Cosmetics microbial safety: a milestone

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Even since the first pages of the European Cosmetic Regulation 1223/2009, it is clear that one of the main objectives of the Regulation is to guarantee the safety of finished products on the market. The **Microbiological safety** of finished Personal Care products is a key aspect: Cosmetics must be well protected from potential microbial spoilage.
This can guarantee their stability as formulations and their safety through end users. In Article 1 of the Regulation, under *Scope and objective*, it is stated: *"This Regulation establishes rules … … in order to ensure the functioning of the internal market and a high level of protection of human health"*. How is the microbiological safety guaranteed? Annex I of the Regulation, reports main indication on how to prepare CPSR (Cosmetic Product Safety Report); specifically, under point 3, needed information about **Microbiological quality** are mentioned: a) Microbiological specifications (substance - mixture - cosmetic product); b) Particular attention for cosmetics used around the eyes, on mucous membranes, on damaged skin, on children <3 years, on elderly people and persons showing compromised immune responses; c)Results of preservation challenge test. (1) In addition to this, the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, under paragraph 4-4 ("Guidelines on Microbiological of the Finished Cosmetic Product") give obligation for carrying out a preservation test with all cosmetic products which could be contaminated under normal storage and usage conditions or if an infection risk for the consumer exists (2).

Although according to the main Cosmetics regulations ww (e.g. US-Europe-Japan) Cosmetics do not have to be sterile, their microbiological quality has to be monitored and guaranteed. In figure 1, causes and consequences of microbial contamination in cosmetic products are reported. This can be done putting in place **preservation strategies**. They include of course the use of synthetic or natural substances able to inhibit the growth of microorganisms (preservatives). In addition, it is possible to control microbial growth also through the use of specific packaging that physically limit the contact of the product with external environment. Another method to control and limit microbial spoilage, consists in adjustments of the formulation pH. Last, but not least, substances able to reduce water activity can certainly reduce the risk of cosmetic contamination.

Watch the Video

MICROBIOLOGICAL TESTS TO ASSESS PRESERVATIVES' EFFICACY
Tests Performed to evaluate if and how Cosmetic preservatives are effective against microbes are basically two: Minimum Inhibitory Concentration test (MIC test) and Microbial Challenge test. What do they are?

M.I.C. Test: How to determine the antimicrobial spectrum of efficacy
The Minimum Inhibitory concentration test aims at finding out the lowest concentration of a product (substance/mixture) that is able to inhibit the growth of one more microorganism/s. In this test, scalar concentration of the product under investigation are added to tubes containing specific microbial suspensions. Tubes containing microbial suspensions + substance/mixture are then mixed by vortexing and placed under incubation for 24h (temperature and condition of growth are the preferable ones for the selected microorganisms). After the incubation period, determination of MIC is based on the evaluation of microbial growth, which causes

