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Date: 20.12.2016

Short-Term Efficacy Test on Wrinkle Depth Reduction Conducted With "Biotulin – Supreme Skin Gel"

(Cosmetic Study)

Summary

Study Sponsor .....: **MyVitalSkin GmbH & Co KG**
Adalbertsteinweg 259
52066 Aachen
Germany

Date of Order.....: 26.10.2016

Performance of Test .....: Derma Consult Concept GmbH
and Evaluation by Hermann-Wandersleb-Ring 4
53121 Bonn
Germany

Supervisors of Study .....: Dr. med. H. Prieur, Dermatologist – Allergist
B. Nissen, Manager Derma Consult Concept

Study Code .....: DCC16K144GR3

Test Product .....: The test product, which was coded as follows, was provided by MyVitalSkin GmbH & Co KG in October 2016:

B. Biotulin - Supreme Skin Gel (1609047)

Subjects .....: Number of individuals.: 20
Sex.....: female
Age range .....: 35-64 years (average: 48,6)

Test Area .....: Crow's Feet

Application.....: Single, controlled Application

Test Period .....: December 2016

Test Parameter.....: Determination of *wrinkle depth* by means of PRIMOS® 5.7 high-res (GFMeßtechnik GmbH, Teltow, Germany)

Design of Study.....: **Start**

- Determination of wrinkle depth (Crow's Feet)
- Controlled test product application by Derma Consult staff member

1 hour after Application

- Determination of wrinkle depth (Crow's Feet)

Evaluation .....: Descriptive statistics (average, median, minimum, maximum, variance, standard error, standard deviation); Wilcoxon Rank Test

Results.....: **Wrinkle Depth**

The test product was found to statistically significantly decrease wrinkle depth in the short-term as measured 1 hour after application; a mean decrease in wrinkle depth by 7% was observed and a positive effect was detected in 75% of the volunteers.

Methods

Measurement of Wrinkle Depth

PRIMOS (Phase-Shifting rapid in vivo measurement of skin) is a non-contact measurement device that allows for real-time three-dimensional in vivo measurement of the micro topography of human skin based on the technology of active image triangulation. The measurement head consists of a digital micromirror device as projection unit and a CCD-camera as recording unit, mounted onto an adjustable rack. For active image triangulation an intensity encoded point M is projected onto the surface under investigation. Its image on the surface is recorded by the CCD-camera from a specific angle. The point M is a function of parameters like intensity, triangulation angle between projection system and camera and some other inner respectively outer coordinates of the camera and projection plane. The height information of the structured surface is coded in the distorted intensity pattern, which is recorded. The resolution and accuracy depends on the optical and topographical characteristics of the measured surface and on the noise characteristics of the measurement system. For accurate in vivo measurements of human skin, depending on the measured part of the human body (inner forearm, forehead, eye zone), different parameters of effective wavelength and amplification factor should be used.
To regard the differences of human skin and avoid undesired distortions by movements, the fast phase-shift technique with settings to accurately detect deeper structures (phase width: 16, 64 & 128 pixels) was used for the measurement. For each measurement, a minimum of 3 recordings were made and the clearest image without movement distortions or artefacts was selected for further processing. On follow-up measurements, the original captured data was overlayed to help in the relocation process of the test area.
At the end of the study, distortions due to body hairs were digitally removed and the macro structure (calculated by polynomial approximation), i.e. the curvature of the entire test area, subtracted to allow a proper analysis of the microstructure, i.e. wrinkles and surface roughness. Wrinkle depth was then assessed by means of the parameter R_{Max} that is defined as the maximum vertical distance from the highest peak to the lowest valley of five segments of equal length. To mitigate locational effects, the evaluation was conducted using the arithmetic average of R_{max} from 50 parallel cuts. System used in this study: PRIMOS compact high-res S/N 108-00041, Software Version 5.7.

