

## Challenge test

**Test aim:** the method allows to determine the preservative system efficacy of cosmetics and parapharmaceutical preparations through inoculation of micro-organisms and the control of their survival time. With this test, the sample is put through quite extreme and severe conditions, since the product is inoculated with a great quantity of micro-organisms, unlikely to develop in ordinary usage conditions. The micro-organisms commonly studied are: *Aspergillus niger*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It is however possible to employ different micro-organisms, even of wild-type strains, based on the customer's needs and the sample matrix.

The aim of the challenge test is to study the preservative system of a product to check the auto preservation of the preparations. Thanks to the method MP-1033-R5/07 it has been developed a specific neutralization system for cosmetic products.

**Procedure:** the test consists in the inoculation at known titration, of micro-organisms into the product and, through their survival time analysis, in the determination of the preservative system efficacy. The sample is put in as many vials as the strains to test.

After the inoculated micro-organism has been homogeneously spread, an aliquot of the product is extracted and diluted in the suitable deactivating dilutant with subsequent dilutions. From each dilution extract 1 ml, put it into a Petri dish, and mix with the agarized soil which is then gelified: the dishes are incubated for 2-4 days at temperatures suitable for each strain. The inoculated product is preserved at ambient temperature for the whole test duration.

The determination of the Units Forming Colonies per gram (UFC/g) is performed when inoculating the organism (Time zero) and at the following times:

### ❖ Bacteria :

Time	Criteria
➤ 48 hours	no variation
➤ 7 days	3-log reduction
➤ 14 days	no variation
➤ 28 days	no variation

### ❖ Mycetes:

Time	Criteria
➤ 7days	2-log reduction
➤ 28 days	no variation

In case of specific needs, it is possible to change the above schedules for the determination of CFU/g. The inoculated product is stored at ambient temperature for the whole test duration.

**NOTE: on specific request, it is possible to perform the Challenge test according to the European Pharmacopoeia.**