ANTİMİKROP

T.C. SAĞLIK BAKANLIĞI

TÜRKAK

AB-1452-T

NAC

TESTING LABORATORY
ISO/IEC 17025:2017
NAC 013 TL

ISO 18184:2019

Tekstil- Tekstil ürünlerinin antiviral aktivite tayini

(Textiles: Determination of antiviral activity of textile products)

Test Sonuç Raporu

ANTİMİKROP AR-GE ve BİYOSİDAL ANALİZ MERKEZİ

Nasuh Akar Mah. Süleyman Hacıabdullahoğlu Cad. No: 37/1
Çankaya/Ankara

tarafından hazırlanmıştır

Müşteri : Bayteks Teknik Tekstil San. Ve Tic. A. Ş.
İrtibat : 0(552) 998 58 73
Email : burcu.turkaslan@tıtınnovatıon.com
Adres : Org. San Böl. 19 No'lu Cad. No:11 Merkez/ KİLİS
Rapor Tarihi : 01.02.2021
Rapor No : R-21-0014

Prof. Dr. Murat ERTÜRK
Sorumlu Yönetici

ANTİMİKROP ANTİMİKROBİYAL MAD. LAB. AR-GE MED. VE DEZ. HİZ. KİMYA SAN. TİC. LTD.ŞTİ.
Nasuh Akar Mah. Süleyman Hacıabdullahoğlu Cad. No:37/1
Çankaya / ANKARA
Hitit V.D. No: 070 031 8715 Mersis No: 0070031871500013

Nihan SEVEN EROĞLU
Kalite Sorumlusu

1. TEST BİLGİSİ

Ürün Örneği:

Üretici	:	Bayteks Teknik Tekstil San. Ve Tic. A. Ş.
Kumaş Kodu	:	-
Kumaş İplik Karışımı	:	MASKE
Kumaş Dokuma/Örme Tipi	:	POLİPROPİLEN NONWOMEN
Kumaş Gramajı	:	30 GSM
Saklama Koşulları	:	Oda ısısı
Ürün Görünümü	:	Beyaz Renkli Maske
Numune Kodu	:	COV-21-0014
Numune Kabul Tarihi	:	15.01.21
Analiz Tarihleri	:	20.01.2021-24.01.2021
Test Adı	:	Bayteks Teknik Tekstil San. Ve Tic. A. Ş. ürünü Maskenin antiviral etkinlik testi
Test Yöntemi	:	ISO 18184:2019 Tekstil- Tekstil ürünlerinin antiviral aktivite tayini (Textiles: Determination of antiviral activity of textile products)
Kontrol Test Maddesi	:	İşlenmemiş Kumaş
Virus	:	SARS-CoV-2 (Covid-19) Klinik izolat (GenBank:MT955161.1)
Maruz Kalma Süresi	:	2 saat
Test Ortamı	:	24 ^{0}C ± 1 ^{0}C
Virüs-Hücre İnkübasyonu	:	37 ^{0}C ± 1 ^{0}C, % 5 CO_2

ÖZET TEST SONUCU:

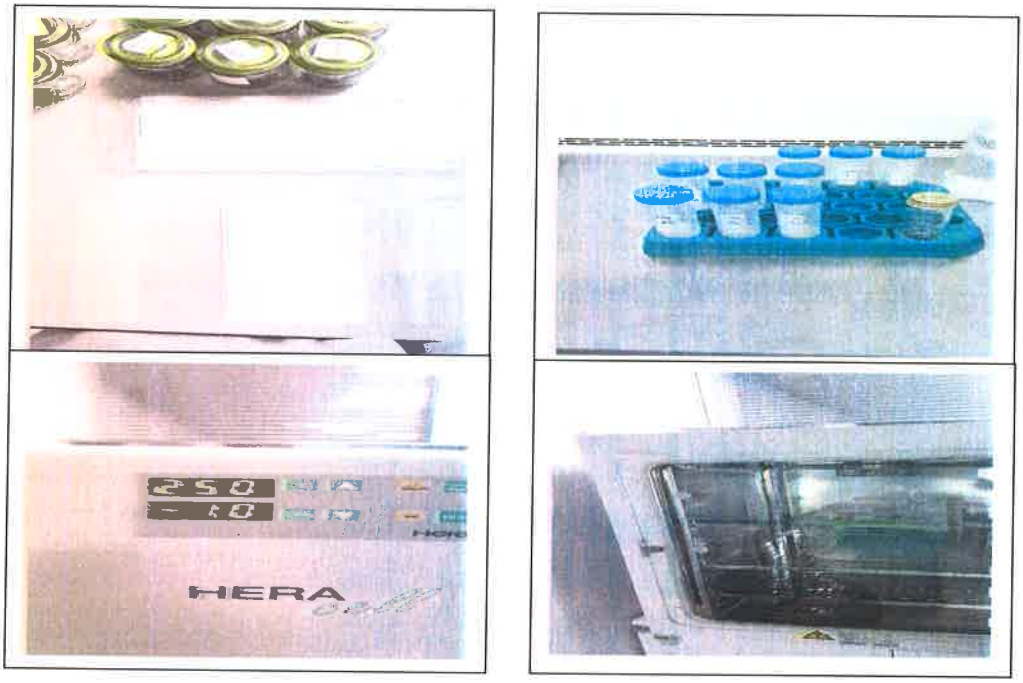
Bayteks Teknik Tekstil San. Ve Tic. A. Ş. Ürünü 3 katlı Maskenin en dış katmanı 2 saatlik zaman zarfında *SARS-CoV-2* (COVID-19) (Klinik izolat) *(GenBank:MT955161.1)* virüsüne karşı % 99,99 oranında VİRÜSİDAL (virüs öldürücü) etki göstermiştir.