Performance of Test

The subjects were selected from the Derma Consult database. They were informed about importance and meaning of the study. Written informed consent was obtained from all the subjects prior to entry into the trial. The following criteria were used for selection of the subjects:

for inclusion in study:
- female (≥ 35 years of age)
- visible Crow's Feet
- ability to comply with the requirements of the study
- fundamentally clinically healthy

for exclusion from study:
- skin diseases or any other medical condition interfering with the objectives of the study
- planned medical treatment during study period
- pregnancy

The subjects of this study were between 35-64 years of age (average: 48,6). They could withdraw from the study at any time without giving any reason.
The subjects were instructed not to use any topical preparations on the test areas starting from seven days prior to testing (preconditioning phase) and until the end of the test with exception of the following decorative cosmetics: lipstick/lipliner, mascara/eyeliner and eyeshadow. For cleansing, water or a mild syndet (Eubos® flüssig – blau; manufacturer: Dr. Hobein, D-53340 Meckenheim-Merl, Germany) was allowed only (whole study inclusive the preconditioning phase).

For the day of testing, the subjects were instructed to cleanse their face at home 4-8 hours before the visit using the mild syndet and not to apply any of the otherwise allowed decorative cosmetics. Prior to the controlled application of the test product by a staff member of Derma Consult (1 pump from the supplied dispensers for the entire face, gently massaging the product into the skin), measurements were taken at a clearly defined site in the Crow's Feet region (randomized side selection). The subjects were instructed to pay special attention not to mechanically remove the product by accident (e.g. by cloth) and to avoid water contact and bodily exertion until the end of the test. Further measuring was performed 1 hour following the product application.
The initial measurement was conducted after adaptation to the controlled environmental conditions of the test institute (room temperature: 21±1°C, relative humidity: 45±5%) and the subjects remained in these controlled conditions until the second measurement was conducted.

Biometry

Measurement data is automatically computerised and after validity check and quality assurance stored centrally in a database. Evaluation is conducted using the software NAG® Statistical Add-Ins for Excel – NAG Ltd., United Kingdom. The data were analyzed by Wilcoxon Rank test. The 0.05 level was selected as the point of minimal acceptance of statistical significance.

Results

Wrinkle Depth

Evaluated is the parameter R_{Max} in comparison to the initial condition. The absolute change is shown below in figure one. A decrease in R_{Max} corresponds to a decrease in wrinkle depth.

