# In vitro virucidal properties of

# <u>Silversol<sup>®</sup> - a nano silver preparation on</u> <u>SARSCov2 virus</u>

Project ID: PRB-NanoS-Pf/20-02

Abstract

Silversol<sup>®</sup>, a nanoparticulate preparation of metallic silver, is has been known to have anti-bacterial properties. Silversol<sup>®</sup> also has been tested earlier for its ability to reduce the viral load *in vitro* studies. In the present study, Silversol<sup>®</sup> (100 ppm), a specifically prepared stable, aqueous nano particulate silver preparation was tested for its ability to inhibit the SARSCov2 replication in a cell culture system.

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### Report No: PRB- PRB-NanoS-Pf/20-2

## Project: In vitro virucidal properties of Silversol® on SARSCov2 virus

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### 1. SUMMARY

### Objective

The objective of this study is to determine the virucidal potential of Silversol<sup>®</sup> on SARSCov2 virus

### Methods

Silversol<sup>®</sup> (100ppm) solution was supplied by Viridis Biopharma Pvt. Ltd. Silversol<sup>®</sup> (100 ppm) was tested on SARSCov2 virus in vero cell culture system for its virucidal properties.

### **Results and Conclusions:**

The results indicate that at 50 ppm Silversol<sup>®</sup> reduced the viral load by more than 60% compared to the untreated viral samples.

#### STATEMENT OF COMPLIANCE

### Concerning Study No. PRB-NanoS-Pf/19-08

We, the undersigned hereby declare that the following report constitutes a true and faithful account of the methods and results of this study. The study that was conducted at CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India, performed essentially in accordance with the general laboratory standards.

Within reason there have been no circumstances that might have affected the quality and integrity of the study.

Submitted By: CSIR-CCMB Date: October, 2020

Dr. Bokara Kiran Kumar (Investigating Scientist)

### QUALITY ASSURANCE STATEMENT

In vitro virucidal properties of Silversol® on SARSCov2 virus

Study No.: PRB-NanoS-Pf/20-2

Date: September 2020

Inspections have been made of various phases of this study and, in the case of repetitive operations, at each stage to assure that the processes are acceptable till that stage.

The final report has been audited according to the appropriate Standard Operating Procedure and is considered to be an accurate presentation of the method and procedures employed and an accurate presentation of the findings.

Date: September, 2020 (Study Director)

# **STUDY REPORT**

# In vitro virucidal properties of Silversol<sup>®</sup> on SARSCov2 virus Trial ID: PRB-NanoS-Pf/20-02

**Sponsor:** 

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### **EXECUTIVE SUMMARY**

Silversol<sup>®</sup>, a formulation of ASAP silver nanoparticles embedded in water, has been demonstrated to have anti-bacterial properties on a variety of bacterial strains as well as antiviral properties. The anti-bacterial property is beneficially used in preparing a wide variety of formulations including dental creams, body lotions/creams, anti bacterial solutions for treatment of textiles, air-purifiers etc. (1-3). The mode of action of silver nanoparticles is primarily by extracting electrons from the pathogens and thereby causing disruption of the lipid membranes. Several other mechanisms of anti-bacterial action have also been proposed. Silversol<sup>®</sup> has also been shown to have anti-viral properties against many viruses including HIV-1, HSV-1, Hepatitis B, Influenza etc. (4, 5). In the present study Silversol<sup>®</sup> was tested for its virucidal properties on SARSCov2 virus cultured on vero cells. The data suggests that at 50 ppm of Silversol<sup>®</sup> was able to decrease the viral load by more than 60% compared to the untreated samples.

### **INTRODUCTION**

Silversol<sup>®</sup> consists of nanosilver particles (NSPs) generally 1 to 100 nm in size in at least one dimension. As the particle size decreases, the surface area-to-volume ratio of the nanoparticles increases dramatically, leading to significant changes in the physical, chemical, and biological properties. Silversol<sup>®</sup> has been shown to have a broad antibacterial effect on a range of Gram-negative and Gram-positive bacteria, antibiotic-resistant bacterial strains and several infectious viruses. Although the antimicrobial effect of Silversol<sup>®</sup> has been widely studied, the exact mechanism of action is still elusive. It is widely accepted that NSPs can anchor to and subsequently penetrate the bacterial cell wall and cell membrane or viral coat. Silversol<sup>®</sup> causes structural changes of the cell membrane, increasing cell permeability, and leading to cell death. It has also been found that NSPs can release silver ions that interact with the thiol groups of many vital enzymes and phosphorus-containing bases, thus inhibiting enzyme function, and preventing DNA replication and cell division.

### MATERIALS AND METHODS

### Materials

All the chemicals used in this study are of analytical grade or more. SARSCov2 virus used in the study was isolated from a patient and proliferated in vero (African green monkey kidney cells, ATCC #CCL81 from *Cercopithecus aethiops*). The isolated virus genome was sequenced at CCMB and found to belong to the clade A2a. Cell culture reagents were from Lonza and Invitrogen. Molecular biology reagents used in the study viz, RT-PCR kits (LabGun COVID-19 RT-PCR Kit) are were purchased from LabGenomics Co., Ltd. Silversol<sup>®</sup> (100 ppm ) solution was supplied by Viridis Biopharma Pvt. Ltd.

### Methods:

All Petri dishes, dilution tube racks, and host-containing apparatus were labeled with the following information: virus, host, test agent, and project number.

