

Challenges of Antimicrobial Testing: Perspectives from the Field

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Senior Scientific Consultant
International Antimicrobial Council



What is the International Antimicrobial Council?

A not-for-profit organization dedicated to promoting the prudent and responsible use of antimicrobial agents and to the development and global harmonization of test methods and procedures that measure antimicrobial activity.





Where is the International Antimicrobial Council?

Regulatory Office

1629 K Street, Suite 300
Washington, DC 20006

Technology Center

7400 Bay Rd. Pioneer Hall 129
University Center, MI 48710





Who are Members of the International Antimicrobial Council?

- Leading Brands and Retailers
- Antimicrobial Supply Companies
- Third Party Testing Laboratories





What is the International Antimicrobial Council?

- Regulatory Claims and EPA Guidance
- Global Test Method Harmonization and Development
 - Test Laboratory Training and Certification





Where are the International Antimicrobial Council Certified Laboratories?



Who is Noble Biomaterials, Inc?

Advanced materials innovator

Textile heritage

Benefit-based technology solutions



VISION

develop

CONSUMER BENEFIT PLATFORMS

designed to

REMOVE BARRIERS

that prevent people from
achieving their potential

Presentation Agenda

Challenges of Antimicrobial Testing: Perspectives from the Field

- Complexities of Antimicrobial Agents
 - Complexities of Textile Processing
- Complexities of Antimicrobial Textile Testing

Examples of how controlling variables in processing and testing can ensure products consistently meet customer expectations

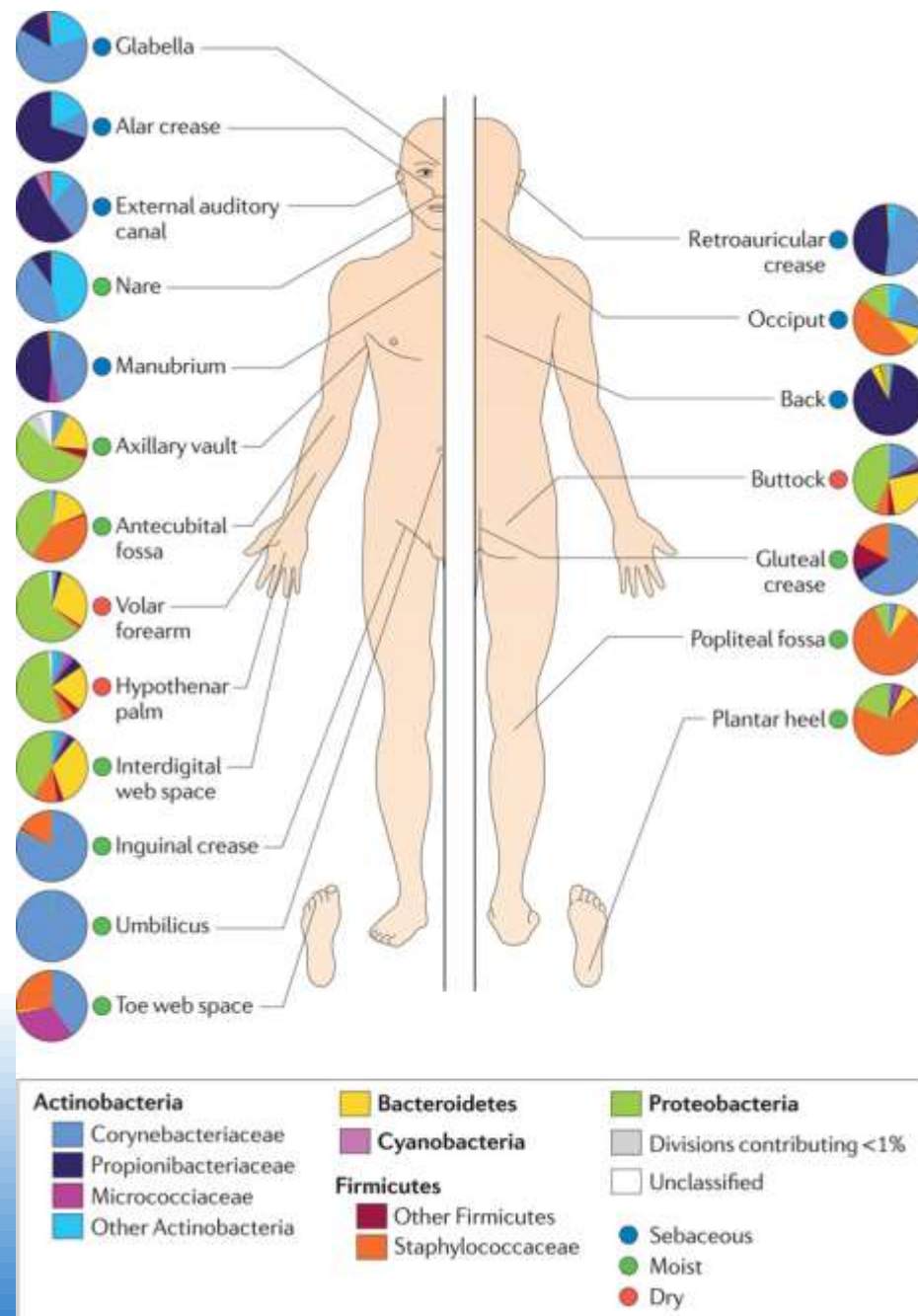
Odor controlling features are demanded by consumers

What is the science behind the stink?

Understanding the “Human Microbiome”

Understanding the “Clothing Microbiome”

The Human Microbiome

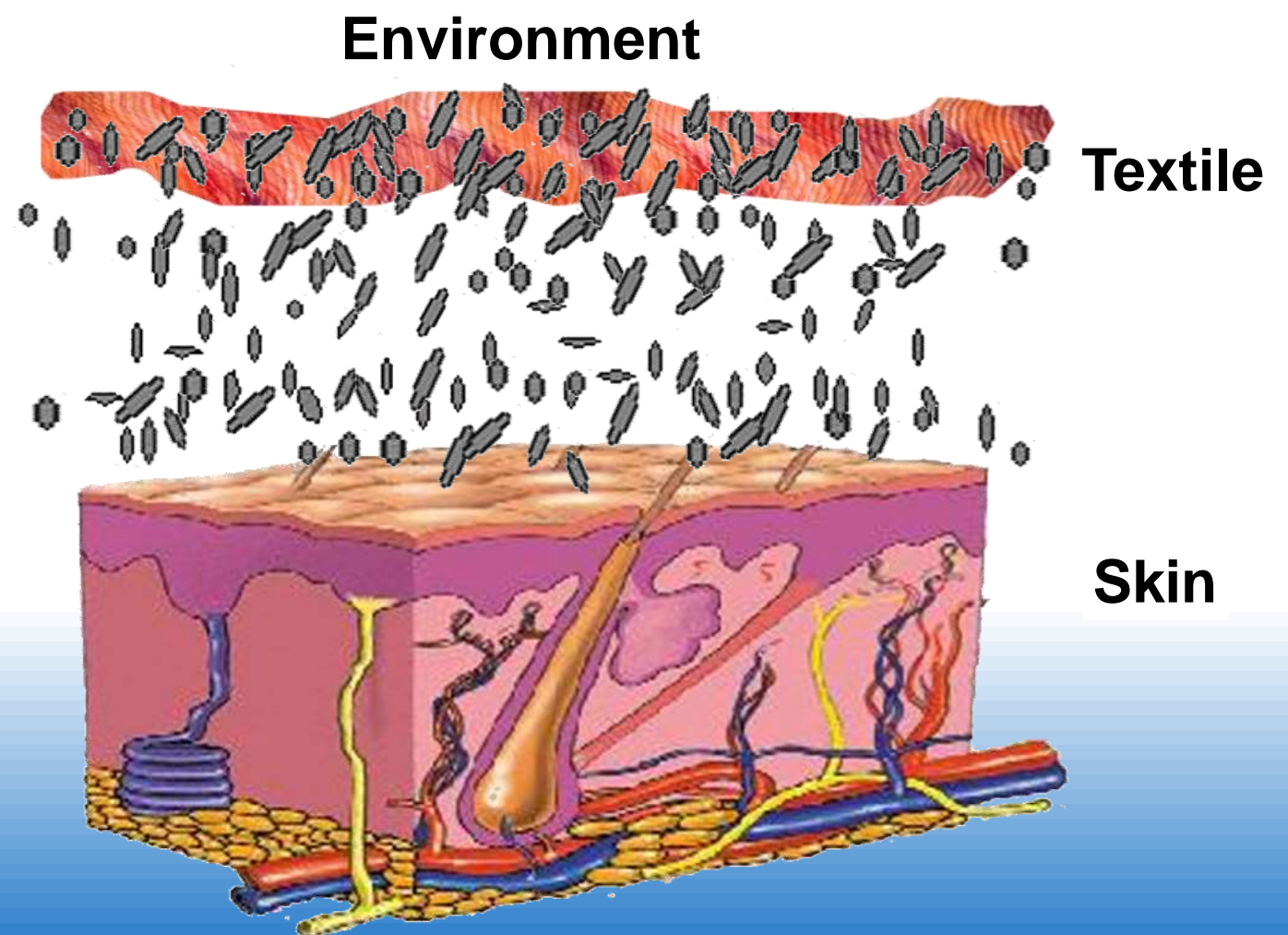


Frequency of the best studied skin microbes[4]

Organism	observations
<i>Staphylococcus epidermidis</i>	Common, occasionally pathogenic
<i>Staphylococcus aureus</i>	Infrequent, usually pathogenic
<i>Staphylococcus warneri</i>	Infrequent, occasionally pathogenic
<i>Streptococcus pyogenes</i>	Infrequent, usually pathogenic
<i>Streptococcus mitis</i>	Frequent, occasionally pathogenic
<i>Propionibacterium acnes</i>	Frequent, occasionally pathogenic
<i>Corynebacterium</i> spp.	Frequent, occasionally pathogenic
<i>Acinetobacter johnsonii</i>	Frequent, occasionally pathogenic
<i>Pseudomonas aeruginosa</i>	Infrequent, occasionally pathogenic

