Introduction

Antimicrobial treatment of textiles has three key benefits:

- Odor control, providing an improved sense of freshness
- Protection against discoloration and degradation of fabrics
- Improving general cleanliness between launderings

To measure the efficacy of antimicrobially-treated textiles, test methods used under controlled conditions for reproducible results are needed. These tests must accelerate the process of microbial growth to allow results to be seen in a timely fashion. Standard test methods that have been developed, peer-reviewed, published, and reproduced by different laboratories fall into two categories, quantitative and qualitative. Quantitative methods involve actual microbe enumeration, with results reported as a percent or log reduction in the contamination level. Qualitative methods are subjective, using ratings and measured zones of inhibition. This article will describe key efficacy tests used in the textile industry with a focus on quantitative bacterial methods, since these are the most broadly applicable.

Quantitative Bacterial Tests

Commonly used quantitative test protocols for antimicrobially-treated textiles are AATCC Test Method (TM) 100 (American Association of Textile Chemists and Colorists), JIS L 1902 (Japanese Industrial Standard), and ISO 20743 (International Standards Organization). These are inoculate and recover methods, with the reported result being a percent or log10 reduction in contamination between either an initial inoculation level of bacteria or against final results on an untreated control. Although organisms used in quantitative tests can vary, most methods call for testing against at least one Gram-negative (e.g., Escherichia coli or Klebsiella pneumoniae) and one Gram-positive (e.g., Staphylococcus aureus). To ensure consistency over time and between laboratories, methods name specific strains of these bacteria. These strains are obtained from the American Type Culture Collection.
Sample swatches of a specified amount (weight, size, and surface area) are inoculated with a specified number of bacteria using a pipette. The inoculum must be completely absorbed into the fabric and in intimate contact with the treated surface. If not, microorganisms multiply in portions that pool apart from the fabric, leading to misleading results for even properly treated samples.

The inoculated sample is incubated in a high humidity environment. Surviving organisms are recovered in a neutralizing broth containing an agent that will stop the action of the antimicrobial. Shaking the sample extracts surviving organisms. The recovery broth is re-plated and re-incubated for 18-24 hours and the number of surviving bacteria counted as colony forming units (CFU). The technician calculates a reduction in bacteria versus either the initial inoculum or the untreated control (if available).

Fig. 1 shows bacterial colonies, ready for counting, for untreated and treated samples. These test methods do not specify an absolute standard for efficacy. Minimum requirements vary from customer to customer and laboratory to laboratory. Quantitative bacterial testing can be used for all antimicrobials. Comparisons can be made between different antimicrobial treatments as well as various treatment levels on the same textile. These methods better simulate real-world conditions, such as a perspiration-soaked shirt or otherwise damp textile thrown into a gym bag or locker, than other methods.

There are also disadvantages to quantitative tests. They are long, involved, and expensive, requiring a number of manipulations to the sample and organisms. Current methods are highly dependent on operator interpretation and technique, and therefore somewhat inconsistent among laboratories nationally and internationally. There are no governing bodies enforcing strict adherence to published methods. Modifications are often made and full customer disclosure is not practiced by all laboratories.

**AATCC TM100**

AATCC TM100 uses a full nutrient broth for dilution to achieve the required testing concentration of bacteria for inoculation. The nutrient level is much higher than expected in most real-world situations, allowing for aggressive bacterial growth and reproduction. Only a single replicate of the test is normally performed, as there is no specificity in the method that requires more than one replicate. Doing the test in triplicate, as a minimum and as specified in JIS L 1902 and ISO 20743, is recommended due to the variability inherent in micro-testing.

AATCC TM100 is cited in military specifications for evaluation of apparel since such apparel may become heavily soiled. Studies sponsored by the US Congress and conducted jointly by Microban International and the US Army Natick Soldier RD&E Center, yielded correlation between positive results in AATCC TM100 and odor control and comfort in the field for army combat uniforms, T-shirts, and socks. Natick has issued clarification to AATCC TM 100 for improving consistency in testing and adherence to protocol. These clarifications include requiring clear documentation of test conditions and requiring use of the method regardless of antimicrobial technology.

**JIS L 1902**

JIS L 1902 was developed in Japan for testing silver-based antimicrobials. It primarily differs from AATCC TM100 in that the nutrient level in the inoculums broth is diluted to 1:20. JIS L 1902 also is explicit about calculating results for treated products versus those for untreated controls and calls for testing in triplicate. The standard for a valid test is that there should be at least a 1.5 log increase on the untreated control.

