The influence of the test product on the key organisms of the respective body region was examined.

Information about the tested product:

Manufacturer:

Börlind GmbH

Lindenstraße 15

75365 Calw

Germany

Name of the product:

ANNEMARIE BÖRLIND | ZZ SENSITIVE | Regenerative Eye Cream

Product class:

(X) Face / Eyes
MyMicrobiome Standard 18.10

( ) Lips
MyMicrobiome Standard 18.10

( ) Body / Neck / Chest / Hands
MyMicrobiome Standard 18.10

( ) Back
MyMicrobiome Standard 18.10

( ) Bottom / Tighs
MyMicrobiome Standard 18.10

( ) Auxillary vault
MyMicrobiome Standard 18.10

( ) Scalp
MyMicrobiome Standard 19.10

( ) Infant skin
MyMicrobiome Standard 20.10

( ) Vaginal tract
MyMicrobiome Standard 21.10

( ) Feet
MyMicrobiome Standard 22.10

( ) Mouth
MyMicrobiome Standard 23.10

Sample receipt:	14 November 2019	Test result:	1,9
Test date/period:	27 November 2019 - 16 December 2019	Approved yes/no:	yes; 07 February 2020

Test description

The MyMicrobiome Standard evaluates cosmetic and personal care products, that come into contact with the skin or mucous membrane, in terms of their influence on the microbiome located at a specific body site.

An intact skin microbiome has a fundamental influence on skin health. Products which are to be skin-friendly must also be Microbiome-friendly in order not to unbalance the skin of the user.

The MyMicrobiome Standard evaluates the influence of cosmetic and personal care products on the microbial key players of a specific skin or mucous membrane area. The human microbiome is very individual from person to person.

Each area, however, harbors a characteristic composition of bacteria, viruses and fungi. The test examines the products influence on the key organisms typical for each skin area and thus offers a standardized procedure.

Various aspects are examined:

- **The microbial quality of the product.**

- **The influence of the product on the natural, healthy skin balance.**

    The skin-commensal bacterium *Staphylococcus epidermidi*s keeps the skin with antimicrobial peptides (so-called bacteriocins) and pH adjustments healthy and keeps skin-harmful germs such as *Staphylococcus aureus* in check. The product should not disturb this balance between skin-friendly and skin-harmful bacteria. This sensitive balance is investigated in conjunction with the product.

- **The influence of the product on the bacterial diversity of the specific body region.**

    Each body region is colonized by a certain microbial composition. For a healthy skin it is particularly important to maintain this biodiversity. The influence of the product on the respective microbial mixture is examined in the test. The aim is to find as many key organisms as possible after contact with the product.

- **The influence of the product on the growth behavior of the microbes of the specific body region.**

    In addition to the diversity of the specific microbiome, the growth or number of different key organisms should not be influenced by the product. This is investigated in a skin-product contact model. The key organisms are brought into **direct** and **indirect** contact with the product and their growth is observed.

Results

• **The microbial quality of the product.**

The prerequisite for the test for microbial friendliness is the microbiological quality of the product. The following table contains the limit values that must be observed.

Types of organisms	Limit values	
	Products specially designed for children under 3 years, eye area or mucous-skins	Other products
Total counts mesophilic, aerobic microorganisms (bacteria, yeasts, molds, (TAMC and TYMC))	≤ 1 x 10^2 cfu/g or ml[a]	≤ 1 x 10^3 cfu/g or ml[b]
Escherichia coli	Not detectable in 1g or 1 ml	Not detectable in 1g or 1 ml
Pseudomonas aeruginosa	Not detectable in 1g or 1 ml	Not detectable in 1g or 1 ml
Staphylococcus aureus	Not detectable in 1g or 1 ml	Not detectable in 1g or 1 ml
Candida albicans	Not detectable in 1g or 1 ml	Not detectable in 1g or 1 ml

a >200 cfu/g or ml, b >2000 cfu/g or ml

Results Microbiological quality:

Determination of TAMC, TYMC, absence of *E. coli, P. aeruginosa* and *S. aureus.*

Parameter	Sample no.: 566.800
TAMC [cfu/0,1 ml]	< 1,0E+01
TYMC [cfu/0,1 ml]	< 1,0E+01
Escherichia coli [in 0,1 ml]	negative
Pseudomonas aeruginosa [in 0,1 ml]	negative
Staphylococcus aureus [in 0,1 ml]	negative

The microbiological quality of the product according to DIN EN ISO 17516 is fulfilled.