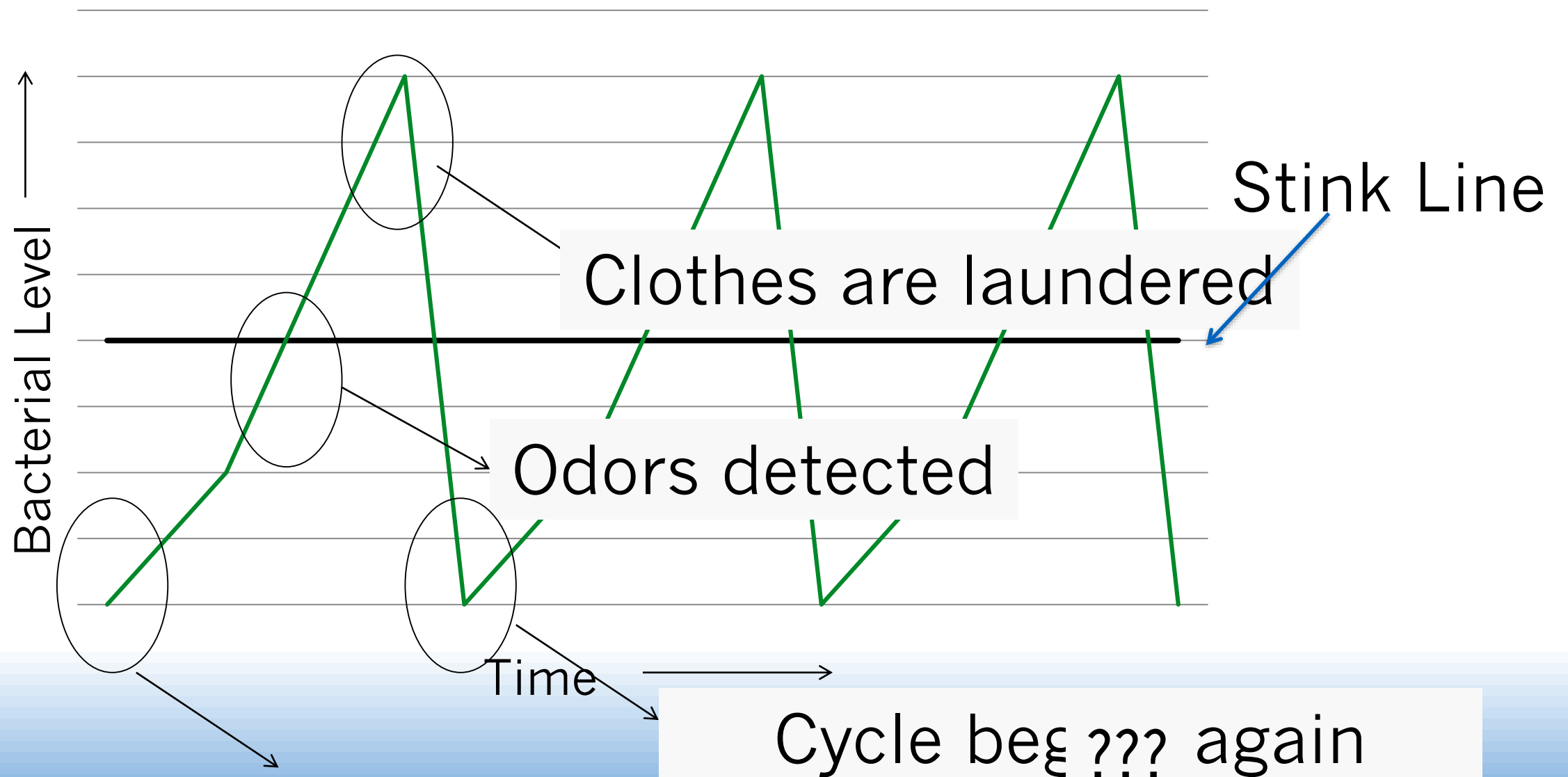
Microorganisms associated with the skin will transfer from the skin to the fabric creating a unique “Clothing Microbiome”

- Bacterial growth generates foul odors
- Textiles provide the perfect conditions for bacterial growth



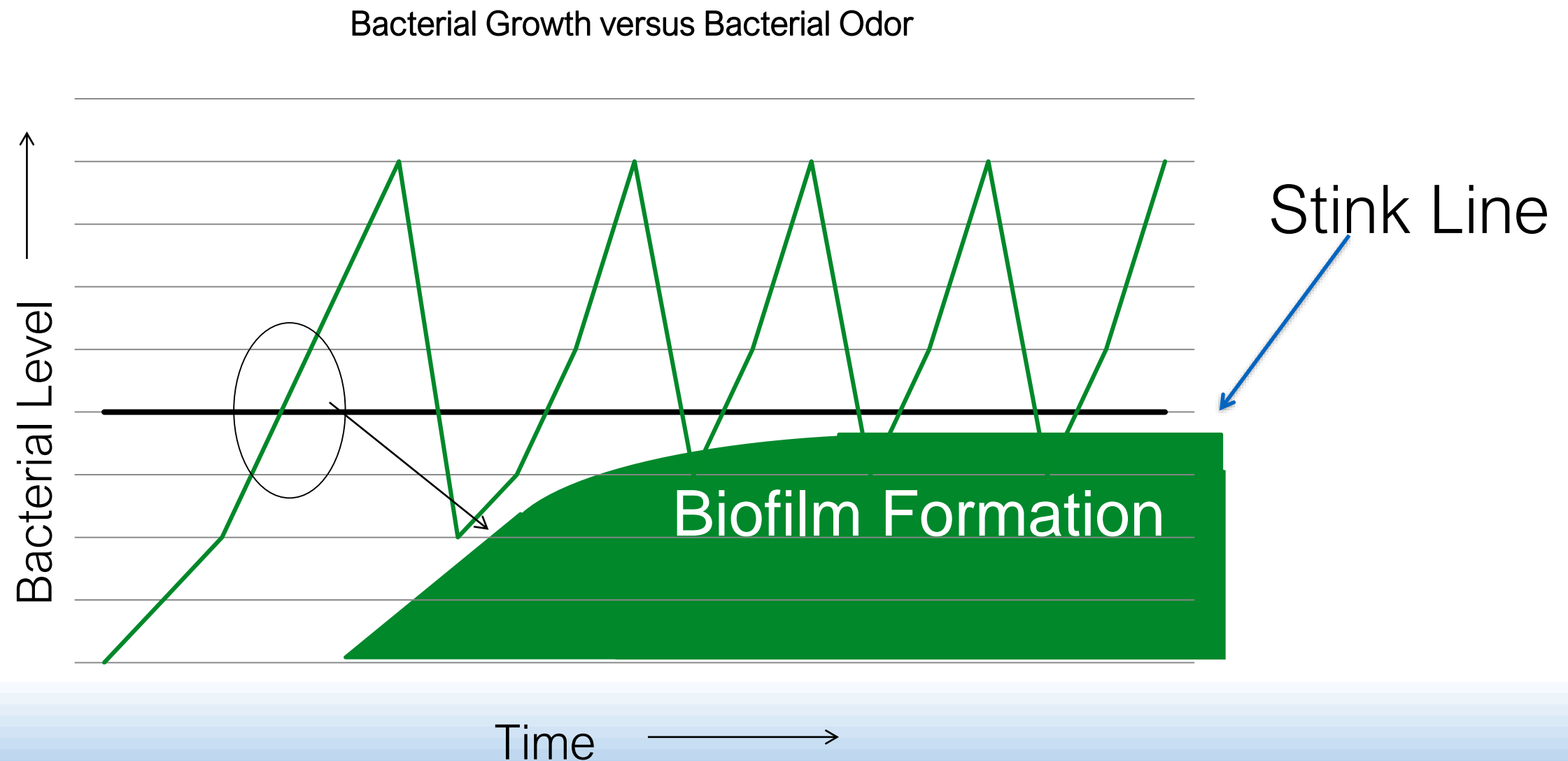
Development of the Clothing Microbiome

Bacterial Growth versus Bacterial Odor



Fresh Smell, Fresh Appearance

Development of the Clothing Microbiome



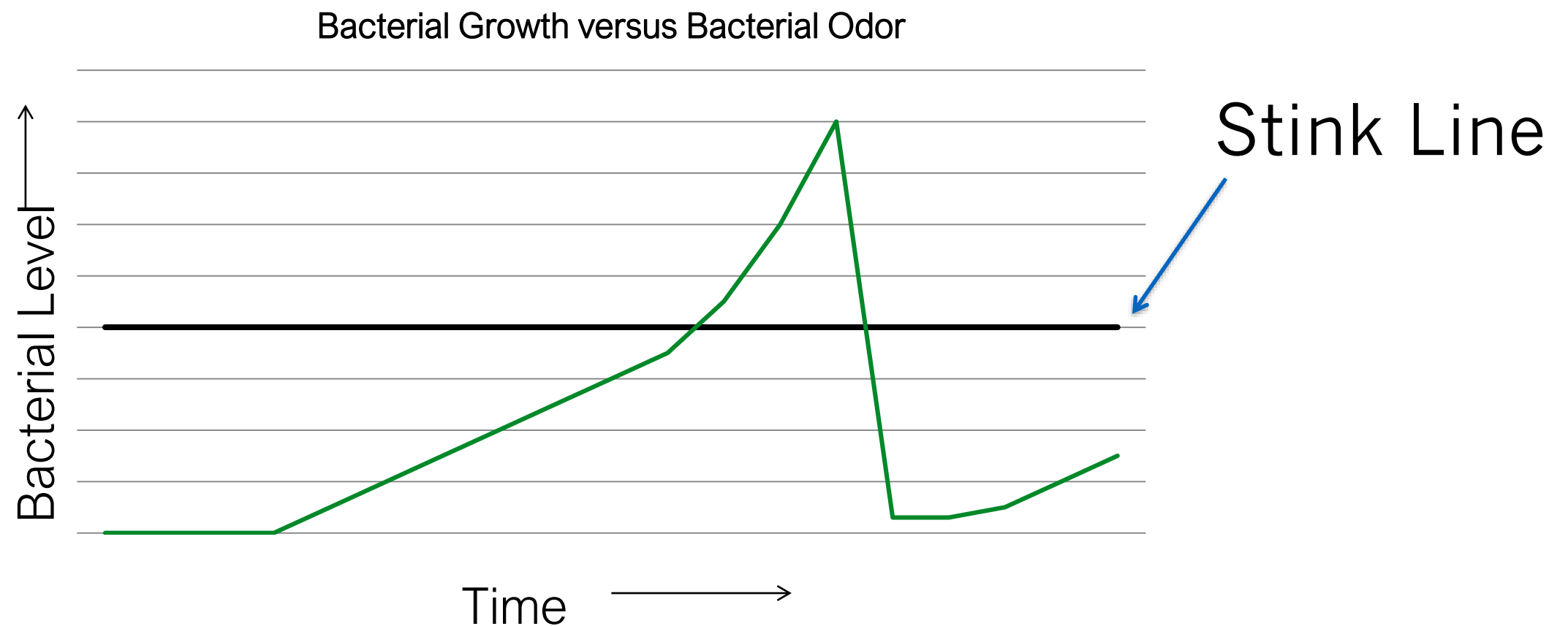
Never quite fresh as new



How can antimicrobial treatments in textiles help?



Controlling the Clothing Microbiome



Fresh Smell, Fresh Appearance
Use after Use



Complexities of Antimicrobial Agents

*What are the types of Antimicrobial Agents
Currently Available?*



Complexities of Antimicrobial Agents

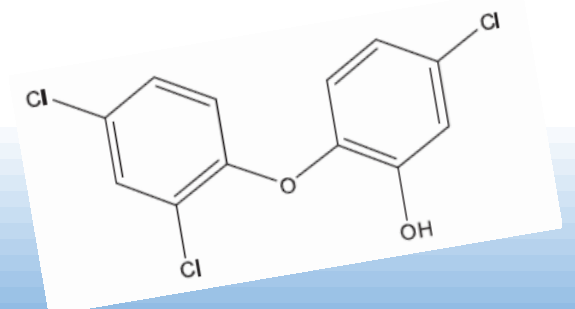
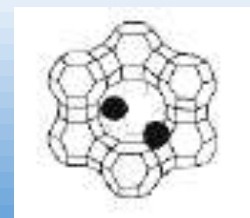
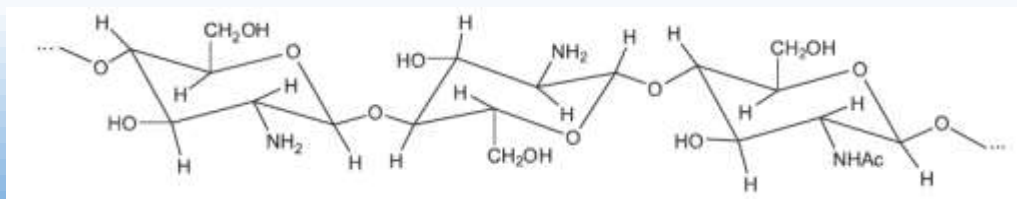
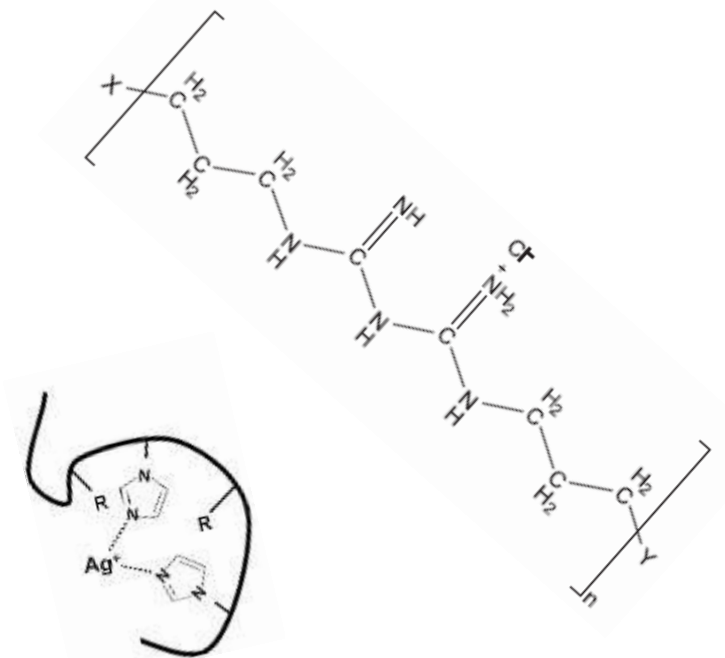
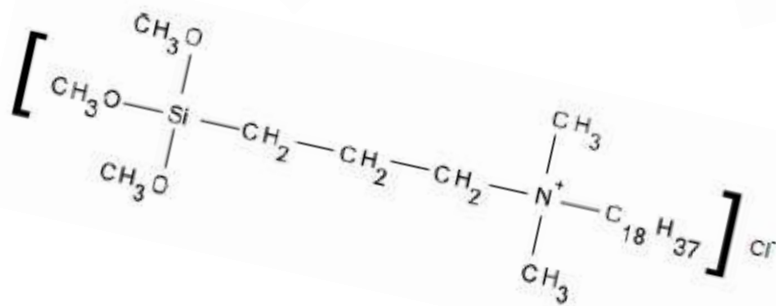
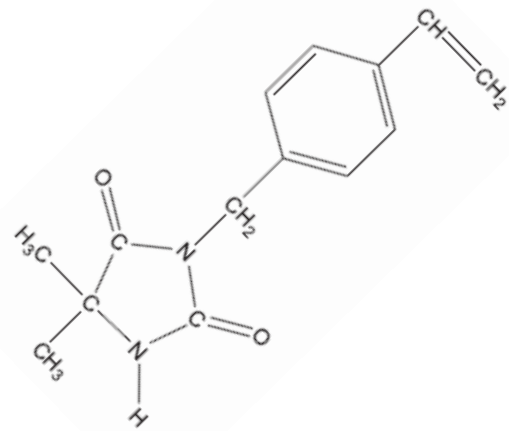
There are many choices for odor control.

particle based
nano-particle based
chemical based
polymer based



Complexities of Antimicrobial Agents

Silver particle
Silver polymer
Quat-Silane
Zinc Pyrithione
Biguanides
Chitosan
Copper
Triclosan
n-Halamines (Chlorine)



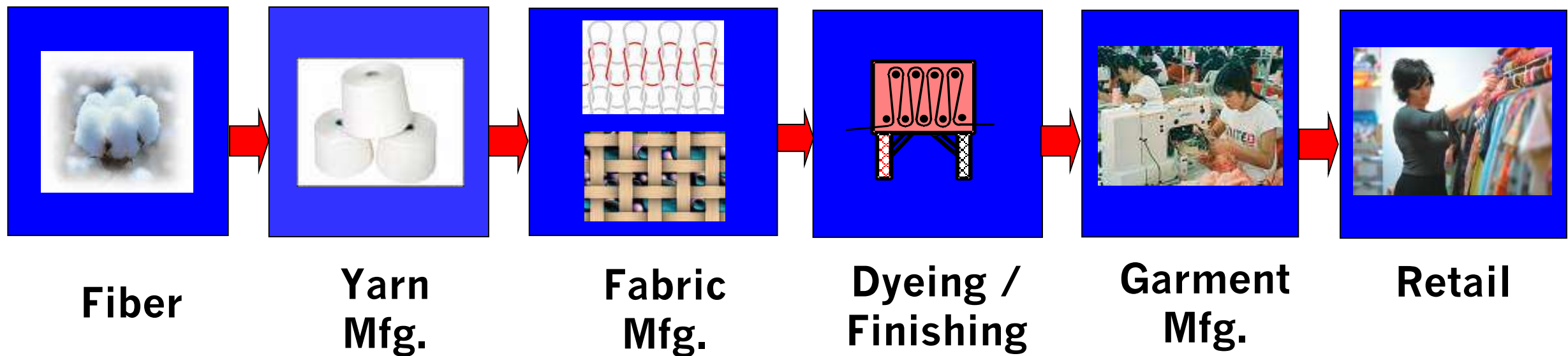
Challenge from the Field:

Many antimicrobial technologies available and all with unique modes of action, fabric compatibility and performance attributes!

Complexities of Textile Processing

What is involved in the manufacturing of Textiles?

Textile Pipeline



Processes



Process Variables

Time

Temperature

Chemistry

Potential Chemistry

wax gas fade inhibitor resin common salt
citric acid silicone softener anti-stat degreaser
nonionic peroxide stabilizer metal Glauber's salt
oil repellent cationic dye wetting agent caustic soda UV absorber
scour thickener reducing agent
acetic acid fixative hydrogen peroxide knitting oils amino functional silicone
detergent disperse dye leveling agent pH buffer
chelating agent lubricant binder pigment sodium hypochloride emulsifiers
Sequestering agent dispersant sodium hydrosulfite chlorine
oxalic acid wicking agent acid dye cationic softener starch retardant agent
softener soda ash defoamer PVA size enzyme water repellent
anti-staining agent soil repellent carrier reactive dye
pigment anionic

Challenge from the Field:

The manufacturing of textiles includes multiple processing steps with a variety of different chemistries!

Complexities of Antimicrobial Testing

There are so many different antimicrobial test methods, which one do I choose?

New and Developing Test Methods for Antimicrobial Treated Textile Articles



AATCC Test Standards

AATCC Test Method 100: Antibacterial Finishes on Fabrics, Evaluation of.

AATCC Test Method 147: Antibacterial Activity Assessment of Textiles materials: Parallel Streak Method





ASTM Test Guides and Standards

ASTM E 2149 Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions

ASTM E 2180 Test Method for Determining the Activity of Incorporated Antimicrobial Agents in Polymeric or Hydrophobic Materials

ASTM E2922-15 Standard Guide for The Use of Standard Test Methods and Practices for Evaluating Antibacterial Activity on Textiles





Common International Standards

JIS L 1902 - Testing for Antibacterial Activity and Efficacy on Textile Products

JIS Z 2801 - Antimicrobial Products: Test for Antimicrobial Activity and Efficacy

ISO 20743 Textiles - Determination of Antibacterial Activity of Antibacterial Finished Products

ISO 22196 Plastics - Measurement of Antibacterial Activity on Plastics Surfaces





Common International Standards

IBRG TEX13/005/1.0 – Quantitative Method for Evaluation
Bactericidal Activity of Textiles and Porous Materials and Articles



OECD – Guidance Document for Quantitative Method for Evaluating
Antibacterial Activity of Porous and Non-porous Antibacterial Treated
Articles (ENV/JM/MOMO(2014)18)



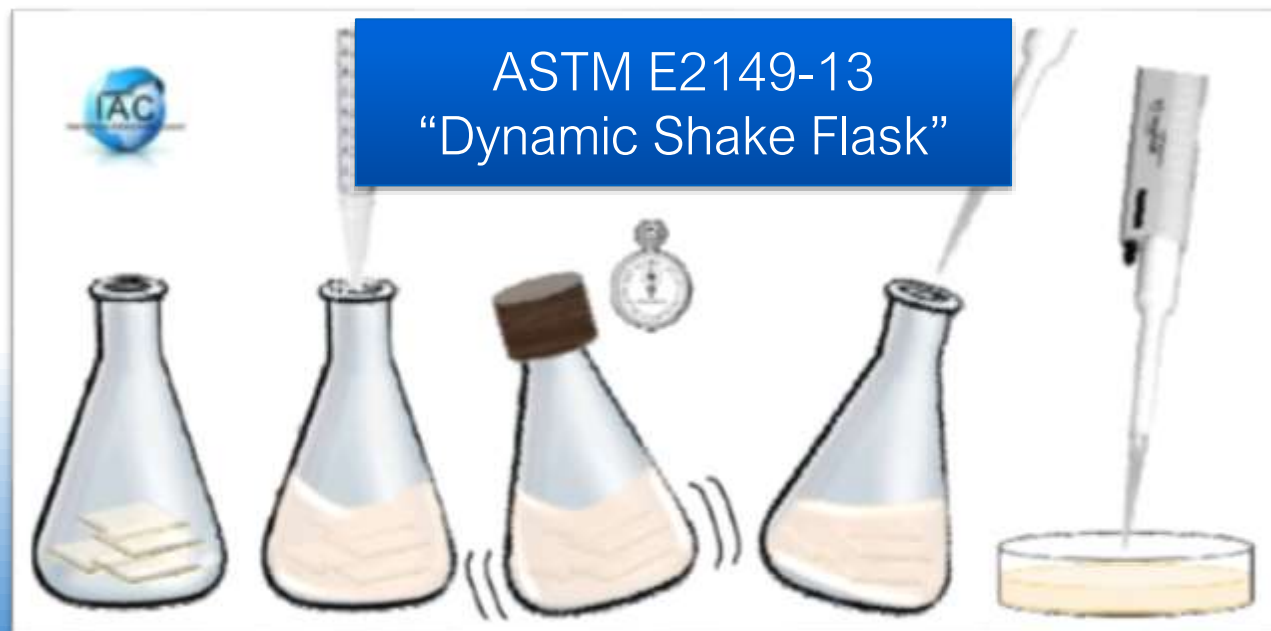
AATCC 147 “ZOI”



“Zone of Inhibition”

“Dynamic Shaking”

“Static Contact”



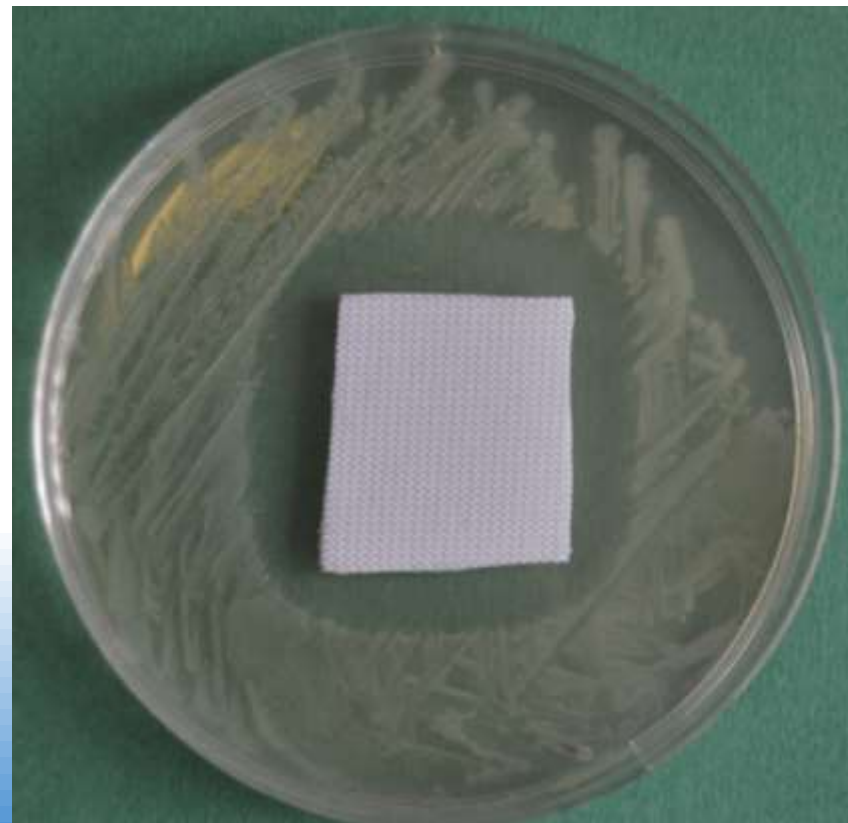
AATCC TM 147

Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method

Kirby-Bauer Antibiotic Testing



Modified Kirby-Bauer Fabric Testing

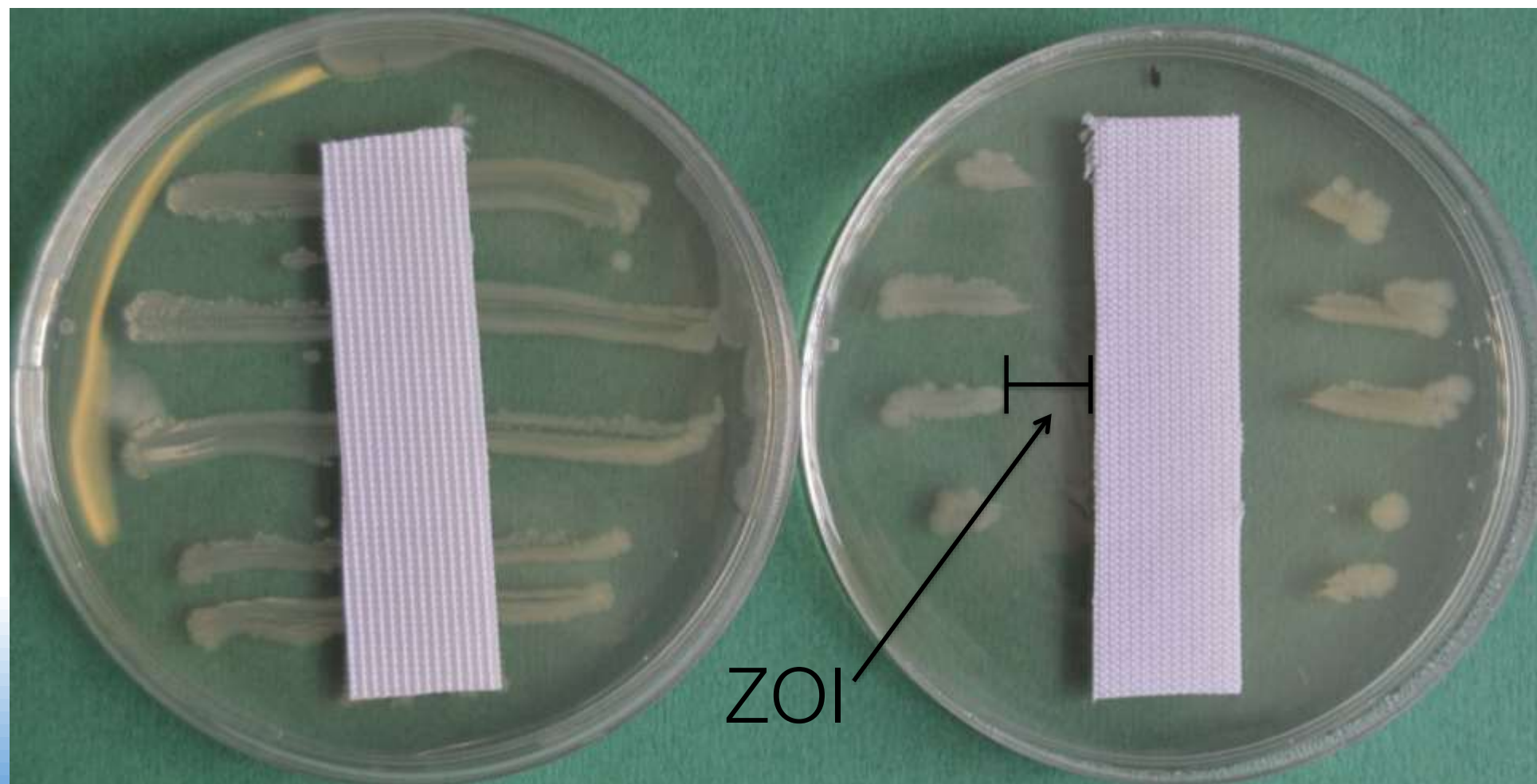


AATCC TM 147 Testing



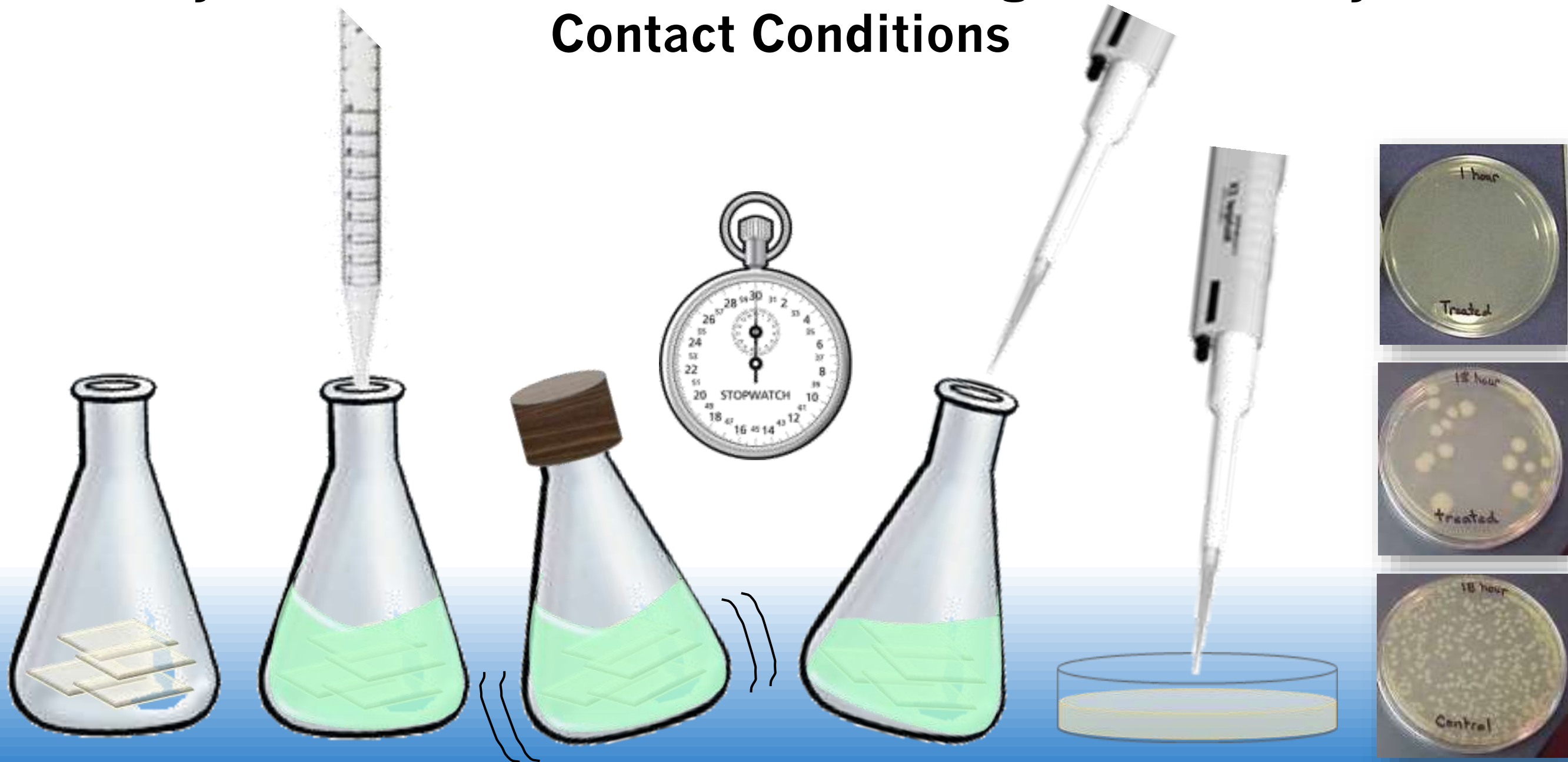
AATCC TM 147

Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method



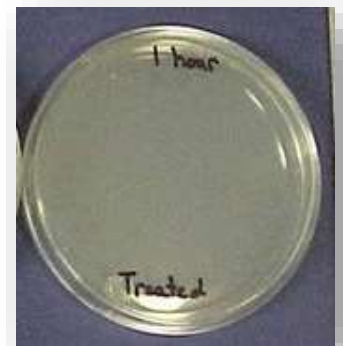
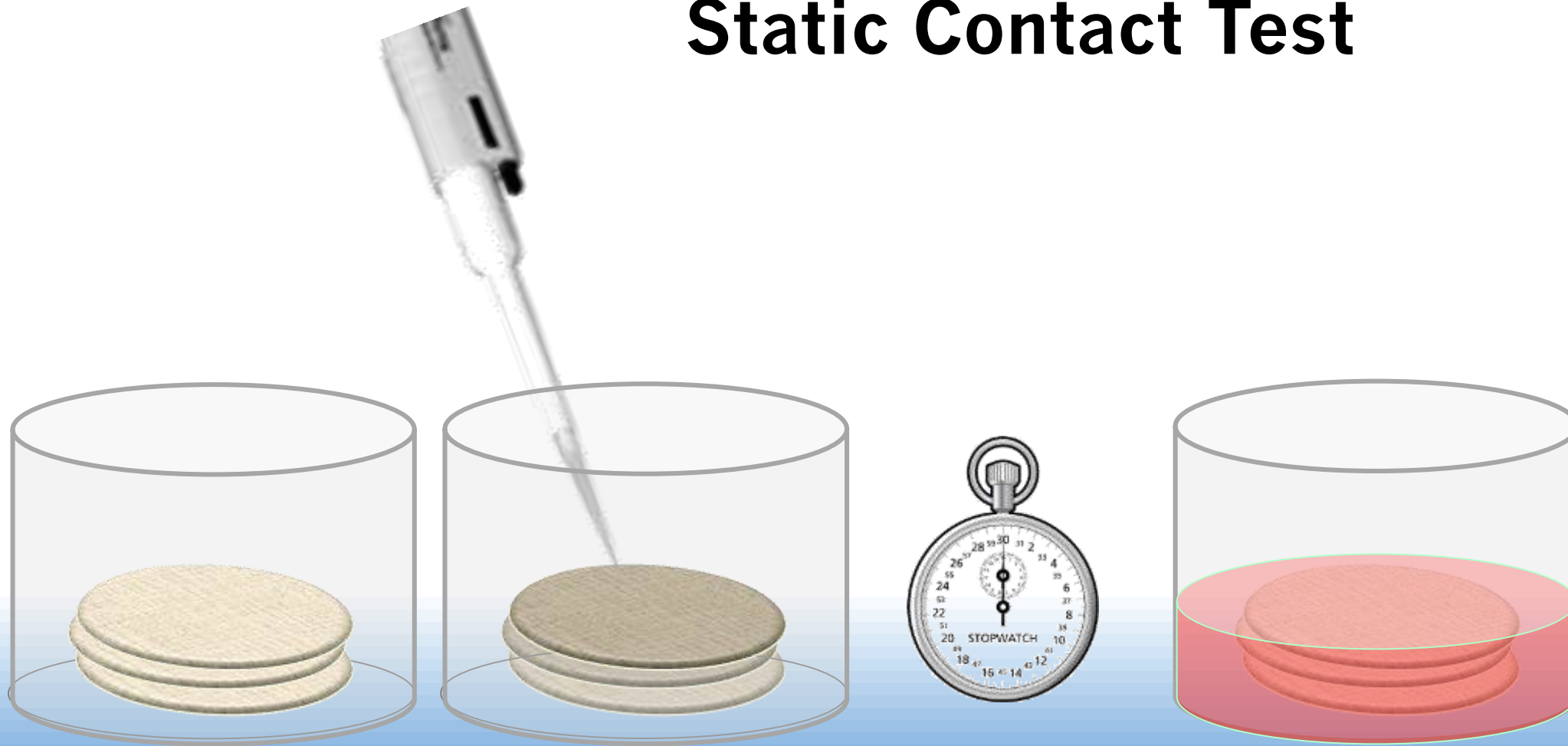
ASTM E2149-13

Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions



AATCC TM 100, JIS L1902, ISO 20743

Static Contact Test



Challenge from the Field:

**Many test methods available and
many different ways to run them!**

Presentation Agenda

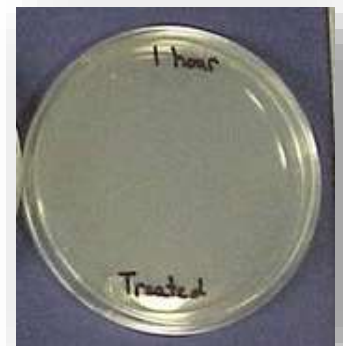
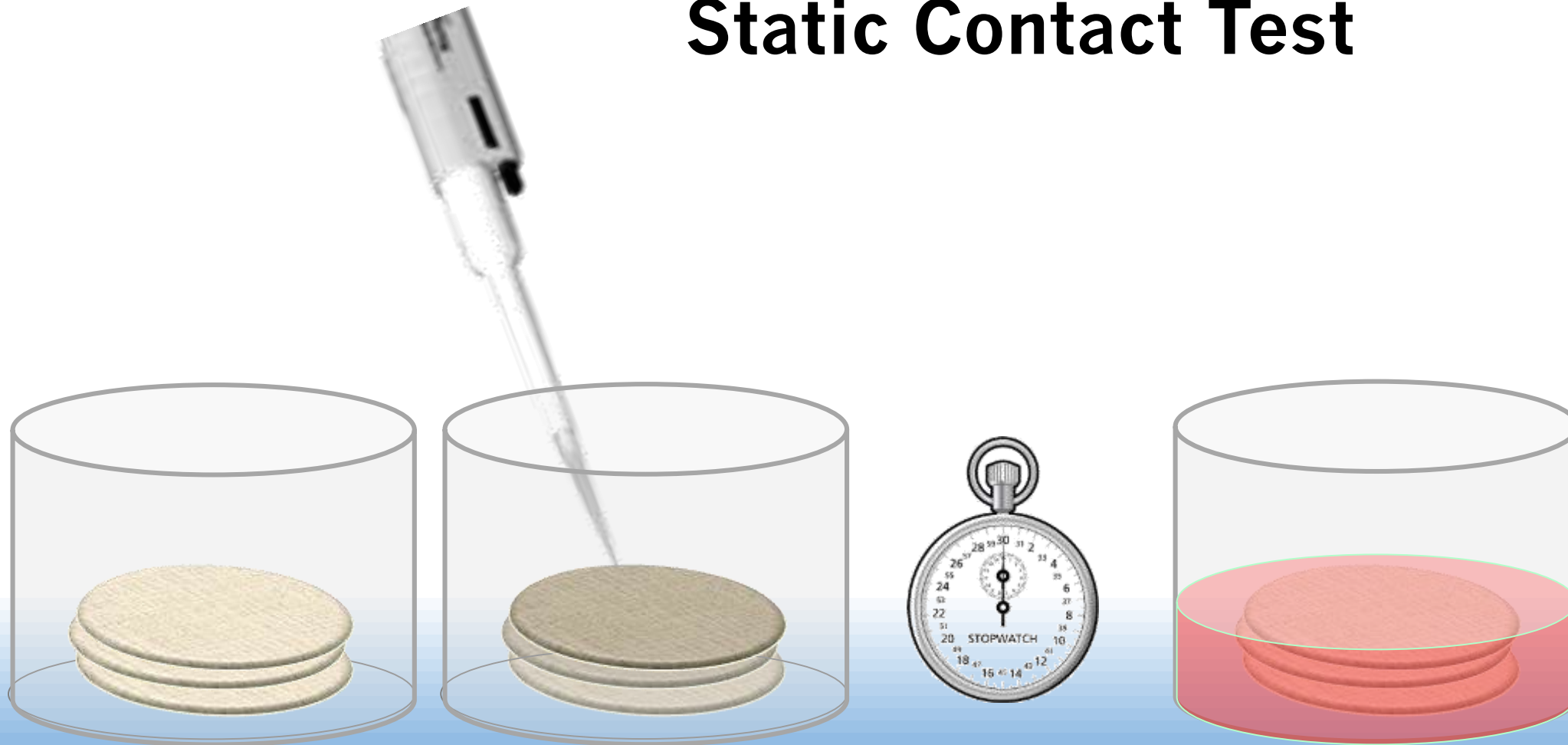
Challenges of Antimicrobial Testing: Perspectives from the Field

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 - Complexities of Textile Processing
- Complexities of Antimicrobial Textile Testing

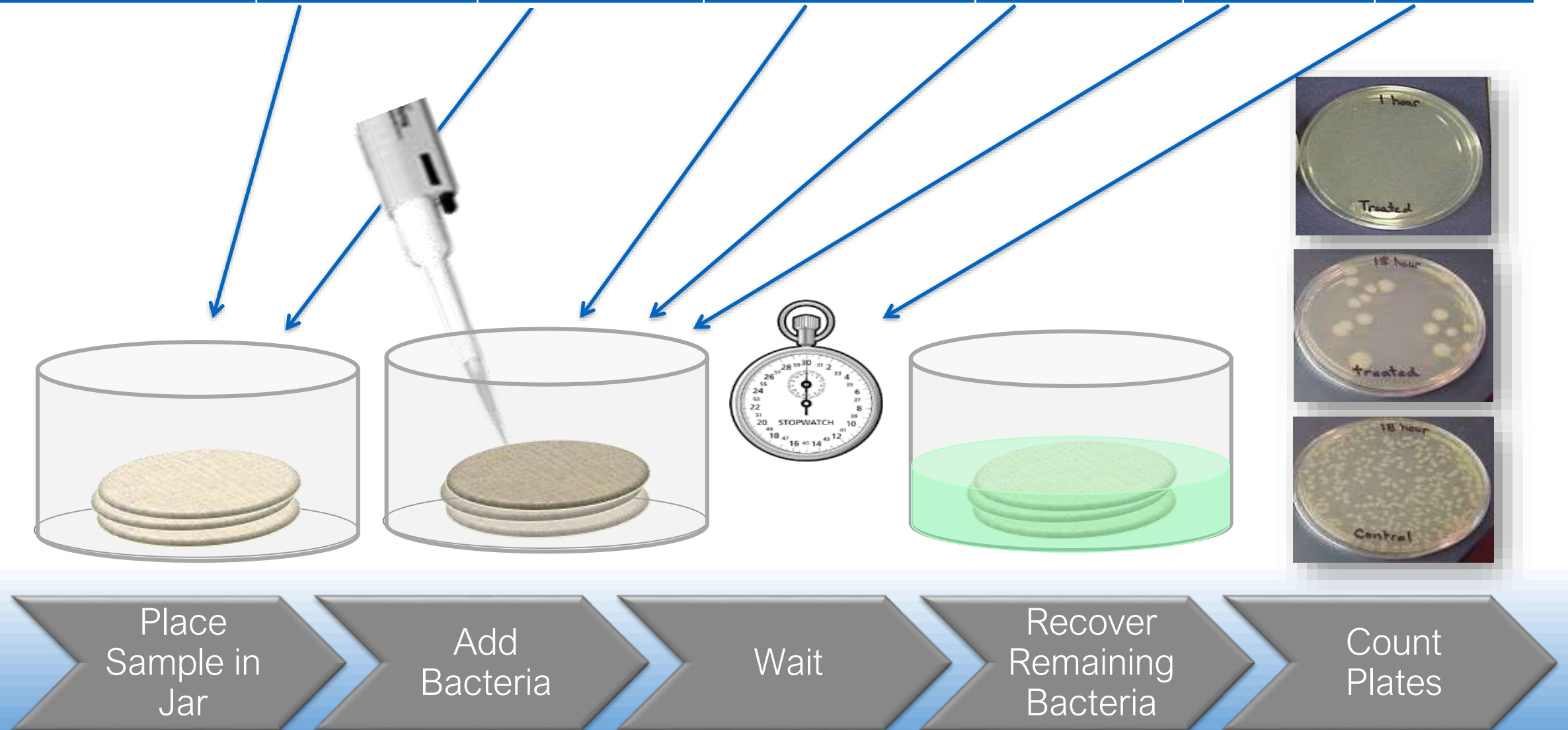
Examples of how controlling variables in processing and testing can ensure products consistently meet customer expectations







AATCC TM 100, JIS L1902, ISO 20743







Static Contact Test



VARIABLES	Sterilization of Test Samples	Size of Test Sample	Specified Organism Type and Concentration	Amount of Nutrient in Bacterial Inoculum	Inoculum volume	Contact Time
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	Sterilization of Test Samples	Size of Test Sample	Specified Organism	Amount of Nutrient in Bacterial Inoculum	Inoculum volume	Contact Time
AATCC100 	Optional	Variable depending on sample absorption	<i>Staph. aureus</i> <i>K. pneumoniae</i>	0% or 100%	1 ml	18-24 hours
ISO 20743/JIS L1902  	Optional	0.4 gram	<i>Staph. aureus</i> <i>K. pneumoniae</i>	5% NB (1:20)	0.2 ml	18-24 hours
IBRG TEX13/OECD TFB 	Not recommended	0.4 gram	<i>Staph. aureus</i> <i>Escherichia coli</i>	0.2% NB (1:500)	0.2 ml	24 hour
ASTM E2149-13 	None	1.0 gram	<i>Escherichia coli</i>	0%	50 ml	1-24 hours
(ASTM E35.15 wk #45351) 	None	0.4 gram	<i>Escherichia coli</i>	0.2% NB (1:500)	0.2 ml	24 hour

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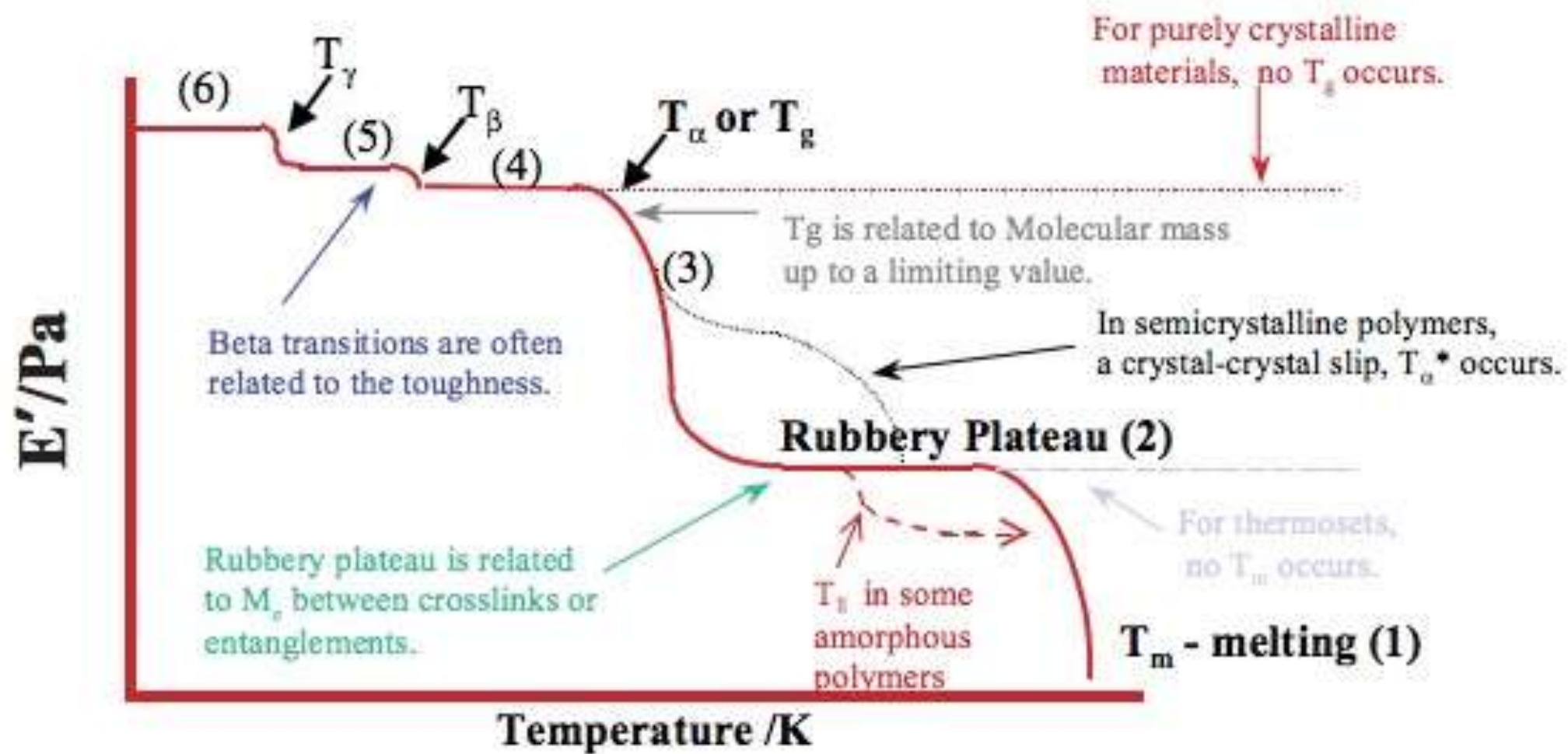
Challenge from the Field:

Sterilization of Test Samples
“Autoclaving”

*How does this affect the fabric and the
microbiological results?*

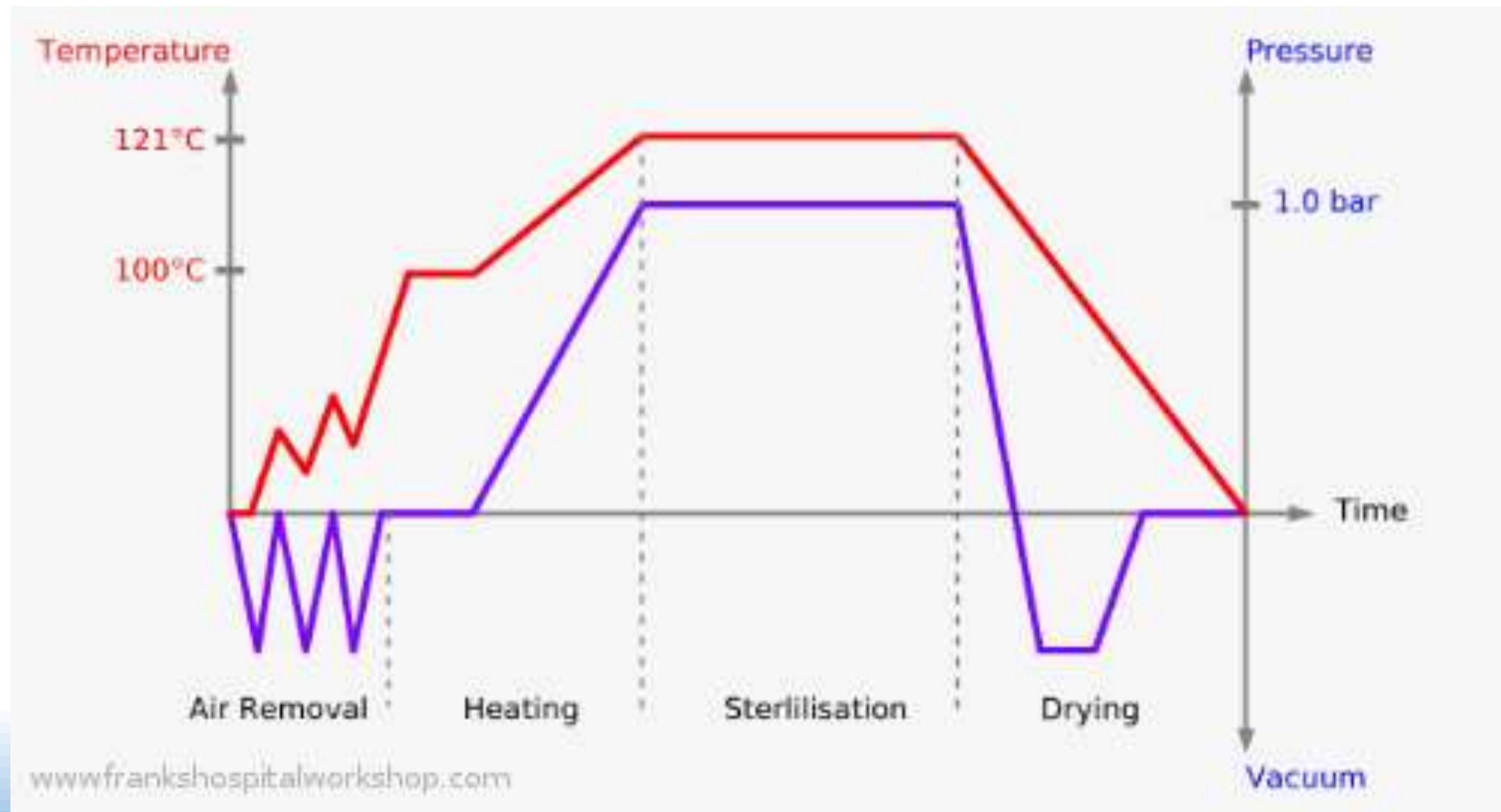
Transition Temperatures

Polyester

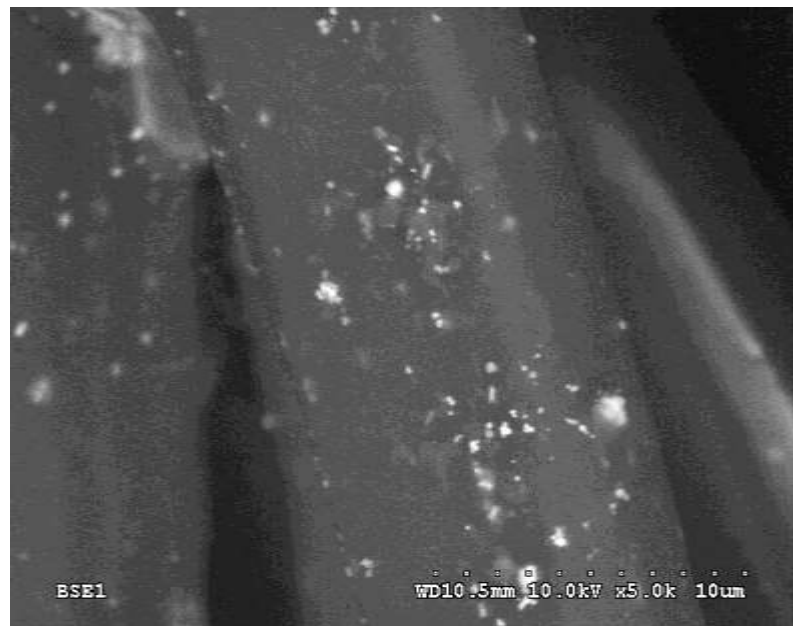


- | | | | | | |
|---------|---------|--------|---------|-------|----------|
| (6) | (5) | (4) | (3) | (2) | (1) |
| local | bend | side | gradual | large | chain |
| motions | and | groups | main | scale | slippage |
| | stretch | | chain | chain | |

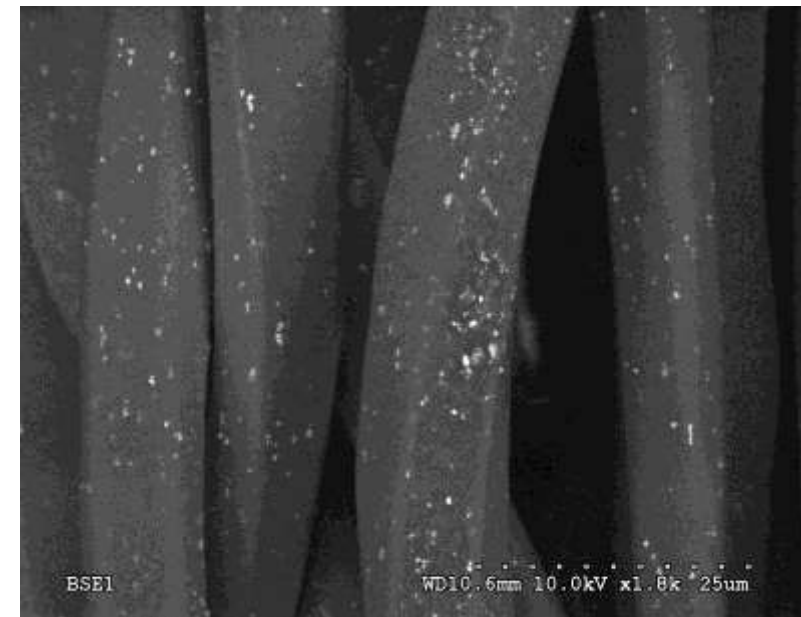
Typical Autoclave Process



Scanning Electron Microscope (SEM) Analysis



**Before
autoclave**

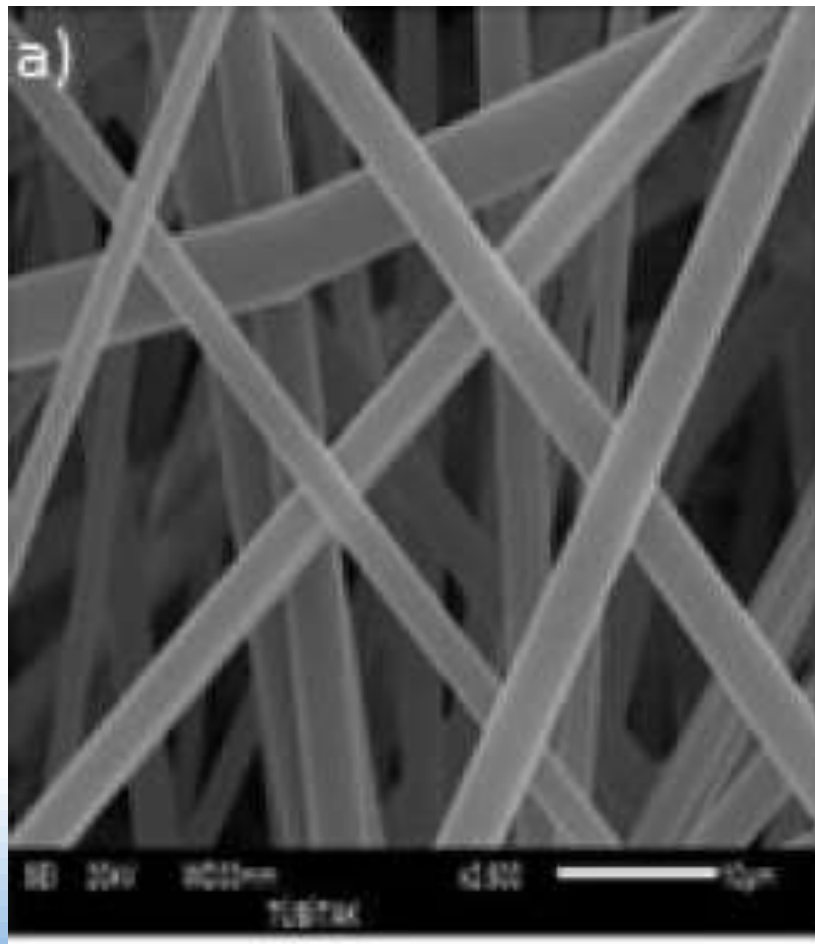


**After
autoclave**



Scanning Electron Microscope (SEM) Analysis

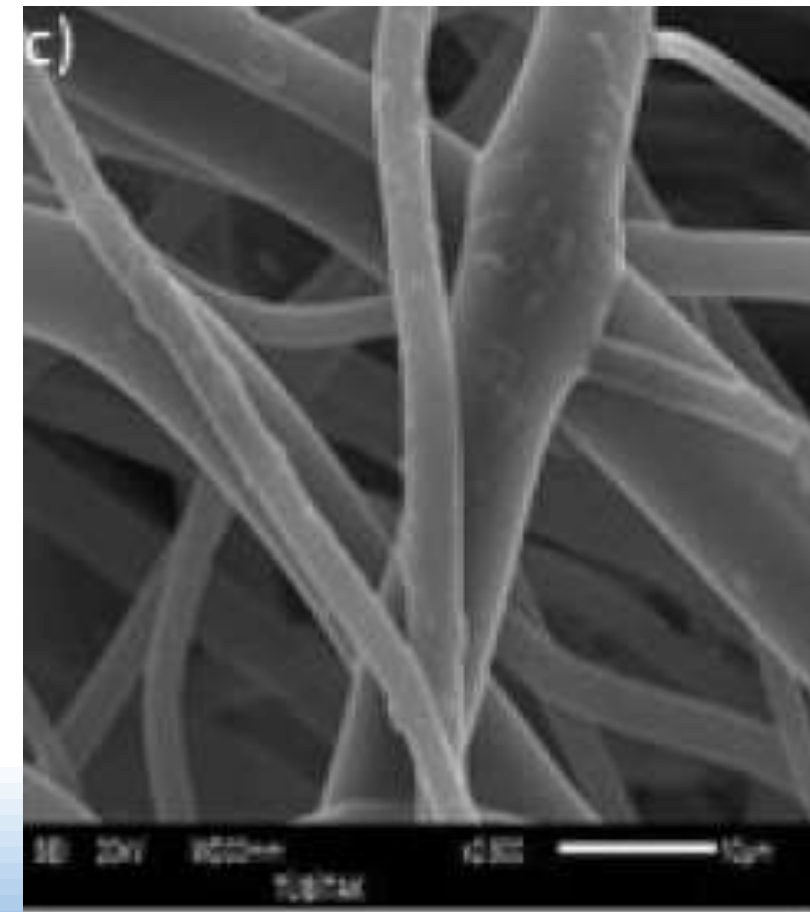
**Before
autoclave**



**Polyester
Nanofibers**



**After
autoclave**



**EFFECTS OF DIFFERENT STERILIZATION METHODS
ON POLYESTER SURFACES**

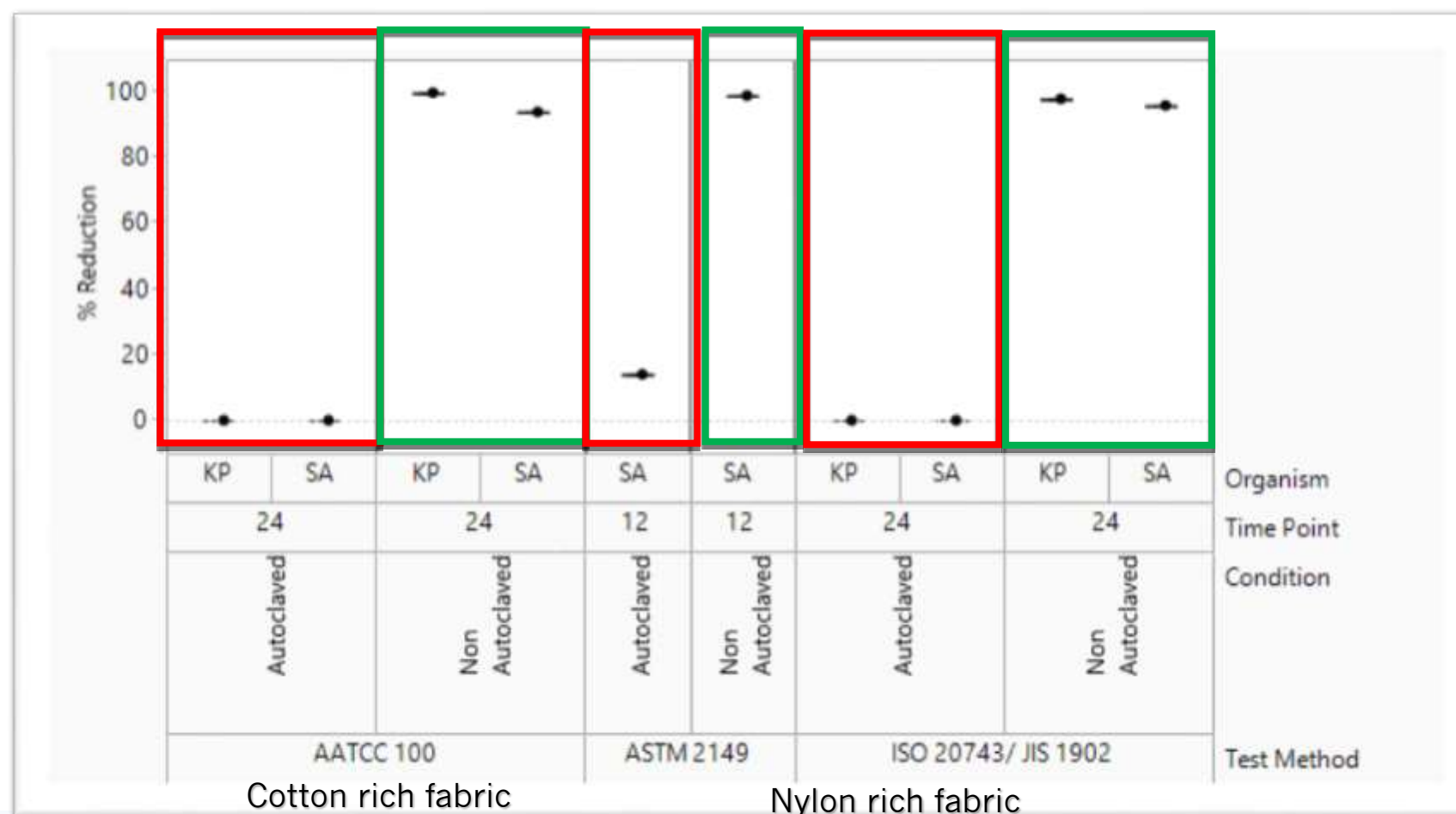
TEKSTİL ve KONFEKSİYON 23(4), 2013

Challenge from the Field:

The autoclaving of textiles significantly alters the physical and chemical composition of the textile

How does this affect the fabric and the microbiological results?

Antimicrobial Activity before and after Autoclave



Autoclaving textile fabrics prior to testing can completely destroy the ability to detect the antimicrobial performance

Summary

- **The need and desire for the addition of antimicrobial agents to textiles for the prevent of odor formation is clearly recognized by the industry**
- **Processing of textiles is very complex with many moving parts. Great care must be taken to understand these potential interactions that might affect the antimicrobial treatment, the application process and the testing of the final product.**

Summary

- **Autoclaving (steam sterilization) subjects the substrate to high pressure, temperature and moisture**
- **Polyester is very susceptible to these high temperatures as are many of the chemistries applied to them.**
- **Resulting antimicrobial test results may not reflect to true antimicrobial performance of the treated fabric due to the autoclave step.**

IAC Testing Recommendations

- To test for the antimicrobial activity of any fabric treated with a properly registered antimicrobial agent, samples **SHOULD NOT** be steam sterilized prior to testing.



IAC Testing Recommendations

- **Only use IAC Trained and Certified Test laboratories**

For a list of IAC Certified laboratories in your area, please contact the IAC
(www.amcouncil.org)

- **Only accept test reports that contain a properly validated IAC Verification number**





SGS

IAC
Certification Laboratory

IAC Certification number: 090012016

Test Report Report No: ASH17-004883-01 Date: Mar 01 2017

Client name: shanghai higcol new material sci&tech development co.,ltd
Client address: room 703,bd3,no.245 jiaochuan rd,shanghai
Sample name: Higcol Mylon Group Mylon
Sample Batch No.:
Product Date:
Manufacturer:
IAC Verification number*: 095-01-00009

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

SGS Sample No.: ASH17-004883-001
SGS reference No.: SL517051926770TX
Date of sample received: Feb 16 2017
Testing period: Feb 16 2017 - Mar 01 2017

TEST(S) REQUESTED:
Selected test(s) as requested by applicant:
Antimicrobial activity test for the submitted sample after 4 cycles (=20 washes; 1 cycle=5 washes)

TEST METHOD(S):
Please refer to the next page(s)

TEST RESULT(S):
Please refer to the next page(s)

Unless otherwise stated the results shown in this test report refer only to the sample(s) tested.
This document cannot be used for publicity, without prior written approval of the SGS.

SGS Authorized Signature

SGS-CSTC Standards Technical Services (Shanghai) Co., Ltd.
Page 1 of 3



Test Verification Number

Allows traceability of all microbiological testing





Test Verification Number

Allows traceability of all microbiological testing

Resinnova LABORATORIES

May 30, 2018

To: Robert
Intern
1629
Wash

From: Matthew
Resinnova
7400
Takoma

Dear Dr. Moore,

Enclosed you will find a report of the antimicrobial testing performed on the fabric samples you provided. The results show that the fabric samples have a high level of antimicrobial activity, which is consistent with the results of the previous testing.

Within this report, you will find a detailed description of the testing procedure, the results of the testing, and a discussion of the results. The report also includes a table of the results, which shows the antimicrobial activity of the fabric samples at different time points.

Sincerely,
Matthew Harlan
President and CEO

Resinnova LABORATORIES

Introduction

The WK #45351 Fabric microbial pathogens. The representative test organism was neutralized and quantified. The survivors are enumerated compared to a population.

Abstract

This study was performed to determine the antimicrobial activity of the fabric samples over time. To this end, the fabric samples were incubated with *E. coli*.

Materials and Method

For each organism, one plate into 10 ml Tryptic orbital shaker at 37°C. The density was determined via spectrophotometer at 600 nm (with an optical density of 0.5). The inoculum solution.

For each time point, the 0.05g swatch was placed in a solution of 100.00% for Fabric #2, 100.00% for Fabric #4, and 100.00% for Fabric #5.

Dilutions were then made (estimated to yield between 10⁵ and 10⁶ CFU/ml) and colonies were counted. Analysis and calculation.

Noted Exceptions to the

- The viable count was on-target, at 6.1

Observations

When placing each fabric sample in the incubator, the curvature of the fabric was kept consistent with the previous testing.

As indicated in section 2.1, the number of colonies should not be more than 3 logs higher than the previous testing.

Results

Comparison of data from the previous testing and the current testing shows a significant reduction in bacterial burden over time for all fabric samples.

Conclusions

Of the provided fabric samples, the fabric samples with the best antimicrobial activity were the fabric samples with the highest level of antimicrobial activity.

Figure 1: Bacterial Burden of *E. coli*

IAC Testing

Sample	Initial Burden (CFU/g)	Final Burden (CFU/g)
Sample #1	1.0 x 10 ⁶	1.0 x 10 ⁵
Sample #2	1.0 x 10 ⁶	1.0 x 10 ⁵
Sample #3	1.0 x 10 ⁶	1.0 x 10 ⁵
Sample #4	1.0 x 10 ⁶	1.0 x 10 ⁵
Sample #5	1.0 x 10 ⁶	1.0 x 10 ⁵

IAC International Antimicrobial Council
Certified Laboratory

IAC International Antimicrobial Council
VERIFIED
20154372





Where are the International Antimicrobial Council Certified Laboratories?



Thank you for your attention

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Robert (Bob) A. Monticello, Ph.D.

International Antimicrobial Council

ramphd@amcouncil.org

