



## CareNow AIRMASK Introduction

CareNow AIRMASK is a germ free & reusable filtration mask designed to protect from harmful pollutants and germs. Polluted atmospheric conditions in urban areas contain infection causing pathogens and particulate matter such as sulfur, manganese, iron dust, Cadmium, Cobalt & several others. CareNow AIRMASK is intended for use for applications involving filtration of air, gas, chemicals, smoke, pollutants and germs. It is not indicated for use involving heavy physical activity like running.

CareNow AIRMASK is a filtration innovation at its best. Its unique, 4-layer antimicrobial cloth mask blocks out more than 95% of harmful particulate matter and instantly kills infection-causing germs (Self Santizing)—all in an advanced, breathable, hypoallergenic design chosen for your comfort and fashion. The adjustable ear loops and nose bridge enables comfort fit for all age groups.

CareNow Airmask consists of 4 layers. The outer and inner layer that comes in contact with the skin is made of 100% Cotton. This fabric is also enhanced with Anti-microbial technology of CareNow called as BLUFENZ that continuously inhibits the growth of pathogen. This ensures that AIRMASK is free from 99.99% of germs at all times.

The science behind BLUFENZ is a unique antimicrobial technology that provides a barrier of protection which is highly effective and everlasting through the life of the fabric. An antimicrobial shield is formed by a long molecular chain of carbon atoms with a +vely charged to a Silica Atom & bonded to the fabric. Pathogen, which are -vely charged comes in contact with the +vely charged molecular chain & undergoes lysis. This mechanism provides continuous defense achieving anti-microbial activity and does not deplete over washes.

## Testing Summary

S.No	Description of Test on AIRMASK	Standard	Results	Testing Authority
1.	Anti-Microbial Effectiveness using bacteria	JISL 1902:2008	Achieved 99.99% kill rate against Bacteria	Intertek
2.	Bacterial Filtration Efficiency Test	ASTM F-2101	Achieved 95% and above Filtration Efficiency	Center of Excellence for Medical Textiles, The South India Textile Research Association, Government of India
3.	Particulate Filtration Efficiency Test	ASTM F2299/F2299 M-03 (2010)	Achieved 95% and above Filtration Efficiency	
4.	Viral Filtration Efficiency Test	—	Achieved less <10 PFU/ml	

PRODUCT SPECIFICATIONS	
<b>Product</b>	CareNow AIRMASK
<b>Product Code</b>	CM001
<b>Product Description</b>	Reusable Cloth Filtration Mask for air, gas, chemicals, smoke, pollutants and germs
<b>Indications for Use</b>	CareNow AIRMASK is intended for everyday use for applications involving filtration of air, gas, chemicals, smoke, pollutants and germs.
<b>Contra Indications</b>	It is not indicated for use involving heavy physical activity like running.
<b>HS Code</b>	63079090
<b>Shelf Life</b>	100 Washes
<b>Colors</b>	Available in Colors of Black and Blue
<b>Sizes</b>	Available in Unisex size of Large for all adults and Small for children under the age of 10
<b>Washing Instructions</b>	    
<b>Material Composition</b>	CareNow AIRMASK consists of 4 layers of 100% Cotton Fabric with treated Anti-microbial property, Filtration layer made of PP and Activated Carbon layer. It also has an exhalation valve and elastic ear loops for comfort wear
<b>Anti-Microbial Effectiveness of Cotton Fabric</b>	99.99% Kill rate against bacteria and fungi
<b>Bacterial Filtration Efficiency</b>	95% of BFE of 3 micron and up
<b>Particulate Filtration Efficiency</b>	95% of 0.3micron and up
<b>Packaging Information</b>	
<b>Inner Box</b>	Each Inner Box of CareNow AIRMASK contains one piece of AIRMASK  Dimension: 140 x 140 x 25 mm
<b>Carton</b>	Each Carton contains 60 pcs of CarNow AIRMASK  Dimension: 300 x 400 x 150 mm

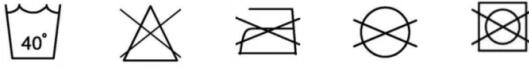
## Instructions For Use



# AIRMASK

CareNow AIRMASK is manufactured in India by CareNow Medical Pvt Ltd, a manufacturer of Persistent Anti-microbial products. CareNow AIRMASK consists of Microbicidal Composite Material, a patented technology of CareNow Medical that continuously inhibits the growth of pathogens on the surface of the material. CareNow AIRMASK is lab-tested for all its functionalities to ensure unparalleled quality and efficiency.

No.	Description of Test on AIRMASK	Standard	Results
1	Anti-microbial Effectiveness using bacteria	JISL 1902:2008	Anti-Bacterial Efficiency achieved 99.99% protection.
2	Bacterial Filtration Efficiency Test	ASTM F-1201	Achieved good performance of 95% and above filtration.
3	Particulate Filtration Efficiency at 0.3 Microns	ASTM F2299/F2299 M-03(2010)	Achieved good performance of 95% and above



CareNow Airmask is a stylish, highly efficient, well fitting, comfortable filtration mask for every day use. It provides best in class filtration against germs, pollutants and reduces odor while actively disinfecting the inner and outer layers of the fabric.

CareNow Airmask consists of 4 layers. The outer and inner layer that comes in contact with the skin is made of 100% Cotton. This fabric is also enhanced with Anti-microbial technology of CareNow that continuously inhibits the growth of pathogen. This ensures that AIRMASK is free from 99.99% of germs at all times. For more information on the technology, please visit [www.carenowmedical.com](http://www.carenowmedical.com).

**Directions for Use:** Position the mask to fit your nose and mouth. Stretch the elastic ear loop bands to go around the ears. Adjust the nose area for a snug fit. **Wash Instructions:** Gently wash the mask in cold water. Line dry only. Do not bleach. Do not iron.