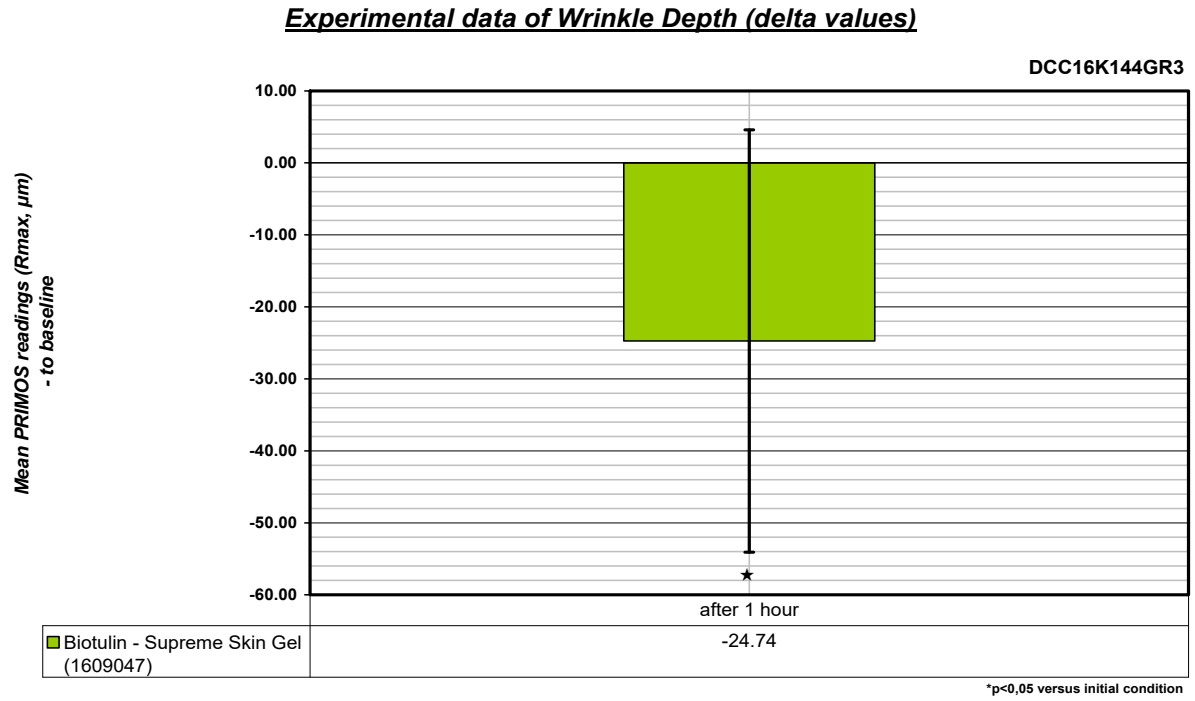


Fig. 1: ΔR_{Max} Values

1 hour after the controlled, single application of the test product, a small, but statistically significant ($p<0.05$), decrease in R_{Max} was observed in the product treated condition as compared to the initial condition.

The test product was found to statistically significantly decrease wrinkle depth in the short-term as measured 1 hour after application; a positive effect of the test product was detected in 75% of the volunteers. The respective percentage change as compared to the respective initial condition is shown in figure two below.

