

Clinical Investigation Final Report FR04A/P273B20

The main objective of this double blinded, randomized and controlled study was to compare *in vivo* the hydrating efficacy of cosmetic products, 2 hours, 8 hours and 24 hours after one single application, in 10 healthy subjects. This report only refers to the results of the comparisons among the three investigational products Moisturizing gel HA (product code: E), Moisturizing gel AL (product code: H) and Moisturizing gel Glycerine (product code: B).

This clinical investigation was performed in accordance with the respective Clinical Investigation Plan and Study Plan and consistent with the main principles of ICH GCP, Helsinki declaration and Portuguese legal requirements.

Identification of the Clinical Investigation

CIP n./ Study Plan n.	EC002_v04/ PHDTA20_v01	Study beginning date:	01/02/2021	Study conclusion/ suspension date:	09/04/2021
Report Date:	02/06/2021	Report Author	Marta de Oliveira Ferreira		

Identification of Study Parties

Sponsor	Swedish Algae Factory Stena Center, Gothenburg, Sweden
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Identification of Investigational Product

Investigational Product (s)			Comparator Product (s)		
Designation	Reference	Batch number	Designation	Reference	Batch number
Moisturizing gel HA	Algica	HA 2021/01/22	-	-	-
Moisturizing gel AL	Algica	AL 2021/01/22	-	-	-
Moisturizing gel Glycerine	Algica	G 2021/01/22	-	-	-

Clinical Investigation Report prepared/changed by**INOVAPOTEK**

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Clinical Investigation Report verified by**INOVAPOTEK**

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HISTORY OF THE DOCUMENT

Revision	Amendment/Deviation	Date
A	First issue	02/06/2021



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Abbreviated terms and definitions

µl	Microliter
A.U.	Arbitrary Units
CEC	Competent Ethics Committee
CEIC	National Ethics Committee for Clinical Research
CIP	Clinical Investigational Plan
CIR	Clinical Investigational Report
cm	Centimeter
CM	Corneometer
cm²	Square Centimeter
CRO	Contract Research Organization
CSI®	Complete Skin Investigation, CK electronic
CV	Curriculum Vitae
EC	Ethics Committee
g	Gram
GHQ	General Health Questionnaire
h	Hour(s)
ICH GCP	International Conference on Harmonisation-Good Clinical Practice
INFARMED	National Authority of Medicines and Health Products I.P.
min	Minute(s)
ml	Milliliter
MS	Microsoft
No	Number
°C	Celsius degrees
PI	Principal Investigator
RH	Relative Humidity
RNEC	National Registry for Clinical Studies
SD	Standard Deviation
t	Time
T	Temperature
vs	Versus

1 – Ethics and Quality Assurance

The CIP EC002 was approved by Ethics Committee for Health of inovapotek on the 17/07/2019. Four subsequent amendments were performed to the CIP, the first one to perform a revision of the Detailed Procedure; the second one to perform recommended alterations by the Ethics Committee of inovapotek; the third one to alter the Principal Investigator and include additional possible test areas and measurements; the fourth to include new recommended alterations by the Ethics Committee of inovapotek.

The CIP EC002 was submitted to INFARMED, I.P. on the 30/05/2019 through the RNEC (National Registry for Clinical Studies) platform under the number 102124.

During trial conduct periodic monitoring was conducted to ensure that the protocol was being followed.

2 – Summary

CIP n./Study Plan n.	EC002_v04 / PHDTA20_v01	Study beginning date:	01/02/2021	Study conclusion/suspension date:	09/04/2021
Title	DOUBLE BLINDED RANDOMIZED CLINICAL STUDY FOR THE EVALUATION OF THE HYDRATING EFFICACY OF COSMETIC PRODUCTS				

This double blinded randomized clinical study aimed to evaluate *in vivo* the hydrating efficacy of cosmetic products 2 hours, 8 hours and 24 hours after one single application, by measurements of the skin capacitance with the equipment Corneometer® compared to one negative control (type II water).

In order to perform this study, one group of 10 female healthy subjects, having 18 years old and over with no skin diseases were enrolled and tested a total of three products: one investigational product (Moisturizing gel AL (product code: H)) and two comparator products: one negative control (type II water) and one positive control (glycerine at 85%, aqueous solution), and another group of 10 female healthy subjects, having 18 years old and over with no skin diseases were enrolled and tested a total of three products: two investigational products (Moisturizing gel HA (product code: E) and Moisturizing gel Glycerine (product code: B)) and two comparator products: one negative control (type II water) and one positive control (glycerine at 85%, aqueous solution).

