organization *de*
SCALENE
SCALENE CYBERNETICS LIMITED

Declaration of Conformity

CE

Classification: Class I

Type of equipment: Scalene Hypercharge Corona Cannon.

Brand Name / Trademark: SHYCOCAN™

Type / Model: SHCC-915

Applicant: Scalene Cybernetics Limited.

Intended Use: The device **S**calene **Hy**percharge **Co**rona **Can**on (**SHYCOCAN**) is intended to be used in an industrial / commercial environment and is designed for active containment by attenuation of Corona family of viruses. **(Laboratories de Especialidades Immunological S.A. de C.V, Report No: 44527- Virucidal Activity concludes 99.9% virus elimination, dated June 16, 2020).** SHYCOCAN operates on a 110/240V – 50/60 Hz to generate extra high tension at a switching frequency of 20 KHz that delivers the necessary signals to a photon mediated electrons emitters (PMEE), that produces hypercharge high velocity electrons that interacts with the negative seeking S-protein of Corona family of viruses thus reducing infectivity and prevent air and surface borne transmission of Corona family of viruses. The Device utilizes viable biological components in the corona family of viruses for its intended purpose (Example: S- protein of the viral Spine). The device does not produce harmful ozone gas **(SSTx LLC, Report No: SHY-OZ-05072020, Test No: SHY-2306-50-1, issue date May 16, 2020.)**

The following harmonized European standards have been applied:

- 2014/30/EU – Electromagnetic Compatibility Directive
- 2014/30/EU – Low Voltage Directive
- IEC 60335-1:2010, COR1:2010, COR2:2011, AMD1:2013, COR1:2014, AMD2:2016, COR1:2016
- IEC 61326-1:2012

Including amendments by the CE Marking Directive 93/68/EEC.

The CE Marking on the products and/or their packaging signifies that Scalene Cybernetics Limited holds the reference technical file available to the European union authorities.

Place and Date of Issue: S-Card Campus, Seegehalli Main Road, Bangalore -560 049 / June 26, 2020.

Authorized Signatory:

Name: Dr. Rajah Vijay Kumar
Title: Director and Chief Scientific Officer

Signature: Jun. 26. 2020

Aug 13th 2020

To,
SCALENE CYBERNETICS LIMITED,
S- Card Campus, Seegehalli Main Road,
Virgonagar Post, Bangalore -560 049.

Sub: SHYCOCAN – Summary of Test Report

We hereby acknowledge that the device SHYCOCAN has been tested under the following standards, and reports were released with appropriate document numbers as mentioned below.

1. *Household and Similar Electrical Appliances – Safety – Part 1 General Requirements* - **IEC 60335 1:2010, COR1:2010, COR2:2011, AMD1:2013, COR1:2014, AMD2:2016, COR1:2016. - Report No: 4789499873, Date of Issue: 26.06.2020**

2. *Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 1: General Requirement* - **IEC 612326-1 Edition 2.0:2012. - Report No: 4789460695-NABL-S1, Project Number: 4789460695 ULR No: TC61682030000215F. Date of Issue: 29 April 2020.**

    a) CISPR 11 – Conducted Disturbance / Radiated Disturbances
    b) IEC 61000-3-2 – Harmonics current emissions.
    c) IEC 61000-3-3 – Voltage fluctuations and flicker emissions.
    d) IEC 61000-4-2 – Electrostatic discharge.
    e) IEC 61000-4-3 - Radiated RF disturbance.
    f) IEC 61000-4-4 – Electrical fast transient.
    g) IEC 61000-4-5 – Surge immunity.
    h) IEC 61000-4-6 – Immunity to conducted disturbance.
    i) IEC 61000-4-8 – Power frequency magnetic field.
    j) IEC 61000-4-11 – Voltage dips & short interruptions.

For UL India Pvt Ltd
Karthik Venkataraman

Karthik Venkataraman

Business Manager
Life & Health Sciences Division
Karthik.venkataraman@ul.com

6 October 2020

Our ref: M3646 - Scaleneanz Corona Cannon Ozone Measurements

Ranjit Varma
Director – Logistics & Purchase
Scaleneanz
23 Flagstaff Cr
Clyde North, VIC 3978

Email: ranjit.v@scaleneanz.com.au

RE: Ozone generation monitoring – Scalene Hypercharge Corona Canon (SHYCOCAN)

Please find enclosed the laboratory monitoring report for ozone generation associated with the *Scalene Hypercharge Corona Canon.* Testing was conducted within an enclosed environment over a 1, 4 and 8 -hour period on 2 October 2020.

Methodology

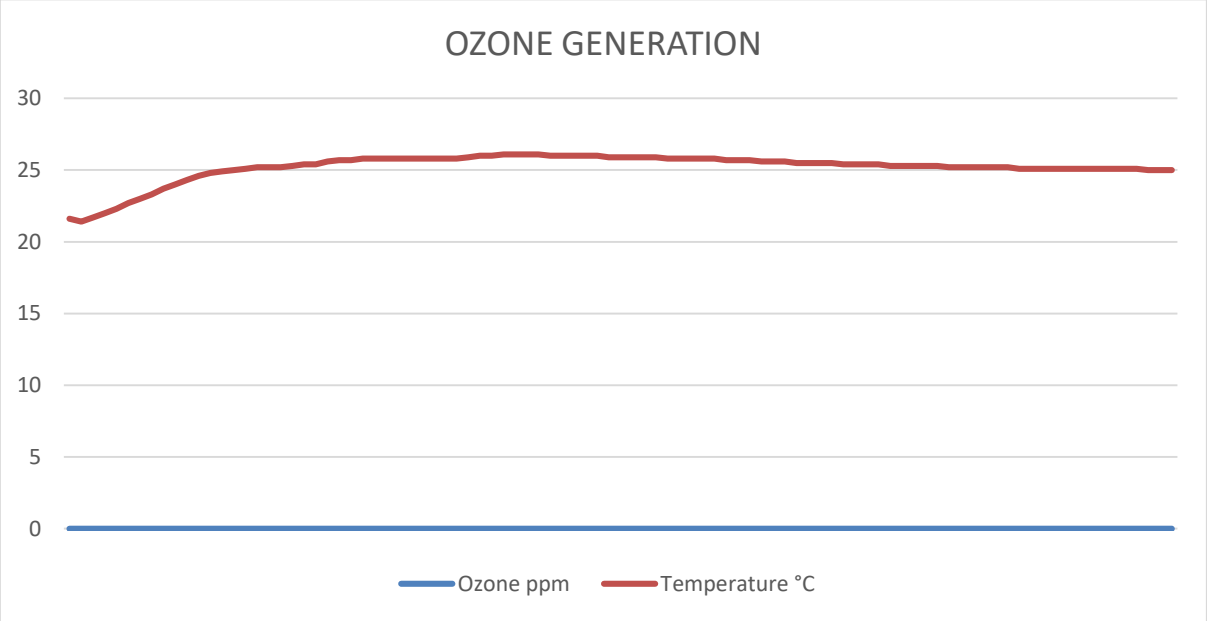
Measurements were obtained by placing the Corona Canon inside an enclosed cabinet. The tests were conducted over an 8-hour period with continuous monitoring to detect the presence of ozone generated by the operation of the unit. A check for electroluminescence was conducted at the commencement of monitoring and at the end of the 8-hour monitoring period. This was to confirm that electron emission was occurring throughout the monitoring period. The Corona Canon operated continuously over the monitoring period.

Test Conditions

Test parameters	Ambient temperature inside enclosure	
	Relative humidity	46%
	Frequency of measurement	Continuous
	Time and time of test	2/10/2020 13:00 – 21:00 hours
Equipment	Grey Wolf Advance Sense Pro with Grey Wolf EC202 probe with ozone sensor (calibrated 03.11.2019) Serial 03-1498 and 2223	No ozone generation detected over the 8-hour monitoring period
Ozone sensor	Measurement uncertainty	± 0.02ppm

Results

Measurement time	Result
13:00	0.0 ± 0.02ppm
14:00	0.0 ± 0.02ppm
15:00	0.0 ± 0.02ppm
16:00	0.0 ± 0.02ppm
17:00	0.0 ± 0.02ppm
18:00	0.0 ± 0.02ppm
19:00	0.0 ± 0.02ppm
20:00	0.0 ± 0.02ppm
21:00	0.0 ± 0.02ppm



Comparison with National Environment Protection Measures (NEPM)

Contaminant	Requirement (ppm)	Averaging period (hours)	Measured (ppm)
Ozone	0.10 NEPM	1	0 ± 0.02
	0.08 NEPM	4	0 ± 0.02
	0.10 Safework Australia	Peak	0 ± 0.02

Attachments:

1. Results spreadsheet
2. Photographs
3. Calibration certificate

Yours sincerely,

Brian Eva.

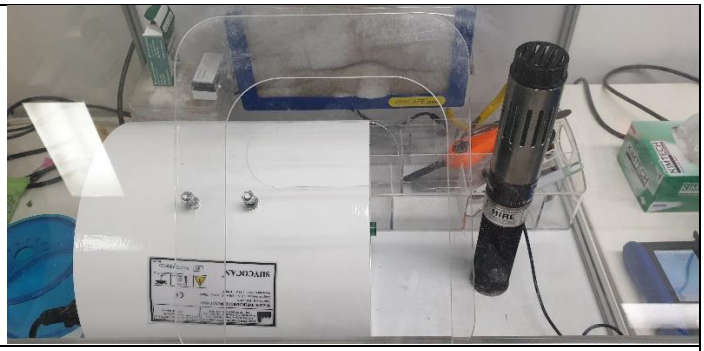
Brian Eva FAIOH, MFAMANZ
Certified Occupational Hygienist (COH)
Certified Air Quality Professional
Dip App Sci (App Chem), Grad Dip Occ Hygiene

Attachment 1: Results Spreadsheet

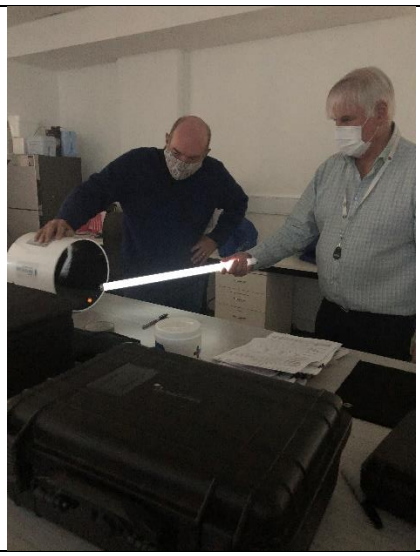
Date Time	Ozone ppm	Temperature °C
2/10/2020 13:00	0	21.6
2/10/2020 13:05	0	21.4
2/10/2020 13:10	0	21.7
2/10/2020 13:15	0	22
2/10/2020 13:20	0	22.3
2/10/2020 13:25	0	22.7
2/10/2020 13:30	0	23
2/10/2020 13:35	0	23.3
2/10/2020 13:40	0	23.7
2/10/2020 13:45	0	24
2/10/2020 13:50	0	24.3
2/10/2020 13:55	0	24.6
2/10/2020 14:00	0	24.8
2/10/2020 14:05	0	24.9
2/10/2020 14:10	0	25
2/10/2020 14:15	0	25.1
2/10/2020 14:20	0	25.2
2/10/2020 14:25	0	25.2
2/10/2020 14:30	0	25.2
2/10/2020 14:35	0	25.3
2/10/2020 14:40	0	25.4
2/10/2020 14:45	0	25.4
2/10/2020 14:50	0	25.6
2/10/2020 14:55	0	25.7
2/10/2020 15:00	0	25.7
2/10/2020 15:05	0	25.8
2/10/2020 15:10	0	25.8
2/10/2020 15:15	0	25.8
2/10/2020 15:20	0	25.8
2/10/2020 15:25	0	25.8
2/10/2020 15:30	0	25.8
2/10/2020 15:35	0	25.8
2/10/2020 15:40	0	25.8
2/10/2020 15:45	0	25.8
2/10/2020 15:50	0	25.9
2/10/2020 15:55	0	26
2/10/2020 16:00	0	26
2/10/2020 16:05	0	26.1
2/10/2020 16:10	0	26.1
2/10/2020 16:15	0	26.1
2/10/2020 16:20	0	26.1
2/10/2020 16:25	0	26
2/10/2020 16:30	0	26
2/10/2020 16:35	0	26
2/10/2020 16:40	0	26
2/10/2020 16:45	0	26
2/10/2020 16:50	0	25.9

2/10/2020 16:55	0	25.9
2/10/2020 17:00	0	25.9
2/10/2020 17:05	0	25.9
2/10/2020 17:10	0	25.9
2/10/2020 17:15	0	25.8
2/10/2020 17:20	0	25.8
2/10/2020 17:25	0	25.8
2/10/2020 17:30	0	25.8
2/10/2020 17:35	0	25.8
2/10/2020 17:40	0	25.7
2/10/2020 17:45	0	25.7
2/10/2020 17:50	0	25.7
2/10/2020 17:55	0	25.6
2/10/2020 18:00	0	25.6
2/10/2020 18:05	0	25.6
2/10/2020 18:10	0	25.5
2/10/2020 18:15	0	25.5
2/10/2020 18:20	0	25.5
2/10/2020 18:25	0	25.5
2/10/2020 18:30	0	25.4
2/10/2020 18:35	0	25.4
2/10/2020 18:40	0	25.4
2/10/2020 18:45	0	25.4
2/10/2020 18:50	0	25.3
2/10/2020 18:55	0	25.3
2/10/2020 19:00	0	25.3
2/10/2020 19:05	0	25.3
2/10/2020 19:10	0	25.3
2/10/2020 19:15	0	25.2
2/10/2020 19:20	0	25.2
2/10/2020 19:25	0	25.2
2/10/2020 19:30	0	25.2
2/10/2020 19:35	0	25.2
2/10/2020 19:40	0	25.2
2/10/2020 19:45	0	25.1
2/10/2020 19:50	0	25.1
2/10/2020 19:55	0	25.1
2/10/2020 20:00	0	25.1
2/10/2020 20:05	0	25.1
2/10/2020 20:10	0	25.1
2/10/2020 20:15	0	25.1
2/10/2020 20:20	0	25.1
2/10/2020 20:25	0	25.1
2/10/2020 20:30	0	25.1
2/10/2020 20:35	0	25.1
2/10/2020 20:40	0	25
2/10/2020 20:45	0	25
2/10/2020 20:50	0	25
3/10/2020 21:00	0	25

Attachment 2 – Photographs



Unit inside sealed enclosure with ozone sensor



Confirming electroluminiscence

Attachment 3: Calibration Certificate

(end of page numbering)

AES
sensing safety

Calibration Certificate

Sensor	Type	Serial No.	Span Gas	Concentration	Traceability Lot #	CF	Reading	
							Zero	Span
Oxygen								
LEL								
PID								
Battery								
Toxic 1	SEN-0-O3. OZONE SENSOR	010013022948078	Nitrogen Dioxide	5.0ppm	WO205490		0	3.00
Toxic 2								
Toxic 3	PT100			20.0C/40.0C	Temperature Chamber/ Thermometer		20.1	40.2
Toxic 4								
Toxic 5								
Toxic 6								

Calibrated/Repaired by: AMEND ROSHAN KUMAR Date: 03.11.2019 Next Due: 03.11.2020

Ozone Safety Test Report

SCALENE CYBERNETICS LIMITED

REPORT No: SHY-OZ-05072020
TEST No: SHY-2306-50-1
REQUIREMENT : USA EPA, FDA, EUA

CONTROLLED COPY ISSUED TO: Scalene ANZ Pty Ltd, Australia.
Tel: +61 1300 SCALENE, Sales@scaleneanz.com.au | www.scaleneanz.com.au
Dated: 25.08.2021

Shreis Scalene Therapeutics LLC

11516 Darnestown Rd,
Gaithersburg MD 20878
USA

Tel: +1 301-926-0566
Fax: +1 301-238-5247

www.shreis.org

email:
john.augustus@shreis.com
maugustus@shreis.com

NATURE OF TEST UNDERTAKEN

OZONE SAFETY TEST

APPLICANT / CUSTOMER	SCALENE CYBERNETICS LIMITED		
MANUFACTURER	SCALENE CYBERNETICS LIMITED S-Card Campus, Seegehalli Main Rd, Virgonagar post, Bangalore-49		
PROGRAM	SHYCOCAN OZONE EMISSION TEST		
TEST FACILITY	Shreis Scalene Therapeutics LLC		
EQUIPMENT UNDER TEST	Scalene Hypercharge Corona Canon (SHYCOCAN)		
DEVICE MODEL	SHCC915		
SAMPLES RECEIVED	02 (Two)		
DEVICE ID	SCL-05082020		
MANUFACTURED SL. No.	SHCC202003001		
DEVICE CONDITION	Good working condition		
DATE OF RECEIPT	10 April 2020		
TESTING STANDARDS	OZONE EMISSION TEST OF EUT WITH CONTROL AS COMMERCIAL OZONE GENERATOR		
TEST DATE	14 MAY 2020	**End Date**	15 MAY 2020
LOCAL AMBIENT CONDITIONS	**Temperature in °C**		21 ±1°C
	Relative humidity in %		<55 %
ISSUE DATE	16 MAY 2020		
TEST INCHARGE	Rayol John Augustus Ph.D. - COT		
TESTS PERFORMED	1) Test carried out by SSTx 2) Proof of non-generation of Ozone by Device 3) Stand Alone Testing & Comparison to Control as Commercial Ozone Generator		

Rayol John Augustus Ph.D. Ex. VP and COO	Meena Augustus President, CEO & CSO
SIGNATURE-1	**SIGNATURE -2**

Disclaimer

This report is issued after testing under the request of the applicant only

This report is the result of the tests carried out at Shreis Scalene Therapeutics LLC for confirming the safety of the SHYCOCAN as a Non-Ozone producing device and as a generator of a steady stream of electrons as claimed by the manufacturer. This report may be reproduced for use by the manufacturer..

DEVICE MODEL UNDER

SUMMARY OF TEST RESULTS

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Dated: 25.08.2021

PARAMETER TESTED	UNITS TESTED	Date of Testing	Result
SHYCOCAN - EMISSION			
Testing for Odor generation	2	1 MAY 2020	**BDL**
Testing for Electron Discharge	2	11 MAY 2020	**ELT Positive**
Electron Discharge with Electroluminescence	2	11 MAY 2020	**YES**
Response with Ozone Detector for presence of Ozone being generated	2	11 MAY 2020	**BDL**
CONTROL OZONE GENERATOR DEVICE COMPARISON			
Testing For Odor Generation	1	12 MAY 2020	**5.6 ppm**
Testing for Electron Discharge	1	12 MAY 2020	**ELT Negative**
Electron Discharge with Electroluminescence	1	12 MAY 2020	**Negative**
Response with Ozone Detector for presence of Ozone being generated	1	12 MAY 2020	**YES**
Visual Test for Corona Discharge	1	12 MAY 2020	**Present in the control device YES (Blue-Violet glow)**
BDL = Below Detection Level, **ELT** = Electroluminescence Test			

Note:

EQUIPMENT UNDER TEST (EUT)

EUT Description

The device **Scalene Hypercharge Corona Canon (SHYCOCAN)** intended to be used in an industrial / commercial / Residential and Transportation environment.

Aim of Device : Device designed for active containment by attenuation of Corona family of viruses.

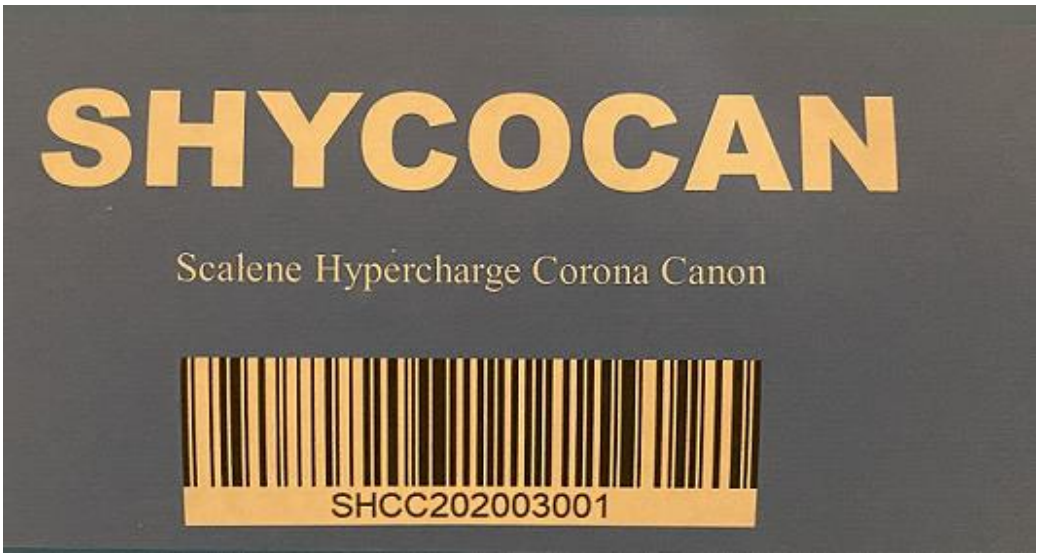
SHYCOCAN : Operational Range : 110-240V – 50/60 Hz

Purpose : To generate extra high tension at a switching frequency of 20 kHz that delivers the necessary signals to a proton mediated electrons radiator that produces hypercharge high velocity electron that interacts with a negative seeking S-protein of Corona family of viruses thus reducing infectivity and prevent air and surface borne transmission of Corona family of viruses.

Engineering Specifications of the EUT as per Manufacturer

Device Name	**SHYCOCAN**
Technical Name	Scalene Hypercharge Corona Canon
Nature of Operation	Hypercharge High Velocity Electron Generation
Estimated Electron Production per second (Manufacturer's Estimate)	10 to 100 trillion per second
Estimated Electron Density (Manufacturer's Estimate)	6 trillion per CC at 12 centimeters
Operating Input Voltage	110V and 240V Standard power supply
Operating Current	0.15 to 0.35 Amps
Switching Frequency	20 kHz

DEVICE : MARKING PLATE (LABEL)



DEVICE USED DURING TESTS

Use*	Product Type	Manufacturer	Model No	Serial No
EUT	Shycocan	Scalene Cybernetics Limited	SHCC915	SHCC202003001

TEST ENCLOSURES

SL No	Name	Type	Dimensions	Comments
1	Perspex Enclosure	CLOSED	4.5'x 1.5' x 1.5'	None
1	Open Room (door Shut)	OPEN	10'x12'x 8'	None

Mode	Voltage (V)	Current (A)	Frequency (AC-Hz)	Phases
Rated	110V – 240V	0.15 – 0.35A	50 – 60 Hz	Single
Tested	230V	0.16 A	50 Hz	Single

1.6 Transformer Used (USA 110V to 220V)

Part Used	Country Used	Model	Make
Step Up & Step-Down Transformer	USA	ST-200	Seven Star CE Compliant

DEVICE OPERATION FREQUENCIES

DESCRIPTION	FREQUENCY
Switching Frequency	20 kHz

DEVICE CONFIGURATION

UNIT	Description
1	• EUT operated at 230V/50Hz 'AC' with max load converted from 110V with Step Up Transformer • Electroluminescence Tube was kept near to the EUT to check the functionality. • Electroluminescence Tube was automatically glowing when it was placed close to the SHYCOCAN that was producing hyper charge high velocity electrons of the EUT.

PERFORMANCE CRITERIA
FOR ODOR / CORONA GLOW/ ELECTRLUMINISCENCE / & OZONE DISCHARGE TESTS

Cat#1: The EUT shall continue to operate as intended during and after the test.

No degradation of performance or loss of function is allowed below a performance level specified by the manufacturer, when the E U T is used as intended.

