EVALUATION OF THE PRODUCT EFFICACY ON HYDRATION, ELASTICITY and pH LEVEL THE BASIS OF INSTRUMENTAL EVALUATION

1. GENERAL INFORMATION

STUDY SPONSOR	Skin Ingredients Pty, Cape Town, South Africa
STUDY CODE	C017/006
NAME AND ADDRESS OF THE ORGANIZATION IN CHARGE OF THE ASSESSMENT	Luamed, Tanja Židan s.p. Lukovica, Slovenia
TEST PRODUCT	Sk.in Marvel
AUTHORIZED BY	Tanja Židan
ADDITIONAL INVESTIGATIONS INVOLVED	Katja Urek
REPORT DATE	23.11.2019

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 level 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	White
FRAGRANCE	Characteristic
INSTRUCTIONS FOR USE	Can be used once daily for 2 weeks then twice daily. 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 dry, damaged skin, and act as an excellent daily moisturiser. sk.in marvel products also include vital skin barrier repair ingredients that improve the skin barrier structure and function. These hydration and revitalization properties in sk.in marvel can be used by all skin types.

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	27.9.2019
FINISHING DATE	22.11.2019

4. METHODOLOGY

PROTOCOL SUMMARY

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

The aim of the test was to determine the direct influence of the tested product on skin hydration, elasticity and pH value. 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 part of the face. The application zone was an area of approximately 283 cm^2. 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 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.

All measurements were carried out in a room with a temperature of 20 ± 3°C and a relative humidity of 50±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.

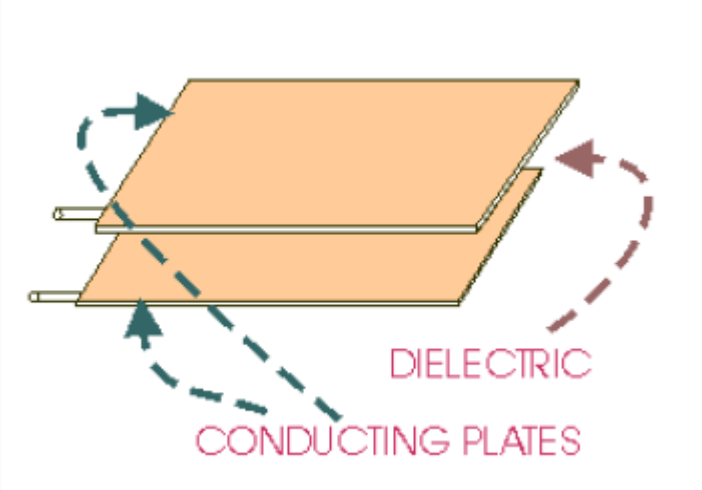
While the amount of water in the inner layers of the skin is relatively constant and is in equilibrium with the other organs of the body (ca. 60–70%), the moisture in the stratum corneum depends on different factors:

- 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.

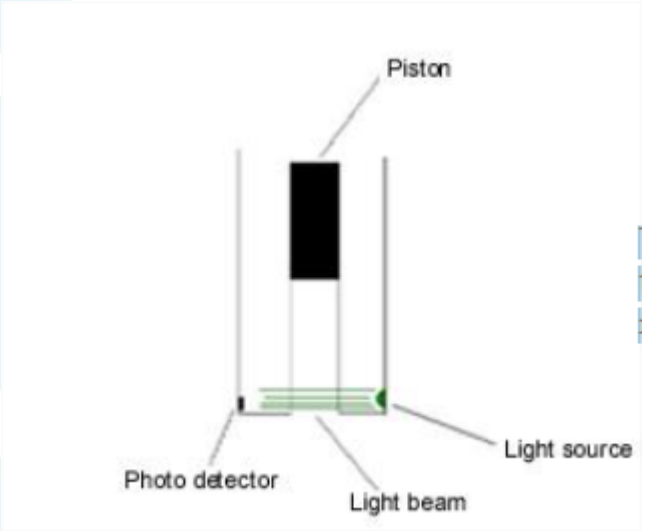
The measurement principle employed in the elasticity probe is based on the suction method.

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).

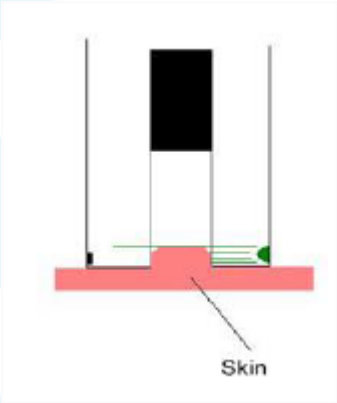
The skin reacts to this stimulus, rising into the chamber of the probe.