A plastic marker was applied on the subjects' forearms to outline the test areas where 2 mg/cm² of each investigational product and 2 µL/cm² of the negative and positive control products were applied directly on the respective test area.

Skin hydration measurements were performed in each test site, in triplicate, before the products' application (t0) and 2 hours (t2), 8 hours (t8) and 24 hours (t24) after the products' application using the Corneometer® CM825 (Courage+Khazaka electronic GmbH, Germany) equipment.

This report only refers to the results of the comparisons among the three investigational products Moisturizing gel HA (product code: E), Moisturizing gel AL (product code: H) and Moisturizing gel Glycerine (product code: B).

Regarding the study results, it can be concluded that the investigational product Moisturizing gel AL has a better effect ($p < 0.05$) on the skin's hydration 24 hours after a

single application when compared to the investigational product Moisturizing gel Glycerine. Regarding the comparisons performed between the investigational products' Moisturizing gel HA and Moisturizing gel AL, as well as the comparisons performed between the investigational products' Moisturizing gel HA and Moisturizing gel Glycerine, it can be concluded that both products' have the same effect on the skin's hydration throughout the 24 hours of evaluation.

The products were well tolerated by the subjects with no uncomfortable symptoms or feelings reported by the subjects.

The benefits of the investigation superposed the risks, as the efficacy degree of the product was assessed and no adverse events were observed.

In conclusion, the investigational product **Moisturizing gel AL** has a better effect ($p < 0.05$) on the skin's hydration 24 hours after a single application when compared to the investigational product **Moisturizing gel Glycerine**, while the remaining product comparisons reveal a similar moisturizing effect for the different investigational products tested.



3 – Introduction

This double blinded randomized clinical study aimed to evaluate *in vivo* the hydrating efficacy of a cosmetic product 2 hours, 8 hours and 24 hours after one single application, by measurements of the skin capacitance with the equipment Corneometer® compared to one negative control (type II water).

In order to perform this study, one group of 10 female healthy subjects, having 18 years old and over with no skin diseases were enrolled and tested a total of three products: one investigational product (Moisturizing gel AL (product code: H)) and two comparator products: one negative control (type II water) and one positive control (glycerine at 85%, aqueous solution), and another group of 10 female healthy subjects, having 18 years old and over with no skin diseases were enrolled and tested a total of three products: two investigational products (Moisturizing gel HA (product code: E) and Moisturizing gel Glycerine (product code: B)) and two comparator products: one negative control (type II water) and one positive control (glycerine at 85%, aqueous solution).

A plastic marker was applied on the subjects' forearms to outline the test areas where 2 mg/cm² of each investigational product and 2 µL/cm² of the negative and positive control products were applied directly on the respective test area.

Skin hydration measurements were performed in each test site, in triplicate, before the products' application (t0) and 2 hours (t2), 8 hours (t8) and 24 hours (t24) after the products' application using the Corneometer® CM825 (Courage+Khazaka electronic GmbH, Germany) equipment.

This report only refers to the results of the comparisons among the three investigational products Moisturizing gel HA (product code: E), Moisturizing gel AL (product code: H) and Moisturizing gel Glycerine (product code: B).

4 – Results and Discussion

4.1 CIP compliance

CIP n./Study Plan no.	EC002_v04 / PHDTA20_v01	Study beginning date:	01/02/2021	Study conclusion/suspension date:	09/04/2021
Title	DOUBLE BLINDED RANDOMIZED CLINICAL STUDY FOR THE EVALUATION OF THE HYDRATING EFFICACY OF COSMETIC PRODUCTS				

No CIP/Study Plan deviations have occurred that can have affected the rights, safety or wellbeing of the subject or the scientific integrity of the clinical investigation.

However, some temperature (T) recorded both for acclimatization and measurements and some relative humidity % (RH) recorded for measurements were out of the specifications (T: 23.0 ± 1.0 °C; RH: 50.0 ± 10.0 %).

Considering all temperature and relative humidity values recorded and the mean values from all time-points both for acclimatization and for measurements, these were considered minor deviations as all procedures were performed under similar conditions for each subject and these conditions did not have impact on the obtained results.

4.2 Test subjects

4.2.1 Subject demographics

10 female subjects were included on each group of this clinical investigation and performed the measurements at time-points t0, t2, t8 and t24.

Full subject demographics data collected through the General Health Questionnaire and concomitant medications and treatments collected through the Concomitant Medications Log are presented on Annex III.

4.2.2 Subject withdrawal and dropouts

For Moisturizing gel AL evaluation (group VI), 11 subjects were screened via telematic methods. Of these, 1 was a screening failure because the subject was not available to participate on this clinical study.

