CONFIRMATION OF THE SKIN COMPATIBILITY AND ABSENCE OF ALLERGENIC POTENTIAL OF COSMETIC PRODUCTS AFTER REPEATED APPLICATION UNDER PATCH IN HUMAN VOLUNTEERS

Human Repeated Insult Patch Test

Report (Version 1):	August 2nd, 2013
Study Code:	**820613.K**
Product:	**LEITE CORPORAL LEITE DE BURRA, REF.ª A657**
Manufacturer:	DMC, DONKEY MILK COSMETICS, Lda. Foros de Mora – 7490-209 Mora PORTUGAL
Study monitor:	Dr. Jorge Leal Barreto
Study Center:	PhD Trials Campo Grande 286, R/C Dto 1724-049 Lisboa
Scientific Manager	Pedro Contreiras Pinto, *PhD*
Principal Investigator	Leonor Girão, *MD*

The present study was performed according the criteria of Good Clinical Practices, and the Quality procedures implemented in PhD Trials.

This report has 25 pages

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Certificado de Controlo de Qualidade

Quality Inspection Certificate

Study number	820613.K/HRIPT
Date of the beginning of the study	June 24th, 2013
Date of the end of study	August 2nd, 2013
Date of audit	July 29th, 2013

O estudo acima referenciado foi realizado de acordo com as regras das Boas Práticas Clínicas e com os procedimentos padronizados da PhD Trials.

The study above listed was performed according with the rules of Good Clinical Practices, and under the standardized procedures of PhD Trials.

O responsável pelo Sistema de Gestão da Qualidade atesta que o presente relatório está de acordo com os dados obtidos e que foi realizado de acordo com os procedimentos e regras acima referenciados.

The Quality System responsible certifies that this report is according with the obtained raw data and respects the procedures and rules above listed.

Name / Surname	Patrícia Costa
Date	August 2nd, 2013
Signature	Patrícia Costa 2.8.2013

I . OBJECTIVE

This study intends to confirm the skin compatibility and absence of allergenic potential of the product **LEITE CORPORAL LEITE DE BURRA, REF.ª A657** after repeated application to the skin under exaggerated experimental conditions in human volunteers.

The product was applied under patch for a defined time. The applications were repeated 9 times over a period of 3 consecutive weeks, period necessary for the possible induction of an allergy.

After a 2-week rest period, with no treatment, a single application of each product under patch, to the induction site and to a virgin site, for a defined time (48 h), enables to reveal a possible induced allergy.

This study enables to justify the claim "*hypoallergenic*" and "*dermatological tested*".

II . STUDY RELEVANCE

Ethics

The study was performed according to the Declaration of Helsinki principles and subsequent amendments.

Although the foreseeable risk incurred by the volunteers that take part in the study are minor, there is suitability between the aim of the study, its possible risks and the potential effects related to the modalities planned in the protocol.

The applications were performed at the Study Center by the dermatologist, helped by the co-investigator or the technician in charge of the study. A clinical examination by the dermatologist, helped by the co-investigator or the technician in charge of the study, was performed at each passage at the Study Center. In case of important reactivity to the product, the applications could be interrupted in the concerned volunteers.

The study was performed under the spirit of Good Clinical Practices. The protocol and test conditions were reviewed by the Internal Review Board (**opinion nº 481/13 from June 21st, 2013**) and submitted to the Ethical Commission of PhD Trials (**opinion nº005/2012 from June, 15th, 2012**).

All the data concerned subject health and clinical data during and after the study performance are subject to medical-patient relationship. The investigational center cannot send to the sponsor/manufacturer the actual identity of the volunteers. The investigational center will present the data fully codified in respect to volunteer's data.

Methodological approach

The skin compatibility of the product was confirmed by the dermatologist, professional with a vast experience in this area.

The experimental conditions adopted create a certain occlusion and favor the penetration of the ingredients through the skin. If some of them have an allergenic potential, this one can be more easily proved by this kind of approach.

The methodology used is an adaptation from the **Marzulli and Maibach** proposal (Human Repeated Insult Patch Test for delayed contact hypersensitivity: HRIPT) and respect the latest recommendations for this kind of studies:

- Marzulli F.N., Maibach H.I., Contact allergy : predictive testing in man, Contact Dermatitis, 1976, 2, pp.1-17
- Draize J.H., Appraisal of the safety of chemicals in Food, Drugs and Cosmetics, FDA (ed), USA, 1959, pp. 46-48
- Frosch P.J. & Kligmann A.M., The Duhring Chamber : an improved technique for epicutaneous testing of irritant and allergic reactions, Contact Dermatitis, 1979, 5, pp 73-81
- AFSSAPS, Test clinique final de securite d'un produit cosmetique en vue de confirmer son absence de potentiel sensibilisant cutane retarde : recommandations aux promoteurs de recherche et aux prestataires de service, Paris, 2009

The patch material and the conditions of use of the products were adapted to the type of test product in accordance with the corresponding procedure.

The experimental area chosen (back) enables to test easily the products. The site of product application was chased at random to get rid of the variability of the skin reactivity dependent from the site.

A different site (with water) serves as control to avoid the possible undercurrent effects not directly related to the test products.

Panel

Referring to the experience acquired in contact allergy to cosmetic products and to the accurate knowledge of the ingredients incorporated into the test products, the number of volunteers, defined in the protocol, was acceptable to confirm, in first approach, the absence of allergenic potential of the test products. The study was performed in a 100 volunteer's panel.

Results

The results are mainly expressed as descriptive data and do not require a statistical treatment.

As the test product has good skin compatibility, under these experimental conditions, by extrapolation it should be safe for human health when applied under normal conditions of use.

III . TYPE OF STUDY

This mono-centric study was performed in open.

The subject was used as its own control.

This study was performed according to the general conditions of PhD Trials, established for the performance of Human test project, summarized in the general protocol (MD.32/01).

IV . STUDY CENTER

IV.1 . Study Center

PhD Trials: Head-office
Rua do Centro, nº23
Casal de Sta Iria
2240-300 Chãos
Ferreira do Zêzere
Portugal
Tel : + (351) 969104007

PhD Trials: Clinical Center

PhD Trials – Clinical Center
Campo Grande, 286, R/C Dto
1749-024 Lisboa
Portugal

IV.2 . Technical Staff

Investigator (dermatologist) : Leonor GIRÃO, *MD*

Scientific manager: Pedro CONTREIRAS PINTO, *PhD*

VI . DATES OF STUDY

Beginning: June 24th, 2013

End: August 2nd, 2013

VII . PRODUCTS

VII.1 . Total number of products

The total number of tested products was three (3).

This number of test products complies with the corresponding procedure which defines the maximal number of test products according to the chosen experimental area and patch material.

This report refers only to the product LEITE CORPORAL LEITE DE BURRA, REF.ª A657.

One control patch, corresponding to the type of patch material used, containing an ad hoc quantity of water for injectable preparation, was applied at the same time.

VII.2 . Identification of the test product

Name	LEITE CORPORAL LEITE DE BURRA
Reference	A657
Batch number	L-330126
Galenic form and organoleptic characteristics	White emulsion
Number and type of samples	1 sample
Volume of samples	250 ml

VII.3 Information concerning the test product

The documents related with the test product, supplied with the samples, are: Letter of Agreement, particularly concerning the conformity of the formulae to the established regulations and their safety, and the Order Form. The qualitative and quantitative formula of the products can be requested by the Investigator in a case by case situation, especially if there are some reactions noted.

VIII . VOLUNTEERS

VIII.1 . Number

The number of volunteers whose data had to be exploitable at the end of the study it is of 50.

Fithy one (51) volunteers were included in the study.

There was one withdrawal (vols # 25 – due to personal reasons) and no exclusion.

The compatibility of the test product was therefore assessed in **fithy** (50) volunteers.

VIII.2 . Specific inclusion criteria

The specific inclusion criteria, defined in the protocol, were the following ones:

- Age: 18 to 65 years,
- Gender: male and female,

- Phototype (Fitzpatrick) : I to IV,
- Type of skin: normal.

