

## EVALUATION OF THE PRODUCT EFFICACY ON HYDRATION, SEBUM LEVEL and WRINKLE REDUCTION ON THE BASIS OF INSTRUMENTAL EVALUATION

### 1. GENERAL INFORMATION

<b>STUDY SPONSOR</b>	Skin Ingredients Pty, Cape Town, South Africa
<b>STUDY CODE</b>	C017/007
<b>NAME AND ADDRESS OF THE ORGANIZATION IN CHARGE OF THE ASSESSMENT</b>	Luamed, Tanja Židan s.p. Lukovica, Slovenia
<b>TEST PRODUCT</b>	Sk.in Pure
<b>AUTHORIZED BY</b>	Tanja Židan
<b>ADDITIONAL INVESTIGATIONS INVOLVED</b>	Katja Urek
<b>REPORT DATE</b>	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, sebum and wrinkle 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

<b>INTENDED USE</b>	Face care product
<b>APPEARANCE</b>	Liquid
<b>COLOR</b>	Colorless transparent
<b>FRAGRANCE</b>	Characteristic
<b>INSTRUCTIONS FOR USE</b>	Only to be used every 3rd night for 3 weeks then if skin can tolerate use every 2nd night. all volunteers continue to use their normal basic skincare regime such as cleanser, moisturiser and SPF and to slot the serums in.
<b>PRODUCT CLAIMS AS DECLARED BY CUSTOMER</b>	sk.in pure helps to diminish the appearance of wrinkles while improving moisture levels, leaving your skin with a straight-from-the-spa glow and smooth texture. sk.in pure products can be used on all skin types, except those suffering from dry and/or sensitive, reactive skin.

### 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

<b>STARTING DATE</b>	24.7.2019
<b>FINISHING DATE</b>	18.9.2019

## 4. METHODOLOGY

### PROTOCOL SUMMARY

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

The aim of the test was to determine the direct influence of the tested product on skin hydration, sebum value and wrinkle 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 cm<sup>2</sup>. 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 sebum level was 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 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.

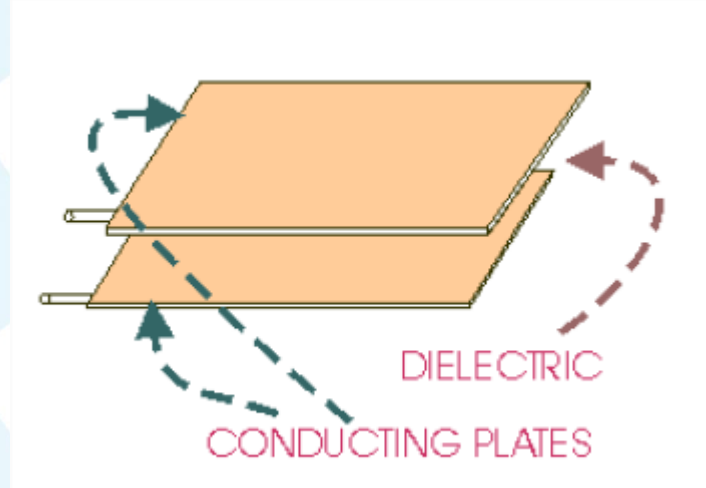
While the amount of water in the inner layers of the skin is relatively constant and is in equilibrium with the other organs of the body (ca. 60–70%), the moisture in the stratum corneum depends on different factors:

- 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.

## SEBUM MEASUREMENT

The skin needs to be soft, smooth and supple, which is achieved via a thin film of hydro lipids on the stratum corneum. This mainly consists of sebum excreted by the sebaceous

glands, which comes up to the skin alongside the hairs and fills the spaces between the cells. The hydrolipidic film also contains moisture components that are excreted with the sweat.

In the Soft Plus, the measurement of sebum is based on the photometric principle.

The sensor is constituted by a light source and four photodetectors that measure light reflected by a mirror behind a mat synthetic tape found on the probe.

When the probe is pressed onto the skin, the tape absorbs the sebum (moisture has different physical properties so it does not interfere with the measure) and becomes more transparent.

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 unable 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.

By pressing the plunger of the probe, the tape runs into the body of the probe where it is lit by the light sources. The light is then reflected by the mirror and measured via the photodiodes. The amount of light transmitted depends on the sebum content on the surface of the measuring area.

