

# MICROBIOLOGICAL QUALITY OF BRAZILIAN LIPSTICKS AFTER NORMAL USE BY CONSUMERS

D.M.M. Pedroso<sup>1</sup>, G.R. Dias<sup>1</sup>, J-L Gesztes<sup>1</sup>

<sup>1</sup> R&D – *Natura Inovação e Tecnologia de Produtos Ltda, Cajamar 07750-000, São Paulo, Brazil*

**Keywords:** microbiological quality, cosmetics, lipsticks, microbiological contamination.

## Summary

Lipsticks are cosmetics which do not contain water and are usually preserved with parabens. When submitted to the Challenge Test, these products did not reach the CTFA criteria, which means that microbiological contamination could occur before the end of its shelf life. The aim of this study was to evaluate the contamination level of 130 lipsticks after its use. Microorganisms were isolated from 14,6 % of the samples. However, only in two samples (1,5%) the contamination level exceeded the 100 CFU/g level, which means that, although the preservative system was not efficient to eliminate bacteria, the lack of free water was enough to prevent the microbial development. Total bacteria and fungi were determined by conventional methodology, according to CTFA Microbiological Guidelines. The microbes were then isolated and characterized as normal skin flora microorganisms. This suggests that products were contaminated by the constant application of lipsticks by consumers. This could lead to cross contamination when the same product is shared by several people. Extra care should be taken into account when this type of products are available to be tested by several consumers in demonstration displays.

## Introduction

The requirements for microbial growth can be divided into two main categories, physical (temperature, pH, osmotic pressure) and chemical (water, sources of nitrogen, carbon, minerals, oxygen, and organic growth factors) (Tortora et al, 1985). Normally, cosmetics are complex mixtures of organic and inorganic materials, highly processed, and could act as culture medium for the development of different microorganisms (bacteria, yeasts and molds) (Toler, 1985).

To prevent the microbes growth, cosmetics industries add preservatives into cosmetics which have some free water levels. Usually, lipsticks do not contain these kind of substances or they are preserved only with parabens (methyl and propylparabens) (Brannan, 1995).

These alkyl esters of p-hydroxybenzoate esters are more effective against yeast and molds than they are against bacteria, and more effective against gram positive bacteria than against gram negative one (Brannan, 1995).

During use, a thin aqueous film could be formed in the lipstick surface. Owing to their high oil solubility, p-hydroxybenzoate have relatively unfavorable partition coefficients, which means that parabens will not migrate to water after been incorporated into the product and the microorganisms could grow in this film (Brannan, 1995).

The aim of this study was to evaluate the microbiological quality of lipsticks after normal use.

## **Materials and Methods**

**Samples:** 130 samples of lipsticks from 27 Brazilian brands after normal use by consumers were analyzed.

**Methods:** Total bacteria and fungi count were determined by conventional methodology, according to CTFA guidelines (1993). Samples were weighted in diluent solution which contained 5% isopropyl miristate solution, to disperse the product (this is a 1:10 dilution). After 20 minutes (to inactivate the preservatives), 1.0 ml of the dilution was transferred by means of a pipette into each of two Petri dishes. Volumes of 15-20 ml of melted agar medium (Sabouraud Dextrose agar – Difco pH 3.5 for fungi and Tryptic Soy Agar- Difco for bacteria) were added and plates were rotated sufficiently to disperse the product. Plates containing TSA were inverted and incubated at  $(35 \pm 2)^{\circ}\text{C}$  for 48 hours. Plates containing Sabouraud Dextrose Agar were incubated at right position at  $(25\pm 2)^{\circ}\text{C}$  for five days. After incubation period, count the colonies under a colony counter (Quebec). The microorganism content per g was calculated by multiplying the colony count by the appropriate dilution factor (10).

Some bacteria colonies were transferred to nutrient agar plates that were incubated at  $(35 \pm 2)^{\circ}\text{C}$  for 24 hours. Gram stains and catalase and oxidase tests of the microbial growth were performed.

## **Results and Discussion**

Microorganisms were isolated from 14,6% of the samples, but only tree samples (2,3%) were contaminated with yeast, seven (5,4%) with molds and 14 (10,8%) with bacteria. Most part of the samples were contaminated with only one type of microorganism, one sample was contaminated with bacteria, yeast and molds and tree samples were contaminated with bacteria and molds. These results could be explained by the preservative system found in the analyzed samples, because parabens are more efficient against yeast and molds (Brannan, 1995).

From 27 different Brazilian brands, contamination was detected in 7 (5,4%). However, only two samples (1,5%) exceeded the allowed limits of  $> 100 \text{ CFU/g}$ .

