

EVALUATION OF THE PRODUCT EFFICACY ON HYDRATION, ELASTICITY and pH LEVEL, WRINKE REDUCTION and REDUCTION IN SIZE AND AMOUNT OF PORES ON THE BASIS OF INSTRUMENTAL EVALUATION

1. GENERAL INFORMATION

STUDY SPONSOR	Skin Ingredients Pty, Cape Town, South Africa
STUDY CODE	C017/003
NAME AND ADDRESS OF THE ORGANIZATION IN CHARGE OF THE ASSESSMENT	Luamed, Tanja Židan s.p. Lukovica, Slovenia
TEST PRODUCT	Sk.in Flash 2
AUTHORIZED BY	Tanja Židan
ADDITIONAL INVESTIGATIONS INVOLVED	Katja Urek
REPORT DATE	22.9.2019

2. PURPOSE AND OBJECTIVE OF THE TEST

The object of this test was to define the direct influence of the tested product on the level of skin hydration, elasticity, pH and wrinkle and pore reduction and to confirm the declared properties and efficacy of the product on the basis of instrumental methods and use test (consumer evaluation).

DESCRIPTION OF THE PRODUCT

INTENDED USE	Face care product
APPEARANCE	Cream
COLOR	Yellowish
FRAGRANCE	Characteristic
INSTRUCTIONS FOR USE	Use every 2nd night for 2 weeks then every night. all volunteers continue to use their normal basic skincare regime such as cleanser, moisturiser and SPF and to slot the serums in.
PRODUCT CLAIMS AS DECLARED BY CUSTOMER	Counteract the signs of UV sun damage and intrinsic skin ageing, improving skin texture, hydrates, minimising pore size, improving fine lines and wrinkles

INCI LIST (QUALITATIVE COMPOSITION) AS DECLARED BY CUSTOMER

The qualitative composition was delivered to the laboratory, by the Sponsor, before the start of the study.

3. TEST SCHEDULE

STARTING DATE	24.7.2019
FINISHING DATE	18.9.2019

4. METHODOLOGY

PROTOCOL SUMMARY

Instrumental test using: Callegari 1930 Company – Soft Plus device (hydration probe, pH, elasticity probe, micro camera)

The aim of the test was to determine the direct influence of the tested product on skin hydration, elasticity, pH, wrinkle and pore reduction. The test was conducted with a special measuring device manufactured by Callegari 1930 Company – Soft Plus.

The instrumental measurements were performed on facial skin. Tested and control zones were indicated on the left and right part of the face. The application zone was an area of approximately 283 cm². The measurements were carried out for each zone in all tested and control places immediately prior to the application of the tested product. Subjects were told not to rinse off the product and to continue with their basic skincare regime one until the end of the study (8 weeks).

The measurement of the hydration effect was performed one, three and five hours after the product application. The arithmetic mean of the measurements of each of the 10 subjects is considered as each final result.

The measurement of the elasticity and pH were performed three times, at the beginning, after 4 weeks and at the end of the study (8 weeks). The arithmetic mean of the measurements of each of the 10 subjects is considered as each final result.

The measurement of depth and degree of wrinkles and amount of pores was performed at the beginning and at the end of the study (8 weeks). Before and after fotos were recorded to evaluate the changes of the 10 subjects.

All measurements were carried out in a room with a temperature of $20 \pm 3^{\circ}\text{C}$ and a relative humidity of $50 \pm 10\%$. Directly before testing, the skin of the volunteers was gently wiped with warm water.

HYDRATION MEASUREMENT

The skin is divided into three layers: the epidermis, the dermis and the subcutaneous tissue.

A very thin hydro-lipid layer is found above the epidermis. This is basically made up of the secretions of the sebaceous glands and it maintains the skin's firmness and prevents the excessive loss of transcutaneous water and the entry of harmful substances.

The epidermis itself is composed of five different layers. In the bottom layer, the stratum basal, the cells divide and push already formed cells into the higher layers. As the cells move into the higher layers, they flatten and eventually die. The top layer of the epidermis, the stratum corneum, is made up of dead, keratinized cells that shed about every two weeks.

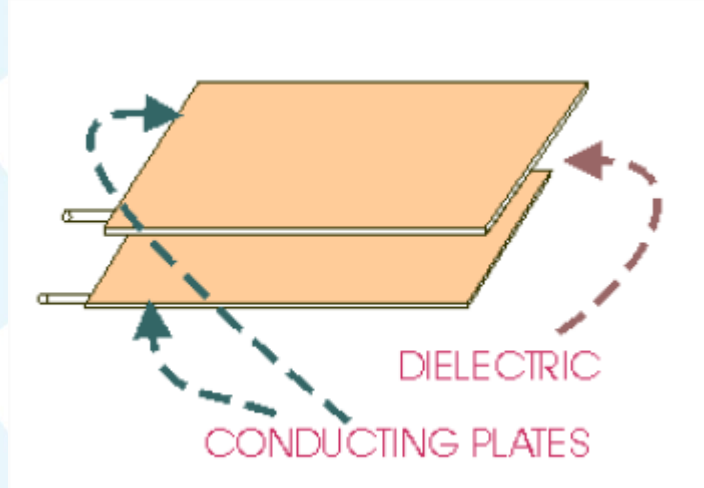
While the amount of water in the inner layers of the skin is relatively constant and is in equilibrium with the other organs of the body (ca. 60–70%), the moisture in the stratum corneum depends on different factors:

- the rate at which the water in the dermis reaches the stratum corneum
- the rate at which the water is eliminated by evaporation (TEWL)
- the ability of the stratum corneum to retain water.

