RGCB
DISCOVERIES FOR A BETTER TOMORROW

Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695014, Kerala State, India.
An Autonomous National Institute for Discovery, Innovation & Translation in Biotechnology and Disease Biology,
Government of India, Ministry of Science & Technology, Department of Biotechnology.

राजीव गाँधी जैव प्रौद्योगिकी केन्द्र, तिरुवनन्तपुरम 695 014, केरल, भारत.
जैवप्रौद्योगिकी और रोग जीवविज्ञान में आविष्कार, नवीनता एवं अनुवाद की स्वायत्त राष्ट्रीय संस्थान,
भारत सरकार विज्ञान एवं प्रौद्योगिकी मंत्रालय, जैवप्रौद्योगिकी विभाग.

Report Title

Anti-Viral testing of "CareNow Antimicrobial Textiles (used in products ECO AIRMASK, BLUFENZ Bed Linen, Personal Protective Kit, Doctor's Coat)" product against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

Test Guideline

ISO 18184:2019 (modified)

Determination of Antiviral activity of Textile Materials.

Test conducted By

RGCB (Rajiv Gandhi Centre for Biotechnology), Trivandrum

Study Director - Dr. Radhakrishnan R. Nair

Study Co-Ordinator - Dr. S. Dayakar

Quality Manager - Ms Heera R. Pillai

Customer Name: CareNow Medical Pvt Ltd
Contact Name: Mr. Akshay Jadhav / Michael Rodrigues
Email: akshay@carenowindia.com/ michael@carenowindia.com
Address: #3/272-5 Neelambur Road, Muthugoundenpudur, Coimbatore, TN, India - 641406
+91 8983938744/+91 9843099609

Sample Submission Date: July 09, 2020
Study date: July 10, 2020
Report date: August, 2020

(Details enclosed)

चेलकत्त पी ओ, पज्ञापुरा, तिरुवनन्तपुरम - 695 014, केरल, भारत, फोन: 0471-2529400 (30 लाईन्स), 2347975, 2348104, 2348753, 2345899

OVERVIEW

1. Objective

The objective of this document is to provide data on antiviral efficacy of CareNow Antimicrobial Textiles, when tested against SARS-CoV-2 using International Standard of ISO 18184 (Determination of the antiviral activity of the textile products against specified viruses)

2. About Rajiv Gandhi Center of Biotechnology

Rajiv Gandhi Center for Biotechnology is an autonomous body of the Department of Biotechnology, Government of India. RGCB is a leading research institution located in Trivandrum, Kerala, India and is involved in Fundamental Research, Technology Development, Translational Science, Education & Intellectual Property development in the field of Biotechnology.

3. Description

CareNow Antimicrobial textiles are reusable textile products that have permanently bonded anti-microbial technology to provide a germ free and clean surface on textiles. The anti-microbial technology works round the clock and provides a barrier of protection against bacteria, fungi and encapsulated virus by inhibiting the growth in textiles.

The science behind the technology is a unique treatment of textile with a permanently lasting anti-microbial technology. The treatment on the textile provides cationic sites with a long molecular chain of carbon atoms with a +vely charged Nitrogen attached to a Silica Atom that is covalently bonded to the fabric. Bacteria, Fungi and Encapsulated Viruses are negatively charged. The cationic sites present on the fabric attract, bind and disrupt the cell membranes of the micro-organisms and provides continuous anti-microbial activity.

In this report, a study of Antimicrobial efficacy of CareNow Antimicrobial Textiles was performed as per ISO 18184:2019 (modified). A regular untreated Textile fabric is used as a control sample. This international standard specifies testing methods for determination of the antiviral activity of the submitted textile samples. CareNow Antimicrobial Textiles are intended to be used in products such as ECO AIRMASK, BLUFENZ Bed Linen, Personal Protective Kit, Doctor’s Coat.

