more elastic the curve.

The means and standard deviations were calculated.

The individual results are expressed:

- in absolute values of the parameter for each experimental time,
- in variation of the parameter against D0 for each experimental time.
- in % change of the parameter values and area variation of D28 against D0.
IV.2. Assessment of skin wrinkles, roughness and general topography

IV.2.1 Principle

3D images of the skin topography were obtained by a digital fringe projection using DLP® micro mirror displays. A fringe pattern is projected on the skin and detected by the CCD camera of the optical system. The 3D effect is calculated by the deflection in the fringes which represent qualitative and quantitative the skin profile. Each ocular and lips area was evaluated (left and right).

IV.2.2 Equipment

A Phase Shifting Rapid In-vivo Measurement of Skin system (PRIMOS 3D 40x30 mm evaluation area, Gfm, Germany) was used.

IV.2.4 Frequency of measurements

The measurements were performed on D0 and D28.

IV.2.5 Expression and interpretation of the results

At each experimental time, the wrinkle parameters and standard roughness was calculated in the full aligned image.

The parameters defined were:

Wrinkle count
Wrinkle volume (µm)

The individual results are expressed:

- in absolute values of the parameter for each experimental time,
- in variation of the parameter against D0 for each experimental time.
- in % change of the parameter values and area variation of D28 against D0.

IV.3. Assessment of the anti-ageing effect by VISIA-CA instrumental evaluation.

IV.3.1 Principle

Standardized photographic images with normal, cross polarized and UV lamp of both hemifaces and the front were obtained before and after the treatment (D0 and 28) with the system VISIA-CA (Canfield Scientific, USA), in order to quantify the evolution of the ageing signs.

IV.3.2 Measuring area

The measuring areas in the face were the chosen automatically by the system after proper alignment. For the imaging system, a mask for the defined area chosen was created and applied in the subsequent images, in order to calculate the differences against D0.
IV.3.3 Equipment

VISIA-CA imaging system (Canfield Scientific, USA) is a standardized imaging photographic system that takes high resolution photos with a Standard light IntelliFlash, a Cross Polarized flash, and under a Ultraviolet lighting. The analysis software allows a definition of a Region Of Interest and the calculation of the areas and number of the following parameters:

- Pores
- Brown spots
- UV Spots

For each parameter, a feature count inside the area and an area in % of pixels was calculated.

IV.3.4 Frequency of measurements

The measurements were performed on D0 and D28.

IV.3.5 Expression and interpretation of the results

Each feature and area inside the defined area were calculated in each evaluation day by the system.

The individual results were expressed:

- in absolute values of the parameter for each experimental time,
- in variation of the parameter against D0 for each experimental time.
- in % change of the parameter values and area variation of D28 against D0.

IV.4. Checking of the acceptability

The results were mainly expressed as descriptive data and do not require a statistical treatment. The test products been well accepted by the subjects, under these experimental conditions, by extrapolation it should be safe for human health when applied by a great panel of consumers.

IV.4. Experimental conditions of use of the test product

The experimental conditions, defined by protocol, were the following ones:

<table>
<thead>
<tr>
<th>Experimental area(s)</th>
<th>Product directions of use</th>
<th>Application(s) at the Institute</th>
<th>Application(s) at home Frequency/duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face</td>
<td>Application of CREME FACIAL LEITE DE BURRA, on clean skin by gentle digital massage until complete absorption, twice a day.</td>
<td>-</td>
<td>From D0 to D28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Application, twice a day</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(morning - after bath, evening - at bedtime)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>for 28 +/- 2 consecutive days</td>
</tr>
</tbody>
</table>
V. RESULTS

V.1. Results from the dermatological evaluation

<table>
<thead>
<tr>
<th>Volunteers codification</th>
<th>Skin reaction</th>
<th>Number and % of subjects exhibiting skin reaction to the test product</th>
</tr>
</thead>
<tbody>
<tr>
<td>/</td>
<td>None</td>
<td>0%</td>
</tr>
</tbody>
</table>

V.2. Results from the questionnaire asked to the volunteers and observation of the individual observation sheet

<table>
<thead>
<tr>
<th>Volunteers codification</th>
<th>Sensations of discomfort noted by the volunteers at home</th>
<th>Number and % of subjects exhibiting sensations of discomfort ascribable to the test product</th>
</tr>
</thead>
<tbody>
<tr>
<td>/</td>
<td>None</td>
<td>0%</td>
</tr>
</tbody>
</table>

No skin reaction was noted after the application of the products. No volunteer experienced any discomfort during the study. Therefore both products presented **very good** skin compatibility during the study.