For Moisturizing gel HA and Moisturizing gel Glycerine evaluation (group VII), 10 subjects were screened via telematic methods and included in this clinical study.

4.3 Investigational Products

4.3.1 Changes to the investigational products & Product deficiencies

No changes to the investigational products were observed during the clinical investigation.

4.3.2 Treatment adhesion

The mean amount of the investigational product **Moisturizing gel HA** weighed during the study was 14.01 ± 0.09 mg, and the mean amount applied per skin area was 2.00 ± 0.01 mg/cm².

The mean amount of the investigational product **Moisturizing gel AL** weighed during the study was 14.07 ± 0.07 mg, and the mean amount applied per skin area was 2.01 ± 0.01 mg/cm².

The mean amount of the investigational product **Moisturizing gel Glycerine** weighed during the study was 13.97 ± 0.08 mg, and the mean amount applied per skin area was 2.00 ± 0.01 mg/cm².

Complete values are presented in Annex IV.

4.3.3. Product codification

The products were coded by inovapotek's personnel not involved in the clinical study, according to the following table:

Investigational and Comparator Products	Product Code
Moisturizing gel HA	E (group VII)
Moisturizing gel AL	H (group VI)
Moisturizing gel Glycerine	B (group VII)

4.4 Measurements

4.4.1 Room conditions

All the study procedures were performed by trained and experienced personnel under controlled atmospheric conditions at all time points. The mean values of temperature (T) and relative humidity (RH) in acclimatization room and in the measurement rooms are presented in the following table. Complete values are presented in Annex V.

Table 1. Mean temperature and relative humidity values recorded during the acclimatization period for the investigational product Moisturizing gel AL

	t0		t24	
	T (°C)	RH (%)	T (°C)	RH (%)
Mean	22.6	45.0	22.7	43.7
SD	0.6	3.7	0.4	3.5

Table 2. Mean temperature and relative humidity values recorded during the measurements for the investigational product Moisturizing gel AL

	t0		t2		t8		t24	
	T (°C)	RH (%)	T (°C)	T (°C)	T (°C)	RH (%)	T (°C)	RH (%)
Mean	22.7	45.5	22.9	44.7	22.8	45.3	23.1	43.8
SD	0.7	3.3	0.5	1.9	0.2	1.4	0.5	3.3

Table 3. Mean temperature and relative humidity values recorded during the acclimatization period for the investigational products Moisturizing gel HA and Moisturizing gel Glycerine

	t0		t24	
	T (°C)	RH (%)	T (°C)	RH (%)
Mean	22.8	40.8	22.1	41.8
SD	0.7	0.7	0.3	1.0

Table 4. Mean temperature and relative humidity values recorded during the measurements for the investigational products Moisturizing gel HA and Moisturizing gel Glycerine

	t0		t2		t8		t24	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
Mean	22.8	41.2	23.1	41.1	22.9	43.4	22.3	41.8
SD	0.6	0.8	0.4	1.0	0.5	1.4	0.4	1.1

4.4.2 Control measurements (if applicable)

Not applicable.

4.4.3 Efficacy evaluations

4.4.3.1 Hydration evaluation

Skin capacity was measured on each test site with a Corneometer® CM825 (Courage+Khazaka electronic GmbH, Germany), applying slight pressure in each test site, in triplicate, before the application of the products (t0) and 2 hours (t2), 8 hours (t8) and 24 hours (t24) after one single application of the products.

The probe head is placed vertically on the skin area to be measured according to the pressure of the spring in the probe. A beep signal is heard if the measurement has been carried out successfully. The probe head is cleaned thoroughly between measurements with a dry paper. Any dirt, water or alcohol on the probe head might influence the measurement values.

Each skin site is measured 3 times not on the exactly same spot but in a neighbouring skin area. Repeated measurements on the same skin area lead to a moisture increase due to occlusion, as water is accumulated under the probe head and cannot evaporate.

The measurement of the skin hydration is based on the internationally recognized capacitance method. This measurement is based on the completely different dielectric constant of water and other substances. The measuring capacitor shows changes of capacitance according to the moisture content of the samples. The changes in capacitance are converted in hydration units varying from 0 to 120, where 0 units correspond to very dry, and 120 units to very humid skin areas. The results are given in arbitrary units (A. U.).

The results obtained at all time-points for the skin hydration with each investigational product are presented on Table 5 and Figure 1. The *p-values* obtained in the statistical analysis performed are also presented in this table and the complete statistical tables of each statistical test performed are presented in Annex VII.

Six extreme outliers (subject 2 at t2, subject 3 at t2, subject 9 at t2 and at t8, and subject 10 at t2 and at t8) were found after statistical analysis of the investigational product Moisturizing gel AL (product H) results and were removed from the differences analysis.