When speaking about the skin's moisture, we refer to the moisture contents of the stratum corneum.

Soft Plus measures the hydration of the stratum corneum through the scientifically acknowledged capacitive method, which measures the amount of electric current passing through a capacitor.

In its simplest form, a capacitor consists of two conducting plates (tracks) that are separated by an insulating material called the dielectric.



In the capacitor constituted by probe and skin, one conducting plate is the probe surface and the other is represented by the deeper (well-hydrated) layer of the skin.

The horny layer, constituted by dead keratinized cells dispersed in a lipid medium, represents an excellent barrier to the passage of both chemical substances and electric current and it can, therefore, be considered a dielectric medium (the dielectric constant of the anhydrous horny layer is usually lower than five). The water dielectric constant is much higher (81), thus, if water is contained in the stratum corneum, the horny layer dramatically changes its dielectric properties.

In measuring the amount of current that flows through the stratum corneum, it is possible to evaluate the dielectric constant of the hydrated stratum corneum and to calculate its moisture content.

ELASTICITY MEASUREMENT

Every day our skin undergoes various mechanical stresses that can lead to its deformation. Different types of forces can deform the skin: lifting, tension, torsion,

pressure and compression. When these forces are halted, the skin returns to its original position because of its elasticity. The measurement of skin elasticity is highly complicated for two reasons:

- the forces involved are very small and so they are measured with difficulty,
- collagen and elastin fibres stabilize the skin and prevent it from being torn in every direction of the space.

For these reasons, all of the methods described in the literature for the measurement of skin elasticity provide an indication of the condition of the skin.

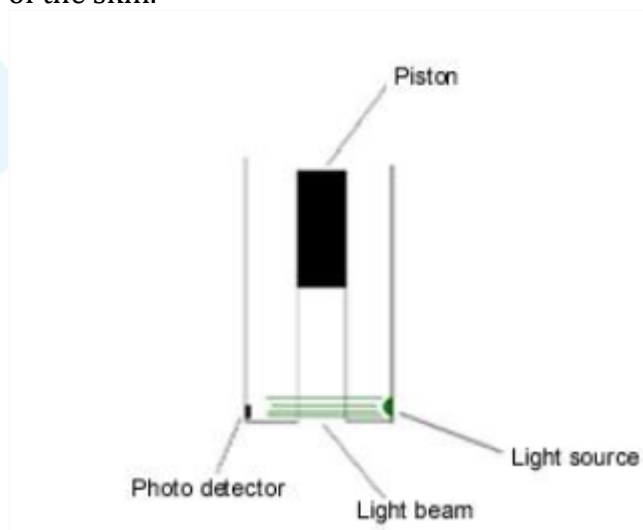
The measurement principle employed in the elasticity probe is based on the suction method.

From a technical point of view, pressing and releasing the piston creates a vacuum inside the probe (the probe is endowed with a small pump, thus, assuring the application of the same and precise vacuum for each measurement).

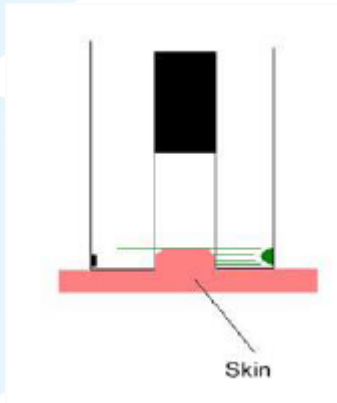
The skin reacts to this stimulus, rising into the chamber of the probe.

The cutaneous deformation is quantified through a sensor developed specifically for this purpose.

The sensor is made up of a light source that produces a transverse light beam on the inside of the probe. The more the skin is deformed on the inside of the probe, the more intense the decrease in the light beam. The decrease of the signal is correlated with the elasticity of the skin.



When the skin does not penetrate the inside of the probe, the quantity of light detected by the photodiode is considered to be maximum.



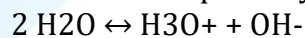
When the skin is not able to oppose resistance to the mechanical stress caused by the suction, it will penetrate the internal part of the probe.

The amount of skin that penetrates is measured by the reduction in the light that reaches the photodiode.

pH MEASUREMENT

The pH of a solution is defined as $-\log_{10} H_3O^+$.

Pure water is partially dissociated in accordance with this equilibrium:



The concentration of ions H_3O^+ is identical to those of ions OH^- and the $pH = 7$.

When acids, bases or salts are dissolved in water, the concentration of ions bearing an opposite charge differs and the $pH \neq 7$.

Skin pH is usually slightly acid, which is mainly due to lactic acid and various amino acids from sweat, free fatty acids from sebum and amino acids and pyrrolidone carboxylic acid from the cornification process of the skin.

The measurement of skin surface pH is used in clinical research to evaluate shifts in pH following external exposures and to evaluate the state of diseased skin with acute or chronic changes.