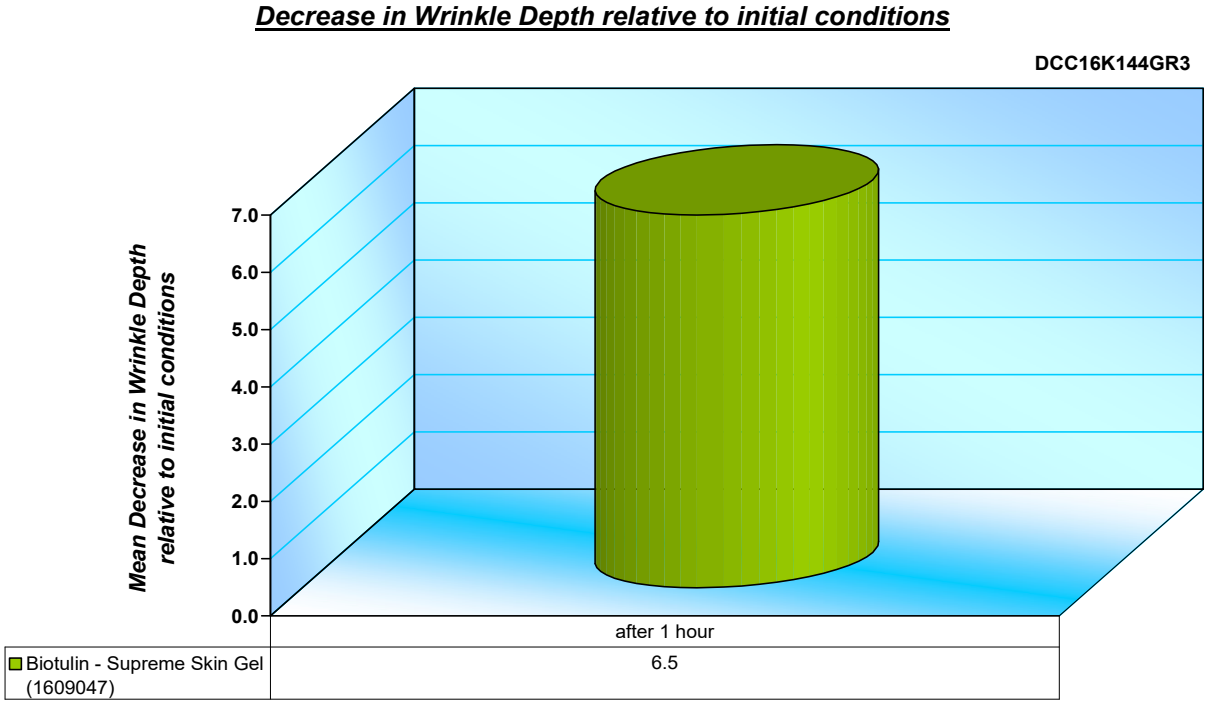


Fig. 2: Decrease in Wrinkle Depth in %

Incompatibility

No incompatibility was observed in or reported by any of the volunteers.

Signature:

B. Nissen
Manager Derma Consult Concept

Signature:

Dr. med. H. Prieur
Dermatologist - Allergist

Enclosures: Measuring values, statistics, summary statistics, graphic representations

Experimental data of Wrinkle Depth, DCC16K144GR3

PRIMOS readings (Rmax, µm)

	start	after 1 hour
	B	B
1	851.72	807.92
2	326.00	300.96
3	374.58	326.68
4	689.66	704.26
5	329.84	333.50
6	517.96	499.68
7	375.02	286.04
8	324.66	294.44
9	424.40	366.72
10	572.26	547.74
11	568.64	559.60
12	529.04	532.52
13	372.74	344.76
14	517.20	476.10
15	293.44	220.00
16	325.52	334.50
17	307.12	262.66
18	336.62	331.54
19	619.50	638.20
20	394.62	387.84
Average	452.53	427.78
S.D.	150.72	160.45
Median	384.82	355.74

Experimental data of Wrinkle Depth, DCC16K144GR3

delta PRIMOS readings (Rmax, µm)

after 1 hour

t1-t0

	B
1	**-43.80**
2	**-25.04**
3	**-47.90**
4	**14.60**
5	**3.66**
6	**-18.28**
7	**-88.98**
8	**-30.22**
9	**-57.68**
10	**-24.52**
11	**-9.04**
12	**3.48**
13	**-27.98**
14	**-41.10**
15	**-73.44**
16	**8.98**
17	**-44.46**
18	**-5.08**
19	**18.70**
20	**-6.78**
Average	**-24.74**
S.D.	**29.34**
Median	**-24.78**

Decrease in Wrinkle Depth relative to initial conditions, DCC16K144GR3

corrected PRIMOS readings (Rmax, µm) [%]

after 1 hour

	B
1	**-5.1**
2	**-7.7**
3	**-12.8**
4	**2.1**
5	**1.1**
6	**-3.5**
7	**-23.7**
8	**-9.3**
9	**-13.6**
10	**-4.3**
11	**-1.6**
12	**0.7**
13	**-7.5**
14	**-7.9**
15	**-25.0**
16	**2.8**
17	**-14.5**
18	**-1.5**
19	**3.0**
20	**-1.7**
Average	**-6.5**
S.D.	**8.1**
Median	**-4.7**
Impr.*	**75**

* % of subjects with relative improvement in test area as compared to initial condition

Descriptive Statistics of Wrinkle Depth, DCC16K144GR3

start

	B
Valid cases	20.0
Mean	452.5
Std. error of mean	33.7
Variance	22717.1
Std. Deviation	150.7
Variation Coefficient	0.3
Minimum	293.4
Maximum	851.7
Median	384.8

after 1 hour

	B
Valid cases	20.0
Mean	427.8
Std. error of mean	35.9
Variance	25744.5
Std. Deviation	160.5
Variation Coefficient	0.4
Minimum	220.0
Maximum	807.9
Median	355.7

