

# 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 2<sup>nd</sup>, 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.

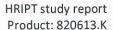
This report has 25 pages





## INDEX

I . OBJECTIVE	. 4
II . STUDY RELEVANCE	. 4
III . TYPE OF STUDY	5
IV . STUDY CENTER	6
IV.1 . Study Center	6
IV.2 . Technical Staff	6
VI . DATES OF STUDY	6
VII . PRODUCTS	6
VII.1 . Total number of products	6
VII.2 . Identification of the test product	7
VII.3 Information concerning the test product	7
VIII . VOLUNTEERS	7
VIII.1 . Number	7
VIII.2 . Specific inclusion criteria	7
VIII.3 . Specific non-inclusion criteria	8
IX. METHODOLOGY	9
IX.1 . Experimental area and sites of application of the test products	9
IX.2 . Experimental conditions of application of the test products	9
IX.3 . Chronology of the study	9
IX.4 . Constraints of the study	10
IX.5 . Control of the observance of the modalities of the protocol	11
IX.6 . Confirmation of the skin compatibility (absence of irritant effect) and absence of allergenic potential	11
X . RESULTS	
XI . CONCLUSION	
Appendix 1	
Appendix 2	
Appendix 3	
Appendix 4	
Appendix 5	





#### Certificado de Controlo de Qualidade

#### **Quality Inspection Certificate**

Study number	820613.K/HRIPT
Date of the beginning of the study	June 24 <sup>th</sup> , 2013
Date of the end of study	August 2 <sup>nd</sup> , 2013
Date of audit	July 29 <sup>th</sup> , 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 2 <sup>nd</sup> , 2013
Signature	Pahulia Colh 2.8.2013

D.45/01 Pág 3 de 25

HRIPT study report Product: 820613.K



#### I. OBJECTIVE

This study intends to confirm the skin compatibility and absence of allergenic potential of the product **LEITE CORPORAL LEITE DE BURRA**, **REF.** 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 21<sup>st</sup>, 2013) and submitted to the Ethical Commission of PhD Trials (opinion nº005/2012 from June, 15<sup>th</sup>, 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.

D.45/0

HRIPT study report Product: 820613.K



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, <u>Contact Dermatitis</u>, 1976, 2, pp.1-17
- Draize J.H., Appraisal of the safety of chemicals in Food, Drugs and Cosmetics, <u>FDA</u> (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, <u>Contact Dermatitis</u>, 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.

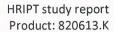
As the test product has good skin compatibility, under these experimental conditions, by extrapolation it should be safe for human health when applied under normal conditions of use.

#### III . TYPE OF STUDY

This mono-centric study was performed in open.

The subject was used as its own control.

10.45/01 Pág 5 de 25





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 2<sup>nd</sup>, 2013

#### VII . PRODUCTS

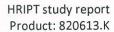
#### VII.1. Total number of products

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.

MD.45/01 Pág 6 de 25





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,

D.45/01 Pág 7 de 25



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

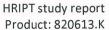
All the volunteers corresponded to these specific inclusion criteria. Their typological characteristics are defined in **Appendix 1/1 to 1/4.** 

#### 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.

MD.45/01 Pág 8 de 25





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 μΙ

Occlusive patch: -Finn Chamber @: aluminium cupula in which the product was put down (20  $\mu$ l 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.

Pág 9 de 25



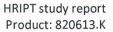
- \* Controls: skin examination and questioning (paragraph IX.6) before patching on D1 and about 15 minutes (or more, if redness appeared after removal of the adhesive), after patch removal on D3, D5, D8, D10, D12, D15, D17, D19, D22.
- Rest period: 2 consecutive weeks at least (4 weeks at the most).
  - \* 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 coinvestigator 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.

MD.45/01 Pág 11 de 25

HRIPT study report Product: 820613.K



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.

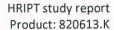
The possible reactions observed during the induction phase were either irritation reactions or revelation of an allergy previously contracted or revelation of an allergy precociously induced by the test product.

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.

