

Micro-challenge test : Useful method for fast and high-throughput preservative efficacy testing

Perrine Cancellieri, Ludovic Landemarre.
GLYcoDiag, F-45520 Chevilly.

Introduction

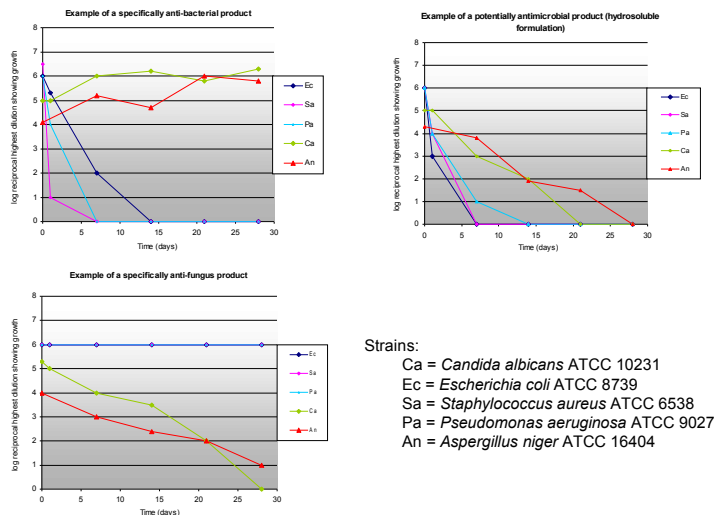
Microbial contamination of cosmetic products is of great importance for the suppliers and the consumers. The microbial growth is at the origin of organoleptic and formulation alterations (viscosity,color). To prevent microbial contamination, addition of system preservatives are needed according to microbial sensibility of the cosmetic product and its use by consumers.

The research and validation of new preservatives system is not an easy project: Most of the scientific information relate to the antibiotics; identification of potential new preservatives products need large screening of samples; preservatives must be used at concentration as low as possible in order to avoid any toxic effects (except against the microbial strains); the new preservative system must be compatible with the formulation and the practices of use of the end product.

The regulatory method used to detect microbial contamination in cosmetics and their raw materials is based on traditional plate count, but even this challenge test method is very accurate, its time consuming and low throughput are the main drawback.

Results

Examples of results obtained with raw material or end product:



Comparative results of micro-challenge test versus regulatory challenge test method:

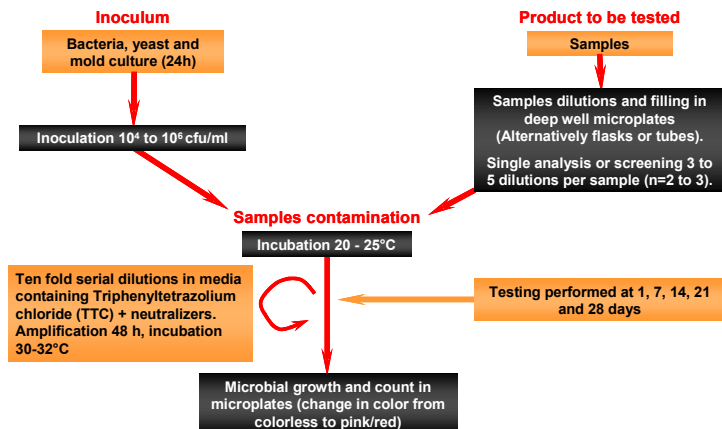
Strains	Micro-challenge test					Agar plate challenge test					
	Inoculum	1 day	7 days	14 days	21 days	28 days	Inoculum	2 days	7 days	14 days	28 days
Formulation without system preservative :											
Ca	1,20E+05	5,05E+04	1,00E+06	5,05E+04	1,00E+04	1,00E+03	2,00E+05	-	1,00E+05	1,00E+05	1,00E+05
Ec	3,20E+06	1,00E+05	1,00E+12	5,50E+04	5,50E+04	1,00E+04	5,00E+05	5,00E+05	2,00E+06	1,00E+06	1,00E+05
Sa	2,00E+06	1,00E+06	5,50E+04	5,50E+04	5,50E+03	1,00E+02	5,00E+05	5,00E+03	< 100	< 100	< 100
Pa	1,30E+06	1,00E+06	5,50E+05	5,50E+05	5,50E+06	1,00E+05	6,00E+05	1,00E+05	1,00E+06	1,00E+06	1,00E+06
An	1,10E+04	-	9,10E+03	5,65E+03	1,70E+03	2,85E+03	1,20E+04	-	1,00E+04	1,00E+04	1,00E+03
Formulation with system preservative :											
Ca	1,20E+05	1,00E+04	1,00E+04	1,00E+04	1,00E+03	1,00E+03	2,00E+05	-	1,00E+04	1,00E+03	1,00E+03
Ec	3,20E+06	1,00E+02	< 100	< 100	< 100	< 100	5,00E+05	< 100	< 100	< 100	< 100
Sa	2,00E+06	1,00E+04	< 100	< 100	< 100	< 100	5,00E+05	< 100	< 100	< 100	< 100
Pa	1,30E+06	1,00E+02	< 100	< 100	< 100	< 100	6,00E+05	< 100	< 100	< 100	< 100
An	1,10E+04	-	6,50E+02	< 100	< 100	< 100	1,20E+04	-	5,00E+02	3,00E+02	< 100

Conclusions

The micro-challenge test is cost effective and the method of choice for research projects aimed the discovery of new preservatives or the control step of anti-microbial efficacy where the regulatory method is not required.

- ➔ Reduced working time
- ➔ Screening capacity could be increased : automatizable
- ➔ High-throughput
- ➔ Low samples quantity required

Method description



Based on previous reports we have developed a miniaturized method of challenge test that allows screening of hundred of samples simultaneously.

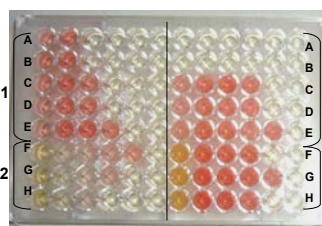
➔ Samples were contaminated in deep well microplate format allowing the study of 90 samples per microplate per microbial strains.

➔ The microorganisms used include all the strains recommended in the current regulatory method for cosmetic preservative efficacy testing.

➔ Counting was performed at each time (day 1, 7, 14, 21 and 28) with a triphenyltetrazolium assay in 96-wells microplates for the yeast and bacteria and with the conventionnal agar plating method for the mold *Aspergillus niger*.

➔ The culture media containing triphenyltetrazolium was designed in order to neutralize the preservative properties (neutralization products included). This adaptation eliminate the need of neutralisation step at each sampling.

➔ The counting of microbial population and the interpretation of the results are fast and easy. Results can be read on microplate reader (data traceability, automatization):



- 4) Controls :
- A: Blanck. Not contaminated sample
 - B: 0,5 % Phénonip®
 - C, D, E: NaCl 0,85 %

- 1) Sample 1, five concentrations (decreasing from A to E) ➔ 2 log reduction at high concentration
- 2) Sample 2, three concentrations (increasing from F to H) ➔ 1 log reduction at high concentration
- 3) Sample 3, three concentrations (increasing from F to H) ➔ No reduction.

➔ Validation conducted on samples of raw material or end products shows a good accuracy with regulatory challenge test.