MICROBIOLOGICAL QUALITY ASSESSMENT OF BABIES' COSMETICS BY USING ISO11930- CHALLENGE TEST

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Abstract - Cosmetics, especially babies' products, must be safe and not contaminated with pathogenic microorganisms.In most cosmetics, there is apreservative compoundused to protect products from microbial contamination. Challenge testis a procedure used for assessing the preservatives efficacy. In this study, five cosmetics used for skin and body care of babieswere collected from different brands in pharmacies in Mecca region – Saudi Arabia.Samples subjected to microbiological analysis, results showthat(bath body, moisturizing lotion, moisturizing cream, shampoo, and hair conditioner)comply with the criteriaAofthestandardsISO11930. Preservative systems were found to be effective against bacteria and fungi during incubation periods at 7, 14, and 28 days.

Keywords - Babies' Cosmetics, Challenge Test, ISO11930, Preservatives.

I. INTRODUCTION

Cosmetics are a large group of products that are aimed at personal care, beauty improvement and hygiene, with skin care, makeup, perfumes, and hair care(Kättström et al., 2022). Most cosmetic products contain several components that are favorable for microbial growth (Siegert et al., 2005).One of the best solutions to reduce microbial growth is adding preservatives to cosmetics to protect them from microbial contamination(Orús and Leranoz ,2005). Neelam et al. (2020) pointed out the important role of preservatives in cosmetics which, preserve of the products from bacteria, viruses, and other microorganisms. The most common preservatives, such as parabens, formaldehyde releasers, isothiazolinones, and organic acids, have been well studied and known for their antimicrobial effects. For example, Parabens have a wide spectrum of activity to kill bacteria and fungi and low sensitizing potential (Mitra et al., 2021). Nevertheless, the activity of parabens sparked active research on alternative preservatives like ethylhexylglycerin, which moisturizes the skin and possesses antimicrobial properties (Mawazi et al., 2022). Challenge test is very important for two reasons. The first is to determine whether the preservative system which is chosen in controlling microbial contamination is effective during the product shelf life. The second is to confirm that the protective system of the cosmetic product continues to be effective against contamination that may increase during and after the production process. ISO 11930 standard isa testused to evaluate the antimicrobial protection of cosmetic products. It consists of three time intervals 7, 14 and 28 days ;five organisms are used in a separate to inoculate the product such as ;Aerobic mesophilic microorganismsPseudomonas aeruginosa DSM 1128 , Staphylococcus aureus DSM 799 , Escherichia coli DSM 1576 , Aspergillus brasiliensis DSM 1988 and yeasts like Candida albicans DSM 1386(ISO 11930,2019).

The main goal of this report is to assess the microbiological quality of babies' cosmetics byusingISO 11930 challenge test.

II. MATERIAL AND METHODS

Five cosmetic products specialists in body and skin care were obtained from local stores, these products are (Bath body, Moisturizing lotion, Moisturizing cream,Shampoo, and Hair conditioner), the cosmetics were preserved with different types of compounds as shown in the table.1 These preservatives have a broad-spectrum activity against bacteria, fungi, and yeasts. Unused products transferred to laboratory in intact case. The experiment is performed in LABORATOIRE MIDAC, Loss, Francehttps://www.midac.fr/

Sample no.	Product	Preservative compounds	Quantity
1	Baby Bath body	Phenoxyethanol, Sodium Benzoate, Ethylhexylglycerin	500ml
2	Baby Moisturizing lotion	Phenoxyethanol	500ml
3	Baby Moisturizing cream	Methylparaben , Dichlorobenzyl Alcohol	500g
4	Baby Shampoo	Sodium Benzoate	400ml
5	Hair conditioner	Dmdm Hydantoin , Methylchloroisothiazolinone, Methylisothiazolinone, Behentrimonium Chloride	350ml

Table 1.Preservative chemicalsin selected cosmetics

Microorganisms	Initial Inoculum volume (CFU/ml)
Pseudomonas aeruginosa DSM 1128	5,80
Staphylococcus aureus DSM 799	5,71
Escherichia coli DSM 1576	5,71
*Candidaalbicans*DSM1386	4,71
*Aspergillusbrasiliensis*DSM1988	4,83

Table 2. Initial Inoculum volume of tested microorganisms

Evaluation of the antimicrobial protection NF ENISO11930 :

In this experiment, 300g or ml is offered from each product,20g is taken and inoculated with various microbial strains 100µLpour10g table.2.Tests are carried out under controlled conditions for the product such as storagetemperatureoftheproduct at 24°C. Cosmetic samples were inoculated with different tested microorganisms. Microbial count is taken atdifferent intervals of 7th, 14th, and 28th days. Challenge tests following NF EN ISO 11930 were carried out on each product complying with Criteria A.