D.45/01 Pág 12 de 25





**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:

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

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

MD.45/01 Pág 13 de 25

HRIPT study report Product: 820613.K



#### XI. CONCLUSION

Under the experimental conditions adopted the repeated applications of the product LEITE CORPORAL LEITE DE BURRA, REF.<sup>2</sup> 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:

218(2015

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 19<sup>th</sup>).

Scientific Manager: Pedro CONTREIRAS PINTO

I the undersigned, Pedro CONTREIRAS PINTO, declare that:

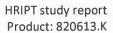
- the final report was examined on August 2<sup>nd</sup>, 2013,

- the results reported accurately and completely reflect the raw data of the study.

P

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

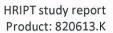
MD.45/01 Pág 14 de 25





# **APPENDICES**

MD.45/01 Pág 15 de 25



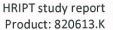


## Appendix 1/1

#### TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

V	olunteers	Age	Sex F=female	Phototype *	Normal skin	
Ref.	Name Surname	(years)	M=male	Phototype	Normalskiii	
1	BARR. SE	29	М	III	Х	
2	SOUS. MA	57	M	1)]	Х	
3	RAMA. AN	18	F	III	Х	
4	RAPO. FI	21	F	- III	Х	
5	RODR. ST	52	F	III	X	
6	RODR. EL	54	F	III	Х	
7	LIMA. AN	42	F	IV	Х	
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	Х	
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	Х	
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	Х	
21	CAIR. MA	27	F	III	X	
22	VAZC. ED	23	M	III	Х	
23	NETO. JO	22	M	III	Х	
24	CRUZ. CL	20	F	III .	X	
25	FERR. CA	29	M	III	X	
26	LOUR. AN	40	M	III	Х	
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	

Pág 16 de 25





Appendix 1/2

#### TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

V	olunteers	Age	Sex F=female	Phototype *	Normal skin	
Ref.	Name Surname	(years)	M=male	Pilototype	ivormai skin	
31	GARC. VI	35	M	III	Χ	
32	RAIM. MA	55	M	III	X	
33	ALME. VA	24	F	III	Χ	
34	PENA. JO	23	F	III	X	
35	TABU. BR	20	М	III	Х	
36	MURT. MA	58	F	III	Х	
37	MOUR. PE	37	М	III	Х	
38	CRAV. DI	23	F	F III		
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	Х	
46	JORG. HU	34	M	III	Х	
47	MART. EL	51	F	III	Х	
48	LEER. IT	20	F	III	Х	
49	AMAR. AN	22	F	III	Х	
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

Type IV

: Burns slightly, always tans easily

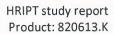
Type V

: Burns rarely, tans intensely

Type VI

: Never burns, strongly pigmented

Pág 17 de 25



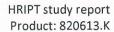


Appendix 2/1

# 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%

MD.45/01 Pág 18 de 25



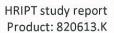


Appendix 2/2

# 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%

MD.45/01 Pág 19 de 25



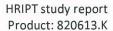


Appendix 3/1

#### 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	/	/	/	1	/	1	1	/	1
2	SOUS. MA	/	/	/	/	/	/	/	/	1
3	RAMA. AN	/	/	1	/	/	/	1	/	/
4	RAPO. FI	/	/	/	/	/	/	/	/	/
5	RODR. ST	/	1	/	/	/	/	1	/	/
6	RODR. EL	/	1	/	/	/	/	1	/	/
7	LIMA. AN	/	/	/	/	/	/	/	/	/
8	DESS. PU	/	/	/	/	/	/	/	/	/
9	TRIN. MA	/	/	/	/	/	/	/	/	/
10	ROSA. SA	/	/	/	/	/	/	/	/	/
11	FILI. CA	/	/	/	/	/	/	/	/	1
12	DUAR. AN	/	/	/	/	/	/	/	/	/
13	BATI. AN	/	/	/	/	/	/	/	/	/
14	PERE. JO	/	/	/	/	/	/	/	/	/
15	LUCA. JO	/	/	/	/	/	/	/	/	/
16	NETO. AN	/	/	/	/	/	/	/	/	/
17	SOUS. CA	1	/	/	1	1	/	/	/	1
18	SILV. PE	/	/	/	1	/	/	/	/	/
19	MARL. HO	/	/	/	1	1	/	/	/	1
20	AGUI. RA	/	/	/	1	1	/	/	/	1
21	CAIR. MA	/	/	/	/	/	/	/	/	/
22	VAZC. ED	/	/	/	1	1	/	/	/	/
23	NETO. JO	/	/	1	1	1	/	/	/	1
24	CRUZ. CL	/	/	1	1	1	/	1	/	1
25	FERR. CA					Withdraw	al			
26	LOUR. AN	1	/	1	/	1	1	/	/	/
27	SILV. CH	1	/	1	/	1	1	1	1	1