If the performance level is not specified by the manufacturer, this may be derived from the product description and documentation and what the user may reasonably expect from the equipment if used as intended.

Cat#2: The E U T shall continue to operate as intended after the test.
No degradation of performance or loss of function is allowed below a performance level specified by the manufacturer, when the E U T is used as intended.

The performance level may be replaced by a permissible loss of performance. However, during the test degradation of performance is allowed but no change of actual operating state or stored data is allowed.

If the minimum performance level or the permissible performance loss is not specified by the manufacturer, either of these may be derived from the product description and documentation and what the user may reasonably expect from the equipment if used as intended.

Cat#3: Temporary loss of function is allowed during the test, provided the function is self- recoverable or can be restored by the operation of the controls.

Environment : Open environment (large room) and Closed Environment (Enclosed chamber made of Perspex)

FUNCTIONS MONITORED
DURING OZONE / ODOR/FLOURESCENT TESTS

The following are the functions monitored during the immunity tests:

- Power ON LED of the EUT shall be glowing
- Electroluminescence Tube was placed very closer to the EUT and the tube used to glow continuously due to the hyper charge high velocity electrons produced by the EUT. (Visual monitoring was carried out throughout the test).
- Olfactory sensitivity of Ozone production
- Forensics Detector – Ozone Detector response to be monitored

EQUIPMENT AND CALIBRATION DETAILS

Test Equipment	Manufacturer	Model No.	Serial No.	Calibration status (Valid up to)
OZONE GAS DETECTOR				
Ozone Gas Detector Professional Series	Forensic Detectors	FD-90A	20204414	May 2020
Range	0-20 PPM		100277	
Calibration	Calibration Sheet attached			
Manufacturer Brochure	Manufacturer Sheet attached			
OZONE GENERATOR (Third Party)				
Alpine Air	Alpineairtec	SKU-Alpine-NF-10000-OG	20200062014	May 2020
Operation Manual	Attached			

SHYCOCAN – CLOSED ENVIRONMENT TEST

COTS EQUIPMENT USED

STEP UP TRANSFORMER – 110V to 200V

FORENSICS DETECTORS – FD-90A

OZONE DETECTOR

DEVICE TEST DETAILS

1. SHYCOCAN STAND ALONE TEST

TEST	**ODOR EMISSION – CLOSED & OPEN ENVIRONMENT**		
Method	Measurements were made by placing the EUT on a non-conductive table 80cm above the horizontal ground plane. The EUT power was connected to the system through a step-up transformer (110 to 220V). The step-up transformer was connected to the main supply that is a stable 110V. The transformed was placed at 1.0 m from the boundary of the unit under test and bonded to a ground reference plane. The EUT was turned on by switching on the step up transformed. The tests were conducted for 15 minutes non-stop to check for presence of any odor.		
TEST ENVIRONMENT			
Parameters recorded during the test	Environment Ambient Temperature	20.56 °C	
	Relative Humidity	54.3 %	
	Frequency	Continuous Operation	
TEST RESULTS	**RESULT : NO ODOR WAS PRESENT DURING ENTIRE TEST FOR CONFIRMATION, TESTS WERE REPEATED 3 TIMES**		
Conforming Standard			
Measurement Uncertainty	NIL		
DEVICE TEST STATUS		**PASSED**	
Supplementary Information: • Test was conducted at Location : SSTx -11516 Darnestown Rd, Gaithersburg, MD 20878			

DEVICE TEST DETAILS

2. SHYCOCAN STAND ALONE TEST

TEST	**CORONA DISCHARGE GLOW – CLOSED AND OPEN ENVIRONMENT**	
Method	Measurements were made by placing the EUT on a non-conductive table 80cm above the horizontal ground plane. The EUT power was connected to the system through a step-up transformer (110 to 220V). The step-up transformer was connected to the main supply that is a stable 110V. The transformed was placed at 1.0 m from the boundary of the unit under test and bonded to a ground reference plane. The EUT was turned on by switching on the step up transformed. The tests were conducted for 15 minutes non-stop to check for presence of any odor.	
TEST ENVIRONMENT		
RECORDED PARAMETERS	Environment Ambient Temperature	20.56 °C
	Relative Humidity	54.3 %
	Frequency	Continuous Operation
TEST RESULTS	**RESULT : NO CORONA GLOW WAS PRESENT DURING ENTIRE TEST. FOR CONFIRMATION, TESTS WERE REPEATED 3 TIMES**	
Measurement Uncertainty	NIL	
DEVICE TEST STATUS		**PASSED**
NO GLOW GENERATED		

Supplementary Information:

- Test was conducted at Location : SSTx -11516 Darnestown Rd, Gaithersburg, MD 20878

DEVICE TEST DETAILS

3. SHYCOCAN STAND ALONE TEST

TEST	**OZONE GENERATION - CLOSED AND OPEN ENVIRONMENT**	
Method	Measurements were made by placing the EUT on a non-conductive table 80cm above the horizontal ground plane. The EUT power was connected to the system through a step-up transformer (110 to 220V). The step-up transformer was connected to the main supply that is a stable 110V. The transformed was placed at 1.0 m from the boundary of the unit under test and bonded to a ground reference plane. The EUT was turned on by switching on the step up transformed. The tests were conducted for over a period of 8 Hours non-stop to check for presence of any Ozone Generation. Measurements were taken every 30 Minutes	
TEST ENVIRONMENT		
Parameters recorded during the test	Environment Ambient Temperature	20.56 °C
	Relative Humidity	54.3 %
	Frequency	Continuous Operation
Forensics Detectors	Ozone Generation Measurement every 30 minutes over 8 Hour period	No Ozone generation was detected over the 8-hour period.
TEST RESULTS	**RESULT : NO OZONE WAS GENERATED DURING ENTIRE TEST of 8 HOURS**	
Measurement Uncertainty	NIL	
DEVICE TEST STATUS		**PASSED**
NON-GENERATION OF OZONE		

Supplementary Information:

- Test was conducted at Location : SSTx -11516 Darnestown Rd, Gaithersburg, MD 20878

SHYCOCAN – NO OZONE OUTPUT REGISTERED

(Repeated Tests)

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Tel: +61 1300 SCALENE, Sales@scaleneanz.com.au | www.scaleneanz.com.au
Dated: 25.08.2021

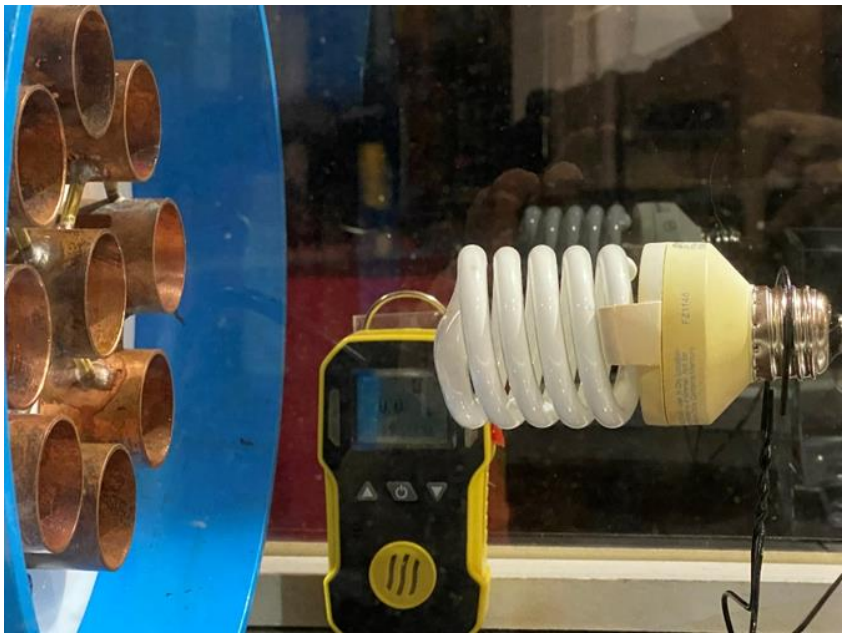
DEVICE TEST DETAILS

4. SHYCOCAN STAND ALONE TEST

TEST	**ELECTROLUMINISCENCE TEST**	
Method	Measurements were made by placing the EUT on a non-conductive table 80cm above the horizontal ground plane. The EUT power was connected to the system through a step-up transformer (110 to 220V). The step-up transformer was connected to the main supply that is a stable 110V. The transformed was placed at 1.0 m from the boundary of the unit under test and bonded to a ground reference plane. The EUT was turned on by switching on the step up transformed. The tests were conducted for 15 minutes non-stop to check for presence of any odor.	
TEST ENVIRONMENT		
Parameters recorded during the test	Environment Ambient Temperature	20.56 °C
	Relative Humidity	54.3 %
	Frequency	Continuous Operation
TEST RESULTS	**RESULT : AN ELECTROLUMINISCENCE EFFECT WAS SEEN WITH A GAS DISCHARGE COILED TUBE AS SOON AS THE DEVICE WAS TURNED ON. THE ELT STOPPED WHEN THE DEVICES TURNED OFF. THIS VALIDATED ELECTRON EMISSION. TEST WAS REPEATED 3 TIMES.**	
DEVICE TEST STATUS		**PASSED**

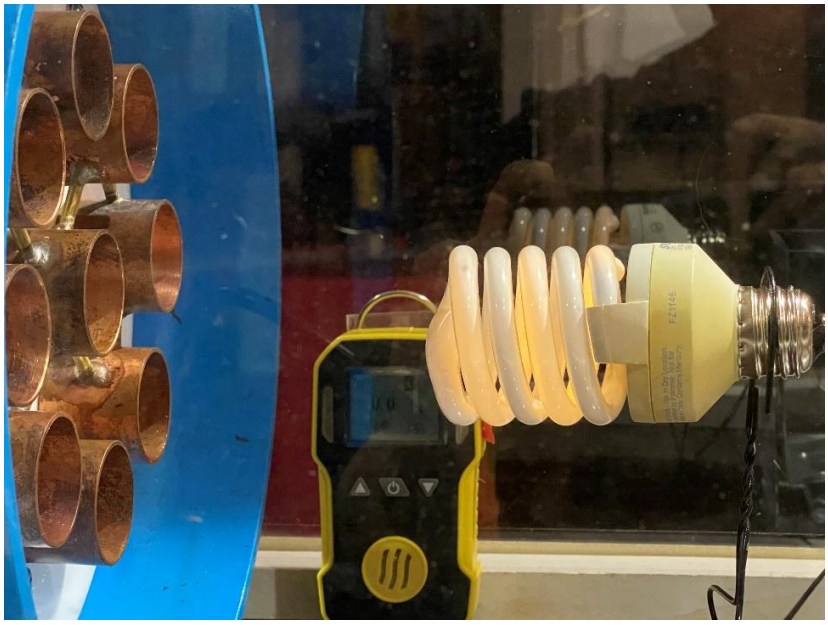
Supplementary Information:

- Test was conducted at Location : SSTx -11516 Darnestown Rd, Gaithersburg, MD 20878



SHYCOCAN Turned OFF – No Electron Discharge

No Electroluminescence



SHYCOCAN Turned ON – Electron Discharge

Electroluminescence

CONTROL OZONE GENERATOR COMPARATIVE TEST DETAILS

5. CONTROL DEVICE (ALPINE AIR) STAND ALONE TEST

TEST	**ODOR EMISSION – CLOSED & OPEN ENVIRONMENT**		
Method	Measurements were made by placing the Third-Party Alpine Air Ozone Generator on a non-conductive table 80cm above the horizontal ground plane. The GEUT power was connected directly to the main supply that is a stable 110V. The transformer was placed at 1.0 m from the boundary of the unit under test and bonded to a ground reference plane. The GEUT was turned on by switching on the step up transformed. The tests were conducted for 5 Seconds to check for presence of any odor.		
TEST ENVIRONMENT			
Parameters recorded during the test	Environment Ambient Temperature	20.56 °C	
	Relative Humidity	54.3 %	
	Frequency	Continuous Operation	
TEST RESULTS	**RESULT : EXTREMELY STRONG ODOR WAS PRESENT DURING ENTIRE TEST. FOR CONFIRMATION, TESTS WERE REPEATED 3 TIMES for 5 SECONDS EACH TIME**		
Measurement Uncertainty	NIL		
DEVICE TEST STATUS		**BEHAVED AS EXPECTED**	
DEVICED PERFORMED AS INTENDED			

Supplementary Information:

- Test was conducted at Location : SSTx -11516 Darnestown Rd, Gaithersburg, MD 20878

CONTROL OZONE GENERATOR COMPARATIVE TEST DETAILS

6. CONTROL DEVICE (ALPINE AIR) STAND ALONE TEST

TEST	**CORONA DISCHARGE GLOW – CLOSED AND OPEN ENVIRONMENT**	
Method	Measurements were made by placing the Third-Party Alpine Air Ozone Generator on a non-conductive table 80cm above the horizontal ground plane. The GEUT power was connected directly to the main supply that is a stable 110V. The transformed was placed at 1.0 m from the boundary of the unit under test and bonded to a ground reference plane. The GEUT was turned on by switching on the step up transformed. The tests were conducted for 5 Seconds to check for presence of any odor.	
TEST ENVIRONMENT		
Parameters recorded during the test	Environment Ambient Temperature	20.56 °C
	Relative Humidity	54.3 %
	Frequency	Continuous Operation
TEST RESULTS	**RESULT : A CLEAR PURPLE/VIOLET GLOWS WAS CLEARLY PRESENT WHEN DEVICES TURNED ON. TESTS WERE REPEATED 3 TIMES FOR 5 SECONDS TIME**	
Measurement Uncertainty	NIL	
DEVICE TEST STATUS		**BEHAVED AS EXPECTED**
DEVICED PERFORMED AS INTENDED		

Supplementary Information:

- Test was conducted at Location : SSTx -11516 Darnestown Rd, Gaithersburg, MD 20878

THIRD PARTY OZONE GENERATOR CLOSED TESTING

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Alpine Air – Ozone Generator

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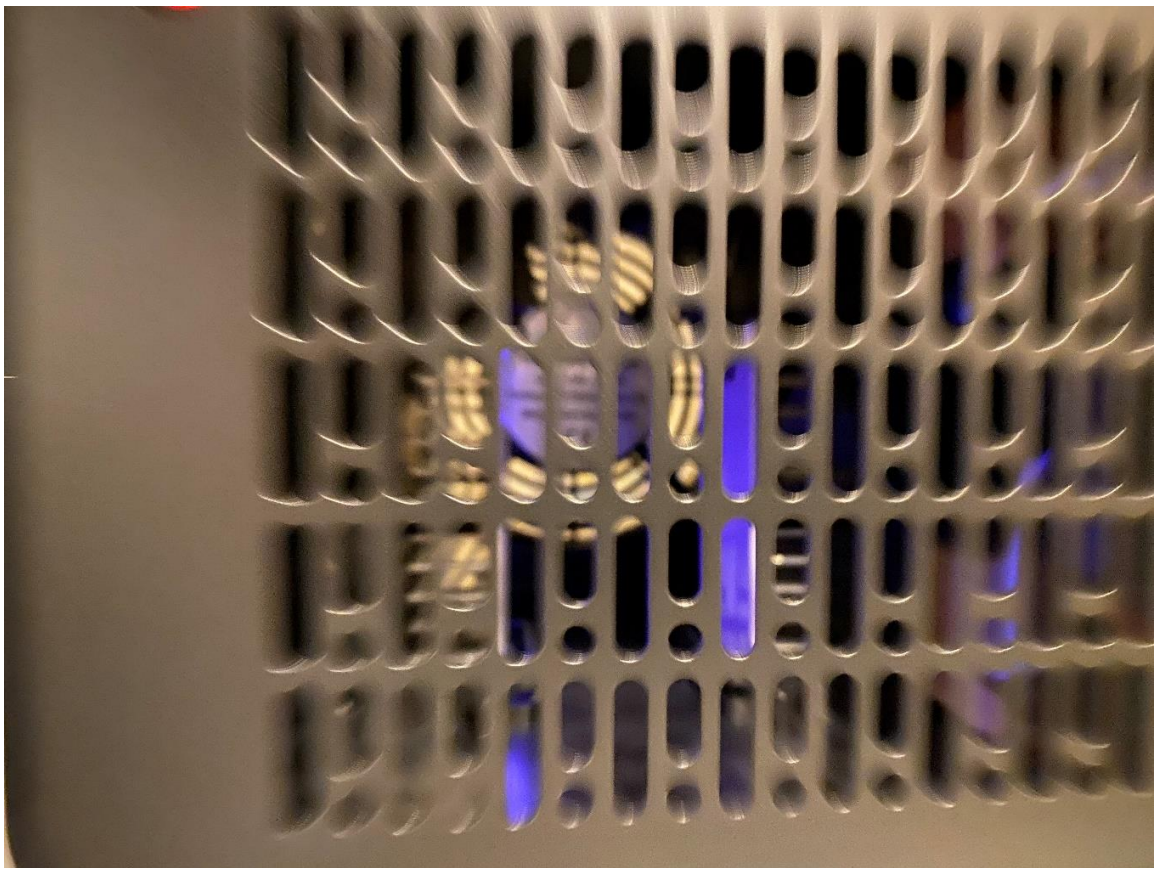
Dated: 25.08.2021



CORONA DISCHARGE GLOW FROM THIRD PARTY OZONE GENERATOR

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CONTROL OZONE GENERATOR COMPARATIVE TEST DETAILS

7. CONTROL DEVICE (ALPINE AIR) STAND ALONE TEST

TEST	**OZONE GENERATION - CLOSED AND OPEN ENVIRONMENT**		
Method	Measurements were made by placing the Third-Party Alpine Air Ozone Generator on a non-conductive table 80cm above the horizontal ground plane. The GEUT power was connected directly to the main supply that is a stable 110V. The transformed was placed at 1.0 m from the boundary of the unit under test and bonded to a ground reference plane. The GEUT was turned on by switching on the step up transformed. The tests were conducted for 5 Seconds to check for presence of any odor.		
TEST ENVIRONMENT			
Parameters recorded during the test	Environment Ambient Temperature	20.56 °C	
	Relative Humidity	54.3 %	
	Frequency	Continuous Operation	
Forensics Detectors	Ozone Generation Measurement every 5 seconds	Strong Ozone generation FD Meter registered beyond limits set	
TEST RESULTS	**RESULT : STRONG OZONE WAS GENERATED DURING ENTIRE TEST - 5 SECONDS MULTIPLE TIMES**		
Measurement Uncertainty	NIL		
DEVICE TEST STATUS		**STRONG GENERATION OF OZONE**	
DEVICE PERFORMED AS INTENDED			

Supplementary Information:

- Test was conducted at Location : SSTx -11516 Darnestown Rd, Gaithersburg, MD 20878

OZONE LEVEL REGISTER VERY FAST WITH THE OZONE GENERATOR

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Dated: 25.08.2021

CONTROL OZONE GENERATOR COMPARATIVE TEST DETAILS

8. CONTROL DEVICE (ALPINE AIR) STAND ALONE TEST 2

TEST	**ELECTROLUMINISCENCE**		
Method	Measurements were made by placing the Third-Party Alpine Air Ozone Generator on a non-conductive table 80cm above the horizontal ground plane. The GEUT power was connected directly to the main supply that is a stable 110V. The transformed was placed at 1.0 m from the boundary of the unit under test and bonded to a ground reference plane. The GEUT was turned on by switching on the step up transformed. The tests were conducted for 5 Seconds to check for presence of electro luminescence effect		
TEST ENVIRONMENT			
Parameters recorded during the test	Environment Ambient Temperature	20.56 °C	
	Relative Humidity	54.3 %	
	Frequency	Continuous Operation	
TEST RESULTS	**RESULT : THE ELECTROLUMINISCENCE TEST FAILED**		
DEVICE TEST STATUS		**DEVICE GENERATED NO ELECTRONS**	
DEVICED PERFORMED AS INTENDED			
Supplementary Information: • Test was conducted at Location : SSTx -11516 Darnestown Rd, Gaithersburg, MD 20878			

OZONE GENERATOR – NO ELECTRON CLOUD DISCHARGE HENCE NO ELECTROLUMINISENCE

SCIENTIFIC EVIDENCE OF THE EFFICACY OF SHYCOCAN IN ATTENUATION OF VIRAL PARTICLES IN THE AIR AND SURFACE

SYNOPSIS

The viral activity studies conducted using a SHYCOCAN device was able to determine its potential to inhibit / attenuate viral particles from air and surfaces without interfering with life forms like bacteria, fungi, plants or animals.

Mammalian cell culture is the basis for these studies as viruses are biological particles that require host cells for their replication. Due to current conditions of biological material control supply and the restrictions in bio security conditions required for handling of many of the viral particles, the environmental and medical agencies allow the use of only phylogenetically similar strains to validate any activity among emerging viruses.

Hence during the various and numerous studies to understand the efficacy of SHYCOCAN, Scalene Hyper Charge Corona technology, the studies used different types of approved and permitted surrogate viruses close to SARS-CoV-2 viral particle.

The studies done are as below:

Virology Studies

Study Title	**Antiviral Efficacy of Surface Disinfection on SARS- CoV-2**
Report Reference	Antiviral testing report, dated 15 July 2021.
Institution Name	**Center for Cellular and Molecular Biology (CCMB), CISR INDIA**
Virus Used	SARS-Cov-2
Justification for the study	SARS-CoV-2 virus remains infective on plastic surfaces like acrylic for upto 4 days according to literatures. Acrylic and other plastics is one of the materials used in most places. The test was done with active SARS -CoV-2 virus on acrylic surfaces.
Study AIM	The aim of the study was to assess viral activity on infected acrylic plates after exposure to SHYCOCAN device.
Study Conclusion	The device shows 90% viral attenuation (reduction) at 10 and 30 minutes, 96% attenuation at 60 minutes of exposure. The difference in the viral particle number between test (exposed under the device) and control (without exposure to the device) is represented as the viral attenuation (%), considering log difference between test and control.

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Study Title	**Viricidal efficacy of surface disinfection system on SARS-CoV-2**
Report Reference	TNO 2021 R10988
Institution Name	**Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (TNO**; English: Netherlands Organisation for Applied Scientific Research)
Virus Used	Infectious SARS-Cov-2
Justification for the study	Infectious SARS-Cov-2 virus, the cause of COVID-19, remains infective on stainless steel surfaces for more than 72 hours. As stainless steel is used extensively in hospitals, homes, offices, schools etc., the test was done with infectious SARS-Cov-2 virus on stainless steel surfaces.
Study AIM	The aim of the experimental work was to gain information on the antiviral efficacy of the Shycocan system in eradicating infectious SARS-CoV-2 virus on stainless steel surface in several different exposure times.
Study Conclusion	Based on the results obtained, a reduction of infectious SARS-CoV-2 virus particles on the stainless-steel disks was observed as a consequence of exposure to the Shycocan system. A 94.9% reduction of infectious SARS-CoV-2 virus particles on the stainless-steel surface was observed after 15 minutes of exposure to the Shycocan system.

