

# Test Report

JOB REF NO. : 2022-03-15-019  
DATE RECEIVED : 15<sup>th</sup> March 2022  
DATE REPORTED : 01<sup>st</sup> April 2022  
PAGE : 1 of 9

**Test Report No. : CPSA/220484116-CA87921**  
**Company : Holista Biotech Sdn. Bhd.**  
**Unit 1201, 12th Floor, Amcorp Trade Centre PJ Tower,**  
**No. 18 Persiaran Barat, 46050 Petaling Jaya, Selangor, Malaysia.**

The following merchandise was (were) submitted and identified by the client as:

Sample Description : Super Bio Nano Silver (Silaeris AgNP)  
Sample Appearance : Opaque, brown solution  
Sample Receiving Date : 15<sup>th</sup> March 2022  
Storage Conditions : Room temperature  
Product Diluent : Distilled water  
Active substances : Nanosilver  
Testing Period : 15<sup>th</sup> March 2022 – 31<sup>st</sup> March 2022  
Test Requested : Determination of the Virucidal Activity  
Test Method : EN 14476:2013+A2:2019 (E)  
Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (phase 2, step 1)

**Test Result** : Please see the next page(s)

Tested by : The test was externally provided.

Remark : -

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LOW ZHEN HUI  
MULTI-BUSINESS LABORATORY MANAGER  
FOOD ANALYST NO. MJMM 0178

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## Test Report No. : CPSA/220484116-CA87921

### Experimental Conditions

Test Organism(s) : *Human coronavirus*, strain 229E, ATCC VR-740  
Concentration/ Contact Time : 150.00ppm / 30 and 60 minutes  
Loading : 0.30 g/L Bovine Albumin Solution  
Test Temperature : 20 ± 1°C  
Incubation Period : 5 days, 36 ± 1°C

### Test Method and Its Validation

Testing Method : Quantal test  
Inactivation Method : Immediate dilution  
Molecular sieving using MicroSpin™ S 400 HR (for formaldehyde only)

The results of validation test A, B, and C proved the viability of the method in all cases.

### Test Results

The results are stated in Tables A and B.

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### Conclusion

**Super Bio Nano Silver (Silaeris AgNP)** showed the required viral reduction of  $\geq 4.0 \log_{10}$  against test strain(s) *Human coronavirus* ATCC VR-740 in accordance with EN 14476:2013+A2:2019 (E) at 150.00 ppm concentration(s) after 30 and 60 minutes under the stated condition. According to the simple acceptance decision rule<sup>†</sup>, there is <50% risk of false acceptance.

### Note

Virucidal Activity : The capability of a product to produce a reduction in the number of viable viruses belonging to reference strains under defined conditions by at least 4 orders ( $10^4$ ).

$R = V_c/N_a$  = the reduction in viability, or  $\lg R = \lg V_c - \lg N_a$

<sup>†</sup>The decision rule applied is simple acceptance rule with no guard band and up to 50% risk of false acceptance or rejection. This rule has been determined by the laboratory and agreed with the client prior to testing.

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## Test Report No. : CPSA/220484116-CA87921

### Table A: Evaluation of the virucidal activity of Super Bio Nano Silver (Silaeris AgNP) on test strains according to EN 14476

Product : Super Bio Nano Silver (Silaeris AgNP)  
 Loading : 0.30 g/L Bovine Albumin Solution  
 Test Strain : *Human coronavirus* ATCC VR-740

Virus control, V <sub>c</sub>	Cytotoxicity effect, CE
V <sub>c1</sub> : 6.63 ± 0.25 V <sub>c2</sub> : 6.88 ± 0.37	CE <sub>1</sub> : 2.50 ± 0.00 CE <sub>2</sub> : 2.50 ± 0.00

