Work Order	3578.3_Rev_1
Setup-Code	2020-03-11-09-30-00

iBrea™

Test Report

Measurement of the antimicrobial activity of dermatological products

Test Object:

iBrea Micro Silver Anti-Bacterial Spray with Pure Silver / iBrea Micro Silver Disinfectant Spray with Pure Silver / iBrea Micro Silver Hand Gel with Pure Silver against Staphylococcus aureus DSM799 ATCC6538

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Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

Test description

Common antimicrobial effectiveness tests described in international pharmacopeia measure the effectiveness of the preservative system in a product by inoculation of a controlled quantity of specific microorganisms. The test then compares the level of microorganisms found on a control sample versus the test sample over a period of 28 days. However, the short term germ-killing effect of dermatological formulations can not be measured within this long time frame, although it is interesting with regard to the anti-microbial properties of a product after application to the skin.

Long term anti-microbial properties, also called preservative effects, are caused by preservatives like parabens. Preservatives mostly kill germs quite slowly. Thus, they are often not able to kill germs within a few hours. Short term anti-microbial properties of dermatological formulations are caused by the fast killing anti-microbial additives, e.g. ethylhexyl glycerine or antiseptic agents, such as polyhexanid, benzalkonium salts or chlorhexidine, triclosan or metals like silver.

The measurement of anti-microbial properties of topical agents in vivo is difficult to perform due to individual variations and because the recovery rate of germs from human skin is associated with a very high range of variation, thus, being difficult to validate. To obtain reproducible results it is mandatory to artificially contaminate larger areas on the skin with a rather high germ concentration. The germ-kill kinetic test was developed in order to obtain reliable kinetic data of fast-killing anti-microbial additives that are supposed to be very similar to in vivo data.

As this test was designed to measure the fast-killing abilities of a dermatological formulation, it does not make any statements about the long-term preservative effectiveness of the formulations. Thus, a dermatological formulation that shows no fast anti-microbial properties may still offer very good long-term preservative properties.

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In order to measure the short term anti-microbial properties as a kinetic, the preservative effectiveness test according to Pharm. Eur. "5.1.3 Efficacy of Antimicrobial Preservation" was modified by applying shorter observation time frames (i.e. 24 hrs) as well as a relevant ratio between germ concentration and product

For this test an amount of 0.5 g product was used which corresponds to a single-use applied onto the human skin. The product sample is challenged with 100 µl of a solution containing 1 x 10^6 germs. This equals the amount of germs on the human hand (see Leyden JJ, Nordstrom KM, McGinley KJ (1991) Cutaneous microbiology. In: Goldsmith L.A., Physiology, biochemistry and molecular biology of the skin, 2nd Edition, Oxford Press, New York, 1403-1424). The germ-product-mixture is incubated at 30°C. Starting with time point zero (t_0) and after given time points, e.g. 1 hour (t_1), 2 hours (t_2) etc. neutralizing medium (SCDLP) is added and thoroughly mixed. The neutralizing medium inactivates fast killing anti-microbial additives as well as preservatives in the formulation, so that these substances do not kill germs during further handling. Aliquots are taken from the mixture and serial dilutions are made, which are then plated on agar plates. These agar plates are incubated at 37°C for 24 hours and, then, the colony forming units on the plates are counted. The short term anti-microbial properties are calculated by comparison of the germ concentration of a sample without antimicrobial additive and the concentration of the sample containing an antimicrobial additive like silver. The reduction can be calculated in percent or in log stages (logarithmic).

Germs double or duplicate every 30 minutes, so after 5 hours a single germ becomes about 1.000 germs. Thus a reduction of germs of about 90% indicates a small efficacy. An effective or strong efficacy starts with a logarithmical reduction of approx. 99.9% (= 3 log10 stages).

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Quality Labs
BIOMATERIAL TESTING

Test Report: Cosmetics-Kill-kinetics

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Test Results

	Sample Name	Sample Code	$t_{0\ h}$	$t_{30\ min}$	t_{1h}	t_{4h}
1	PBS (Reference)	6831103207530	$2{,}7 \times 10^5$	$2{,}6 \times 10^5$	$2{,}7 \times 10^5$	$2{,}6 \times 10^5$
2	iBrea Micro Silver Hand Gel with pure Silver	6831103207531	$< 1{,}0 \times 10^1$	$< 1{,}0 \times 10^1$	$< 1{,}0 \times 10^1$	$< 1{,}0 \times 10^1$
3	iBrea Micro Silver Anti-Bacterial Spray with Pure Silver / iBrea Micro Silver Disinfectant Spray with Pure Silver	6831103207532	$< 1{,}0 \times 10^1$	$< 1{,}0 \times 10^1$	$< 1{,}0 \times 10^1$	$< 1{,}0 \times 10^1$

Test strain	*Staphylococcus aureus DSM 799 ATCC6538*
Initial cell count / ml	**1.0×10^7**
Initials of the editor	CG CG
Measurement ended on	Mar-16-2020

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The result apply only to the samples tested.

Comments on test objects

NONE

Interpretation of the results based on the measurements

Already after addition of the germ solution to the samples 2 and 3 no viable cells were detected. No significant germ reduction was detected in the reference sample (1x PBS buffer).

Editor: Ms. Görgey

Crosschecked: Mr. Shendi

References

Pierre G. Agache, Philippe Humbert, Howard I. Maibach (2011). Measuring the skin. Springer, Berlin.

Leyden JJ, Nordstrom KM, McGinley KJ (1991) Cutaneous microbiology. In: Goldsmith L.A., Physiology, biochemistry and molecular biology of the skin, 2nd Edition, Oxford Press, New York, 1403-1424

Pharm. Eur. 6.6, 5.1.3 Efficacy of Antimicrobial Preservation

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