Study AIM	**The virucidal/neutralizing of SARS-COV-2, Surrogate viral particles when exposed to SHYCOCAN**
Report Reference	44527 – Virucidal Activity Equine Arteritis
Institution Name	Laboratorios de Especialidades Immunológicas S.A de C.V - Mexico
Virus Used	Equine arteritis virus ATCC VR-76
Virus Description	EAV is a virus of the genus *Enterovirus*. It is an enveloped RNA virus, it has limited host range and generally known to affect equids.
Justification for Surrogate	Both SARS-COV-2 and EAV are enveloped RNA viruses that belong to the *Nidovirales* order. (REF:*US EPA-2020 List n: Disinfectants for use against SARS-COV-2*)
Study Conclusion	During the analysis, it was determined that the SHYCOCAN device has virucidal activity a logarithmic reduction of 3.134 for EAV during a 15-minute exposure at 50 cm from the device and 3.435 reduction during a 120-minute exposure at 500 cm from the equipment under test (EUT). The interpretation of this result is that within the exposure times tested, the SHYCOCAN eliminates more than 99% of the viral particles

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Study AIM	**To assess the effect of SHYCOCAN and resultant viral activity after exposure of the device to capsid with spike proteins.**
Report Reference	RN44527 – Virucidal Activity virus with Influenza B
Institution Name	Laboratorios de Especialidades Immunológicas S.A de C.V - Mexico
Virus Used	INFLUENZA-B virus ATCC VR-1535
Virus Description	INFLUENZA-B virus belong to the Beta Influenza virus in the family Orthomyxoviridae is known to effect humans. It is an enveloped RNA virus that has spike proteins.
Justification for Surrogate	SARS-COV-2 and Influenza-B are both enveloped RNA virus with capsid and spike proteins.
Study Conclusion	During the Analysis it was determined that the device had caused a reduction of 47287,67313 and 25212 CFID 50 of Influenza B virus corresponding to distances and times evaluated (100 cm from the device for 15-minute, 100 cm from the device for 45-minute and 300 cm from the device for 45-minute). As comparison a symptomatic seasonal flue patient provides between 12000 and 38000 CFID50 every 30-minute (REF. YAN et.al (2018, Infectious virus in exhaled breath of symptomatic of seasonal influenza cases from a college community. PNAS, 115(5, PP1081-1086))). These experimental results were obtained with the previous equipment activation for 120-minute in a 6 m X 3m X 2m room. The interpretation of the result is that within the exposure times tested the SHYCOCAN device eliminates the INFLUENZA-B virus, a virus that has spike proteins as corona virus, in similar magnitude of the exhaled virus by a symptomatic influenza patient for 30 minutes

Study AIM	**To assess the microbial reduction capability with reference to e-coli emptycc 68 with MS2 phage 80CC15597B1 (approved surrogate virus) at different distances and different times of exposure continuously using the SHYCOCAN device**
Report Reference	AWRTCL/17618A/20-21 dated 26.10.2020
Institution Name	Aquadiagnostics an IAPMO group -USA
Virus Used	Bacteriophage – MS2 Phage 80CC15597B1
Virus Description	ESCHERICHIA Virus MS2 is an icosahedral, positive cells single strand RNA virus that infects the bacterium ESCHERICHIA COLI (E-COLI) and other members of the ENTEROBACTERIACEAE. MS2 is a

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	member of a family of closely related bacterial virus that includes bacteriophage f2, bacteriophage qβ, R17 and GA.
Justification for Surrogate	MS2 Phage is a surrogate virus used for the study the efficacy of environmental disinfectants and environmental control devices
Study Conclusion	Acrylic sheets smeared with MS2 Phage culture at 12ft distance from the EUT has clearly established 99.976%,99.994%,99.996% pfu in 30 minutes, 1 hr, 2 hrs respectively.

Study AIM	**Fast Response (60-seconds ,5-minute,15-minute) of MS2 Phage contaminated planks exposed to SHYCOCAN device installed in an L-shaped chamber at various distances covering an area of 1000 sq ft. (samples placed at 4 corners and middle of the room)**
Report Reference	AWRTCL/17819/20-21 dated 27/01/2021
Institution Name	Aquadiagnostics an IAPMO group -USA
Virus Used	Bacteriophage – MS2 Phage 80CC15597B1
Virus Description	ESCHERICHIA Virus MS2 is an icosahedral, positive cells single strand RNA virus that infects the bacterium ESCHERICHIA COLI (E-COLI) and other members of the ENTEROBACTERIACEAE. MS2 is a member of a family of closely related bacterial virus that includes bacteriophage f2, bacteriophage qβ, R17 and GA.
Justification for Surrogate	MS2 Phage is a surrogate virus used for the study the efficacy of environmental disinfectants and environmental control devices
Study Conclusion	The tested unit of SHYCOCAN was capable of reducing MS2 Phage counts to the tune of 99.771 to 99.780% in a span of 15 mins duration, sampled between 5 min to 60 mins elapsed times corresponding initial counts (without exposure to SHYCOCAN device) were also taken outside the chamber in which SHYCOCAN was installed. Total area covered for testing was 74 feet X 20 feet X 11 feet (Height), which is 16,280 cubic feet. Relevant equipment used were calibrated to National/International traceability. Analysis was done as per published US EPA / APHA methods as applicable. Microbial cultures used were MS2 Phage 80CC15597B1 and E.Coli 80CC15597 as host.

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Study AIM	**Study on effect of SHYCOCAN exposure on MS2 phage in 17000 cubic feet test chamber with a pillar in the middle to cause shadow area at different heights and different exposure times**
Report Reference	AWRTCL/PRTR/17819A/20-21 dated 12/11/2020
Institution Name	Aquadiagnostics an IAPMO group -USA
Virus Used	Bacteriophage – MS2 Phage 80CC15597B1
Virus Description	ESCHERICHIA Virus MS2 is an icosahedral, positive cells single strand RNA virus that infects the bacterium ESCHERICHIA COLI (E-COLI) and other members of the ENTEROBACTERIACEAE. MS2 is a member of a family of closely related bacterial virus that includes bacteriophage f2, bacteriophage qβ, R17 and GA.
Justification for Surrogate	MS2 Phage is a surrogate virus used for the study the efficacy of environmental disinfectants and environmental control devices
Study Conclusion	MS2 Phage contaminated planks were exposed at a height of 7 ft and 11 ft from the floor for 5 mins, 15 mins,30 mins and 45 mins. It was noted that the %age reduction at 7ft from the floor at various exposure times from 5 mins to 45 mins was 99.179% to 99.397% respectively and at 11 ft the reduction was 99.11% to 99.358%. It is concluded that exposure at different heights from floor to 11ft did not have significant effect on the efficacy of the device.

Study AIM	**To study the neutralizing and disinfection efficacy of the SHYCOCAN against Avian Corona Virus in the air**
Report Reference	CLE-EFL-SS11 dated 06-Nov-2020
Institution Name	Indian Institute of Technology – Guwahati, India
Virus Used	Avian Coronavirus
Virus Description	The Avian Coronavirus is a positive sense, linear single-stranded RNA virus of the family *Coronaviridae* and Genera *Coronavirus*. Avian Coronavirus causes respiratory syndrome and renal damage in broilers as well as drop in egg production in laying hens.
Justification for Surrogate	Like in SARS-CoV-2 In Avian Coronavirus also the S-Protein is an important target of infectivity, it is also a positive sense single stranded RNA virus within the same family.
Study Conclusion	It was established by the study that the unit under test has a notable effect of inactivation of Avian Corona Virus in the air within the chamber

	exposed to the device between 15 mins and 120 mins. The viral inactivation was 100%.

Bacterial and Fungal Studies

Study AIM	**To determine if the SHYCOCAN exposure has any bactericidal effect on non- pathogenic and useful bacterium in the environment.**
Report Reference	SCRI073120-B/2020 dated 31/07/2020
Institution Name	Microbiology Laboratory - Scalene Energy Research Institute, Bangalore, India
Bacteria /Fungus Used	Bacillus. Subtilis
Bacteria /Fungus Description	Bacillus. Subtilis, is a gram positive, catalase positive bacterium found in soil and the gastrointestinal track of ruminants and humans. B. Subtilis is considered a benign organism as it does not possess traits that cause diseases. It is not considered pathogenic or toxigenic to humans, animals, or plants. The potential risk associated with this bacterium is low
Justification for use of the species	The B. Subtilis is a naturally occurring useful bacterium in the daily lives of humans. It is commonly found in pasteurized milk, dairy and other products. Moreover B. Subtilis is an important organism use for the production of fermented food. Destruction of useful bacterium can cause long term health and environmental impact
Study Conclusion	This study was performed to check the effectiveness of bacterial growth or destruction after exposure to SHYCOCAN During the study and subsequent analysis, it was observed that the device had no bactericidal activity. Assays were setup for exposure to SHYCOCAN at a distance of 50 cm for 15-minute and 280 cm for 120-minute against corresponding controlled culture plates, after 48 hrs. of incubation there was no decrease in the number of colonies and the number of bacterial cells (cfu per ml) in the exposed plates when compared to the controlled plates

Study AIM	**To assess if exposure to SHYCOCAN would affect the viability of fungal reduction.**
Report Reference	SCRI073120-F/2020 dated 31/07/2020
Institution Name	Microbiology Laboratory - Scalene Energy Research Institute, Bangalore, India

Bacteria /Fungus Used	Saccharomyces Cerevisiae
Bacteria /Fungus Description	Saccharomyces Cerevisiae is a small single cell fungal organism with a doubling time at 30 degrees centigrade of 1.5-2 hrs. The species is instrumental in wine making, baking and brewing since ancient times. It is a environmentally useful fungi that sporulates
Justification for use of the species	Saccharomyces Cerevisiae is a model organism for such studies as it possesses nuclear genome of 12068 kb that are organized in 16 chromosomes. This organism was selected because of its ability to reproduce spores in large numbers apart from its benefits in environment.
Study Conclusion	The study was performed by exposing cultures with 99.78% and 99.52% live spores set at a specific distance from the SHYCOCAN device and timepoint were evaluate. 50 cm from the device for 15-mins, 280 cm from the device for 120 mins. Controlled plates were positioned in similar conditions of temperature and humidity but not exposed to SHYCOCAN. The cultures were incubated for 24 hrs. The cell viability between the controlled (93.47%) and exposed (93.48%) was not significant. The conclusion is that the EUT does not interfere with the physiology/viability of the tested fungus within the exposure times tested.

Toxicology Studies

Study AIM	**To assess the toxicity of the SHYCOCAN device on human cell lines**
Report Reference	CLE-EFL-SS014 dated 25-Nov-2020
Institution Name	Indian Institute of Technology – Guwahati, India
Cells Used	Human Alveolar Basal Epithelial Cells (A549)
Cells Description	Lung Parenchymal Cells are cells exposed to external gases along with suspended particles from the air in the lung of an animal. These cells form thin-walled alveoli, forming an enormous surface which serves to maintain proper gas exchange. Any Toxicity from breathing air affects these cells most
Justification	Human Lung Parenchymal cells are most susceptible when a human spends a large amount of time in an environment of atmospheric toxicity . The study was done to assess if SHYCOCAN device could have any adverse effects in these cells during a continuous exposure period of 12 hrs
Study Conclusion	The effect if the SYCOCAN device was tested on human alveolar basal epithelial cells. The cells were seeded into 96-wells plates and were incubated overnight for attachment. After that the cells were exposed to

	SHYCOCAN device. The cells were then added with MTT reagent and further incubated for 3 hours at 37 degrees centigrade. Later, the MTT reagent was removed from the wells and formazan crystals were dissolved with 100 µL of Di-methyl sulfoxide (DMSO). The MTT results indicated the non-toxic nature of SHYCOCAN device as the percentage of cell viability was found to be almost same as controlled group. Even after the prolonged exposure for 12 hrs, the cell viability was found to be more than 87%. Subsequently, the microscopic images showed no morphological differences between test and control groups. Conclusively, these results showed that the SHYCOCAN device is non-toxic to human cells

Study AIM	**To assess the toxicity of the SHYCOCAN device on Vero Cells (African green monkey kidney epithelial cells)**
Report Reference	Report dates 10-Dec-2020
Institution Name	University of Madras, India
Cells Used	Vero Cells (African green monkey kidney epithelial cells)
Cells Description	The Vero lineage are isolated from epithelial cells extracted from a African green monkey (Chlorocebus-sp)
Cells Justification	Vero cells are used in many laboratories for production of both live and inactivated viral vaccines. Throughout the world Vero cells are used to produce vaccines of different disease and extensively used for its research purpose. Vero cells were used in the study as these are very sensitive and also to see if exposure to shycocan as any adverse effect as may be used in vaccine manufacturing environment.
Study Conclusion	The viability of the Vero Cells upon exposure to SHYCOCAN device was evaluated to scrutinize the impact of electron emission from the SYHCOCAN device on cell lines. Vero cells were exposed to SHYCOCAN for different time periods (1 hr, 6hrs, 12hrs, 18hrs and 24hrs) and viability was analyzed using cell counting kit-8 (CCK-8) (Sigma Aldrich, USA). The results obtained showed no significant difference between the viability of control and exposed cells indicating that the exposure to SHYCOCAN device had no impact on the viability of cells upon exposure for all time periods. These results demonstrated clearly that the SHYCOCAN device is non-toxic to the Vero Cells (African green monkey kidney epithelial cells).

Note: SHYCOCAN, SCALENE SHYCOCAN, CORONAGUARD Powered by SHYCOCAN etc., are all based on the Scalene Hypercharged Corona Canon Technology using PMEE.

Organization de Scalene Foundation,

Authorized Signatory

Dated: 25 Aug 2021

SCALENE CYBERNETICS LIMITED

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Issued date: 25.08.2021

सी सी एम बी CCMB
कोशिकीय एवं आणविक जीव विज्ञान केन्द्र
CENTRE FOR CELLULAR AND MOLECULAR BIOLOGY
उप्पल रोड़, हैदराबाद – 500 007, भारत
Uppal Road, Hyderabad - 500 007, India

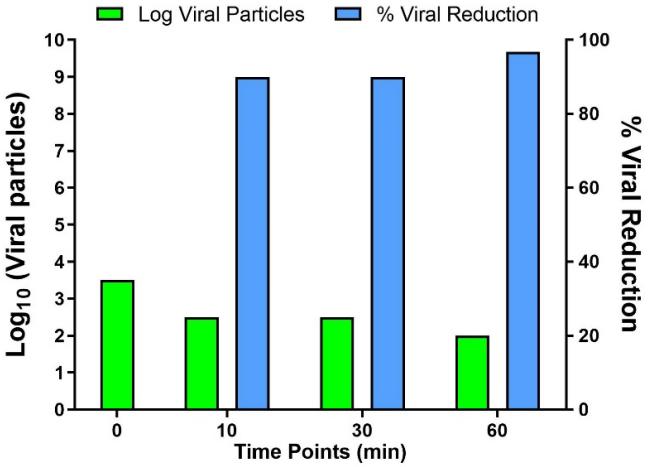
Anti-viral Testing Report

Date: 15th July, 2021

Name of the company: Shycocare Technologies Private Limited
Specifics of the Product: Shycocan
Name of the virus tested: SARS-CoV2
Time Points Tested (min):10, 30, 60

Assay Details:

Assay Performed	Materials used	Manufacturer
Viral RNA Extraction	MagMAX™ Viral/Pathogen Extraction Kit	Applied Biosystems by Thermo Fisher
qRT-PCR	MERIL COVID-19 One-Step RT-PCR Kit	MERIL Diagnostics



The Virus sample was dried for 45 minutes on the acrylic plate prior to exposure to the device (Shycocan). Shycocan was pre-heated for 15 minutes before conducting the experiment. The dried virus sample on the acrylic plate was exposed to Shycocan for 10, 30 and 60 min along with the viral control (without exposure to device).

Results: The device showed 90% viral reduction at 10 and 30 minutes. 96% reduction at 60 minutes of exposure. The viral particles decreased from $10^{3.5}$to $10^{2.5}$at 10 minutes, $10^{2.5}$at 30 minutes &$10^{2.0}$at 60 minutes.

The $TCID_{50}$ was calculated using the Spearman-Karberformula

Proportional Distance (PD) between 2 dilutions**:** (% next above 50% endpoint)- 50% / (% next above 50% endpoint) – (% next below 50% endpoint)

$LogTCID_{50}$ = log dilution next above 50% + PD

The difference in the viral particle number between the test (exposed under the device) and control (without exposure to device) is represented as the Viral Reduction (%) considering log difference between test and control.

(Dr B Kiran Kumar)
Scientist-in-charge, CCMB

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Dated: 25.08.2021

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- CCMB is neither responsible nor liable for any advice, course of treatment or any other information that is obtained hereafter from this report.

Date:16.07.2021

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TNO report

TNO 2021 R10988

Viricidal efficacy of surface disinfection system on SARS-CoV-2

Date	20 April 2021
Author(s)	JMBM van der Vossen F Schaafsma A Kreikamp M. Heerikhuisen
Copy no	-
No. of copies	-
Number of pages	10 (incl. appendices)
Number of appendices	-
Sponsor	Scalene EU Ltd. Mr. Anton Jongbloed
Project name	Scalene # BSL3 antiviral treatment
Project number	060.48172

Management summary

Title :	Viricidal efficacy of surface disinfection system on SARS- CoV-2
Authors :	JMBM van der Vossen F Schaafsma A Kreikamp M. Heerikhuisen
Date :	20 april 2021
Assignment nr. :	060.48172
Report nr. :	TNO 2021 R10988

At the request of Scalene Europe Ltd. (Scalene), TNO, The Netherlands, tested the viricidal efficacy of Shycocan disinfection system against SARS-CoV-2.

The aim of the experimental work was to gain information on the antiviral efficacy of the Shycocan system in eradicating infectious SARS-CoV-2 virus on stainless steel surface in several different exposure times.

For testing, the virus suspension was deposited on insulated stainless steel disks and dried on the surface prior exposure to the Shycocan system made available by Scalene. Virus inactivating efficacy was studied during different exposure times ranging from 1 minute to 15 minutes. All efficacy tests were performed in triplicate and the numbers of infectious virus particles were compared to triplicate controls of dried virus on stainless steel disks without exposure to the Shycocan system. The difference in between exposed and non-exposed indicates the virus inactivating effect of the Shycocan system.

Based on the results obtained, a reduction of infectious SARS-CoV-2 virus particles on the stainless steel disks was observed as a consequence of exposure to the Shycocan system. A 94.9% reduction of infectious SARS-CoV-2 virus particles on the stainless steel surface was observed after 15 minutes of exposure to the Shycocan system.

Contents

1 Introduction

Scalene Europe Ltd. (Scalene), requested TNO, The Netherlands, to test the viricidal efficacy of Shycocan disinfection system against SARS-CoV-2.

The Shycocan disinfection system has been tested for surface and air disinfection purposes. This system distributes electrons/photons into the atmosphere that impairs infectious properties of viral particles. Antiviral effects of the system were previously reported against bacteriophage MS2 and enveloped avian corona virus. The current Covid-19 pandemic urged Scalene to evaluate the antiviral effect of the Shycocan system against SARS-CoV-2 present on surfaces.

Based on Scalene's request TNO offered to evaluate the Shycocan systems' performance on a stainless-steel surface in a series of several different exposure times. All tests were done in triplicate including the negative exposure controls.

The aim of the experimental work was to gain information on the antiviral effect of the Shycocan system in eradicating infectious SARS-CoV-2 virus on a surface.

2 Technical approach

High density suspensions of SARS-CoV-2 virus were prepared in BSL-3 facility by propagation of the virus on Vero E6 cell line. A virus density of 7 log 50% Tissue Culture Infectious Dose per millilitre ($TCID_{50}$/mL) was obtained.

A volume of 50 µL virus suspension was deposited on stainless steel disks (grade 316, 2B finish with diameter of 20 mm) and dried on the surface during 45 minutes prior exposure to the Shycocan system made available by Scalene present at 85 cm distance from the disks that were present on an insulated surface. The virus inactivating effect was studied 1, 5 and 15 minutes after 15 minutes pre-running of the Shycocan system. In addition, a 5 minute exposure was measured without pre-running of the system (thus with cold start). All tests were performed in triplicate. Triplicate controls of dried virus on stainless steel disks without exposure to the Shycocan system were included.

The difference between the recovered infectious counts ($TCID_{50}$/mL) of non-exposed virus and exposed virus was used to calculate the level of reduction of the number of infectious virus particles as a consequence of the treatments. During the experiments the temperature was 20°C and relative humidity was about 70%.

The disks collected from all experiments were individually vortexed in 5 mL of Soybean Casein Digest broth with Lecithin and Poly oxyethylene sorbitan mono oleate (SCDLP) broth and 5 g glass beads (diameter 3 mm) for sampling of the viruses from each disk. The virus titers in the collected SCDLP broth were analyzed by $TCID_{50}$ determination. For this, serial dilutions of the collected individual virus samples in SCDLP broth were added to pretreated monolayers of Vero E6 cell line in microtiter plates. The plates containing 8 replicate dilutions of each individual sample were analyzed for cytopathological effects (CPE) after 6 days of incubation at 37°C in a CO_2 incubator (5% CO_2).

The cytopathological effect (CPE) was analyzed microscopically and expressed as a value from 0 to 4 per well per dilution. The meaning of the numbers are:

0. About 0% CPE
1. About 25% CPE
2. About 50% CPE
3. About 75% CPE
4. About 100% CPE.

For the $TCID_{50}$ calculation, '1' to '4' CPE is scored as 'virus present' (positive) and '0' as 'virus absent' (negative). Thus, as examples, a score of 4444 4444 means 100% positive, 0000 0000 means 0% positive, 4000 0200 means 2 out of 8 replicates = 25% positive.
The percentages per dilution from all negative up to 4 backward dilutions are used in the $TCID_{50}$ calculations. The $TCID_{50}$ calculations were done by using the Spearmann-Karber method (1). The formula is shown below.

The formula is:

Negative logarithm of the 50 % end point = Negative logarithm of the highest virus concentration used - [(Sum of % affected at each dilution / 100 - 0,5) x (lg of dilutions)]

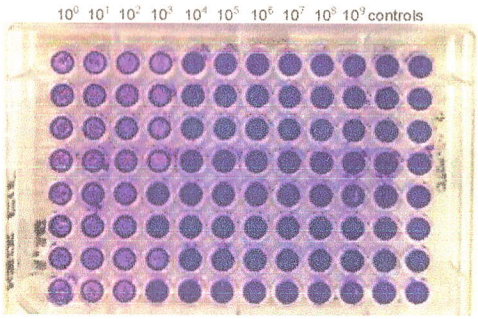

Figure 1 A $TCID_{50}$ result visualized by crystal violet staining of cells in 96 wells. Above the picture of the 96 well plate, the dilution range is shown including negative controls of cells not exposed to the SARS-CoV-2 virus (latter two columns). Each row represents a replicate of one of the triplicate exposures or negative exposure control. Cytopathological effects are visible in the lower dilutions based on clearance of the blue color in the wells.

3 Results and discussion

The antiviral tests with the Shycocan system were performed divided over two days of testing. At each day, the average non-exposed SARS-CoV-2 virus count on the stainless steel disks was determined as baseline for the comparison to the treated samples (5.25 log units $TCID_{50}$ on day 1 and 4.17 log $TCID_{50}$ units at day 2). The non-exposed results and those obtained with the Shycocan system are shown in table 1.

Table 1 Viral SARS-CoV-2 counts expressed as log $TCID_{50}$ on stainless steel disks non-exposed and exposed to the Shycocan system during indicated conditions. All counts are shown including the standard deviation (SD) of triplicate analysis. ND indicates not determined conditions at that particular day.

Exposure conditions	log $TCID_{50}$ / SD (day 1)	log $TCID_{50}$ / SD (day 2)
Non-exposed (15 minutes)	5.25 / 0.53	4.17 / 0.38
Preheated 1 minute	ND	4.04 / 0.13
Preheated 5 minutes	4.75 / 0.18	ND
Cold start 5 minutes	ND	3.63 / 0.40
Preheated 15 minutes	3.96 / 0.33	ND

Both the log $TCID_{50}$ reduction and percentage reduction of the number of infectious SARS-CoV-2 virus particles are shown in Table 2.

Table 2 Reduction in viral SARS-CoV-2 counts by the Shycocan system expressed as log $TCID_{50}$ and as a percentage compared to the non-exposed control.