V.3. Assessment of qualities and efficacy (self-assessment) of the products

<table>
<thead>
<tr>
<th>Item</th>
<th>Q1. The product has a pleasant fragrance?</th>
<th>Q2. The product is easy to spread?</th>
<th>Q3. The product has a nice texture?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>0 2 0 6 7</td>
<td>0 1 0 7 7</td>
<td>0 2 0 8 5</td>
</tr>
<tr>
<td>% of satisfied subjects</td>
<td>86.7%</td>
<td>93.3%</td>
<td>86.7%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>Q4. The product is easily absorbed by the skin?</th>
<th>Q5. The product has a nice colour?</th>
<th>Q6. The product does not leave the skin greasy?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>0 3 0 7 5</td>
<td>0 0 4 7 4</td>
<td>0 2 0 11 2</td>
</tr>
<tr>
<td>% of satisfied subjects</td>
<td>80%</td>
<td>73.3%</td>
<td>86.7%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>Q7. The product is pleasant to touch?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>0 2 0 9 4</td>
</tr>
<tr>
<td>% of satisfied subjects</td>
<td>86.7%</td>
</tr>
</tbody>
</table>
Figure 1: Self-assessment of qualities. % of total number of volunteers (n = 15), the favorable responses to the questionnaire cosmetic quality. It is also indicated that the responses were statistically significant (* p <0.05)

The product evaluation showed that, for a significant percentage of volunteers, the product:
- Has a pleasant fragrance,
- It is easy to spread,
- Has a nice texture,
- Is easily absorbed by the skin,
- Leaves no greasy skin,
- Is pleasant to touch.

The following tables show in summary the results for each item, the number of volunteers, and % of satisfied volunteers:

<table>
<thead>
<tr>
<th>Item</th>
<th>Q1. After using the product, I feel the skin more moisturized</th>
<th>Q2. After using the product, I feel the skin softer and smoother</th>
<th>Q3. After using the product, I notice an improvement in the overall appearance of my skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>1 1 2 6 5</td>
<td>1 1 1 8 4</td>
<td>0 1 5 5 4</td>
</tr>
<tr>
<td>% of satisfied subjects</td>
<td>73.3%</td>
<td>80%</td>
<td>60%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>Q4. The skin looks smoother, with a more uniform appearance</th>
<th>Q5. After using the product, I noticed reduced wrinkles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>0 1 4 6 4</td>
<td>0 2 5 7 1</td>
</tr>
<tr>
<td>% of satisfied subjects</td>
<td>66.7%</td>
<td>53.3%</td>
</tr>
</tbody>
</table>
Figure 2: % of total number of volunteers (n=15), the favorable responses to the cosmetic efficacy questionnaire. It is also indicated that the responses were statistically significant (* p<0.05).

The product evaluation showed that for a significant percentage of volunteers, the product:
- Leaves skin softer and smoother.

V.4. Assessment of the firming effect and skin elasticity recovery

The results of skin elasticity and firmness before and after use of the product expressed in % change relative to D0 and are summarized in the following charts:

Figure 3 and 4: (Mean + std. Standard) graphic showing the variation of firmness parameter (R0) and of elasticity parameter (R2) relative to D0. It is also indicated statistically significant (* p<0.05).
V.4.1 Evaluation of skin relief - Eye Zone

The results of the skin relief, before and after using the product, expressed as the number of wrinkles and volume μm are summarized in the chart below:

![Graph showing the evolution of wrinkles and volume over 28 days.]

Figure 5: (Mean ± std. Standard) graphic showing the evolution of wrinkles in the ocular area during the study. It is also indicated statistically significant (* p <0.05).

![Graph showing the evolution of wrinkles volume.]

Figure 6: (Mean ± std. Standard) graphic showing the evolution of wrinkles volume (ocular) during the study. It is also indicated statistically significant (* p <0.05).

<table>
<thead>
<tr>
<th></th>
<th>ocular</th>
<th>Left side</th>
<th>Right side</th>
<th>Global</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Change in the number of wrinkles after 28 days</td>
<td></td>
<td>-42.3</td>
<td>-39.6</td>
<td>-41.0</td>
</tr>
<tr>
<td>% Change in the wrinkles volume after 28 days</td>
<td></td>
<td>-18.3</td>
<td>-20.6</td>
<td>-19.5</td>
</tr>
</tbody>
</table>
The results of the skin relief, before and after using the product, expressed as the number of wrinkles and volume μm are summarized in the chart below:

Figure 7: Representative graphic of wrinkle reduction (Vols 1 and # 3) before and after applying the product. Figure obtained after detection of the common area and standard automatic alignment.

**V.4.2 Evaluation of skin relief – Labial area**

Figure 8: (Mean ± std. Standard) graphic showing the evolution of wrinkles in the labial area during the study. It is also indicated statistically significant (* p <0.05).