The low nutrient level for JIS L 1902 biases testing to provide more positive results for antimicrobials such as silver and cationic antimicrobials, which can be neutralized by proteins in the nutrient. The test can be used with other antimicrobials, such as triclosan,
but surprisingly triclosan seems to provide better results at the higher nutrient level used in AATCC TM100. AATCC TM100 is more aggressive since it gives organisms far more resources for growth and reproduction. However, actual nutrient levels on a fabric under normal use would be closer to those used in JIS L 1902. The lower nutrient level increases the necessity for an operator skilled in running the test to get valid results.

ISO 20743
There is now an International Standards Organization standard, ISO 20743, modeled largely on the JIS L 1902, but allowing more flexibility in the conditions of the test. This is as much a curse as a blessing when trying to compare results between laboratories.

ASTM E2149 Shake Flask Method
The Shake Flask Method is a quantitative screening test, developed by Dow Corning for quaternary (quat) silane (poly-3-siloxy-propyldimethloctadecyl ammonium chloride) antimicrobial treatment, generally known as the “Dow Shake Flask Method.” The Shake Flask Method can be faster than AATCC TM100 or JIS L 1902 and requires considerably less technique to do consistently, but differs radically from the other quantitative tests.

The fabric sample (~1 g), is immersed in 50 mL of an inoculated buffer solution in a flask, which is then agitated using a wrist action shaker. The bacterial concentration is $\sim 10^5$ mL$^{-1}$ but with no nutrient beyond that transferred with the organisms from the original culture. The time period for the exposure is 1 h. After the specified time, the technician plates an aliquot of the buffer from the flask onto nutrient agar without neutralization, incubates for 18-24 h, and then counts the number of colonies. A reduction is calculated using the known initial bacterial concentration and the final count after exposure to the test sample or is calculated versus an untreated control. It is recommended that an untreated control be tested in parallel to ensure a valid test and to run the test in triplicate. The “inoculum only” flask, as specified in the method, should be tracked alongside all treated and untreated samples with each test, to ensure there is no bactericidal effect resulting from the non-nutritive test buffer or due to surfactants added for wetting.

From a practical commercial perspective, the Shake Flask Method is only applicable to quat silanes and cannot be used to compare results with or among other commercial antimicrobial treatments. It has also been implied that only the Shake Flask Method can be used to test grafted quat silane technologies, contrary to at least one report on grafted quat silanes and experience in this lab.

There is little or no correlation between the Shake Flask Method and other quantitative tests. In this laboratory, untreated cotton fabrics have yielded reductions as high as 95% (~1 log) after 1-hr contact time, while showing 2-3 log increases over the standard 24-h exposure time using AATCC TM100. Quick reductions for a quat silane treated sample after 1-h exposure and then growth over the next 24 h have also been observed in this lab, indicating that bacteria may be removed from the liquid buffer, but are still viable and capable of reproducing.

The conditions of the Shake Flask Method, a small piece of fabric in a large amount of fluid with samples being taken from the fluid and not from the fabric surface, do not replicate real-life use conditions. As a result, the test is not widely accepted by professionals as an indicator of efficacy.

Qualitative Bacterial Tests
AATCC TM147 is a qualitative, zone of inhibition test adapted from the Kirby-Bauer test used in the medical field for decades. Both JIS L 1902 and ISO 20743 have qualitative sections modeled on AATCC TM147.

The bottom of a Petri dish is filled with nutrient agar that is streaked with the organism of interest. The test sample, a strip of fabric, is then placed over the streaks. Fig. 2 shows the results of an AATCC

Fig. 2. AATCC Test Method 147.
TM147 test (the light colored streaks are the bacterial organism). For the treated sample, the area of no growth at the sample edge is called the zone of inhibition and reported in millimeters. Some samples will not show a zone of inhibition, but the streaks will stop at the edge of the sample; as long as there is no growth under the sample, the result will be reported as a pass and is commonly referred to as contact inhibition.

AATCC TM147 is quick (done over a 24-h period), cheap, simple, and well-defined. In this researcher’s experience, the primary difference among laboratories reporting results using AATCC TM147 has been at the subjective level of determining contact inhibition, where it is easily possible for one laboratory to fail the sample, while another may pass it.