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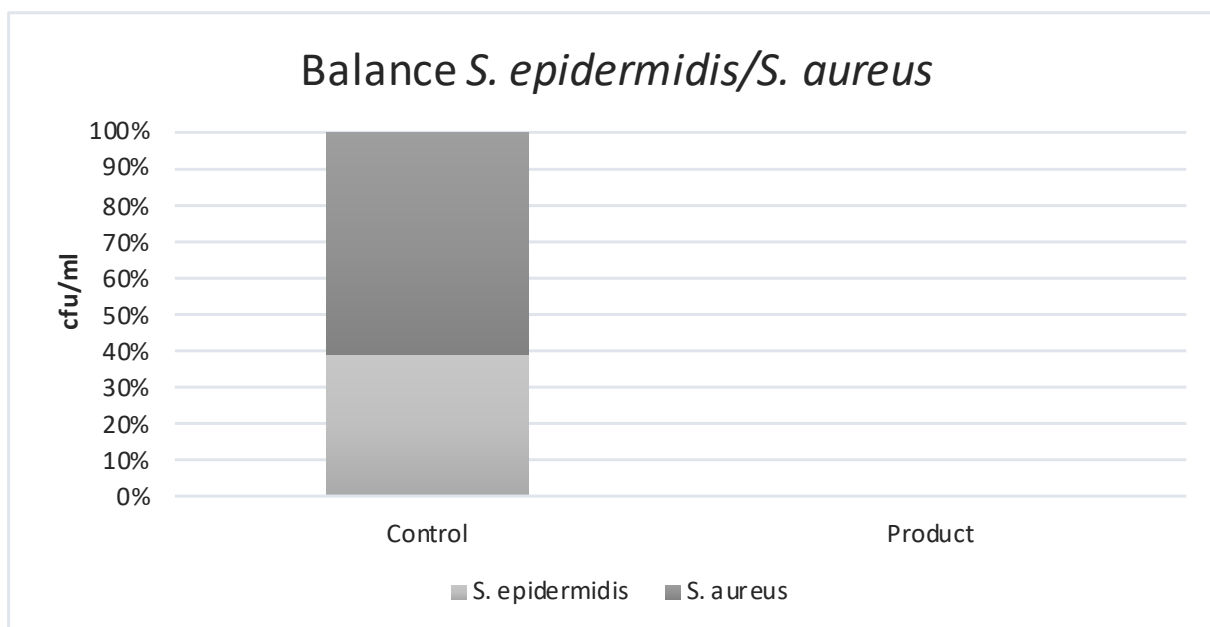
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Microbiome *friendly*

Results

- **The influence of the product on the natural, healthy skin balance.**

A co-culture of *S. epidermidis* and S*. aureus* is incubated with the product for 4 h.
The ratio of the two microbes to each other is determined.

Determination of the bacterial count at time t = 4 h

Balance *S. epidermidis/S. aureus*

	cfu/ml	
	S. epidermidis	*S. aureus*
Control	2,8E+02	4,4E+02
Product	< 10	< 10

Results

• **The influence of the product on the microbial diversity of the specific body region.**

A co-culture of key organisms of the specific body region is incubated with the product for 4 h. The ratio of the bacteria compared to the control (PBS) is determined.


Diversity in the presence of the product
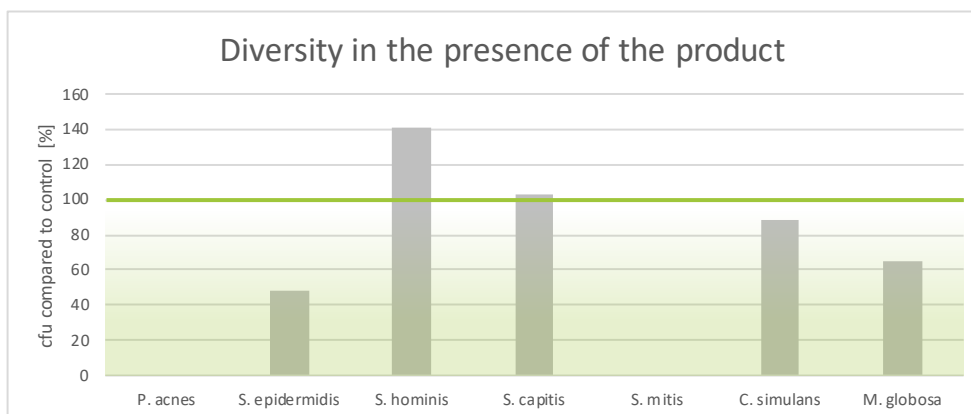
Key-Microbe	Time t=4 h	Rating
P. acnes	cfu/ml	
Control	8,0E+02	3
Product	1,0E+01	
S. epidermidis		
Control	4,5E+02	3
Product	2,2E+02	
S. hominis		
Control	2,0E+02	2
Product	2,8E+02	
S. capitis		
Control	2,6E+02	1
Product	2,7E+02	
S. mitis		
Control	2,5E+02	3
Product	< 10	
C. simulans		
Control	2,5E+02	1
Product	2,2E+02	
M. globosa		
Control	1,5E+02	2
Product	1,0E+02	
Overall rating:		2,1

Microbiome *friendly*

Results

- **The influence of the product on the growth behavior of the microbes of the specific body region – directly.**

