



**MICROCHEM**  
L A B O R A T O R Y

## STUDY REPORT

### Study Title

Antimicrobial Efficacy of Respilon Textile Materials Modified  
for Viruses

### Test Method

American Association of Textile Chemists and Colorists Method 100  
Assessment of Antibacterial Finishes on Textile Materials

### Study Identification Number

NG7125

### Test Facility

Microchem  
Laboratory 1304 W.  
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Rock, TX 78681  
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## AATCC 100: General Information

The American Association of Textile Chemists and Colorists (AATCC) is a well established non-profit organization that provides education, develops test methods, and sets standards for the textile industry. The AATCC method 100 is a quantitative test method designed to assess the performance of antimicrobial finishes on textiles. It can be conducted using contact times ranging from ten minutes up to 24 hours. For an AATCC 100 test, non-antimicrobial control textiles are used as the baseline for calculations of microbial reduction. The method is versatile and can be used to determine the antimicrobial activity of a diverse array of porous materials in addition to textiles. Because the method allows a great degree of latitude with regard to how the procedure is carried out, some scientists consider it to be more similar to a testing guideline than a test method.

## Laboratory Qualifications Specific to the AATCC 100

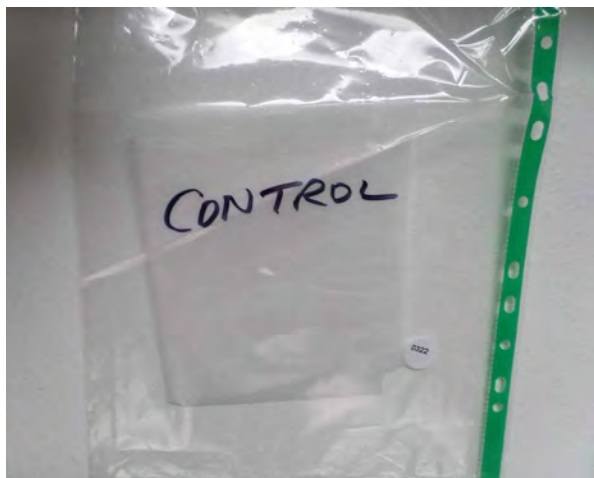
Microchem Laboratory began conducting the AATCC 100 test method in 2007. Since then, the laboratory has performed thousands of AATCC 100 tests on a broad array of test substances, against a myriad of bacterial, fungal, and viral species. The laboratory may also modify the AATCC 100 test method as needed in order to accommodate customer needs. Every AATCC 100 test at Microchem Laboratory is performed in a manner appropriate to the test substances submitted by the Study Sponsor, while maintaining the integrity of the method.

## Study Timeline



## Test Substance Information

The test substances were received on 26 APR 2016 and the following pictures were taken.

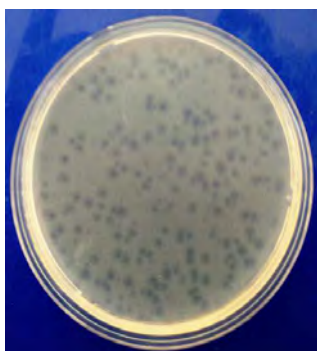


Test Substances Received: **Control, RESPILON NW16004-08 Polypropelene, Non-Woven with accelarated copper oxide**

Test Substances arrived in dimensions that were not optimal for the conduct of the Study. Test substances were cut down to ideal sizes for the Study.

## Test Microorganism Information

The test microorganism(s) selected for this test:



### **MS2 Bacteriophage (MS2), ATCC 15597-B1**

This virus is a non-enveloped positive-stranded RNA virus of the bacteriophage family Leviviridae. Bacterial cells are the hosts for bacteriophages, and *E. coli* 15597 serves this purpose for MS2 bacteriophage. Its small size, icosohedral structure, and environmental resistance has made MS2 ideal for use as a surrogate virus (particularly in place of picornaviruses such as poliovirus and human norovirus) in water quality and disinfectant studies.

**Permissive Host Cell System for MS2: *Escherichia coli*, 15597**

## Diagram of the Procedure



## Summary of the Procedure

- Stock virus is thawed and standardized to prepare a test inoculum. The test inoculum supplemented with an organic soil load, if requested.
- Test and control materials cut into appropriately-sized swatches and stacked. The number of swatches per stack is that which is required to absorb the entire liquid inoculum. Alternately, test and control materials are cut to achieve a mass requested by the Study Sponsor (e.g. 1 g), and tested in a single layer.
- Test and control materials are inoculated with the test virus, and incubated in a humid environment at room temperature for the determined contact time.
- An additional control is implemented to verify neutralization effectiveness of the antimicrobial agent.
- The viral concentration is determined at “Time Zero” to verify the target inoculum.
- Following neutralization, the carrier suspensions are quantified to determine the levels of infectious virus using standard cell culture (e.g. TCID<sub>50</sub>) or plaque assay techniques.
- Assay trays/plates are incubated for the period most suitable for the virus-host cell system (e.g. 7 days).
- After the incubation period, the assay is scored for the presence/absence of test virus and cytotoxic effects. The appropriate calculations are performed (e.g. Spearman-Kärber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- Log<sub>10</sub> and percent reductions are computed for test surfaces relative to the Time Zero enumeration(s), and reported to the Study Sponsor.

## Criteria for Scientific Defensibility of a Modified AATCC 100 Study

For Microchem Laboratory to consider a modified AATCC 100 virus study to be scientifically defensible, the following criteria must be met:

1. The average number of infectious virus recovered from the time zero and parallel control samples must be a minimum of 4-Log<sub>10</sub>.
2. Viral cytopathic effects are distinguishable from cytotoxic effects caused by test material exposure.
3. Effectiveness of the neutralization method is demonstrated.
4. Assay wells/plates designated as sterility controls are absent of infectivity, contamination, and cytotoxicity (if applicable).

## Passing Criteria

AATCC does not specify performance criteria, therefore it may be established by the Study Sponsor. A similar test method, ISO 20743, recommends a 2-Log<sub>10</sub> or 99% reduction. The United States Environmental Protection Agency (US EPA) often recommends a 3-Log<sub>10</sub> or 99.9% reduction. Federal regulatory agencies such as the US EPA specify the following passing criteria for virucidal efficacy:

Complete inactivation of the test virus at all dilutions.

If cytotoxicity is observed, a  $\geq 3$ -Log<sub>10</sub> reduction in viral titer is observed past the level of cytotoxicity relative to the virus control.

## Testing Parameters used in this Study

Test Substance Swatch Size:	4.8 cm diameter	Number of Swatches per Stack:	10
Replicates:	One		
Viral Inoculum Volume:	1.0 ml	Target Inoculum:	5-log <sub>10</sub> /carrier
Dilution Medium:	PBS	Soil Load:	none requested
Contact Time:	3 hours	Contact Conditions:	ambient
Host Cell Line:	<i>E. coli</i> ATCC 15597	Cell Passage Number:	N/A
Assay Medium:	50% TSA	Neutralizer:	D/E broth (10 ml)
Incubation Period:	18-24 hours	Incubation Conditions:	36 ± 1°C

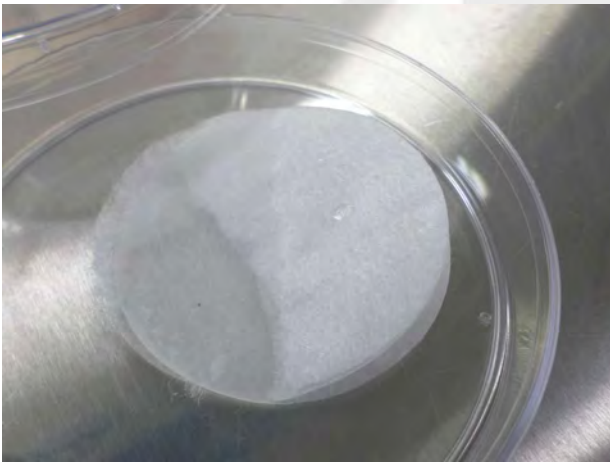
## Study Modifications

No further modifications were made to the method for this study.

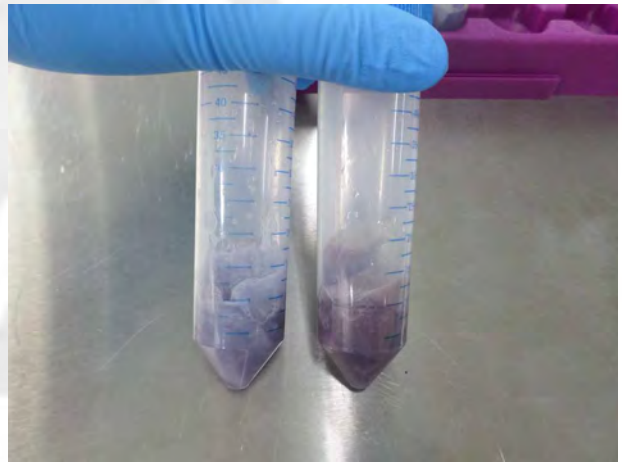
## Study Notes

One portion of the sponsor-provided control textile demonstrated significant hydrophobicity, evidenced by beading of the viral inoculum on the textile surface and lack of absorption (see study photographs).