It is possible to use different methods for the measurement of pH:

- the colourimetric method, which uses a particular chemical indicator (a shift in pH is paralleled with a visible shift in colour)
- the potentiometer pH measurement, which is the most precise and easy method used today.

The measurement of pH employed for the Soft Plus is based on the potentiometer principle.

In this procedure, electrical impulses are measured using an electrode and the difference in potential is measured with a potentiometer. The sensor is a glass electrode with

selective hydrogen-ions sensitivity and has the highest sensitivity and reliability. This is formed by a glass bulb in which the active electrode (which is in contact with the skin) and the reference electrode (plunged into a solution of KCl) are combined. The small amount of water lying between the electrode and the skin surface is sufficient to dissolve the ions that are present on the skin surface (lactate, etc.) and to create adequate measuring conditions.

When the pH probe is in contact with a solution containing ions H_3O^+ , a flux of electrons moves between the active electrode and the reference electrode: the potentiometer measures the difference in potential, which is directly proportional to the pH of the test solution.

The reactions that occur at the electrodes are shown below:

$H_3O^+ + e^- \leftrightarrow \frac{1}{2} H_2 + H_2O$ active electrode

$Cl^- \leftrightarrow \frac{1}{2} Cl_2 + e^-$ reference electrode.

A planar electrode was developed for the specific requirements of skin pH measurements. The planar electrode has several advantages. The electrode membrane is flat and the contact between the electrode and the skin surface covers a relatively large area. Use of an electrode is non-invasive and the electric current is small and constant and causes no damage.

DESCRIPTION OF VOLUNTEERS

INCLUSION CRITERIA	GENERAL Skin without irritation and changes requiring pharmacological treatment	SPECIFIC Age: 25-65 Skin type: Normal skin Amount: 10
EXCLUSION CRITERIA	- Skin diseases or any other medical condition requiring systemic medical treatment or which may interfere with the objectives of the study.	

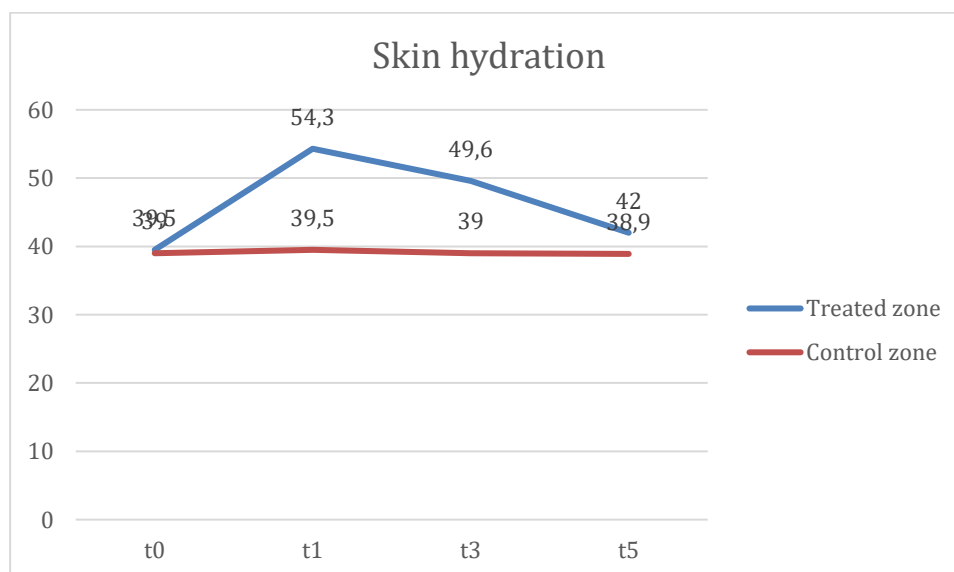
5. RESULTS

PRESENTATION OF RESULTS-HYDRATION MESUREMENT

Table 1: The results of skin hydration measurement before application (t0) and 1, 3 and 5 hours after the product application in arbitrary units. Each result is the average of 5 individual measurements.

SUBJECT CODE	Treated zone				Control zone			
	t0	t1	t3	t5	t0	t1	t3	t5
001	37	50	46	39	35	35	37	37
002	34	46	43	37	35	35	37	33
003	56	77	70	59	53	56	55	58
004	24	32	30	26	24	21	23	24
005	60	84	75	64	63	64	61	62
006	40	56	51	43	40	40	43	40
007	32	46	40	34	32	29	29	29
008	56	74	71	59	56	59	53	55
009	20	27	25	21	18	21	19	17
010	36	51	45	38	34	35	33	34
MEAN	39,5	54,3	49,6	42	39	39,5	39	38,9
MIN	20	27	25	21	18	21	19	17
MAX	60	84	75	64	63	64	61	62
MEDIAN	36,5	50,5	45,5	38,5	35	35	37	35,5
SD	13,7	18,9	17,3	14,4	14,3	15,3	14,0	15,0

Graph I: The average results of skin hydration measurement before application (t0) and 1, 3 and 5 hours after the product application in arbitrary units.

