



STUDY/TEST ELEMENT REFERENCES: 02/TC-PTS_150_15-001

Study Report - Version nº 1

**ASSESSMENT IN HUMAN WITH SENSITIVE SKIN OF THE CUTANEOUS
COMPATIBILITY OF A COSMETICAL PRODUCT AFTER A SINGLE
UNDER PATCH APPLICATION UNDER DERMATOLOGICAL CONTROL**



**SPONSOR: NUTRITAPE, S.L.
TESTED ELEMENT: L-LEUCINE
REFERENCE: BCAA**

Madrid, 21st August 2015

This report contains 17 pages, including 3 Annex pages

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1. IDENTIFICATION OF THE STUDY

Name of the study: Assessment in human with sensitive skin of the cutaneous compatibility of a cosmetic product after a single under patch application under dermatological control (Patch test).

Director of the laboratory: Irene Zaldívar Notario

Director of the study: Irene Zaldívar Notario

Sponsor: NUTRITAPE, S.L.

Tested element: L-LEUCINE, reference: BCAA, batch: L201501290103

2. OBJECTIVE AND PRINCIPLE OF THE STUDY

This study had as an objective verifying the cutaneous compatibility of a cosmetic product (Reference: BCAA), after a single application on the sensitive skin under exaggerated experimental conditions.

The product was applied, only once, over the skin of the back and under an occlusive patch.

The compatibility of the product with the skin was verified, after at least 15 minutes of removing the patches and by means of visual exam of the experimental area, by the responsible technical expert, as well as by a dermatologist in charge of the study.

3. TYPE OF STUDY

This study has been carried out in the Experimental Center.

Each volunteer that participated was his/her own controller. The negative control excluded false positives.

The study was carried out following general conditions in Zurko Research, established for the execution of study project on humans (Structure and Content of Clinical Study Reports from ICH Harmonised Tripartite Guideline; International Recommendations ICH Topic E6, CPMP/ICH/135/95 of May 1st 1996, European Parliament and Council Guideline 2001/20/CE - DOCE OF May 1st 2001).

Previously, Zurko Research assessed the suitability of the product for the type of study and methodology to be employed.

4. RESEARCH CENTER AND TECHNICAL TEAM

4.1 Research Center

ZURKO RESEARCH S.L.

Gran Vía, nº 62, left. 4^a

28013 Madrid (Spain)

Tel: (+34) 91.521.15.88

4.2 Technical Team

Director of the study: Irene Zaldívar Notario, PhD in Biochemistry.

Researcher: Isabel Garzón de la Iglesia, Chemist.

Dermatologist: Javier Pedraz Muñoz. Medical license number: 283706434.

5. STUDY EXECUTION SCHEDULE

Beginning of the experimental phase: August 5th, 2015

Finalizing of the experimental phase: August 7th, 2015

6. TESTED PRODUCT

6.1 Identification of the tested product

PRODUCT

Reference of product

Batch of product

Zurko Research Reference

Cosmetic Form and organoleptic characteristics

Number, type and volume of container

L-LEUCINE

BCAA

L201501290103

02/TC-PTS_150_15-001

White powder

Container 100 g

The tested element was stored at room temperature, following the indications of the sponsor.

The identity and stability of the tested element are in the product data sheet provided by NUTRITAPE, S.L.

6.2 Information regarding the tested product

The documents related to the tested product that were supposed to accompany the samples were the qualitative formula and the letter of commitment from the Sponsor, particularly the one referring to the consistency of the formula with the current regulations and its safety.

7. VOLUNTEERS

7.1 Number

11 volunteers with sensitive skin were included in the study. The number of volunteers required at the end of the study was 10. Considering that the number of volunteers used in this type of study is sufficient to verify compatibility of a cosmetic dermal product.

No withdrawal was registered and no exclusion was decided by the Researcher.

The compatibility of the tested product was therefore verified on 11 volunteers.

All volunteers continued the following recommendations of the study's principal investigator:

- Intense sun exposure prediction (direct sun or UV rays), during the study.
- Vaccination prediction during the study period, having had the last vaccine within the 3 weeks previous to the study.
- Intention to bath in the bath, swimming pool or the sea, or having sauna or Turkish baths during the study.
- Intention to practice intense sport while the patch is on the back, that could produce intense sweating and affect the patch.
- No apply other cosmetic product in the experimental area

7.2 Specific inclusion criteria

The specific inclusion criteria, defined in the protocol, were as follow:

- Age: 18-70 years old
- Sex: both
- Photo-type (Fitzpatrick): I to VI
- Sensitive skin according to scale of the Center.

All volunteers responded to the specific inclusion criteria. Their typology is defined in **Annex 1**.