All the volunteers corresponded to these specific inclusion criteria. Their typological characteristics are defined in **Appendix 1/1 to 1/4.**

VIII.3 . Specific non-inclusion criteria

The specific non-inclusion criteria were the following ones:

- cutaneous marks on the experimental area which could interfere with the assessment of skin reactions (pigmentation problems, scar elements, over-developed pilosity, ephelides and naevi in too great quantity, sunburn, etc),
- eczematoid reaction still visible, scar or pigmentary sequelae of previous tests on the experimental area,
- allergy to colophony, to nickel,
- allergy or reactivity to cosmetic products of the same category than the one tested,
- skin hyper-reactivity,
- reactivity to adhesive plaster,
- participation in more than 5 tests under exaggerated use conditions (under patch) within 12 months before the study, including 3 HRIPT tests at the most,
- intensive sun exposure within the month before the study,
- forecast of intensive sun or UVA exposure (UV lamps) during the test period,
- forecast of bath (bathtub, sea or swimming-pool), sauna or hammam sessions during the test period,
- intensive or regular practice of one or several sports whose temporary interruption creates difficulties,
- treatment with Vitamin A acid or its derivatives within 3 months before the beginning of the study,
- treatment with topical corticoids on the experimental area within 8 days before the study,
- treatment with PUVA or UVB within 1 month before the study,
- forecast of vaccination during the test period or last vaccination within 3 weeks before the study.

All the volunteers corresponded to these specific non-inclusion criteria.

IX. METHODOLOGY

IX.1 . Experimental area and sites of application of the test products

The chosen experimental area was the back.

The site of application of the products was chosen by the dermatologist, the co-investigator or the technician in charge of the study. Skin appearance was taken into account and the areas of friction with clothes were avoided.

Each product was applied by the dermatologist, the co-investigator or the technician in charge of the study, to one of the sites localized by a clockwise distribution, altering of one rank from a subject to another.

IX.2 . Experimental conditions of application of the test products

Patch material	Experimental conditions of use	Quantity applied
Occlusive patch Finn Chamber large	As it is.	20 µl

Occlusive patch: *-Finn Chamber® : aluminium cupula in which the product was put down (20 µl or approximately 20 mg), kept in position by an hypoallergenic adhesive: Scanpor® (inner diameter: 8 mm, surface: 50 mm²)*

The quantities of product were measured with a micropipette with single use tips.

All the experimental conditions, defined in the protocol, were fully respected.

IX.3 . Chronology of the study

The applications of the test product and the removal of the patches were performed by the dermatologist, the co-investigator or the technician in charge of the study.

- **Induction phase :** 3 consecutive weeks

  * Application of the product to a perfectly delimited site, under patch on D1, D3, D5, D8, D10, D12, D15, D17, D19.

  * Patch removal
    - after 48 h of contact on D3, D5, D10, D12, D17, D19.
    - after 72 h of contact on D8, D15, D22.

* Controls: skin examination and questioning (paragraph IX.6) before patching on D1 and about 15 minutes (or more, if redness appeared after removal of the adhesive), after patch removal on D3, D5, D8, D10, D12, D15, D17, D19, D22.

- **Rest period :** 2 consecutive weeks at least (4 weeks at the most).

  * no application of product.

- **challenge :** 1 week.

  * application of the product to a perfectly delimited virgin site and to the site defined for the induction phase, under patch on D36.

  * patch removal after 48 h of contact on D38.

  * controls: skin examination and questioning (see IX.6) before patching on D36 and about 15 minutes (or more, if redness appeared after removal of the adhesive), after patch removal on D38, D39, D40 (48, 72, 96 h after application).

All the experimental conditions of application, defined in the protocol, were respected.

IX.4 . Constraints of the study

The constraints imposed on the volunteers were those defined for this kind of methodology in accordance with the corresponding procedure and those specifically defined for each study:

- no application of other products (than the tested ones) to the experimental area,
- no wearing of too thigh or restraining clothes on the experimental area, liable to produce frictions and to cause unsticking of the patches,
- no bath (bathtub or swimming-pool or sea), no hammam or sauna sessions during the study,
- if shower, protection of the experimental area or no violent projection of water and no application of soap to the experimental area to avoid patch removal or appearance of intercurrent phenomena and very gentle wiping if necessary,
- no excessive sweating and no intensive sport liable to cause unsticking of the patches,
- no intensive sun or UVA exposure (UV lamps) during the study, especially after patch removal,
- neither anti-allergic, anti-inflammatory (systemic or topical corticotherapy...) treatment nor treatment with patent medicines containing vitamin A acid or its derivatives during the study (if therapeutic requirement : exclusion foreseen),
- no vaccination during the study,

- at least 14 passages at the Institute, 15 if a pre-inclusion visit is necessary.

IX.5 . Control of the observance of the modalities of the protocol

The investigator checked about the respect of the **constraints**.

The volunteers were questioned at the end of the study. The Investigator assessed the importance of the possible deviations in comparison with the experimental conditions required at the beginning of the study.

The synthesis of the answers obtained was enclosed in **Appendices 2/1 and 2/2**.

All the deviations from the protocol were analyzed and the investigator assessed their effect on the validity of the results.

All the constraints of the study, defined in the protocol, were respected by the volunteers.

IX.6 . Confirmation of the skin compatibility (absence of irritant effect) and absence of allergenic potential

IX.6.1 . Frequency of the examinations

The skin examination and joint questioning was performed by the dermatologist helped by the co-investigator or the technician in charge of the study.

The examination was performed, visually under standard "daylight", according to the frequency mentioned on paragraph IX.3.

All the examinations were performed in accordance with the conditions defined in the protocol.

IX.6.2 . Expression of the results of the skin examination and questioning

The expression of the results of the skin examination and questioning was that defined for this type of study in accordance with the corresponding procedure.

In case of reactivity:

- **the main visible signs were noted, i.e. :**

Erythema, Œdema, Vesicle, Bulla, Papule, Scab, Dryness, Coloration, Soap effect.

The intensity of the **erythema and oedema** was assessed according to an ordinal scale: slight, moderate, severe.

The appearance of the **erythema** was specified: diffuse, punctuated, peripheral (around the application site).

The importance of the number of **vesicles and papules** was assessed according to an ordinal scale: 1 to 2 vesicles or papules, more than 2 vesicles or papules.

Bulla, scab, dryness, coloration and soap effect were described.

The importance of the **dryness and coloration** was assessed according to an ordinal scale: slight, moderate, severe.

– **the main sensations of discomfort were described, i.e. :**
Heating, Stinging, Pruritus (itching).

The results were expressed:

- **in percentage of reactive volunteers** : for this calculation only the visible signs of reactivity were taken into account : erythema, oedema, vesicle, bulla, papule, scab.

- **in a descriptive manner** for the other visible signs or for the sensations of discomfort : when the frequency of appearance of these signs justified it, the percentage of reactive volunteers was possibly calculated.

IX.6.3 . Interpretation of the results of the skin examination and questioning

All the volunteers included in the study were taken into account to confirm the skin compatibility of the test product as long as they were submitted at least to one post application examination at the defined time.

All the volunteers included in the study were taken into account to confirm the absence of allergenic potential of the test product (in absence of allergic reaction during the induction phase) as long as they were submitted to the challenge.

The interpretation of the results of the skin examination and questioning was that defined for this type of study in accordance with the corresponding procedure.

The possible reactions observed during the induction phase were either **irritation reactions** or **revelation of an allergy previously contracted or revelation of an allergy precociously induced** by the test product.

The possible reactions observed during the challenge on the "virgin" site were compared to those observed on the "induction" site at the same times. They were either **irritation reactions** or **revelation of an allergy induced during the induction phase** by the test product.

The nature, intensity, time of appearance, time of disappearance, location (induction site and/or virgin site) of the skin reaction was taken into account for the interpretation of the results.

To appreciate the skin compatibility and possible irritation reactions, the interpretation of the results, performed by the dermatologist helped by the co-investigator, was absolute (referring to **the experience of the investigator centre** in this field and especially to the **data acquired** on products of same cosmetic category tested under similar conditions). The test product could therefore have a **very good, good, moderate or bad skin compatibility**.

To appreciate the allergenic potential, the interpretation of the results was partly based on the allergenicity evaluation scale established by the **ICDRG** (International Contact Dermatitis Research Group) and took into account the visible reactions (clinical signs) and the possible reactions appeared on the control site :

NT	: non tested
?+	: doubtful reaction, only slight erythema
+	: positive reaction (with no vesicle) : erythema, infiltration, sometimes some papules
++	: strong positive reaction: presence of erythema, papules, vesicles
+++	: violent positive reaction: with presence of bullae
-	: negative reaction
IR	: irritation reaction

X . RESULTS

The individual data of the skin examination and questioning of the volunteers are enclosed in **Appendices 3/1 to 4/2.**

In summary:

Induction phase	
Type of reactivity on the induction site	**Number and percentage of reactive volunteers**
None	0 / 0%