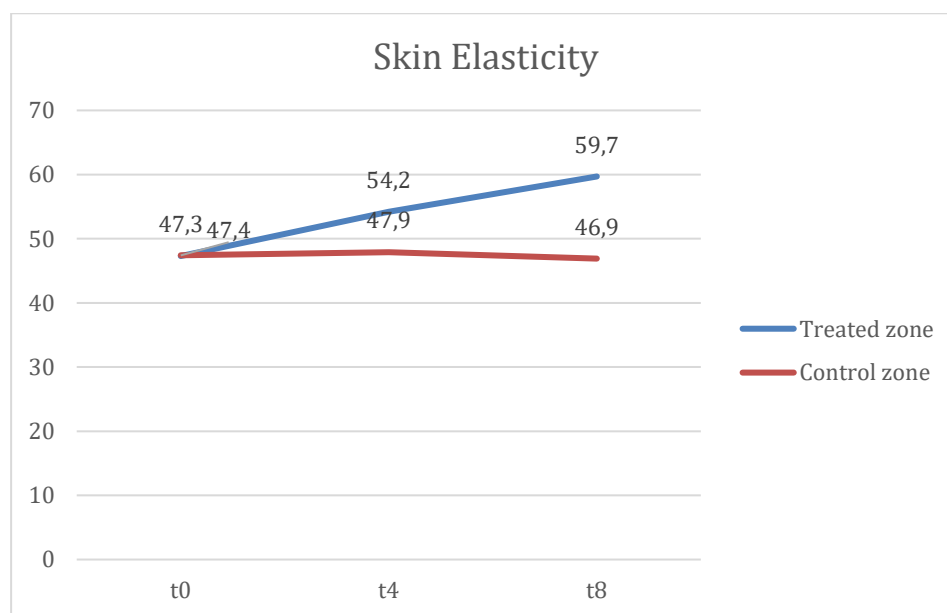


PRESENTATION OF RESULTS- ELASTICITY

Table 2: The results of skin elasticity measurement before application (t0), after 4 weeks (t4) and 8 weeks (t8) after the product application in arbitrary units. Each result is the average of 5 individual measurements

SUBJECT CODE	Treated zone			Control zone		
	t0	t4	t8	t0	t4	t8
001	63	68	78	62	62	62
002	23	28	32	22	27	24
003	50	54	55	52	50	52
004	18	20	21	18	16	18
005	48	57	64	47	51	48
006	31	35	39	34	30	27
007	69	84	94	71	69	69
008	68	78	82	65	70	66
009	31	34	39	31	31	30
010	72	84	93	72	73	73
MEAN	47,3	54,2	59,7	47,4	47,9	46,9
MIN	18	20	21	18	16	18
MAX	72	84	94	72	73	73
MEDIAN	49	55,5	59,5	49,5	50,5	50
SD	20,4	24,0	26,4	20,2	20,6	20,6

Graph II: The average results of skin elasticity measurement before application (t0), after 4 weeks (t4) and 8 weeks (t8) after the product application in arbitrary units.

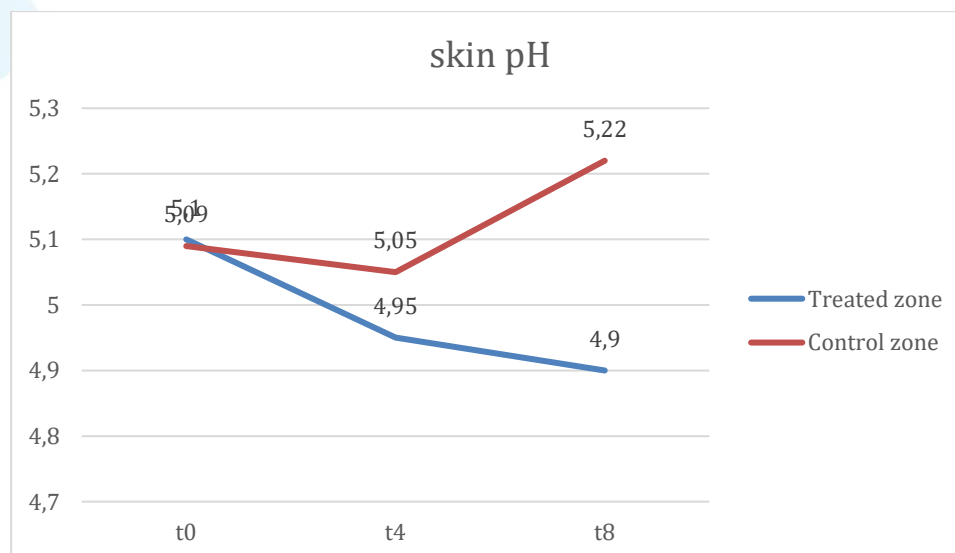


PRESENTATION OF RESULTS- pH

Table 3: The results of skin pH measurement before application (t0), after 4 weeks (t4) and 8 weeks (t8) after in arbitrary units. Each result is the average of 5 individual measurements

SUBJECT CODE	Treated zone			Control zone		
	t0	t4	t8	t0	t4	t8
001	6,0	5,7	6,7	6,1	6,1	6,0
002	5,0	5,5	4,9	5,2	4,9	4,8
003	5,0	4,7	4,5	4,8	5,3	5,2
004	6,0	6,1	5,6	5,7	6,1	6,1
005	4,0	3,7	4,1	4,2	3,6	4,2
006	5,0	5,0	5,1	5,4	4,4	5,2
007	4,0	3,5	3,9	3,9	3,9	4,4
008	4,0	4,1	3,5	3,9	4,1	4,1
009	6,0	5,8	4,9	5,7	5,9	6,4
010	6,0	5,4	5,8	6,0	6,2	5,8
MEAN	5,1	4,95	4,9	5,09	5,05	5,22
MIN	4	3,5	3,5	3,9	3,6	4,1
MAX	6	6,1	6,7	6,1	6,2	6,4
MEDIAN	5	5,2	4,9	5,3	5,10	5,20
SD	0,9	0,9	1,0	0,8	1,0	0,8

Graph III: The average results of skin pH measurement before application (t0), after 4 weeks (t4) and 8 weeks (t8) after the product application in arbitrary units.