Exposure conditions	log $TCID_{50}$ reduction	percentage reduction
Preheated 1 minute	0.1	24.1%
Preheated 5 minutes	0.5	68.4%
Cold start 5 minutes	0.5	71.2%
Preheated 15 minutes	1.2	94.9%

The results indicate an anti-viral effect of the Shycocan system against SARS-CoV-2. The observed reduction of 1.2 log unit or 94.9% of infectious virus particles was obtained after 15 minutes exposure.

Compared to previous experimental work (3, 4), the virus eradicating effect on surfaces by using the Shycocan system was better against SARS-CoV-2 than against the Influenza B virus (also an enveloped RNA virus with spike proteins). For the latter virus only a 0.3 log (49.9%) reduction was observed after 45 minutes exposure to the system. However, the Equine arteritis virus (also enveloped RNA virus with spike proteins) on a surface 50 cm distant from the Shycocan showed a 3.1 log (99.9%) reduction after 15 minutes exposure.

4 Conclusions

The following can be concluded:

- Based on the results obtained, a reduction of infectious SARS-CoV-2 virus particles on stainless steel disks was observed as a consequence of exposure to the Shycocan system.
- A 94.9% reduction of infectious SARS-CoV-2 virus particles was obtained after 15 minutes exposure to the Shycocan system.

5 References

1. Ramakrishnan MA. Determination of 50% endpoint titer using a simple formula. World J Virol. 2016 May 12;5(2):85-6. doi: 10.5501/wjv.v5.i2.85. PMID: 27175354; PMCID: PMC4861875.
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3. AM Bianciotto, Tres Monos Lab, Mexico, Shycocan-InfluenzaB-Mexico-Report-English-Color-HQ.pdf
4. AM Bianciotto, Tres Monos Lab, Mexico, Shycocan-EAV-Report-English-Color-HQ.pdf

6 Signature

Zeist, 31-05-2021

ValidSigned by Frank Schuren on 31-05-2021

FHJ Schuren, PhD
Senior Scientist

TNO

ValidSigned by Jos van der Vossen on 31-05-2021

JMBM van der Vossen, PhD
Author

Mexico City, June 16, 2020

REPORT FOR THE VIRUCIDAL ACTIVITY TEST (EQUINE ARTERITIS) FOR DEVICE

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CACIQUE, ANDRÉS M. BIANCCIOTTO
GENERAL MANAGER

INTRODUCTION

The virucidal activity test for device is able to determine its potential to remove viral particles from surface.

Mammalian cell culture is the base for this test because virus are biological entities that require host cells for their reproduction.

Due to the current conditions of biological material supply and the restrictive biosecurity conditions required for the handling of many virus, the Environmental Protection Agency (EPA, 2020) allows the use of phylogenetically similar strains to validate virucidal activity against emerging virus.

During the study, the next viral equivalences were used:

Virucidal activity evaluation	Virus used	Explanation
SARS-CoV-2	Equine arteritis virus ATCC VR-796	Both are enveloped RNA virus that belong to the Nidovirales order.

REAGENTS

- Cell culture media EMEM, ATCC, Lot: 80913222, Expiration date: 10/20.
- DPBS, pH 7.4, CORNING, Lot: 34518003, Expiration date: 12/21.
- Trypsin 0.25%, GIBCO, Lot: 2177694, Expiration date: 01/22.
- 2-Propanol (isopropanol) J.T. Baker, Lot: A04C70, Expiration date: 11/11/23.
- Thiazolyl Blue Tetrazolium Bromide SIGMA ALDRICH, Lot: MKCG3023, Expiration date: 09/23.
- MTT 5 mg/mL, LEI, Lot: CC-0857, Expiration date: 22/10/20.

BIOLOGICAL MATERIAL

- Equine arteritis virus ATCC, Lot: 59681624, Expiration date: VALID.
- Vero Cell line ATCC, Lot: 60150897, Expiration date: VALID.

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REPORT No. 44527 – Virucidal activity (equine arteritis) for device

EQUIPMENT AND INSTRUMENTS

- Biosafety Hood (Level II) ESCO, ID: CF013, Upcoming service: 08/20.
- CO_2 Incubator NUAIRE, ID: IN014, Upcoming service: 07/22.
- Plate reader MOLECULAR DEVICES, ID: LM002, Upcoming service: 06/20.
- Inverted microscope LEICA, ID: MO003, Upcoming service: 02/21.
- Water bath THERMO, ID: BM013, Upcoming service: 06/20.
- Multichannel micropipette 30-300 µL SARTORIUS, ID: PI198, Upcoming service: 08/20.
- Micropipette 100-1000 µL EPPENDORF, ID: PI107, Upcoming service: 03/21.
- Micropipette 10-100 µL EPPENDORF, ID: PI136, Upcoming service: 12/20.

SAMPLE

The analyzed sample was SHYCOCAN with lot N/A and described as Electron emission device for environmental and surface sanitization versus coronavirus and analogues.

PERSONNEL

The personnel that executed the test was trained in cell line and virus handling and cytopathogenic agent detection. The personnel have developed the methodologies in Laboratorios de Especialidades Inmunológicas S.A. de C.V.

METHODOLOGY

The methodology was executed according to the proposal from LABORATORIOS DE ESPECIALIDADES INMUNOLÓGICAS, S.A. DE C.V.

The objective of the executed methodology was to quantify the device virucidal activity.

Day 1

The morphology was reviewed in order to confirm a higher than 95% confluence before the assay.

In aseptic conditions, Vero cell suspension was obtained.

Cell density was determined and adjusted to 1,2 x 10^5 cells/mL in a final volume of 45 mL.

Adjusted cell suspension was added to the well indicated in **Figure 1** (8 plates):

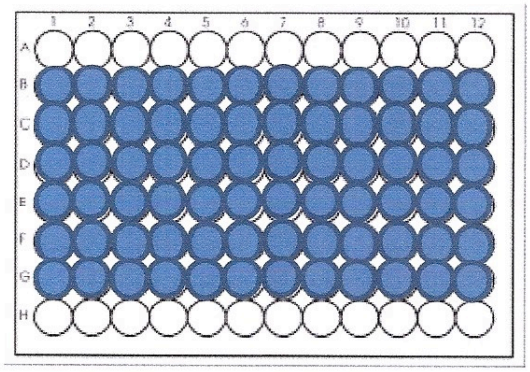

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Figure 1. Cell suspension addition scheme Day 1.

Plates were incubated for 48 hours at 37 °C and 5% CO_2.

Day 2

After the incubation and in aseptic conditions, 3 equine arteritis working bank vials were thawed.

100 µL of Antibiotic Antimycotic (100X) were added to each vial.

330 µL of the vial content were added to 9 sterile 10 cm Petri plates labeled according to **Table 1**:

10 cm Petri plate	Treatment
1 a 3	50 cm from the device for 15 minutes
4 a 6	500 cm from the device for 120 minutes
7 a 9	Viral Control

Table 1. Plate scheme according to treatment.

In the Cell Culture and Bioassay area, SHYCOCAN equipment was turned on during two hours with the doors closed.

After the period was over, plate 1 to 6 were placed at the distance mentioned in **Table 1** inside biosafety cabinets.

After the plates were placed in the exposition point, the plates with the viral suspension were opened for the period mentioned in **Table 1**.

After the closure of the last plate, each well from the plates seeded in **Day 1** were verified to have a minimum 95% confluence.

In aseptic conditions, 6 dilution plates were prepared according to **Table 2**:

Dilution plate number	Treatment
1 y 2	50 cm from the device for 15 minutes
3 y 4	500 cm from the device for 120 minutes
5 y 6	Viral control

Table 2. Dilution plate preparation.

120 µL of EMEM media were added according to **Figure 2**:

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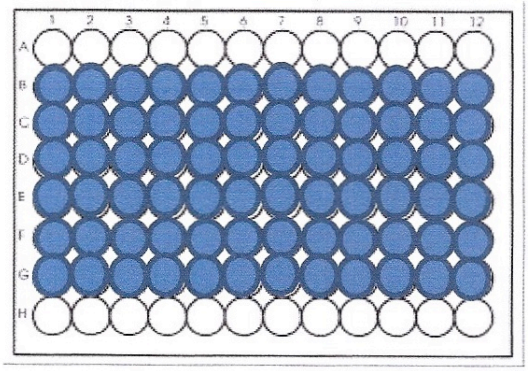

Figure 2. Cell culture media addition to dilution plates Day 2.

Viral recovery

9 mL of EMEM media were used to recover each treatment viral suspension and were transferred to a sterile 15 mL conic tube.

Por cada tratamiento se utilizaron 9 mL de medio EMEM para recuperar la suspensión viral resultante y se colocó en un tubo cónico estéril de 15 mL.

120 µL from this solution were added to column1 wells for dilute plates 1 to 4.

1:2 serial dilutions were performed according to **Figure 3**:

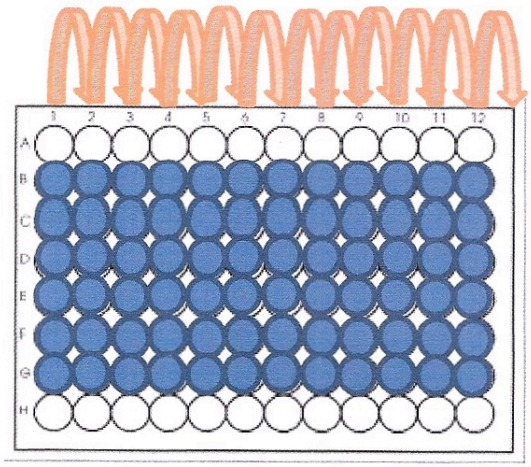

Figure 3. Serial dilution Day 2.

100 µL were transferred from the corresponding dilution plate to the plate with cells according to **Table 3**:

Plate with cells number	Treatment	Description
1 y 2	50 cm from the device for 15 minutes	100 µL were transferred from each column to dilution plates 1 and 2.
3 y 4	500 cm from the device for 120 minutes	100 µL were transferred from each column to dilution plates 3 and 4.
5 y 6	Viral control	100 µL were transferred from each column to dilution plates 5 and 6.
7 y 8	Negative control	100 µL were added of EMEM media.

Table 3. Dilution addition to plates with cells.

Plates were incubated at 37 °C and 5% CO_2 for 4 days.

Day 3

In aseptic conditions, media was removed for every well in the plates with cells by inversion.

100 µL of MTT solution were added to each well with cells.

Plates were incubated at 37 °C and 5% CO_2 for 2 hours.

After the incubation, media was removed for every well in the plates with cells by inversion.

100 µL of isopropanol were to each well with cells and the plates were incubated in agitation for 1 hour.

Plates were read at 570 nm.

Statistical analysis

The absorbance data were copied to an Excel spreadsheet for the plates Negative Control, Viral Control, 50 cm from device for 15 minutes and 500 cm from device for 120 minutes.

Absorbance average was calculated for the first row of Negative Control plate. This value was named "Blank Average".

Absorbance average was calculated for each well with cells for the Negative Control plate. This value was named "Negative Control Average".

Average viability was determined for each concentration by using the next formula:

$$Average\ viability = \frac{Absorbance\ average\ by\ concentration - Blank\ average}{Negative\ Control\ Average - Blank\ average} x100$$

For each concentration, mortality ratio was determined by using the next formula:

$$Mortality\ ratio = \frac{100 - Average\ viability}{100}$$

LD50 was determined by using the next procedures:

a) When inside the concentrations tested a 50% viability is detected, the average of the superior and inferior concentration is used as the LD50.
b) When each concentration has a higher than 50% viability, the LD50 is the highest concentration tested.

Viral titre was determined by calculating the inverse of the LD50.

The remaining viral titre was compared to the recovered viral titre by using the Logarithmic reduction formula:

$$Logarithmic\ reduction = \log_{10} \frac{Recovered\ viral\ titre}{Remaining\ viral\ titre}$$

The assay is valid when the logarithmic reduction from the viral control is less than 3.

For the plates for each treatment (50 cm from the device for 15 minutes and 500 cm from the device for 120 minutes) the absorbance average was calculated for every concentration.

Remaining viral viability diminution was determined by subtracting the Averages for each concentration to the Negative Control average.

With this value Remaining viral viability was determining by using this formula:

$$Average\ viability = \frac{Negative\ Control\ Average - Remaining\ viability\ diminution - Blank\ average}{Negative\ Control\ Average - Blank\ average} x100$$

Mortality rate of the remaining virus was determined by using the next formula:

$$Mortality\ rate = \frac{100 - Average\ viability}{100}$$

LD50 was determined by using the next procedures:

a) When within the concentration a 50% viability is reached, the LD50 is the average from the superior and inferior concentration identified.
b) When within the concentration a 50% viability is not reached, the Spearman-Karber formula is used to determine LD50:

$$Spearman - Karber\ Coefficient = \log_{10} \frac{1}{Lowest\ dilution\ used} - \frac{\log_{10} 2}{2} + \log_{10}(2) * \sum Mortality\ rate$$

$$DL50 = 10^{-Spearman-Karber\ Coefficient}$$

Viral titre was determined by calculating the inverse of the LD50.

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REPORT No. 44527 – Virucidal activity (equine arteritis) for device

The remaining viral titre was compared to the recovered viral titre by using the Logarithmic reduction formula:

$$Logarithmic\ reduction = \log_{10} \frac{Recovered\ viral\ titre}{Remaining\ viral\ titre}$$

When the LD50 is lower than the highest concentration tested, the logarithmic reduction is reported as higher than the logarithmic reduction calculated.

A device has virucidal activity when a logarithmic reduction higher than 3 is obtained when the substance has cytotoxic activity and higher than 4 when it has not.

RESULTS

Viral Control												
Used dilution	0.05	0.025	0.0125	0.0063	0.0031	0.0016	0.0008	0.0004	0.0002	1E-04	5E-05	2E-05
% Remaining virus viability	6.4327	6.506	6.0174	5.9523	5.4556	5.7731	5.7406	7.9391	11.139	21.896	41.243	43.246
Mortality rate	0.9357	0.9349	0.9398	0.9405	0.9454	0.9423	0.9426	0.9206	0.8886	0.781	0.5876	0.5675
Results	**Viral control titre**										$4x10^7$	

Virucidal activity 50 cm from the device for 15 minutes												
Used dilution	0.05	0.025	0.0125	0.0063	0.0031	0.0016	0.0008	0.0004	0.0002	1E-04	5E-05	2E-05
% Remaining virus viability	7.0271	6.9131	6.4327	6.05	5.7976	5.4474	5.5614	5.7569	12.361	16.88	39.468	57.17
Mortality rate	0.9297	0.9309	0.9357	0.9395	0.942	0.9455	0.9444	0.9424	0.8764	0.8312	0.6053	0.4283
Results	**Recovered titre**		$4x10^7$		**Remaining titre**		27307		**Logarithmic reduction**		3.134	

Virucidal activity 500 cm from the device for 120 minutes												
Used dilution	0.05	0.025	0.0125	0.0063	0.0031	0.0016	0.0008	0.0004	0.0002	1E-04	5E-05	2E-05
% Remaining virus viability	5.5533	5.366	4.853	4.5843	4.3319	5.6266	7.1004	9.2989	34.036	40.249	64.156	65.369
Mortality rate	0.9445	0.9463	0.9515	0.9542	0.9567	0.9437	0.929	0.907	0.6596	0.5975	0.3584	0.3463
Results	**Recovered titre**		$4x10^7$		**Remaining titre**		13653		**Logarithmic reduction**		3.435	

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CONCLUSIONS

During the analysis it was determined that the device has virucidal activity and causes a logarithmic reduction of 3.134 for equine arteritis virus during a 15 minutes exposure at 50 cm from the device and a 3.435 reduction during a 120 minutes exposure at 500 cm from the equipment.

This experimental result was obtained with a previous equipment activation for 120 minutes at a 6 m x 3 m x 2 m room. The interpretation of the result is that within the exposure times tested the equipment eliminates more than 99.9% of the viral particles.

REFERENCES

United States Environmental Protection Agency EPA (2020). *List N: Disinfectants for Use Against SARS-CoV-2.* Recovered from the Environmental Protection Agency website: https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2

Kind regards

M. in Sc. Israel Velázquez Martínez
Sanitary Responsible Party

Mexico City, July 27, 2020

REPORT FOR THE VIRUCIDAL ACTIVITY TEST (INFLUENZA B) FOR DEVICE

TRES MONOS LAB S. DE R.L. DE C.V.
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ANDRES MARCOS BIANCIOTTO
GENERAL MANAGER

INTRODUCTION

The virucidal activity test for device is able to determine its potential to remove viral particles from surface.

Mammalian cell culture is the base for this test because virus are biological entities that require host cells for their reproduction.

Due to the current conditions of biological material supply and the restrictive biosecurity conditions required for the handling of many virus, the Environmental Protection Agency (EPA, 2020) allows the use of phylogenetically similar strains to validate virucidal activity against emerging virus.

During the study, the next viral equivalences were used:

Virucidal activity evaluation	**Virus used**	**Explanation**
SARS-CoV-2	Influenza B virus ATCC VR-1535	Both are enveloped RNA virus that have spike proteins.

REAGENTS

- Cell culture media EMEM, ATCC, Lot: 80913222, Expiration date: 10/20.
- DPBS, pH 7.4, CORNING, Lot: 34518003, Expiration date: 12/21.
- Trypsin 0.25%, GIBCO, Lot: 2177694, Expiration date: 01/22.
- 2-Propanol (isopropanol) J.T. Baker, Lot: A04C70, Expiration date: 11/11/23.
- Thiazolyl Blue Tetrazolium Bromide SIGMA ALDRICH, Lot: MKCG3023, Expiration date: 09/23.
- Antibiotic-Antimycotic (100X) GIBCO, Lote: 2112697, Expiration date: 11/20
- MTT 5 mg/mL, LEI, Lot: CC-0898, Expiration date: 11/26/20.

BIOLOGICAL MATERIAL

REPORT No. 44527 – Virucidal activity (influenza B) for device

- Influenza B virus ATCC, Lot: 59681563, Expiration date: VALID.
- MDCK Cell line ATCC, Lot: 70020331, Expiration date: VALID.

EQUIPMENT AND INSTRUMENTS

- Biosafety Hood (Level II) ESCO, ID: CF013, Upcoming service: 08/20.
- CO_2 Incubator NUAIRE, ID: IN014, Upcoming service: 07/22.
- Plate reader MOLECULAR DEVICES, ID: LM002, Upcoming service: 06/21.
- Inverted microscope LEICA, ID: MO003, Upcoming service: 02/21.
- Water bath THERMO, ID: BM013, Upcoming service: 06/22.
- Multichannel micropipette 30-300 µL SARTORIUS, ID: PI198, Upcoming service: 08/20.
- Micropipette 100-1000 µL EPPENDORF, ID: PI107, Upcoming service: 03/21.
- Micropipette 10-100 µL EPPENDORF, ID: PI136, Upcoming service: 12/20.

SAMPLE

The analyzed sample was SHYCOCAN with lot N/A and described as Electron emission device for environmental and surface sanitization versus coronavirus and analogues.

PERSONNEL

The personnel that executed the test was trained in cell line and virus handling and cytopathogenic agent detection. The personnel have developed the methodologies in Laboratorios de Especialidades Inmunológicas S.A. de C.V.

METHODOLOGY

The methodology was executed according to the proposal from LABORATORIOS DE ESPECIALIDADES INMUNOLÓGICAS, S.A. DE C.V.

The objective of the executed methodology was to quantify the device virucidal activity.

Day 1

The morphology was reviewed in order to confirm a higher than 95% confluence before the assay.

In aseptic conditions, MDCK cell suspension was obtained.

Cell density was determined and adjusted to 1,7 x 10^5 cells/mL in a final volume of 45 mL.

Adjusted cell suspension was added to the well indicated in **Figure 1** (10 plates):

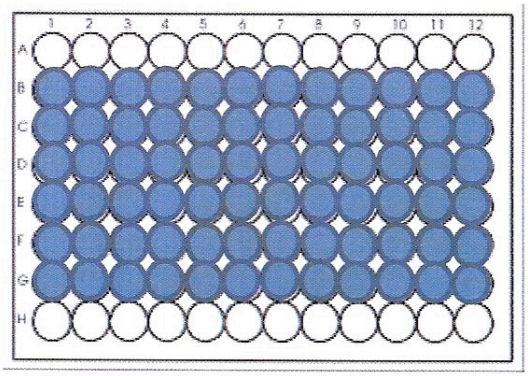

Figure 1. Cell suspension addition scheme Day 1.

Plates were incubated for 48 hours at 37 °C and 5% CO_2.

Day 2

After the incubation and in aseptic conditions, 4 influenza B working bank vials were thawed.

100 µL of Antibiotic Antimycotic (100X) were added to each vial.

330 µL of the vial content were added to 12 sterile 10 cm Petri plates labeled according to **Table 1**:

10 cm Petri plate	Treatment
1 to 3	100 cm from the device for 15 minutes
4 to 6	100 cm from the device for 45 minutes
7 to 9	300 cm from the device for 45 minutes
10 to 12	Viral Control

Table 1. Plate scheme according to treatment.

In the Cell Culture and Bioassay area, SHYCOCAN equipment was turned on during two hours with the doors closed.

After the period was over, plate 1 to 9 were placed at the distance mentioned in **Table 1** inside biosafety cabinets.

After the plates were placed in the exposition point, the plates with the viral suspension were opened for the period mentioned in **Table 1**.

After the closure of the last plate, each well from the plates seeded in **Day 1** were verified to have a minimum 95% confluence.

In aseptic conditions, 8 dilution plates were prepared according to **Table 2**:

REPORT No. 44527 – Virucidal activity (influenza B) for device

Dilution plate number	Treatment
1 and 2	100 cm from the device for 15 minutes
3 and 4	100 cm from the device for 45 minutes
5 and 6	300 cm from the device for 45 minutes
7 and 8	Viral control

Table 2. Dilution plate preparation.

120 µL of EMEM media were added according to **Figure 2**:

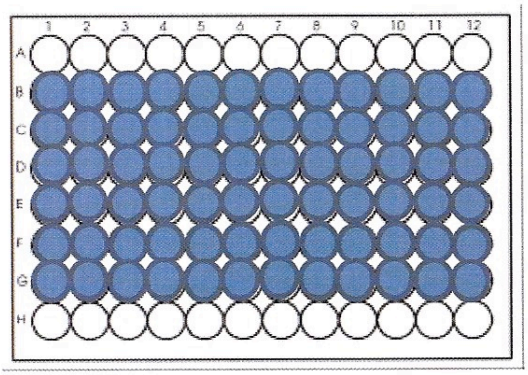

Figure 2. Cell culture media addition to dilution plates Day 2.

Viral recovery

9 mL of EMEM media were used to recover each treatment viral suspension and were transferred to a sterile 15 mL conic tube.

120 µL from this solution were added to column1 wells for dilute plates 1 to 4.

1:2 serial dilutions were performed according to **Figure 3**:

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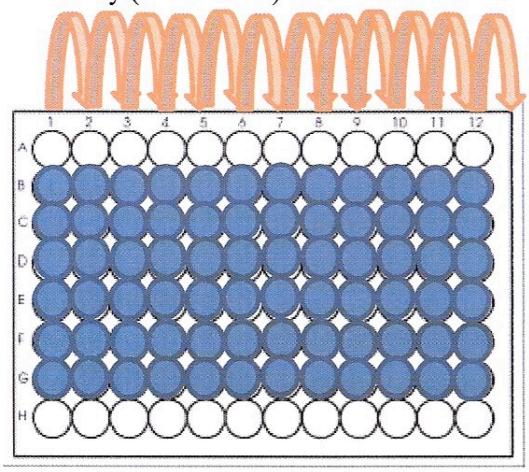

Figure 3. Serial dilution Day 2.