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 –log10 H3O+.

Pure water is partially dissociated in accordance with this equilibrium:
2 H2O ↔ H3O+ + OH-
The concentration of ions H3O+ 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 is ≠ 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.	

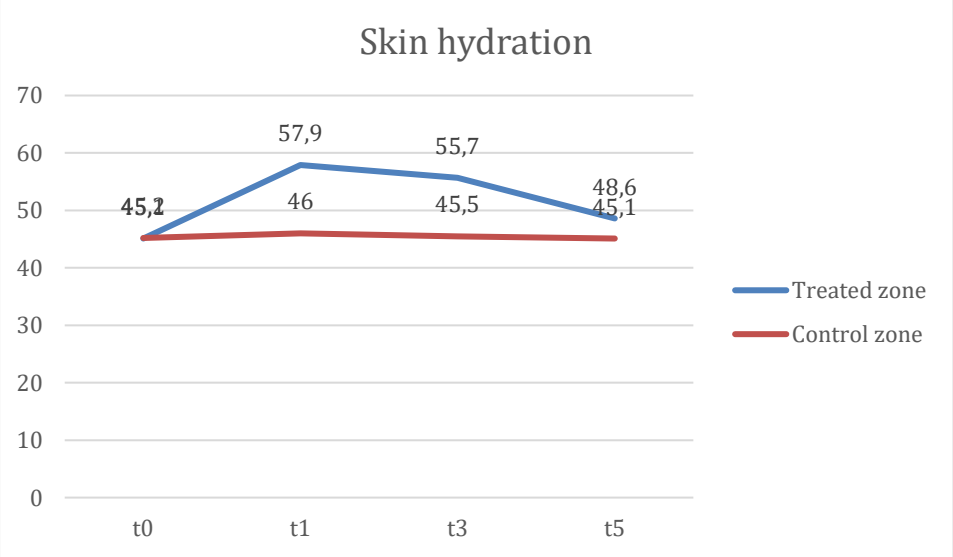
5. RESULTS

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.

	Treated zone				Control zone			
SUBJECT CODE	t0	t1	t3	t5	t0	t1	t3	t5
001	62	82	75	67	63	62	60	63
002	74	91	92	80	75	77	76	73
003	19	25	24	21	17	22	21	20
004	63	79	78	68	64	66	62	60
005	15	20	19	16	10	20	16	17
006	45	57	55	49	46	46	42	42
007	23	31	28	24	24	21	24	26
008	59	76	73	64	62	59	60	57
009	72	94	89	76	72	69	73	74
010	19	24	24	21	19	18	21	19
MEAN	45,1	57,9	55,7	48,6	45,2	46	45,5	45,1
MIN	15	20	19	16	10	18	16	17
MAX	74	94	92	80	75	77	76	74
MEDIAN	52	66,5	64	56,5	54	52,5	51	49,5
SD	23,8	30,1	29,3	25,6	25,2	23,5	23,4	23,0

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.