In this test, the antiviral activity of one of the CareNow Antimicrobial Textiles and Untreated Textiles was identified by inoculating with E and S gene of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with 120 minutes of treatment time and the samples were evaluated by analyzing the reduction of viral load in percentage. The sample color is not representative of the entire gamut of colors that are offered for different end uses but essentially represents the technology and efficacy of antiviral property of textile

4. Summary

The test method involves the inoculation of SARS-CoV-2 viral genes (E gene and S gene) on to the test sample (CareNow Antimicrobial Textiles & Untreated Textiles). In 120 minutes of treatment time, viral load on the samples were observed at the time frequency of 0, 30, 45, 60 and 120 minutes. Proceeding with neutralization to retrieve the inoculated SARS-CoV-2 viral genes. The retrieved RNA content was evaluated by performing qRT-PCR technique for further confirmation of antiviral activity of the selected test sample.

5. Result Summary:

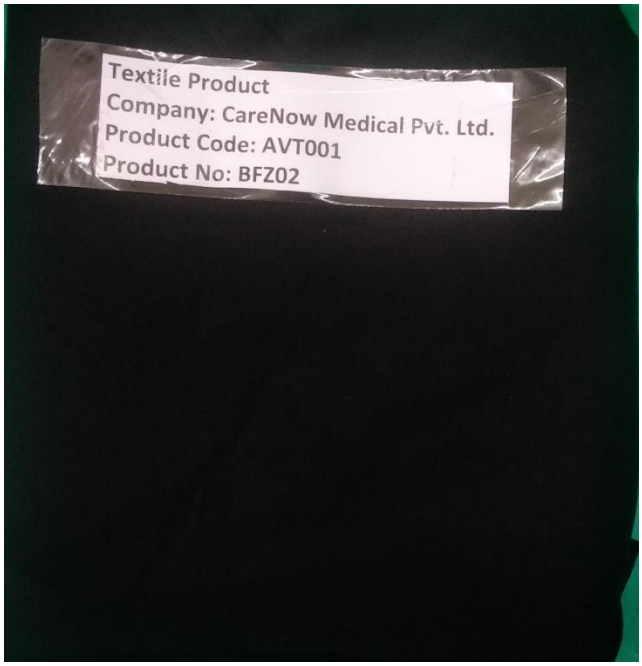
Sr. No	Time (min)	CareNow Antimicrobial Textile		Untreated Textile	
		Reduction in Viral Load %		Reduction in Viral Load %	
		E Gene	S Gene	E Gene	S Gene
1	45	>95	>95	NS	NS
2	60	99	99	NS	NS

Where, NS: Non-Significant

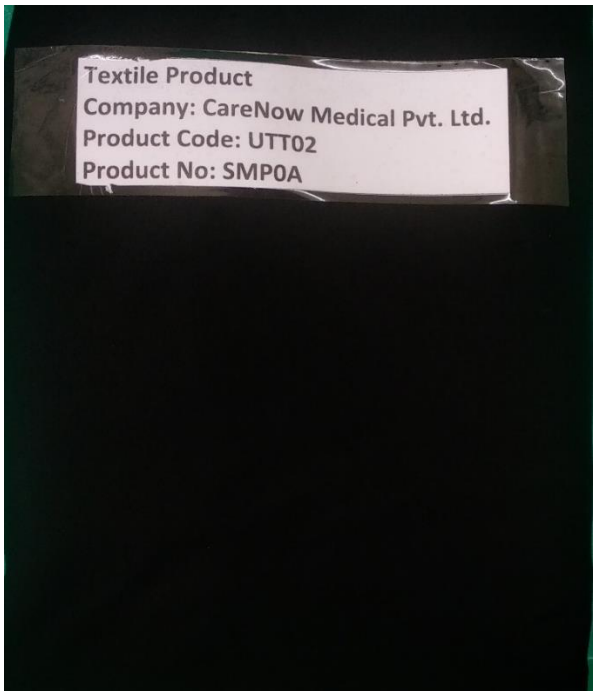
For detailed tabulated result, refer appendix 3, Tabular representation of viral reduction rate

6. Inference & Conclusion

1. In the present study, SARS-CoV-2 specific RNA (E&S target gene) was not detected after 45 minutes of contact time, indicating rupturing of viral envelope with >95% of reduction in viral load in test sample whereas the Control sample of Untreated Textile exhibited Not Significant reduction of Viral Load.
2. It has also significantly enhanced the antiviral log reduction and reduces viral infectivity by 99% reduction of viral load in 60 minutes of contact time.
3. It indicates, test sample (CareNow Antimicrobial Textiles) shows effective antiviral activity against encapsulated SARS-CoV-2 virus within 45 minutes of contact time.