100 µL were transferred from the corresponding dilution plate to the plate with cells according to **Table 3**:

Plate with cells number	Treatment	Description
1 and 2	100 cm from the device for 15 minutes	100 µL were transferred from each column to dilution plates 1 and 2.
3 and 4	100 cm from the device for 45 minutes	100 µL were transferred from each column to dilution plates 3 and 4.
5 and 6	300 cm from the device for 45 minutes	100 µL were transferred from each column to dilution plates 5 and 6.
7 and 8	Viral control	100 µL were transferred from each column to dilution plates 7 and 8.
9 and 10	Negative control	100 µL were added of EMEM media.

Table 3. Dilution addition to plates with cells.

Plates were incubated at 37 °C and 5% CO_2 for 7 days.

Day 3

In aseptic conditions, media was removed for every well in the plates with cells by inversion.

100 µL of MTT solution were added to each well with cells.

Plates were incubated at 37 °C and 5% CO_2 for 2 hours.

After the incubation, media was removed for every well in the plates with cells by inversion.

100 µL of isopropanol were to each well with cells and the plates were incubated in agitation for 1 hour.

REPORT No. 44527 – Virucidal activity (influenza B) for device

Plates were read at 570 nm.

Statistical analysis

The absorbance data were copied to an Excel spreadsheet for the plates Negative Control, Viral Control, 100 cm from device for 15 minutes, 100 cm from device for 45 minutes and 300 cm from device for 45 minutes.

Absorbance average was calculated for the first row of Negative Control plate. This value was named "Blank Average".

Absorbance average was calculated for each well with cells for the Negative Control plate. This value was named "Negative Control Average".

Average viability was determined for each concentration by using the next formula:

$$Average\ viability = \frac{Absorbance\ average\ by\ concentration - Blank\ average}{Negative\ Control\ Average - Blank\ average} x100$$

For each concentration, mortality ratio was determined by using the next formula:

$$Mortality\ ratio = \frac{100 - Average\ viability}{100}$$

LD50 was determined by using the next procedures:

a) When inside the concentrations tested a 50% viability is detected, the average of the superior and inferior concentration is used as the LD50.
b) When each concentration has a higher than 50% viability, the LD50 is the highest concentration tested.

Viral titre was determined by calculating the inverse of the LD50.

The remaining viral titre was compared to the recovered viral titre by using the Logarithmic reduction formula:

$$Logarithmic\ reduction = \log_{10} \frac{Recovered\ viral\ titre}{Remaining\ viral\ titre}$$

The assay is valid when the logarithmic reduction from the viral control is less than 3.

For the plates for each treatment (100 cm from device for 15 minutes, 100 cm from device for 45 minutes and 300 cm from device for 45 minutes) the absorbance average was calculated for every concentration.

Remaining viral viability diminution was determined by subtracting the Averages for each concentration to the Negative Control average.

REPORT No. 44527 – Virucidal activity (influenza B) for device

With this value Remaining viral viability was determining by using this formula:

$$Average\ viability = \frac{Negative\ Control\ Average - Remaining\ viability\ diminution - Blank\ average}{Negative\ Control\ Average - Blank\ average} x100$$

Mortality rate of the remaining virus was determined by using the next formula:

$$Mortality\ rate = \frac{100 - Average\ viability}{100}$$

LD50 was determined by using the next procedures:

a) When within the concentration a 50% viability is reached, the LD50 is the average from the superior and inferior concentration identified.
b) When within the concentration a 50% viability is not reached, the Spearman-Karber formula is used to determine LD50:

$$Spearman - Karber\ Coefficient = \log_{10} \frac{1}{Lowest\ dilution\ used} - \frac{\log_{10} 2}{2} + \log_{10}(2) * \sum Mortality\ rate$$

$$DL50 = 10^{-Spearman-Karber\ Coefficient}$$

Viral titre was determined by calculating the inverse of the LD50.

The eliminated virus titre was determined by subtracting the recovered viral titre minus the remaining virus titre per treatment:

$$Eliminated\ titre = Recovered\ titre - Remaining\ titre$$

RESULTS

Viral Control												
Used dilution	0.05	0.025	0.0125	0.0063	0.0031	0.0016	0.0008	0.0004	0.0002	1E-04	5E-05	2E-05
% Remaining virus viability	55.069	71.137	83.268	85.999	84.572	92.692	94.931	96.038	89.813	90.034	85.433	61.442
Mortality rate	0.4493	0.2886	0.1673	0.14	0.1543	0.0731	0.0507	0.0396	0.1019	0.0997	0.1457	0.3856
Results	**Viral control titre (CFID50)**											123794

Virucidal activity 100 cm from the device for 15 minutes												
Used dilution	0.05	0.025	0.0125	0.0063	0.0031	0.0016	0.0008	0.0004	0.0002	1E-04	5E-05	2E-05
% Remaining virus viability	53.273	66.043	71.358	86.614	92.84	104.21	101.16	106.96	103.69	108.83	96.924	67.963
Mortality rate	0.4673	0.3396	0.2864	0.1339	0.0716	-0.0421	-0.0116	-0.0696	-0.0369	-0.0883	0.0308	0.3204
Results (CFID50)	**Recovered titre**		123794		**Remaining titre**		76507		**Eliminated titre**		47287	

REPORT No. 44527 – Virucidal activity (influenza B) for device

Virucidal activity 100 cm from the device for 45 minutes												
Used dilution	0.05	0.025	0.0125	0.0063	0.0031	0.0016	0.0008	0.0004	0.0002	1E-04	5E-05	2E-05
% Remaining virus viability	59.867	66.511	85.63	88.214	101.21	97.933	107.28	115.58	105.12	105.41	99.016	71.875
Mortality rate	0.4013	0.3349	0.1437	0.1179	-0.0121	0.0207	-0.0728	-0.1558	-0.0512	-0.0541	0.0098	0.2813
Results (CFID50)	**Recovered titre**		123794		**Remaining titre**		56481		**Eliminated titre**		67313	

Virucidal activity 300 cm from the device for 45 minutes												
Used dilution	0.05	0.025	0.0125	0.0063	0.0031	0.0016	0.0008	0.0004	0.0002	1E-04	5E-05	2E-05
% Remaining virus viability	62.156	74.434	79.232	84.818	90.773	94.562	92.938	94.513	96.875	97.293	92.495	63.189
Mortality rate	0.3784	0.2557	0.2077	0.1518	0.0923	0.0544	0.0706	0.0549	0.0313	0.0271	0.075	0.3681
Results (CFID50)	**Recovered titre**		123794		**Remaining titre**		98583		**Eliminated titre**		25212	

CONCLUSIONS

During the analysis it was determined that the device has a virucidal activity and causes a reduction of 47287, 67313 and 25212 CFID50 of influenza B virus corresponding to the distances and times evaluated (100 cm from device for 15 minutes, 100 cm from device for 45 minutes and 300 cm from device for 45 minutes).

As a comparison a symptomatic seasonal influenza patient produces between 12000 and 38000 CFID50 every 30 minutes (Yan et al, 2018).

This experimental result was obtained with a previous equipment activation for 120 minutes at a 6 m x 3 m x 2 m room. The interpretation of the result is that within the exposure times tested the SHYCOCAN device eliminates the influenza B virus, a virus that has spike proteins as Coronavirus, in similar magnitudes of the exhaled virus by a symptomatic influenza infected patient for 30 minutes.

REFERENCES

United States Environmental Protection Agency EPA (2020). *List N: Disinfectants for Use Against SARS-CoV-2.* Recovered from the Environmental Protection Agency website: https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2

Yan et al (2018). *Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community.* PNAS, 115(5), pp. 1081-1086.

REPORT No. 44527 – Virucidal activity (influenza B) for device

Kind regards

M. in Sc. Israel Velázquez Martínez
Sanitary Responsible Party

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GSTIN: 29AAHCA0185G1ZP

TEST REPORT

Report No: AWRTCL/17618A/20-21 **Date: 29.08.2020 (Updated 26.10.2020)**

CUSTOMER DETAILS	SAMPLE DETAILS	TEST DETAILS
Name & Address: **Kind Attn:** **The Manager** **M/s. Eureka Forbes Limited, R&D Centre** **Bangalore**	**Sample received:13.08.2020**	**Method:** **As agreed between the Testing Laboratory and the customer**
	Sample code no: AWRTCL/17618A /20-21	
	Sample Description: Forbes Coronaguard powered by SHYCOCAN	
	Sample Quantity for Testing: 1 No	
	Submitted by : M/s. Eureka Forbes Limited, R&D Centre	
	Date of Analysis started : 17.08.2020	
	Date of Analysis Completed:29.08.2020	
	Subcontract : Not applicable	
	Sample condition when received : Intact	

EXECUTIVE SUMMARY:

One unit of Coronaguard powered by Shycocan, manufactured and marketed by M/s Eureka Forbes Limited, was assessed for its microbial reduction capability with reference to E.coli MTCC 68 and a bacteriophage MS2 phage ATCC15597B1 (a surrogate virus) at different distances and at different time of exposures , continuously. The data are presented in tables 1 & 2. The test data inferred that when the Coronaguard was kept ON continuously, MS2 phage bacteriophage, under the influence of Coronaguard, was found to be reduced by > 99.9% at all the elapsed time intervals of sampling. Whereas E.coli bacterial strain did not show reduction in counts.

The current testing was conducted in a chamber of 1700 cft (approx) in a closed condition.

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TEST REPORT

TEST DATA OF CORONAGUARD IN DECONTAMINATION OF DIFFERENT MICROORGANISMS ON SURFACES

SCHEMATIC DIAGRAM OF CORONAGUARD INSTALLATION AT 7' HEIGHT FROM GROUND.

TEST CHAMBER : (13' x 12' x 11' W-D-H) ≈ 1700 cft ROOM

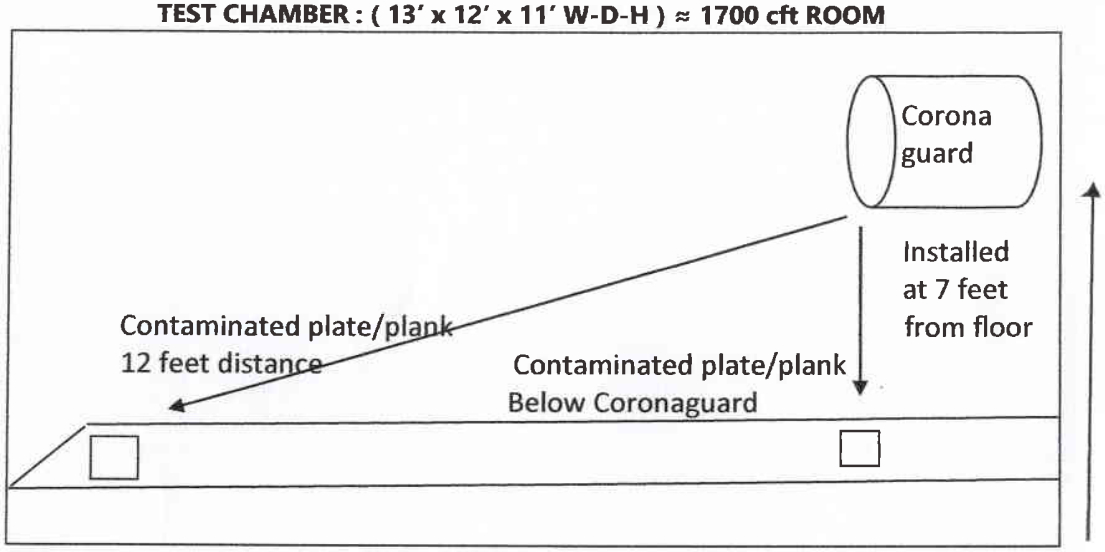

Table – 1 E.coli MTCC 68 reduction : Installation of Coronaguard at 7 feet from the floor

#	Test Details	Microbial counts with Coronaguard in OFF condition	Microbial counts with Coronaguard in ON condition		% Reduction
			1 hr exposure Short distance	1 hr exposure Farthest distance	
1	Test organism was taken in petri plates at two different distances: In the same line (short distance) below the Coronaguard in the in the farthest distance of 12 feet.	5 x 10^6 cfu/ swab	TNTC / 0.1 ml = TNTC/swab	TNTC/0.1 ml= TNTC/swab	**No reduction**
2	Test organism smeared on Acrylic sheets (planks) : At 1 hr	4 x 10^6 cfu/swab	1hr exposure Short distance TNTC / 0.1 ml = TNTC/swab	1 hr exposure Farthest distance TNTC/0.1 ml= TNTC/swab	**No reduction**
			2 hr exposure : Short distance TNTC / 0.1 ml = TNTC/swab	2 hr exposure : Farthest distance TNTC/0.1 ml= TNTC/swab	**No reduction**

TNTC: Too numerous to count

INFERENCE: Test data reveal no reduction in E.coli counts due to exposure to Coronaguard for 1 hr & 2 hr duration also.

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TEST REPORT

TEST DATA OF CORONAGUARD IN DECONTAMINATION OF DIFFERENT MICROORGANISMS ON SURFACES
SCHEMATIC DIAGRAM OF CORONAGUARD INSTALLATION AT SAME PLANE AT 12 FEET DISTANCE
TEST CHAMBER : (13' x 12' x 11' W-D-H) ≈ 1700 cft ROOM

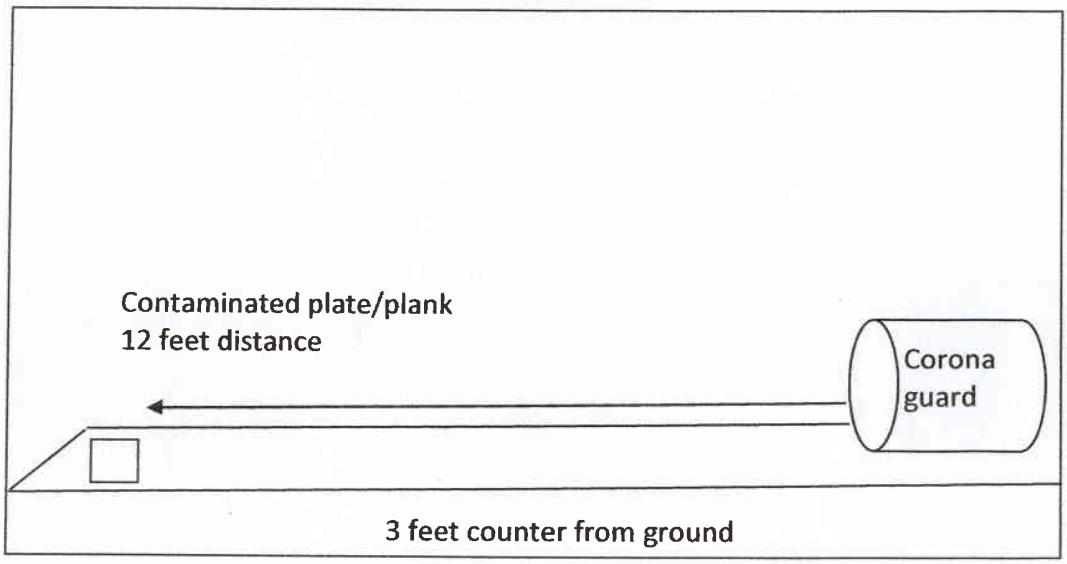

Table – 2 MS2 PHAGE ATCC 15597B1 reduction : Installation of Coronaguard on a counter

Type of Test	Microbial Count with Coronaguard in OFF condition	Sampling Elapsed Time	Microbial counts with Coronaguard i ON condition	% Reduction
In the same plane of Coronaguard, Acrylic sheets smeared with MS2 phage culture are kept at 12 feet distance from the Coronaguard.	9 x 10^6 pfu/swab	30 minutes	2.11x 10^3 pfu/swab	99.976%
		1 hr	4.8x 10^2 pfu/swab	99.994%
		2 hr	3.2 x 10^2 pfu/swab	99.996%

INFERENCE: Above test data reveal that Coronaguard has capability of decontaminating MS2 phage (Bacteriophage- a surrogate Virus)

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TEST REPORT

METHODOLOGY:

Contaminant bacterial (E.coli) culture taken in Petriplates:

Two identical sterile petriplates of 100 mm diameter were taken. In one of the plates, 1 ml of broth culture of (24 hr old) was added + 1 ml 0.9% physiological saline and retained in the test chamber for 1 hr (Coronaguard was OFF). The plate was retrieved, and the sample was serially diluted and plated on to M Endo agar media. Incubation was done at 37 ^{0}C/24 hr. Similarly two contaminated plates each were exposed to Coronaguard (Switched ON) for mentioned running time at short distance and farthest distance respectively. The plates were retrieved at designated intervals and the samples were enumerated for E.coli counts.

Contaminant taken on Acrylic planks:

Two identical acrylic planks with ½' x ½' dimensions were smeared with 1 ml of 24 hr broth culture separately, The process of before running and after running Coronaguard was followed as above.

Contaminant bacteriophage (MS2 phage) culture taken on Acrylic planks:

The smears of MS2 phage were also prepared by using 1 ml inoculum. Further process of exposure, sampling before and after running the Coronaguard was done as explained above. Sample extraction was done by swabbing an area of 10cm x 10cm using 10 ml 0.9% physiological saline.

MS2 phage counts were analysed by two layer agar method. Incubation was done at 37 ^{0} C / 24 hr.

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TEST REPORT

IMPORTANT
Reagent grade water
Analytical grade Chemicals
NIST traceable standard chemicals for calibration

Microbial cultures: ATCC and MTCC standard cultures
MS2 phage ATCC 15597B1
E.coli ATCC15597 (host)
E.coli MTCC 68

Table – 3 Microbial growth media used for the current testing

#	Name of the growth media	Make	Number
1	Nutrient broth	HiMedia	M002
	Nutrient agar	HiMedia	M001
2	M Endo agar	HiMedia	M1106
3	Soya Casein Digest Agar (Tryptone Soya Agar)	HiMedia	M290
4	Soya bean Casein digest medium (Tryptone Soya broth)	HiMedia	M011
5	Agar Agar powder	HiMedia	GRM666

All the relevant equipment was calibrated by accredited agencies as applicable.

REFERENCES:
NSF/ANSI 55 Ultraviolet Microbiological Water treatment systems
IAPMO-IWPSC-01:2019 Evaluation of Point use Drinking water purification systems – specification
APHA 23rd edition Standard Methods for the examination of Water & Waste Water

Dr S.MURALIDHARA RAO
Head – Laboratory

00--------End of the Test Report ------00

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TEST REPORT

Report No: AWRTCL/PRTR/ 17819/20-21 **Date: 27.01.2021**

CUSTOMER DETAILS	SAMPLE DETAILS	TEST DETAILS
Name & Address **Kind Attn:** **Mr.Nishchay Miterr** **M/s EMDET Engineers Pvt** **Plot No:F-4, Phase – III** **Chakan MIDC,** **PUNE – 410501** **M:9536666666**	**Sample received: 19.10.2020** **Sample code no: AWRTCL/17819 /20-21** **Sample Description: Shycocan** **Sample Quantity for Testing: 1No** **Submitted by: M/s EMDET Engineers Pvt Ltd** **Date of Analysis started : 25.01.2021** **Date of Analysis Completed : 27.01.2021** **Subcontract : Not Applicable** **Sample condition when received: Intact**	**Method:** **As agreed between the Testing Laboratory and the customer**

SHYCOCAN installed at the entrance above 7 feet from floor

Total Area covered for the testing 74 feet x 20 feet (1480 sft area with 11 feet height ceiling)

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TEST REPORT

Methodology:
Contaminated planks at different distances were placed and Shycocan was run electrically. At intervals of 5,15,30,45 and 60 minutes of exposure the contaminated planks were retrieved, and microbial counts were enumerated.

To determine whether contaminated planks show survival of microorganisms up to 60 minutes without treatment (exposure of Shycocan) was also monitored.

Table – 1 MS2 Phage reduction on Entire Office premises at 7 feet height from the Floor and Shycocan installed at entrance (1480 sft area with 11 feet height ceiling)

Time	AWRTCL/17819/ 20-21 Shycocan			
	Distance	Before Treatment MS2 Phage Counts	After Treatment MS2 Phage Counts	% Reduction
5 minutes	10 feet from entrance	$7.0x10^6$Pfu/Swab	$1.63x10^4$ Pfu/Swab	99.767
15 minutes			$1.60x10^4$ Pfu/Swab	99.771
30 minutes			$1.58x10^4$ Pfu/Swab	99.774
45 minutes			$1.62x10^4$ Pfu/Swab	99.768
60 minutes			$1.59x10^4$ Pfu/Swab	99.773

Pfu: Plaque forming units

Table – 2 MS2 Phage reduction on Entire Office premises at 7 feet height from the Floor and Shycocan installed at entrance (1480 sft area with 11 feet height ceiling)

Time	AWRTCL/17819/ 20-21 Shycocan			
	Distance	Before Treatment MS2 Phage Counts	After Treatment MS2 Phage Counts	% Reduction
5 minutes	18 feet from entrance	$7.0x10^6$Pfu/Swab	$1.70 x10^4$ Pfu/Swab	99.757
15 minutes			$1.68x10^4$ Pfu/Swab	99.760
30 minutes			$1.56x10^4$ Pfu/Swab	99.762
45 minutes			$1.63x10^4$ Pfu/Swab	99.767
60 minutes			$1.60x10^4$ Pfu/Swab	99.771

Pfu: Plaque forming units

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TEST REPORT

Table – 3 MS2 Phage reduction on Entire Office premises at 7 feet height from the Floor and Shycocan installed at entrance (1480 sft area with 11 feet height ceiling)

Time	AWRTCL/17819/ 20-21 Shycocan			
	Distance	Before Treatment MS2 Phage Counts	After Treatment MS2 Phage Counts	% Reduction
5 minutes	24 feet from entrance	8.0 x10^6Pfu/Swab	1.84x10^4 Pfu/Swab	99.770
15 minutes			1.83x10^4 Pfu/Swab	99.771
30 minutes			1.80x10^4 Pfu/Swab	99.775
45 minutes			1.77x10^4 Pfu/Swab	99.778
60 minutes			1.76x10^4 Pfu/Swab	99.780

Pfu: Plaque forming units

IMPORTANT

Microbial cultures: MS2 phage ATCC 15597B1 (bacteriophage)
Host: E.coli ATCC 15597
Method: Published USEPA /NSF /ANSI55

Dr S.MURALIDHARA RAO
Head – Laboratory

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Note:

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TEST REPORT

Report No: AWRTCL/PRTR/ 17819A/20-21 **Date: 12.11.2020**

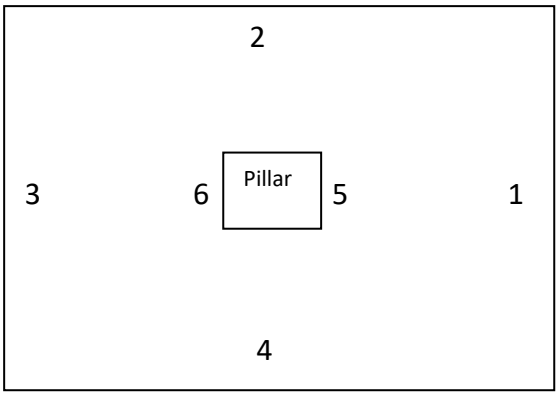
CUSTOMER DETAILS	SAMPLE DETAILS	TEST DETAILS
Name & Address Kind Attn: Mr.Nishchay Miterr EMDET Engineers Pvt Ltd Plot No:F-4, Phase – III Chakan MIDC, PUNE – 410501 M:9536666666	Sample received: 19.10.2020 Sample code no: AWRTCL/17819A /20-21 Sample Description: Sycocan Unit Sample Quantity for Testing: 1No Submitted by: M/s Shycocan Date of Analysis started : 10.11.2020 Date of Analysis Completed :12.11.2020 Subcontract : Not Applicable Sample condition when received: Intact	Method: As agreed between the Testing Laboratory and the customer

