



Proof of concept:

The types of bacteria used for studies of the antibacterial effects of Appeartex' surface modifications are primarily *E.coli* and *S.aureus* (representing gram negative and gram positive bacteria), but *Pseudomonas aeruginosa* has also been tested in certain experiments.

ASTM E2149-01 (American standard that is frequently used in the industry)

1g material (modified or unmodified textile, nonwoven etc.) is placed in a 50 ml bacterial suspension (~10⁵ cfu/ml) and is shaken for e.g. 1h. 100 µl suspension is retrieved before and after shaking and is plated with and without dilution. The number of colonies is counted and the reduction in viable bacteria calculated.

This test has been done for a large number of materials, such as cotton, polyester and mixed textiles, microfibre cloths and nonwoven materials, and the reduction in viable bacteria is generally in the interval 99 – 99.99%.

JIS L 1902:2002 (Japanese standard that is frequently used in the industry and is recommended by the renowned German test institute Hohenstein)

"Absorption method":

200 µl bacterial suspension (~10⁵ cfu/ml in diluted nutrient broth) is added to 0.4g material (modified or unmodified textile, nonwoven, etc.). The bacteria are then allowed to grow in 37°C for 18h, upon which they are shaken out of the material in sterile physiological saline that is then plated for colony count and comparison between modified and unmodified material.

This test method is more recent in the laboratory and has therefore not been performed for that many materials, but the effect seems to generally be over log 5 (the S value in the standard, corresponding to a 99.999% reduction)

Wiping test: (For this type of more realistic test, there is no appropriate standard, but the tests are done in cooperation with and under supervision of a big company with competence in the field)

Bacterial suspension is pipetted into a Petri dish and the liquid is then wiped around with an initially dry cloth (modified or unmodified). Upon the wiping, ~40°C LB-agar (nutrient agar) is poured into the Petri dish for quantification of residual bacteria. Liquid is squeezed out of the cloth and further plated for colony count (100 µl on an agar plate) and the cloth is then laid on (in close contact with) an agar plate. Upon 37°C incubation overnight, colonies are counted.

<i>S.aureus</i>	Residual liquid	Squeezed-out liquid	Cloth
Red. in modified cloth compared unmodified cloth	>98%	>99%	>99%

Aerosolized bacteria, test on hard surfaces: (Neither for this type of more realistic test is there an appropriate standard, but simple and well-known methods have been used both for depositing bacteria on the surfaces and for quantification of the viability)

Bacterial suspension is sprayed onto a surface (e.g. glass, plastic or stainless steel) upon which the sample is laid to dry in room temperature (~20-30 min). Thereafter the viability is assayed either by an agar pressure stick (Hygicult TPC from Orion Diagnostica) that is pressed against the surface for a few seconds and then sealed or by agar (~40 °C) overlay. Colonies are counted after overnight incubation.

In this test, the reduction is generally over 99% and often over 99.9%, both when assayed with pressure stick and with agar overlay.