MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antimicrobial Efficacy of Treated Medical Masks
Modified for Viruses

Test Method

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

Study Identification Number

NG6983

Study Sponsor

Suzhou Letian Protective Products, Ltd.
Zhangjiagang City
Nanfeng Town, Nanfeng Village
c/o AP Goldshield LLC

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378

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

Carriers Inoculated	Carriers Treated	Carriers Neutralized	Assay Initiated	Assay Scored	Report Delivered
12 APR 2016* 14 APR 2016**	12 APR 2016 14 APR 2016	12 APR 2016 14 APR 2016	12 APR 2016 14 APR 2016	19 APR 2016 21 APR 2016	28 APR 2016

** Study timeline for Influenza A (H1N1)*
***Study timeline for Human Coronavirus and Poliovirus*

Test Substance Information

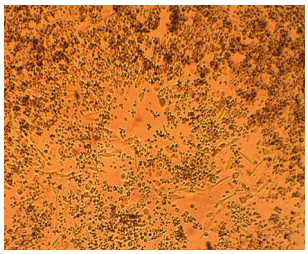
The test substances were received on 05 APR 2016 and the following picture was taken.

Test Substances Received: Treated – Blue Mask (Left), Control – White Mask (Right)

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:



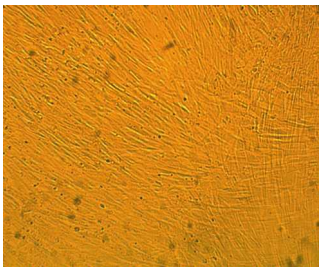
Influenza A (H1N1)

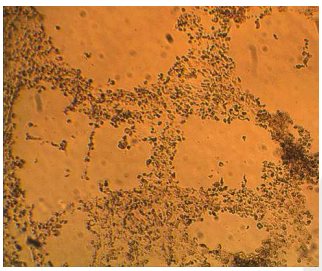
Influenza A virus is an enveloped, minus-stranded member of the family *Orthomyxoviridae*, and causative agent of the illness influenza (which is more widely recognized by the term 'flu'). Influenza is more serious than other seasonal mild, respiratory tract infections (e.g. the common cold) with symptoms that can last for upwards of several weeks. Young children and the elderly are particularly susceptible to severe illness and death due to infection. Influenza is readily transmitted via infective aerosols direct contact with infective respiratory secretions. Potential transmission by contaminated environmental surfaces (fomites) has increasingly become of interest, and Influenza virus is highly vulnerable to inactivation by drying and exposure to variety of disinfectant actives.

Permissive Host Cell Line Selected for Influenza A (H1N1): MDCK (Madin Darby Canine Kidney Cells), ATCC CCL-34

Test Microorganism Information (Cont.)

The test microorganism(s) selected for this test:

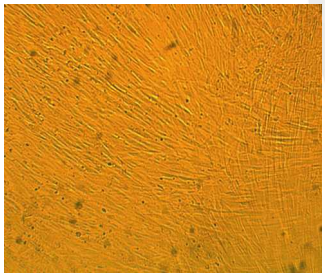


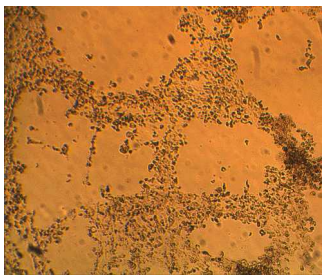


Human coronavirus 229E (HCoV), ATCC VR-740

HCoV is an enveloped, positive-sense RNA virus in the *Coronaviridae* family. Coronaviruses cause mostly mild to moderate upper respiratory infections year-round in humans. Zoonotic strains of coronavirus have also recently emerged (e.g. SARS-CoV and MERS-CoV) and become of great concern due to their virulence and high mortality rates. Similar to other respiratory viruses, coronaviruses are transmitted by inhalation of infective aerosols and person-to-person contact. Symptoms include cough, runny nose, sore throat, and mild fever. Immunocompromised persons and those with poor cardiovascular health may also develop pneumonia. Relative to other enveloped, respiratory viruses, coronaviruses are less vulnerable to inactivation during dessication, yet are similarly inactivated by a number of disinfectants.