Picture of Sample



Control Samples

CONTENTS:

APPENDIX 1

ISO 18184:2019 (modified) test procedure

a) Principle:

The viruses are inoculated to a specimen. After specific contacting time, the remaining infectious virus is counted, and the reduction rate is calculated by the comparison between antiviral test specimen and the reference (control) specimen by common logarithm.

b) Materials / Apparatus / Chemicals required:

Test sample	:	CareNow Antimicrobial Textiles
Test organism	:	*SARS-CoV-2- RGCB Isolate*
Chemicals	:	Neutralizing buffer
Enzyme	:	Ribonuclease
Materials / Apparatus:		Incubator, capable of maintaining at (25 ± 2^{o}C, 34 ± 2^{o}C, or 37 ± 2^{o}C)

c) Test isolate:

SARS-CoV-2-RGCB Isolate is the CoV-2 viral strain employed to study antiviral efficacy of CareNow Antimicrobial Textiles used in products such as ECO AIRMASK, BLUFENZ Bed Linen, Personal Protective Kit, Doctor's Coat etc.

d) Details of the test sample and control sample

Sl. No	Specification	CareNow Antimicrobial Textiles	Control Sample
	Sample Number	Textile 1 (fully blinded)	Textile 2 (fully blinded)
	Batch Number	BFZ02	SMP0A
	Product Code	AVT001	UTT02
	Sample		

e) Test Procedure:

- Test samples was obtained by punching a hole in the fabric.
- 50 µl of virus (SARS-CoV-2- RGCB Isolate) was spotted on the fabric (Δ Ct 26).
- The samples were incubated for 60 minutes.
- 150 µl of neutralization buffer (1X) was added to retrieve the virus.
- RNAase treatment performed as per manufacturers instruction (Genelink,40-5101-01)
- RNA was isolated as per manufacturers instruction (ADT Biotech-Malaysia,811801/811803) qRT-PCR was performed to quantify the RNA content using Kit (Real Star SARS-CoV-2 RT-PCR kit 1.0, altona Diagnostics GmbH-Germany, 023005) as per manufacturers instruction.

APPENDIX 2

qRT-PCR for quantitative analysis of retrieved RNA

a. Sample preparation

- The isolated RNA after the RNAase treatment is the starting material for Real Time PCR technique.
- The quality of the isolated RNA has a profound impact on the performance of the entire test system.
- In case of kits and systems for nucleic acid extraction, the test performer should follow the instructions given by the manufacturers
- If using spin column based sample preparation including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube prior to the elution of the nucleic acid

b. Mixture and Reaction setup

- All reagents and samples should be thawed completely, mixed and centrifuged briefly before use.
- The kit used for qRT-PCR contains a heterologous Internal Control (IC) which can either be used as RT-PCR Inhibition Control or as a control of sample preparation procedure and as RT-PCR Inhibition Control.
- Prepare the master mix with IC (Internal Control). If IC was added during the sample preparation procedure, set up the Master mix according to the following pipetting scheme

Number of Reactions	**1**	**12**
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	20 µl	240 µl

- Pipette 20 µl of the Master mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube

- Add 10 µl of sample (eluate from the nucleic acid extraction) or 10 µl of controls (Positive or Negative control). So, the reaction setup will be

Master mix + Sample or control = Total volume.

- There should be one positive and one Negative control per run and thoroughly mix the samples and controls with the Master mix by pipetting up and down.
- Close the 96-well reaction plates and tubes with appropriate lids or optical adhesive film
- Centrifuge the 96-well reaction plates in the centrifuge with microtiter plate rotor for 30 seconds approximately 1000 x g (~ 3000rpm).

c. Dyes

Dyes are the fluorescence Detectors in RT-PCR, the following table tells the targets with detector name, reporter and quencher

Target	**Detector Name**	**Reporter**	**Quencher**
B-βCoV specific RNA	Target E gene	FAM™	(None)
SARS-CoV-2 specific RNA	Target S gene	Cy5	(None)
Internal Control	IC	JOE™	(None)

d. Result Interpretation in RT-PCR

Detection channel			
FAM™	Cy5	JOE™	B-βCoV (target E gene) and SARS-CoV-2 (target S gene) specific RNA detected.
+	**+**	**+***	B-βCoV (target E gene) specific RNA detected.
+↑	**-**	**+***	SARS-CoV-2 (target S gene) specific RNA detected.
-	**-**	**+**	Neither B-βCoV (target E gene) nor SARS-CoV-2 (target S gene) specific RNA detected. The sample does not contain detectable amounts of B-βCoV (target E gene) or SARS-CoV-2 (target S gene) specific RNA.
-	**-**	**-**	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

APPENDIX 3

Test results - Test product against SARS-CoV-2

a) Raw data of E and S gene against CareNow Antimicrobial Textiles and Untreated Textiles

- Control Untreated Textiles - E Gene

No.	Color	Name	Type	Ct
1		Before expose-UTT02	Textile material	-
2		Before expose-UTT02	Textile material	-
3		0 min -UTT02	Textile material	26
4		0 min -UTT02	Textile material	26
5		30 min -UTT02	Textile material	27
6		30 min -UTT02	Textile material	27
7		45 min -UTT02	Textile material	28
8		45 min -UTT02	Textile material	28
9		60 min -UTT02	Textile material	29
10		60 min -UTT02	Textile material	29
11		120 min -UTT02	Textile material	29
12		120 min -UTT02	Textile material	29
13		Negative control	Negative Control	-
14		Positive control	Positive Control	30

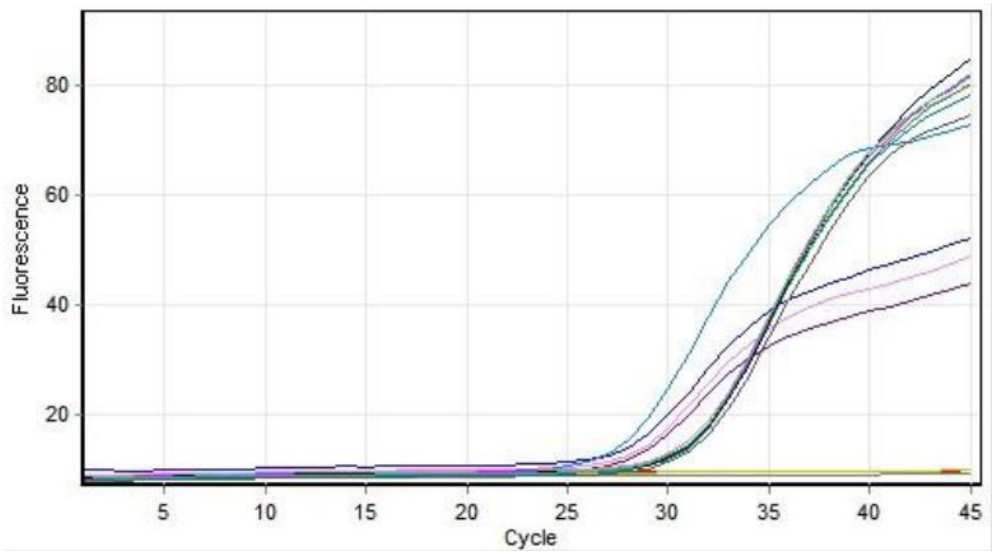


Figure: Graphical representation of activity in Control Untreated Textiles - E Gene

- **Control Untreated Textiles - S Gene**

No.	Color	Name	Type	Ct
1		Before expose-UTT02	Textile material	-
2		Before expose-UTT02	Textile material	-
3		0 min -UTT02	Textile material	24
4		0 min -UTT02	Textile material	25
5		30 min -UTT02	Textile material	25
6		30 min -UTT02	Textile material	26
7		45 min -UTT02	Textile material	26
8		45 min -UTT02	Textile material	27
9		60 min -UTT02	Textile material	26
10		60 min -UTT02	Textile material	27
11		120 min -UTT02	Textile material	28
12		120 min -UTT02	Textile material	28
13		Negative control	Negative Control	-
14		Positive control	Positive Control	30

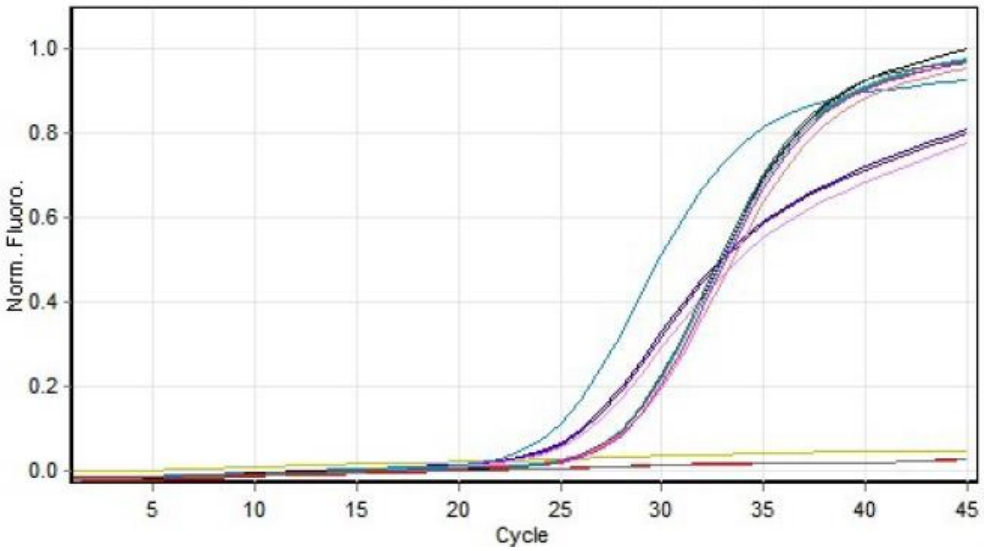


Figure: Graphical representation of activity in Control Untreated Textiles - S Gene

- **AVT001 (CareNow Antimicrobial Textiles) against E Gene:**

No.	Color	Name	Type	Ct
1		before expose -AVT001	Textile material	-
2		before expose - AVT001	Textile material	-
3		before expose - AVT001	Textile material	-
4		0 min - AVT001	Textile material	25
5		0 min - AVT001	Textile material	26
6		0 min - AVT001	Textile material	25
7		30 min - AVT001	Textile material	29
8		30 min - AVT001	Textile material	28
9		30 min - AVT001	Textile material	29
10		45 min - AVT001	Textile material	31
11		45 min - AVT001	Textile material	31
12		45 min - AVT001	Textile material	30
13		60 min - AVT001	Textile material	-
14		60 min - AVT001	Textile material	-
15		60 min - AVT001	Textile material	-
16		120 min- AVT001	Textile material	-
17		120 min - AVT001	Textile material	-
18		120 min- AVT001	Textile material	-
19		Negative control	Negative Control	-
20		Positive control	Positive Control	29