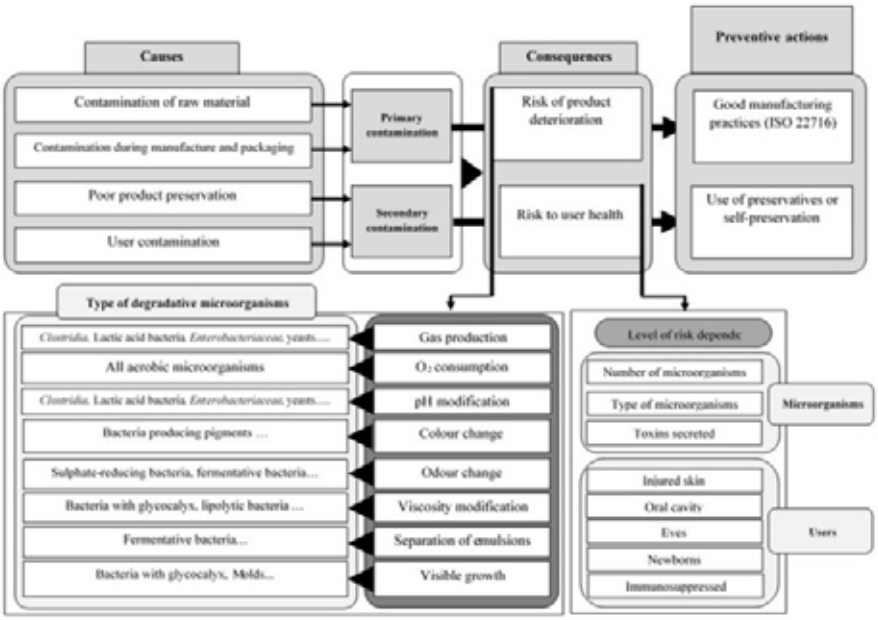

Figure 1. Causes, consequences and preventing cosmetics contamination (3).

tubes turbidity: where no turbidity is observed it means that no microbial proliferation occurred. The no-turbid tube containing the lowest concentration of the product under evaluation represents the minimum concentration of tested product able to inhibit microorganism growth. Figure 2 gives a visual representation of MIC test.

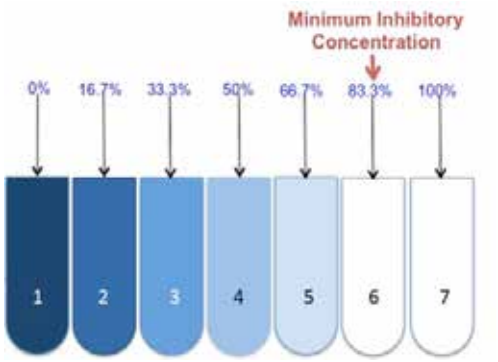

Figure 2. Visual representation of MIC test.

Microbial Challenge test: Evaluation of the preservative efficacy in a finished cosmetic product

Preservative Challenge test is doubtless the method recommended by international Regulations to evaluate the preservative efficacy in the finished cosmetic formulation. Different protocols on how to perform this test (main differences lies in acceptance criteria that will be later described), can be found in the European, American, and Japanese pharmacopoeia, as well as other organizations, such as PCPC (Personal Care Products Council) (from CTFA-M1 to CTFA-M7), ASEAN (Association for Southeast Asian Nations), ASTM (American Society for Testing and Materials) and International Organization for Standardization (ISO 11930 standard), among others (3).

While the above described MIC test uniquely predicts the antimicrobial spectrum of efficacy of the preservative, the challenge test is able to tell whether the preservative is effective in every single specific finished cosmetic formulation. A challenge test is a procedure in which a product is challenged by exposure to specified types of bacteria and fungi to determine whether it is adequately preserved. Test organisms should be representative of those likely to occur as contaminants during use (4).

In addition to standard used microorganisms (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Candida albicans, Aspergillus brasiliensis*), it is also possible to inoculate in house factory isolates, that typically come from earlier contaminations. The organisms, as single suspension or mixed pools, are into samples of the product and aliquots removed at appropriate intervals for the determination of survivors (4). Best procedure would be to perform Challenge tests at the beginning, during and at the end of the shelf life of the product.

The following steps summarize challenge test procedure (5):

- The product is separated into different containers;
- Each container is inoculated with a microorganism's suspension at a fairly high concentration (in some cases pools of different microbes are inoculated)
- After mixing, an aliquot from each container in analysed to determine the initial product contamination (cfu/ml or cfu/g)
- Each container is then placed under incubation for the whole duration of the challenge test (temperature and condition of incubation are set out in accordance with the type of microorganisms under evaluation)
- At pre-determined time intervals aliquots from each container are analysed to determine the level of contamination (usually at 48h – 7days – 14 days – 28 days).

Generally, Preservative Challenge tests last 28 days, and logically a decrease of the initial microbial load is expected at every interval of analysis. Optional:

- The product can be re- incubated and analysed further
- The product can be re-inoculated with even more microorganisms and allowed to incubate further (sometimes up to one year)

In Table 1 are reported Challenge test acceptance criteria, according to most known and used methods.