Permissive Host Cell Line for HCoV: MRC-5 (Human Lung Fibroblast Cells), ATCC CCL-171





Poliovirus 1 (PV 1), ATCC VR-1562

Poliovirus 1 is a non-enveloped, positive-sense RNA virus in the *Picornoviridae* family. PV 1 is one of three Poliovirus serogroups, and acts as a causative agent of the neurological disease poliomyelitis – commonly known as polio. Polio has multiple well known symptoms, including paralysis and difficulty breathing. However, the majority of polio infections are either asymptomatic or result in less severe symptoms such as fever, myasthenia, and back pain or stiffness. Polio is primarily transmitted through the fecal-oral route, and viable viral particles may be detected in the stool of infected persons for several weeks after they stop showing symptoms. According to the World Health Organization (WHO), global efforts have contributed to a 99% decrease in polio cases since 1988. Despite this significant reduction polio is still endemic in several countries, presenting a hazard to non-endemic countries if carriers of the virus cross borders.

Permissive Host Cell Line for PV 1: Vero (African Green Monkey Kidney Cells), ATCC CCL-81

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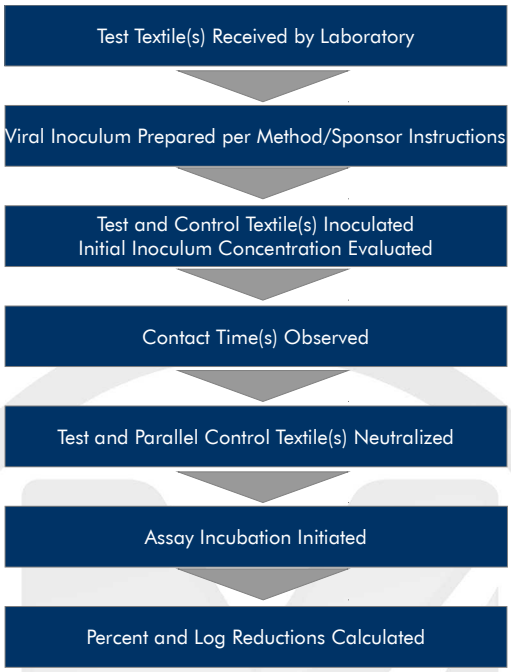
Diagram of the Procedure



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 singlet 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. $TCID_{50}$) 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-Karber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- Log_{10} 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_{10}.
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.

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_{10} or 99% reduction. The United States Environmental Protection Agency (US EPA) often recommends a 3-Log_{10} 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 ≥3-Log_{10} 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:	4
Replicates:	One		

Viral Inoculum Volume:	1.0 ml	Target Inoculum:	5-log_{10}/Carrier
Dilution Medium:	PBS	Soil Load:	No Soil
Contact Time:	10 minutes	Contact Conditions:	Ambient
Host Cell Line:	See study notes	Cell Passage Number:	See study notes
Assay Medium:	See study notes	Neutralizer:	See study notes
Incubation Period:	7 days	Incubation Conditions:	See study notes

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Study Modifications

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

Study Notes

Host Cell Lines and Passage Numbers

Influenza A (H1N1) Host Cells: MDCK, passage 155
Human Coronavirus Host Cells: MRC-5, passage 27
Poliovirus 1 Host Cells: Vero, passage 218

Neutralizer

Neutralization of the test and control substrates was achieved via vortexing in 2.0 ml of 10% FBS EMEM supplemented to a final concentration of 0.1% lecithin, followed by Sephacryl gel column filtration.

Assay Medium and Incubation Conditions

Influenza A (H1N1) : Influenza Infection Medium, incubated at 34 ± 2 °C, 5 ± 1% CO_2.
Human Coronavirus : 2% FBS EMEM, incubated at 37 ± 2 °C, 5 ± 1% CO_2.
Poliovirus 1 : 2% FBS EMEM, incubated at 37 ± 2 °C, 5 ± 1% CO_2.

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Control Results

Sterility:	Confirmed	Titer:	See study results
Neutralization:	Confirmed	Cytotoxicity Titer:	No cytotoxicity observed

Calculations

Viral and cytotoxicity titers ($TCID_{50}$/$TCLD_{50}$ and $TCCD_{50}$, respectively) were determined according to the method developed my Spearman-Karber:

$$-\text{Log}_{10} \text{ of 1st Dilution} - \left(\frac{\text{sum of \% mortality at each dilution}}{100}\right) - 0.5$$

Log Reduction of Virus is determined according to the following formula:

$$\text{Log}_{10} \text{ Reduction} = B - A$$

Where:
B = Plate Recovery Control Surface $TCID_{50}$
A = Virus Test Surface $TCLD_{50}$