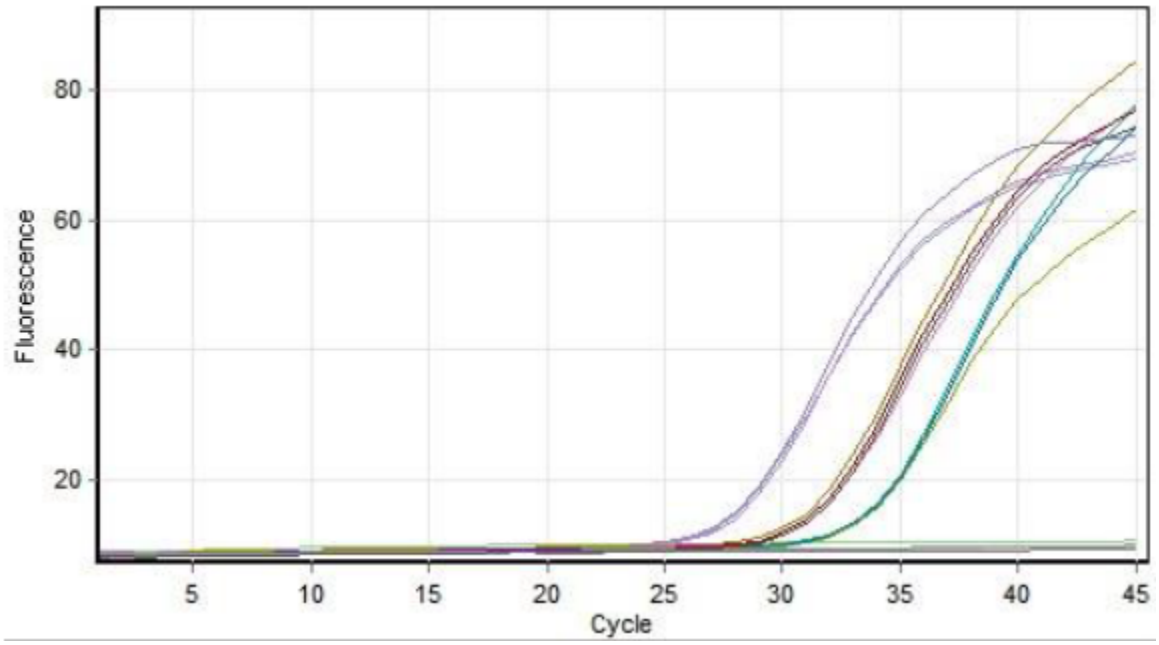


Figure: Graphical representation of activity in CareNow Antimicrobial Textiles (E Gene)

- **AVT001 (CareNow Antimicrobial Textiles) against S Gene:**

No.	Color	Name	Type	Ct
1		before expose - AVT001	Textile material	-
2		before expose - AVT001	Textile material	-
3		before expose - AVT001	Textile material	-
4		0 min - AVT001	Textile material	24
5		0 min - AVT001	Textile material	24
6		0 min - AVT001	Textile material	25
7		30 min - AVT001	Textile material	27
8		30 min - AVT001	Textile material	27
9		30 min - AVT001	Textile material	27
10		45 min - AVT001	Textile material	29
11		45 min - AVT001	Textile material	29
12		45 min - AVT001	Textile material	29
13		60 min - AVT001	Textile material	-
14		60 min - AVT001	Textile material	-
15		60 min - AVT001	Textile material	-
16		120 min- AVT001	Textile material	-
17		120 min - AVT001	Textile material	-
18		120 min- AVT001	Textile material	-
19		Negative control	Negative Control	-
20		Positive control	Positive Control	29