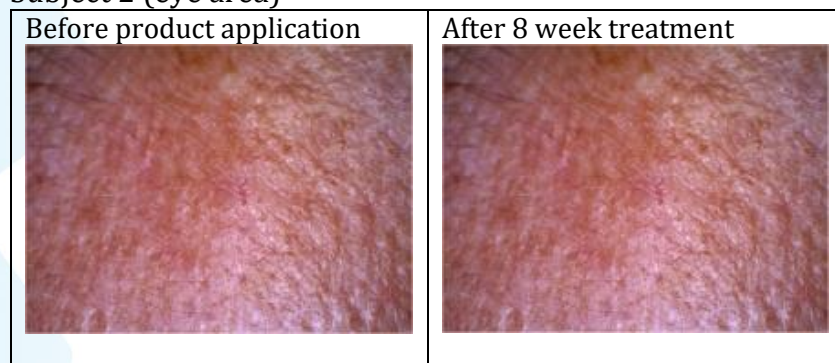


PRESENTATION OF RESULTS- DEPTH AND AMOUNT OF WRINKLES

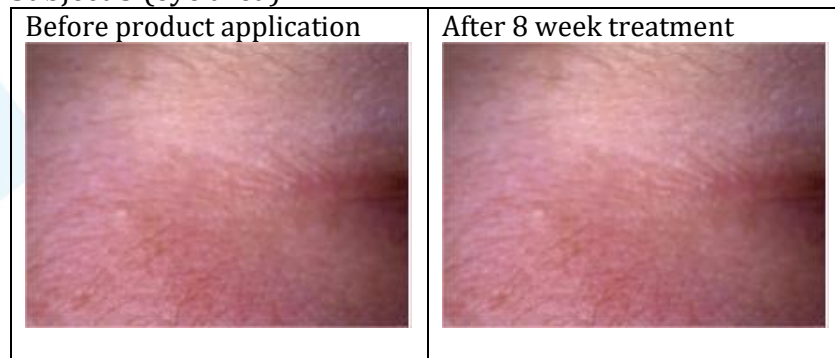
Subject 1 (forehead)



Subject 2 (eye area)



Subject 3 (eye area)



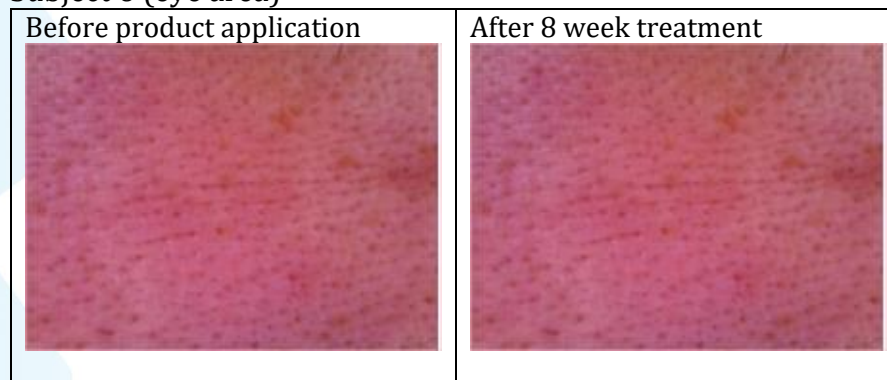
Subject 4 (forehead)



Subject 5 (mouth area)



Subject 6 (eye area)



Subject 7 (forehead)



Subject 8 (forehead)



Subject 9 (mouth area)

