Micro-challenge test : Miniaturized, reliable and totally customizable method for fast and high-throughput preservative efficacy testing

Introduction

Our micro-challenge test method is the result of the adaptation, optimization and miniaturization of the regulatory method of challenge test (ISO11930).
The research and validation of new preservative system needs high screening capabilities allowing testing number of samples. Concentrations, associations and synergy of each product are key parameters to built current strategies of systems preservatives and boosters development. Moreover, 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.

Reduced working time

From research material to formulated end products

High-throughput screening

Low samples quantity required (1/10)

Low cost

Main characteristics

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

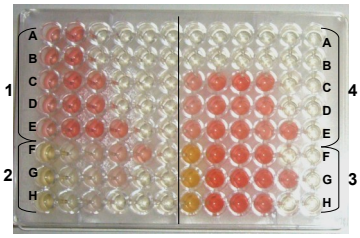
Samples were contaminated in deep well microplate format allowing the study up to 90 samples per microplate per microbial strains.

The microorganisms used include all the strains recommended in the current regulatory method for cosmetic preservative efficacy testing (ISO11930): *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404.

Counting was performed at each time (day 1, 7, 14, 21 and 28) with a triphenyltetrazolium assay in 96-wells microplates.

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 is fast and easy. Results can be read on microplate reader (data traceability, automatization)



4) Controls :
A: Blank. 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 materials or end products shows results in total agreement with those obtained with the regulatory challenge test.**

Versatility / Improvement

Rechallenge testing. Repeated inoculation with specific test micro-organisms can show the number of challenges a product can withstand before the preservative system fails for the micro-organism studied. The rechallenge testing consists of consecutive inoculation of a product (t=0h, 24h, 48h and 72h) followed by sampling after 7, 14, 21 and 28 days to determine preservative antimicrobial activity.

Mixed challenging. The goal is to compare the efficiency of a preservative with a mixture of the microbial strains usually used for challenge testing and not only in a mono-culture. Mixed challenge testing consists of a single inoculation of the product to be analysed with a mixture of microorganisms followed by sampling after 7, 14, 21 and 28 days to determine antimicrobial activity, regarding fungi and/or bacteria, in a more complex environment model.

For research and early stage development :
The **micro-challenge test intended for research** is completely customizable regarding the microbial strain (**up to 40 strains validated**), the time points and the duration of the test. It can be adapted depending on the goal of each customer.

Conclusions / Bibliography

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 absolutely required.

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