Microbiology proficiency-testing scheme in cosmetics

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INTRODUCTION

The current context of reducing preservatives in cosmetics, especially antimicrobials compounds, is compelling the cosmetics industry to find new solutions to ensure the health security of raw materials and finished products while respecting the expectations of consumers of more natural products. Laboratories performing microbiological analyses on cosmetics therefore have a key role to play in this process and must ensure their performance through regular proficiency-testing schemes (PTSs). The complexity and diversity of cosmetic matrices is a factor to be taken into account in developing a PTS, especially during the preparation of stable and homogeneous samples.
This work describes the design and the implementation of PTS for the analyses of cosmetic samples spiked with three different strains. The goal of this PTS is to allow laboratories to demonstrate the reliability of their results and to compare each other analytical data and protocols used for the enumeration of yeast, mould and mesophilic aerobic bacteria in cosmetics.

METHODOLOGY

The setting up of a proficiency test can be schematized by 3 main steps: preparation of the samples, analyses by the laboratories and statistical treatment of the data.

SAMPLES PRODUCTION

One of the fundamental aspects for the implementation of a PT is the preparation of homogenous and stable samples.
For this PT, samples were prepared by spiking individually each sample of dermatological cream with a suspension of *Aspergillus niger, Candida albicans* and *Bacillus cereus* in well controlled proportions.
According to the requirements of the ISO 13528 standard [1], homogeneity of the samples was verified by experimental studies on 10 samples *in duplo* taken randomly across a batch of samples for *mesophilic aerobic bacteria* parameter (the results of the homogeneity check are summarized graphically in Figure 1). Stability of the product was proved by analyzing a sample after 7 and 14 days.

ANALYSES

Samples were shipped at (5±3) °C to the laboratories participating to the test together with a standard sample for monitoring the temperature.
A reply form was made available to allow the laboratories to return their analysis results.
Moreover, participants were invited to enter in the reply form some complementary information such as the date of the beginning of the analysis, method used, growth medium used, incubation temperature and time and the type of plating.
Given the stability of the product, the participants were invited to analyze the samples as soon as possible after the reception.

STATISTICAL TREATMENT

The statistical treatments were conducted according to ISO 13528 standard [1]. The assigned values (x_{pt}) were estimated using the robust means of the results transformed in log(CFU/g). The proficiencies of each laboratory were evaluated thanks to tolerance values (TV) of twice the standard deviations.
The results (x) could be evaluated and classified through z-scores:

- $z \leq |2|$: satisfactory,
- $|2| \leq z \leq |3|$: questionable,
- $z \geq |3|$: unsatisfactory,

Where : $z = \frac{x - x_{pt}}{\left(\frac{TV}{2}\right)}$

RESULTS and DISCUSSION

The results of the homogeneity check are summarized graphically in Figure 1. These data show that the samples are homogenous enough to meet the requirements of the test, with a gap between the minimal and maximal values lower than 0,5 CFU/g in log for mesophilic aerobic bacteria parameter .
The analyses results of the stability checks showed a satisfactory recovery rate considering the expected concentration after storing the samples at (5±3) °C for 14 days (see Table 1). The variability of the performed method can explain the difference between the results collected from D_0 to D_{14}.
The first PT on microbiological analyses of cosmetics was set up in March 2018, gathering fourteen laboratories around the world. Ten to thirteen laboratories out of fourteen gave their results together with useful information for the interpretation of the data.The main statistical parameters of this PT are summarized in Table 2.
An assigned value (x_{pt}) of 3.575 log(CFU/g) was calculated for mesophilic aerobic bacteria parameter and 3.458 log(CFU/g) was calculated for "yeast + mould" parameter. These values were estimated from the robust mean of the all returned results except those considered as incoherent.
The laboratories' results are shown on Figures 2 and 3. On the histograms, assigned value and tolerance interval are indicated in the x-axis and the results of the laboratories are shown in different colours as a function of the performed method.
Most of the laboratories followed the standard methods. For mesophilic aerobic bacteria parameter (figure 2), 9 laboratories used ISO 21149 [2] method (in orange) and 4 laboratories used an other method (in purple). For "yeast + mould" parameter (figure 3), 10 laboratories used ISO 16212 [3] method (in orange) and 2 laboratories used an other method (in purple). However no tendency was highlighted as a function of the method used.

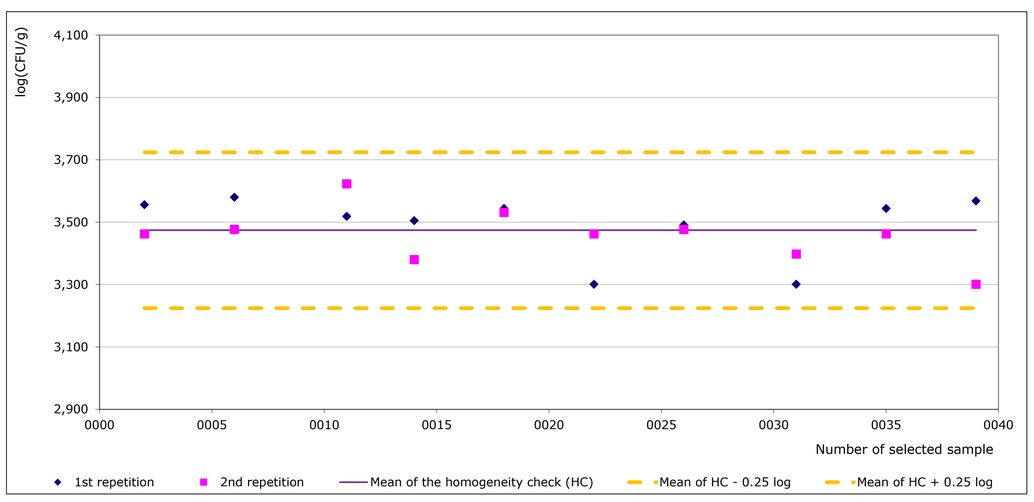

Figure 1. Results of the homogeneity check of the samples as a function of the production order for mesophilic aerobic bacteria parameter