Challenge phase	
Type of reactivity on the induction site and virgin site	**Number and percentage of reactive volunteers**
None	0 / 0%

XI . CONCLUSION

Under the experimental conditions adopted the repeated applications of the product **LEITE CORPORAL LEITE DE BURRA, REF.ª A657,** under occlusive patch, on a panel of 50 volunteers, induced no irritative reactions and the product **has a very good skin compatibility.**

Moreover, the repeated applications induced **no allergic reactions.**

Signatures and dates:

21/8/2013

Investigator : Leonor GIRÃO (dermatologist)

I the undersigned, Leonor GIRÃO, declare that the overall conduct of the study was carried out under my responsibility having in mind the basic principles and spirit of Good Clinical Practices ("Avis aux promoteurs et aux investigateurs pour les essais cliniques des médicaments" : principes généraux – FR.OB – 1987, international recommendations ICH E 6, step 4, of 1/5/1996 and general principals of the Portuguese law 46/2004 from August 19th).

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.

21/8/2013

PharDevelopment Trials, Lda
NIF: 509753914
Rua do Centro, 23 2240-300 Chãos

APPENDICES

Appendix 1/1

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female M=male	Phototype *	Normal skin
Ref.	Name Surname				
1	BARR. SE	29	M	III	X
2	SOUS. MA	57	M	III	X
3	RAMA. AN	18	F	III	X
4	RAPO. FI	21	F	III	X
5	RODR. ST	52	F	III	X
6	RODR. EL	54	F	III	X
7	LIMA. AN	42	F	IV	X
8	DESS. PU	31	M	IV	X
9	TRIN. MA	23	F	III	X
10	ROSA. SA	22	F	III	X
11	FILI. CA	21	F	III	X
12	DUAR. AN	20	F	III	X
13	BATI. AN	22	F	III	X
14	PERE. JO	22	M	III	X
15	LUCA. JO	27	F	III	X
16	NETO. AN	63	F	III	X
17	SOUS. CA	22	F	III	X
18	SILV. PE	25	M	III	X
19	MARL. HO	33	F	III	X
20	AGUI. RA	21	F	III	X
21	CAIR. MA	27	F	III	X
22	VAZC. ED	23	M	III	X
23	NETO. JO	22	M	III	X
24	CRUZ. CL	20	F	III	X
25	FERR. CA	29	M	III	X
26	LOUR. AN	40	M	III	X
27	SILV. CH	29	F	III	X
28	CARB. SA	46	F	III	X
29	LOPE. FL	38	F	III	X
30	CARB. MA	52	F	III	X

Appendix 1/2

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female M=male	Phototype *	Normal skin
Ref.	Name Surname				
31	GARC. VI	35	M	III	X
32	RAIM. MA	55	M	III	X
33	ALME. VA	24	F	III	X
34	PENA. JO	23	F	III	X
35	TABU. BR	20	M	III	X
36	MURT. MA	58	F	III	X
37	MOUR. PE	37	M	III	X
38	CRAV. DI	23	F	III	X
39	CARD. JO	28	M	III	X
40	CARL. LI	39	M	III	X
41	LOPE. RU	19	F	III	X
42	FERN. RI	21	M	III	X
43	PINT. OL	43	F	III	X
44	VERO. LI	53	F	III	X
45	AMAR. IN	24	F	III	X
46	JORG. HU	34	M	III	X
47	MART. EL	51	F	III	X
48	LEER. IT	20	F	III	X
49	AMAR. AN	22	F	III	X
50	MANU. GU	63	F	III	X
51	SANT. CA	21	F	III	X

Legends: / = no x = yes

***phototype according to Fitzpatrick**, established on the principle of a first 30 to 40-minute sun exposure after the winter or a period without exposure of an equivalent duration:*

Type I	*: Always burns easily, never tans*
Type II	*: Always burns easily, tans minimally*
Type III	*: Burns moderately, tans gradually*
Type IV	*: Burns slightly, always tans easily*
Type V	*: Burns rarely, tans intensely*
Type VI	*: Never burns, strongly pigmented*