Table 5. Skin hydration results obtained before (t0) and after 2 hours (t2), 8 hours (t8) and 24 hours (t24) of product's application for the investigational products Moisturizing gel HA (Product code: E), Moisturizing gel AL (Product code: H) and Moisturizing gel Glycerine (Product code: B)

	Moisturizing gel HA (Product code: E)				Moisturizing gel AL (Product code: H)				Moisturizing gel Glycerine (Product code: B)			
	t0 (n=10)	t2 (n=10)	t8 (n=10)	t24 (n=10)	t0 (n=10)	t2 (n=10)	t8 (n=10)	t24 (n=10)	t0 (n=10)	t2 (n=10)	t8 (n=10)	t24 (n=10)
Skin hydration (A.U.) mean values	33.18	32.43	33.50	29.72	33.81 (n=10)	37.73 (n=10)	38.53 (n=10)	36.35 (n=10)	31.93	32.49	33.88	27.61
±SD	5.29	5.99	3.54	8.09	7.58	13.52	18.73	8.97	6.95	5.82	6.07	8.12
Mean differences		-0.75	0.32	-3.46		0.71 (n=6)	-0.60 (n=8)	2.54		0.56	1.95	-4.31
±SD		3.68	4.01	6.31		0.97	3.08	4.32		4.04	3.55	6.41
Mean differences (%)		-2.03%	2.35%	-10.53%		2.19%	-1.89%	7.58%		3.24%	7.54%	-
±SD (%)		10.52%	12.72%	18.90%		3.14%	9.53%	12.79%		14.59%	13.25%	20.81%
No. of subjects with Skin hydration (A.U.) increase		5	5	3		4	3	7		5	7	2
% of subjects with Skin hydration (A.U.) increase		50.00%	50.00%	30.00%		66.67%	37.50%	70.00%		50.00%	70.00%	20.00%
Maximum increase (%)		16.88%	22.56%	14.25%		5.63%	14.71%	37.40%		33.21%	31.70%	23.27%
Mean increase among subjects with positive effects (%)		6.35%	12.94%	11.81%		3.92%	7.60%	12.73%		13.40%	12.82%	13.41%
p value (E vs H)						0.072**	0.876**	0.211**				
p value (E vs B)										0.478*	0.301*	0.628*
p value (H vs B)										0.730**	0.076**	0.035**
p value (before – t0 vs after – other time-points)		0.538*	0.804*	0.117*		0.401*	0.786*	0.096*			0.115*	0.062*

* Paired t-test ** Independent t-test *** Mann-Whitney test

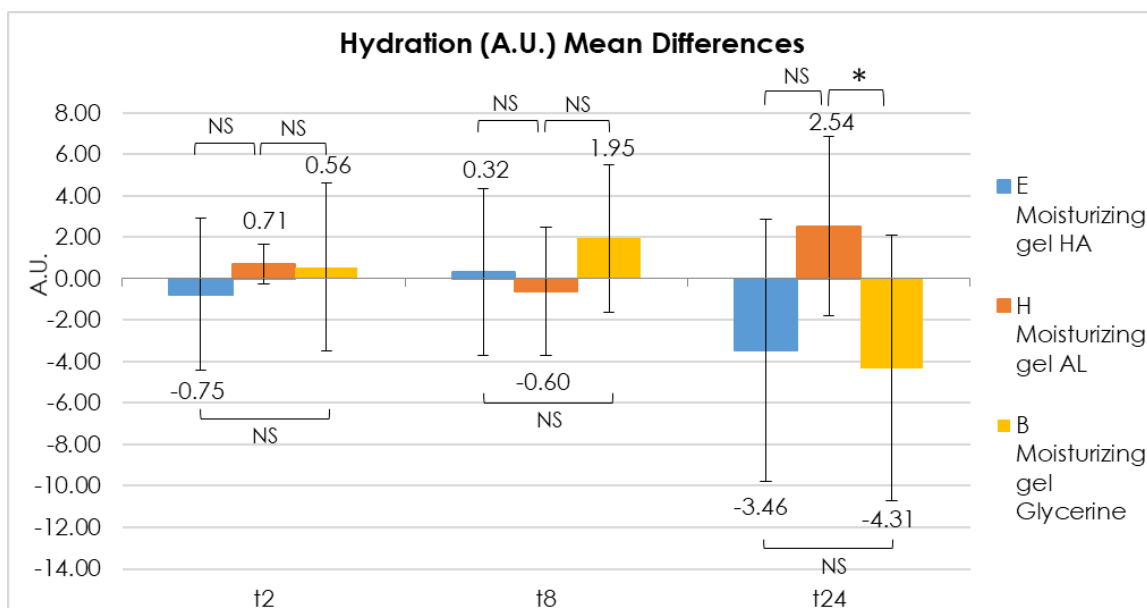


Figure 1. Skin hydration mean differences to the baseline obtained after 2 hours (t2), 8 hours (t8) and 24 hours (t24) of application of the investigational products Moisturizing gel HA (Product code: E), Moisturizing gel AL (Product code: H) and Moisturizing gel Glycerine (Product code: B).