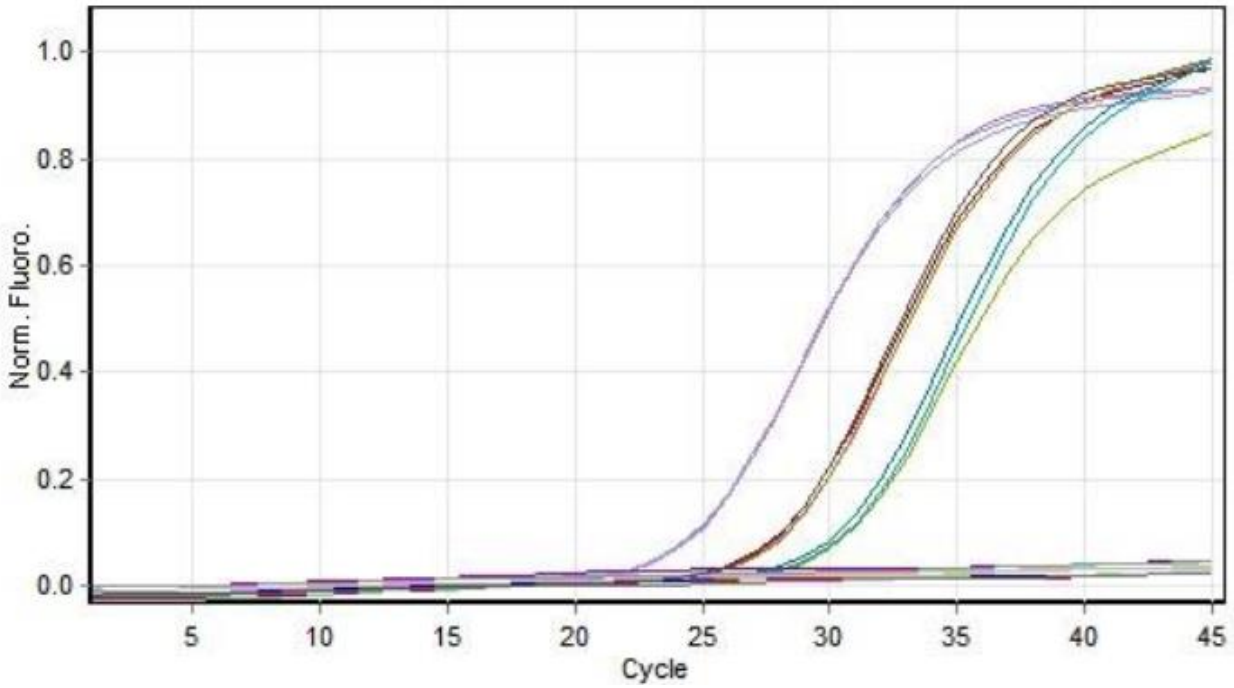


Figure: Graphical representation of activity in CareNow Antimicrobial Textiles (S Gene)

b. Tabular representation of viral reduction rate

Sl. No.	Time (min)	AVT001						UTT02					
		SARS-CoV-2											
		E Gene			S Gene			E Gene			S Gene		
		Ct Value*	Delta Ct**	%Reduction in Viral load	Ct Value	Delta Ct	%Reduction in Viral load	Ct Value	Delta Ct	%Reduction in Viral load	Ct Value	Delta Ct	%Reduction in Viral load
1	0	25	-	-	24	-	-	26	-	-	24	-	-
2	30	29	4	-	27	3	-	27	1	NS[##]	25	1	NS
3	45	31	2	>95	29	2	>95	28	1	NS	26	1	NS
4	60	ND[#]	-	99	ND	-	99	29	1	NS	27	1	NS
5	Positive control	29	-	-	29	-	-	30	-	-	30	-	-
6	Negative control	ND	-	-	-	-	-	-		-	-	-	-

* means triplicates ** Delta CT of 3-4 corresponds to 1 log difference #ND -Not Detected ## NS-Non-significant

c. Inference and Conclusion:

1. In the present study, SARS-CoV-2 specific RNA (E&S target gene) was not detected after 45 minutes of contact time, indicating rupturing of viral envelope with >95% of reduction in viral load in test sample whereas the Control Sample of Untreated Textile exhibited Not Significant reduction of Viral Load.
2. It has also significantly enhanced the antiviral log reduction and reduces viral infectivity by 99% reduction of viral load in 60minutes of contact time.
3. It indicates, test sample (CareNow Antimicrobial Textiles) shows effective antiviral activity against encapsulated SARS-CoV-2 virus within 45 minutes of contact time.

d. Quality Control:

In accordance with the ISO 15189:2012-certified Quality Management System, each lot of SARS-CoV-2 RT-PCR assay is tested against predetermined specifications to ensure consistent product quality.