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Scientific Evidence Of The Efficacy of Shycocan In Attenuation Of Viral Particles In The Air And Surface

SHYCOCAN

Synopsis

The viral activity studies conducted using a SHYCOCAN device was able to determine it's 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 require 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 the SARS-CoV-2 viral particle.

The studies done follow

VIROLOGY STUDIES

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Virology Study #1

STUDY AIM	The virucidal/ neutralizing of SARS-CoV-2, surrogate viral particles when exposed to the 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 a 3.435 reduction during a 120-minute exposure at 500cm from the equipment under test (EUT). The interpretation of this result is that within the exposure times tested, the SHYCOCAN eliminates more than 99.9% of the viral particles.

Virology Study #2

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 belonging to the Beta Influenza virus in the family 'Orthomyxoviridae' is known to affect humans. It has 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-minutes, 100 cm from the device for 45-minutes and 300 cm from the device for 45-minutes). As comparison, a symptomatic seasonal flu 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-minutes in a 6m 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 coronavirus, in a similar magnitude of the exhaled virus by a symptomatic influenza patient for 30 minutes.

Virology Study #3

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 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 of 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.

Virology Study #4

STUDY AIM	Fast Response (60-seconds, 5 minutes, 15 minutes) 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/PRTR/17822/20201 dated 22/10/2020
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 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 of 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 99.61% to 99.74% in a span of 15 minutes duration, sampled at 60-seconds, 5-minutes, 15-minutes elapsed times corresponding initial counts (without exposure to SHYCOCAN device) were also taken outside the chamber in which SHYCOCAN was installed. Relevant equipment used 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.

Virology Study #5

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 of 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.

Virology Study #6

STUDY AIM	To study the neutralizing and disinfection efficacy of the SHYCOCAN against the Avian Coronavirus in the air
Report Reference	CLE-EFL-SS11 dated 06-Nov-2020
Institution Name	Indian Institute Of Technology (IIT) – 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 Coronavirus in the air within the chamber exposed to the device between 15 minutes and 120 minutes. The viral inactivation was 100%.

BACTERIAL AND FUNGAL STUDIES

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Bacterial and Fungal Studies #1

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 the use of the species	The B. Subtilis is a naturally occurring useful bacterium useful 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 products. 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 the 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-minutes against corresponding controlled culture plates, after 48 hours 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.

Bacterial and Fungal Studies #2

STUDY AIM	To assess if exposure to the 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 centigrade of 1.5-2 hrs. The species is instrumental in wine-making, baking and brewing since ancient times. It is an environmentally useful fungi that sporulates.
Justification for the 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 it's ability to reproduce spores in large numbers apart from it's benefits in the 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-minutes, 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 hours. 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

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TOXICOLOGY STUDY #1

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 (IIT) – 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 atmosphere 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 SHYCOCAN device was tested on humans 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 the 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 the 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.

TOXICOLOGY STUDY #2

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 an African green monkey (Chlorocebus-sp)
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 it's 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 the 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 SHYCOCAN device on cell lines. Vero Cells were exposed to SHYCOCAN for different time periods (1hr, 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 the SHYCOCAN device had no impact on the viability of cells upon exposure for all time periods. These demonstrated clearly that the SHYCOCAN devices are non-toxic to the Vero Cells (African green monkey epithelial cells).

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