Figure 9: (Mean ± std. Standard) graphic showing the evolution of wrinkles volume (labial area) during the study. It is also indicated statistically significant (* p <0.05).
<table>
<thead>
<tr>
<th>lips</th>
<th>Left side</th>
<th>Right side</th>
<th>Global</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Change in the number of wrinkles after 28 days</td>
<td>-40.0%</td>
<td>-34.4%</td>
<td>-37.2%</td>
</tr>
<tr>
<td>% Change in the wrinkles volume after 28 days</td>
<td>-28.9%</td>
<td>-25.8%</td>
<td>-27.3%</td>
</tr>
</tbody>
</table>

Figure 10: Representative graphic of wrinkle reduction (Vols #4 and 15) before and after applying the product. Figure obtained after detection of the common area and standard automatic alignment.
V.4.2 Evaluation of photoaging – standard image

The evaluation results of photoaging, expressed as UV spots, brown spots and pores before and after the use of the product, expressed in number of spots / pores, are summarized in the chart below:

Figure 11: (Mean + std. Standard) graphic showing the evolution of spots and pores during the study. It is also indicated statistically significant (* p <0.05).

Figure 12: (Mean + std. Standard) graphic showing the area occupied by spots and pores during the study. It is also indicated statistically significant (* p <0.05).

<table>
<thead>
<tr>
<th>% Change in the number of spots and pores after 28 days</th>
<th>UV spots</th>
<th>Brown spots</th>
<th>Pores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-10,57%</td>
<td>-4,61%</td>
<td>-2,65%</td>
</tr>
<tr>
<td>% Change in the area of spots and pores after 28 days</td>
<td>-8,15%</td>
<td>1,54%</td>
<td>-6,24%</td>
</tr>
<tr>
<td>Vol # 7 – D0 (left)</td>
<td>Vol #7 – D28 (left)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vol # 15 – D0 (right)</th>
<th>Vol #15 – D28 (right)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vol # 15 – D0 (left)</th>
<th>Vol #15 – D28 (left)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure 13:** Representative pictures of Wrinkle Reduction (Vols # 7 and 15), before and after applying the product. Pictures obtained by the VISIA system.
V.5. Discussion

The results of instrumental evaluation show a significant increase in skin firmness and elasticity 27.1% and 18.7% improvement. This result is consistent with that expected for a product that modulates the elasticity.

The evaluation results of the skin relief suggested a statistically significant reduction in wrinkles count of 41.0% and in the wrinkles volume of 19.5% in the ocular area; and a statistically significant reduction in wrinkles count of 37.2% and in the wrinkles volume of 27.3% in the labial area.

For the analysis of the effects of photoaging, represented by the UV and brown spots, there is a significant reduction in the number of spots detected by the VISIA system. This reduction is 10.6% in the UV spots and 4.6% in brown spots.

Likewise, there is a reduction in the area occupied by the spots, although not statistically significant. This suggests the complete disappearance of some spots and the maintenance of the size of those which did not disappear.

In terms of pores, there is a slight decrease in the count, although not significantly. However there is a significant reduction of pore area of 6.2%, suggesting the non comedogenicity of the product, confirmed by analysis of its acceptability.

VI. CONCLUSION

Under the experimental conditions and taking into account the grading scale established by the researcher center, the product CREME FACIAL LEITE DE BURRA, batch 330127/A 655 has a very good acceptability. A very good skin tolerance was thus confirmed.

Regarding other features, the product was well assessed for its cosmetic qualities, notably its:
- Pleasant fragrance,
- Ease of spreading,
- Nice texture,
- Easier to be absorbed by the skin,
- Does not leave the skin greasy,
- Pleasantness to the touch.

Likewise, the product was much appreciated for its cosmetic efficacy, including:
- Capability to leave the skin softer and smoother.

In quantitative terms:

The application of the product significantly increased the skin firmness by 27.1%.

The application of the product significantly increased the skin elasticity by 18.7%.

The application of the product significantly reduced the number of wrinkles in the ocular area by 41.0%.
The application of the product significantly reduced the volume of skin wrinkles in the ocular area by 19.5%.

The application of the product significantly reduced the number of wrinkles in the nasolabial area by 37.2%.

The application of the product significantly reduced the volume of skin wrinkles in the ocular area by 27.3%.

The application of the product significantly reduced the number of brown and UV spots in the skin by 4.6% and 10.6%, respectively.

The application of the product maintained the number of pores, but significantly reduced its area by 6.2%.

The results suggest that the product CREME FACIAL LEITE DE BURRA, batch 330127/A 655 - after application for 28 days on 15 volunteers under the conditions described - improved skin elasticity and has the capability of reducing the wrinkles' appearance. Moreover, the product CREME FACIAL LEITE DE BURRA, batch 330127/A 655 significantly reduces some of the parameters of skin aging.

Scientific Manager: Pedro CONTREIRAS PINTO

I, the undersigned, Pedro CONTREIRAS PINTO, declare that:
- the final report was examined on August 2nd, 2013.
- the results reported accurately and completely reflect the raw data of the study.