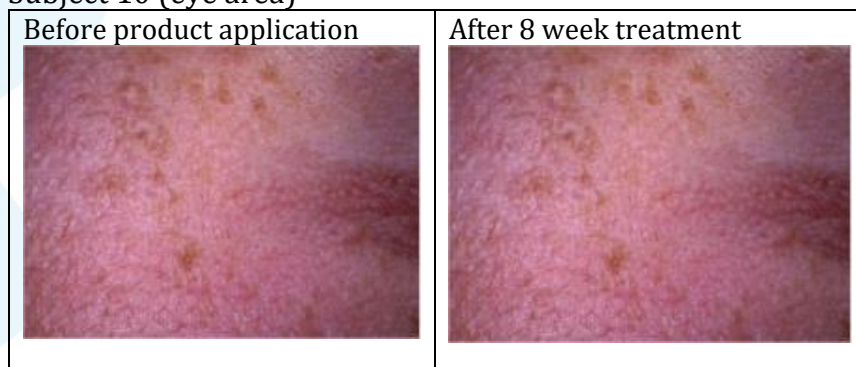
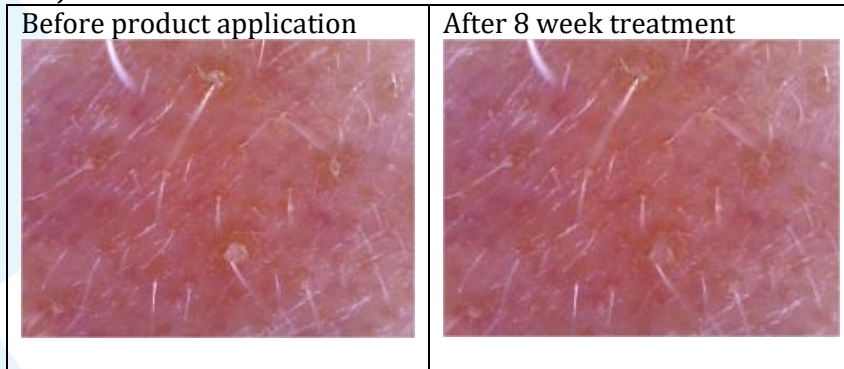
Subject 10 (eye area)

ASSESSMENT OF PRODUCT PROPERTIES

Table 4: Assessment of product anti-wrinkle effect:

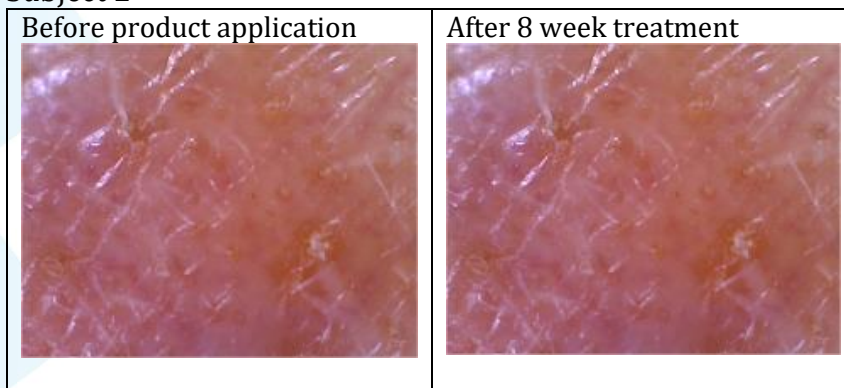
The product corrects skin elasticity	85% positive responses
The product corrects skin firmness	85% positive responses
The product improves appearance of the skin	80% positive responses
The product reduces the appearance of wrinkles	75% positive responses