Test concentration (ppm) / contact time (min)	First assay, N <sub>a1</sub>	Second assay, N <sub>a2</sub>	Average reduction
150.00 / 30	N <sub>a1</sub> : ≤2.50 ± 0.00 lg R <sub>1</sub> : ≥4.13 ± 0.25	N <sub>a2</sub> : ≤2.50 ± 0.00 lg R <sub>2</sub> : ≥4.38 ± 0.37	lg R: ≥4.26 ± 0.32
150.00 / 60	N <sub>a1</sub> : ≤2.50 ± 0.00 lg R <sub>1</sub> : ≥4.13 ± 0.25	N <sub>a2</sub> : ≤2.50 ± 0.00 lg R <sub>2</sub> : ≥4.38 ± 0.37	lg R: ≥4.26 ± 0.32

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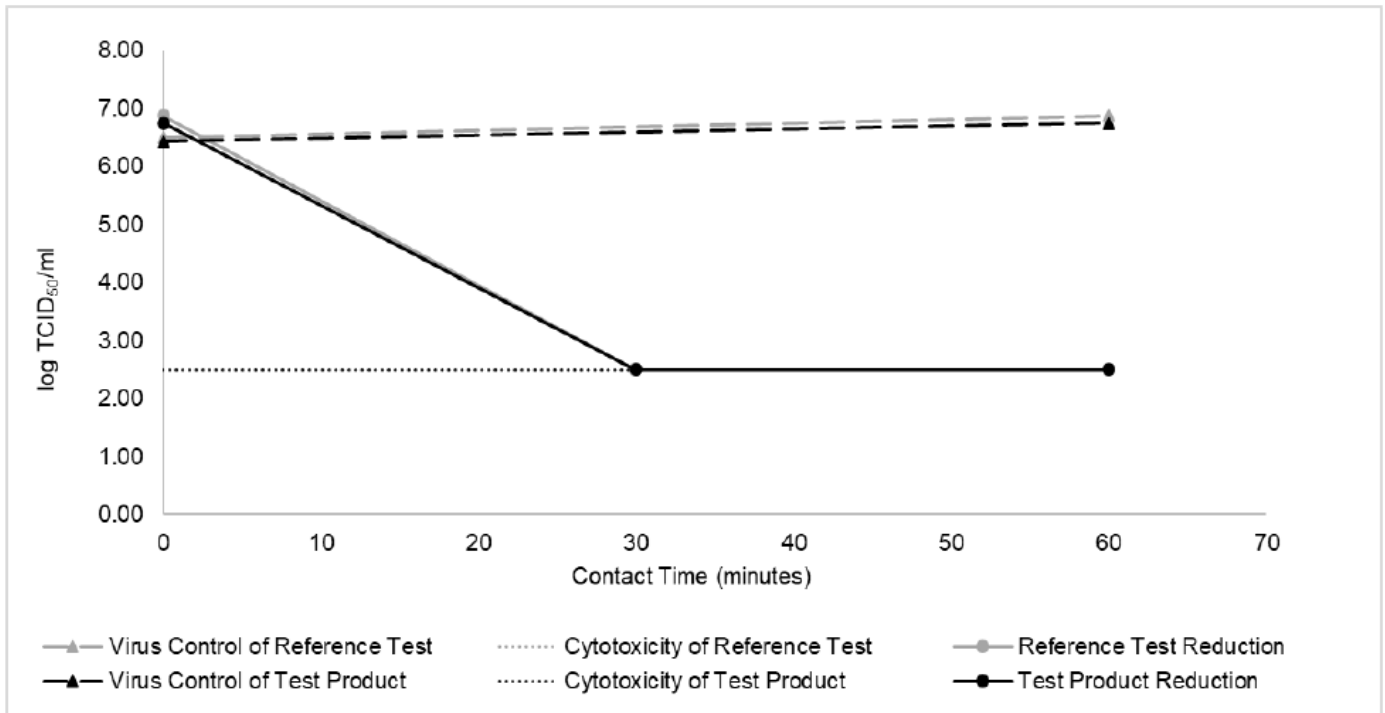
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## Test Report No. : CPSA/220484116-CA87921

**Table B: Control tests and method validation for Table A**

Test strain	Cell susceptibility control	Suppression efficiency control	Reference test for virus inactivation
<i>Human coronavirus ATCC VR-740</i>	A: 7.00 ± 0.38 A <sub>PBS</sub> : 6.75 ± 0.33	B: 7.00 ± 0.38 V <sub>C</sub> : 6.63 ± 0.25	C <sub>30</sub> : ≥4.38 ± 0.37 C <sub>60</sub> : ≥4.38 ± 0.37

### Note

- TCID<sub>50</sub> : The dilution of the virus suspension that induces a cytopathic effect (CPE) in 50% of cell culture units.  
 CPE : The morphological alteration of cells and/or their destruction caused by the cytopathic effect of virus multiplication.  
 V<sub>C</sub> : log<sub>10</sub> TCID<sub>50</sub> per mL in the viral test suspension at the beginning and at the maximum contact time.  
 N<sub>a</sub> : log<sub>10</sub> TCID<sub>50</sub> per mL in the test mixture at the end of the contact time.  
 CE : The morphological alteration of cells caused by the cytotoxicity effect of the product test solution.  
 A : log<sub>10</sub> TCID<sub>50</sub> per mL in the cell susceptibility control as compared to PBS.  
 B : log<sub>10</sub> TCID<sub>50</sub> per mL in the suppression efficiency control as compared to the virus control.  
 C : log<sub>10</sub> TCID<sub>50</sub> per mL in the reference test for virus inactivation after 30 and 60 minutes (5 and 15 minutes for vaccinia virus).

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### Table C: Summary of the log reductions of the quantitative suspension test according to EN 14476

Test strain	Test concentration (ppm) / contact time (min)	Log reduction (TCID <sub>50</sub> /mL)	Associated risk <sup>†</sup>
<i>Human coronavirus</i> ATCC VR-740	150.00/ 30	≥ 4.26 ± 0.32	<50% risk of false acceptance
	150.00/ 60	≥ 4.26 ± 0.32	<50% risk of false acceptance

<sup>†</sup>The decision rule applied is simple acceptance rule with no guard band and up to 50% risk of false acceptance or rejection. This rule has been determined by the laboratory and agreed with the client prior to testing.

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## Test Report No. : CPSA/220484116-CA87921

**Efficacy of Super Bio Nano Silver (Silaeris AgNP) against *Human coronavirus*, strain 229E, ATCC VR-740 in a quantitative suspension test at 20°C according to EN 14476:2013+A2:2019 (E) under clean condition**

### Expert Opinion\*

This expert opinion is based on the test report CPSA/220484116-CA87921 dated 01<sup>st</sup> April 2022.

The virucidal activity of the disinfectant Super Bio Nano Silver (Silaeris AgNP) of Holista Biotech Sdn Bhd against *Human coronavirus* ATCC VR-740 was investigated by a quantitative suspension test according to EN 14476:2013+A2:2019 (E) under clean condition (0.30 g/L Bovine Albumin Solution).

According to this suspension test, a disinfectant or a disinfectant solution at a particular concentration is considered as having virucidal activity if the virus titre is reduced by  $\geq 4 \log_{10}$  (inactivation  $\geq 99.99\%$ ) within the recommended exposure period.

Super Bio Nano Silver (Silaeris AgNP) was examined at 20°C at the concentration(s) of 150.00ppm for the exposure time(s) of 30 and 60 minutes. After the exposure time(s), the viral reduction exceeded 4  $\log_{10}$ -steps in all assays. According to the simple acceptance decision rule<sup>†</sup>, there is <50% risk of false acceptance. Therefore, a virucidal activity against *Human coronavirus* ATCC VR-740 was measured as follows:

Clean condition	150.00 ppm	30 minutes
Clean condition	150.00 ppm	60 minutes

\*Opinions and interpretations expressed here are outside the scope of SAMM (Laboratory Accreditation Scheme of Malaysia) accreditation.

†The decision rule applied is simple acceptance rule with no guard band and up to 50% risk of false acceptance or rejection. This rule has been determined by the laboratory and agreed with the client prior to testing.

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### Test Part Description

Sample Description : Super Bio Nano Silver (Silaeris AgNP)



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\*\*\*\*End of Test Report\*\*\*\*

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