### **Enumeration of the virus:**

Virus stock was prepared by growing the virus on vero cells and by centrifuging the culture supernatant. The virus stock was enumerated by estimating the plaque forming units (PFU) in the serial dilutions of the virus stock. In 96 well plates, the serial dilutions of the viral stock were added to vero cells and the plates were incubated for 5-6 days. On observation of

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cytopathic effects by microscopic examination, the cells were fixed by para-formaldehyde. The fixed cells were stained with crystal violet (0.1%) and the PFUs were counted to arrive at the concentration of virus in the stock based on the factor of serial dilution.

### Establishing the Limits of Detection (LOD) of the assay method:

RT-PCR kit efficiency was established by performing the assay on the RNA isolated from the enumerated stock. Known concentration of the viral stock was processed for RNA extraction as per the RNA extraction kit provided by Qiagen. The extracted RNA was serially diluted to estimate the LOD of the PCR kit. The figure below shows the efficiency of the RT-PCR kit used in the study.



### **Suspension Time-Kill Test:**

A 25 cm<sup>2</sup> flask of Vero cells were grown in DMEM media containing 10% fetal bovine serum (FBS). A day before the experiment, the vero cells were trypsinized and the cell count was determined using a hemocytometer. Approximately 20,000 cells were added in each of the 96 well plates and incubated at  $37^{0}$ C in CO<sub>2</sub> incubator (5%).

The antiviral efficacy test for Silversol<sup>®</sup> was carried out using, Suspension Time-Kill Test for Virus. Pre-quantified virus, stored at -20<sup>0</sup>C were incubated according to the table given below. After incubating the viral particles with the Silversol<sup>®</sup> for 15 min, the mixture was transferred to the vero cells, after removing the medium. The density of vero cells at the time of infection was estimated to be 40,000 cells per well. Each treatment was done in triplicates. The

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Multiplicity of infection (MOI) used was 0.1. After incubating for 3-4 days, viral titre in the culture supernatant was determined using RT-qPCR as described earlier.

The concentrations of the Silversol<sup>®</sup> employed in this study are 100, 75, 50, 25, and 10 ppm

### **Test agent preparation:**

Parameters	Summary	Replicates
Cell culture	Medium alone	3/group
Virus control	1 part virus + 9 parts medium	3/dilution
Virucidal/disinfectant	1 part virus + 9 parts	3/dilution
Treatment	disinfectant	

- All the four combinations were incubated, followed by elution, plating and incubation with the Vero cells.
- Untreated cells were considered as control.

### **RESULTS:**

Viral titre estimated by RT-PCR obtained from the supernatants of the untreated wells was considered as 100%. The reduction in the virus, as estimated by RT-PCR, is plotted against the ppm of Silversol<sup>®</sup>. There is a graded decrease in the viral load with increase in the ppm concentration of Silversol<sup>®</sup> used in the treatment of the virus. 15 min treatment with 75 ppm showed a 83% reduction in the viral load; while the treatment with 50 ppm yielded 60% reduction in the viral count.



The virucidal property of Silversol<sup>®</sup> was retested in an independent experiment at 50 ppm in triplicates. Based on the log reduction of the viruses that were exposed to one million virus particles, at 50 ppm Silversol<sup>®</sup> brought about 87% decrease in viral load after 15 min exposure.

### Cell viability in the presence of silversol:

Cell viability of Silversol<sup>®</sup> on vero cells is investigated using MTT assay. MTT (3-(4,5-<u>dimethylthiazol</u>-2-yl)-2,5-di<u>phenyl</u>tetrazolium bromide) is reduced to its formzaon in the presence of viable cells. The xtent of reduction and associated color change is used to estimate the viability of cells. The data below demonstrates that at 50 ppm Silversol<sup>®</sup> could decrease the viability by 25%.

Partial decrease in the virucidal property at 100 ppm may be partly due to the reduction in viability of the cells in the presence of  $Silversol^{\$}$ .



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### **Conclusion**:

At 50 ppm Silversol<sup>®</sup> reduces the viral load more than 60% compared to untreated samples in a in vitro cell culture assay using vero cells. The cell viability of vero cells is partially reduced in the presence of Silversol<sup>®</sup>.

### References

- Zhang, X.-F.; Liu, Z.-G.; Shen, W.; Gurunathan, S. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. *Int. J. Mol. Sci.* 2016, *17*, 1534.
- Burduşel, A.-C.; Gherasim, O.; Grumezescu, A.M.; Mogoantă, L.; Ficai, A.; Andronescu, E. Biomedical Applications of Silver Nanoparticles: An Up-to-Date Overview. *Nanomaterials* 2018, *8*, 681.
- Morris, D.; Ansar, M.; Speshock, J.; Ivanciuc, T.; Qu, Y.; Casola, A.; Garofalo, R.P. Antiviral and Immunomodulatory Activity of Silver Nanoparticles in Experimental RSV Infection. *Viruses* 2019, *11*, 732.
- Salleh, A.; Naomi, R.; Utami, N.D.; Mohammad, A.W.; Mahmoudi, E.; Mustafa, N.; Fauzi, M.B. The Potential of Silver Nanoparticles for Antiviral and Antibacterial Applications: A Mechanism of Action. *Nanomaterials* 2020, *10*, 1566.
- Galdiero,S et.al. Silver nanoparticles as potential antiviral agents. Molecules (2011) 16, 8894-8918