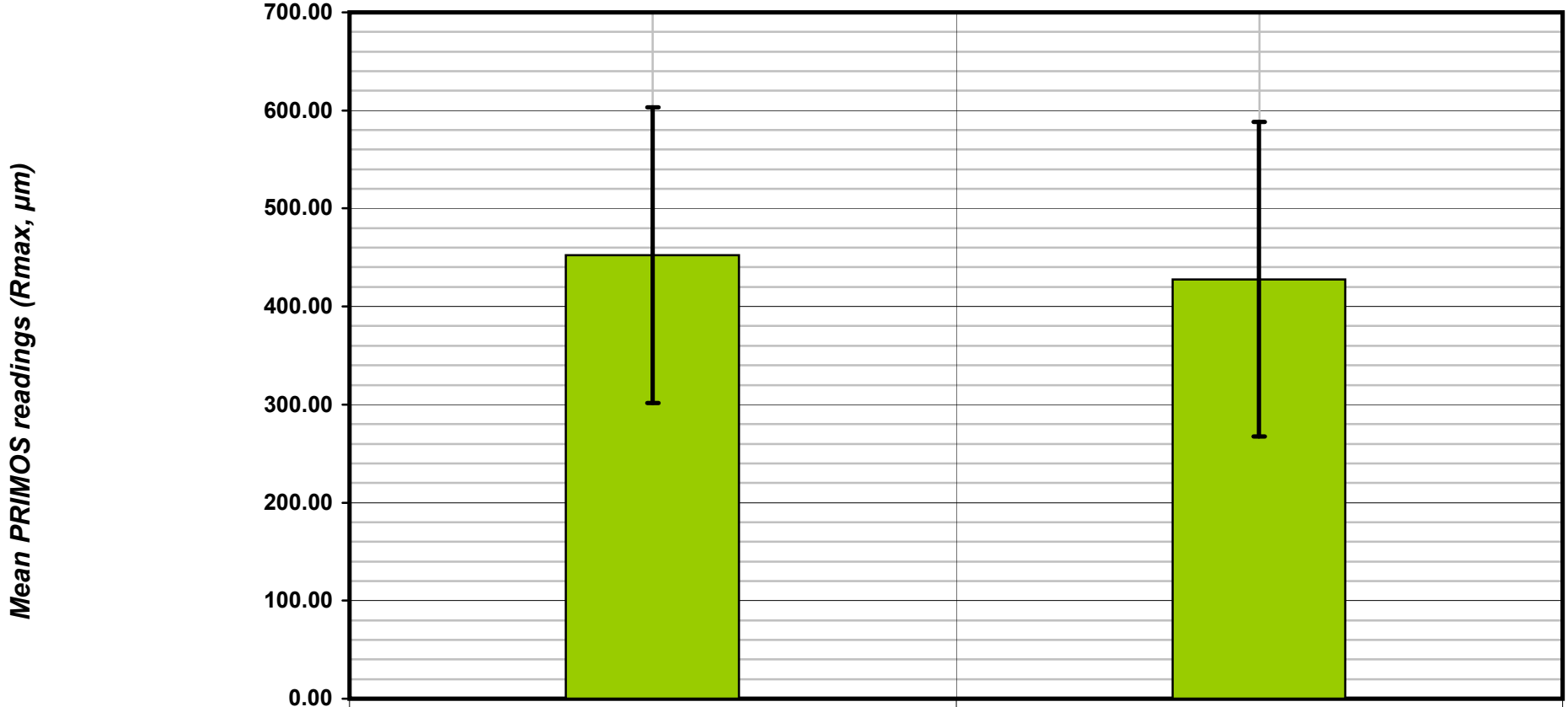
Wilcoxon Rank Test of Wrinkle Depth, DCC16K144GR3

start - after 1 hour

	B
Rank sum (positive)	186
Z-value	3.0053
Significance	0.0014
non-zero observations	20