III. RESULTS

Microbiological Analysis:

Samples were examined to detect if they are microbiologically stable or not, bacteria were Inoculated on TSA mediumand incubatedat35°C for 48h, while Candidaalbicans inoculated on SDA mediumand incubated at 30 °C for 48h. Aspergillusbrasiliensis inoculated on PDA mediumIncubation at25°Cfor3hours. Results in table.3, show that all products contained no detectable contamination (less than 10 colony forming units per g).

Table 3. Microbiological analysis of cosmetics samples tested on TSA, SDA, and PDA media.

Evaluation of the antimicrobial protection: NF ENISO11930 (Challenge test):

The evaluation of the antimicrobial protection was carried out according to the methods of the standard NF EN ISO 11930 challenge test. Results show that samples comply with the criteriaAofthestandardNFENISO11930.In Tables(4, 5,6,7, and 8), baby bath body, baby moisturizing lotion, babymoisturizing cream, baby shampoo and hair conditioner,Preservative systems found effective against bacteria and fungi during incubation period at 7, 14 , and 28 days. The products are inoculated with initial volume of inoculum (5,80, 5,71, 5,71, 4,71, 4,83) for P.aerogenosa , S.aures ,E.coli.C.albicans and A. brasiliensis respectively .

Sample ID	Product Type	Aerobic mesophilicmicroorganisms (CFU/g)	Yeasts /Molds (CFU/g)
Sample 1	Baby Bath body	<10	<10
Sample 2	BabyMoisturizing lotion	<10	<10
Sample 3	Baby Moisturizing cream	<10	<10
Sample 4	Baby Shampoo	<10	<10
Sample 5	Hair conditioner	<10	<10

Table 4.Baby Bath body inoculated with tested microorganisms during 7, 14, and 28days.

Strains	*P.aeruginosa*	*S.aureus*	*E.coli*	*C.albicans*	*A.brasiliensis*
Actualtests					
Inoculum N0 **log(N0)**	6,30E+05 **5,80**	5,10E+05 **5,71**	8,80E+05 **5,94**	5,15E+04 **4,71**	6,75E+04 **4,83**
T7/DAYS /microorganisms(CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	
T14/DAYS microorganisms(CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	<1,00E+01 <1,00 **>3,83**
T28 /DAYS microorganisms(CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	<1,00E+01 <1,00 **>3,83**

Table 5. Baby Moisturizing lotioninoculated with tested microorganisms during 7, 14, and 28 days

Strains	*P.aeruginosa*	*S.aureus*	*E.coli*	*C.albicans*	*A.brasiliensis*
Actualtests					
Inoculum N0 **log(N0)**	6,30E+05 **5,80**	5,10E+05 **5,71**	8,80E+05 **5,94**	5,15E+04 **4,71**	6,75E+04 **4,83**
7 DAYS microorganisms (CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	
14JOURS/DAYS microorganisms (CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	<1,00E+01 <1,00 **>3,83**
28JOURS/DAYS microorganisms (CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	<1,00E+01 <1,00 **>3,83**

Table 6. baby moisturizing creaminoculated with tested microorganisms during 7, 14, and 28 days

Strains	*P.aeruginosa*	*S.aureus*	*E.coli*	*C.albicans*	*A.brasiliensis*
Actualtests					
Inoculum N0 **log(N0)**	6,30E+05 **5,80**	5,10E+05 **5,71**	8,80E+05 **5,94**	5,15E+04 **4,71**	6,75E+04 **4,83**
T7/DAYS microorganisms(CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	
T14/DAYS microorganisms(CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	<1,00E+01 <1,00 **>3,83**
T28/DAYS microorganisms(CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	<1,00E+01 <1,00 **>3,83**