Statistical parameter \ Analytical parameter	Mesophilic aerobic bacteria	Yeast	Mould	Yeast + Mould
Number of returned results (p_{ca})	13	10	11	13
Assigned values for proficiency testing (x_{pt})	3.575	3.156	3.047	3.458
Standard uncertainty of the assigned value (u_{xpt})	0.064	0.099	0.199	0.064
Robust standard deviation of the results (x_{pt}), from all the results which participated to the estimation of the assigned values	0.178	0.239	0.503	0.176
Number of results taken into account for the estimation of the assigned value (p_{xpt})	12	9	10	12
Coefficient of variation (%) (CV x_{pt})	5	8	17	5
Standard deviation for proficiency assessment σ_{pt}	0.178	0.239	0.503	0.176
Tolerance value (TV)	0.356	0.478	1.006	0.352
Number of results out of the tolerance interval p_D	2	0	1	1

Table 2. Summary of the statistical treatment of the data, log (CFU/g)

log(CFU/g) \ Day of analysis	D_0	D_7	D_{14}
Mould	3.00	3.06	3.07
Yeast	3.02	2.95	2.80
Mesophilic aerobic bacteria	2.83	2.88	2.67

Table 1. Average results of the enumeration of mould, yeast and mesophilic aerobic bacteria in dermatological cream after 14 days at (5±3) °C

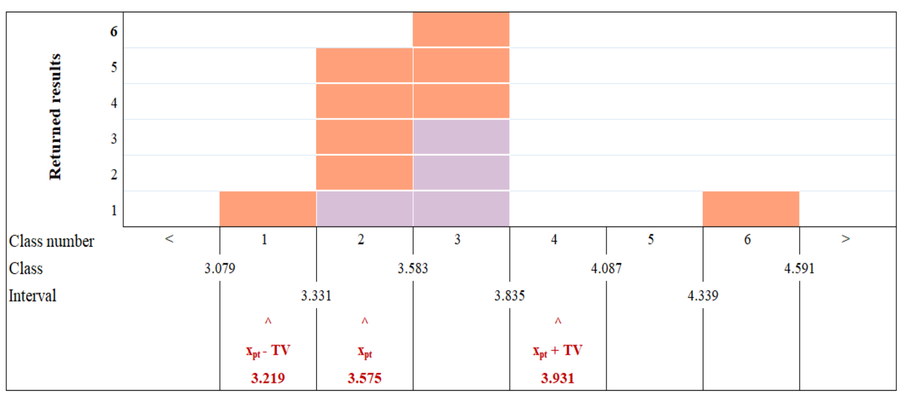

Figure 2. Distribution of the results for "mesophilic aerobic bacteria" parameter

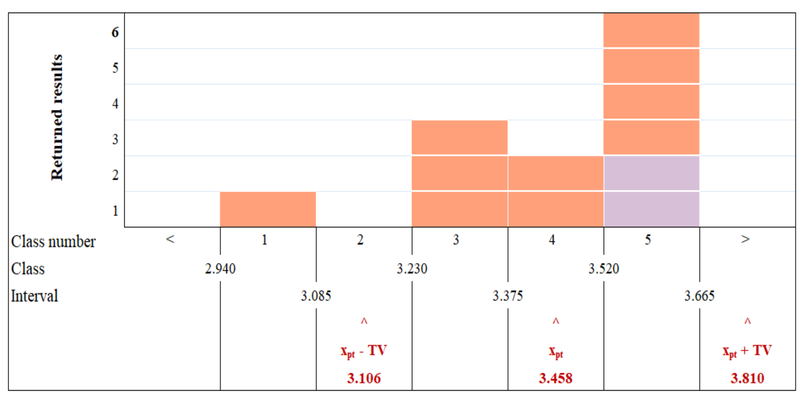

Figure 3. Distribution of the results for "yeast + mould" parameter

CONCLUSION

A PT for microbiology analysis in cosmetics, gathering fourteen laboratories around the world, was successfully implemented and the results were published in an interlaboratory comparison report distributed to the participants. This first PT has been transformed into a regular PTS, including 2 tests per year. Laboratories can now monitor punctually and/or continuously through time the reliability of their results for microbiological analyses of cosmetics.

REFERENCES

(1) **ISO 13528:2015** - Statistical methods for use in proficiency testing by interlaboratory comparisons
(2) **ISO 21149**: Cosmetics - Microbiology - Enumeration and detection of aerobic mesophilic bacteria
(3) **ISO 16212** : Cosmetics -- Microbiology -- Enumeration of yeast and mould