The influence of the product on the growth of each individual microbe of the key organisms of the specific body region is investigated and put in relation to the control (PBS). Product contact with the microorganisms is directly.
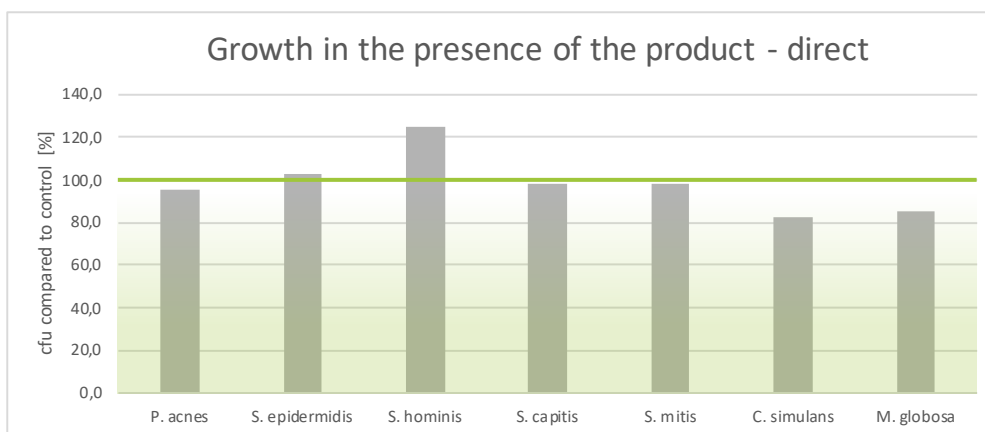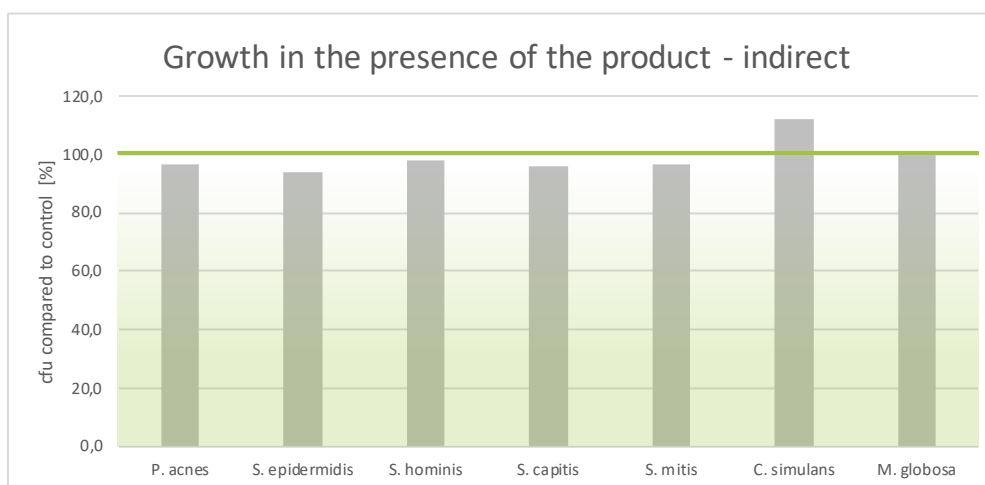

Key-Microbe	cfu /Plate		Rating
P. acnes	Control	352	
	Product	336	1
S. epidermidis	Control	92	
	Product	95	1
S. hominis	Control	102	
	Product	127	2
S. capitis	Control	93	
	Product	91	1
S. mitis	Control	57	
	Product	56	1
C. simulans	Control	202	
	Product	167	2
M. globosa	Control	53	
	Product	45	2
Overall rating:			1,4

Results

- **The influence of the product on the growth behavior of the microbes of the specific body region** – **indirectly.**

The influence of the product on the growth of each individual microbe of the key organisms of the specific body region is investigated and put in relation to the control (PBS). The product contact to the microorganisms is indirect.

Key-Microbe	cfu /Plate		Rating
P. acnes	Control	408	
	Product	394	1
S. epidermidis	Control	135	
	Product	127	2
S. hominis	Control	105	
	Product	103	1
S. capitis	Control	75	
	Product	72	1
S. mitis	Control	64	
	Product	62	1
C. simulans	Control	194	
	Product	218	1
M. globosa	Control	54	
	Product	54	1
Overall rating:			1,1

Microbiome
friendly

Results

The results are evaluated with grades from 1 (one) to 3 (three). If the product shows no or positive influence to the above-mentioned aspects, a grade of 1 is awarded respectively.

If only a very weak negative influence can be detected in the tests, the grade 2 is awarded and in case of a clearly negative influence, the product receives the grade 3.

The product has passed up to grade 2.0.

Here the grade means

1 = Microbiome-friendly   2 = Microbiome-neutral   3 = Microbiome-damaging.

Test	Grade
Balance of the skin microbiome	3,0
Diversity of the corresponding skin microbiome (x2)	2,1
Skin-product contact direct (x2)	1,4
Skin-product contact indirect	1,1
Overall grade	**1,9**

With an overall grade of 1,9 the seal „Microbiome-friendly" is awarded according to MyMicrobiome Standard 18.10.

Place, Date: Balzers, 07 February 2020

Responsible person: Dr. Kristin Neumann

Signature: