

Test Methods and Protocols for SILVADUR™ Treated Articles

May 13, 2020

The following four test methods are currently approved for evaluating application efficiency, technology performance, and wash durability for SILVADUR™ treated fabrics.

-
- 1 Colorimetric Test for Rapid Identification of SILVADUR™ in Textiles

 - 2 Antimicrobial Test Method for Antibacterial Performance: ASTM E3160-18 or equivalent such as AATCC 100 - 2019 test method

 - 3 Performance Durability by Washing Methods: ASTM E3162-18 or AATCC 61-2A

 - 4 Odor Control Sensory Test Method IACM 0710: Biofouling of textiles from milk

When requesting performance evaluation from Third Party Facilities, it is very important that the exact specifications and requirements are followed. A minimum of 20 grams each of SILVADUR™ treated and untreated fabrics are required to complete the evaluations.

1. Colorimetric Test for Rapid Identification of SILVADUR™ in Textiles

This on-site qualitative method can be used to detect silver in fabric of any color and composition. Detection is performed using a commercially available reagent kit.

Materials

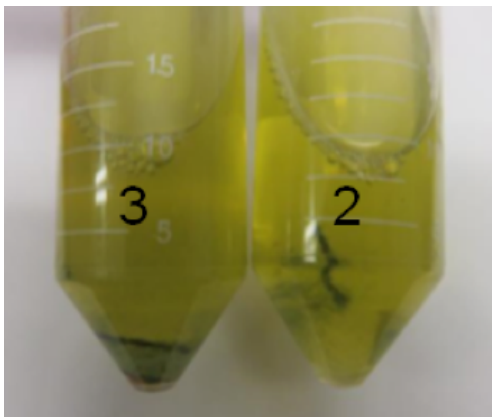
Latex Gloves, Goggles, Scissors

- A. Solution A 0.1 M Nitric Acid (Sigma-Aldrich 32042)
- B. Solution B 1 N Sodium Hydroxide Solution (Sigma-Aldrich 71463)
- C. Solution C 20X borate buffer (Fisher Scientific PI28341)
- D. HACH product code: 2296600
 - Reagent 1 - Powder Sachet
 - Reagent 2 - Liquid Sachet
 - Sodium Thiosulfate Sachet
- E. Three 50 ml Clear Vials Labeled vial # 1, vial # 2, and vial # 3
- F. Fabric (2 grams) cut into small pieces

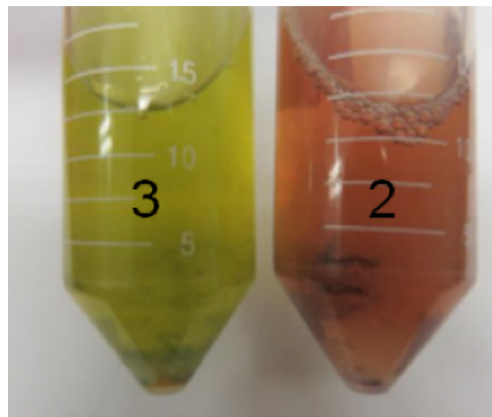
Protocol

The following protocol should be conducted for SILVADUR™ treated and untreated samples to ensure no false positive results are caused by any other finishing materials applied on fabrics.

1. Cut 2 gram of textile sample into small sections and place in a vial #1 with lid.
2. Add 50 ml of Solution A to the vial # 1 on top of the fabric. Securely close and gently invert for 5 minutes to ensure the fabric is saturated.
3. Add 3.5 ml of Solution B to the vial #1. Securely close and gently Invert the vial for 2 -3 times
4. Add 2.0 ml of Solution C to the vial #1. Securely close and gently Invert the vial for 2 -3 times
5. Wear nitrile glove and goggle. Open and add the content of the powder sachet followed by the liquid sachet to vial # 2. Gently swirl to mix thoroughly. Results will be compromised if Reagent 1 is exposed to liquid prior to mixing with Reagent 2.
6. Decant the liquid in vial #1 into vial #2. Securely close Vial # 2 and invert gently for 1 minute. The solution may contain small particles after mixing, which will not affect the method results
7. Decant half of the liquid from Vial 2 into Vial 3. The volumes in each vial should be approximately the same (+/- 5 mL).
8. Open and add the content of the Sodium Thiosulfate sachet into vial # 3. Securely close and gently invert the vial 2-3 times to ensure mixing.
9. A color difference between vial #2 and vial #3 indicates the present of SILVADUR™ on the fabric. The final solution color in Vial 2 is typically observed to be brown, maroon, orange, or red, and the solution color in Vial 3 is observed to be yellow or green.
10. Recommended optional negative control: Repeat steps 1-7 with untreated fabric. Vial 2 and Vial 3 should match in color (typically yellow or green), indicating no silver is present in the control
11. See below for example results.



No SILVADUR™



SILVADUR™ detected

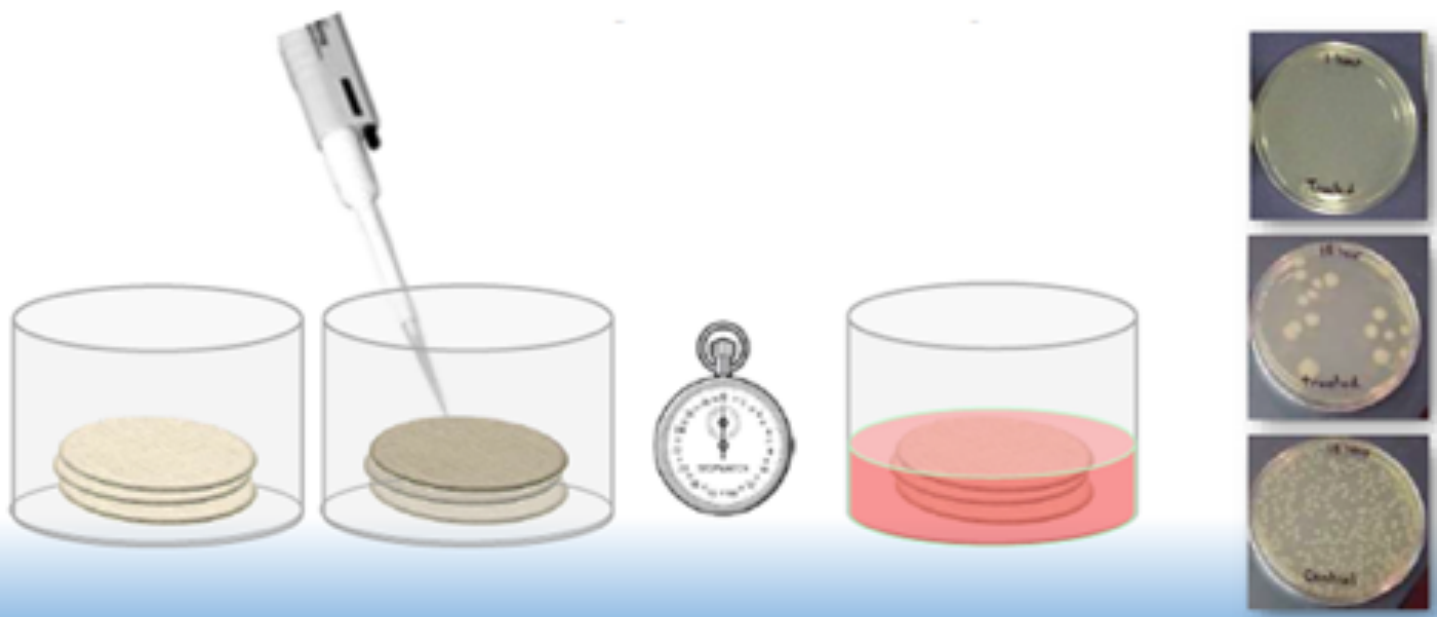
This analysis is fast, qualitative, and reproducible. This method can be used to ensure the presence of SILVADUR™ product in fabrics down to approximately 10 ppm silver.

2. Antimicrobial test methods for antibacterial performance: ASTM E3160 - 2018 and AATCC 100-2019

Real life activities may change the balance of one's microbiome and produce more microorganisms that cause malodor. Controlling the presence of microorganisms is one way to control the generation of malodor. This antimicrobial evaluation measures whether a SILVADUR™ treated fabric can directly eradicate or reduce bacteria or inhibit their growth when compared to untreated fabric. ASTM E3160 - 2018 and AATCC 100-2019 are the two standard methods that have been validated for consistency and reproducibility for SILVADUR™ treated fabrics.

The protocol written in ASTM E3160 - 2018 and AATCC 100-2019 standard methods can be conducted using the following **SPECIFICATIONS that MUST BE** met to ensure consistency and reproducibility of the test results.

1. The evaluation must include **SILVADUR™** treated and **UNTREATED** textiles.
2. Textile samples **MUST NOT** be sterilized prior to testing.
3. Overnight bacterial culture should come from a shaking broth solution.
4. Sample weight of **0.4 grams for ASTM** and **1.0 gram for AATCC**.
5. Test inoculum volume of **0.2 ml for ASTM** and **1.0 ml for AATCC**.
6. For ASTM method, test inoculum should contain bacteria diluted in 1:500 nutrient broth and sterile distilled water for unwashed sample and 1:20 nutrient broth for washed sample. For AATCC, test inoculum should contain 1:20 nutrient broth and sterile distilled water.
7. Bacterial concentration of inoculum should contain $1-3 \times 10^5$.
8. Contact time should be 24 hours (+/- 15 minutes).
9. Samples should be neutralized in D/E broth after contact time.
10. Bacteria should include *Escherichia coli* (ATCC # 25922) for ASTM, and both *Staphylococcus aureus* (ATCC # 6538) and *Klebsiella pneumoniae* (ATCC # 4352) for AATCC (tested separately).



3. Performance durability by washing methods: ASTM E3162-18 or AATCC 61-2A

ASTM E3162-18 is an accelerated laundering test method to measure the durability of antibacterial agents applied to textiles under simulated home laundering conditions. The common equipment used in this evaluation is a Launder-Ometer. The basic principle of the evaluation is subjecting ~ 10 grams of treated fabric to wash conditions with 500 ml defined detergent solution at ~ 50° C under abrasive action using stainless steel balls to simulate five home launderings during a 45-minute laundering cycle at ~ 40 rpm. After each cycle the fabric is removed, rinsed thoroughly by hand in water, and returned to a fresh solution of detergent and clean steel balls. The laundering cycles are repeated depending on the total number of washes required.

AATCC 61-2A is also acceptable within this program. All wash methods must follow the testing protocols written in the standard methods.



The performance of fabrics that require good durable odor control is usually validated by performing antimicrobial testing on SILVADUR™ fabrics that are washed according to the requested number of laundering cycles. Successful SILVADUR™ treatment is established when the treated fabric reduces the bacterial loading by at least 99% from the untreated fabric. Below are some examples of antimicrobial test reports after antimicrobial testing was completed. The tested fabrics below demonstrated successful SILVADUR™ treatment even after the treated fabrics were subjected to multiple laundering cycles. Bacterial reduction more than 99.9% was observed. The fabrics have successful SILVADUR™ treatment.

Example of Antimicrobial Test Results performed using ASTM E3160 - 2018 test method

The tested samples below demonstrated significant antimicrobial activity performance after multiple laundering cycles.

Type of material	100% Polyester
Antimicrobial agent	3% SILVADUR™ 960Flex
Application Process	Padding

Testing Parameters	
Method	ASTM E3160
Microorganism	<i>Escherichia coli</i> (ATCC # 25922)
Amount of test sample	0.4 gram
Contact time	24 hours
Initial bacterial inoculum	<i>E. coli</i> 2.36E+05
Nutrient dilution in inoculum	1:20
Wash protocol	ASTM E3162-18

Test Results	
Sample ID	Bacterial reduction from supplied control <i>E. coli</i>
Control- untreated after 40 washes	-
Silvadur Treated After 40 washes (8 cycles)	>99.9%

Example of Antimicrobial Test Results performed using AATCC 100 test method

The tested samples below demonstrated significant antimicrobial activity performance after multiple laundering cycles.

Type of material	85% Polyester/15% Cotton
Antimicrobial agent	2% SILVADUR™ 930Flex
Application Process	Exhaust

Testing Parameters		
Method	AATCC 100	
Microorganism	<i>Staphylococcus aureus</i> (ATCC 6538), <i>Klebsiella pneumoniae</i> (ATCC 4352)	
Amount of test sample	1.0 gram	
Contact time	24 hours	
Initial bacterial inoculum	<i>S. aureus</i> 1.52E+05	<i>K. pneumoniae</i> 2.44E+05
Nutrient dilution in inoculum	1:20	1:20
Wash protocol	ASTM E3162-18	

Test Results		
Sample ID	Bacterial reduction from supplied control <i>S. aureus</i> <i>K. pneumoniae</i>	
Control- non-treated after 20 washes	-	-
Treated sample A after 20 washes	99.6%	>99.9%
Treated sample B after 20 washes	>99.9%	>99.9%

4. Odor Sensory Test Method IACM 0710: Biofouling of textiles from milk

It is clear that by controlling or inhibiting the growth of bacteria, the odors that they produce can also be controlled. Odor generated by growing (metabolizing) bacteria on clothing is recognized by the consumer's sensory experience, and producing a reproducible, scientifically designed laboratory test method to mimic the wide variation in these sensory experiences has been challenging. There are several quantitative methods under development but there are no current industry standard test methods available that give a true measure of microbial odor production yet.

Bacteria metabolize certain proteins, fats, carbohydrates and other nutrients to generate odor, and cows' milk, which is rich in nutrients, is a good surrogate to generate fouling odor. Odor associated with bacterial growth found in the fouling of cows' milk as well as the biofouling of raw milk in the dairy industry is common and can occur over very short periods of time. This simple form of odor generation has been used in many instances as examples of odor control on textiles.

IACM 0710 is a qualitative method that measures the generation of odor due to the addition of Pasteurized cows' milk. **Odor is measured subjectively via human sensory odor panels and requires trained personnel to conduct the evaluation.**

This odor control test standard is only required when seeking Intellifresh™ branding Certificates of Compliance and must be conducted in an IAC laboratory.

Protocol

1. Cut 1 gram of both treated and untreated fabrics into 1.5 inch x 1.5 inch samples and place flat in separate sealable containers.
2. Apply 1 ml of Fat Free, Grade A, Pasteurized Cows' Milk (within expiration date) evenly directly onto the fabric sample making sure that all the milk gets absorbed into the fabric sample and NO milk gets deposited on the container.
3. Tightly seal containers and place in warm area, preferably in incubator set to 37° C.
4. After 24-48 hours, carefully remove the lid and rate presence of odor by smelling (see rating scale below).
5. A treated fabric is considered to pass the test if the rating after 48 hours is ≤ 3 .

Odors produced on untreated textiles can be extremely pungent. It is recommended to begin smelling the antimicrobial treated samples first before proceeding to the untreated control samples so as to not overwhelm the senses.

Note: All milk products will contain microorganisms at different stages of development and concentration. Because of the differences in initial concentration of bacteria, odor generation and differences between treated and untreated textiles may occur within 12 hours of incubation. In other cases, this differentiation may not occur until several days after inoculation. Odors must be measured periodically in order to determine the point at which differentiation occurs.

Example of the odor evaluation

Test Method: IACM 0710: Qualitative Evaluation for Odor/Stain Resistance of Antimicrobial Treated Articles

Test Results			
IAC #	Sample Description	24 Hrs. Rating	48 Hrs. Rating
5698-1	SILVADUR™ treated sample after 40 washes (ASTM E3162-18 – 8 cycles)	1	1
5698-2	Untreated sample	2.3	3.7
	IAC Standard Untreated Control	3	5

Rating Scale

1 = Faint Odor Present – Not Offensive

2 = Odor Present – Not Offensive

3 = Noticeable Odor – Somewhat Offensive

4 = Strong Odor – Offensive

5 = Very Strong Odor – Extremely Offensive