Experimental data of Wrinkle Depth

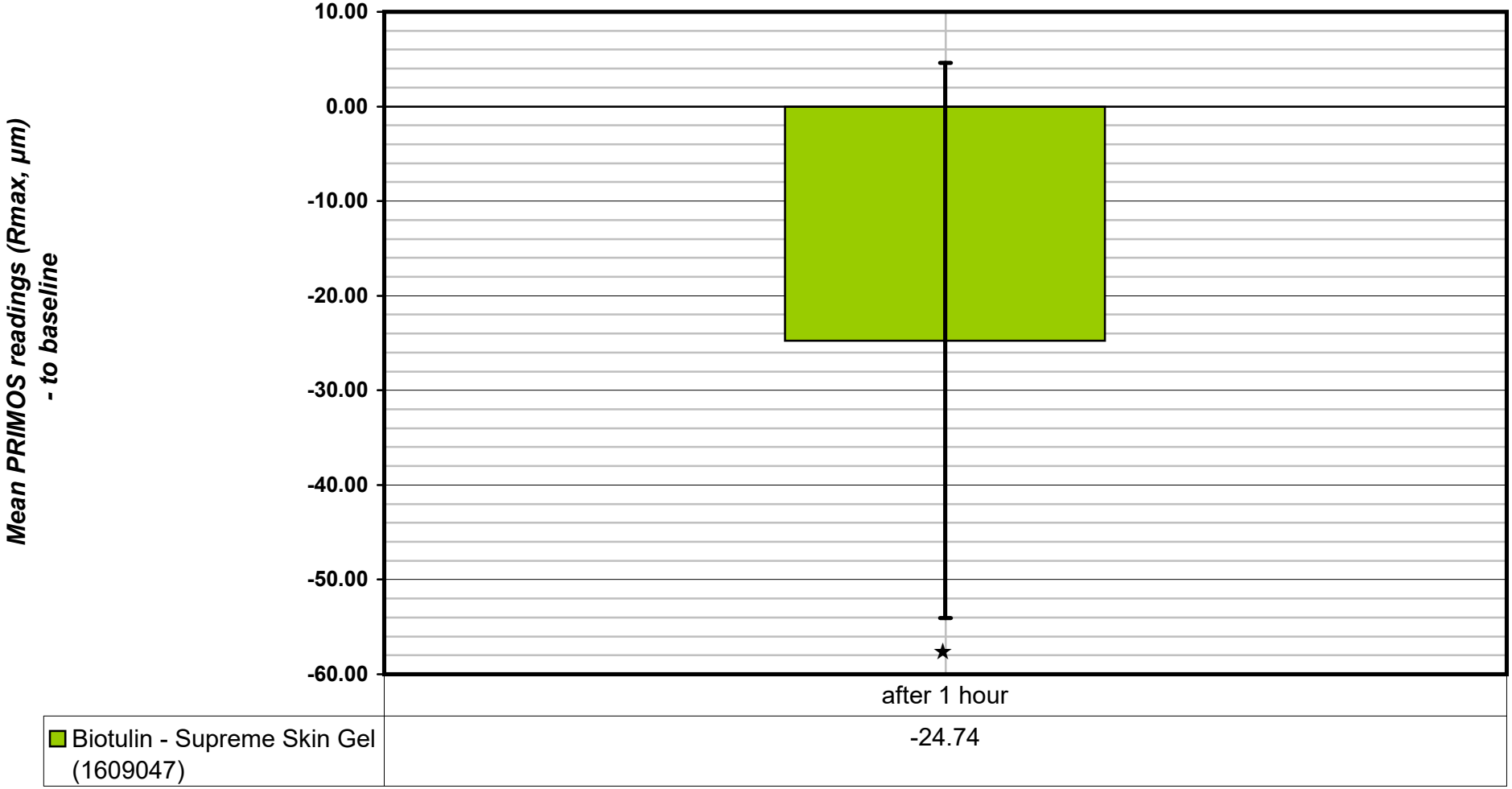
DCC16K144GR3



	start	after 1 hour
Biotulin - Supreme Skin Gel (1609047)	452.53	427.78

Experimental data of Wrinkle Depth (delta values)

DCC16K144GR3



***p<0,05 versus initial condition**

Decrease in Wrinkle Depth relative to initial conditions

DCC16K144GR3