Table – 1 Summary of MS2 phage reduction at different heights

Time of Exposure	% Reduction of MS2 phage at different Height	
	Contamination at At 7 feet from Floor	Contamination at 11 feet from Floor
5 minutes	99.179	99.115
15 minutes	99.328	99.142
30 minutes	99.443	99.222
45 minutes	99.397	99.358

% reduction is average of 6places

Placement of contaminated planks in 17000 cft Test Chamber with a pillar in the middle



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TEST REPORT

Table - 2 MS2 Phage reduction at 7 feet height from the Floor and Shycocan installed at 7 Feet

Time	Sampling Locations	AWRTCL/17819A/ 20-21 Shycocan		
		MS2 phage counts Before Treatment	MS2 Phage counts After Treatment	% Reduction
5 minutes	1	$6.0x10^6$Pfu/Swab	$1.27x10^4$ Pfu/Swab	99.788
	2		$1.13x10^4$ Pfu/Swab	99.812
	3		$1.20x10^4$ Pfu/Swab	99.80
	4		$1.36x10^4$ Pfu/Swab	99.773
	5 (Pillar Front Side)		$1.09x10^4$ Pfu/Swab	99.818
	6 (Pillar Back Side)		$2.35x10^5$ Pfu/Swab	96.083

Pfu: Plaque forming units; TNTC: Too Numerous to Count

Table – 3 MS2 phage reduction at 7 feet height from the floor and Shycocan installed at 7 feet

Time	Sampling Locations	AWRTCL/17819A/ 20-21 Shycocan		
		Before Treatment MS2 Phage Counts	After Treatment MS2 Phage Counts	% Reduction
15 minutes	1	$7.0x10^6$Pfu/Swab	$1.21x10^4$ Pfu/Swab	99.827
	2		$1.08x10^4$ Pfu/Swab	99.845
	3		$1.19x10^4$ Pfu/Swab	99.830
	4		$1.34x10^4$ Pfu/Swab	99.808
	5 (Pillar Front Side)		$9.7x10^3$ Pfu/Swab	99.861
	6 (Pillar Back Side)		$2.24x10^5$ Pfu/Swab	96.80

Pfu: Plaque forming units; TNTC: Too Numerous to Count

Table – 4 **MS2 phage reduction at 7 feet height from the floor and Shycocan installed at 7 feet**

Time	Sampling Locations	AWRTCL/17819A/ 20-21 Shycocan		
		Before Treatment MS2 Phage Counts	After Treatment MS2 Phage Counts	% Reduction
30 minutes	1	$8.0x10^6$Pfu/Swab	$9.3x10^3$ Pfu/Swab	99.883
	2		$8.4x10^3$ Pfu/Swab	99.895
	3		$1.01x10^4$ Pfu/Swab	99.873
	4		$1.20x10^4$ Pfu/Swab	99.850
	5 (Pillar Front Side)		$7.3x10^3$ Pfu/Swab	99.908
	6 (Pillar Back Side)		$2.20x10^5$ Pfu/Swab	97.25

Pfu: Plaque forming units; TNTC: Too Numerous to Count

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Table – 5 MS2 phage reduction at 7 feet height from the floor and Shycocan installed at 7 feet

Time	Sampling Locations	AWRTCL/17819A/ 20-21 Shycocan		
		Before Treatment MS2 Phage Counts	After Treatment MS2 Phage Counts	% Reduction
45 minutes	1	$7.0x10^6$Pfu/Swab	$6.7x10^3$ Pfu/Swab	99.904
	2		$7.0x10^3$ Pfu/Swab	99.90
	3		$8.0x10^3$ Pfu/Swab	99.885
	4		$9.1x10^3$ Pfu/Swab	99.870
	5 (Pillar Front Side)		$5.4x10^3$ Pfu/Swab	99.923
	6 (Pillar Back Side)		$2.17x10^5$ Pfu/Swab	96.90

Pfu: Plaque forming units; TNTC: Too Numerous to Count

Table – 6 MS2 phage reduction at 11 feet height from the floor and Shycocan installed at 7 feet

Time	Sampling Locations	AWRTCL/17819A/ 20-21 Shycocan		
		Before Treatment MS2 Phage Counts	After Treatment MS2 Phage Counts	% Reduction
5 minutes	1	$6.0x10^6$Pfu/Swab	$1.47x10^4$ Pfu/Swab	99.755
	2		$1.35x10^4$ Pfu/Swab	99.775
	3		$1.29x10^4$ Pfu/Swab	99.785
	4		$1.36x10^4$ Pfu/Swab	99.773
	5 (Pillar Front Side)		$1.20x10^4$ Pfu/Swab	99.80
	6 (Pillar Back Side)		$2.52x10^5$ Pfu/Swab	95.80

Pfu: Plaque forming units; TNTC: Too Numerous to Count

Table – 7 MS2 phage reduction at 11 feet height from the floor and Shycocan installed at 7 feet

Time	Sampling Locations	AWRTCL/17819A/ 20-21 Shycocan		
		Before Treatment MS2 Phage Counts	After Treatment MS2 Phage Counts	% Reduction
15 minutes	1	$6.0x10^6$Pfu/Swab	$1.40x10^4$ Pfu/Swab	99.766
	2		$1.24x10^4$ Pfu/Swab	99.793
	3		$1.19x10^4$ Pfu/Swab	99.802
	4		$1.23x10^4$ Pfu/Swab	99.795
	5 (Pillar Front Side)		$1.10x10^4$ Pfu/Swab	99.817
	6 (Pillar Back Side)		$2.47x10^5$ Pfu/Swab	95.880

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Table – 8 MS2 phage reduction at 11 feet height from the floor and Shycocan installed at 7 feet

Time	Sampling Locations	AWRTCL/17819A/ 20-21 Shycocan		
		Before Treatment MS2 Phage Counts	After Treatment MS2 Phage Counts	% Reduction
30 minutes	1	$7.0x10^6$Pfu/Swab	$1.21x10^4$ Pfu/Swab	99.827
	2		$1.08x10^4$ Pfu/Swab	99.845
	3		$1.00x10^4$ Pfu/Swab	99.846
	4		$1.06x10^4$ Pfu/Swab	99.850
	5 (Pillar Front Side)		$9.4x10^3$ Pfu/Swab	99.865
	6 (Pillar Back Side)		$2.34x10^5$ Pfu/Swab	96.10

Pfu: Plaque forming units; TNTC: Too Numerous to Count

Table – 7 MS2 phage reduction at 11 feet height from the floor and Shycocan installed at 7 feet

Time	Sampling Locations	AWRTCL/17819A/ 20-21 Shycocan		
		Before Treatment MS2 Phage Counts	After Treatment MS2 Phage Counts	% Reduction
45 minutes	1	$7.0x10^6$Pfu/Swab	$9.9x10^3$ Pfu/Swab	99.858
	2		$9.1x10^3$ Pfu/Swab	99.870
	3		$8.7x10^3$ Pfu/Swab	99.876
	4		$8.9x10^3$ Pfu/Swab	99.872
	5 (Pillar Front Side)		$7.8x10^3$ Pfu/Swab	99.888
	6 (Pillar Back Side)		$2.25x10^5$ Pfu/Swab	96.785

Pfu: Plaque forming units; TNTC: Too Numerous to Count

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TEST REPORT

PRODUCT PICTURE11

Dr S.MURALIDHARA RAO
Head – Laboratory

00--------End of the Test Report --------00

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Indian Institute of Technology Guwahati
Guwahati – 781 039, Assam, INDIA
Date: Friday, 06 November 2020
Project Reference No:CLE-EFL-SS11

For Scalene
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Report of Disinfection Efficiency of

FORBES CORONAGUARD powered by SHYCOCAN

Against Avian Coronavirus in the Air

Objective

Study the disinfection efficiency of the FORBES CORONAGUARD powered by SHYCOCAN against Avian Coronavirus in the Air.

Scope

- Fabrication of disinfection efficiency testing chamber specific to test Forbes coronaguard air disinfection capacity
- Design of experiment for evaluating the Forbes Coronaguard disinfection efficiency
- Testing of Forbes Coronaguard air disinfection efficiency against the Avian coronavirus which is from the same family of SARS CoV-2
- Submission of a technical report to Eureka Forbes Limited on FORBES CORONAGUARD air disinfection against Avian coronavirus

Testing methodology

Step1 : The newly constructed chamber with a dimension of 1850x1850x1820mm(~6300 lit) show in Figure 1 was used to generate the aerosol of Avian Coronavirus (300 µl) with appropriate air mixing units. The leak proof chambers made of HDPE sheets with the support of aluminium pipe structure. Temperature and relative humidity of air inside the champer was maintained at 25±1°C and 60±4 %.

Step 2: The aerosol generator used for medical application is fixed inside the chamber and particle size analyser i.e. particulate matter (PM) sensor was fixed at wall 4 (refer Figure 1)to measure the uniformity of aerosol mixing and its stability. It is loaded with virus solution.

Step 3: Air filtration system is used to filter the air + virus particle. it was kept outside the chamber having inlet 30 cm suction pipe from chamber. The filter air is circulated back to chamber to maintain same chamber pressure. The membrane filter with average pore size of 0.01 micron used to filter air at 10LPM capacity using vacuum pump.

For Scalene

Step 4: Air disinfection unit (Forbes Coronaguard) installed at wall 4 (refer Figure 1) facing towards wall 2 &3 and at 60-degree angle with wall 4.

Step 5: After fixing all the units mentioned above, chamber was sealed with sealant.

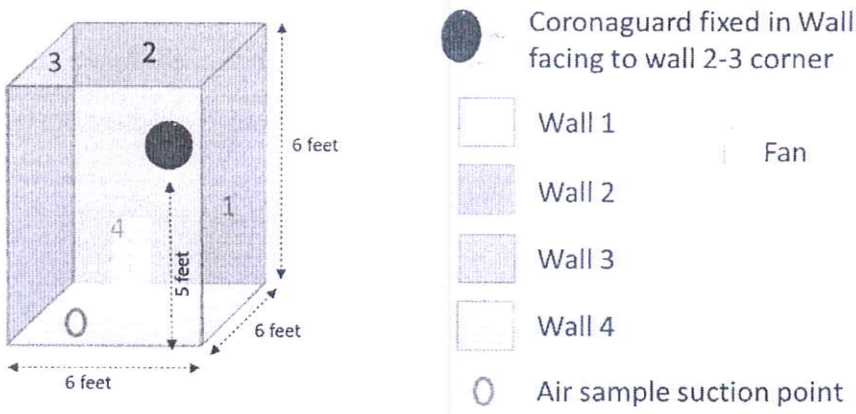


Figure 1: Chamber used to test the Corona Guard

Step 6: The aerosol generator and air circulation fan are switched on inside the chamber and the experiment was continued till PM levels came to steady-state, i.e. ~ 15 min

Step 7: Collect the air sample by filtering them through membrane filter at fixed air flowrate 10LPM for 10 mins

Step 8: Collect membrane filter with virus loaded at the control environment (sample No 1) for analysis of baseline value at controlled environment.

Step 9: Switched on the Forbes Coronogarurd for 15 mins and switch off and repeat the step 8 for sample collection (sample No2).

Step 10 : After collecting sample 2, switch on the Coronogarurd for another 45 mins and switch off and repeat the step 8 for sample collection (sample No 3).

Step 11: After collecting sample 3, switch on the Coronogarurd for another 60 mins and switch off and repeat the step 8 for sample collection (sample No 4).

Step 12: 100 µl of the filtrate (PBS or Media) collected from the sample 1 to 4 will inoculated into the chorioallantoic fluid of a nine-day old embryonated chicken egg.

Step 13: The eggs will be inoculated in duplicates and initial virus concentration will be used as a control.

Step 14: The eggs will be incubated at 37°C and constantly checked for motility and realtime PCR will be carried out at 48 hrs to check the presence of virus particle in the filtrate.

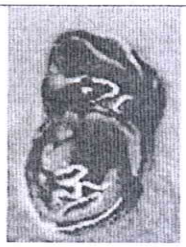
Step 15: Fold changes of the virus concentration will be calculated with respect to the control and plotted on a graph.

Performance Analysis

Analysis of air sample for Avian coronavirus removal efficiency

The samples collected from the air within the chamber was inoculated in embryonated chicken eggs, and the virus analysis result is present in Table 1. The embryos did not present any characteristic lesions because of the low concentration of the virus, but upon performing Real Time PCR we were able to titrate the virus and the results show significant reduction in the virus titre.

Table 1: Summary of Air sample analysis

Sample No	1	2	3	4
Conditions	FORBES CORONAGUARD OFF	After FORBES CORONAGUARD ON for 15 min	After FORBES CORONAGUARD ON for 60 min	After FORBES CORONAGUARD ON for 120 min
Virus Presence in Air	100*	9.96×10^{-10}	3.08×10^{-12}	9.829×10^{-13}
% of Virus disinfection	0	100	100	100
Visualization of motility of eggs				
Note@ Above summarised results are average of two experimental results *The intital RNA genome number of avian coronavirus was 16905 (Taken as 100%)				

Conclusion

The above experiments have shown that the FORBES CORONAGUARD powered by SHYCOCAN has a notable effect on the inactivation of Avian Coronavirus in the air within the chamber as shown in the table below:

Forbes CoronaGuard	Antiviral effect (Air) (%)
0 min	0
15 min	100
60 min	100
120min	100

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BACTERIAL BEHAVIOR TO THE EXPOSURE OF SHYCOCAN DEVICE

Introduction:

The bacterial activity experiment was performed to determine the proficiency of the equipment.

Bacterial culture was used as the foundation for this experiment because bacteria shows favorable growth characteristics, its ability to produce and secrete fine chemicals outside the cell, with current conditions and facilities provided by Scalene Energy Research Institute, the experiment was executed accordingly.

During this study the bacteria that was cultured,

Bacteria Used	Explanation
Non- Pathogenic *Bacillus spp.*	Biological and Growth Characteristics, morphology and other biochemical conditions of the bacterial culture can be distinguished.

TABLE 1: Study of Bacterial culture

Materials: Culture Media.

- Peptone-5.0g (TLK CHEMIKA, CAS No.91079-40-2 Batch 002)
- Beef Extract-3.0g (LOBA Chemie, LOT #L17055A1601)
- Sodium Chloride-5.0g (NICE)
- Agar-15.0g (BACTO, 43004 K05), pH-7.0.
- Simmon Citrate Agar(SCA) is containing the following chemicals, Ammonium Di-hydrogen Phosphate-1 g, Di-potassium Hydrogen Phosphate - 1 g, Di-potassium Hydrogen Phosphate-1 g, Sodium Chloride-5g, Sodium Citrate-2g, Magnesium Sulphate-0.2g, Agar-15g, Bromothymol Blue (pH indicator)- 0.08g.
- Urea Broth is containing the following chemicals, Urea-20 g, Di-sodium Hydrogen Phosphate- 9.5 g, Potassium Di-hydrogen Phosphate- 9 g, Yeast Extract- 0.1 g, Phenol Red-0.01 g.
- Nitrate Broth is containing the following chemicals, Potassium Nitrate-1 g, Peptone-5 g, Beef Extract- 3g
- Motility Soft Agar Medium is containing the following chemicals, Beef Extract- 3 g, Peptone-10 g, Sodium Chloride-5 g, Agar-15 g, Triphenyl Tetrazolium Chloride 1 %- 5 ml or 0.025 g.
- Starch agar media is containing the following chemicals, Starch – 20g, Beef Extract-3g, Peptone-5g, Agar-15g

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Other reagents:

- 70% Ethanol
- Distilled water
- RAPID Biochemical Identification Test Kit [KB001 HiIMViC]

Equipment and Instruments:

- Laminar air flow chamber
- Autoclave
- Hot Air Oven
- Incubator
- Microscope
- Colony counter

Biological Material:

Non- Pathogenic Bacillus spp. was used as the biological material in this experiment.

Sample

The examined device was SHYCOCAN described as a photon mediated electron emitter and device for environment and surrounding surface and arial containment from corona family of virus.

Methodology:

The experiment was carried out as per the requirements from Scalene Energy Research Institute, Laboratory, Bangalore, Karnataka, India 560049.

The objective of the implemented methodology was to quantify the effect of exposure of the device on Bacterial culture.

Day 1:

1ml of the bacterial strain was inoculated into the petri dishes and nutrient agar medium was poured into these plates and was allowed to solidify under aseptic condition, later the plates were incubated for 24 hours at 37°C.

Day 2:

After 24 hours of incubation period the colonies were examined for morphological characterization that was stained by gram staining method and which showed gram positive rod shaped structure, as the colonies from this plate was confirmed positive, the strain was then subjected to continuous streaking on a solidified nutrient agar plate which was placed inside the incubator and incubated for 24 hours at 37°C.

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Day 3:

A tube of strain labelled as *Bacillus* continuous streak test (BCST) from day 2 plate was inoculated in a 5ml nutrient broth tube and incubated for 24 hours at 37°C, for reviewing the biochemical characterization for the following two methods which was executed, one being the biochemical test which was conducted manually and another was biochemical identification kit which contains 12 small wells.

Day 4:

Upon completion of 24 hours incubation period, the kit was opened under aseptic conditions and about 50µl of broth culture was pipetted from day 3 broth culture tubes and inoculated into each well of the kit. This kit was incubated for 24 hours at 37°C.

Day 5:

1) Biochemical Identification Kit:

The kit was removed from the incubator that was placed inside a sterilized laminar air flow chamber and opened carefully. Later 2 drops of Kovac's reagent was added to the first well, which showed a reddish pink colour within 10 seconds and indicated positive result for indole test where as the reagent remains pale coloured if the test was negative. In this similar manner 2 drops of methyl red reagent was added to the second well which showed colour change from yellow to red and this test indicated positive for MR test, in the same way 2 drops of Baritt reagent A and 2 drops of Baritt reagent B was added to the third well, there was a gradual colour change after 5-10minutes which showed pinkish red that indicated positive for VP test. In the fourth well that shows citrate test can be performed in manual method as well. The remaining wells that is from the 5th well until 12th well shows the test results for carbohydrates which can be observed in (Fig 2). Which showed the control kit as comparison for the test kit.

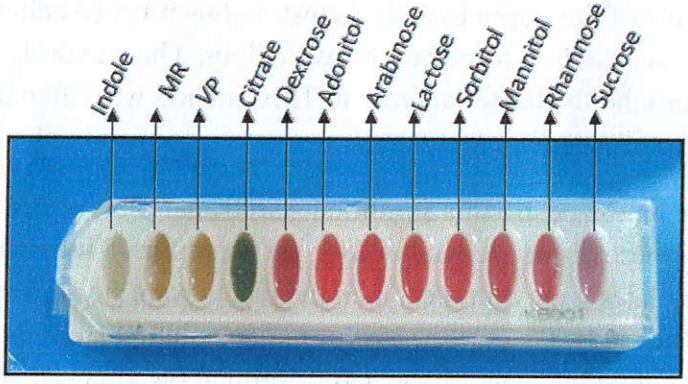

Figure 1: Description of biochemical identification kit

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2) Biochemical Test Performed Manually:

- **Urease Test:**

The urease test is used to differentiate organisms based on their ability to hydrolyse urea with the presence of urease enzyme. The phenol red indicator detects the production of ammonia by breaking down urea. The culture from the broth was then inoculated into sterile tube containing 5ml of urea broth media and one uninoculated urea broth tube was used as a control. Then the inoculated and uninoculated tubes were then incubated at 37°C for 24 hours, after the incubation process the tubes were analyzed that showed the phenol red indicator changes colour to deep pink due to alkaline nature of the medium that indicates positive test for urease, in case the colour remains yellow it implies absences of urease and hence it is negative which can be observed in (Figure 3).

- **Nitrate Test:**

Nitrate reductase enzyme was utilized to detect the ability of an organism to reduce Nitrate(NO^-_3) to Nitrite (NO^-_2)or other nitrogenous compounds, such as molecular nitrogen(N2). The broth cultures were inoculated into sterile tube containing 5ml of nitrate broth and one uninoculated nitrate broth tube was used as a control, both the inoculated as well as uninoculated tubes were incubated at 37°C for 24 hours, after the incubation process the tubes were removed in which 3 drops of solution A and solution B was added. A red colour appearance in the tubes were observed that indicates the reduction of the nitrate which showed the test was positive. In case there was no colour change the culture showed no reduction in the nitrate and that the test indicated negative which can be visualized in (Figure 4).

- **Starch Hydrolysis:**

This test is used to identify bacteria that can hydrolyse starch using the enzymes a-amylase and oligo-1,6-glucosidase. In order to use starch as a carbon source, bacteria must secrete a-amylase and oligo-1,6- glucosidase into the extracellular space. These enzymes break the starch molecules into smaller glucose subunits which can then enter directly into glycolytic pathway. In order to interpret the results of the starch hydrolysis test, iodine must be added to the agar plate then iodine reacts with the starch to form dark brown colour. Thus, hydrolysis of the starch will create a clear zone around the bacterial growth. This method was often used to differentiate species from the genera *Clostridium* and *Bacillus*.

A single continuous streak was made on the centre of a solidified starch agar plate with a sterilized loop containing the culture, these plates were allowed to incubate for 48 hours at 37°C. After the incubation period the plates were removed, then the plates were flooded with iodine solution with a dropper for 30 seconds. Discard the excess iodine solution from the plate into a beaker and continue to examine the plate for clear zone around the streaked area, as mentioned above if a clear zone appearances the test is positive and absences of the clear zone indicates negative for starch hydrolysis that can be observed in (Figure 5).

- **Citrate Test:**

Simmons citrate agar (SCA) consists of carbon and ammonium phosphate salt as nitrogen sources, to determine the ability of the organism to ferment citrate as a carbon source, the organism should produce an enzyme called citrate which shows the initial pH as 6.9 and upon addition of bromothymol blue reagent which was used as a pH indicator showed pH change of 7.6 with the help of citrate enzyme. The bacteria has the potential to convert ammonium phosphate to ammonia and ammonium hydroxide which are alkaline end products; that raised the pH levels causing bromothymol turn into blue colour.

Sterilized SCA slants were prepared in boiling tubes and the strains were inoculated on the SCA slant tubes in a single continuous streak manner on one slant tube where as the other slant tube of SCA was left uninoculated which was used as control. These slant tubes were incubated at 37°C for 24-48 hours. Later the tubes were examined for the growth and change of colour in the tubes after incubation period. Presence of growth and a change of colour from green to blue represents a positive test for citrate and absence of colour change from blue to green indicates citrate negative which can be viewed in (Figure 6 and in Table 2).

- **Motility Test:**

This test was performed to mainly determine the movement of the bacterial growth. The strains were stab inoculated with a sterilized loop into the soft agar tube and uninoculated soft agar tubes were utilized as a control and was incubated at 37°C for 24 hours; the tube that showed the presence of growth around the stabbed inoculation and the area away from the stabbed inoculation indicates positive, where as the growth that was observed along the stabbed inoculation showed negative for motility test which was indicated in (Figure 7).

- **Catalase Test:**

The test was executed to show the presence of catalase with an enzyme that catalysis the release of oxygen from hydrogen peroxide (H_2O_2), while most aerobic and facultative anaerobes utilize oxygen to produce hydrogen peroxide that was used differentiate aerotolerant strains of Clostridium and Bacillus species, which showed positive results. Now a small amount of colony was scrapped from the culture plate with the help of a platinum loop and applied on a clean sterilized glass side, a drop of 3% H_2O_2 was added on the applied colony that was present on the clean glass side. Presence of oxygen effervescence on the colony within 1minute indicated the test was positive on the other hand the absence of oxygen effervescence within 1 minute was examined to be negative for catalase test which could be visualized in (Figure 8).

