



ANTIMICROBIAL EFFECTIVENESS TEST

WHAT IS THE ANTIMICROBIAL EFFECTIVENESS TEST?

Referenced in the current USP <51>, the Antimicrobial Effectiveness Test demonstrates the effectiveness of the preservative system in a product. A product is inoculated with a controlled quantity of specific microorganisms. The test then compares the level of microorganisms found on a control sample versus the test sample over a period of 28 days.

WHAT IS AN ANTIMICROBIAL PRESERVATIVE?

Antimicrobial preservatives are substances added to non-sterile dosage forms to protect them from microbiological growth or from microorganisms that are introduced inadvertently during or subsequent to the manufacturing process. In the case of sterile articles packed in multiple-dose containers, antimicrobial preservatives are added to inhibit the growth of microorganisms that may be introduced from repeatedly withdrawing individual doses. Examples of antimicrobial preservatives include alcohol, formaldehydes and iodine.

PRODUCT CATEGORIES

For testing purposes, the USP has divided test articles into four separate categories:

- Category 1 – Injections, other parenterals including emulsions, otic, sterile nasal products made with aqueous bases or vehicles.
- Category 2 – Topically used products made with aqueous bases or vehicles, non-sterile nasal products, and emulsions, including those applied to mucous membranes.
- Category 3 – Oral products other than antacids made with aqueous bases or vehicles.
- Category 4 – Antacids made with an aqueous base.

TEST ORGANISMS

When performed according to USP <51>, five indicator organisms are utilized for the purpose of challenging the preservative system in a product. Three of the five USP indicator organisms, Escherichia Coli, Pseudomonas aeruginosa, and Staphylococcus aureus, address the growth of bacteria. Candida albicans is the representative yeast, while Aspergillus niger is a mold.

The above listed microorganisms are ATCC cultures and must be harvested under current USP guidelines to assure viability. (Other organisms can be incorporated into the test as customer and product needs dictate.)

A product is inoculated (contaminated) with a number of organisms between 1×10^5 (100,000) to 1×10^6 (1,000,000) colony forming units (CFU) per mL of product. At various intervals, depending on the category, the product is tested to determine its ability to control reproduction or destroy the microorganisms.

PRODUCT CRITERIA

A logarithmic reduction is evaluated at each test interval required for the category. By test definition, any growth over the allotted amount for any of the indicated microorganisms renders the preservative in the product **not effective**.

WHEN DOES A PRODUCT NEED AN ANTIMICROBIAL EFFECTIVENESS TEST?

As part of a stability study, it is beneficial to determine if a preservative system will stand up to the product's shelf life. It may also be beneficial to determine if the preservative system chosen for a product is compatible with the formulation of the product. The USP procedure is intended for a self-contained finished product.

NOTE: It is necessary to retest the effectiveness of the preservative system any time the formulation is changed or when significant product or packaging changes occur.

VALIDATION

The first time a product is tested for Antimicrobial Effectiveness, a validation is necessary to show the microorganisms are able to withstand the formulation. A full validation is performed in three independent studies with each of the studies recovering not less than 70% of the growth inoculum versus the control.

It is **necessary to revalidate** a product whenever a formulation change has occurred, when the manufacturing process has been changed, or when changes in packaging occur.

HOW MUCH PRODUCT IS REQUIRED?

If a product is submitted in liquid form, a volume of not less than 20 mL is preferred. When submitting granular or powdered dosage forms, a weight of 20 grams is preferred. The validation of this product requires an additional 100 mL or grams. At the time of product submittal, it is necessary to note the category to be tested for the product.

Testing Time Intervals; under normal condition, the turnaround time to perform the Antimicrobial Effectiveness test is 7 weeks due to incubation requirements. However, if scheduling arrangements are made prior to product submittal, it is possible to reduce the time needed to prepare the necessary organisms used in testing.

REFERENCES:

USP <51> Antimicrobial Effectiveness

USP <1227> Validation of Microbial Recovery from Pharmacopeial Articles

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