ANTİMİKROP ANTİMİKROBİYAL ARAŞ. GEL. LAB. AR-GE HİZ. SAN. TİC. LTD.ŞTİ. Naşeh Ahmet Mah. Süleyman Hacıabdullahoğlu Cad. No:37/1 Çankaya / ANKARA VD. No: 070 031 8715 Mersis No: 0070031871500013

02 1 0 14

2. TEST YÖNTEMİ

2.1. *SARS-CoV-2* (COVID-19) virüs stok kültürü (~ 10^7 $TCID_{50}$) DMEM-10 (% 10 FBS (fetal dana serumu) içeren pen/strep/fungizonlu Dulbecco's Minimum Essential Medium ile çoğaltılan Vero E6 hücre hattı kullanılarak hazırlandı.



2.2. Test maddesi Bayteks Teknik Tekstil San. Ve Tic. A. Ş. Ürünü 3 katlı maskenin en dış katı ve kontrol kumaşlardan 20 mm x 20 mm boyutlarında kesildi. 9 adet kontrol ve 6 adet test kumaş için 0,45±0,05'er gram ağırlığında kütleler hazırlandı. 3'er adet parça sitotoksisite ve nötralizasyon testi için, 3 adet kumaş parçası başlangıç (t0) virüs titre tayini kullanıldı. Diğerleri 0.2 ml stok virüs inoküle edilerek maruz kalma süresi olan t2 saat süresince bekletildi. Süre sonunda, her bir parça kumaş 20 ml DMEM-10 içeren tüplere aktarıldı ve vortekslendikten sonra log dilüsyonlar yapılıp geri kazanılan virüs tayini için hücre içeren 96-kuyucuklu pleytlere aktarıldı. Pleytler 37 ^0C ±1 ^0C % 5 CO_2 şartlarında inkübe edildi. 4 günlük inkübasyon sonrasında invert mikroskop altında sitopatik etki değerlendirilip $TCID_{50}$ Spearman-Karber formülüne göre hesaplandı. Virüsidal (virüs öldürme) aktivitesi kontrol kumaş $TCID_{50}$ değeri ile test kumaş $TCID_{50}$ değeri karşılaştırılarak Log ve % aktivite cinsinden hesaplandı.

ANTİMİKROP ANTİMİKROBİYAL MAD. LAB.
AR-GE MÜH. VE SAN. HİZ. TEKS. SAN. VE TİC. LTD.ŞTİ.
Nasuh Akar Mah. Süleyman Hakkı Abdullahoğlu Cad. No:37/1
Çankaya / ANKARA
Hüsl V.D. No: 070 031 8715 Mersis No: 0070031871500013