- **Oxidase Test:**

To achieve the ability of microbes to produce Oxidase enzyme , a cytochrome oxidase catalyzes the oxidation of reduced cytochrome by molecular oxygen that resulted in the formation of water and hydrogen peroxide. A strip of Whatman's filter paper was placed inside a sterile laminar air flow chamber and to this strip a speck of culture was rubbed on it

by using a platinum loop, a drop of 1% of Dimethyl-p-phenylenediamine (DMPD) solution was added and allowed it stay on for 5-10 seconds, now the filter paper was examined where in the culture would show dark purple colour that would indicate the positive; absence of dark purple colouration could show negative test for oxidase test which was observed in (Table 2).

Result:

1) Biochemical Identification kit:

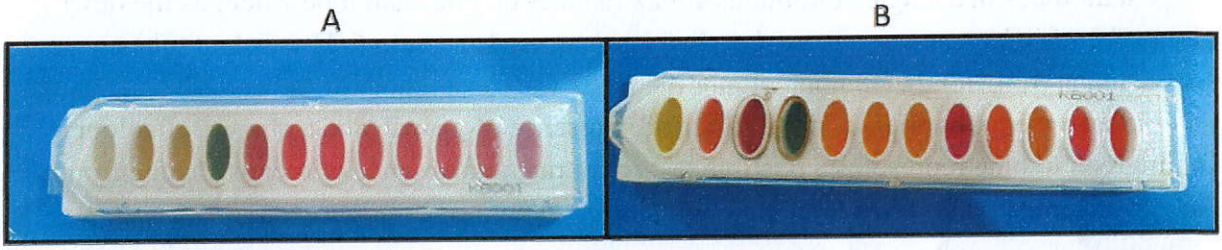

Figure 2: In this figure**(A)** is shown as Control kit where as **(B)** shows the test.

Biochemical Test	*Bacillus spp.*
Indole	Negative
MR test	Negative
VP test	Positive
Simmons's citrate test	Positive
Dextrose	Positive
Adonitol	Positive
Arabinose	Positive
Lactose	Negative
Sorbitol	Positive
Mannitol	Positive
Rhamnose	Positive
Sucrose	Positive
Urease	Negative
Nitrate	Positive
Motility	Positive
Catalase test	Positive
Oxidase test	Negative
Starch Hydrolysis	Positive

TABLE 2: Complete result for the biochemical Identification Test kit.

2) Biochemical Test that was performed manually:

Figure 3: In figure **(A)** it indicates the control tube and **(B)** indicates that the test tube was negative for urease test.

3) Nitrate Test:

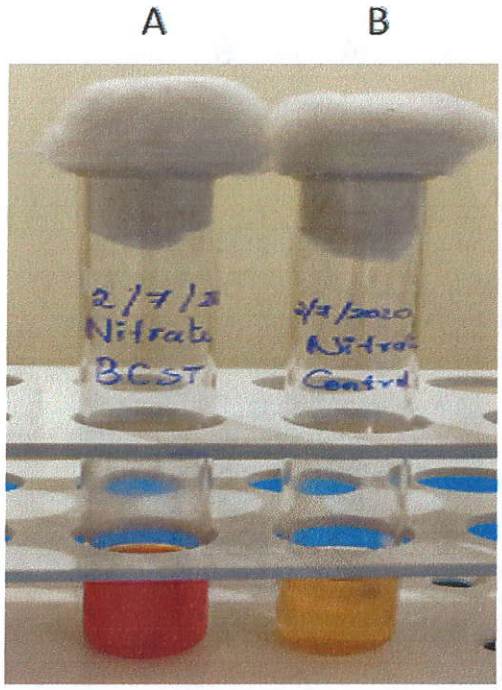

Figure 4: In figure **(A)** it indicates that the test tube showed colour change from pale yellow to red colour indicating positive result for nitrate test while **(B)** indicates the control tube as pale yellow.

4) Starch Hydrolysis Test:

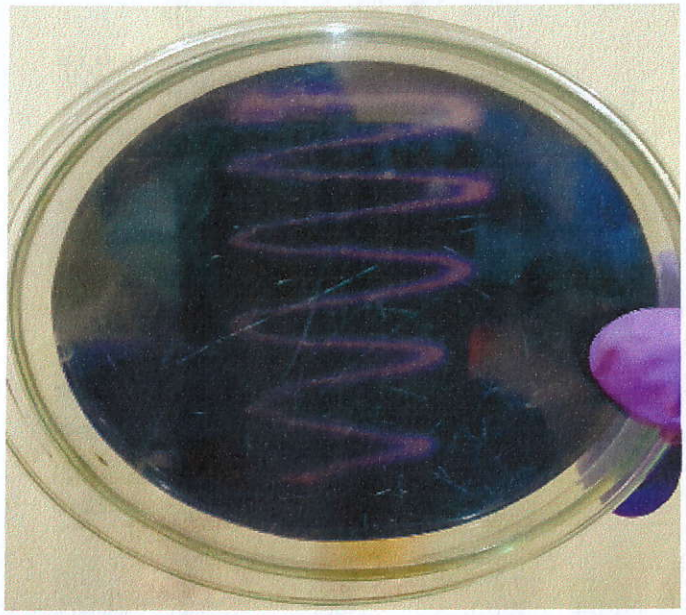

Figure 5: Appearance of clear zone around the culture that was grown showed positive for Starch Hydrolysis test.

5) Citrate Utilization Test:

A B

Figure 6: The figure **(A)** showed the control test tube that remained green where as in **(B)** the test tube showed the colour that changed gradually from green to blue at 48 hours of incubation showed positive test for citrate utilization.

6) Motility test:

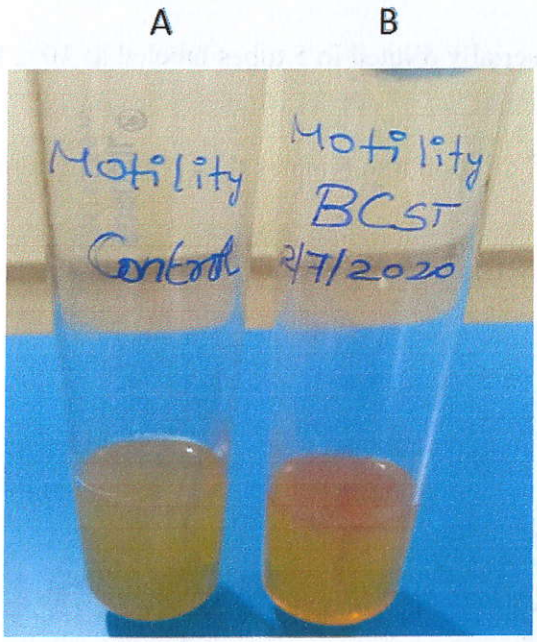

Figure 7: In figure **(A)** it shows the control tube and **(B)** showed the growth of the culture away from the centre stab and hence indicates that motility test was positive.

7) Catalase test:

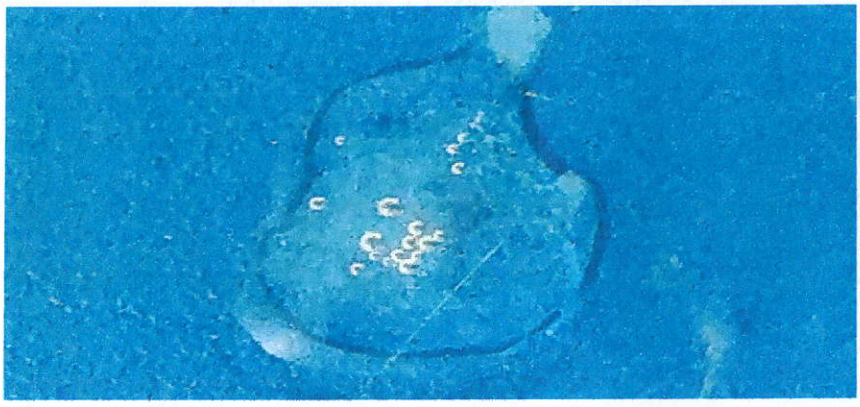

Figure 8: This figure indicates the presence of oxygen effervescences on the bacterial culture that was positive for catalase test.

Day 6:

1ml of the broth cultures were serially diluted in 5 tubes labeled as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}.

We transferred each 1ml of 10^{-5} dilution into 8 plates then poured nutrient agar and incubated for 24 hours at 37 ° C.

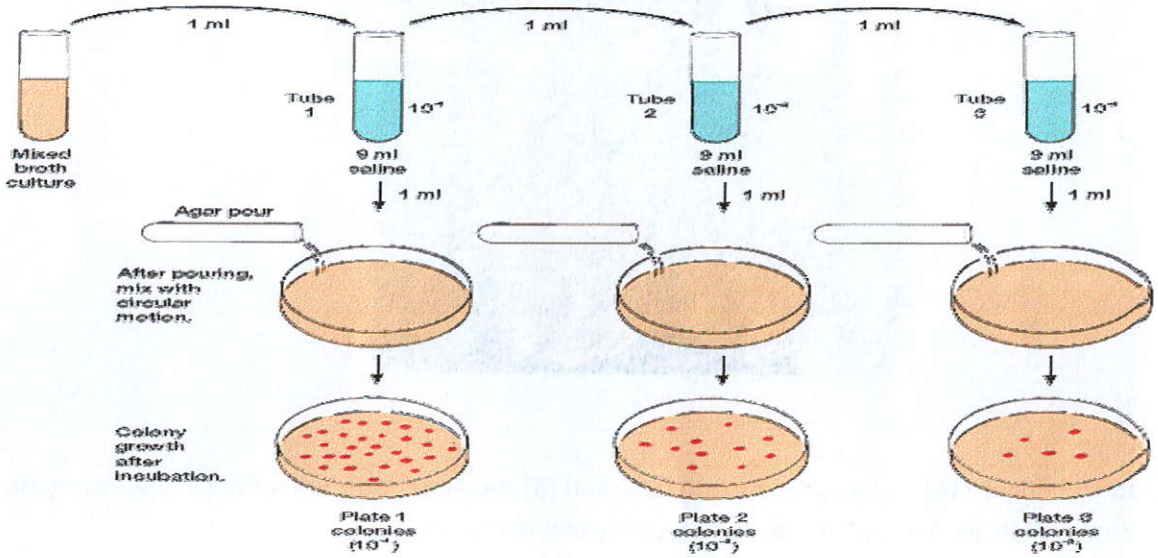

Figure 9: An image that describes the process of serial dilution.

Plate: A1 and A2 were exposed to the device at a distance of 50cm for 15 minutes.

Plate: B1 and B2 were placed in the controlled room for 15 minutes which was non-exposed to device.

Plate: C1 and C2 were exposed to the device at a distance of 280cm for 120 minutes.

Plate: D1 and D2 were placed in the controlled room for 120 minutes which was non-exposed to device.

After 15 minutes Plate A1, A2, B1 and B2 were incubated for 24-48 hours at 37 ° C.

After 120 minutes Plate C1, C2, D1 and D2 were incubated for 24-48 hours at 37 ° C.

Test Plates	Experiment
Plate A1 and A2	50 cm from the device for 15 minutes
Plate B1 and B2	15 minutes in controlled room
Plate C1 and C2	280 cm from the device for 120 minutes
Plate D1 and D2	120 minutes in controlled room

TABLE 3: Plates treated according to the experiments that was executed.

Day 7:

All 8 plates were observed after 24-48 hours of incubation period.

PLATES	EXPERIMENT	INCUBATION	NUMBER OF COLONIES COUNTED
Plate A	50 cm from the device for 15 minutes	48 hours	22
Plate B	15 minutes in controlled room	48 hours	11.5
Plate C	280 cm from the device for 120 minutes	48 hours	20.5
Plate D	120 minutes in controlled room	48 hours	18.5

TABLE 4: The estimated colonies counted from the plates at different time period.

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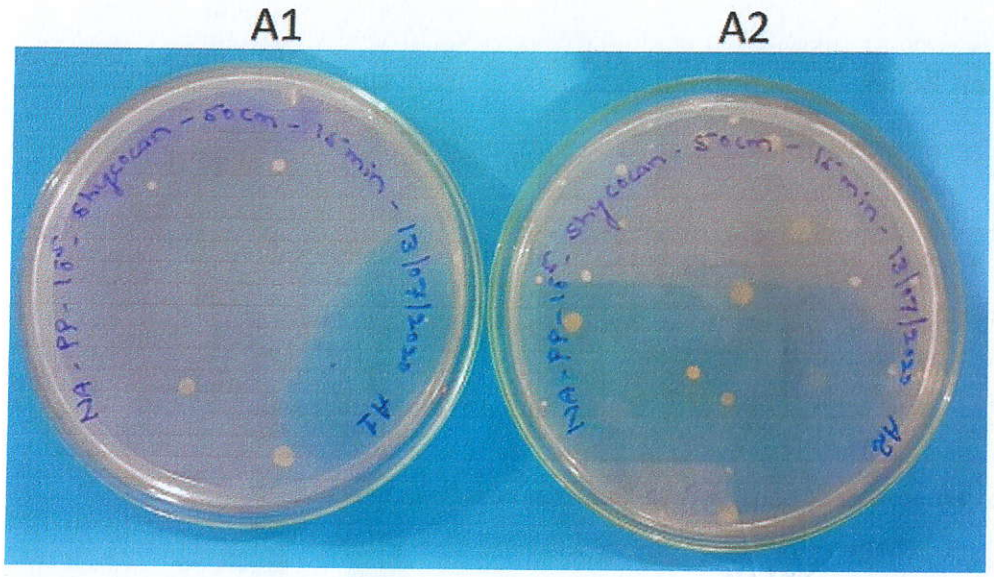

Figure 10: The image above shows 48hour test plates of Bacillus spp. that was placed open at 50cm from the device and exposed for 15 minutes **(A1)** and **(A2)** being its duplicate plate.

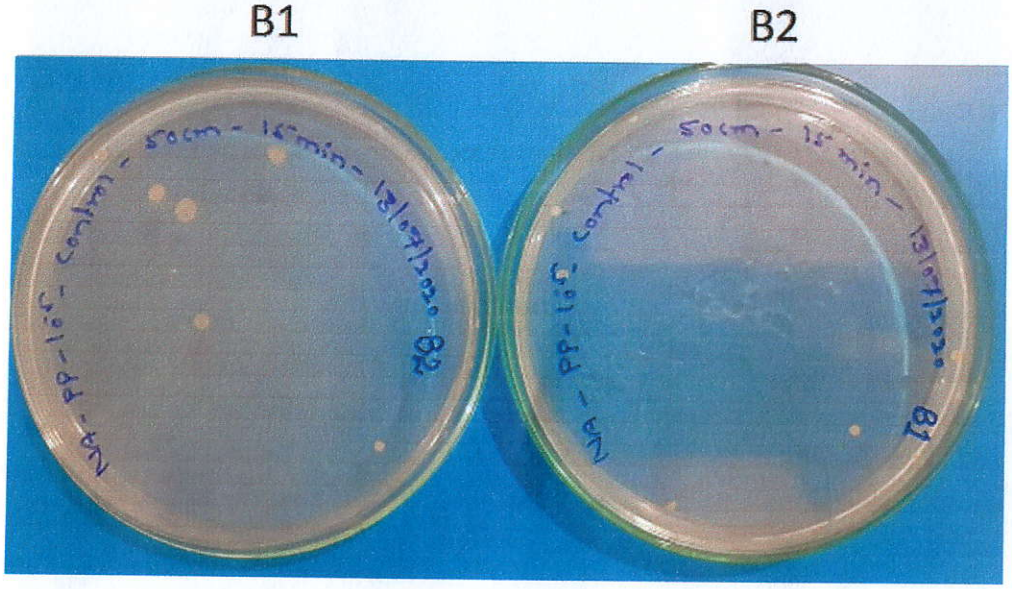

Figure 11: The image above indicated 48hour control plates of Bacillus spp. that was non exposed to the device and placed open at 50 cm inside a controlled room for 15 minutes **(B1)** and **(B2)** being its duplicate plate.

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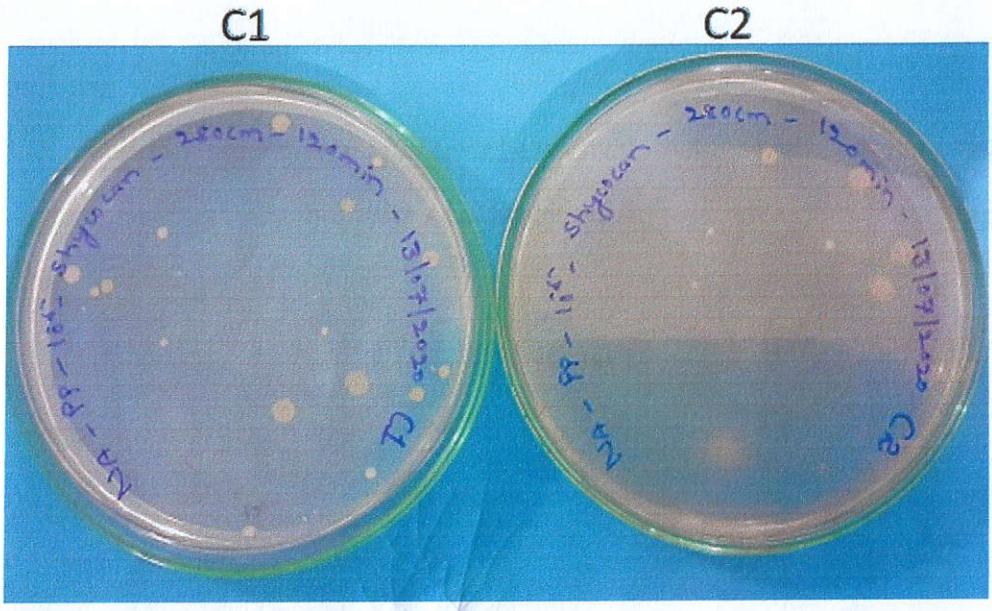

Figure 12: The image above shows 48hour test plates of Bacillus spp. that was placed open at 280cm from the device and exposed for 120 minutes **(C1)** and **(C2)** being its duplicate plate.

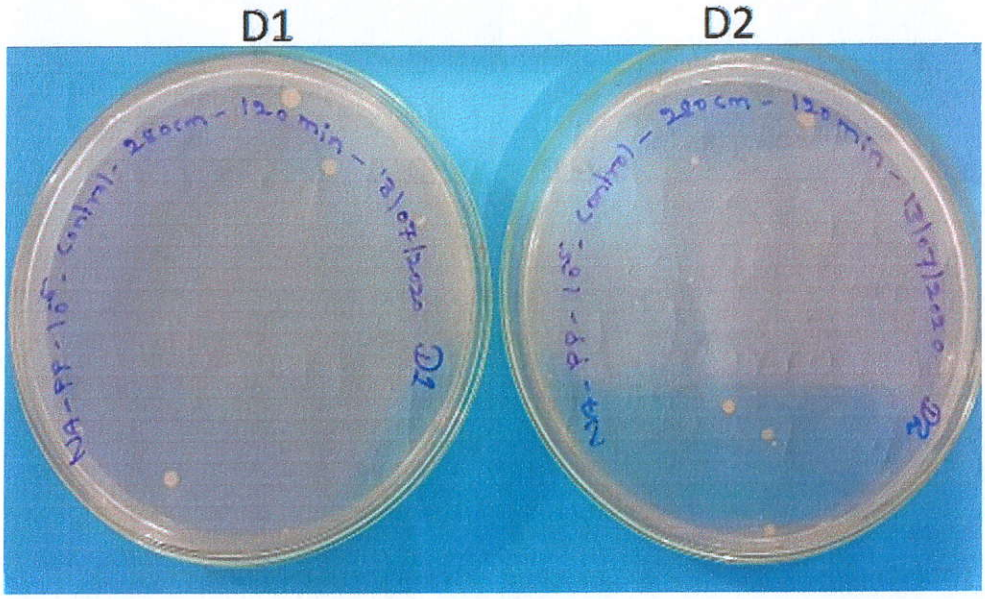

Figure 13: The image above indicated 48hour control plates of Bacillus spp. that was non exposed to the device and placed open at 280 cm inside a controlled room for 120 minutes **(B1)** and **(B2)** being its duplicate plates.

Calculation:

Microorganisms per milliliter/ gram of the sample = Number of colonies (Average of duplicates) / Amount plated x Dilution

Plate A $= \frac{A_1+A_2}{2}$

$= \frac{13+31}{2}$

$= \frac{22}{10^{-5}}$

$= 22 \times 10^5$ CFU/ml

Plate B $= \frac{B_1+B_2}{2}$

$= \frac{14+9}{2}$

$= \frac{11.5}{10^{-5}}$

$= 11.5 \times 10^5$ CFU/ml

Plate C $= \frac{C_1+C_2}{2}$

$= \frac{28+13}{2}$

$= \frac{20.5}{10^{-5}}$

$= 20.5 \times 10^5$ CFU/ml

Plate D $= \frac{D_1+D_2}{2}$

$= \frac{25+12}{2}$

$= \frac{18.5}{10^{-5}}$

$= 18.5 \times 10^5$ CFU/ml

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PLATES	EXPERIMENT	INCUBATION	Number of bacterial cells (CFU/ml)
Plate A	50 cm from the device for 15 minutes	48 hours	22×10^5
Plate B	15 minutes in controlled room	48 hours	11.5×10^5
Plate C	280 cm from the device for 120 minutes	48 hours	20.5×10^5
Plate D	120 minutes in controlled room	48 hours	18.5×10^5

TABLE 5: Number of bacterial cells per ml present in 10^{-5} dilution.

Conclusion:

During the analysis it was determined that the device had no effect on the bacterial activity and showed 22×10^5 CFU/ml and 20.5×10^5 CFU/ml that was positioned at a distance and times that was estimated (50cm from the device for 15 minutes and 280cm from the device for 120minutes).

A comparison was made by placing the control plates in a similar condition but not exposed to SHYCOCAN in which 11.5×10^5 CFU/ml and 18.5×10^5 CFU/ml was shown according to the distance and times that was calculated (50cm at15 minutes and 280cm for 120minutes).

The interpretation of the result is that within the exposure times tested, the device (SHYCOCAN) does not interfere with the mechanism of the bacteria during this test. It is concluded that "SHYCOCAN" does not have effects on the bacteria that is found in the environment, on the other hand it is seen to promote healthier growth in these organism.

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2) Dubey, R.C., and Maheshwari, D.K. (2012). Practical Microbiology (II Ed.) S. Chand & Co. P Ltd, New
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Report No: 521

Dated : 31-07-2020

EFFECT OF SHYCOCAN DEVICE EXPOSURE ON FUNGAL SPORES

Introduction:

The Fungal test was performed to determine the competency of the Equipment.

The base that was utilized for this test was fungus that produced spores, as we looked into the fungus category and choose *Saccharomyces cerevisiae.*as it was a suitable organism for executing this experiment.

Saccharomyces cerevisiae a model organism and most valuable aspects for all basic research, as it possesses nuclear genome of 12068 kilobases that are organized in 16 chromosomes. *S. cerevisiae* was selected for this test because of its ability to reproduce spores in large numbers apart from its benefits.

During this study the fungus that was cultured,

Fungus used	Explanation
Saccharomyces cerevisiae	*S. cerevisiae* have the capability to multiply within 1-2hours at 32°C, they are sporulating fungus as well.

Table 1: Study of the Fungus culture.

Materials: Culture Media.

- Yeast Extract Peptone Dextrose Broth (YPD) that contains the following chemicals:
- Yeast Extract - 10g,
- Peptone – 20g,
- Dextrose – 20g.