## DESCRIPTION OF VOLUNTEERS

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

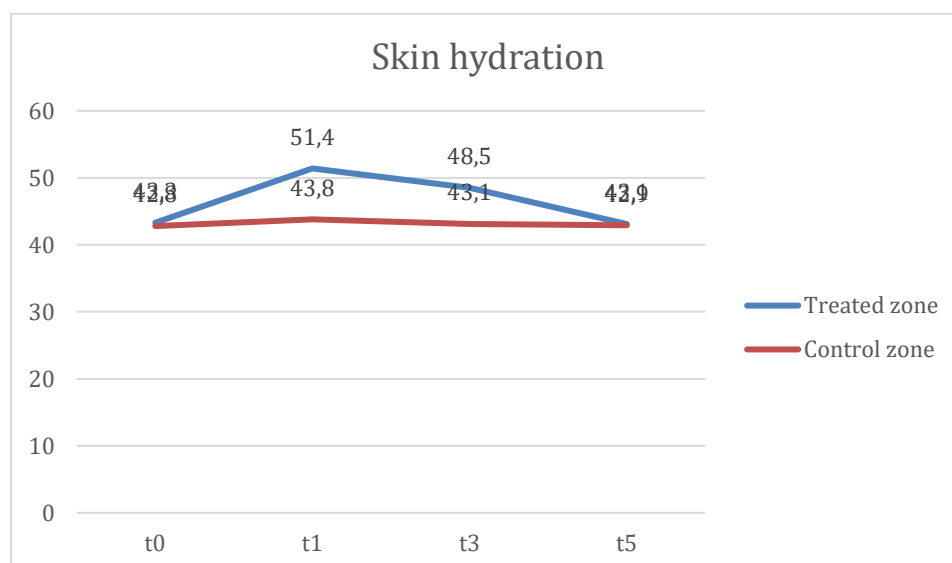
## 5. RESULTS

### 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	63	74	70	62	63	66	64	63
002	37	40	42	37	38	40	35	36
003	31	39	35	31	30	29	33	30
004	26	30	30	26	27	29	27	25
005	28	32	31	28	28	28	25	29
006	19	21	21	19	20	21	18	23
007	50	55	55	50	48	49	49	46
008	74	91	83	74	74	73	74	75
009	56	70	63	55	55	56	56	52
010	49	62	55	49	45	47	50	50
MEAN	43,3	51,4	48,5	43,1	42,8	43,8	43,1	42,9
MIN	19	21	21	19	20	21	18	23
MAX	74	91	83	74	74	73	74	75
MEDIAN	43	47,5	48,5	43	41,5	43,5	42	41
SD	17,9	22,6	19,9	17,7	17,4	17,5	18,3	17,3

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.



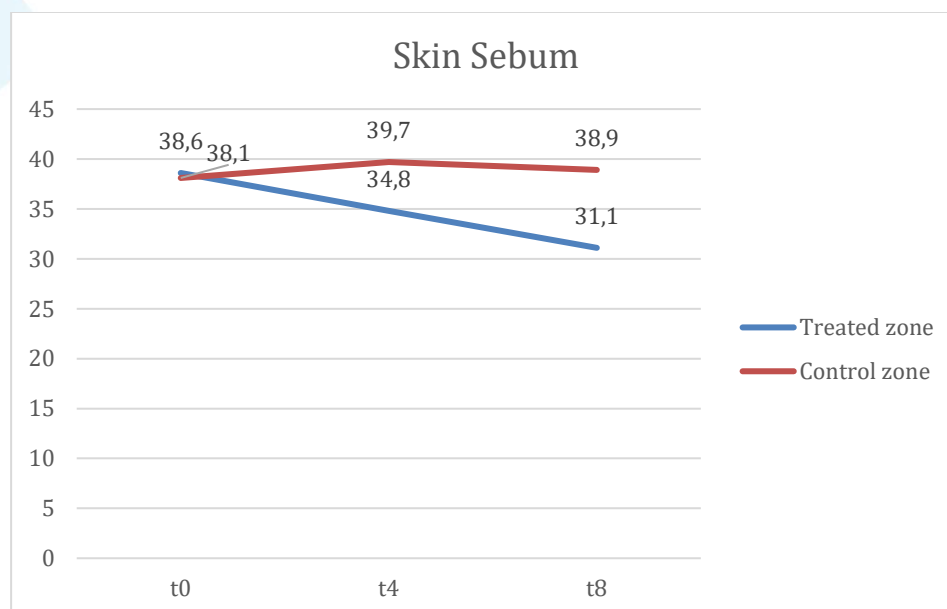


## PRESENTATION OF RESULTS- SEBUM

Table 2: The results of skin sebum level 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	20	18	16	19	21	20
002	40	35	33	38	38	43
003	69	59	57	70	72	68
004	30	25	21	30	31	29
005	30	29	26	28	31	27
006	37	32	27	39	39	41
007	43	39	35	41	46	45
008	29	28	25	32	29	29
009	60	59	49	59	62	60
010	28	24	22	25	28	27
MEAN	38,6	34,8	31,1	38,1	39,7	38,9
MIN	20	18	16	19	21	20
MAX	69	59	57	70	72	68
MEDIAN	33,5	30,5	26,5	35	34,5	35
SD	15,3	14,0	12,9	15,7	16,1	15,6