NB21014

3. SONUÇLAR

3.1. Ham veriler (Log $TCID_{50}$)

TEST	Materyal	Log sulandırma- cpe değerlendirme						
		-1	-2	-3	-4	-5	-6	-7
Virüs Titrasyon	Stok Virüs	444444	444444	444444	444444	444044	430032	010000
t0 Enfektivite doz	Kontrol 1	4444 4444	4444 4444	4444 4444	0404 0340	0000 0000	-	-
	Kontrol 2	4444 4444	4444 4444	4444 4444	0400 4404	0000 0000	-	-
	Kontrol 3	4444 4444	4444 4444	4444 4444	0403 0404	0000 0000	-	-
Sitotoksisite	Kontrol 1	0000 0000	0000 0000	0000 0000	-	-	-	-
	Kontrol 2	0000 0000	0000 0000	0000 0000	-	-	-	-
	Kontrol 3	0000 0000	0000 0000	0000 0000	-	-	-	-
	Test 1	0000 0000	0000 0000	0000 0000	-	-	-	-
	Test 2	0000 0000	0000 0000	0000 0000	-	-	-	-
	Test 3	0000 0000	0000 0000	0000 0000	-	-	-	-
Nötralizasyon	Kontrol 1	4444 4444	4444 4444	4444 4444	4444 0404	0000 0000	-	-
	Kontrol 2	4444 4444	4444 4444	4444 4444	0304 4040	0000 0000	-	-
	Kontrol 3	4444 4444	4444 4444	4444 4444	0044 0034	0020 0000	-	-
	Test 1	4444 4444	4444 4444	4444 4444	4440 0403	0000 0000	-	-
	Test 2	4444 4444	4444 4444	4444 4444	0404 4004	0000 0000	-	-
	Test 3	4444 4444	4444 4444	4444 4444	4444 0000	0000 0000	-	-
T2 Saat	Kontrol 1	4444 4444	4444 4444	0404 0000	0000 0000	0000 0000	-	-
	Kontrol 2	4444 4444	4444 4444	4000 4404	0000 0000	0000 0000	-	-
	Kontrol 3	4444 4444	4444 4444	0044 0440	0000 0030	0000 0000	-	-
	Test 1	0000 0400	0000 0000	0000 0000	0000 0000	-	-	-
	Test 2	0040 0004	0000 0000	0000 0000	0000 0000	-	-	-
	Test 3	0040 0000	0000 0000	0000 0000	0000 0000	-	-	-

ANTİMİKROP ANTİMİKROBİYAL M.O. LAB.
AR-GE MÜH. VE SAN. TİC. LTD.ŞTİ.
Nasuh Akar Mah. Süleyman Hacıabdullahoğlu Cad. No:37/1
Çankaya / ANKARA
Hük. V.D. No: 670 031 3715 Mersis No: 0670031371500014

62 1 0 1 4

3.2. Test Sonuçları

TEST	Test Materyali	(Log $TCID_{50}$)	Log		Yorum/Sonuç
		Sonuç	Sonuç	Ortalama	
Virüs Titrasyonu	Stok Virüs		7.17	-	UYGUN
Sitotoksisite	Kontrol Kumaş	+			UYGUN
	Test Kumaş	+			UYGUN
Nötralizasyon Kontrol	Kontrol Kumaş 1	5,25	5,9	5,8	UYGUN
	Kontrol Kumaş 2	5,00	5,7		
	Kontrol Kumaş 3	5,00	5,7		
	Test Kumaş 1	5,13	5,80	5,70	
	Test Kumaş 2	5,00	5,70		
	Test Kumaş 3	5,00	5,70		
t0 Saat Virüs Enfektivite Testi	Kontrol Kumaş 1	5,00	6,30	6,30	UYGUN
	Kontrol Kumaş 2	5,00	6,30		
	Kontrol Kumaş 3	5,00	6,30		
Virüsidal Test (t2 saat)	Kontrol Kumaş 1	3,75	5,1	5,30	UYGUN
	Kontrol Kumaş 2	4,00	5,30		
	Kontrol Kumaş 3	4,13	5,4		
	Test Kumaş 1	0,63	1,90	2,00	R=Log Kt0-Log Vt2 R=4,30 (%99,99)
	Test Kumaş 2	0,75	2,10		
	Test Kumaş 3	0,63	1,90		

ANTİMİKROP ANTİMİKROBİYAL AR-GE LAB. TİC. LTD. ŞTİ.
Çankaya / ANKARA
Vergi V.D. No: 670 031 8715 Mersis No: 0670031871500013

4. ÇALIŞMANIN KAYITLARININ SAKLANMASI

4.1. Kayıtların Saklanması: Bu çalışma için özel olarak geliştirilen tüm orijinal ham veriler arşivlenecektir. Bu orijinal veriler, yalnız bunlarla sınırlı olmamak üzere aşağıdaki bilgileri içerir: Defterler, veri formları ve hesaplamalar, elle yazılmış tüm ham veriler ile son çalışma raporunun onaylı kopyası.

