

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

### Information about the tested product:

#### Manufacturer:

ICHIMARU PHARCOS Co., Ltd,  
318-1, Asagi, Motosu-shi  
Gifu 501-0475  
Japan

#### Name of the product:

Wamino-BONBON (1% v/w)



#### Product class:

Dry skin

MyMicrobiome Standard 18.10

Sebaceous skin

MyMicrobiome Standard 18.10

Moist skin

MyMicrobiome Standard 18.10

Feet

MyMicrobiome Standard 22.10

Vaginal tract

MyMicrobiome Standard 21.10

Mouth

MyMicrobiome Standard 23.10

Scalp

MyMicrobiome Standard 19.10

Infant skin

MyMicrobiome Standard 20.10

Sample receipt: 01 October 2020

Test date/period: 18 March - 22 March 2021

Test result: 1,5

Approved yes/no: yes; 07 May 2021

### 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 epidermidis* 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))	$\leq 1 \times 10^2$ cfu/g or ml <sup>a</sup>	$\leq 1 \times 10^3$ cfu/g or ml <sup>b</sup>
<i>Escherichia coli</i>	Not detectable in 1g or 1 ml	Not detectable in 1g or 1 ml
<i>Pseudomonas aeruginosa</i>	Not detectable in 1g or 1 ml	Not detectable in 1g or 1 ml
<i>Staphylococcus aureus</i>	Not detectable in 1g or 1 ml	Not detectable in 1g or 1 ml
<i>Candida albicans</i>	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.: 210.322.59
TAMC [cfu/0,1 ml]	< 1,0E+01
TYMC (incl. <i>Candida albicans</i> ) [in 0,1 ml]	negative
<i>Escherichia coli</i> [in 0,1 ml]	negative
<i>Pseudomonas aeruginosa</i> [in 0,1 ml]	negative
<i>Staphylococcus aureus</i> [in 0,1 ml]	negative

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

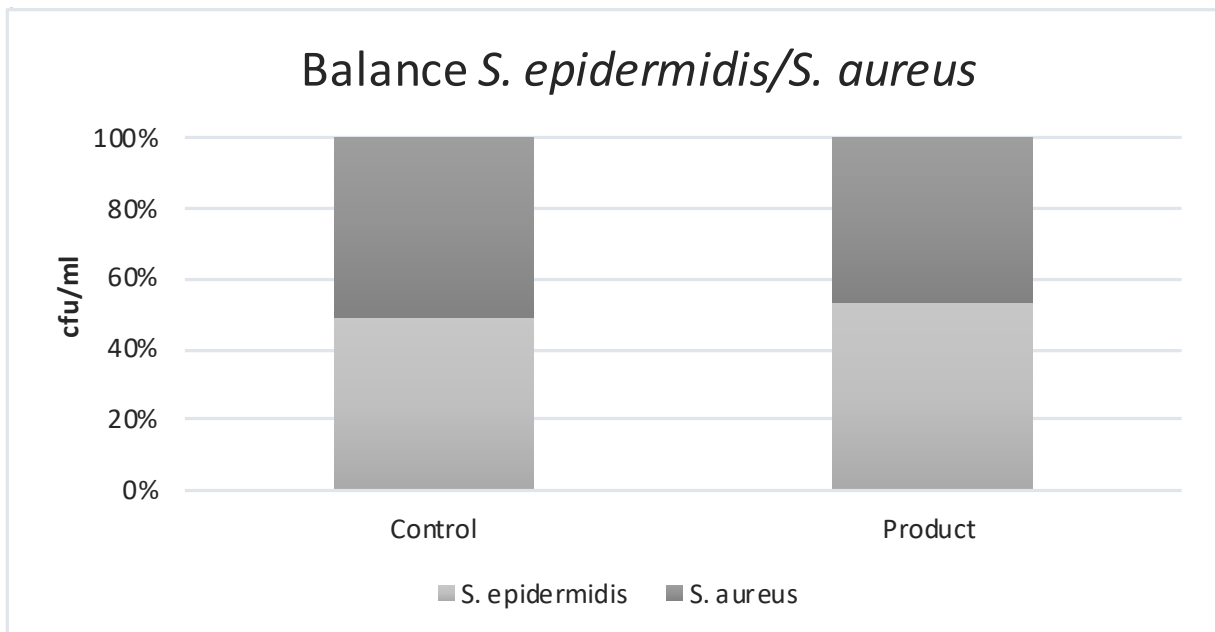
**Results**

- **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

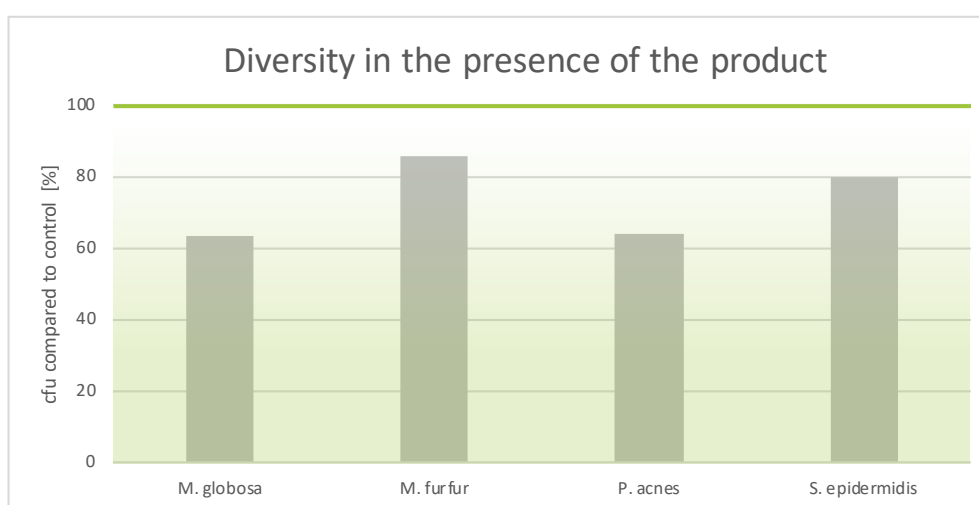


	cfu/ml		Ratio Product/ Control	Grade
	<i>S. epidermidis</i>	<i>S. aureus</i>		
<b>Control</b>	3,3E+02	3,4E+02	1,2	1
<b>Product</b>	3,4E+02	3,0E+02		

### 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.

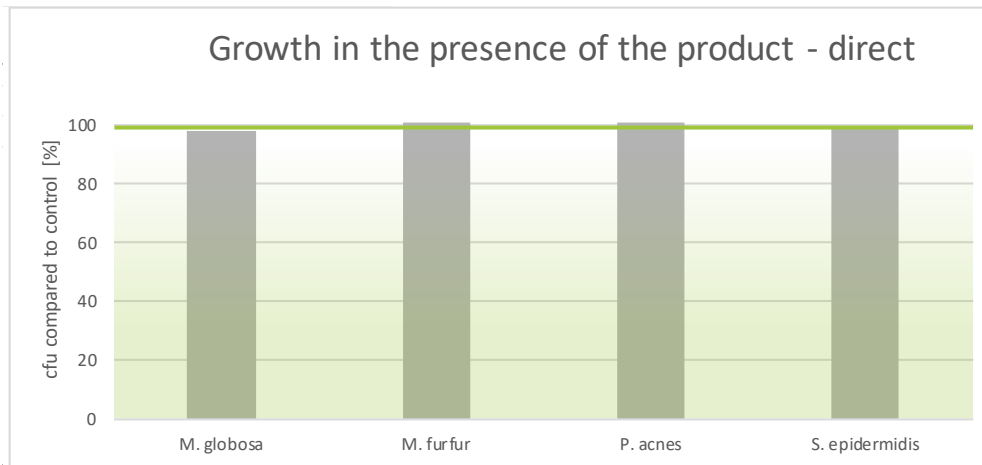


Key-Microbe	Time t=4 h	Rating
<b>M. globosa</b>	cfu/ml	
Control	3,2E+03	3
Product	2,0E+03	
<b>M. furfur</b>		
Control	1,7E+03	2
Product	1,4E+03	
<b>P. acnes</b>		
Control	3,2E+03	3
Product	2,0E+03	
<b>S. epidermidis</b>		
Control	2,1E+03	2
Product	1,7E+03	
<b>Overall rating:</b>		2,5

### 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.

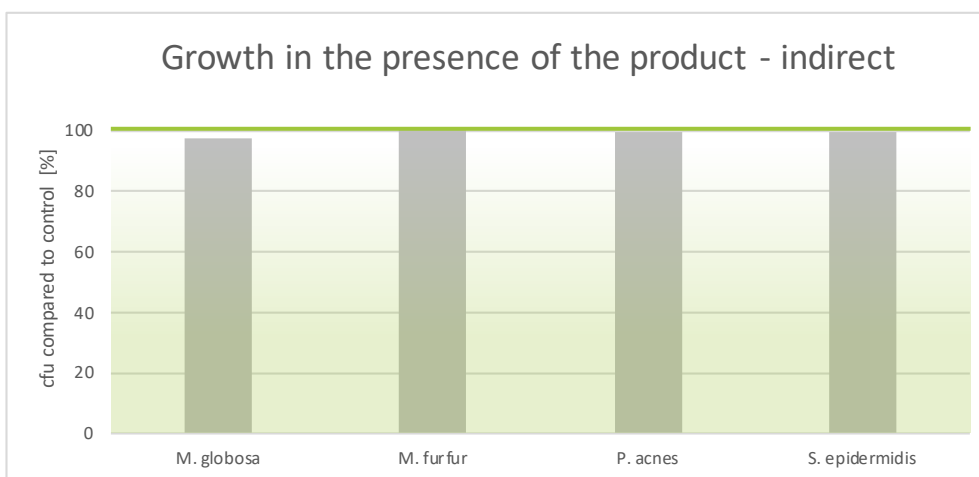


Key-Microbe	cfu /Plate		Rating
	Control	Product	
<i>M. globosa</i>	Control	728	1
	Product	716	
<i>M. furfur</i>	Control	1084	1
	Product	1092	
<i>P. acnes</i>	Control	1844	1
	Product	1856	
<i>S. epidermidis</i>	Control	916	1
	Product	904	
Overall rating:			1,0

### 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
	Control	756	
<i>M. globosa</i>	Product	736	
<i>M. furfur</i>	Control	1076	1
	Product	1080	
<i>P. acnes</i>	Control	1924	1
	Product	1920	
<i>S. epidermidis</i>	Control	876	1
	Product	872	
Overall rating:			1,0

### 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	1,0
Diversity of the corresponding skin microbiome (x2)	2,5
Skin-product contact direct (x2)	1,0
Skin-product contact indirect	1,0
<b>Overall grade</b>	<b>1,5</b>

**With an overall grade of 1,5 the seal „Microbiome-friendly“ is awarded.**

Place, Date: Balzers, 07 May 2021

Responsible person: Dr. Kristin Neumann

Signature:

