Preservative Efficacy Testing in Personal Care Products

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1.0 OBJECTIVE

The overall purpose of this study is to confirm the preservative efficacy of personal care products, based on the procedure outlined in USP <51>.

2.0 PROTOCOL OVERVIEW

Several different isolates of potential contaminants were inoculated into personal care products. Samples of each product were held at room temperature and the amount of each challenge organism remaining in each product was assayed after predetermined storage intervals. The population of each microorganism at each testing interval was compared to the population present at the inoculation point to determine the ability of each product to reduce or eliminate each challenge microorganism.

3.0 MATERIALS AND METHODS

3.1 Challenge microorganisms and stock solution preparation

The following challenge microorganisms were prepared for this study:

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

Each culture was prepared from a lyophilized preparation (KWIK-STIK™, Microbiologics, St. Cloud, MN) according to manufacturer's instructions or from stock plates. The bacterial cultures were transferred into Tryptic Soy Broth (TSB, Neogen) and incubated at 35 ± 2°C for 24 ± 2 hours. After incubation, the cultures were centrifuged (Multifuge X1R, ThermoScientific, Waltham, MA), washed in sterile peptone water and resuspended to their original volume. Fungal cultures were transferred into Sabouraud Dextrose Broth (SDB, Neogen) and incubated at 25 ± 2°C for 3-6 days. After incubation, the cultures were centrifuged (Multifuge X1R, ThermoScientific, Waltham, MA), washed in sterile peptone water and resuspended to their original volume.

3.2 Inoculation and storage of samples

Products were provided by Century Systems, including the following:

- Miracle Lotion (Lot #2018-10-25)
- Miracle Lotion (Lot #2018-11-15)

Each product was aseptically subdivided into five sterile subsamples. The products were inoculated at a final target level of 10^5 – 10^6 cfu/g of product for each challenge organism. Samples were stored at room temperature and evaluated immediately after inoculation (Day 0) as well as after 14 and 28 days of storage.

3.3 Sample plating and enumeration

Each sample was directly plated (or at appropriate dilutions) onto the following agars and incubated at the indicated temperatures and intervals, based on the challenge organism to be recovered:

- All bacterial cultures (i.e. *E. coli*, *P. aeruginosa*, and *S. aureus*) – TSA, 35°C for 48-72 hours
- All fungal cultures (i.e. *C. albicans*, and *A. brasiliensis*) – SDA, 25°C for 5 days

After incubation, plates were enumerated using a Quebec colony counter (Model #3325, Reichert Technologies, Depew, NY). The number of observed colonies typical for each challenge organism was multiplied by the dilution factor to determine the total count in cfu/g.

3.4 Data analysis

The raw count observed for each sample was converted to $\log_{10}$ cfu/g. The amount of each challenge organism present at each testing interval was compared to the amount present at the initial inoculation point to determine whether the challenge organisms are able to be reduced or eliminated by the products. If a minimum reduction of 1 log is observed in the samples inoculated with the bacterial cultures after 14 days, with no subsequent increases observed after 28 days, and no increases in the amount of fungal organisms recovered after either 14 or 28 days, a product was deemed to have successfully demonstrated antimicrobial activity.

4.0 RESULTS AND DISCUSSION

Results for the lotion samples are shown in Tables 1 and 2, below, including organism inoculated, time of storage, observed result (in cfu/mL, $\log_{10}$ of the

observed result, and (for samples after Day 0), the reduction (in log_{10} cfu/mL) seen from the point of inoculation.

Table 1. Sample results (Miracle Lotion – Lot #2018-10-25)

Sample	*E. coli*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. brasiliensis*
Day 0	960,000	1,640,000	2,100,000	170,000	210,000
Log_{10}	5.98	6.21	6.32	5.23	5.32
Day 14	<10	<10	<10	<10	<10
Log_{10}	<1.00	<1.00	<1.00	<1.00	<1.00
Reduction	>4.98	>5.21	>5.32	>4.23	>4.32
Day 28	<10	<10	<10	<10	<10
Log_{10}	<1.00	<1.00	<1.00	<1.00	<1.00
Reduction	>4.98	>5.21	>5.32	>4.23	>4.32

Table 2. Sample results (Miracle Lotion – Lot #2018-11-15)

Sample	*E. coli*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. brasiliensis*
Day 0	630,000	1,250,000	680,000	116,000	63,000
Log_{10}	5.80	6.10	5.83	5.06	4.80
Day 14	10	<10	<10	<10	<10
Log_{10}	1.00	<1.00	<1.00	<1.00	<1.00
Reduction	4.80	>5.10	>4.83	>4.06	>3.80
Day 28	<10	<10	<10	<10	<10
Log_{10}	<1.00	<1.00	<1.00	<1.00	<1.00
Reduction	>4.80	>5.10	>4.83	>4.06	>3.80

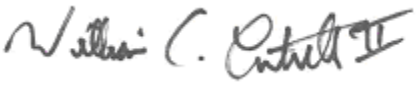
After 14 days of storage, reduced amounts of all challenge organisms were observed in both products. With the exception of minimal recovery for *E. coli* in the Lot #2018-11-15 product at this test point, no challenge organism was recoverable from this test point forward. Both products showed more than the required reduction after 14 days of storage, with no subsequent outgrowth of any challenge organism. Therefore, the preservative efficacy of these products can be demonstrated according to USP <51>.

5.0 REFERENCES

U.S. Pharmacopeia Convention. 2018. USP<51>, U.S. Pharmacopeia – National Formulary, USP 41-NF36, Online Edition.

6.0 SUMMARY REPORT APPROVAL

Prepared By:



1/04/2019

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