4.2. Test Maddesinin Saklanması: Arta kalan test kumaş örneği oda ısısında Şahit Numune olarak 3 ay süre ile saklanacaktır.

BU RAPOR ÖN SAYFA DÂHİL OLMAK ÜZERE 6 SAYFADAN İBARETTİR.

ANTİMİKROP ANTİMİKROBİYAL TİC. LTD. ŞTİ.
AR-GE MÜH. VE KİM. SAN. TİC. LTD. ŞTİ.
Nasuh Akar Mah. Süleyman Hacıabdullahoğlu Cad. No:37/1
Çankaya / ANKARA
Hitit V.D. No: 070 031 8715 Mersis No: 0070031871500013

62 1 0 1 6

STUDY REPORT 349.2021.102
Version 02

IN VITRO STUDY OF ANTIVIRAL ACTIVITY OF TEXTILE PRODUCTS

Sample NV.499.02

Company	BAYTEKS TEKNİK TEKSTİL SAN VE TİC A.Ş
Address	Başpınar Organze O.S.B Mahallesi 2.Bölge Şehitkamil /Gaziantep
Institution that conducted the study	Núcleo Vitro Serviços Cientificos Ltda. Rua da Várzea, 22, Jardim São Pedro, Porto Alegre-RS, Brasil, CEP 91040-600
Product code	NV.499.02
Product name	Nonwoven fabric after sterilization
Sample receiving	27/01/2021
Report issuance	08/02/2021

1. INTRODUCTION

It is increasing the search for products that offer protection against illness causing agents. According to this demand, agents with antiviral properties are being applied to textile industries with the goal to incorporate functional properties with quality in order to promote health and wellbeing to the population.

The study according to ISO 18184:2019 is the reference in order to evaluate textile products with antiviral activity. It is evaluated whether the contact with the product reduces the number of viral particles.

This study was conducted with a virus from the coronavirus family and it is a member of SARS-CoV-2 family. The coronavirus is a virus with a RNA genome that belongs to the family Coronaviridae. The subfamily Orthocoronaviridae has 4 genus: alpha, beta, gamma and deltacoronavirus. This study was conducted with an alphacoronavirus (CCov – VR 809) as a viral particle that represents a good model in order to study virus.

2. PURPOSE

Evaluate if the sample reduces the alphacoronavirus viral viability following the methodology present at ISO 18184:2019.

3. STUDY RELEVANCE

The experimental conditions used herein are accepted and in accordance with methodologies currently used by ISO 18184:2019.

4. SAMPLE DESCRIPTION

Sample information	Product code	Storage temperature
Nonwoven fabric after sterilization	**NV.499.02**	Room temperature

5. METHODOLOGY

5.1 Cell culture

It was used VERO cells cultured with DMEM (Dulbecco's Modified Eagle's Medium) with addition of supplements in an incubator at 37°C and 5% of CO_2. At cell passage 6 after thawing, cells were plated in appropriate plates in order to establish a monolayer.

5.2 Virus culture

It was used the alphacoronavirus (CCoV – VR809) previously titred according to the following calculation. This virus was selected since it belongs to the SARS-CoV-2. For this assay, the virus titre was $10^{5.0}$.

5.3 Calculation of the virus titre according to TCID50:

$Y = X \times 10^{a}$

$a = \sum p - 0,5$

x= base dilution – it was used base 10

Calculation of virus stock:
Calculation of p value according to number of replicates with 7 dilutions with base of 10.
$a = \sum 7.0 - 2.0$; $a = 5.0$

Virus titre used in the assay: $10^{5.0}$

5.4 Sample preparation

Sample **NV.499.02**

- Sample conditions in the assay: Pieces of fabric following the dimensions 20mm x 20 mm (±2 mm). Samples were autoclaved according to ISO 18184:2019.

.

5.5 Control groups preparation

- Cell control group: culture media supplemented;
- Viral control group: culture media supplemented;

5.6 Cytotoxic analysis

The cell control group and the group NV.499.02 were incubated with supplemented culture media in the same conditions as in the viral assay in order to establish if the samples could induce a cytotoxic effect in the cell culture. The culture media was added to the cell monolayer at a 1:1 and at a 1:10 dilution in duplicate to each group. After 24 hours, it was evaluated the cell culture and images were taken.

