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ASSESSMENT OF THE SOOTHING EFFECT OF A COSMETIC PRODUCT ON SKIN DISCOMFORTS INDUCED BY A CHEMICAL IRRITANT (LACTIC ACID)

RIVOLI COSMÉTIQUES SA
RIVOLI GENÈVE Le Visage TONIC
Formula Reference: lab-00003.8

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Centro di Saggio operante in BPL - Sistema di gestione qualità certificato ISO 9001
Iscritto al Registro Regione Lombardia ai fini dell'autocontrollo alimentare (N 030015309008)

KEY PERSONNEL

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STUDY DESIGN

1.1 Title

Assessment of the soothing effect of a cosmetic product on skin discomforts induced by a chemical irritant (lactic acid).

1.2. Aim of the study

The study is aimed to assess the effect of a cosmetic product in decreasing the skin discomforts induced by a chemical irritant (lactic acid). In order to reach this goal, a clinical study is carried out on 35 healthy female subjects aged over 18 years old with sensitive skin (positive to stinging test*). The study in carried out in comparison to a negative control skin area treated with demineralized water.

*10% lactic acid aqueous solution is applied to the sensory innervation-rich (hypersensitive) skin of both nasolabial fold sides, in order to detect stinging sensation that typically arise in sensitive subjects within few minutes.

1.3. Tested product

1.3.1. Information provided by the Customer

- ☒ Product name: **RIVOLI GENÈVE Le Visage TONIC Formula Reference: lab-00003.8**
- ☒ Way of use: tested product is applied by the experimenter, by means of a soaked cotton swab, on right/left nasolabial fold according to a previously defined randomization list. The contralateral side is treated with demineralized water and acts as negative control.
- ☒ The test cosmetic product conforms to Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast) (Text with EEA relevance) and to its annexes.
- ☒ The cosmetic product to be tested is safe under normal or reasonably foreseeable conditions of use.
- ☒ Qualitative INCI formula: AQUA (WATER), PENTYLENE GLYCOL, BUTYLENE GLYCOL, PPG-1-PEG-9 LAURYL GLYCOL ETHER, SODIUM BENZOATE, ALLANTOIN, POTASSIUM SORBATE, CITRIC ACID, ADENOSINE TRIPHOSPHATE, HYDROLYZED OAT PROTEIN, NIACINAMIDE, TAMARINDUS INDICA SEED GUM, BISABOLOL, PARFUM (FRAGRANCE), DIPOTASSIUM PHOSPHATE, PVP, DISODIUM EDTA

1.4. Ethical requirements

The study was carried out in compliance with the following ethical requirements.

I. All the subjects participating in the study are healthy volunteers of at least 18 years old.
II. All the subjects participating in the study, are selected with the supervision of a dermatologist according to inclusion/non-inclusion criteria.
III. The volunteer's participation in the study is free.
IV. All the subjects participating in the study are informed of the aim and the design of the study.
V. All the subjects participating in the study are informed of the possible risk involved in the study execution.
VI. All the subjects participating in the study give their informed consent signed at the beginning of the study.
VII. Before the volunteers are exposed to the product to be tested, all relevant safety information about the product itself and each ingredient are collected and evaluated.
VIII. 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).
IX. All the precautions are taken in consideration in order to avoid excessive skin reactions.
X. In case of non-expected/adverse skin reaction occurrence, the medical experimenter evaluates the severity of the reaction (reporting it in the data collecting sheet) and as a consequence he proceeds with the appropriate therapy.

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1.5. Subjects participating in the study

1.5.1. Subjects' enrolment

The subjects participating in the study are screened and enrolled in the study under the supervision of a board-certified dermatologist from a panel of healthy subjects, in accordance with the inclusion and non-inclusion criteria reported in the sections here below.

1.5.1.1. Inclusion criteria

- ☑ Healthy female subjects
- ☑ Caucasian ethnicity
- ☑ Aged over 18 years old
- ☑ Subjects with sensitive skin (positive to stinging test, score ≥2)
- ☑ Subjects registered with National Health Service (NHS)
- ☑ Subjects certifying the truthfulness of the personal data disclosed to the investigator
- ☑ Subjects able to understand the language used in the investigation center and the information given by the investigator
- ☑ Subjects able to respect the instructions given by the investigator as well as able to respect the study constraints and specific requirements
- ☑ The pharmacological therapy (except for the pharmacological therapy in the non-inclusion criteria) should be stable for at least one month without any changes expected or planned during the study
- ☑ Commitment not to change the daily routine or the lifestyle
- ☑ Subjects who have not been recently involved in any other similar study
- ☑ Subjects informed about the test procedures and who have signed a consent form.