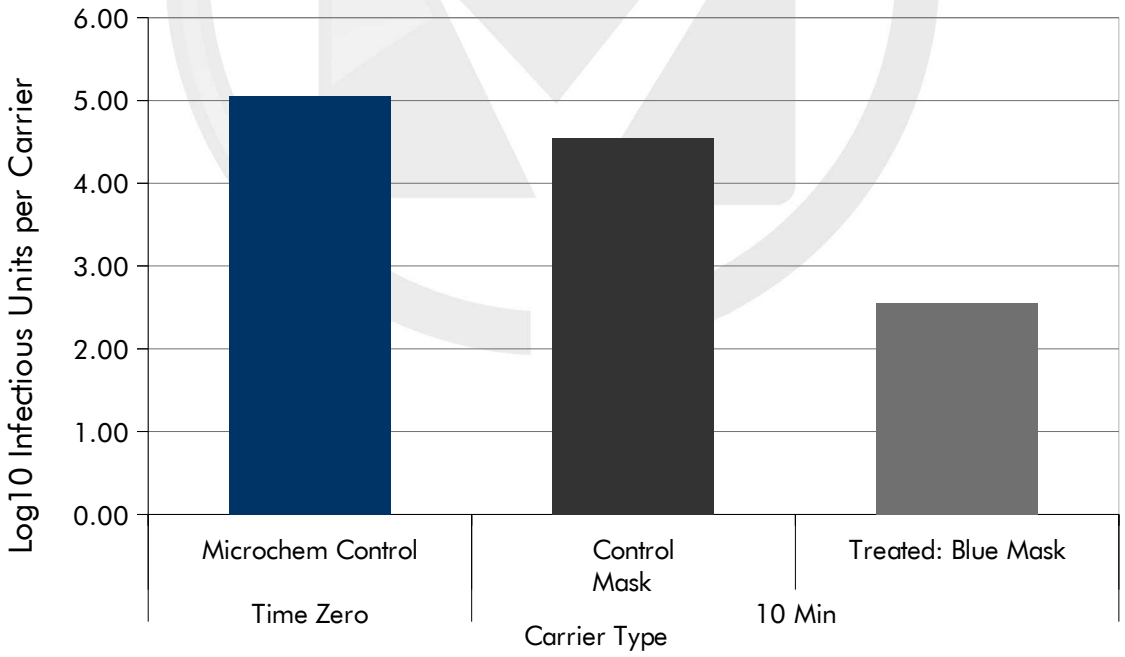
Percent Reduction of Virus is determined according to the following formula:

$$\text{Percent Reduction} = 1 - \left(\frac{C}{B}\right) * 100$$

Where:
B = Log_{10} of Virus Control Carrier
C = Log_{10} of Virus Test Carrier

Results of the Study

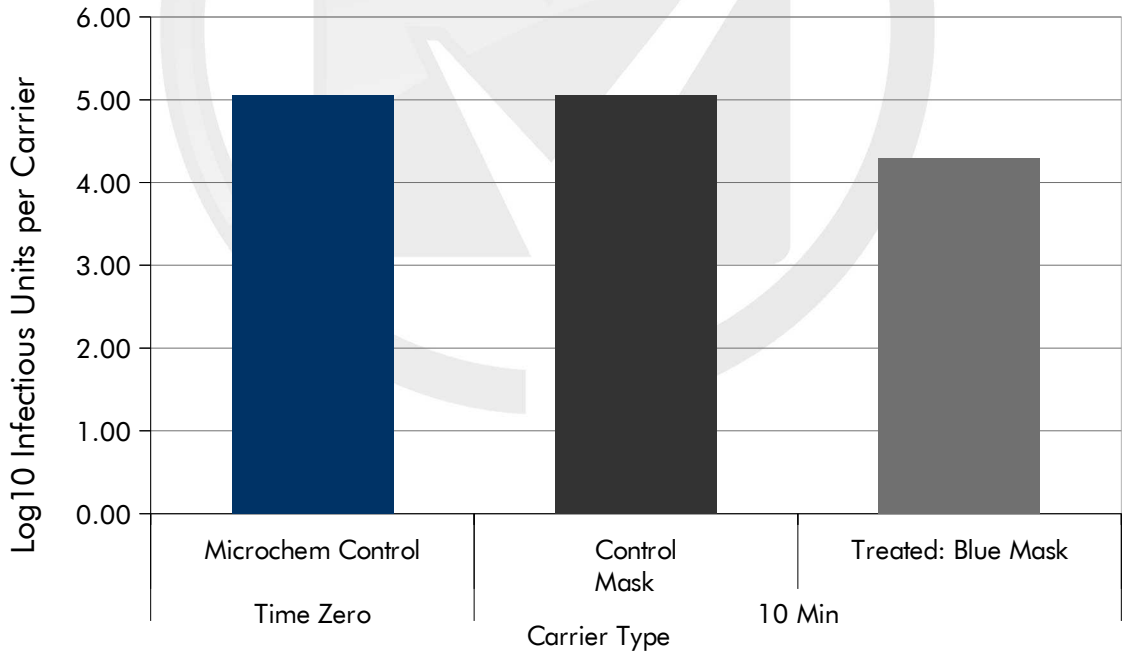
Test Microorganism	Contact Time	Carrier Type	Log_{10} Infectious Units per Carrier	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero	Percent Reduction Compared to Control at 10 Min	Log_{10} Reduction Compared to Control at 10 Min
Influenza A (H1N1) ATCC VR-1736	Time Zero	Microchem Control	5.05	N/A		N/A	
	10 Min	Control Mask	4.55				
		Treated: Blue Mask	2.55	99.68%	2.50	99.00%	2.00