Other reagents:

- Crystal violet,
- 0.4% Trypan Blue stain
- Phosphate buffer saline (PBS)
- 70% Ethanol
- Distilled water

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EQUIPMENT AND INSTRUMENTS:

- Laminar air flow chamber
- Autoclave
- Hot Air Oven
- Weigh Balance
- Incubator
- Microscope
- Haemocytometer and cover slips.

BIOLOGICAL MATERIAL:

Saccharomyces cerevisiae was used as the biological material in this experiment.

Sample

The examined device was SHYCOCAN described as a photon mediated electron emitter device for environment and surrounding surface and arial containment from corona family virus.

Methodology:

The experiment was carried out as per the requirements from Scalene Energy Research Institute, Laboratory, Bangalore, Karnataka, India 560049.

The objective of the implemented methodology was to quantify the containment of the device.

a) Preparation of a uniform cell suspension:

Each 0.5ml of this strain was inoculated into sterile 8 tubes containing 4.5ml of YPD broth. The cells were resuspended in the tubes for 5-7 times by using a pipette to make a uniform cell suspension, before the experiment was executed a known concentration of cells/ml was measured. Then the resuspended cells were transferred to 8 plates labelled as below,

Plate: A1 and A2 were exposed to the device at a distance of 50cm for 15 minutes.

Plate: B1 and B2 were placed in the controlled room for 15 minutes which was non-exposed to device.

Plate: C1 and C2 were exposed to the device at a distance of 280cm for 120 minutes.

Plate: D1 and D2 were placed in the controlled room for 120 minutes which was non-exposed to device.

After 15 minutes Plate A1, A2, B1 and B2 were incubated for 2 hours and 24 hours at 32 ° C.

b) Preparation of 1:1 dilution of the cell suspension with trypan blue:

In a sterile vial, about 10µl of 0.4% trypan blue was pipetted, later 10µl of cell suspension was added and resuspended to form a uniform solution of cells along with trypan blue. This solution was incubated at room temperature for 2-3minutes.

c)Diluted suspension to be loaded into haemocytometer:

Firstly, the haemocytometer and coverslip was rinsed with sterile distilled water and then cleaned with 70% ethanol, then 10µl of the previously prepared solution (trypan blue+ cell suspension) was transferred to one side by touching the edge cover slip that was placed on the haemocytometer chamber with a pipette tip and allowing the suspension to be sucked in by capillary action, the cells can be counted using a microscope with a magnification of 400x.

Calculation:-

- % Cell Viability = [Total Viable cells (Unstained) / Total cells (Viable +Dead)] X 100.
- Viable Cells/ml = Average viable cell count per square x Dilution Factor x 10^4
- Average viable cell count per square = Total number of viable cells in 5 squares / 5.
- Dilution Factor = Total Volume (Volume of sample + Volume of diluting liquid) / Volume of sample.
- Total viable cells/Sample = Viable Cells/ml x the original volume of fluid from which the cell sample was removed.

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PLATES	EXPERIMENT	INCUBATION	NUMBER OF CELLS COUNTED					
			Live cells			Dead cells		
			Plate1	Plate2	Avg.	Plate1	Plate2	Avg.
Sample (Known concentration of cells)	Cell concentration Before experiment	Immediate	17	17	17	2	2	2
Plate A	50 cm from the device for 15 minutes	2 hours	22	22	22	2	2	2
Plate B	15 minutes in controlled room	2 hours	42	24	33	1	2	1.5
Plate C	280 cm from the device for 120 minutes	2 hours	43	37	40	5	1	3
Plate D	120 minutes in controlled room	2 hours	37	34	35.5	2	2	2

Table 2: Spores observed and counted in sample after 2 hours incubation

PLATES	EXPERIMENT	INCUBATION	NUMBER OF CELLS COUNTED					
			Live cells			Dead cells		
			Plate1	Plate2	Avg.	Plate1	Plate2	Avg.
Sample (Known concentration of cells)	Cell concentration Before experiment	Immediate	17	17	17	2	2	2
Plate A	50 cm from the device for 15 minutes	24 hours	269	235	252	0	1	0.5
Plate B	15 minutes in controlled room	24 hours	210	241	225.5	0	1	0.5
Plate C	280 cm from the device for 120 minutes	24 hours	387	288	337.5	1	2	1.5
Plate D	120 minutes in controlled room	24 hours	100	184	142	0	2	1

Table 3: Spores observed and counted in sample after 24 hours incubation.

Results:

10 microliters of cell suspension taken in 10 microliters of 0.4% trypan blue solution (1:1).

Dilution Factor = Total Volume (Volume of sample + Volume of diluting liquid) / Volume of sample.

Dilution Factor = 10+10 / 10 = 20/ 10 = 2.

Hence the dilution factor for all the below sample is 2.

Known concentration of cells/ml sample (Before experiment):

Average viable cell count per square = Total number of viable cells in 5 squares / 5.

= 6+1+3+1+6 / 5 = 3.4

Viable Cells/ml = Average viable cell count per square x Dilution Factor x 10^4

= 3.4 x 2 x 10^4 = 6.8 x 10^4 cells/ml

Average dead cell count per square = Total number of dead cells in 5 squares / 5.

= 1+1/ 5 = 0.4

Dead cells/ml = Average dead cell count per square x Dilution Factor x 10^4

= 0.4 x 2 x 10^4 = 0.8 x 10^4 cells/ml

% Cell Viability = [Total Viable cells (Unstained) / Total cells (Viable +Dead)] X 100.

= 17/19 x 100 = 89.47%

% Dead Cell = 2/19 x 100 = 10.53%

Total viable cells/Sample = Viable Cells/ml x the original volume of fluid from which the cell sample was removed.

= 6.8 x 10^4 x 5 = 34 x 10^4

In this manner the remaining plates A1,A2,B1,B2,C1,C2 and D1,D2 were calculated by using the same formula as provided above.

Calculation	*Sample	Plate A1	Plate A2	Plate B1	Plate B2	Plate C1	Plate C2	Plate D1	Plate D2
Average viable cell count per square	6+1+3+1 +6 /5 = 3.4	3+2+1+8 +8/5 =4.4	2+2+3+1 0+5/5 =4.4	5+15+15 +4+3/5 =8.4	2+8+6+4 +4/5 =4.8	9+7+5+1 0+12/5 =8.6	6+8+4+5 +14/5 =7.4	10+8+4+ 12+3/5 =7.4	4+9+6+1 +14/5 =6.8
Viable Cells/ml	3.4 x 2 x 10^4 = 6.8 x 10^4 cells/ml	4.4 x 2 x 10^4 = 8.8 x 10^4 cells/ml	4.4 x 2 x 10^4 = 8.8 x 10^4 cells/ml	8.4 x 2 x 10^4 = 16.8 x 10^4 cells/ml	4.8 x 2 x 10^4 = 9.6 x 10^4 cells/ml	8.6 x 2 x 10^4 = 17.2 x 10^4 cells/ml	7.4 x 2 x 10^4 = 14.8 x 10^4 cells/ml	7.4 x 2 x 10^4 = 14.8 x 10^4 cells/ml	6.8 x 2 x 10^4 = 13.6 x 10^4 cells/ml
Average dead cell count per square	1+1/ 5 = 0.4	1+1/ 5 = 0.4	1+1/ 5 = 0.4	1/ 5 = 0.2	1+1/ 5 = 0.4	1+2+2/ 5 = 1	1/ 5 = 0.2	2+1/ 5 = 0.6	1+1/ 5 = 0.4
Dead cells/ml	0.4 x 2 x 10^4 = 0.8 x 10^4 cells/ml	0.4 x 2 x 10^4 = 0.8 x 10^4 cells/ml	0.4 x 2 x 10^4 = 0.8 x 10^4 cells/ml	0.2 x 2 x 10^4 = 0.4 x 10^4 cells/ml	0.4 x 2 x 10^4 = 0.8 x 10^4 cells/ml	1 x 2 x 10^4 = 2 x 10^4 cells/ml	0.2 x 2 x 10^4 = 0.4 x 10^4 cells/ml	0.6 x 2 x 10^4 = 1.2 x 10^4 cells/ml	0.4 x 2 x 10^4 = 0.8 x 10^4 cells/ml
% Cell Viability	17/19 x 100 =89.47%	22/24 x 100 =91.67%	22/24 x 100 =91.67%	42/43 x 100 =97.67%	24/26 x 100 =92.31%	43/48 x 100 =89.58%	37/38 x 100 =97.37%	37/40 x 100 =92.5%	34/36 x 100 =94.44%
% Dead Cell	2/19 x 100 = 0.53%	2/22 x 100 = 8.33%	2/22 x 100 = 8.33%	1/43 x 100 = 2.33%	2/26 x 100 = 7.69%	5/48 x 100 = 10.42%	1/38 x 100 = 2.63%	3/40 x 100 = 7.5%	2/36 x 100 = 5.56%
Total viable cells/Sample	6.8 x 10^4 x 5 = 34 x 10^4	8.8 x 10^4 x 5 = 44 x 10^4	8.8 x 10^4 x 5 = 44 x 10^4	16.8 x 10^4 x 5 = 84 x 10^4	9.6 x 10^4 x 5 = 48 x 10^4	17.2 x 10^4 x 5 = 86 x 10^4	14.8 x 10^4 x 5 = 74 x 10^4	14.8 x 10^4 x 5 = 74 x 10^4	13.6 x 10^4 x 5 = 68 x 10^4

Table 4: Results for 2 hours incubation:

*Sample : Known concentration of cells taken for the experiments

Calculation	Sample	Plate A1	Plate A2	Plate B1	Plate B2	Plate C1	Plate C2	Plate D1	Plate D2
Average viable cell count per square	6+1+3+1 +6 /5 = 3.4	42+60+59 +53+55/5 =269/5 =53.8	37+50+28 +52+68/5 =235/5 =47	22+41+4 4+51+52 /5 =210/5 =42	47+51+6 0+38+45 /5 =241/5 =48.2	87+81+8 2+85+52 /5 =387/5 =77.4	62+61+6 0+53+52 /5 =288/5 =57.6	15+18+2 1+24+22 /5 =100/5 =20	21+54+4 8+26+35 /5 =184/5 =36.8
Viable Cells/ml	3.4 x 2 x 10^4 = 6.8 x 10^4 cells/ml	53.8 x 2 x 10^4 = 107.6 x 10^4 cells/ml	47 x 2 x 10^4 = 94 x 10^4 cells/ml	42 x 2 x 10^4 = 84 x 10^4 cells/ml	48.2 x 2 x 10^4 = 96.4 x 10^4 cells/ml	77.4 x 2 x 10^4 = 154.8 x 10^4 cells/ml	57.6 x 2 x 10^4 = 115.2 x 10^4 cells/ml	20 x 2 x 10^4 =40 x 10^4 cells/ml	36.8 x 2 x 10^4 = 73.6 x 10^4 cells/ml
Average dead cell count per square	1+1/ 5 = 0.4	0/5 =0	1/ 5 = 0.2	0/ 5 = 0	1/ 5 = 0.2	1/ 5 = 0.2	2/ 5 = 0.4	2/ 5 = 0.4	0/ 5 = 0
Dead cells/ml	0.4 x 2 x 10^4 = 0.8 x 10^4 cells/ml	0 x 2 x 10^4 =0 x 10^4 cells/ml	0.2 x 2 x 10^4 = 0.4 x 10^4 cells/ml	0 x 2 x 10^4 = 0 x 10^4 cells/ml	0.2 x 2 x 10^4 = 0.4 x 10^4 cells/ml	0.2 x 2 x 10^4 = 0.4 x 10^4 cells/ml	0.4 x 2 x 10^4 = 0.8 x 10^4 cells/ml	0.4x 2 x 10^4 = 0.8 x 10^4 cells/ml	0 x 2 x 10^4 = 0 x 10^4 cells/ml
% Cell Viability	17/19 x 100 = 89.47%	269/269 x 100 =100%	235/236 x 100 =99.57%	210/210 x 100 =100%	241/242 x 100 =99.59%	387/388 x 100 =99.74%	288/290 x 100 =99.31%	100/102 x 100 =98.04%	184/184 x 100 =100%
% Dead Cell	2/19 x 100 = 10.53%	0/269 x 100 =0%	1/236 x 100 = 0.43%	0/210 x 100 = 0%	1/242 x 100= 0.41%	1/388 x 100 = 0.26%	2/290 x 100 = 0.69%	2/102 x 100 = 1.96%	0/184 x 100 = 0%
Total viable cells/Sample	6.8 x 10^4 x 5 = 34 x 10^4	107.6x 10^4 x 5 = 538 x 10^4	47 x 10^4 x 5 = 235 x 10^4	42 x 10^4 x 5 = 210 x 10^4	48.2 x 10^4 x 5 = 241 x 10^4	154.8 x 10^4 x 5 = 774 x 10^4	57.6 x 10^4 x 5 = 288 x 10^4	40 x 10^4 x 5 = 200 x 10^4	73.6 x 10^4 x 5 = 368 x 10^4

Table 5: Results for 24 hours incubation

*Sample : Known concentration of cells taken for the experiments

Average Calculation	Sample (Before treatment)	Plate A (A1+A2) Device (50cm 15minutes)	Plate B (B1+B2) Control (15 minutes)	Plate C (C1+C2) Device(280cm 120 minutes)	Plate D (D1+D2) Control (120 minutes)
Total viable cells/ 5ml Sample	34×10^4	44×10^4	66×10^4	80×10^4	71×10^4
Cells/5ml	3,40,000	4,40,000	6,60,000	8,00,000	7,10,000
% Cell Viability	89.47%	91.67%	94.99%	93.48%	93.47%
% Dead Cell	10.53%	8.33%	5.01%	6.52%	6.53%

Table 6: Final Result based on average (duplicate) for 2 hour incubation:

Average Calculation	Sample (Before treatment)	Plate A (A1+A2) Device (50cm 15minutes)	Plate B (B1+B2) Control (15 minutes)	Plate C (C1+C2) Device(280cm 120 minutes)	Plate D (D1+D2) Control (120 minutes)
Total viable cells/ 5ml Sample	34×10^4	386.5×10^4	225.5×10^4	531×10^4	284×10^4
Cells/5ml	3,40,000	38,65,000	22,55,000	53,10,000	28,40,000
% Cell Viability	89.47%	99.78%	99.79%	99.52%	99.02%
% Dead Cell	10.53%	0.22%	0.21%	0.48%	0.98%

Table 7: Final Result based on average (duplicate) for 24 hour incubation

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Conclusion:

During the analysis it was determined that the device (Shycocan) had no effect on the fungal activity and showed 91.67% and 93.48% live spores corresponding to the distance and times that was evaluated (50cm from the device for 15 minutes and incubated for 2hours and 280cm from the device for 120minutes and incubated for 2hours).

In a similar manner 99.78% and 99.52% live spores corresponding to the distance and time for which it was evaluated (50cm from the device for 15 minutes and incubated for 24hours and 280cm from the device and incubated for 120minutes for 24hours).

A comparison was made by placing the control plates in a similar condition that was not exposed to SHYCOCAN. In which 94.99% and 93.47% similar to the distance and times as the exposed spores was calculated (50cm at15 minutes and incubated for 2 hours and 280cm at 120minutes and incubated for 2 hours). However under same condition 99.79% and 99.02% of spores was detected at the time and distance that was calculated (50cm at 15 minutes and incubated for 24 hours and 280cm at 120minutes and incubated for 24 hours respectively).

The interpretation of the result is that within the exposure times tested, the equipment does not interfere with the mechanism of the fungus after the exposure to the Shycocan device. Finally, this concluded that "SHYCOCAN" is proved to be not harmful or lethal to the fungus that is found in the environment.

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3) https://viable.amrita.edu/sub=3&brch=188&sim=336&cnt=2

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SCALENE ENERGY RESEARCH INSTITUTE * BANGALORE * R&D SCL

Report No: 520

Dated : 31-07-2020

Indian Institute of Technology Guwahati
Guwahati – 781 039, Assam, INDIA
Date: Wednesday, 25 November 2020
Project Reference No:CLE-EFL-SS014

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Date 8:12:2020

Report of Cell Toxicity Study of

Forbes Coronoguard powered by SHYCOCAN

Objective

Study the Cellular toxicity of Forbes Coronoguard in human alveolar basal epithelial cells.

Scope

- **Cell viability study at different time post treatment.**
- **Cytotoxicity study on human cell lines**

Testing methodology

The effect of Forbes Coronaguard powered by SHYCOCAN will be tested on the human alveolar basal epithelial cells (A549). The cells will be seeded into the 96-wells plates and exposed to the guard for 0-12 hours. The cells will be then added with MTT reagent and further incubated for 3 hrs at 37°C. Later, the MTT reagent will be removed from the wells and cell viability will be determined using plate reader at 570 nm.(Mosmann, 1983)

Performance Analysis

The effect of Forbes CoronaGuard powered by SHYCOCAN has been tested on the human alveolar basal epithelial cells (A549). The cells were seeded into the 96-wells plates and were incubated overnight for attachment. After that, cells are exposed to the Forbes Coronaguard for 0-12 hours. The cells were then added with MTT reagent and further incubated for 3 hours at 37°C. Later, the MTT reagent was removed from the wells and formazan crystals were dissolved with 100 µL of Di-methyl sulfoxide (DMSO). Cell viability has been determined using plate reader at 570 nm. Additionally, the microscopic images were also taken to validate the non-toxic nature of the guard and to look for any morphological changes due to the Forbes Coronaguard powered by SHYCOCAN.

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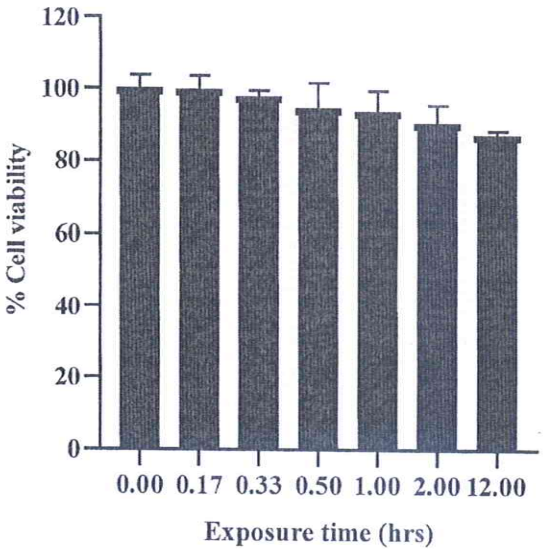

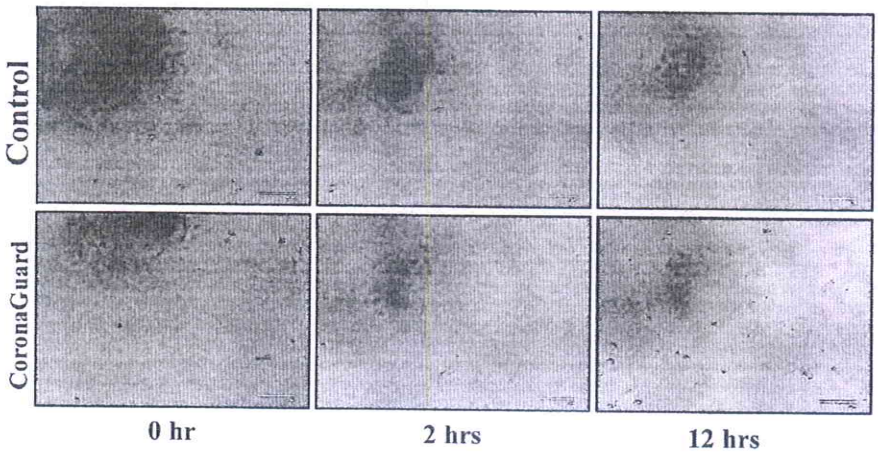

Conclusion

The MTT results indicated the non-toxic nature of Forbes Coronaguard powered by SHYCOCAN as the percentage cell viability was found to be almost same as the controlled group (Mosmann, 1983). Even after the prolonged exposure for 12 hrs, the cell viability was found to be more than 87% (Mondal, 2020). Substequently, the microscopic images showed no morphological differences between test and control groups. Conclusively, these results showed that the Forbes Coronaguard powered by SHYCOCAN is non-toxic to the human cells.

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10/12/2020

Report on evaluation of cell viability assay for Forbes Coronaguard powered by Shycocan

Objective:

- Cell viability study of Forbes Coronaguard powered by Shycocan in Vero cells (African green monkey kidney epithelial cells)

Scope:

- Cell viability study at different time post treatment of Forbes Coronaguard powered by Shycocan

Testing methodology:

- The viability of the Vero cells (African green monkey kidney epithelial cells) upon exposure to electrons was evaluated to scrutinize the impact of electron emission from Forbes Coronaguard on cell lines. Vero cells were exposed to emanated electron from Forbes coronaguard powered by Shycocan for different time period (1hr, 6hrs, 12hrs, 18hrs and 24hrs) and viability was analysed using cell counting kit-8 (CCK-8) (Sigma Aldrich, USA) following manufacturer's protocol.

Performance Analysis:

- The cell viability was calculated as percentage of viable cells and then plotted on a graph.

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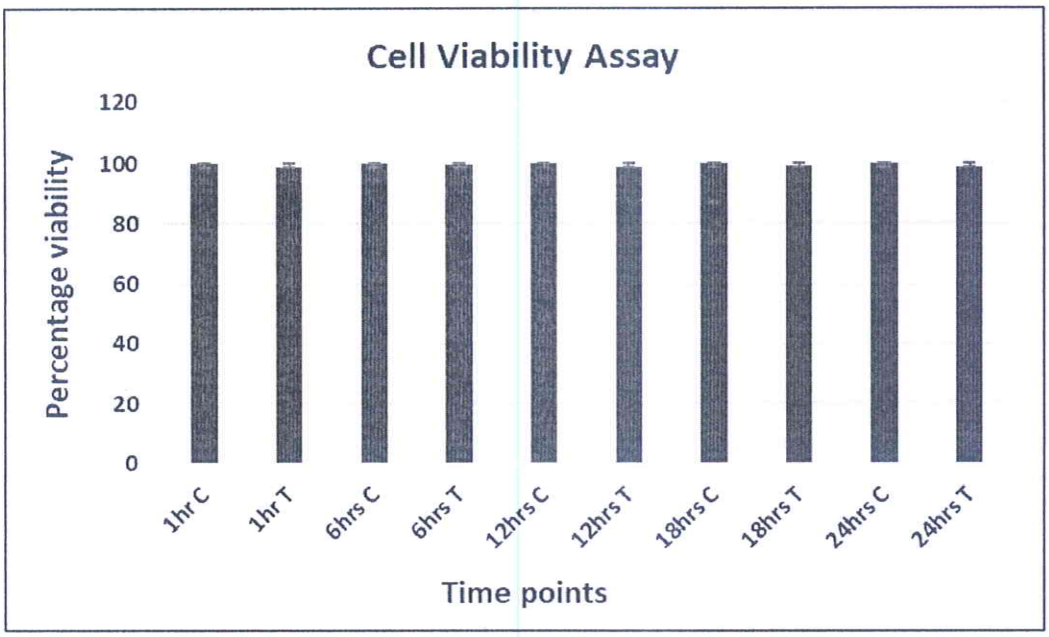

Fig 1. Viability of Vero cells upon exposure to electron emission from Forbes Coronaguard for different time points. 'X' axis denotes the Time points in hours. 'Y' axis denotes the viability of cells in percentage. C= control; T= treated

Conclusion:

- The obtained results showed no significant difference between the viability of control and exposed cells indicating that the emitted electrons from Forbes Coronaguard had no impact on the viability of cells upon exposure for all the time points. Categorically, these results showed that the Forbes Coronaguard powered by Shycocan is non-toxic to the Vero cells (African green monkey kidney epithelial cells).

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