7.3 Specific non-inclusion criteria

The specific non-inclusion criteria, defined in the protocol, were as follow:

- Cutaneous marks on the experimental area that could interfere with the evaluation of the skin reactions (pigmentation disruptions, scars, excessive hair areas, excessive freckles and moles, solar skin burns ...).
- Tattoos in the experimental area.
- Injuries or infection in the experimental area.
- Pathologies in the experimental area.
- Eczematous reaction which has not fully disappeared, scar or pigmentation complications from previous studies in the experimental area.
- Allergies to metals.
- Reactivity to medical tape.
- Participation during the previous 30 days in any study under exaggerated conditions (under a patch).
- Intense sun exposure during the month previous to the study.
- Carrying out a treatment containing acid vitamin A or its by-products, during the 3 months previous to the study.
- Carrying out a treatment containing topical corticoids, on the experimental area during the 8 days previous to the beginning of the study.
- Carrying out UVA or UVB treatments during the month previous to the study.
- Treatment with any medicine for psoriasis, vitiligo, within one month before the study.
- Being pregnant or breastfeeding.

All volunteers responded to these specific non inclusion criteria.

8. EQUIPMENT

- Finn Chamber Std® occlusive patch
- Curatest® semioclusive patch
- Finn Chamber Aqua® occlusive patch
- Finn chambers filter paper discs
- Pasteur pipettes 1 ml
- Sterile containers
- Finn chamber tray
- Distilled water
- Micropipette Siner lab (Ref. HG20566) 20-200µl
- Tweezers
- Sanitary alcohol 70°
- Cotton
- Precision Balance Model: PS 750. R2. Radwag

9. METHODOLOGY

9.1 Criteria for application the product

Type of product: rinse off ingredient.

Experimental area: UpperBack

Product preparation: use of a small amount of water to adhere the sample to the patch.

Applied quantity: 20 mg of product preparation over occlusive patch (Finn Chamber Aqua® occlusive patch)

Contact time: 48 hours

Control time after patch removal: 15-30 minutes

9.2 Experimental procedure

The first day of the test, instructions of the study were given to the volunteers. Before starting the study they filled the informed consent and the exclusion criteria.

Day 1 (0 hours) – Sample preparation and application. The principal investigator examined the study area each participant and verify the inclusion criteria and none of the exclusion criteria.

After cleaning the experimental area, the product under study was applied to the patch on top of the back in occlusive conditions for 48 hours. One patch without product was applied in the same experimental conditions (negative control).

Day 3 (48 hours) – Clinical Examination and Scoring. Skin reactions were evaluated 15-30 minutes after patch removal according to the scores reported in table 1, which describes the severity of Erythema (E) and Oedema (OE) parameters.

Score	Assessment of reaction	PARAMETERS EVALUATED	
		Erythema (E)	Oedema (OE)
-: 0	Absence	No erythema	No oedema
-/+: 0.5	Doubtful	Very slight erythema (barely perceptible: quiet pinked coloration of one part of the tested area)	-
+: 1	Slight	Slight erythema (quiet pinked coloration of the complete tested area or rather visible on one part of the tested area)	Slight oedema (palpable and visible)
++: 2	Moderate	Obvious erythema (clear erythema covering all of the tested area)	Obvious oedema with or without papule/s or vesicle/s
+++: 3	Severe	Intense erythema (severe erythema covering all the tested area or erythema diffusing outside the tested area)	Intense oedema (extended area outside the tested area) with or without vesicle/s or blister/s

Table 1. Clinical Examination and Scoring

Other types of skin irritation could be observed (Dryness (D); Detergency (DT); Thickness (T); Reflectivity (R)) according to the following scale:

- 0.5 = doubtful
- 1 = Slight
- 2 = Moderate
- 3= Severe

The volunteers verified absence of reaction at 24 hours after patch removal. In the case that exits visible reaction, the subject must return to the center performing subsequent reads until his disappearance.

9.3 Interpretation of Results

The analysis and the interpretation of the results were carried out according to the results obtained in the experimental conditions.

They were descriptive and completed by the calculation of the Mean Irritation Index (M.I.I.).

$$\text{M.I.I.} = \frac{\Sigma \text{ of the grade erythema and oedema}}{\text{Number of volunteers}}$$

The obtained index was used to classify the studied cosmetic product according to the following scale:

M.I.I.	Product Classification
M.I.I.=0.0	Non Irritating (NI)/Very Good Cutaneous Compatibility
M.I.I.<0.20	Non Irritating (NI) / Good Cutaneous Compatibility
$0.20 \leq \text{M.I.I} < 0.50$	Slightly Irritating (SI) / Intermediate Cutaneous Compatibility
$0.50 \leq \text{M.I.I} < 1$	Moderately Irritating (NI) / Bad Cutaneous Compatibility
M.I.I. ≥ 1	Irritating (I) / Very Bad Cutaneous Compatibility

Individual values and the product class were taken into account to write a suitable conclusion under the study conditions (48 hours single patch test under occlusion).




10. RESULTS

The individual reading results was presented in Annex II.