These results are lower than the results obtained by the FDA laboratories which had made a research to evaluate the frequency and level of microbiological contamination of used cosmetics in the United States (1988- 1989). They found contamination on 50% of the faces, eyes and lips products, and 5% of the samples exceeded the CTFA limits. In 1990, the FDA analyzed 407 closed products and microbiological contamination was not found in any sample. So they concluded that the products are adequately preserved while are closed, but the preservative does not support successive contamination made by the consumers (Tran et al, 1994).

In another study, FDA investigators collected a total of 3027 cosmetics samples from 171 US retailers. Bacteriological data were obtained for 2892 samples of liquid mascara, eye, shadows, liquid eyeliners, lipsticks, lip glosses, facial blushes, rouges, foundation and other products.. Microbial contamination was found in only 50% of 2802 shared-use cosmetics, and positive results generally were obtained only by enrichment. In our study, we found contamination in 14,6% of the

samples. The difference found between the works could be explained by the different applied methodologies and the type of the samples, since FDA worked with shared use cosmetics.

Sixteen strains were submitted to Gram stains and catalase and oxidase tests. Fourteen strains (87,5%) were characterized as gram-positive cocci, arranged as tetrads or grapelike clusters (staphylococci), catalase positive. This kind of microorganisms is common of normal skin flora (Roth & James, 1989), which could indicate that samples were probably contaminated by the user.

Only one colony (6,25%) was classified as gram-negative bacillus and it was oxidase negative. Gram-negative bacillus does not belong to the resident skin flora, since it is more sensible to desiccation, but it could be part of the transient flora. Baird (1977) also isolated gram-negative bacilli in 6,1% of cosmetics. In our study we found only one strain (6,25%) that was characterized as gram-positive bacillus with endospore.

## Conclusions

Microorganisms were isolated from 14,6% of used lipsticks. However, only 1,5% of the samples showed a contamination level superior the 100 CFU/g level, which means that, although the preservative system was not efficient to eliminate bacteria, the product does not contain enough water to support microbiological growth.

## References

- [1] Baird, R.M. Microbial contamination of cosmetics products. *J. Soc. Cosmet. Chem.* **28** (1977): 17-20.
- [2] Brannan, D.K. Cosmetic preservation. *J. Soc. Cosmetic Chem.* **46** (1995): 199-220.
- [3] CTFA. Microbiology Guidelines. Curry, A. S., Graf, J. G; McEwen, G.N. Eds. Cosmetic, Toiletries and Fragrance Association, Washington, Table M –1, 1993.
- [4] Kabara, J.J. Cosmetic Preservation – the problems and the solutions. In: COSMETIC AND DRUG PRESERVATION PRINCIPLES AND PRACTICE. Kabara, J.J. Ed. Marcel Dekker, Inc. New York and Basel. P 3-6, 1984.
- [5] Romanowski, P. & Schueller, R. Microorganisms and personal-care products. *Cosmetics & Toiletries* **110**(1995): 71-78.
- [6] Roth, R.R. & James, W.D. Microbiology of the skin: resident flora, ecology, infection. *J.Am. Academy of Dermatology* **20** (1989): 367-390.
- [7] Spooner, D.F. Hazards associated with the microbiological contamination of non-sterile pharmaceuticals, cosmetics and toiletries. In: MICROBIAL QUALITY ASSURANCE IN PHARMACEUTICALS, COSMETICS AND TOILETRIES. Boolmfield, S.F.: Baird, R.; Leak, E.E.; Leech, R. Eds. Ellis Horwood Limited Publishers, New York, Chapter 2, p 15-34, 1988.
- [8] Steinberg, D.C. Preservatives For Cosmetics. *Cosmetics & Toiletries* (1996): 13-14.
- [9] Toler, J.C. Preservative stability and preservative systems. *Int. J. Cosmet Sci.* **7** (1985): 157-164.
- [10] Tortora, G.J.; Funke, B.R.; Case, C.L. Microbiology an introduction. 5<sup>th</sup> edition. The Benjamin/Cummings Publishing Company, California. 1985.
- [11] Tran, T.T.; Hurley, F.J.; Shurbaji, M.; Koopman, L.B. Adequacy of cosmetic preservation: chemical analysis, microbial challenge and in-use testing. *Int. J. of Cosmetic Science* **16** (1994): 61-76.
- [12] Tran, T.T. & Hitchins, A D. Microbial survey of shared-used cosmetics test kits available to the public. *J. Industrial Microbiology* **13** (1994): 389-391.