PRESENTATION OF RESULTS- NUMBER AND SIZE OF PORES

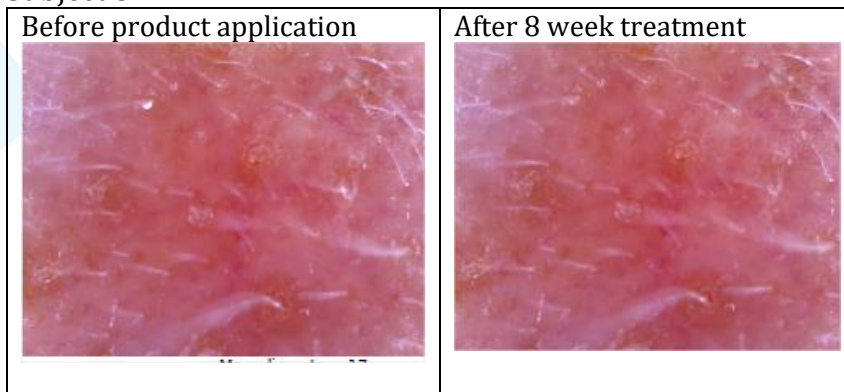
Subject 1



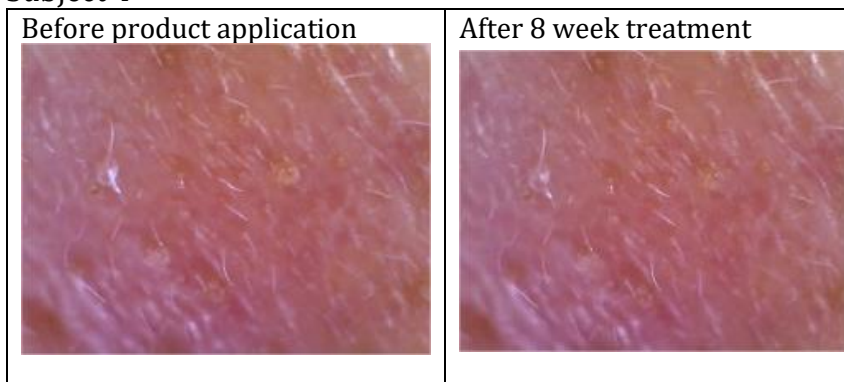
Subject 2

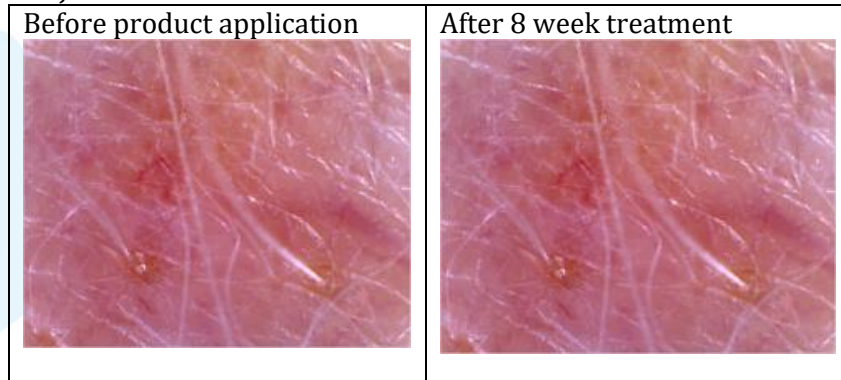
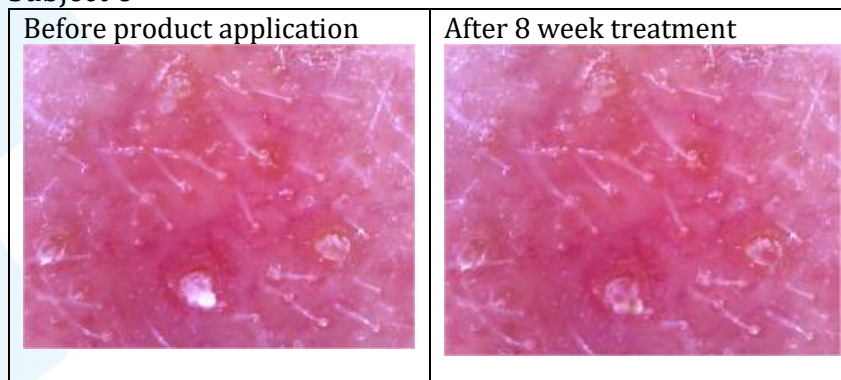
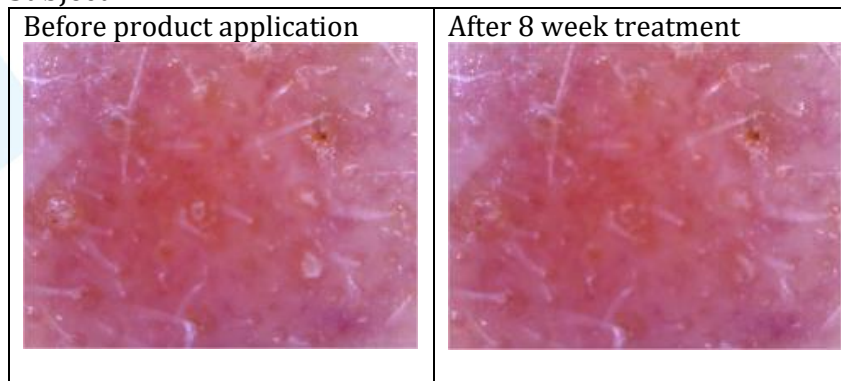
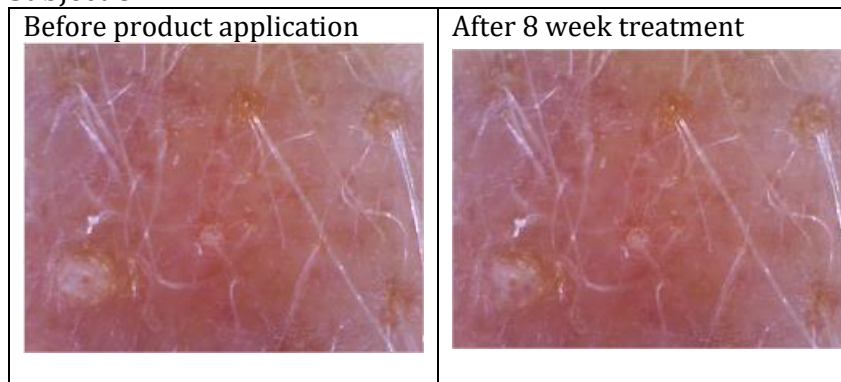


