

Repeat Challenge Test

Test aim: the method allows to determine the preservative system efficacy of cosmetics and parapharmaceutical preparations through the repeated inoculation of micro-organisms and the control of their survival time. 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. Thanks to the method it has been developed a specific neutralization system for cosmetic products..

Procedure: the inoculation at known titration, of micro-organisms into the product is repeated after 14 days and, through the control of their survival time, it is possible to determine the preservative system efficacy. The sample is put in as many vials as the strains to test.

An aliquot of the product is extracted and diluted in 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 then incubated for 2-4 days at the temperatures suitable for each strain.

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

❖ *Bacteria :*

<i>Time</i>	<i>Criteria</i>
➤ 48 hours	<i>no variation</i>
➤ 7 days	<i>3-log reduction</i>
➤ 14 days	<i>no variation</i>
➤ 28 days	<i>no variation</i>

❖ *Mycetes:*

<i>Time</i>	<i>Criteria</i>
➤ 7days	<i>2-log reduction</i>
➤ 28 days	<i>no variation</i>

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.