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



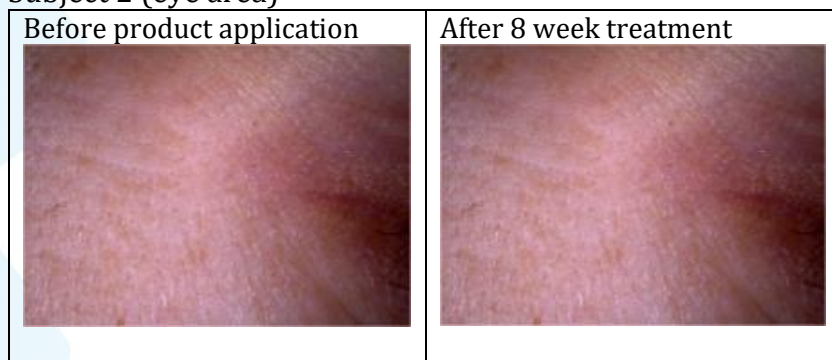


## PRESENTATION OF RESULTS- DEPTH AND DEGREE OF WRINKLES

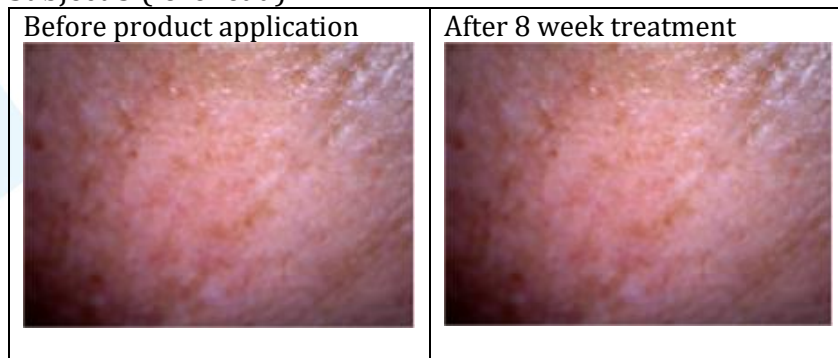
### Subject 1 (eye area)



### Subject 2 (eye area)



### Subject 3 (forehead)



### Subject 4 (eye area)



**Subject 5 (eye area)**



**Subject 6 (forehead)**

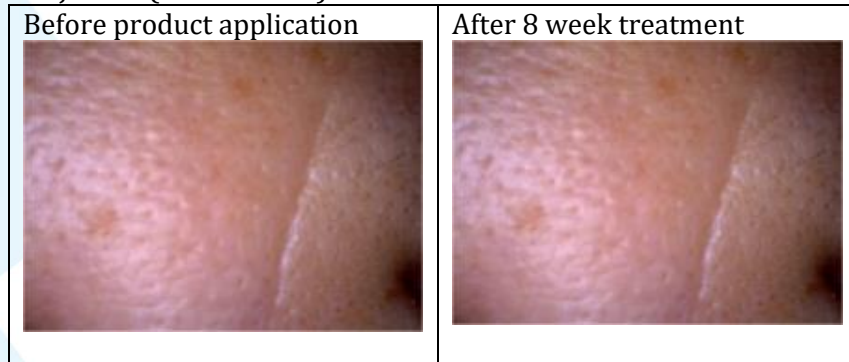


**Subject 7 (mouth area)**



**Subject 8 (forehead)**



**Subject 9 (mouth area)**

**Subject 10 (forehead)**

**ASSESSMENT OF PRODUCT PROPERTIES**

Table 3: assessment of the product anti wrinkle effect

The product corrects skin elasticity	75% positive responses
The product corrects skin firmness	75% positive responses
The product improves appearance of the skin	85% positive responses
The product reduces the appearance of wrinkles	70% positive responses

## 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 and 3 hours after application.**

### SKIN SEBUM EVALUATION

Assumption:

The product reduces sebum level of the skin, if the parameter value decreases over time.

Conclusion:

**The product was found to decrease skin sebum value within 8 weeks after application.**

### DEPTH AND DEGREE 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.**



## 7. SUMMARY OF THE REPORT

### SKIN HYDRATION EFFECT

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

Table 4: 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	7,1	4,9	-0,3

### SKIN SEBUM

Under the study conditions, after continuous daily application of the product, can be concluded that the tested product reduces skin sebum level.

Table 5: Values of skin sebum before application (t0), 4 weeks (t4) and 8 weeks (t8) after the product application in arbitrary units in comparison to the control zone.

TIME	$\Delta t4$	$\Delta t8$
AVERAGE	-5,4	-8,3

### 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.

**Product Sk.in Pure confirmed to hydrate, and decrease the amount of sebum. The reduction of depth and amount of wrinkles has been confirmed based on the in use test.**

## 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