Next table showed the M.I.I. at 15-30 minutes after removal of the patch.

M.I.I.	Results	Number of reactive volunteers	Reactive volunteers %
0.0000	Non Irritating/ Very Good Cutaneous Compatibility	0	0%

Next figure included the pictures after 48h.

V1	V2	V3	V4	V5	V6
					
V7	V8	V9	V10	V11	
					

11. CONCLUSION

Under adopted experimental conditions, the product, **L-LEUCINE, REFERENCE: BCAA** is **Non Irritating. In conclusion, it product has Very Good Cutaneous Compatibility.**

12. SAMPLES AND DOCUMENTS TO BE STORED

The following documents will be stored in the Zurko Research archive:

- Signed informed consents.
- Signed non-inclusion criteria.
- Laboratory notebook containing the evaluation data collected by the technician.
- Final report.
- Documents provided by the sponsor.

The documents will be stored during 5 years. After 5 years the sponsor will be asked about the possibility of extension because of the commercialization of the tested element.

The sample of the tested product will be stored in the Zurko Research archive for samples, during 1 year.

13. BIBLIOGRAPHY REFERENCES

1. The SCCS'S Notes of Guidance for the Testing of Cosmetic Substances and their Safety Evaluation 8th Revision
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4. North American Contact Dermatitis Group Patch-Test Results, 2001-2002 Study Period. Dermatitis: December 2004. Marks, James G. Jr; Belsito, Donald V.; DeLeo, Vincent A.; Fowler, Joseph F. Jr; Fransway, Anthony F.; Maibach, Howard I.; Mathias, Toby C.G.; Nethercott, James R.; Rietschel, Robert L.; Rosenthal, Lawrence E.; Sherertz, Elizabeth F.; Storrs, Frances J.; Taylor, James S.
5. Nueva Clasificación de tipos piel y sus implicaciones en Dermatología Cosmética. Revisión Dermatología Venezolana. Vol. 43, N° 4, 2005. Leslie Baumann, Sadegh Amini, Eduardo Weiss

SIGNATURES

Researcher: Isabel Garzón de la Iglesia. Chemist. I, the undersigned, Isabel Garzón de la Iglesia, declare that this study has been carried out under my responsibility and in the essence of the Clinical Good Practices (International Recommendations ICH Topic E6, CPMP/ICH/135/95 of May 1st 1996, European Parliament and Council Guideline 2001/20/CE - DOCE OF May 1st 2001).

-The results here presented reflect accurately and completely the raw data of the study.

Signature:



Dermatologist: Javier Pedraz Muñoz. Medical license number: 283706434

Signature:



Director of the study: Irene Zaldívar Notario, PhD in Biochemistry. I, the undersigned, Irene Zaldívar Notario, declare that this study has been carried out under my responsibility and in the essence of the Clinical Good Practices (International Recommendations ICH Topic E6, CPMP/ICH/135/95 of May 1st 1996, European Parliament and Council Guideline 2001/20/CE - DOCE OF May 1st 2001).

-The results here presented reflect accurately and completely the raw data of the study.

-This report is a complete, reliable and precise presentation of the study and its results.

Signature:



Quality manager: Sara Rodriguez Olmo, Pharmacist. I, the undersigned, Sara Rodriguez Olmo, declare that this study has been checked according to the procedures of the Quality Unit.

The inspections that have been made, allow confirming that the final report reflects accurately the primary data of the study.

Signature:

 zurko
bio research
CIF: B84273911

ANNEXES

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Annex I: Information relating to volunteers

Volunteers		Age (Years)	Sex F= Female M= male	Phototype	Skin Type (R/S)
Ref.	Acronym				
1	V1	52	M	IV	S
2	V2	26	F	III	S
3	V3	53	M	III	S
4	V4	23	F	II	S
5	V5	62	F	II	S
6	V6	18	F	III	S
7	V7	55	F	III	S
8	V8	33	F	II	S
9	V9	19	F	II	S
10	V10	53	F	II	S
11	V11	48	M	II	S

R = Resistant

S= Sensitive

Annex II: Individual Results at 15-30 minutes after removal patch

Volunteers		Erythema (E)		Oedema (OE)		Other Skin Irritation (D; DT; T; R)
Ref.	Acronym	Tested Product	Control	Tested Product	Control	
1	V1	-	-	-	-	-
2	V2	-	-	-	-	-
3	V3	-	-	-	-	-
4	V4	-	-	-	-	-
5	V5	-	-	-	-	-
6	V6	-	-	-	-	-
7	V7	-	-	-	-	-
8	V8	-	-	-	-	-
9	V9	-	-	-	-	-
10	V10	-	-	-	-	-
11	V11	-	-	-	-	-
M.I.I.		0		0		
M.I.I. Total		0.00				

D =Dryness

DT = Detergency

T= Thickness

R = Reflectivity

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