Subject 3

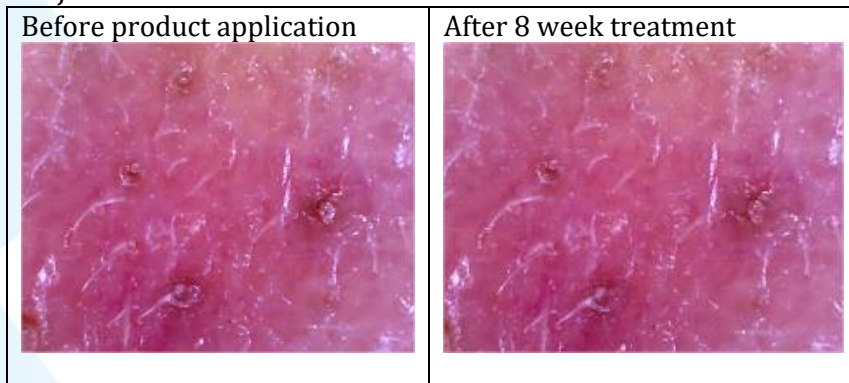


Subject 4

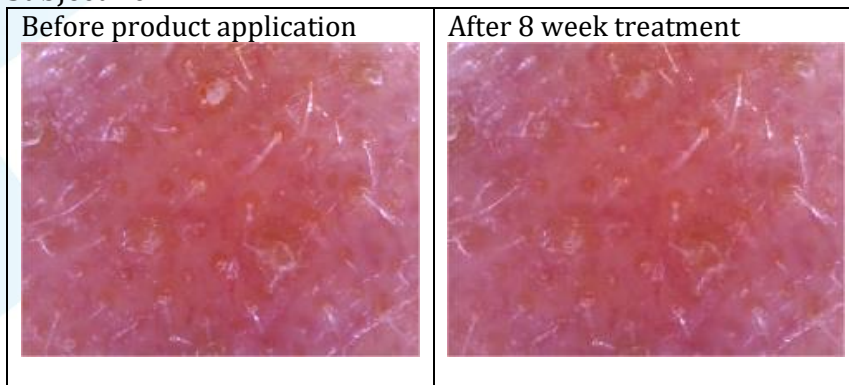


Subject 5**Subject 6****Subject 7****Subject 8**

Subject 9



Subject 10



6. CONCLUSION

SKIN HYDRATION EFFECT

Assumption:

The product hydrates the skin, if the parameter value increases over time.

Conclusion:

The product was found to increase skin hydration level within 1 hour, 3 hours and 5 hours after application.

SKIN ELASTICITY EVALUATION

Assumption:

The product increases elasticity of the skin if the skin, if the parameter value increases over time.

Conclusion:

The product was found to increase skin elasticity level within 8 weeks after application.

SKIN pH EVALUATION

Assumption:

The product strengthens skins barrier function if the pH values are below 5,0. This is in line with existing literature, where a relatively large number of reports (c. 50%) actually describes pH values below 5,0 as optimal; this is in contrast to the general assumption, that skin surface pH is on average between 5,0 and 6,0. Not only prior use of cosmetic products, especially soaps, have profound influence on skin surface pH, but the use of plain tap water, in Europe with a pH value generally around 8,0, will increase skin pH up to 6 h after application before returning to its 'natural' value of on average below 5,0. It is demonstrated that skin with pH values below 5,0 is in a better condition than skin with pH values above 5,0, as shown by measuring the biophysical parameters of barrier function, moisturization and scaling. An acid skin pH (4-4.5) keeps the resident bacterial flora attached to the skin, whereas an alkaline pH (8-9) promotes the dispersal from the skin.

Conclusion:

The product was found to slightly reduce pH value of the skin after 8 weeks of using it once per day.

DEPTH AND AMOUNT OF WRINKLES

Assumption:

The product slightly reduces depth and amount of wrinkles. Both parameters are measured and determined with micro camera and in use test.

Conclusion:

The product was found to slightly reduce the depth and amount of wrinkles after 8 weeks of usage, based on the measurements and consumer evaluation of the product.

NUMBER AND SIZE OF PORES

Assumption:

The product reduces number and size of pores. Both parameters are measured and determined with micro camera.

Conclusion:

The product was found to reduce the number and size of pores after 8 weeks of usage, based on the measurements with micro camera.

7. SUMMARY OF THE REPORT

SKIN HYDRATION EFFECT

Under the study conditions, after single application, can be concluded that the tested product hydrates the skin within 1 hour, 3 hours and 5 hours after product application.

Table 5: Values of skin hydration rate before application and 1, 3 and 5 hours after application in arbitrary units (AU) in comparison to the control zone.

TIME	$\Delta t1$	$\Delta t3$	$\Delta t5$
AVERAGE	14,3	10,1	2,6

SKIN ELASTICITY

Under the study conditions, after continuous daily application of the product, can be concluded that the tested product increases skin elasticity.

Table 6: Values of skin elasticity before application (t0), 4weeks (t4) and 8 weeks (t8) after the product application in arbitrary units in comparison to the control zone.

TIME	$\Delta t4$	$\Delta t8$
AVERAGE	6,5	12,9

pH MEASUREMENT

Under the study conditions, after eight week daily application, can be concluded that the tested product decreased pH value to 4,90

REDUCTION OF AMOUNT AND DEPTH OF WRINKLES

Under the study conditions and continuous daily application of the product, can be concluded that the tested product slightly decreased the amount and depth of wrinkles.

REDUCTION OF NUMBER AND SIZE OF PORES

Under the study conditions and continuous daily application of the product on 10 subjects it can be concluded that the tested product decreases the size and amount of pores.

Product Sk.in Flash 2 confirmed to hydrate, increase skin elasticity, optimize skin pH value and reduce the appearance of wrinkles and size and number of pores.

8. REFERENCES

- Regulation of the European Parliament and of the Council (EC) No. 1223/2009 of 30 November 2009 on cosmetic products.
- Cosmetics Europe – The Personal Care Association (previously COLIPA) Guidelines "Product Test Guidelines for the Assessment of Human Skin Compatibility 1997."
- Cosmetics Europe – The Personal Care Association (previously COLIPA) Guidelines for the Evaluation of the Efficacy of Cosmetic Products 2008.
- SOP 014 at Luamed company
- Joachim W. Fluhr. Practical Aspects of Cosmetic Testing. ISBN 978-3-642-05067-1