

Time Kill

The time kill test is an in vitro suspension test method that demonstrates the potential speed of antimicrobial activity of a product against selected representative bacteria. Samples are taken at time points that are relevant to the use situation for that product. Test organisms and test conditions are standardized to allow comparability of results. The test determines that, as formulated, the product has the potential for antimicrobial activity in a time frame comparable to its proposed use. The spectrum of activity of an ingredient, having been determined previously during drug review for Category I status, should not be re-evaluated by the time kill method.

In the 1994 Tentative Final Monograph the Agency proposed the use of twenty-six bacterial species, set forth several evaluation points up to 30 minutes, and fixed the test dilution at 10%. In addition, no standard method was proposed in the tentative rule. The Industry Coalition recommends that:

- A limited number of culture collection strains of bacteria, representative of various types of bacteria with well-documented use, should be tested.
- At least three samples should be taken at times that reflect how a product is used.
- Product should be tested at concentrations that reflect the use directions.
- The time kill protocol adopted by ASTM should be used for the evaluation of topical antimicrobial products.

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Confirmation that a formulated product exhibits a speed of activity in a time-frame consistent with its proposed use can be determined using a limited number of bacteria that are: i) species representative of organisms of concern and ii) strains known to be stable and robust under normal laboratory conditions.

Because it is impossible to test against all potential bacteria, testing is done on representative strains. Strains that can be consistently cultured and do not require special incubation or nutritional requirements are chosen. Test results from the use of organisms that require special handling, such as anaerobes and micro-aerophils like *Haemophilus*, *Bacteroides*, etc. are unlikely to provide an accurate assessment of the product's activity against common aerobic organisms found on the skin. The aim is to use robust organisms that are representative strains of the various classes of bacteria of concern and that can be expected to perform consistently under standardized test conditions.

There is precedence for this approach by FDA and other U.S. agencies. In the First Aid Antiseptic monograph (56 Fed. Reg. 33644, July 22, 1991) *Staphylococcus aureus*

ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, and *Escherichia coli* ATCC 8739 are used to demonstrate efficacy. The US Environmental Protection Agency (EPA) requires the use of *S. aureus* ATCC 6538, *Salmonella choleraesuis* ATCC 10708, and *P. aeruginosa* ATCC 15442 to demonstrate effectiveness of disinfectants and sanitizers for human health purposes under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

The European Union (Vol. 1, Tab 6) has also recognized the principle of using representative organisms in setting standards for the testing of disinfectants and antiseptics:

- EN 1040, the test method for a demonstration of basic bactericidal activity of disinfectants and antiseptics utilizes only *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 15442.
- EN 1499, the test method used to demonstrate efficacy of hygienic handwashes utilizes *E. coli* NCTC 10538 as the sole test organism.
- EN 1276, the quantitative suspension test method for the evaluation of bactericidal activity of disinfectants and antiseptics used in food, industrial, domestic and institutional settings, uses *S. aureus* ATCC 6538, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 15442, and *Enterococcus hirae* ATCC 10541.

By using specified laboratory strains of Gram positive and Gram negative bacteria, it is possible to create a database for comparison of formulations. These bacteria are known to be stable in the laboratory. In the September 1999 submission, the Industry Coalition recommended the use of the following test organisms: *S. aureus* ATCC 6538, *E. coli* ATCC 11229, *S. choleraesuis* ATCC 10708, *P. aeruginosa* ATCC 9027, and *S. epidermidis* ATCC 12228, and continues to do so.

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The rate at which the bacteria die is usually represented by a first order reaction. The time points selected for the study should be sufficient in number to provide an accurate assessment consistent with the proposed use of the formulation. For example, for topical antimicrobial products where application times may vary from several seconds to several minutes, three time points covering this time span are sufficient. A zero (0) time sample should not be required. Due to sampling and test constraints a true zero time point sample can never be achieved.

Product should be tested at concentrations that reflect the use directions.

The formulations should be tested at a dilution consistent with actual product use conditions. Testing at a fixed 10% concentration may be inconsistent with actual usage and therefore would be inappropriate. For example, pre-operative skin preparations

and alcohol rubs may be used "as-is" without additional water and therefore should be tested undiluted. Handwashes or surgical scrubs that may be diluted with water before application should be tested at a 50%-75% concentration to be consistent with usage patterns. Bars should be tested at a 90%-95% dilution (5%-10% concentration). Other forms should be tested based on the type of antibacterial product being tested and its label indications.

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A standardized ASTM test protocol is in draft format. The test specifics of organism growth (broth vs. agar), inoculum preparation (washed vs. unwashed cells), use of log phase or stationary phase organisms, as well as effective and immediate neutralization of the active ingredient at all time points are addressed. This is necessary in order to provide accurate evaluation and comparability of different test materials. Variability in methodology and lack of comparability of data lead to inaccurate assessment of formulations.

Summary

The primary purpose of the time kill kinetic test is to assess the potential speed of action of formulated topical antimicrobial products consistent with their proposed use under standardized testing conditions. Use of standardized organisms in a published ASTM method will assure standardization of the critical parameters of the test protocol, such as the bacterial strain and media for growth, that will result in consistently reproducible testing and facilitate comparison of topical antimicrobial products. The use of a limited number of representative test organisms is a well established concept and is currently in use in the United States and the European Union.