1.5.1.2. Not-inclusion criteria

- ✘ Subject does not meet the inclusion criteria
- ✘ Subjects with acute or chronic diseases able to interfere with the outcome of the study or that are considered dangerous for the subject or incompatible with the study requirements
- ✘ Subjects participating or planning to participate in other clinical trials
- ✘ Subjects deprived of freedom by administrative or legal decision or under guardianship
- ✘ Subjects not able to be contacted in case of emergency
- ✘ Subjects admitted to a health or social facility
- ✘ Subjects planning a hospitalisation during the study
- ✘ Subjects who participated in a similar study without respecting an adequate washout period
- ✘ Subjects having an acute, chronic or progressive illness liable to interfere with the study data or considered by the Investigator hazardous for the subject or incompatible with the study requirements
- ✘ Subjects under pharmacological treatments that are considered incompatible with the study requirement by the investigator
- ✘ Subjects having a skin disease or condition liable to interfere with the study data or considered by the Investigator hazardous for the subject or incompatible with the study requirements
- ✘ Subjects that have shown allergies or sensitivity to cosmetic products, drugs, patch or medical devices
- ✘ Subject breastfeeding, pregnant or not willing to take necessary precautions to avoid pregnancy during the study (for the women of childbearing potential).

1.5.1.3. Withdrawal of subjects

A subject enrolled in the study can be withdrawn and considered as a drop-out when: (i) adverse reactions, judged severe and attributable to the tested product, occur, (ii) the subject is no longer eligible to participate in the study, (iii) the subject develops a pathological condition, not related to the study, but appearing during the study period, (iv) it is required the prescription of a concomitant treatment, (v) the study requirements are not satisfied (significant deviation from the protocol), (vi) significant non-compliance with respect to product use or to the study protocol.

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1.6. Study development

The study is carried out as follow:

- Enrolment of 35 subjects according to inclusion/non-inclusion criteria.
- Application of a 10% lactic acid aqueous solution to the skin of both nasolabial fold sides, in order to induce the stinging sensation.
- **Tmax:** when the discomfort peaks its maximum sensation (approximately few minutes after the application of the 10% lactic acid aqueous solution) the experimenter scores the intensity of the perceived discomfort and applies the tested product by means of a soaked cotton stick, on right/left nasolabial fold according to a previously defined randomization list. The contralateral side is treated with demineralized water and acts as negative control.
- **Timm:** the experimenter scores the intensity of the perceived discomfort immediately after the first product/water application
- **T30'':** the experimenter scores the intensity of the perceived discomfort 30 seconds after product/water application
- **T1'/T2'/T3'/T4'/T5':** the experimenter scores the intensity of the perceived discomfort 1/2/3/4/5 minutes after product/water application

1.7. Materials and methods

In the sections here below are reported the materials and the methods employed in the study.

1.7.1. Clinical scoring of the stinging sensation

At each monitored time the experimenter registers, with the collaboration of the subject, the intensity of the perceived stinging sensation according to the scores reported in the table below.

Table A. Clinical scoring of the stinging sensation	**Score**
No reaction	**1**
Mild reaction	**2**
Moderate reaction	**3**
Severe reaction	**4**

1.8. Results and Statistics

1.8.1. Results

The results are reported in tables in their respective units.

1) The mean values are calculated as:

$$m = \frac{\sum_{1}^{20} p}{20} \qquad [1]$$

where: p is the value of the parameter to be analysed.

2) The standard error of the mean is calculated as:

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

where: p is the value of the parameter to be analysed, n is the number of subjects

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

1.8.2. Statistical analysis

Data were submitted to Wilcoxon signed test. The intra-group statistical analysis was carried out vs Tmax. The inter-group statistical analysis was carried out on treated vs. control area data, at each experimental monitored time. Variations are considered statistically significant when p value is ≤ 0,05.
Statistical analysis is performed using NCSS 10 software.

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1.9. 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 analyzed 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 (e.g. active vs. placebo) 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 evaluated 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 exist, those values are used to validate product claim(s).

Our studies are conducted inspiring by the general principle of the Good Clinical Practice and in Compliance with declaration of Helsinki. In general for all clinical in vivo studies we follow the best practice for claim substantiation evidence expressed by Technical document on cosmetic claims- ANNEX II and if present we follow or take inspiration by normative (as ISO for sunscreen) or guidelines as EEMCO guidance (European Expert Group on Efficacy Measurement of Cosmetics and other Topical Products) or cosmetic Europe guidelines (Guidelines for the evaluation of the efficacy of cosmetics products, May 2008) as well as by the instruction of use provided by the supplier of each instrument or from publication. Thanks to our experience we defined our internal standard operative procedures for each skin evaluation, instrumental, clinical and self-assessment.
Our quality Management System is certified according to ISO 9001:2015.