CareNow Airmask हर दिन उपयोग के लिए एक स्टाइलिश, अद्यतिक और अच्छी तरह से फिटिंग, आरामदायक नियंत्रण मास्क है। यह कौटुम्बिक, प्रदूषकों के खिलाफ वर्ग नियंत्रण में बहुसे अच्छा प्रदर्शन करता है और कपड़े की अद्यतिक और बाहरी परतों को स्क्रिप्ट रूप से कौटुम्बिक रूप से चुनौती देता है। बाहरी और भीतरी परत जो त्वचा के संपर्क में आती है वह 100% कॉटन से बनी होती है। इस कपड़े को CareNow की एंटी-माइक्रोबियल तरलीक से भी बढ़ाया जाता है जो लतातार रोजाना के विकास को रोकता है। यह सुनिश्चित करता है कि AIRMASK हर समय 99.99% कौटुम्बिक से बुनत हाँ। प्रोयोगिकों के बारे में अधिक जानकारी के लिए, कृपया [www.carenowmedical.com](http://www.carenowmedical.com) पर जाएं।

CareNow Airmask 是一款时尚，高效，合身，舒适的过滤面罩，可每天使用。它提供了一流的过滤能力，可防止细菌，污染物和气味，同时可对织物的内层和外层进行积极消毒。

CareNow Airmask由4层制成。与皮肤接触的内外层由100%棉制成。CareNow的抗微生物技术还可以连续抑制病原体的生长，从而增强了这种面料。这样可以确保AIRMASK始终都不含99.99%的细菌。有关该技术的更多信息，请访问[www.carenowmedical.com](http://www.carenowmedical.com)

使用说明：放置好口罩以适合您的鼻子和嘴巴。拉伸松紧的耳圈带，以环绕耳朵。调整鼻部区域以贴身。清洗说明：用冷水轻轻清洗口罩。仅手洗。不要漂白。不要熨烫。

## Packaging



## TEST REPORT

NUMBER : TRPT16069851  
 DATE : 28-Aug-2016

TEST CONDUCTED (AS REQUESTED BY THE APPLICANT)

**1. Anti Bacterial Test JIS L 1902: 2008**

**ANTIBACTERIAL ACTIVITY**

1. Test Bacteria: **Staphylococcus aureus ATCC 6538**

<b>Quantitative Assessment of Activity - JIS L 1902: 2008</b>				
<b>UNTREATED FABRIC:</b> Concentration of Inoculum (Ma): $1.06 \times 10^5$		Log = 5.02		
<b>UNTREATED FABRIC:</b> Concentration after 18 hour incubation (Mb): $5.00 \times 10^6$		Log = 6.69		
Growth Value (F= Mb-Ma): 1.67				
Sample Identification	No. Bacteria Recovered	Log Bacteria Recovered (Mc)	Bacteriostatic Activity (S) = Mb - Mc	Percentage Reduction of Bacteria
<b>TRPT16069851 – BLACK COLOR FABRIC</b>	< 10	< 1	<b>&gt;5.69</b>	<b>&gt;99.99</b>

s2. Test Bacteria: **Klebsiella pneumoniae ATCC 4352**

<b>Quantitative Assessment of Activity - JIS L 1902: 2008</b>				
<b>UNTREATED FABRIC:</b> Concentration of Inoculum (Ma): $1.14 \times 10^5$		Log = 5.05		
<b>UNTREATED FABRIC:</b> Concentration after 18 hour incubation (Mb): $5.80 \times 10^6$		Log = 6.76		
Growth Value (F= Mb-Ma): 1.71				
Sample Identification	No. Bacteria Recovered	Log Bacteria Recovered (Mc)	Bacteriostatic Activity (S) = Mb - Mc	Percentage Reduction of Bacteria
<b>TRPT16069851 – BLACK COLOR FABRIC</b>	< 10	< 1	<b>&gt;5.76</b>	<b>&gt;99.99</b>

The Standard Antimicrobial value of Evaluation = S  $\geq$  2.0

Where-

Ma=Logarithm of Concentration of Starting bacterial inoculum (CFU/ml)

Mb=Logarithm of number of bacteria after 18 hour incubation on untreated sample (Average of 3 specimens)

Mc=Logarithm of number of bacteria after 18 hour incubation on treated sample (Average of 3 specimens)

**COMMENT:**

When tested as specified, Garment labeled **TRPT16069851 – BLACK COLOR FABRIC**; **PASSES** the Quantitative Assessment of activity for **Staphylococcus aureus** and **Klebsiella pneumoniae** by **JIS L 1902: 2008 Test Method**.

## END OF THE TEST REPORT ##

*This report is made solely on the basis of your instructions and/or information and materials supplied by you. It is not intended to be a recommendation for any particular course of action. Intertek does not accept a duty of care or any other responsibility to any person other than the Addressee in respect of this report and only accepts liability to the Addressee insofar as is expressly contained in the terms and conditions governing Intertek's provision of services to you. Intertek makes no warranties or representations either express or implied with respect to this report save as provided for in those terms and conditions. We have aimed to conduct the Review on a diligent and careful basis and we do not accept any liability to you for any loss arising out of or in connection with this report, in contract, tort, by statute.*



# THE SOUTH INDIA TEXTILE RESEARCH ASSOCIATION

P.B.No.3205, 13/37, Avinashi Road, Aerodrome P.O., Coimbatore - 641 014, INDIA

Ph: (0422) 2574367-9, 4215333

Fax : (0422) 2571896, 4215300

E-mail : info@sitra.org.in

Website : <http://www.sitra.org.in>

Address all correspondence to the Director

## CoE - Microbiology Laboratory

Test Report No	: M1900241	Report Date	: 05-03-2020
SITRA Ref No	: XXVII / M / E / 1606 / 20	No of Samples	: 1
Customer Ref No	: DT-18.02.2020	Received On	: 04-03-2020

To

**Care Now Medical Pvt Ltd**

3/272-5 Neelambur Road,  
Muthugounden Pudur,  
Coimbatore, Tamil Nadu, India  
Pincode : 641406  
Contact No(s) : 0422-2914949, 9047111714

Tested on: 25.02.2020 to 27.02.2020

Dear Sir / Madam,

This has reference to the sample(s) submitted by you for testing vide your Requisition Form reference no : **DT-18.02.2020**.