AATCC TM147 cannot be used to measure the relative efficacy of one antimicrobial versus another because antimicrobials diffuse through agar at different rates or not at all. Since the amount of antimicrobial present is usually based on the weight of the fabric, a light fabric may not show activity despite being appropriately treated, and diffusion seen in the test does not represent antimicrobial behavior in actual use. The method provides a nice visual demonstration of inhibition, but also engenders confusion. Silver and cationic antimicrobials like quat silane and polyhexamethylene biguanide (PHMB) will not diffuse through agar because they bind with proteins in the agar; while other antimicrobials simply see the agar as a polymeric continuum with the fabric matrix. A zone of inhibition does not measure the tendency of the antimicrobial to migrate from the fabric or to transfer to skin or other contact surfaces any more than the lack of a zone of inhibition shows that it will not. Transfer of the antimicrobial by contact has already been shown to be undetectable in the case of the most thoroughly-studied antimicrobial in the market.\textsuperscript{10} Migration is only relevant to laundering durability, which can be readily determined.

**Applicability of Bacterial Tests**

JIS L 1902 is the most universal of the bacterial efficacy tests (and ISO 20743 that is modeled on it). It uses a reasonable level of nutrients, simulates real-world use and behavior, and allows for comparisons among antimicrobials. In Table I, ☒ means that the test should not be used with a specific antimicrobial and ☐ marks those recommended by the author.

### Table I. Applicability of Test Methods

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>JIS L 1902\textsuperscript{a}</th>
<th>AATCC TM147</th>
<th>ASTM E2149</th>
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</thead>
<tbody>
<tr>
<td>PHMB</td>
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<tr>
<td>Quat Silane</td>
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<tr>
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<td>n-Halamine</td>
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<td>Chitosan</td>
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\textsuperscript{a}ISO 20743 considered equivalent to JIS L 1902.

**Fungal Tests**

**AATCC TM30 (Part III)**

Standard fungal tests are typically qualitative. In AATCC TM30 (Part III),\textsuperscript{11} the sample is placed on the surface of potato dextrose agar nutrient that has been lawn struck with spores of *Aspergillus niger*. The organism and sample are incubated for seven days, the typical time for the fungus to mature, and then subjectively-rated based on the amount of fungal activity on the product surface. The ratings are 0 for no growth, 1 for growth that can only be seen with a microscope, and 2 for growth that is visible to the unaided eye. If a zone of inhibition is present, which is rare, it is measured and reported as well. Fig. 3 shows an AATCC TM30 (Part III) sample at the end of testing.

The AATCC TM30 (Part III) is fast relative to other fungal tests (7 days versus a more common 28 days in length) but more aggressive than normally seen in real life; only the most

![Fig. 3. AATCC Test Method 30, Part III.](image-url)
effective treatment completely prevents growth, though no growth means that the sample will be highly resistant to fungal attack. The test is not very discriminating with regards to performance; even the minutest amount of microscopic or macroscopic growth will result in a rating other than 0. In this laboratory, standard ratings are applied to the test, but comments related to percent coverage of the fungus on the sample surface are added and photographs included as documentation.

**AATCC TM30 (Part IV)**

In AATCC TM30\(^\text{11}\) (Part IV), a dry, treated and untreated 1 × 3 in. strip of nutrient saturated fabric, sprayed with a mixed-spore suspension of mildew-causing organisms, is I-suspended and incubated in a closed jar with sterile water in the bottom to provide moist conditions (Fig. 4). After the incubation period, the technician grades percent coverage by fungal growth. The organism must germinate and establish itself on the treated fabric, whereas in the Part III test, the agar provides an antimicrobial-free zone for the fungus to establish itself and then overgrow the sample. Thus, the Part IV method is less aggressive than the Part III method and allows for somewhat better discrimination between treated and untreated samples.

**Testing Issues**

Antimicrobial treatment of textiles provides benefits in odor control and freshness, as well as protection against degradation due to bacteria and mold. However, differences in test results between laboratories and claims of performance through the use of unspecified and inappropriate test methods continually create confusion in the antimicrobial marketplace. The comparative information available on different antimicrobial technologies is often misleading due to differences in test methodologies, inappropriate methodologies, sometimes honest confusion in running methods, and differing levels of expertise. Just because two laboratories cite AATCC TM100 or JIS L 1902 does not mean that the laboratories are actually running those protocols in exactly the same way. The quality of testing is distressingly poor; in blind round robin testing\(^\text{12}\) less than half of the laboratories evaluated were capable of running a dependable quantitative test protocol. The only way to be sure of comparisons is by running tests side-by-side in the same laboratory with the same series of tests. Where differences occur between laboratories, only detailed review of the test protocol will enable one to understand results.

**References**

4. AATCC TM 100 clarification, Amy Johnson (contact), US Army Natick Soldier RD&E Center, Natick, MA, USA.

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