Chemlab CHEMLAB
Teknologi Kimia Chemical Technology 化学工艺

CHEMICAL LABORATORY (MALAYSIA) SDN BHD (27822-K)
81-85 (2nd & 3rd Floor), Jalan SS25/2, Taman Bukit Emas, 47301 Petaling Jaya, Selangor Darul Ehsan, Malaysia.
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ASSAYERS, ANALYTICAL CHEMISTS, MICROBIOLOGISTS AND INDUSTRIAL CONSULTANTS

CERTIFICATE OF ANALYSIS

Test Method
EN 14476:2013+A2:2019
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)

Client
Health West Pty Ltd
153 Hicks Street,
Mundijong 6123 WA Australia

Testing Laboratory
Chemical Laboratory (M) Sdn Bhd
81-85(2nd & 3rd Floor), Jalan SS25/2,
Taman Bukit Emas
47301 Petaling Jaya, Selangor
Malaysia

IDENTIFICATION OF TEST ITEM

Test item name:	Silver Oxygen Complex Oxygen > 500PPM Silver Ions> 16PPM
Lab ID:	PJ-D/FD/1365/22
Batch no.:	Not Specified
Expiry date:	16 August 2026
Manufacturer:	Health West Pty Ltd
Receipt date:	26th April 2022
Storage conditions:	Room temperature away from sunlight
Product diluent recommended by manufacturer:	Not specified
Active substances:	Silver oxygen complex
Product appearance:	Clear, colourless solution

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TEST METHOD & VALIDATION

Test method:	EN 14476:2013+A2:2019 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)
Titration method:	Quantal tests (TCID50 method)
Inactivation method:	Molecular sieving (microspin)

EXPERIMENTAL CONDITIONS

Date of test:	26th April 2022- 15th June 2022
Product diluent:	Distilled water
Concentration / contact time:	100%* / 5 minutes ± 10 seconds
Test temperature:	(30 ± 2) °C
Interfering substance:	Clean condition (0.3 g/L bovine serum albumin)
Test organism / passage no.:	Human coronavirus (HCoV-229E), strain 229E, ATCC VR-740 / P16
Cell line / passage no.:	MRC-5 ATCC CCL-171 / P11
Growth medium:	DMEM supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin
Incubation temperature:	(36 ± 1) °C, 5% CO2
Incubation period:	2 to 5 days
Appearance of the solution:	Clear, colourless liquid dilutions
Stability and appearance of product dilutions during test:	Homogenous without any precipitate

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TEST PROCEDURE

1. Test Procedure *Na*: Determination of Virucidal Concentrations

1.1 100 µL of interfering substance was pipetted into a tube. 100 µL of virus test suspension was addedto the tube and mixed.

1.2 800 µL of the product test solution was added to tube. The tube was mixed and the stopwatch wasstarted at once. The tube was placed in a water bath controlled at the chosen test temperature θ for the contact time t.

1.3 Immediately at the end of t, the tube was mixed and the activity of the product test solution was inactivated or removed using one of the following methods:

1.3.1 Dilution in ice-cold maintenance medium. 50 µL of the mixture was transferred into 450 µL ice-cold maintenance medium and put in an ice bath, or if the cytotoxicity of the product test solution is too high,

1.3.2 Ultrafiltration using MicroSpin™ S-400 HR columns. 100 µL of the mixture is transferred to the column and the residual virus was obtained as per manufacturer's instructions.

1.4 Within 30 minutes of product inactivation, a series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium.

1.5 The virus titre for *Na* was determined using quantal test (endpoint titration). 100 µL of each dilutionwas transferred into 8 wells of a microtitre plate containing a confluent (>90%) cell monolayer without any medium. 100 µL of maintenance medium was added to the last row of wells to serve as the cell control.

1.6 After 1 hour incubation at 37 °C, 100 µL of maintenance medium was added to each well.

1.7 The cells were incubated for the appropriate incubation period until cytopathic effect (CPE; morphological alteration of cells and/or their destruction as a consequence of virus multiplication) was observed. The results were recorded as '0' for no CPE, or '1' to '4' for approximately 25%, 50%, 75%, and 100% CPE, respectively.

1.8 The virus titre was calculated using the Spearman-Kärber method and expressed as lg TCID50/mL,i.e., the 50% infecting dose of a virus suspension that induces a CPE in 50% of cell culture units.

2. Virus Control *N*

2.1 The virus control *N* was performed in parallel to the test *Na* at two contact times: at 0 minute and the longest contact time used in the test *Na*. The product test solution was substituted with hard water (distilled water for ready-to-use products).

2.2 The inactivation method chosen must be the same as the one chosen in *Na*. A series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium.

2.3 The virus titre for *N* was determined using quantal test according to Sections 1.5 to 1.8.

3. Cytotoxicity Effect *CE*: Verification for Possible Morphological Alteration of Cells by the Test Product

3.1 100 µL of hard water (distilled water for ready-to-use products) and 100 µL of interfering substance were mixed with 800 µL of the product test solution.