To compare the hydration efficacy of the investigational products Moisturizing gel HA, Moisturizing gel AL and Moisturizing gel Glycerine, the differences obtained at each time-point of analysis (without the baseline values) for each investigational product were compared with each other. Statistically significant differences between the investigational product Moisturizing gel AL and the investigational product Moisturizing gel Glycerine were observed only at t24 ($p \leq 0.05$) when comparing its differences, but not at t2 and t8. Regarding the comparison of the skin hydration differences between the investigational product Moisturizing gel HA and the investigational product Moisturizing gel AL, no statistically significant differences were found at all evaluated time-points. Also, no statistically significant differences between the results obtained for the investigational product Moisturizing gel HA and the investigational product Moisturizing gel Glycerine were found at all evaluated time-points ($p \leq 0.05$) when comparing its differences.

It can therefore be concluded that the investigational product **Moisturizing gel AL** has a better effect on the skin's hydration 24 hours after a single application when compared to the investigational product **Moisturizing gel Glycerine**. Regarding the comparisons performed between the investigational products' **Moisturizing gel HA** and **Moisturizing gel AL**, as well as the comparisons performed between the investigational products

Moisturizing gel HA and **Moisturizing gel Glycerine**, it can be concluded that both products have the same effect on the skin's hydration throughout the 24 hours of evaluation.

4.4.4 Tolerance evaluations

Not applicable.

4.5 Adverse Events

No adverse events were reported.



5 – Conclusion

This double blinded randomized clinical study aimed to evaluate *in vivo* the hydrating efficacy of cosmetic products 2 hours, 8 hours and 24 hours after one single application, by measurements of the skin capacitance with the equipment Corneometer® compared to one negative control (type II water).

This report only refers to the results of the comparisons among the three investigational products Moisturizing gel HA (product code: E), Moisturizing gel AL (product code: H) and Moisturizing gel Glycerine (product code: B).

Regarding the study results, it can be concluded that the investigational product Moisturizing gel AL has a better effect ($p < 0.05$) on the skin's hydration 24 hours after a single application when compared to the investigational product Moisturizing gel Glycerine. Regarding the comparisons performed between the investigational products Moisturizing gel HA and Moisturizing gel AL, as well as the comparisons performed between the investigational products Moisturizing gel HA and Moisturizing gel Glycerine, it can be concluded that both products have the same effect on the skin's hydration throughout the 24 hours of evaluation.

The products were well tolerated by the subjects with no uncomfortable symptoms or feelings reported by the subjects.

The benefits of the investigation superposed the risks, as the efficacy degree of the product was assessed and no adverse events were observed.

In conclusion, the investigational product **Moisturizing gel AL** has a better effect ($p < 0.05$) on the skin's hydration 24 hours after a single application when compared to the investigational product **Moisturizing gel Glycerine**, while the remaining product comparisons reveal a similar moisturizing effect for the different investigational products tested.

6 – Bibliographic References

1	Clinical investigation of medical devices for human subjects — Good clinical practice. International Standard ISO 14155. 3rd edition. 20/07/2020
2	Guideline for Good Clinical Practice – ICH harmonised tripartite guideline E6 (R2). International Conference on Harmonization (ICH). 9 November 2016
3	Regulation (EC) no 1223/2009 of the European Parliament and of the Council and its amendments; 30/11/2009
4	Mod.075.04 Clinical Investigation Plan_v004_EC002, inovapotek, 27/03/20.

7 – Data Handling and Record Keeping

The documents and records supporting the clinical investigation will be archived in the Study Master File at CRO for 10 years. The CRO must obtain Sponsor written permission before disposing of any records even if the retention requirements have been met.



ANNEXES

ANNEX I - CVs of the Investigators

ANNEX II - Randomization table

ANNEX III - Subject Demographics, Concomitant Medication & Treatments

ANNEX IV - Investigational Product Weight

ANNEX V - Temperatures and Relative Humidity

ANNEX VI - Skin Hydration Results

ANNEX VII - Statistical Analysis Results

ANNEX VIII - Study Plan