## Study Photographs



**Photo 1.** Control textile demonstrating hydrophobicity on one portion of the prepared carrier. Despite this full absorption of the viral inoculum was achieved by recovering un-absorbed inoculum and re-inoculating absorbent portions of the product.



**Photo 2.** Control and test swatches directly following harvest in D/E broth at the end of the selected contact time.

## Control Results

Sterility:	Confirmed	Titer:	See study results
Neutralization:	N/A	Cytotoxicity Titer:	N/A

## Calculations

$$\text{Percent Reduction} = \left( \frac{B - A}{B} \right) \times 100$$

Where:

B = Number of viable test microorganisms on the control carriers immediately after inoculation

A = Number of viable test microorganisms on the test carriers after the contact time

$$\text{Log}_{10} \text{Reduction} = \text{Log} \left( \frac{B}{A} \right)$$

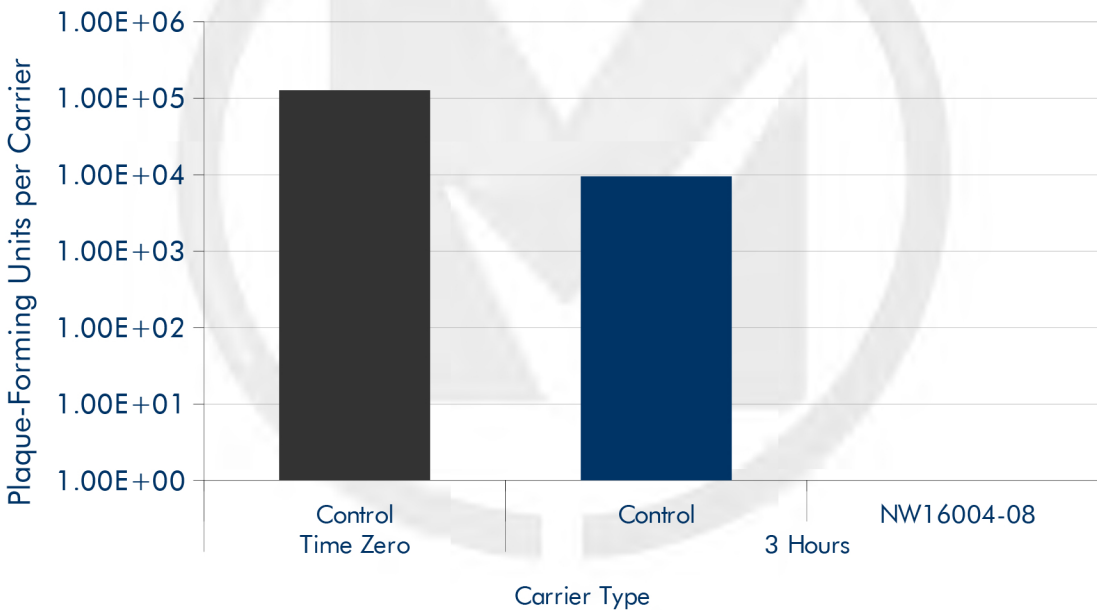
Where:

B = Number of viable test microorganisms on the control carriers immediately after inoculation

A = Number of viable test microorganisms on the test carriers after the contact time

## Results of the Study

Test Microorganism	Contact Time(s)	Carrier Type	Plaque-Forming Units (PFU) per Carrier	Percent Reduction Compared to Control at Contact Time	Log <sub>10</sub> Reduction Compared to Control at Contact Time
MS2 Bacteriophage ATCC 15597-B1	Time Zero	Control	1.28E+05	N/A	N/A
	3 Hours	Control	9.50E+03		
		NW16004-08	<5.00E+00	>99.95%	>3.28



Note: Values below the limit of detection for this assay are designated <5.00E+00 in the table above, and depicted as a value of zero in the graph above.

*The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.*

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