The results pertaining to your sample(s) are enclosed herewith.

*Yours faithfully,*

Authorized Signatory

*Dr. E. Santhi*

Encl : Bill [ T-COE-1920-01425 ]



## IMPORTANT

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Address all correspondence to the Director

Test Report No : M1900241

Report Date : 05-03-2020

Care Now Medical Pvt Ltd

Ref : DT-18.02.2020

**Test Name :** Bacterial Filtration Efficiency ASTM F 2101-Standard Test Method for Evaluating the Bacterial Filtration Efficiency (BFE) of Medical Face Mask Materials, Using a Biological Aerosol of *Staphylococcus aureus*

**Test Condition:**

Inoculum size : *Staphylococcus aureus* ATCC 6538 =  $5 \times 10^5$  CFU/ mL

Media used : Tryptic soya agar

Dilution medium used : Peptone water

Incubation conditions :  $37^\circ\text{C}$  for 24 h

**Observation :**

Test Parameter	M1900241-1
	Described by the customer : Fabric: CareNow Mask Batch No:CM0220
Area of test specimen	Facemask
Sample exposure side	Faceside
Flow rate of aerosol	28.5 L/min
Mean particle size of challenging aerosol	$3.0 \pm 0.3$ micron
Average plate count of positive control	2221
Average plate count of negative control	0
BFE of test specimen (%)	95.1

**Result :** The sample showed 95.1% bacterial filtration efficiency against *Staphylococcus aureus* ATCC 6538 when tested according to ASTM F 2101 test method.

5/3/2020

Authorized Signatory

Dr. E. Santhi.

- End of Report -





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## CoE - PHYSICAL LABORATORY

XXVII / P / E / 1603 / 20

Inward / Report No : P1900362

Inward Date : 05-03-2020

Report Date : 05-03-2020

To

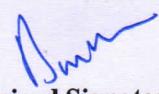
Care Now Medical Pvt Ltd  
3/272-5neelambur Road,  
Muthugounden Pudur,  
Coimbatore,Tamil Nadu ,India  
Pincode : 641406  
Contact No(s) : 0422-2914949,9047111714

Dear Sir / Madam,

This has reference to the sample(s) submitted by you for testing vige your letter reference no :  
**DT-18.02.2020.**

The results pertaining to your sample(s) are enclosed herewith.

*Yours faithfully,*

  
**Authorized Signatory**

Encl : Bill



### IMPORTANT

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E-mail : info@sitra.org.in

Website : <http://www.sitra.org.in>

Address all correspondence to the Director

Care Now Medical Pvt Ltd, Ref : DT-18.02.2020

(Sample Tested at : R.H. 65% +/- 2% and Temp. 21 Degree C +/- 1 Degree C)

Particulate Filtration Efficiency at 0.3 Microns ASTM F2299/F2299M-03 (2010)	P1900362-1 Described by the Customer : Carenow mask Batch No:CM0220
Percentage	98.78

Test Side : Face Side (Outside)

Area Tested : 33.17cm<sup>2</sup>

Particle Size : 0.3μm

Laboratory Condition : 21°C, 50%RH

- End of Report -



*[Signature]*



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E-mail : info@sitra.org.in

Website : <http://www.sitra.org.in>

Address all correspondence to the Director

## CoE - Microbiology Laboratory

Test Report No	: M1900250	Report Date	: 05-03-2020
SITRA Ref No	: XXVII / M / E / 1610 / 20	No of Samples	: 1
Customer Ref No	: Dt.18.02.2020	Received On	: 18-02-2020

To

**Care Now Medical Pvt Ltd**

3/272-5neelambur Road,  
Muthugounden Pudur,  
Coimbatore,Tamil Nadu ,India  
Pincode : 641406  
Contact No(s) : 0422-2914949,9047111714

Tested on: 27.02.2020 to 04.03.2020

Dear Sir / Madam,

This has reference to the sample(s) submitted by you for testing vide your Letter reference no :  
**Dt.18.02.2020.**

The results pertaining to your sample(s) are enclosed herewith.

*Yours faithfully,*

Authorized Signatory

**Dr. E. Santhini**

Encl : Bill | T-COE-1920-01427 |



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Website : http://www.sitra.org.in

Address all correspondence to the Director

Test Report No : M1900250

Report Date : 05-03-2020

Care Now Medical Pvt Ltd

Ref : Dt.18.02.2020

Test Name : Viral Filtration Efficiency In house method

**Test Condition:**

Inoculum size	: Bacteriophage phi x 174 (ATCC 13706) = $2 \times 10^8$ PFU/mL; <i>E.coli</i> ATCC 13706
No .of test articles tested	: 6
No .of test articles passed	: 6
Media used	: Bacterio phage Nutrient agar
Method of plating	: Spread plate
Inoculum / plate	: <i>E.coli</i> : 0.1 ml; Bacteriophage: 0.5 mL
Incubation conditions	: 37°C for 24 h
Pressure & Exposure Duration	: 1.6 psi for 3 min
Sample Size	: 47mm (Dia)

**Observation :**

Observation	M1900250-1		
	Pre- Challenge Assay Titer (PFU/mL)	Post- Challenge Assay Titer (PFU/mL)	Filtration
Positive Control (0.22 µm cellulose nitrate membrane Filter)	$2.0 \times 10^8$	$1.9 \times 10^8$	No
Sample - Trial 1	$2.0 \times 10^8$	<10	Yes
Sample - Trial 2	$2.0 \times 10^8$	<10	Yes
Sample - Trial 3	$2.0 \times 10^8$	<10	Yes
Sample - Trial 4	$2.0 \times 10^8$	<10	Yes
Sample - Trial 5	$2.0 \times 10^8$	<10	Yes
Sample - Trial 6	$2.0 \times 10^8$	<10	Yes