1.10. Start/end date of the study

The table here below reports the dates of the study.

Start of study management and enrolment	End of study management and enrolment	Test execution (first included subject)	Test execution (last included subject)	Study End Date (draft report delivery)
10/10/2022	28/10/2022	31/10/2022	18/11/2022	25/11/2022

1.11. 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	05/12/2022	First release
01	13/12/2022	Dates of the study added

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RESULTS
CLINICAL SCORING OF THE STINGING SENSATION

Table 1. Table below reports the clinical scores registered for each subject participating in the study in treated area. Raw data are expressed as: 1. No reaction; 2. Mild reaction; 3. Moderate reaction; 4. Severe reaction.

TREATED AREA									
n.	Vol ID	Tmax	Timm	T30''	T1'	T2'	T3'	T4'	T5'
01	O3607C	4	4	4	4	3	2	2	2
02	I6107C	4	3	2	2	2	2	1	1
03	L3353C	4	4	3	3	2	2	1	1
04	I5138R	4	3	2	2	2	2	2	2
05	P1219P	4	2	1	1	1	1	1	1
06	F6445G	4	3	3	3	2	1	1	1
07	C5420F	4	4	3	3	2	2	1	1
08	S4700M	4	3	2	1	1	1	1	1
09	P2325G	4	4	3	2	1	1	1	1
10	B4852D	4	4	3	2	2	1	1	1
11	C4532M	4	3	3	3	2	2	2	2
12	M4622F	3	2	1	1	1	1	1	1
13	F1334T	4	3	3	3	2	2	1	1
14	R4724M	4	3	3	3	3	2	2	2
15	S1741G	4	4	3	2	1	1	1	1
16	C1498S	4	4	4	3	3	2	2	2
17	P1979M	4	3	2	2	2	1	1	1
18	M4075E	4	3	3	3	2	2	2	1
19	M4805M	4	4	3	3	3	2	2	1
20	V1860G	4	4	3	2	2	1	1	1
21	A1177M	4	4	3	3	2	2	1	1
22	F1437A	4	3	3	3	3	2	2	2
23	P4253T	4	3	3	2	1	1	1	1
24	M1104S	4	4	4	4	3	2	2	2
25	F2352F	3	2	1	1	1	1	1	1
26	R0923S	4	3	2	2	2	2	2	2
27	B4232N	4	3	3	3	2	2	2	2
28	S6976M	4	4	3	3	2	2	1	1
29	C4150L	4	3	3	2	2	2	2	2
30	L2355S	3	2	2	1	1	1	1	1
31	L2381A	4	4	3	3	3	3	3	2
32	M4263R	4	3	3	3	2	2	2	2
33	G4961N	4	4	3	3	3	3	3	3
34	Q3571P	4	4	4	4	4	4	4	4
35	C4491P	4	4	4	3	3	3	2	2
	Mean	3,9	3,3	2,8	2,5	2,1	1,8	1,6	1,5
	SEM	0,0	0,1	0,1	0,1	0,1	0,1	0,1	0,1
	Wilc. vs Tmax	--	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	Wilc. vs CTR	0,317	**0,007**	**0,001**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**

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Table 2. Table below reports the clinical scores registered for each subject participating in the study in control area (treated with water). Raw data are expressed as: 1. No reaction; 2. Mild reaction; 3. Moderate reaction; 4. Severe reaction.

CONTROL AREA

n.	Vol ID	Tmax	Timm	T30''	T1'	T2'	T3'	T4'	T5'
01	O3607C	4	4	4	4	3	4	3	3
02	I6107C	4	3	3	3	2	2	2	1
03	L3353C	4	4	4	4	3	2	2	2
04	I5138R	4	3	3	3	3	3	3	3
05	P1219P	4	3	3	2	2	1	1	1
06	F6445G	4	3	3	3	3	3	3	3
07	C5420F	4	4	3	3	3	2	2	2
08	S4700M	4	4	3	3	3	2	2	1
09	P2325G	4	4	3	3	2	2	2	1
10	B4852D	4	4	3	3	3	3	3	3
11	C4532M	4	3	3	2	2	1	1	1
12	M4622F	3	2	2	2	2	1	1	1
13	F1334T	4	4	4	4	4	4	4	3
14	R4724M	4	4	4	4	4	4	4	4
15	S1741G	4	4	3	2	2	2	2	2
16	C1498S	4	4	4	3	3	3	3	2
17	P1979M	4	4	4	3	2	2	2	1
18	M4075E	4	3	3	3	3	2	2	2
19	M4805M	4	4	4	4	3	3	2	1
20	V1860G	4	4	3	3	3	2	1	1
21	A1177M	4	4	3	3	3	2	2	2
22	F1437A	4	4	4	4	4	4	3	3
23	P4253T	4	4	4	3	3	3	2	2
24	M1104S	4	4	3	3	2	2	2	2
25	F2352F	3	3	3	2	2	1	1	1
26	R0923S	4	4	3	3	3	3	3	3
27	B4232N	4	4	4	4	4	4	3	3
28	S6976M	3	3	2	2	1	1	1	1
29	C4150L	4	3	3	3	2	2	2	2
30	L2355S	3	2	2	1	1	1	1	1
31	L2381A	4	4	4	4	3	3	3	3
32	M4263R	4	3	3	3	3	3	2	2
33	G4961N	4	4	3	3	3	3	3	3
34	Q3571P	4	4	4	4	4	4	4	4
35	C4491P	4	4	4	4	4	3	3	3
	Mean	3,9	3,6	3,3	3,1	2,8	2,5	2,3	2,1
	SEM	0,1	0,1	0,1	0,1	0,1	0,2	0,2	0,2
	Wilc. vs Tmax	--	0,002	0,000	0,000	0,000	0,000	0,000	0,000