Table 7. baby Shampoo inoculated with tested microorganisms during 7, 14, and 28 days

Strains	*P.aeruginosa*	*S.aureus*	*E.coli*	*C.albicans*	*A.brasiliensis*
Actualtests					
Inoculum N0 **log(N0)**	6,30E+05 **5,80**	5,10E+05 **5,71**	8,80E+05 **5,94**	5,15E+04 **4,71**	6,75E+04 **4,83**
7/DAYS microorganisms(CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	
T14/ DAYS microorganisms(CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	<1,00E+01 <1,00 **>3,83**
T28 /DAYS microorganisms(CFU/mL)Log **Reduction**	<1,00E+01 <1,00 **>4,80**	<1,00E+01 <1,00 **>4,71**	<1,00E+01 <1,00 **>4,94**	<1,00E+01 <1,00 **>3,71**	<1,00E+01 <1,00 **>3,83**

Table 8.hair conditioner inoculated with tested microorganisms during 7, 14, and 28 days

IV. DISCUSSION

In general, cosmetics with high water content are more exposed tomicrobial contaminationthan dry products. Microorganisms can damage the content of the product or cause a health risk to the consumer (Lundov et al.,2009). Liquid products are real homes for microorganisms,such as, Staphylococcus aureus, Pseudomonas aeruginosa, fungi such as, Aspergillus, Penicillium and yeasts(Alshehrei,2024).

The effectiveness of preservatives in skincare encounters certain issues, especially microorganisms developing resistance to them. Recent studies

highlight the growing issue of the resistance pattern among some bacteria and fungi species with reduced sensitivity to common preservatives, such as parabens and isothiazolinones (Nowak-Lange et al., 2022). The use of preservatives in cosmetics is very important to prevent any microbial contamination and therefore maintain safety and prolong product shelf life.

In this study,five samples are shown to be in good condition and there is no detectable contamination in these products.Samples were inoculated with tested microorganisms and incubated atthree intervals 7, 14, and 28 days. All samples found comply with criteria A according to ISO11930 - challenge test.The number of microorganisms decreased from zero time until the end of the test. In case of inoculating with bacteria, the product must show no less than a three-logarithms reduction from the initial count at seven days, and no increase from the seventh day count at days 14 and 28. For fungi,Aspergillus brasiliensismust show no increase in count intwo weeks and no less than a onelogarithm reduction at three weeks.Candida albicansare reduced by no less than onelogarithm at seven days and show no increase from the seven-day count aftertwo and three weeks.

Different preservative compounds are used in tested products.In baby bath body product, three preservatives are in ingredients: Ethylhexylglycerin , Sodium Benzoate, and Phenoxyethanol.Ethylhexylglycerin is a preservative that increases the shelf life of a wide variety of products (Aerts et al.,2016). In baby moisturizing lotion,phenoxyethanol (PE), is an effective preservative against microbial contaminationand it has less inhibition effect on skin mycoflora (Dreno et al.,2019). Inbaby moisturizing cream, the preservative compound is methylparaben which is derivates of parabens, it is usually used for preserving cosmetics and pharmaceutical products.It isgenerally safe in cosmetics and has a low sensitization potential.It is also effective against various microorganisms (Sandle, 2017).

In babies' shampoo, sodium benzoate is a preservative compound. This product is approved by the Food and Drug Administration (FDA) and it is safe when it is added to hair cosmetics. It is also used in personal care products as a preservative. It has antimicrobial properties, and it is effective in preventing the growth of microorganisms and it is safe and extends the cosmetic shelf life. Due to its natural origin and preservation effectiveness,sodium benzoate is a good additive in different personal care products such as makeup items,creams, shampoos,and lotions.

In hair conditioner, Methylchloroisothiazolinone, Methylisothiazolinone, Behentrimonium Chloride, and DmdmHydantoin are preservative compounds. Methylisothiazolinone Methylchloroisothiazolinoneare organicheterocyclic compounds and derivates ofisothiazolinones. Theyregularlyare used as a preservative in lotions, skin creams, and shampoos (Yim et al., 2014). DMDM Hydantoin is an antimicrobial formaldehydedonor and a very effective preservative against microorganisms. In industry, manufacturers addit to cosmetics in very low percentage to inhibit growth of microbes (Alshehrei ,2024).

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