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International Standards of Microbiological Quality in Cosmetic Products

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Abstract

Blue and green cosmetics are sensitive products for microbial contamination and reducing the contamination producing according to Cosmetic Good Manufacturing Practice (ISO 22716), providing proper storage condition and packaging are effective factors. In addition antimicrobial effective preservatives can be added to protect the cosmetic products.[1,2] Generally, preservatives are chemical substrates that are effective on cytoplasm (conjugation mechanism, ribosomes, nucleic acids, thiol groups, amino groups), cell wall and cytoplasm membrane (membrane potential, enzymes, membrane penetration).[3] The neutralization of the antimicrobial effect of the preservatives in microbiological quality control of cosmetics is essential for the safety of the tests. The antimicrobial preservative in the cosmetic which is transferred to the medium affects the test negatively. The neutralization process can be carried out according to international standards used in microbiological analysis of cosmetic products. The neutralization of preservatives in cosmetics can be performed by adding of neutralizing agents in solvent, medium and/or washing solution. The agents which are reacted with the preservative groups for the removal of antimicrobial affect are used for this neutralization process which is necessary for the removal of antimicrobial effect. According to European Pharmacopeia 2.6.12. sodium bisulfite for glutaraldehyde and mercury compounds; dilution for sorbates and aldehydes; lecithin, polysorbate 80, saponin for quaternary ammonium compounds, parabens and iodines; thioglycollate for mercurial; thiosulfate for halogens can be used as neutralizing agents.[4] Lecithin, polysorbate 80 for parabens, phenoxyethanol, phenylethanol; Lecithin, saponin, polysorbate 80, sodium dodecyl sulphate for quaternary ammonium compounds and cationic surfactants; glycine, histidine for aldehydes formaldehyde-release agents; sodium thiosulphate for oxidizing agents; sodium bisulphate, L-cysteine and thioglycollic acid for metallic salts can be added to the medium, solvent and/or washing solutions according to ISO 21149.[5] For the removal of antimicrobial effect, neutralizing agents in proper concentrations should be added to medium, solvents and washing solutions. The addition of these agents with antimicrobial effect should be proved by the studies of conformity. Neutralizing agents react with antimicrobial agents to eliminate the antimicrobial effect.

Key Words: Cosmetic product, Antimicrobial preservative, Standards, Quality control

1. Introduction

Blue and green cosmetics constitute a suitable medium for the growth of microorganisms due to their contents. Microbiological limit that have to be exist and pathogen microorganisms that have not be exist in cosmetics are identified by international standards and are given by regulations. The tests that have to be applied in cosmetics within regulations are the determination of total aerobic mesophilic

microorganism count, total number of yeast and mould count and specific microorganisms. The determination of total aerobic mesophilic microorganism count (TAMC), total yeast and mould conut (TMYC) depends on the transfer of product after the dissolved in the buffer solution. The cosmetic product specimen, in which it was dissolved the buffer soution, pH was balanced and antimicrobial activity was neutralized, was transfered in Tyriptic Soy Agar (TSA) for TAMC according to proper dissoution factors and Sabouraud Dextrose Agar (SDA) medium for TMYC parameter. TSA and SDA mediums are incubated in specific conditions. Bioburden was determined by pour plate method, membrane filtration method and the most probable number method. The bioburden in gram or mL was determined by counting the colonies in the media after incubation. For specific microorganisms, the specimen equal to gram or mL was incubated by transferring in Tyriptic Soy Broth (TSB) medium for preenrichment. The microorganism identification was performed in this selective medium in which preenrichment process was performed.

2. Methods

2.1. Microbial Enumeration Tests

For TAMC and TMYC parameter, dissolve or dilute (usually a 1 in 10 dilution is prepared) the product to be examined in Buffered Sodium Chloride Peptone Solution pH 7.0, Phosphate Buffer Solution pH 7.2, or Soybean–Casein Digest Broth. The cosmetic product specimen, in which it was dissolved the buffer soution, pH was balanced and antimicrobial activity was neutralized. Unless otherwise directed, use 10 g or 10 mL of the product to be examined taken with the precautions referred to above. The sample solution was trasnfered to spesific media.

Pour Plate Method: Prepare for each medium at least two Petri dishes for each level of dilution. Incubate the plates of Soybean–Casein Digest Agar at 30° to 35° for 3 to 5 days for TAMC parameter and the plates of Sabouraud Dextrose Agar at 20° to 25° for 5 to 7 days for TMYC parameter. Take the arithmetic mean per culture medium of the counts, and calculate the number of cfu per g or per mL of product.

Most Probable Number Method: The sapmle solution transfered spesific media tubes.Incubate all tubes for 3 to 5 days at 30° to 35°. Record for each level of dilution the number of tubes showing microbial growth. Determine the most probable number of microorganisms per g or mL of the product to be examined from MPN statistical calculation chart.

Membran Filtration Method: Use a filtration apparatus designed to allow the transfer of the filter to the medium., The sample solution transfer the appropriate amount to each of two membrane filters, and filter immediately. Wash each filter with diluent or neutralising solutions. For the determination of TAMC, transfer one of the membrane filters to the surface of Soybean–Casein Digest Agar. For the determination of TYMC, transfer the other membrane to the surface of Sabouraud Dextrose Agar. Incubate the plate of Soybean– Casein Digest Agar at 30° to 35° for 3 to 5 days and the plate of Sabouraud Dextrose Agar at 20° to 25° for 5 to 7 days. Calculate the number of cfu per g or per mL of product.

2.2. Tests For Specified Microorganisms

For specific microorganisms, the specimen equal to gram or mL was incubated by transferring in Tyriptic Soy Broth (TSB) medium for preenrichment. The microorganism identification was performed in this selective medium in which preenrichment process was performed. The sample transfered Maccokey Agar for E. coli, Cetrimide Agar for P. aeruginosa, Mannitol Salt Agar or Baird Parker Agar for S. aurues, Sabouraud Dextrose Agar for C. albicans parameters. The possible presence of all spesific microorganisms is indicated by typical growth on media and all spesific microorganisms confirmed by identification tests.

Table.1 Standard test parameter cosmetic product

Test Pamameter	International Standard
TAMC	ISO 21149 , EP 2.6.12, USP 61
TMYC	ISO 16212 , EP 2.6.12, USP 61
E. coli	ISO 21150, EP 2.6.13, USP 62
S. aurues	ISO 22718, EP 2.6.13, USP 62
P. aeruginosa	ISO 22717, EP 2.6.13, USP 62
C. albicans	ISO 18416, EP 2.6.13, USP 62

3. Discussion

The microbiological quality is determined according to ISO 17516 and Cosmetic Regulation. The limit is $<10^2$ cfu in TAMC and TMYC for the cosmetics for mucosal membrane, eye contour and children younger than 3, $<10^3$ cfu for other cosmetic product. *E. coli, S. aureus, P. aruginosa* and *C. albicans* should not exist in all cosmetics. Microbiological quality control tests for cosmetic products are performed according to EP, USP and ISO methods. The tests and requirements are defined under the specified standards.

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