STUDY/PRODUCTS REFERENCES : Il 538-Il 538* / 05.2317 and 05.2318

CHECKING IN HUMAN OF THE SKIN COMPATIBILITY OF A COSMETIC PRODUCT AFTER SINGLE APPLICATION UNDER PATCH

Patch test under dermatological control

SPONSOR : ANIOS

TEST PRODUCT : ANIOSGEL 85 Lot G22061
CONTROL PRODUCT : SOLUTION D'ETHANOL A 80 % v/v lot G22062

Study report

CTF / AmC

Bordeaux, September 06th, 2005

18 pages in this report including 7 in Appendices

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CHECKING IN HUMAN OF THE SKIN COMPATIBILITY OF A COSMETIC PRODUCT AFTER SINGLE APPLICATION UNDER PATCH

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I . AIM AND PRINCIPLE OF THE STUDY

This study intended to check the skin compatibility of the product **ANIOSGEL 85 Lot G22061**, after single application to the skin under exaggerated experimental conditions.

The product was applied, once, to the skin under patch.

A control product **SOLUTION D'ETHANOL A 80 % v/v lot G22062,** supplied by the Sponsor, was concurrently applied under the same conditions as the test product.

The skin compatibility of the test products was checked, after patch removal and visual examination of the experimental area by the dermatologist.

II . RELEVANCE OF THE STUDY

Ethics

The study aiming at a better knowledge of the skin compatibility of the test product and the foreseeable risk incurred by the volunteers who took part in the study being minor, there was a suitability between the aim of the study, its possible risks and the possible troubles related to the modalities planned in the protocol.

Methodological approach

The skin compatibility of the test product was checked by the dermatologistr who has an appropriate experience.

The experimental conditions adopted created a certain occlusion and favoured the penetration of the ingredients through the skin. If some of them had an irritative potential, this one was more easily proved by this kind of approach.

Numerous publications supported this methodology, notably :

- 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

- Matthies W., Test strategies for development of cosmetic products using dermatological test models, Seifen-Öle-Fette-Wachse, 1991, 117, pp 42-43

- Mikulowska A., Reactive changes in human epidermis following simple occlusion with water, Contact Dermatitis, 1992, 26, pp 224-227

The patch material, the contact time with skin, and the conditions of use of the product depended on the test product in accordance with the corresponding procedure.

The experimental area chosen (back) enabled to test easily the product.

Panel

The panel was representative of a large population.

Moreover, as it was a first approach in skin compatibility and as the application and examinations were perfectly controlled, the number of volunteers defined in the protocol was sufficient to check the cutaneous tolerance and appreciate the possible irritation.

Results

The results were mainly expressed as descriptive data and did not require a statistical treatment.

If the test product had a good skin compatibility under these experimental conditions, by extrapolation it would be safe for human health when applied under normal conditions of use.

III . TYPE OF THE STUDY

This monocentric study was performed in open.

The subject was used as own control.

It was performed according to the general conditions of Evic France - Idec department, established for the performance of Human test project.

The test project was submitted to the previous agreement of the internal committee of Evic France before its performance (opinion n° 640/05 of August 19th, 2005).

IV . INVESTIGATOR CENTRE AND TECHNICAL STAFF

IV.1 . Investigator centre

Evic France – Idec department
57, rue Ulysse Gayon
33 000 Bordeaux – France

tel : 05 57 14 00 80

IV.2. Technical staff

Investigator : Doctor Melissa MIGNARD-GUILLAUME (dermatologist)

Co-investigator : Doctor Clotilde TRARIEUX-FOURAULT (general practitioner)

Responsible technician : Sylvie POMMIER

V . DATES OF PERFORMANCE OF THE STUDY

Beginning on : August 23rd, 2005

End on: August 25th, 2005

VI . TEST PRODUCT

VI.1 . Number of test product simultaneously tested in the study

One product was tested in this study.

Two control patches, corresponding to the type of patch material used for the test product concerned, were applied at the same time : one containing an ad hoc quantity of water for injectable preparation, the other (supplied by the Sponsor) containing an ad hoc quantity of solution of ethanol at 80 %.

VI.2 . Identification of the test product

Denomination	ANIOSGEL 85
Batch number	G22061
Evic France reference	05.2317
Galenic form and organoleptic characteristics	Blue viscous solution
Number and type of sample	1 glass flask
Content of the samples	125 ml

VI.3 . Identification of the control product supplied by the Sponsor

Denomination	SOLUTION D'ETHANOL A 80 % v/v
Batch number	G22062
Evic France reference	05.2318
Galenic form and organoleptic characteristics	Transparent liquid
Number and type of samples	1 glass flask
Content of the samples	125 ml

VI.4 . Information concerning the test product

The documents relating to the test product supplied with the samples were the qualitative and quantitative formula and the Sponsor's letter of agreement particularly concerning the conformity of the formula to the regulations in force and its safety.

