

The Comparison of Microbial Challenge Test and In-Use Test Method on Preservative Efficacy Testing in Skincare Products

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Introduction:

The size of cosmetic market in the world and Indonesia is predicted increase, which will reach \$463.5 billion by 2027. Consisted increasing public awareness of maintaining skin health, the average age of Indonesian people among Gen-Z and Millennials who have a high awareness of skin care, and social media that has contributed greatly. With the increase in the cosmetic industry sector, the control of cosmetic products must always be carried out, including the four products studied in this study encompassed day cream, face mask, gel, and face mist. These skincare product formulations are mostly susceptible to microbial growth due to their high water content and the source of their ingredients.



Fig. 1 DMDM Hydantoin

Preservatives are usually added to avoid growth of microbial contaminants in skincare products. The type of preservative used in cosmetic products and also used in this study is an aldehyde-formaldehyde compound in the form of DMDM Hydantoin and also phenolic compound in the form of phenoxyethanol and chlorphenesin.

In addition to preservative compounds, the process of evaluating the safety of new cosmetic product formulas is generally carried out as a way to determine the stability of the formula that has an impact on product quality or safety. Three steps that are commonly carried out, especially in preservation efficacy testing (PET) used are (1) physical, chemical, and microbiological testing; (2) microbial challenge test; and (3) in-use testing. The steps taken in this study are steps (2) and (3).

Based on the background, this study aims to confirm the adequate concentration of preservatives in the skincare product resulting from PET by comparing the microbial challenge test and the in-use test method. This confirmation is necessary to ensure the concentration of optimum preservatives in the skincare products resulting in a more cost-effective and safer product.

Results & Discussion:

Table 1. Comparative Data on the Results of Challenge Test and In-use Test

Products	Variation	Challenge Test		In-Use Test
		EP	IP	
Day Cream (A)	A-01	Passed A	Passed	M (passed) - tube P (failed) - pot
	A-02	Passed B	Passed	M (passed) - tube P (failed) - pot
	A-03	Passed B	Passed	M (passed) - tube P (failed) - pot
Face Mask (B)	B-01	Passed A	Passed	W (passed) - tube M (passed) - pot
	B-02	Passed A	Passed	W (passed) - tube G (failed) - tube
	B-03	Passed B	Passed	M (passed) - pot M (passed) - tube
Gel (C)	C-01	Passed B	Passed	M (passed) - tube M (passed) - pot
	C-02	Passed B	Passed	M (passed)
Face Mist (D)	D-01	Passed A	Passed	M (passed)
	D-03	Passed B	Passed	M (passed)

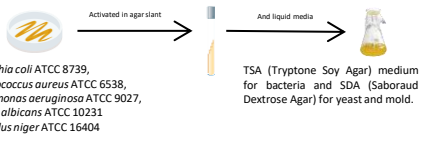
Based on Table 1 for the challenge test, for all products variation 01 (which has the highest concentration of preservatives) has the highest EP acceptance status, namely "passed A" for day cream, face mask, and face mist products, and "passed B" for gel products.

This is in accordance with the literature that higher concentrations of preservatives can better inhibit microbial growth. But based on the data, the results were "failed" for variations 2 and 3 in the gel formula. This might happened because the formulation in gel preparations consists of various plant extracts and high water content, causing the gel preparation to be a good growth medium for microorganisms. The next step commonly taken is an in-use test to determine the minimum shelf life of the product and its period after opening. The number of microbial contaminants can also later be correlated as a confirmation test result.

On the result of the in-use test, it can be observed that there is a higher number of microbial contaminants in pot packaging than in tube packaging in each type of product which can occur due to tube packaging that supports minimizing attachment to the environment compared to pot packaging. Dermatologist Victor Georgescu also said that tube packaging is also the safest to use for cosmetic products other than single-dose products because the contamination with air or surrounding pollution can be minimized thereby reducing the possibility of contaminant microorganisms entering the product.