Appendix 2/1

CONTROL OF THE OBSERVANCE
Constraints

Constraints (50 exploitable results)	Number of volunteers who respected the constraints	Percentage of volunteers who respected the constraints
No application of other products than the tested ones to the experimental area Deviation : none	50	100%
No wearing of too tight or restraining clothes on the experimental area, liable to produce frictions and to cause unsticking of the patch Deviation : none	50	100%
No bath (bathtub, swimming pool or sea), no hammam or sauna sessions during the study Deviation : none	50	100%
If shower, protection of the experimental area or no violent projection of water and no application of soap to the experimental area to avoid patch removal or appearance of intercurrent phenomena and very gentle wiping if necessary Deviation : none	50	100%
No excessive sweating and no intensive sport liable to cause unsticking of the patch Deviation : none	50	100%

Appendix 2/2

CONTROL OF THE OBSERVANCE
Constraints

Constraints **(50 exploitable results)**	**Number** of volunteers who respected the constraints	**Percentage** of volunteers who respected the constraints
No intensive sun or UVA exposure (UV lamps) during the study, especially after patch removal Deviation : none	50	100%
Neither anti-allergic, anti-inflammatory (systemic or topical corticotherapy...) treatment nor treatment with patent medicines containing Vitamin A acid or its derivatives during the study (if therapeutic requirement : exclusion foreseen) – no medical treatment which could interfere with the study Deviation : none	50	100%
No vaccination during the study Deviation : none	50	100%
At least 14 passages at the Institute (15 if a pre-inclusion visit was necessary) Deviation : none	50	100%

Appendix 3/1

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Volunteers		Reactivity								
Ref.	Name Surname	D3	D5	D8	D10	D12	D15	D17	D19	D22
1	BARR. SE	/	/	/	/	/	/	/	/	/
2	SOUS. MA	/	/	/	/	/	/	/	/	/
3	RAMA. AN	/	/	/	/	/	/	/	/	/
4	RAPO. FI	/	/	/	/	/	/	/	/	/
5	RODR. ST	/	/	/	/	/	/	/	/	/
6	RODR. EL	/	/	/	/	/	/	/	/	/
7	LIMA. AN	/	/	/	/	/	/	/	/	/
8	DESS. PU	/	/	/	/	/	/	/	/	/
9	TRIN. MA	/	/	/	/	/	/	/	/	/
10	ROSA. SA	/	/	/	/	/	/	/	/	/
11	FILI. CA	/	/	/	/	/	/	/	/	/
12	DUAR. AN	/	/	/	/	/	/	/	/	/
13	BATI. AN	/	/	/	/	/	/	/	/	/
14	PERE. JO	/	/	/	/	/	/	/	/	/
15	LUCA. JO	/	/	/	/	/	/	/	/	/
16	NETO. AN	/	/	/	/	/	/	/	/	/
17	SOUS. CA	/	/	/	/	/	/	/	/	/
18	SILV. PE	/	/	/	/	/	/	/	/	/
19	MARL. HO	/	/	/	/	/	/	/	/	/
20	AGUI. RA	/	/	/	/	/	/	/	/	/
21	CAIR. MA	/	/	/	/	/	/	/	/	/
22	VAZC. ED	/	/	/	/	/	/	/	/	/
23	NETO. JO	/	/	/	/	/	/	/	/	/
24	CRUZ. CL	/	/	/	/	/	/	/	/	/
25	FERR. CA	Withdrawal								
26	LOUR. AN	/	/	/	/	/	/	/	/	/
27	SILV. CH	/	/	/	/	/	/	/	/	/

Appendix 3/2

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Volunteers		Reactivity								
Ref.	Name Surname	D3	D5	D8	D10	D12	D15	D17	D19	D22
28	CARB. SA	/	/	/	/	/	/	/	/	/
29	LOPE. FL	/	/	/	/	/	/	/	/	/
30	CARB. MA	/	/	/	/	/	/	/	/	/
31	GARC. VI	/	/	/	/	/	/	/	/	/
32	RAIM. MA	/	/	/	/	/	/	/	/	/
33	ALME. VA	/	/	/	/	/	/	/	/	/
34	PENA. JO	/	/	/	/	/	/	/	/	/
35	TABU. BR	/	/	/	/	/	/	/	/	/
36	MURT. MA	/	/	/	/	/	/	/	/	/
37	MOUR. PE	/	/	/	/	/	/	/	/	/
38	CRAV. DI	/	/	/	/	/	/	/	/	/
39	CARD. JO	/	/	/	/	/	/	/	/	/
40	CARL. LI	/	/	/	/	/	/	/	/	/
41	LOPE. RU	/	/	/	/	/	/	/	/	/
42	FERN. RI	/	/	/	/	/	/	/	/	/
43	PINT. OL	/	/	/	/	/	/	/	/	/
44	VERO. LI	/	/	/	/	/	/	/	/	/
45	AMAR. IN	/	/	/	/	/	/	/	/	/
46	JORG. HU	/	/	/	/	/	/	/	/	/
47	MART. EL	/	/	/	/	/	/	/	/	/
48	LEER. IT	/	/	/	/	/	/	/	/	/
49	AMAR. AN	/	/	/	/	/	/	/	/	/
50	MANU. GU	/	/	/	/	/	/	/	/	/
51	SANT. CA	/	/	/	/	/	/	/	/	/

Legend:

/ : nothing to report
E : Erythema
Oe : Œdema
V : Vesicle
D : Dryness
S : Soap effect

Na : Not applied
Bu : Bulla
Pa : Papule
Sc : Scab
C : Coloration
Pr : Pruritus
Hea : Heating
St : Stinging

1 : Slight intensity
2 : Moderate intensity
3 : Severe intensity
d : diffuse
p : punctuated
peri : peripheral
Vesicles or papules
1 : nb = 1 or 2

Appendix 4/1

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Volunteers		Reactivity								According to the ICDRG criteria
		Induction site				Virgin site				
Ref.	Name Surname	D36	D38	D39	D40	D36	D38	D39	D40	
1	BARR. SE	/	/	/	/	/	/	/	/	-
2	SOUS. MA	/	/	/	/	/	/	/	/	-
3	RAMA. AN	/	/	/	/	/	/	/	/	-
4	RAPO. FI	/	/	/	/	/	/	/	/	-
5	RODR. ST	/	/	/	/	/	/	/	/	-
6	RODR. EL	/	/	/	/	/	/	/	/	-
7	LIMA. AN	/	/	/	/	/	/	/	/	-
8	DESS. PU	/	/	/	/	/	/	/	/	-
9	TRIN. MA	/	/	/	/	/	/	/	/	-
10	ROSA. SA	/	/	/	/	/	/	/	/	-
11	FILI. CA	/	/	/	/	/	/	/	/	-
12	DUAR. AN	/	/	/	/	/	/	/	/	-
13	BATI. AN	/	/	/	/	/	/	/	/	-
14	PERE. JO	/	/	/	/	/	/	/	/	-
15	LUCA. JO	/	/	/	/	/	/	/	/	-
16	NETO. AN	/	/	/	/	/	/	/	/	-
17	SOUS. CA	/	/	/	/	/	/	/	/	-
18	SILV. PE	/	/	/	/	/	/	/	/	-
19	MARL. HO	/	/	/	/	/	/	/	/	-
20	AGUI. RA	/	/	/	/	/	/	/	/	-
21	CAIR. MA	/	/	/	/	/	/	/	/	-
22	VAZC. ED	/	/	/	/	/	/	/	/	-
23	NETO. JO	/	/	/	/	/	/	/	/	-
24	CRUZ. CL	/	/	/	/	/	/	/	/	-
25	FERR. CA	Withdrawal								
26	LOUR. AN	/	/	/	/	/	/	/	/	-
27	SILV. CH	/	/	/	/	/	/	/	/	-
28	CARB. SA	/	/	/	/	/	/	/	/	-
29	LOPE. FL	/	/	/	/	/	/	/	/	-
30	CARB. MA	/	/	/	/	/	/	/	/	-

Appendix 4/2

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Volunteers		Reactivity								According to the ICDRG criteria
		Induction site				Virgin site				
Ref.	Name Surname	D36	D38	D39	D40	D36	D38	D39	D40	
31	GARC. VI	/	/	/	/	/	/	/	/	-
32	RAIM. MA	/	/	/	/	/	/	/	/	-
33	ALME. VA	/	/	/	/	/	/	/	/	-
34	PENA. JO	/	/	/	/	/	/	/	/	-
35	TABU. BR	/	/	/	/	/	/	/	/	-
36	MURT. MA	/	/	/	/	/	/	/	/	-
37	MOUR. PE	/	/	/	/	/	/	/	/	-
38	CRAV. DI	/	/	/	/	/	/	/	/	-
39	CARD. JO	/	/	/	/	/	/	/	/	-
40	CARL. LI	/	/	/	/	/	/	/	/	-
41	LOPE. RU	/	/	/	/	/	/	/	/	-
42	FERN. RI	/	/	/	/	/	/	/	/	-
43	PINT. OL	/	/	/	/	/	/	/	/	-
44	VERO. LI	/	/	/	/	/	/	/	/	-
45	AMAR. IN	/	/	/	/	/	/	/	/	-
46	JORG. HU	/	/	/	/	/	/	/	/	-
47	MART. EL	/	/	/	/	/	/	/	/	-
48	LEER. IT	/	/	/	/	/	/	/	/	-
49	AMAR. AN	/	/	/	/	/	/	/	/	-
50	MANU. GU	/	/	/	/	/	/	/	/	-
51	SANT. CA	/	/	/	/	/	/	/	/	-