MD.45/01 Pág 20 de 25





Appendix 3/2

#### SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

	Volunteers					Reactivity	1			
Ref.	Name Surname	D3	D5	D8	D10	D12	D15	D17	D19	D22
28	CARB. SA	/	/	/	/	/	/	/	/	1
29	LOPE. FL	/	1	/	/	/	/	/	/	1
30	CARB. MA	1	/	/	/	/	/	/	/	/
31	GARC. VI	1	/	/	/	/	/	/	/	/
32	RAIM. MA	/	1	/	/	/	/	/	/	1
33	ALME. VA	1	/	/	/	/	/	/	/	/
34	PENA. JO	/	/	/	/	1	/	/	/	1
35	TABU. BR	/	/	/	/	/	/	/	/	1
36	MURT. MA	/	/	/	/	/	/	/	/	/
37	MOUR. PE	/	/	/	/	/	/	/	/	/
38	CRAV. DI	1	/	/	/	/	/	1	/	/
39	CARD. JO	/	/	/	/	1	/	1	/	/
40	CARL. LI	1	/	/	/	/	/	/	/	/
41	LOPE. RU	/	/	/	/	/	/	1	/	/
42	FERN. RI	1	/	/	/	/	/	/	/	/
43	PINT. OL	/	/	/	1.	1	/	1	/	/
44	VERO. LI	/	/	/	/	/	/	/	/	/
45	AMAR. IN	/	/	/	/	1	/	1	/	1
46	JORG. HU	1	/	/	/	/	/	1	1	1
47	MART. EL	/	/	/	/	/	/	1	/	/
48	LEER. IT	/	/	/	/	/	/	/	/	/
49	AMAR. AN	/	/	/	/	/	/	/	/	1
50	MANU. GU	/	/	1	/	1	/	1	/	1
51	SANT. CA	/	/	1	/	/	/	/	/	/

Legend:

/ : nothing to report

Na : Not applied

E: Erythema
Oe: Œdema
V: Vesicle
D: Dryness
S: Soap effect

Bu: Bulla
Pa: Papule
Sc: Scab
C: Coloration
Pr: Pruritus
Hea: Heating

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

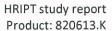
1 : Slight intensity

2 : Moderate intensity

St: Stinging Vesicles or paper

1: nb = 1 or 2

MD.45/01 Pág 21 de 25

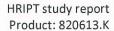




Appendix 4/1

## SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

			Reactivity							
	Volunteers		Induction site				Virgi	According to the ICDRG		
Ref.	Name Surname	D36	D38	D39	D40	D36	D38	D39	D40	criteria
1	BARR. SE	/	/	/	/	/	/	/	/	-
2	SOUS. MA	/	/	/	/	/	/	/	/	
3	RAMA. AN	/	/	/	/	/	/	/	/	-
4	RAPO. FI	/	/	/	/	/	/	/	/	
5	RODR. ST	/	/	/	/	/	/	/	/	-
6	RODR. EL	/	/	/	/	/	/	/	/	-
7	LIMA. AN	/	/	/	/	/	/	/	/	-
8	DESS. PU	1	/	/	/	/	/	/	/	-
9	TRIN. MA	/	/	/	/	/	/	/	/	-
10	ROSA. SA	/	/	/	/	/	/	/	/	
11	FILI. CA	/	/	/	/	/	/	/	/	- L
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	/	/	/	1	/	/	/	/	-
23	NETO. JO	/	/	/	/	/	/	/	/	-
24	CRUZ. CL	/	/	/	/	/	/	/	/	-
25	FERR. CA					With	ndrawal			
26	LOUR. AN	/	/	/	/	/	/	/	/	-
27	SILV. CH	/	/	/	/	1	/	/	/	-
28	CARB. SA	/	/	1	/	1	/	/	/	=
29	LOPE. FL	/	/	1	/	/	/	1	/	-
30	CARB. MA	1	1	1	1	1	1	1	1	-