RESULTS

Results of the Study (Cont.)

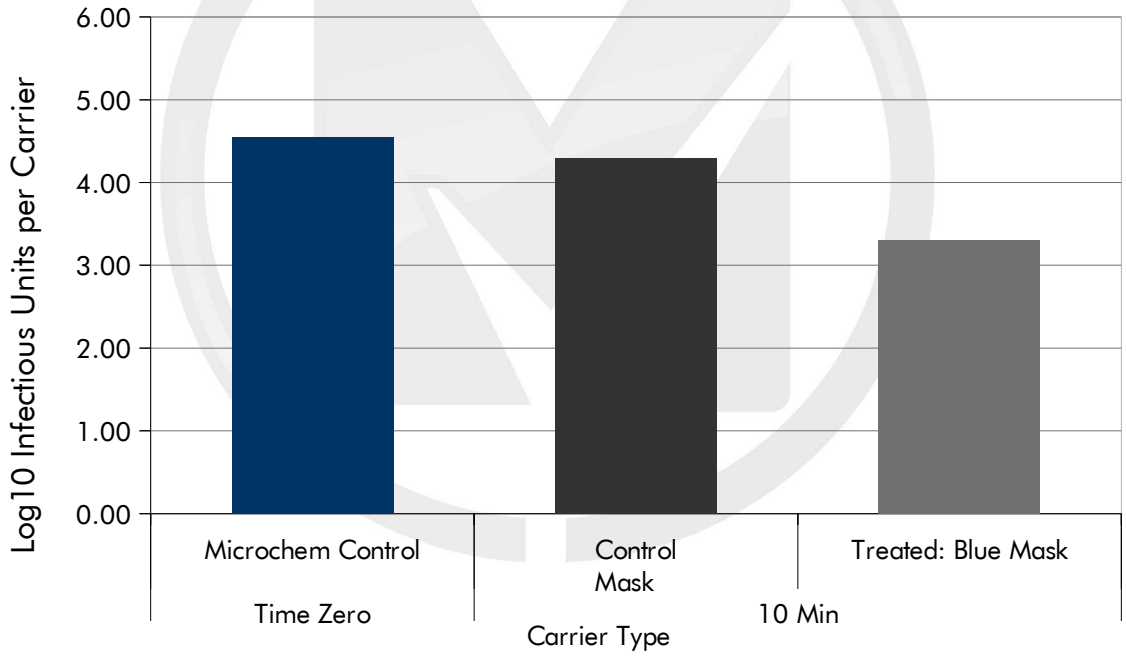
Test Microorganism	Contact Time	Carrier Type	Log_{10} Infectious Units per Carrier	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero	Percent Reduction Compared to Control at 10 Min	Log_{10} Reduction Compared to Control at 10 Min
Poliovirus 1 ATCC VR-1562	Time Zero	Microchem Control	5.05	N/A		N/A	
	10 Min	Control Mask	5.05				
		Treated: Blue Mask	4.30	82.22%	0.75	82.22%	0.75



RESULTS

Results of the Study (Cont.)

Test Microorganism	Contact Time	Carrier Type	Log_{10} Infectious Units per Carrier	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero	Percent Reduction Compared to Control at 10 Min	Log_{10} Reduction Compared to Control at 10 Min
Human Coronavirus ATCC VR-740	Time Zero	Microchem Control	4.55	N/A		N/A	
	10 Min	Control Mask	4.30				
		Treated: Blue Mask	3.30	94.38%	1.25	90.00%	1.00



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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