VII . VOLUNTEERS

VII.1 . Number

The number of volunteers whose data had to be exploitable at the end of the study was 10.

10 volunteers were included in the study.
No volunteer discontinued and no exclusion was decided by the investigator.

The compatibility of the test product was therefore assessed in 10 volunteers.

VII.2 . Specific inclusion criteria

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

- age : 18 to 70 years old,
- sex : female and/or male,
- phototype (Fitzpatrick) : I to V,
- all types of skin on body.

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

VII.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 troubles, scar elements, over-developed pilosity, ephelides and naevi in too great quantity, sunburn…..),
- 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,
- skin hyper-reactivity,
- reactivity to ethanol,
- reactivity to adhesive plaster,
- participation in more than 5 tests under exaggerated use conditions (under patch) within 12 months before the study, including 3 hypoallergenicity 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 created 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.

VIII . METHODOLOGY

VIII.1 . Experimental area and application site of the product

The experimental area was the back.

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

VIII.2 . Experimental conditions of application of the test product

The experimental conditions defined in the protocol were the following ones :

Patch material	Experimental conditions of use	Quantity applied
Finn Chamber standard®	As it is. Evaporation 15 minutes at least onto the patch	20 µl

Occlusive patch

-Finn Chamber standard® : 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 had to be measured with a micropipette with single use tips.

The same experimental conditions were applied for the control patch with the product **SOLUTION D'ETHANOL A 80 % v/v lot G22062**.

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

VIII.3 . Contact time of the test product with skin

The product had to be in contact with skin, under patch, for 48 ± 5 hours.

The patch removal had to be performed by the dermatologist.

The contact time and the modalities of removal, defined in the protocol, were respected.

VIII.4 . Constraints of the study

The constraints imposed on the volunteers were the following ones :

- no application of other products (than the tested one) 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 patch,
- 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 patch,
- 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.

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

The investigator checked 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 is enclosed in **Appendices 2/1 and 2/2**.

All the deviations from the protocol were analysed 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.

VIII.6 . Checking of the skin compatibility

VIII.6.1. Frequency of the examinations

The skin examination and joint questioning had to be performed by the dermatologist.

This examination had to be performed visually under standard "daylight" source, 15 minutes (or more if some redness appeared after patch removal) after patch removal.

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

VIII.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, Oedema, Vesicle, Bulla, Papule, Scab, Dryness, Coloration, Soap effect.

The intensity of the **erythema and œdema** was assessed according to an ordinal scale : very slioght, 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.

Bullae, 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 : erythema, oedema, vesicle, bulla, papule, scab, were taken into account.

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

- **in score of skin irritation**, calculated from the "marks" allocated to the visible signs : erythema, oedema, vesicle, papule (from 1 to 2 or 3) and bulla, scab (2 if presence) which took into account the intensity of the skin reactions.

For each volunteer, an **individual daily irritation score (Idis)** was calculated : sum of all the marks obtained for the observed signs.

For the panel, a **mean daily irritation score (Mdis)** was calculated according to the formula :

Mdis = Σ (Idis) / number of volunteers (exploitable data)

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

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

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.

This interpretation, performed by the dermatologist, was absolute. The test product could therefore have a **very good, good, quite good, moderate or bad skin compatibility.**

The possible reactions observed were either reactions of irritation or the revelation of an allergy previously acquired.

IX . RESULTS

The individual data of the skin examination and questioning of the volunteers are enclosed in **Appendices 3**.

In brief :

Control time after patch removal	Number of reactive volunteers	Types of reaction	Mean daily irritation score Mdis	% of reactive volunteers
T15 minutes	1	Very slight punctuated erythema (vol. ref. 5)	0.05	10%

To be noted : No skin reaction was noted with the control product **SOLUTION D'ETHANOL A 80 % v/v lot G22062** supplied by the Sponsor.

X . CONCLUSION

Under the experimental conditions adopted, the product **ANIOSGEL 85 Lot G22061 has a good skin compatibility.**

Signatures and dates

Investigator : Doctor Melissa MIGNARD-GUILLAUME (dermatologist) 09-09-05

I the undersigned, Melissa MIGNARD-GUILLAUME, declare that the overall conduct of the study was carried out under my responsibility and in accordance with the principles of Good Clinical Practices ("Avis aux promoteurs et aux investigateurs pour les essais cliniques des médicaments" : principes généraux – FR.OB – 1987 and international recommendations ICH E 6, step 4, of 1/5/1996).

09.09.05 Travaux - Fourault

Co-Investigator : Doctor Clotilde TRARIEUX-FOURAULT (general practitioner)

I the undersigned, Clotilde TARIEUX-FOURAULT, declare that the overall conduct of the study was carried out under my responsibility and in accordance with the principles of Good Clinical Practices ("Avis aux promoteurs et aux investigateurs pour les essais cliniques des médicaments" : principes généraux – FR.OB – 1987 and international recommendations ICH E 6, step 4, of 1/5/1996).