**Result :** : The test sample filtered test organism when tested according to In-House test method

*B. A. N.*  
6/3/2020

Authorized Signatory

Dr. E. Santhini

- End of Report -



**CARENOW MEDICAL PRIVATE LIMITED**  
**CARENOW AIRMASK**

Author: Akshay Jadhav

Reviewed by: R. Kathiresan

Approved by: Anand Venkatachalam

## Revision History:

CARENOW MEDICAL PRIVATE LIMITED		
CARENOW AIRMASK		
<b>Title:</b> Report on Viricidal activity of CareNow AIRMASK	<b>Doc:</b> CNAM1600	
<b>Date:</b> 30 <sup>th</sup> Jan 2020	<b>Revision No:</b> 1.0	Page 2 of 6

## A. Scope

The scope of this document is to report viricidal activity of CareNow AIRMASK

## B. Reference

- “The effectiveness of antiviral agents with broad-spectrum activity against chikungunya virus varies between host cell lines” *Evelyn J Franco*1,2, *Jaime L Rodriguez*1, *Justin J Pomeroy*1, *Kaley C Hanrahan*1 and *Ashley N Brown*.
- “Apoptotic induction of lung adenocarcinoma A549 cells infected by recombinant RVG Newcastle disease virus (rL-RVG) *in vitro*” *YULAN YAN*1, *BING LIANG*2, *JIN ZHANG*1, *YANG LIU*1 and *XUEFENG BU*.

## C. Procedure:

1. Sample – Fabric of CareNow AIRMASK
2. Control – Blank Solution
3. 1 sq.cm sample was infected with 10<sup>9</sup> PFU/mL of bacteriophage phi X 174 for 24h at 37 Deg C.
4. Viable bacteriophage was extracted from the sample by shaking at 200 rpm in PBS for about 3h.
5. The filtrate was filtered through 0.45 micron syringe filter.
6. In parallel, A549 cells at the density of 1x 10<sup>4</sup> and 10<sup>5</sup> was seeded in 96 and 24 well plate respectively in DMEM (10 %) for 24 h at CO<sub>2</sub> incubator.
7. Cells after reaching 90 % confluence was infected with bacteriophage (positive control) and different volume of filtrate.
8. Incubation was continued in CO<sub>2</sub> incubator for 24h.
9. At the end of incubation, morphology of cells infected with and without bacteriophage were observed under Inverted Phase Contrast Microscopy.
10. MTT assay was performed to quantify the viable cells.

## D. Observation Table

Test groups	Cytotoxicity	Cell viability	Virucidal activity
control	0	100	NA
Phage 10 <sup>9</sup>	95.4	4.6	Observed
10 <sup>5</sup>	81.6	18.4	Observed
10 <sup>1</sup>	59.8	40.2	Not observed
10 microL	31.8	68.2	Observed
20 microL	32.0	68.0	Observed
30 microL	28.6	71.4	Observed

CARENOW MEDICAL PRIVATE LIMITED			
CARENOW AIRMASK			
<b>Title:</b> Report on Viricidal activity of CareNow AIRMASK		<b>Doc:</b> CNAM1600	
<b>Date:</b> 30 <sup>th</sup> Jan 2020	<b>Revision No:</b> 1.0		Page 3 of 6

40 microL	72.9	27.1	Not observed
50 microL	78.1	21.9	Not observed
60 microL	94.8	5.2	Not observed
70 microL	94.8	5.2	Not observed
80 microL	94.7	5.3	Not observed
90 microL	96.0	4.0	Not observed
100 microL	95.3	4.7	Not observed

#### E. Inference

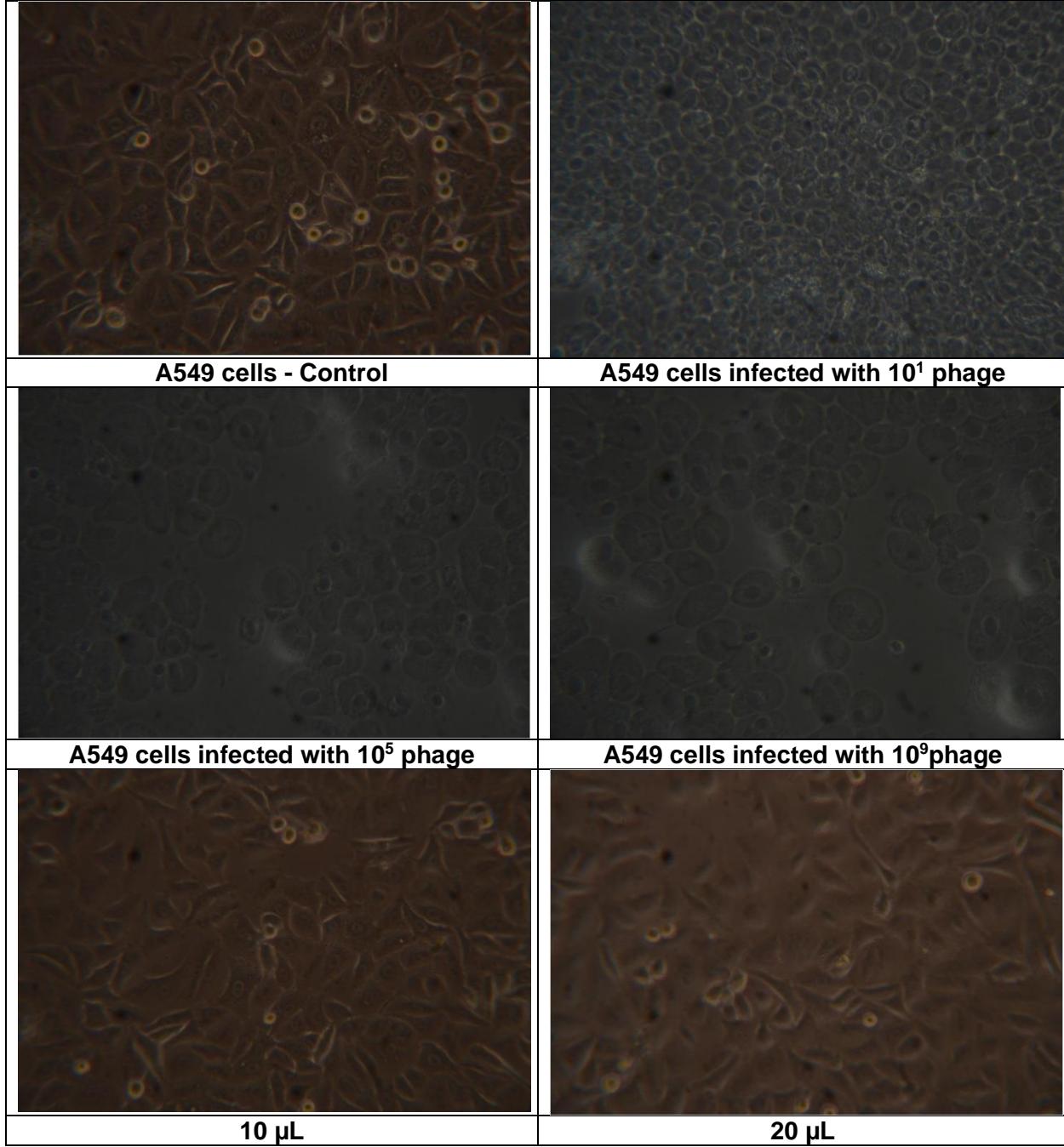
Morphology:

Elongated cells are viable and healthy; round and cells with disturbed membrane are dead cells. Morphology of the cells infected with 10 to 30 microL of filtrate was comparable with control cells suggesting the viricidal activity or the available virus is not sufficient enough to infect A549 cells. But when the volume of filtrate increases, no. of viable cells decreased. This in turn suggest the ability of virus to infect the cells after incubating in fabric.

Similar trend was observed in MTT assay as well where the number of viable cells decreased when increasing the volume of the filtrate.

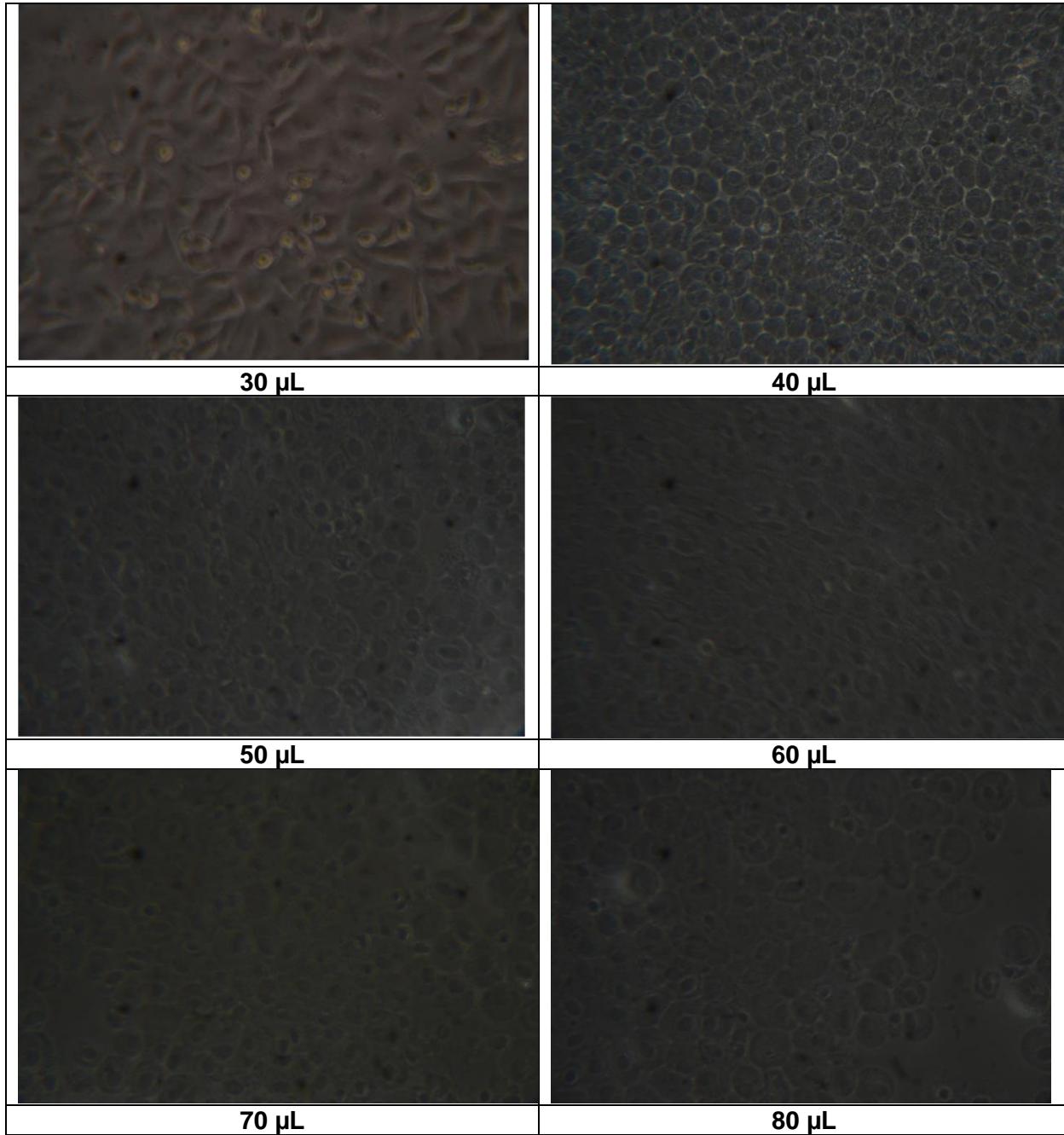
**CARENOW MEDICAL PRIVATE LIMITED****CARENOW AIRMASK****Title:** Report on Viricidal activity of CareNow AIRMASK**Doc:** CNAM1600**Date:** 30<sup>th</sup> Jan 2020**Revision No:** 1.0

Page 4 of 6

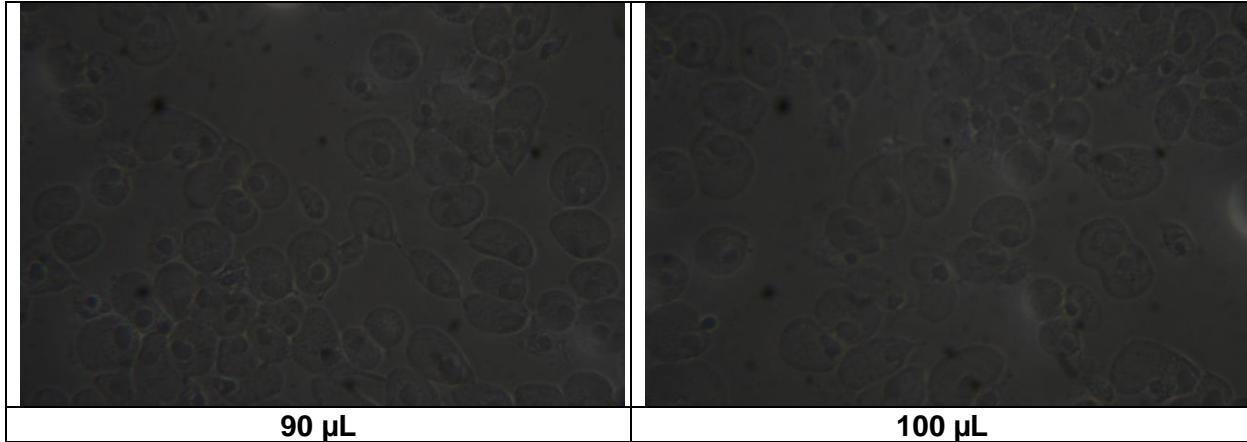
**F. Images**

**CARENOW MEDICAL PRIVATE LIMITED**  
**CARENOW AIRMASK**

**Title:** Report on Viricidal activity of CareNow AIRMASK      **Doc:** CNAM1600  
**Date:** 30<sup>th</sup> Jan 2020      **Revision No:** 1.0      **Page 5 of 6**



CARENOW MEDICAL PRIVATE LIMITED		
CARENOW AIMASK		
<b>Title:</b> Report on Viricidal activity of CareNow AIMASK	<b>Doc:</b> CNAM1600	
<b>Date:</b> 30 <sup>th</sup> Jan 2020	<b>Revision No:</b> 1.0	Page 6 of 6



#### G. Conclusion

1 Sq Cm of Fabric is found to exhibit antiviral activity on the challenge loads of  $10^9$  PFU/mL when the cells were infected at concentrations of 10-30  $\mu$ L.

#### G. Remarks

The test conditions of Viral Load of  $10^9$  PFU/mL is an extreme condition. Under normal use conditions, the viral load will be significantly less. The current test item has performed satisfactorily under the extreme conditions. Hence usage on the normal conditions will have a good viricidal activity on the Test Fabric. The Test product can now be further tested under actual conditions and actual/surrogate of different viruses including H1N1, SARS-COV1, SARS-COV2 and other influenza viruses.

**CARENOW MEDICAL PVT LTD**  
**CARENOW AIRMASK**

Author: Akshay Jadhav

Reviewed by: R. Kathiresan

Approved by: Anand Venkatachalam

## Revision History:

CARENOW MEDICAL PVT LTD		
CARENOW AIRMASK		
<b>Title:</b> To Study performance of CareNow AIRMASK after washing		<b>Doc.No.:</b> CNAM100
<b>Date:</b> 13/01/2020	<b>Rev. No.:</b> 1.0	<b>Page 2 of 6</b>

#### **Scope:**

The scope of this report is to document study performance of CareNow AIRMASK after washing.

#### **Background:**

CareNow AIRMASK is a germ free & reusable filtration mask designed to protect from harmful pollutants and germs. is intended for use for applications involving filtration of air, gas, chemicals, smoke, pollutants and germs. It is not indicated for use involving heavy physical activity like running. CareNow AIRMASK is a filtration innovation at its best. Its unique, 4-layer antimicrobial cloth mask blocks out more than 95% of harmful particulate matter and instantly kills infection-causing germs (Self Sanitizing)—all in an advanced, breathable, hypoallergenic design chosen for your comfort and fashion.

This fabric is also enhanced with Anti-microbial technology of CareNow called as BLUFENZ that continuously inhibits the growth of pathogen. This ensures that AIRMASK is free from 99.99% of germs at all times. This mechanism provides continuous defense achieving anti-microbial activity and does not deplete over washes.

Thus, to verify, the study was conducted, whether CareNow AIRMASK provides same protection after washes. In the study CareNow AIRMASK will be washed and tested at an interval of 25 washes, the study continued till 110 washes. The results received were after 25 wash, 50 wash, 75 wash and 110 wash

#### **Reference**

- ASTM E2315
- BS EN ISO 11737

#### **Procedure of ASTM E2315:**

1. AIRMASK were washed under tap water and dried in indoor atmosphere. Wash AIRMASK 25 times
2. Prepare nutrient agar plates and prepare nutrient broth tubes
3. Transfer a loop full of test organisms into tube containing 5 ml of sterile nutrient broth marked with date, name of the bacterium, strain number. prepare one UN-inoculated tubes as control tubes
4. Incubate all the tubes at 37°C for 24 hours
5. Prepare the test specimen by cutting the specimen in square shapes which will be equivalent to 1 gm.
6. Prepare test inoculum by transferring  $1.0 \pm 0.1$  mL of 24 hours old bacterial culture into tubes containing  $9.0 \pm 0.1$  mL of sterile distilled water and then mix it with the help of vortex mixer.

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7. Mark the plates and screw cap bottles, such as test date, organisms, sample particulars, and individual signature.
8. Place the test specimens inside the Sterile screw cap bottles.
9. Using a sterile Micro pipette (1000µl), add 1 ml of test inoculum (which is prepared in step 5) on the surface of the test specimen.
10. Make sure that the test specimen is completely contacted with the test inoculum.
11. Immediately transfer the swatches in to sterile 250 mL culture bottle and add  $100 \pm 1$  mL of 0.05 % neutralizing solution, shake bottle vigorously for 1 minutes and make serial dilutions (  $10^0, 10^1, 10^2, 10^3$  ) with sterile water.
12. Incubate the inoculated plates at  $37^\circ\text{C}$  for 24 – 48 hours. After incubation, count the number of bacterial colonies, the average number of bacterial colonies is noted as number of bacteria presented in 0<sup>th</sup> contact time.
13. Repeat the step 7 and for antibacterial treated swatches, transfer the swatches in to sterile 250 mL culture bottle close the lid tightly and incubate it at  $37^\circ\text{C}$  for 24hours. After incubation repeat step 8 and 9,
14. count the number of bacterial colonies. Number of recovered bacterial colonies is noted as number of bacteria presented in 1<sup>st</sup> hour contact time.
15. Incubate the bottles with inoculated Test specimens at  $37 \pm 2^\circ\text{C}$  for 24 hours.
16. After 24 hours of incubation count the bacterial colonies for results.
17. Similar procedure has to be performed after 50 wash, 75 wash and 110 wash.

#### **Procedure of BS EN ISO 11737:**

##### **Media preparation**

1. Weigh accurately the required quantity of Buffered Sodium chloride peptone solution pH 7.
2. Pour the required quantity of DM water and mix it, boil it to dissolve and close it tightly.
3. Perform the above step for Total Plate Count Agar and Agar medium c.
4. Sterilization will be proceed using autoclave at  $121^\circ\text{C}$  for 20 mins under 15 LBS pressure.

##### **Sample preparation**

1. AIRMASK samples was washed 25 times under tap water and dried in indoor atmosphere
2. The sample has been exposed to room temperature before testing it.
3. Take a number sample (ex: Single swabs, wipes etc...) and immerse it in a 250 mL pf

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sterile buffered sodium chloride peptone solution along with sterile magnetic beads and close the container.

4. Stir the solution in Magnetic stirrer at appropriate room temperature for 5 mins.
5. Similarly, samples were prepared after 50 wash, 75 wash and 110 wash.

### Sample inoculation

1. Perform Membrane filtration followed by sample preparation at sterile condition.
2. Repeat the above step for triplicate in random sample and also in anaerobic condition also if required.
3. Place the filtered membrane on the Plate Count Agar and Agar Medium C and Incubate for 37°C for 24 to 48 hours. In Bacteriological incubator and 28°C for 5 to 7 days respectively in BOD incubator.

After incubation enumerate the CFU (Colony Forming Units) and report the results.

### Conclusion:

In Table 1 to 5, product CareNow AIRMASK was tested as per ASTM E2315 at wash interval of 0, 25, 50, 75, 110 washes. Result shows no changes in log reduction %

In Table 6 and figure 1 product CareNow AIRMASK was tested as per BS EN ISO 11737 at wash interval of 0, 25, 50, 75, 110 washes, obtained result shown bioburden is increasing up to 19.2 CFU/100cm<sup>2</sup> when AIRMASK was washed 110 times.

At 110 wash log reduction % was 99.9999% and bioburden was 19.2 CFU/100cm<sup>2</sup>. Thus, it can be concluded that product CareNow AIRMASK can be used up to washes not more than 100

### Result:

**Table 1 CareNow AIRMASK performance after 0 wash**

ASTM E-2315 After 0 wash					
S.NO	Test Organisms	% of Reduction			
		1 hour	4 hours	24 hours	
1.	<i>Staphylococcus aureus</i> ATCC 6538	99.99	99.99	99.9999	
2.	<i>Listeria monocytogenes</i> ATCC 19115	99.99	99.99	99.9999	
3.	<i>Enterococcus faecalis</i> ATCC 29212	99.99	99.99	99.9999	
4.	<i>Escherichia coli</i> ATCC 25922	99.99	99.99	99.9999	
5.	<i>Pseudomonas aeruginosa</i> ATCC 15442	99.99	99.99	99.9999	
6.	<i>Klebsiella pneumoniae</i> ATCC 4352	99.99	99.99	99.9999	
7.	<i>Candida albicans</i> ATCC 10231	90	90	99.9999	
8.	<i>Aspergillus niger</i> ATCC 6275	90	90	99.9999	
9.	Positive Control	99.9999	99.9999	99.9999	
10.	Negative Control	TNTC	TNTC	TNTC	

Where TNTC = Too Numerous To Count ( Above 300 CFU)

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**Table 2 CareNow AIRMASK performance after 25 wash**

ASTM E-2315 After 25 wash					
S.NO	Test Organisms	% of Reduction			24 hours
		1 hour	4 hours	24 hours	
1.	<i>Staphylococcus aureus</i> ATCC 6538	99.99	99.99	99.9999	
2.	<i>Listeria monocytogenes</i> ATCC 19115	99.99	99.99	99.9999	
3.	<i>Enterococcus faecalis</i> ATCC 29212	99.99	99.99	99.9999	
4.	<i>Escherichia coli</i> ATCC 25922	99.99	99.99	99.9999	
5.	<i>Pseudomonas aeruginosa</i> ATCC 15442	99.99	99.99	99.9999	
6.	<i>Klebsiella pneumoniae</i> ATCC 4352	99.99	99.99	99.9999	
7.	<i>Candida albicans</i> ATCC 10231	90	90	99.9999	
8.	<i>Aspergillus niger</i> ATCC 6275	90	90	99.9999	
9.	<i>Positive Control</i>	99.9999	99.9999	99.9999	
10.	<i>Negative Control</i>	TNTC	TNTC	TNTC	