Appendix 4/2

#### SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

	Volunteers		Reactivity							
			Induction site			Virgin site				According to the ICDRG
Ref.	Name Surname	D36	D38	D39	D40	D36	D38	D39	D40	criteria
31	GARC. VI	/	/	/	/	1	/	/	/	-
32	RAIM. MA	/	/	/	/	1	/	/	/	-
33	ALME. VA	/	/	/	/	1	/	/	/	-
34	PENA. JO	/	/	/	/	/	/	/	/	- 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
35	TABU. BR	/	/	/	/	/	/	/	/	
36	MURT. MA	/	/	/	/	/	/	/	/	-
37	MOUR. PE	1	/	/	/	/	/	1	/	7 - Table 1
38	CRAV. DI	1	1	/	/	/	/	/	/	-
39	CARD. JO	/	/	/	/	/	/	1	/	
40	CARL. LI	/	/	/	/	/	/	/	/	-
41	LOPE. RU	/	/	/	/	/	/	/	/	-
42	FERN. RI	/	/	/	/	/	/	/	/	-
43	PINT. OL	/	/	/	/	/	/	/	/	
44	VERO. LI	1	/	/	/	/	/	/	/	-
45	AMAR. IN	/	/	/	/	1	/	/	/	
46	JORG. HU	/	/	/	/	/	/	/	/	-
47	MART. EL	/	/	/	/	/	/	/	/	-
48	LEER. IT	/	/	/	/	/	/	/	/	-
49	AMAR. AN	/	/	/	/	/	/	/	/	-
50	MANU. GU	/	/	/	/	/	/	/	/	-
51	SANT. CA	/	/	/	/	/	/	/	/	-

Legend: /: nothing to report Na : Not applied

E: Erythema

De: Œdema

V: Vesicle

D: Dryness

S: Soap effect

Eu: Bulla

Pa: Papule

Sc: Scab

C: Coloration

Pr: Pruritus

Hea: Heating

St: Stinging Vesicles or papules 1: nb = 1 or 2

1 : Slight intensity

3 : Severe intensity

d : diffuse

p:punctuated

peri : peripheral

2 : Moderate intensity

#### ICDRG

NT : non tested

?+ : uncertain reaction, only slight erythema

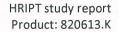
+ : positive reaction (with no vesicle) : erythema, infiltration, sometimes some papules

++ : strong positive reaction: presence of erythema, papules, and vesicles

+++ : violent positive reaction: with presence of bullae

- : negative reaction IR : irritation reaction

D/45/01 Pág 23 de 25





Appendix 5

#### **ETHICAL COMISSION PROTOCOL OPINION**



# Comissão de Ética Independente

#### Independent Ethics Committee

Ay

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			
Name da 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	010200.01			

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

#### Decisão / Decisions

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:

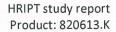
Aceite / Accepted	Motivo da rejeição / Motif to reject
Aceite com modificações / Accepted with modifications	
Rejekado / Rejected	

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	Versão nº : 01	Data: 29.07.2011	
Code	Version or	Date	
Titulo : Parecer da Comissão de Title: Ethics Committee Decision			Page 1/2

D.45/01 Pág 24 de 25





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 spansor(s).

Assinatura e data Date and Signature	July 15062012					
· 中一年的一个	Amilcar ROBERTO, PhD					
Responsible for the review	Presidente da Comissão de Ética					
	Chairman of the Ethical Committee					

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