Quality Assurance Personnel : Danièle PICARD

09.09.05

I the undersigned, Danièle PICARD, declare that:
- this kind of study was audited according to the procedure of the investigator centre on August 22nd, 2005,
- the report of the audits was transmitted to the Management of Evic France and to the Investigator,
- the final report was examined on September 08th, 2005,
- the results reported accurately and completely reflect the raw data of the study.

APPENDICES

Appendix 1

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female M=male	Phototype *	Reactive skin	Allergic and/or atopic
Ref.	Name surname					
1	DI G. S	23	M	III	x	/
2	GATU. R	19	F	IV	/	/
3	DUCH. M	57	F	II	/	/
4	LAPE. J	20	M	III	/	/
5	AMES. V	24	F	IV	/	x
6	HOUE. A	56	F	III	x	x
7	COUR. C	19	F	IV	/	/
8	CASS. M	57	F	III	/	/
9	BARR. V	39	F	III	/	/
10	DIAZ. J	37	M	IV	/	/

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 **(10 exploitable results)**	**Number** of volunteers who respected the constraints	**Percentage** of volunteers who respected the constraints
No application of other products than the tested one to the experimental area Deviation : none	10	100%
No wearing of too thigh or restraining clothes on the experimental area, liable to produce frictions and to cause unsticking of the patch Deviation : none	10	100%
No bath (bathtub, swimming pool or sea), no hammam or sauna sessions during the study Deviation : none	10	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	10	100%
No excessive sweating and no intensive sport liable to cause unsticking of the patch Deviation : none	10	100%

Appendix 2/2

CONTROL OF THE OBSERVANCE
Constraints

Constraints **(10 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	10	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 – no medical treatment which could interfere with the study Deviation : none	10	100%
No vaccination during the study Deviation : none	10	100%

Appendix 3/1

CHECKING OF THE SKIN COMPATIBILITY
Treated site

Volunteers		T15 minutes	
Ref.	**Name Surname**	**skin examination**	**Idis**
1	DI G. S	/	0
2	GATU. R	/	0
3	DUCH. M	/	0
4	LAPE. J	/	0
5	AMES. V	E 0.5 p	0.5
6	HOUE. A	/	0
7	COUR. C	/	0
8	CASS. M	/	0
9	BARR. V	/	0
10	DIAZ. J	/	0
Mdis		**0.05**	

Legends :

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

Bu : Bulla
Pa : Papule
Sc : Scab
C : Coloration
Pr : Pruritus
St : Stinging
Hea : Heating
Vesicles and papules

0.5 : Very slight intensity
1 : Slight intensity
2 : Moderate intensity
3 : Severe intensity
d : diffuse
p : punctuated
peri : peripheral
1 : nb = 1 or 2
2 : nb > 2

Appendix 3/2

CHECKING OF THE SKIN COMPATIBILITY
Control site : Water for injectable preparation

Volunteers		T15 minutes	
Ref.	**Name Surname**	**skin examination**	**Idis**
1	DI G. S	/	0
2	GATU. R	/	0
3	DUCH. M	/	0
4	LAPE. J	/	0
5	AMES. V	E 0.5	0.5
6	HOUE. A	/	0
7	COUR. C	/	0
8	CASS. M	/	0
9	BARR. V	/	0
10	DIAZ. J	/	0
Mdis		**0.05**	

Legends :

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

Bu : Bulla
Pa : Papule
Sc : Scab
C : Coloration
Pr : Pruritus
St : Stinging
Hea : Heating
Vesicles and papules

0.5 : Very slight intensity
1 : Slight intensity
2 : Moderate intensity
3 : Severe intensity
d : diffuse
p : punctuated
peri : peripheral
1 : nb = 1 or 2
2 : nb > 2

Appendix 3/3

CHECKING OF THE SKIN COMPATIBILITY
Control site : Solution of ethanol at 80 %

Volunteers		T15 minutes	
Ref.	**Name Surname**	**skin examination**	**Idis**
1	DI G. S	/	0
2	GATU. R	/	0
3	DUCH. M	/	0
4	LAPE. J	/	0
5	AMES. V	/	0
6	HOUE. A	/	0
7	COUR. C	/	0
8	CASS. M	/	0
9	BARR. V	/	0
10	DIAZ. J	/	0
Mdis		**0**	

Legends :

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

Bu : Bulla
Pa : Papule
Sc : Scab
C : Coloration
Pr : Pruritus
St : Stinging
Hea : Heating
Vesicles and papules

0.5 : Very slight intensity
1 : Slight intensity
2 : Moderate intensity
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d : diffuse
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1 : nb = 1 or 2
2 : nb > 2