Challenge Tests – different methods; different acceptance criteria: how to interpret the results?

When having a look at the above reported challenge test acceptance criteria, it is evident that none of the cited methods requires a complete kill of the test organisms in 28 days. According to some studies, it is possible that the survivors will be able to adapt and grow if given the right circumstances (6); as a consequence, it would be important to consider that although preservative system of the formula is very important, it is not the only parameter that contributes in determining the microbiological

Method	DAY 2		DAY 7		DAY 14		DAY 28	
	B	YM	B	YM	B	YM	B	YM
JP	-	-	-	-	2	NI	NI	NI
USP	-	-	-	-	2	NI	NI	NI
EP – A	2	-	3	-	-	2	NI	NI
EP – B	-	-	-	-	3	1	NI	NI
CTFA –M3	-	-	2	1	NI	NI	NI	NI
CTFA-M4	-	-	3	1	CR	CR	CR	CR
ASEAN	-	-	3	NI	NI	NI	NI	1
ISO11930-A	-	-	3	Y1 M-	NI	Y NI M0	NI	YNI M1
ISO11930-B	-	-	-	Y- M-	3	Y1 M0	NI	YNI MNI
INHOUSE	Acceptance criteria are often in line or even strictest than CTFA method M4; they can vary case by case							

B= Bacteria (gram+ and gram-)
YM= Yeasts and Moulds
JP= Japanese Pharmacopoeia
USP= United States Pharmacopoeia
EP= European Pharmacopoeia
CTFA= Cosmetic Toiletry, and Fragrance Association (M3: Water-Miscible Personal Care Products); (M4: Eye Area Cosmetics)
ASEAN= Association for Southeast Asian Nations
INHOUSE= Test performed according to protocols set out by private companies
NI= No Increase
CR= Continued Reduction

Table 1. Challenge test acceptance criteria, based on needed Log Reduction of Microbial load, in time.

safety for a product: also the type of packaging, its closure and type of consumer use/abuse are relevant (6).

I've been asked several times, and I ask myself as well, whether Preservative challenge test is a suitable way to mime the risk of potential contamination that a product can face during its life cycle. According to some experts, the volunteer inoculation at time zero of the test is a too high stress for the product, if compared to the real contamination risk during usage (always remember that preservatives are intended to protect the product during its use by consumers, and not also to reduce potential contamination deriving from production, filling and storage before its use). On the contrary, some others think that the type of stress induced by standard challenge tests underestimates the real risk for product to get spoiled during its whole life cycle. Therefore, these last ones prefer to re-adapt the above described standard protocol, with multiple inocula and prolonged incubation time. Already in 1987, someone tried to solve this doubt: Brannan et al. compared results of microbial challenge test (shampoo and skin lotion), to consumer use testing. What they found out was that product that resulted **well and moderately preserved** according to challenge test results, were less prone to consumer in use contamination. On the contrary, those products that failed the challenge test, and thus were labelled as **poorly preserved,** had higher rate (46-90%) of returning contaminated after consumers' use. Few experts concluded that Microbial challenge test could be accurately used to predict the risk of consumer contamination of cosmetic product (7).

CONCLUSIONS

Keeping in mind this article's starting point: **products safety always at first**, our position on this topic is to always adopt a case by case formula evaluation. From one side the acceptance of borderline challenge test results, which are at the limit of acceptability and do not show complete reduction of the microbial load at 28 days, could be risky, especially for those products where packaging and type of end usage could overexpose the formulation to spoilage risk. On the other hand, we consider no sense to always expect a complete reduction of the microbial load in 48 hours. In fact, this second approach could be useless for those products that are less prone to microbial contamination thanks to their INCI composition, the intended condition of usage and the type of packaging. In this case, in order to obtain a super-fast contamination recovery, the preservative overdosing would be not only useless, but also risky for the safety profile of the product. Case by case evaluation, together with weighted analysis of challenge test results is always the answer and a one-way ticket for product safety!

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