Where TNTC = Too Numerous To Count ( Above 300 CFU)

**Table 3 CareNow AIRMASK performance after 50 wash**

ASTM E-2315 After 50 wash					
S.NO	Test Organisms	% of Reduction			24 hours
		1 hour	4 hours	24 hours	
1.	<i>Staphylococcus aureus</i> ATCC 6538	99.99	99.99	99.9999	
2.	<i>Listeria monocytogenes</i> ATCC 19115	99.99	99.99	99.9999	
3.	<i>Enterococcus faecalis</i> ATCC 29212	99.99	99.99	99.9999	
4.	<i>Escherichia coli</i> ATCC 25922	99.99	99.99	99.9999	
5.	<i>Pseudomonas aeruginosa</i> ATCC 15442	99.99	99.99	99.9999	
6.	<i>Klebsiella pneumoniae</i> ATCC 4352	99.99	99.99	99.9999	
7.	<i>Candida albicans</i> ATCC 10231	90	90	99.9999	
8.	<i>Aspergillus niger</i> ATCC 6275	90	90	99.9999	
9.	<i>Positive Control</i>	99.9999	99.9999	99.9999	
10.	<i>Negative Control</i>	TNTC	TNTC	TNTC	

Where TNTC = Too Numerous To Count ( Above 300 CFU)

**Table 4 CareNow AIRMASK performance after 75 wash**

ASTM E-2315 After 75 wash					
S.NO	Test Organisms	% of Reduction			24 hours
		1 hour	4 hours	24 hours	
1.	<i>Staphylococcus aureus</i> ATCC 6538	99.99	99.99	99.9999	
2.	<i>Listeria monocytogenes</i> ATCC 19115	99.99	99.99	99.9999	
3.	<i>Enterococcus faecalis</i> ATCC 29212	99.99	99.99	99.9999	
4.	<i>Escherichia coli</i> ATCC 25922	99.99	99.99	99.9999	
5.	<i>Pseudomonas aeruginosa</i> ATCC 15442	99.99	99.99	99.9999	
6.	<i>Klebsiella pneumoniae</i> ATCC 4352	99.99	99.99	99.9999	
7.	<i>Candida albicans</i> ATCC 10231	90	90	99.9999	
8.	<i>Aspergillus niger</i> ATCC 6275	90	90	99.9999	
9.	<i>Positive Control</i>	99.9999	99.9999	99.9999	
10.	<i>Negative Control</i>	TNTC	TNTC	TNTC	

Where TNTC = Too Numerous To Count ( Above 300 CFU)

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**Table 5 CareNow AIRMASK performance after 110 wash**

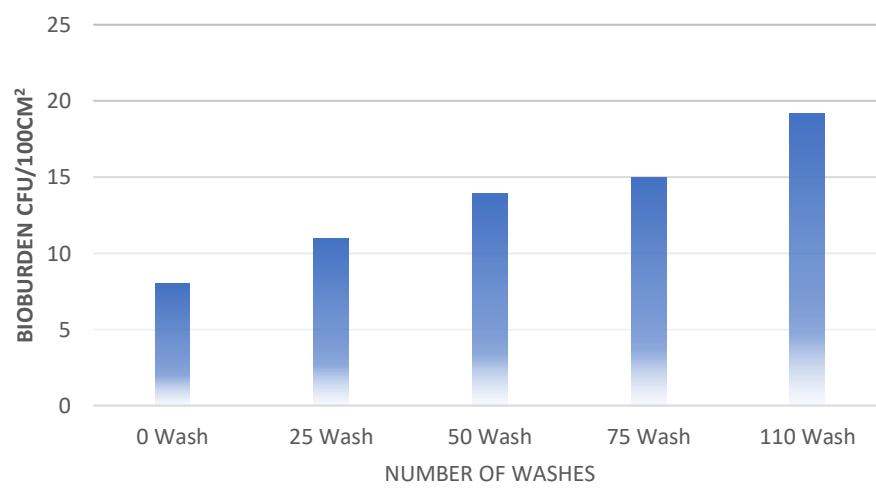
<b>ASTM E-2315 After 110 wash</b>					
<b>S.NO</b>	<b>Test Organisms</b>	<b>% of Reduction</b>			
		<b>1 hour</b>	<b>4 hours</b>	<b>24 hours</b>	
1.	<i>Staphylococcus aureus</i> ATCC 6538	99.99	99.99	99.9999	
2.	<i>Listeria monocytogenes</i> ATCC 19115	99.99	99.99	99.9999	
3.	<i>Enterococcus faecalis</i> ATCC 29212	99.99	99.99	99.9999	
4.	<i>Escherichia coli</i> ATCC 25922	99.99	99.99	99.9999	
5.	<i>Pseudomonas aeruginosa</i> ATCC 15442	99.99	99.99	99.9999	
6.	<i>Klebsiella pneumoniae</i> ATCC 4352	99.99	99.99	99.9999	
7.	<i>Candida albicans</i> ATCC 10231	90	90	99.9999	
8.	<i>Aspergillus niger</i> ATCC 6275	90	90	99.9999	
9.	<i>Positive Control</i>	99.9999	99.9999	99.9999	
10.	<i>Negative Control</i>	TNTC	TNTC	TNTC	

Where TNTC = Too Numerous To Count ( Above 300 CFU)

**Table 6 Bioburden analysis on CareNow AIRMASK as per BS EN ISO 11737**

<b>Product</b>	<b>Bioburden CFU/100cm<sup>2</sup></b>				
	<b>0 Wash</b>	<b>25 Wash</b>	<b>50 Wash</b>	<b>75 Wash</b>	<b>110 Wash</b>
CareNow AIRMASK	8	11	13.9	15	19.2

### AIRMASK BIO BURDEN ANALYSIS



**Figure 1 Bioburden analysis of CareNow AIRMASK**