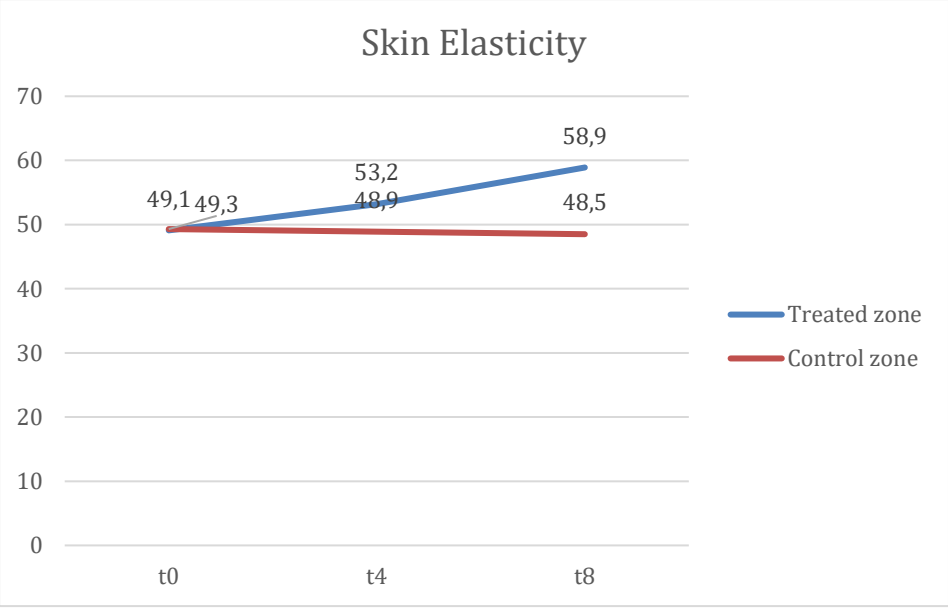


PRESENTATION OF RESULTS- ELASTICITY

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

	Treated zone			Control zone		
SUBJECT CODE	t0	t4	t8	t0	t4	t8
001	53	56	56	52	48	51
002	51	53	59	50	52	51
003	53	54	60	53	52	51
004	74	81	89	71	73	73
005	41	45	48	44	43	44
006	40	47	54	40	40	40
007	24	26	30	27	23	22
008	56	63	69	57	60	55
009	61	67	76	61	60	59
010	38	40	48	38	38	39
MEAN	49,1	53,2	58,9	49,3	48,9	48,5
MIN	24	26	30	27	23	22
MAX	74	81	89	71	73	73
MEDIAN	52	53,5	57,5	51	50	51
SD	13,9	15,2	16,4	12,6	13,9	13,6

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.

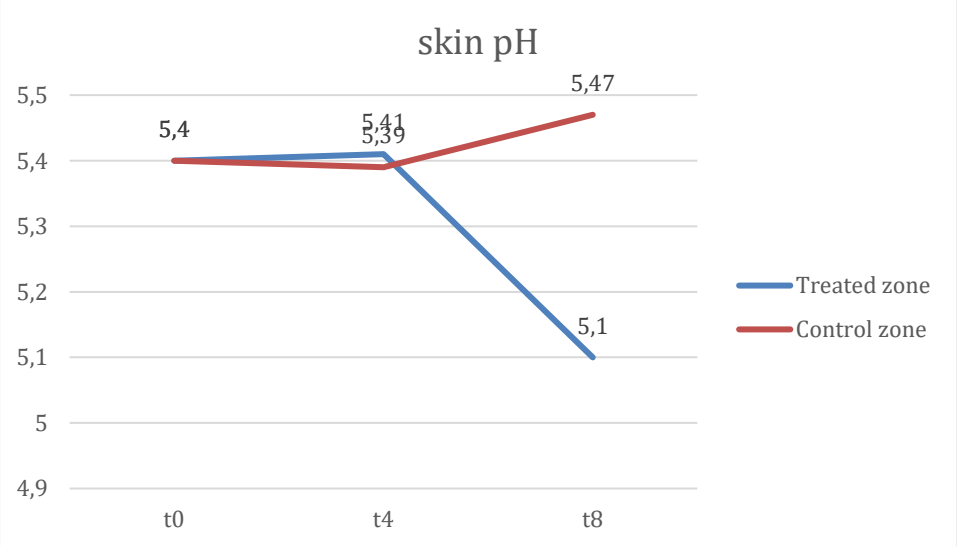


PRESENTATION OF RESULTS- pH

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

	Treated zone			Control zone		
SUBJECT CODE	t0	t4	t8	t0	t4	t8
001	6,0	6,4	5,9	5,9	6,0	6,1
002	5,0	4,6	4,6	5,4	4,8	4,7
003	6,0	5,9	6,1	5,7	5,9	6,1
004	5,0	5,1	4,8	5,2	4,9	5,0
005	5,0	4,9	4,4	4,6	4,8	4,9
006	5,0	5,4	4,0	5,4	5,3	5,3
007	5,0	4,5	4,9	5,0	5,1	4,9
008	5,0	5,4	4,4	4,6	4,9	5,1
009	6,0	5,8	6,3	6,2	6,1	6,2
010	6,0	6,1	5,6	6,0	6,1	6,4
MEAN	5,4	5,41	5,1	5,4	5,39	5,47
MIN	5	4,5	4	4,6	4,8	4,7
MAX	6	6,4	6,3	6,2	6,1	6,4
MEDIAN	5	5,4	4,85	5,4	5,20	5,20
SD	0,5	0,6	0,8	0,6	0,6	0,7

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.



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 reduce pH value of the skin after 8 weeks of using it once per day.

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, 3 hours and 5 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	Δt1	Δt3	Δt5
AVERAGE	12,0	10,3	3,4

SKIN ELASTICITY

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

Table 5: 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	Δt4	Δt8
AVERAGE	4,5	10,6

pH MEASUREMENT

Under the study conditions, after eight week daily application, can be concluded that the tested product decreased pH value to 5,10

Product Sk.in Marvel confirmed to hydrate, increases skin elasticity, and reduce skin pH value.

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."
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- Joachim W. Fluhr. Practical Aspects of Cosmetic Testing. ISBN 978-3-642-05067-1