Legend:

/ : nothing to report
E : Erythema
Oe : Œdema
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Hea : Heating
St : Stinging

1 : Slight intensity
2 : Moderate intensity
3 : Severe intensity
d : diffuse
p : punctuated
peri : peripheral
Vesicles or papules
1 : nb = 1 or 2

ICDRG

NT	*:*	*non tested*
?+	*:*	*uncertain reaction, only slight erythema*
+	*:*	*positive reaction (with no vesicle) : erythema, infiltration, sometimes some papules*
++	*:*	*strong positive reaction: presence of erythema, papules, and vesicles*
+++	*:*	*violent positive reaction: with presence of bullae*
-	*:*	*negative reaction*
IR	*:*	*irritation reaction*

Appendix 5

ETHICAL COMISSION PROTOCOL OPINION

Comissão de Ética Independente

Independent Ethics Committee

Opinion nº 005/2012

A Comissão de Ética reviu o seguinte projecto / *The Ethics Committee reviewed the following project:*

Tipo de estudo / *Type of Study*	**Human Repeated Insult Patch Test**
Nome do protocol / *Protocol name*	CONFIRMATION OF THE SKIN COMPATIBILITY AND ABSENCE OF ALLERGENIC POTENTIAL OF COSMETIC PRODUCTS AFTER REPEATED APPLICATION UNDER PATCH IN HUMAN VOLUNTEERS
Código do protocol / *Protocol Code*	010203.01

Documentos revistos / *Reviewed Documents*	☒ **Protocolo do estudo / Study protocol**
	☒ **Declaração de Consentimento Informado / Informed Consent Form**
	☒ **Formulário de Submissão / Submission form**

Decisão / *Decision:*
Após o cumprimento dos requisitos processuais e outras condições impostas por esta comissão, o projecto é: / *Subsequent to the fulfillment of the procedural requirements and other conditions imposed by the committee the project is hereby:*

Aceite / *Accepted*	☒	**Motivo da rejeição / *Motif to reject***
Aceite com modificações / *Accepted with modifications*	☐	
Rejeitado / *Rejected*	☐	

O investigador principal e os promotores deverão ler e cumprir com o seguinte: / The principal investigator and the sponsors are required to read and comply with the following:

- A Comissão examinou os procedimentos para o trabalho aqui proposto. Estes não apresentam impedimentos éticos à sua realização pelo os aprova neste estrito sentido. Todos os outros requisitos relacionados com o trabalho (p.ex. científicos, processuais, legais, financeiros e regulamentares, etc) mantêm-se da única responsabilidade do investigador principal e/ou do promotor(es).

Código : IMP 01/05/02.01 *Code*	**Versão nº :** 01 *Version nr*	**Data :** 29.07.2011 *Date*
Título : Parecer da Comissão de ética *Title: Ethics Committee Decision*		Page 1/2

- *The Committee examined the proceedings for the work proposed concerning the ethical aspects and did not found any impeachment for their execution, therefore, approved them in this strict sense. All other concerns related to the work (e.g. scientific, procedural, legal, financial and regulatory etc) remain the sole responsibility of the principal investigator and/or the sponsor(s).*

Assinatura e data **Date and Signature**	15062012
Responsible for the review	**Amilcar ROBERTO, PhD** Presidente da Comissão de Ética Chairman of the Ethical Committee

Código : IMP 01/02/01.01 *Code*	**Versão nº :** 01 *Version nr*	**Data :** 29.07.2011 *Date*
Título : Parecer da Comissão de Ética *Title: Ethics Committee Decision*		Page 2/2