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Microbial Control

Microbiology Laboratory Report

Bacterial and Fungal In-can Challenge test according to ISO 11930:2012.

EVLY PHARMA KOZMETİK SANAYİ VE TİCARET LİMİTED ŞİRKETİ
ŞERİFALİ MAH. SÖYLEŞİ SK. -
SİTESİ - BLOK NO: 41A
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Laboratory Microbiologists:
Independent Laboratory

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Summary / Recommendations.

The sample both preserved was all free from microbial contamination as received.

The sample labelled

THE PUREST SOLUTİONS PEPTİDE COMPLEX - preserved by manufacturer

All achieved a pass in the ISO 11930-2012 test procedure.

Did not exhibit sufficient protection to meet the pass criteria of the ISO 11930-2012 test.

1. Introduction

The aim of the conducted test was to evaluate antimicrobial protection of a skin cosmetic product produced by EVLY PHARMA KOZMETİK from Turkey. The test was performed according to ISO 11930:2012.

2. Materials tested

Sample of skin product produced by EVLY PHARMA KOZMETİK, Istanbul Turkey were received for testing. Sample was unpacked in the laboratory and internal laboratory number was assigned to sample.

Sample identification:

THE PUREST SOLUTİONS PEPTİDE COMPLEX - Preserved by manufacturer

Sterility Test

To assess the initial contamination a Z-streak was applied with sterile cotton-tipped applicator on Tryptic Soy Agar (TSA) petri dishes to check for bacterial contamination (incubation 30°C for upto 7 days) and on Malt Extract Agar (MEA + Gentamycin) to check for fungal contamination (incubation 28°C for upto 7 days). Gentamycin is an antibiotic to suppress bacterial growth.

Table 1

B = bacteria, F = filamentous fungi (mould), Y = yeast

pH	Sample Description	B	F	Y
5.36	THE PUREST SOLUTİONS PEPTİDE COMPLEX **Preserved by manufacturer**	0	0	0

Rating Legend:

Plating Results	Score
No detectable growth	0
1–10 colonies	1
11–100 colonies	2
101–1000 colonies	3
>1000 colonies	4

Test methodology

The test was run separately for each micro-organism. For each product 20 ml of each formulation was dispensed into five sterile containers, one per each strain of micro-organism. In accordance with ISO standard 11930:2012 requirements the following strains of test organisms were used:

- *Pseudomonas aeruginosa* (ATCC 9027)
- *Staphylococcus aureus* (ATCC 6538)
- *Escherichia coli* (ATCC 8739)
- *Candida albicans* (ATCC 10231)
- *Aspergillus brasiliensis* (ATCC 16404)

The initial concentration of inoculum per micro-organism was as follows:

- *Pseudomonas aeruginosa* – 2.45 x 10^5 CFU/ml
- *Staphylococcus aureus* – 1.30 x 10^5 CFU/ml
- *Escherichia coli* – 1.75 x 10^5 CFU/ml
- *Candida albicans* – 6.05 x 10^4 CFU/ml
- *Aspergillus brasiliensis* – 6.50 x 10^4 CFU/ml

At T0, T7, T14 and T28 1 g of each tested product was taken for assessment of the survival of test microorganisms. 1 g of each sample was mixed with 9 ml of Dey-Eagle Neutralizing broth to neutralize the activity of the biocides. Neutralized samples were then serially diluted (10^0 to 10^-4) and plated on TSA for bacteria, PDA for *A. brasiliensis*, and SDA for *C. albicans*. The plates were then incubated at (32.50 ± 2.50)°C for 48h to 72h for the bacteria and *C. albicans* and at (22.50 ± 2.50)°C for 3 to 5 days for *A. brasiliensis.*

3. Enumeration of the microorganism and interpretation of the results

Plates with the growth of 30 to 300 colonies were taken for enumeration of bacteria and *C. albicans* and 15 to 150 colonies for *A. brasiliensis*. Then the numbers of surviving microorganisms in test samples were calculated and demonstrated as CFU/ml.

For each sample the log of reduction of total viable count was calculated. Based on the log reduction the preservation efficacy of each tested sample was evaluated according to the following criteria:

Log reduction values

Microorganisms	Bacteria			*C. albicans*			*A. brasiliensis*	
Sampling time	T7	T14	T28	T7	T14	T28	T14	T28
Criteria A	≥3	≥3 and NI*	≥3 and NI	≥1	≥1 and NI	≥1 and NI	≥0	≥1 and NI
Criteria B	Not performed	≥3	≥3 and NI*	Not performed	≥1	≥1 and NI	≥0	≥0 and NI

*No increase in the count from the previous contact time

The ISO 11930:2012 standard describes two criteria of product protection:

- Criterion A, whereby the formulation is protected against microbial proliferation that may present a potential risk for the user and no additional factors are considered;
- Criterion B, whereby the level of protection is acceptable if the risk analysis demonstrates the existence of control factors not related to the formulation indicating that the microbiological risk is tolerable for the cosmetic product.

Test results presented below include the log reduction values for each sample and microorganism and also the pass/fail data for both criteria A and B.

Bacterial strains	**Initial TVC (CFU/ml)**	**Log reduction values**			**Criteria A result**	**Criteria B result**	**Final result**
		T7	**T14**	**T28**			
Escherichia coli	2.45 x10^5	>5	>5	>5	Passed	Passed	**Passed**
Staphylococcus aureus	1.30 x10^5	>5	>5	>5	Passed	Passed	
Pseudomonas aeruginosa	1.75 x10^5	>5	>5	>5	Passed	Passed	
Candida albicans	6.50 x 10^4	>5	>5	>5	Passed	Passed	
Aspergillus brasiliensis	6.50 x 10^4	>5	>5	>5	Passed	Passed	