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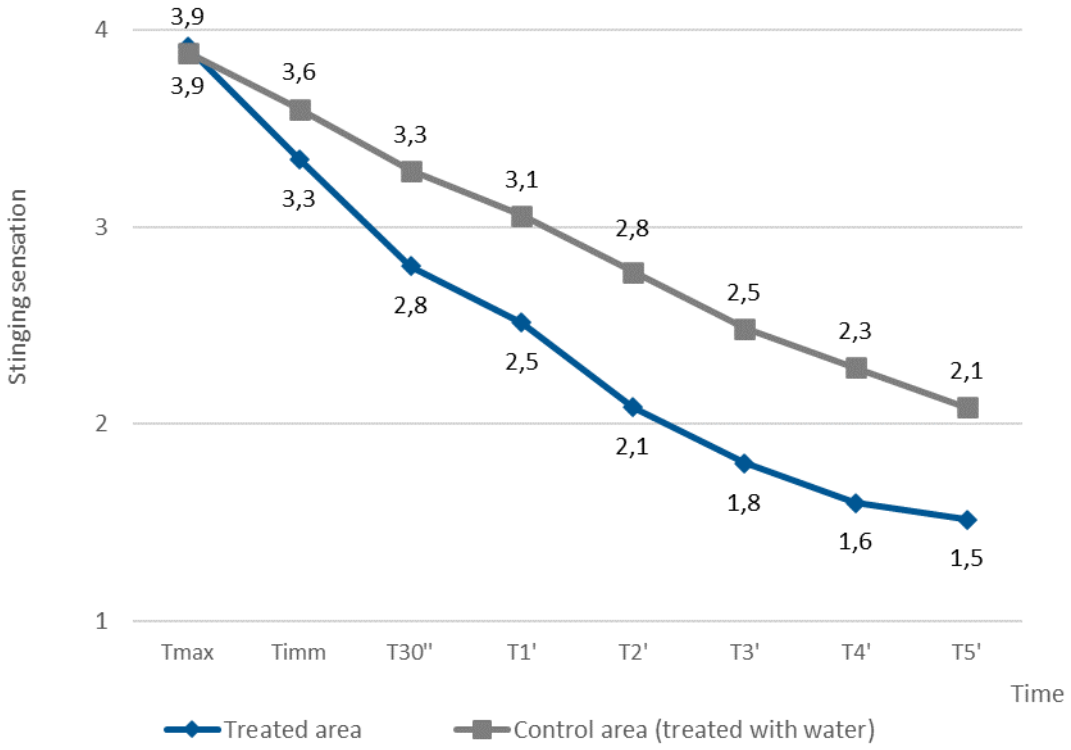
Graph 1. The graph shows the mean data obtained at each monitored check for the parameter under analysis.



COMMENT. As it is possible to notice, the single product application determines a statistically significant decrease of the stinging sensation induced by the lactic acid solution, starting from Timm (and at each following monitored time).
A physiological decrease of the stinging sensation is recorded also in the skin site treated with demineralized water, but the inter-group statistical analysis highlights that the decrease of the sensation is faster due to the application of the tested product (Wilcoxon test TREATED vs CONTROL AREA $p<0.05$) starting from Timm.

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CONCLUSION

According to the obtained results we can conclude that the tested product:

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RIVOLI GENÈVE Le Visage TONIC
Formula Reference: lab-00003.8

shows a soothing effect on the lactic acid-induced stinging sensation.
Its single application determines a statistically significant decrease of the stinging sensation soon after its first application (Timm) and at each following monitored time.
Moreover, starting from Timm, the decrease of stinging sensation is faster due to the application of the tested product, compared to results obtained in the control area.

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