5.7 Antiviral activity analysis

Viral aliquot was diluted in cell culture media and the fragments of fabric from group NV.499.02 were embedded in closed tubes for 5, 30 and 120 minutes in sterile work conditions. After the times periods, one aliquot was added to a new tube in order to end the contact with the samples. The viral control group was executed in the same way, but it had no contact with any sample. It was then added the work solutions to the cell monolayer in quadruplicated to each group in order to evaluate viral multiplication.

5.8 Results analysis

The cell culture with the viral particles was evaluated according to the cytopathic effect (CPE) caused by the virus. It is compared the cell control group with the viral control group and the sample's group NV.499.02 in order to evaluate the viral replication. The result is evaluated with the TCID50 method with logarithm dilution series. The result is expressed as logarithm reduction of viral particles and calculated the difference of viral particles between the viral titre and the group NV.499.02

6. RESULTS

6.1 Cytotoxic analysis

In order to evaluate the viral titre, it is needed to evaluate cell cytotoxic from the samples. Therefore, it is evaluated the culture monolayer and the cell morphology. It was observed a confluent monolayer with morphology establishment in all groups at a 1:10 dilution.

.

6.2 Antiviral analysis activity

The virus evaluated causes cell morphology alterations that are called cytophatic effect (CPE). Through CPE, it is possible to analyze and quantify the viral multiplication in each group. It is executed the viral titre identification to each group in order to calculate the antiviral activity value. The results in viral logarithm reduction and percentage observed in the group NV.499.02 is presented at Table1:

Product	Contact time (minutes)	Log reduction	Percentage
Nonwoven fabric after sterilization	5	2.5	99.68%
	30	4.0	99.99%
	120	4.5	99.996%

Table 1: Results of viral titre at the contact time evaluated. It is shown the log reduction and the viral reduction percentage.

The reduction of 4.5 logarithms in the viral titre by the group NV.499.02 after 120 minutes of contact demonstrated that it has antiviral activity and that is related to a 99.996% reduction of viral particles.

7. CONCLUSION

According to the results obtained herein, is can be concluded that:

- The sample NV.499.02, Nonwoven fabric after sterilization reduced 2.5 logarithms after 5 minutes and this is related to a 99.68% reduction of viral particles;

- The sample NV.499.02, Nonwoven fabric after sterilization, reduced 4.0 logarithms after 30 minutes and this is related to a 99.99% reduction of viral particles;

- The sample NV.499.02, Nonwoven fabric after sterilization, reduced 4.5 logarithms after 120 minutes and this is related to a 99.996% reduction of viral particles;

8. FINAL CONCLUSION

In the study entitled "***IN VITRO* STUDY OF ANTIVIRAL ACTIVITY OF TEXTILE PRODUCTS"** regarding the product **Nonwoven fabric after sterilization,** code **NV.499.02**, sent by the company **BAYTEKS TEKNİK TEKSTİL SAN VE TİC A.Ş** it is observed that:

The product Nonwoven fabric after sterilization, code NV.499.02, has antiviral activity.

This report is exclusively intended to the **National Agency of Sanitary Surveillance of the Ministry of Health** and to the internal use of **BAYTEKS TEKNİK TEKSTİL SAN VE TİC A.Ş.** No information of this report can be disclosed in any communication vehicle without the written authorization of the author. The only part that can be presented is the results of this study report for communication purposes.

9. NOTES

The results described herein are regarding the sample evaluated in the conditions and concentrations evaluated in this study.

The results demonstrated herein are exclusively of *in vitro* studies.

10. REFERENCES

Alberts, B. et al. Biologia molecular da célula. Artmed: 6ª edição. 2017.
Carlucci, et al. Antiherpetic activity and mode of action of natural carregeenans of diverse structural types. Antiviral Research: 1999: 93-102.
ISO 18184:2019 –Textiles – determinal of antiviral activity of textile products.
Su,et al. Modes of antiviral action of chemical portions and constituents from woad root extract against influenza virus A FM1. Evidence-based complementary and alternative medicine. 2016.
Buonavoglia C, Decaro N, Martella V, Elia G, Campolo M, Desario C, et al. Canine coronavirus highly pathogenic for dogs. Emerg Infect Dis. 2006;12(3):492–4.
Whitley RJ, Roizman B. Herpes simplex virus infections. Vol. 357, Lancet. Elsevier Limited; 2001. p. 1513–8.