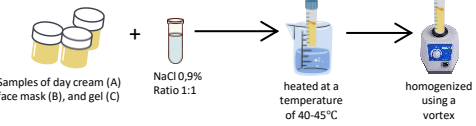
Materials & Methods:

1. Preparation of microbes

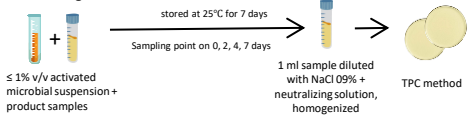


- Escherichia coli* ATCC 8739,
- Staphylococcus aureus* ATCC 6538,
- Pseudomonas aeruginosa* ATCC 9027,
- Candida albicans* ATCC 10231,
- Aspergillus niger* ATCC 16404

2. Sample preparation



3. The challenge test



4. The in-use test

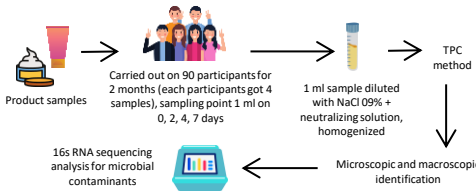


Table 2. Microbial Contaminant Identification Results

Isolate	Type of isolate	GRAM stain	Microscopic identification	Macroscopic colony	Genus/Species
G	Bacteria	(+)			<i>Micrococcus</i> sp.
B	Bacteria	(+)			<i>Staphylococcus</i> sp.
C	Bacteria	(-)			<i>Pseudomonas</i> sp.

Table 2 shows one of the identification of microbial contaminants found in each samples. In total, there were 21 types of isolates found as contaminants.

Based on the observation of macroscopic and microscopic characteristics of microbial isolates, the results of the estimation of the genus/species were obtained with a percent identity value exceeding 95% for all types of isolates base on BLAST results. The isolates that have been identified have habitats on human skin or in environments. The majority of the contaminant isolates came from a group of Gram-positive bacteria, especially those in the form of cocci and staphylococci, but there were also isolate of fungi and yeasts. Meanwhile, *Pseudomonas* sp. and *Staphylococcus* sp. are often found as the contaminants because they are widely found in the environment and types of bacteria that acts as a significant nosocomial infection agent (ex: *Pseudomonas aeruginosa* and *Staphylococcus aureus*)

Conclusions:

The four types of cosmetic products tested have the result that the acceptance criteria of Passed B based on EP and IP microbial challenge test could be considered an adequate preservative since it gives an almost similar result to Passed A criteria during the in-use test. This can be applied to the cosmetic company in the manufacturing of products with several positive benefits such as the costs incurred by the company will certainly be less and the use of fewer preservatives can reduce allergic reactions and is safer for consumers who have allergies to the preservatives compounds. Meanwhile, the recommended and safer packaging to use is tube packaging compared to pot packaging

Acknowledgements:

The authors gratefully acknowledge to PT Paragon Technology and Innovation and School of Life Sciences and Technology, Institut Teknologi Bandung for the facilities and financial support.

References:

Benson, H. A., Roberts, M. S., Leite-Silva, V. R., & Walters, K. (Eds.). (2019). *Cosmetic Formulation: Principles and Practice*. CRC Press.
BPOM, 2011. Keputusan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia Nomor HK.00.05.4.1745 Tentang Kosmetik. Jakarta. Halaman 1-5.
Cinelli, P., Coltellì, M. B., Signori, F., Morganti, P., & Lazzari, A. (2019). Cosmetic packaging to save the environment: Future perspectives. *Cosmetics*, 6(2), 26.
European Pharmacopoeia. (2019). *European pharmacopoeia*. Strasbourg: Council of Europe. Halaman 300-350.
Giorgio, A., Miele, L., Bonis, S.D., Conforti, I., Palmiero, L., Guida, M., Libralato, G., & Aliberti, F. (2018). Microbiological Stability of Cosmetics by using Challenge Test Procedure. *Journal of Pure and Applied Microbiology*, 12, 23-28.
Papageorgiou, S., Varvaresou, A., Tsihrivas, E., & Demetzos, C. (2010). New alternatives to cosmetics preservation. *Journal of cosmetic science*, 61(2), 107.
Yulianti, N. M. D. R., Oktaviani, P. N., Yoga, I. M. S., Wisudawati, N. N. S., & Sari, P. R. J. (2021). Perilaku Pembelian Kosmetik Secara Online Selama Pandemi. *Ganaya: Jurnal Ilmu Sosial Dan Humaniora*, 4(2), 420-437.