11. APPROVAL

Bibiana Franzen Matte, phD
CRO 23877

ISO 18184:2019 Textiles- Determination of antiviral activity of textile products

Microbiological Solutions Limited (MSL)
Gollinrod, Walmersley, Bury, BL9 5NB, UK

Angela Davies, CEO

Customer: Bayteks Teknik Tekstil A.S
Contact name: Yunus Emre Bilgin
Email: yunus.bilgin@bayteks.com
Address: ORG. SAN. BÖL. 19 NOLU CADDE NO:9 MERKEZ
KİLİS
TURKEY
PO/Quote number: Q003560
Report Date: 11/11/2020
Issue Number: 1

Megan Barrett
Laboratory Manager

Peter Thistlethwaite
Technical Projects Manager

Test information		Deviation
Name of Product	Test 1 – 1	
Batch Number & Expiry Date	N/S	
Date of Delivery	13/08/2020	
Period of Analysis	05/11/2020-11/11/2020	
Manufacturer / Supplier	TIT Innovation	
Storage Conditions	Ambient	
Appearance of the Product	Antiviral nonwoven fabric	
Neutralisation Method	Dilution	
Test Concentrations	As supplied	
Test Temperature	25^0C ± 1°C	
Temperature of Incubation	37°C ±1°C	
Identification of the Viral Strains:	Feline corona virus, Strain Munich	
Contact Times	2 hour ± 10s	

Test Result Summary

The test fabrics showed the following log reductions when tested against Feline coronavirus with a 2 hour contact time:
Test 1 – 1.92log (98.78%)

The test results on this report refer only to the items tested as supplied by the customer. This report shall not be reproduced except in full and with written approval of Microbiological Solutions Ltd. All reports are archived for a minimum of 2 years.
The sample will be retained for 1 month unless otherwise requested in writing.

	Feline coronavirus	**COVID-19 (SARS—CoV2)**
Realm	Riboviria	Riboviria
Order	Nidovirales	Nidovirales
Family	Coronaviridae	Coronaviridae
Genus	Alphacoronavirus	Betacoronavirus
Species	Alphacoronavirus 1	COVID-19

The members of the family Coronaviridae are enveloped and have a positive sense RNA genome. Coronaviruses have a distinct morphology with an outer 'corona' of embedded envelope spikes. These viruses cause a broad spectrum of animal and human disease.

Andrew M.Q. King, Michael J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz 'Virus Taxonomy,
Classification and Nomenclature of Viruses, Ninth Report of the International Committee on Taxonomy of Viruses' 2012 ISBN 9780123846846

Scope

This standard outlines the test method for the determination of the antiviral activity of the textile products against specified viruses.

Method

A 20mmx20mm sample of test material is cut (overall mass should be 0.40g and can be made up with extra material if required). 9 control pieces are required and 6 test pieces.

3 pieces of each material are used to test the effect of the fabric on cells without virus (cytotoxicity), 3 control pieces are used to recover the starting titre of virus. The remaining pieces ate inoculated with 200µl of virus at a concentration of ~10^7 $TCID^{50}$ (giving a final concentration of 10^5) and left for the contact time.

Following the contact time, the fabric is recovered in 20ml of cell culture media and enumerated onto an appropriate cell line. TCID50 is calculated following the appropriate incubation time. Antiviral activity is calculated by comparison of the antiviral test material to the immediate recover from the control fabric.

Microbiological Solutions Ltd
Gollinrod
Walmersley
Bury, BL9 5NB

Tel: 0844 824 6003
Email: info@mls.io
Web: www.msl.io
Company number: 4218514

Test identification Reference: **J002290** ISO 18184:2019

Test Results

0 hours		
Sample	Log recovery	Average
Control 1	5.46	
Control 2	5.46	
Control 3	5.50	5.47

Controls		
Initial inoculum	7.04	Valid
Cytotoxicity Control	4.04	Valid
Cytotoxicity Test 1	3.88	Valid

Test 1

Contact time:2 hour				
Sample	Log recovery	Average	Reduction	Percentage
Control 1	4.79			
Control 2	4.75			
Control 3	4.54	4.69	0.78	83.32%
Test 1	3.83			
Test 2	3.38			
Test 3	3.46	3.56	1.92	98.79%

***Control fabric must not show >1 log reduction**

Controls

Microbiological Solutions Ltd
Gollinrod
Walmersley
Bury, BL9 5NB

Tel: 0844 824 6003
Email: info@mls.io
Web: www.msl.io
Company number: 4218514