3.2 The product test solution was inactivated or removed using the same method as the one chosen in *Na*.

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TEST PROCEDURE

3.3 A series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenancemedium.

3.4 The cytotoxicity of the product test solution was determined using quantal test according toSections 1.5 to 1.8.

3.5 The results were recorded as 't' for cytotoxicity, i.e., the morphological alteration of cells and/or their destruction or their reduced sensitivity to virus multiplication caused by the product.

4. Interference Control *A*: Verification that the Susceptibility of the Cells for the Virus Infection is Not Influenced Negatively by the Treatment with the Test Product

4.1 To check the reduction of the sensitivity of the cells to virus, comparative virus titrations were performed in cells that have or have not been treated with product test solution.

4.2 For the test *AT*, 100 µL of the lowest apparently non-cytotoxic dilution (determined from the cytotoxicity effect test *CE*) of the product test solution and 100 µL of maintenance medium were transferred into each 8 wells of a microtitre plate containing a confluent (>90%) cell monolayer without any medium.

4.3 In parallel, the negative interference control *APBS* was performed using PBS instead of the product test solution.

4.4 After 1 hour incubation at 37 °C, the supernatant was discarded. A series of ten-fold dilutions of the virus test suspension was prepared in maintenance medium. 100 µL of each dilution was titrated to each well. The virus titre for *AT* and *APBS* was determined using quantal test according to Sections 1.5 to 1.8.

5. Suppression Control *B*: Validation of the Inactivation Method

5.1 100 µL of interfering substance was pipetted into a tube. 100 µL of maintenance medium was added to the tube and mixed.

5.2 800 µL of the product test solution of the highest concentration used in the test *Na* was added totube and mixed.

5.3 The activity of the product test solution was inactivated or removed using the same method employed for the test *Na* using one of the following methods:

5.3.1 Dilution in ice-cold maintenance medium. 50 µL of the mixture was transferred into 400 µL ice-cold maintenance medium. 50 µL of the virus test suspension was added to the mixture.The tube was mixed and put in an ice bath for 30 minutes ± 10 seconds, or if the cytotoxicityof the product test solution is too high,

5.3.2 Ultrafiltration using MicroSpin™ S-400 HR columns. 100 µL of the mixture is transferred to the column and the eluate was obtained as per manufacturer's instructions. 50 µL of the eluate was transferred into 400 µL ice-cold maintenance medium. 50 µL of the virus test suspension was added to the mixture. The tube was mixed and put in an ice bath for 30 minutes ± 10 seconds.

5.4 At the end of the 30 minutes incubation, a series of ten-fold dilutions of the inactivated mixture *BT* was prepared in ice-cold maintenance medium.

5.5 The virus titre for *BT* was determined using quantal test according to Sections 1.5 to 1.8.

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TEST PROCEDURE

5.6 200 µL of the virus test suspension and 800 µL of PBS were mixed with 1 mL of 1.4% (w/v)formaldehyde.

5.7 The tube was mixed and the stopwatch was started at once. The tube was placed in a water bathcontrolled at the chosen test temperature θ for the contact time t.

5.8 Immediately at the end of t, the tube was mixed and the activity of the product test solution was inactivated or removed using one of the following methods:

5.8.1 Dilution in ice-cold maintenance medium. 20 µL of the mixture was transferred into 180 µL ice-cold maintenance medium and put in an ice bath, or if the cytotoxicity of the formaldehyde is too high,

5.8.2 Ultrafiltration using MicroSpin™ S-400 HR columns. 100 µL of the mixture is transferred to the column and the residual virus was obtained as per manufacturer's instructions.

5.9 Within 30 minutes of product inactivation, a series of ten-fold dilutions of the inactivated mixturewas prepared in ice-cold maintenance medium.

5.10 The virus titre for C was determined using quantal test according to Sections 1.5 to 1.8.

5.11 The cytotoxicity effect for formaldehyde was determined according to Section 3, using the same inactivation method chosen for the reference test C

CONTROLS AND VALIDATION

Test Organism	Cytotoxicity Effect	Interference Control	Suppression Control	Reference Test
HCoV-229E ATCC VR-740	N: 6.50 ± 0.00 CE: 2.50 ± 0.00	APBS: 7.00 ± 0.38 AT: 6.75 ± 0.33	BN: 6.50 ± 0.00 BT: 6.13 ± 0.37	C5: ≥2.00 ± 0.00 C15: ≥2.00 ± 0.00

The control and validation tests A, B, and C were within the basic limits:

- The difference between the virus control N and the cytotoxicity effect CE must be ≥4.00 to verify that the cytotoxicity of the product does not affect cell morphology and growth or susceptibility for the test organism which are necessary to demonstrate a 4-log reduction of the virus
- The difference APBS and AT must be <1.00 to verify that the susceptibility of the cells for the virus infection is not influenced negatively by the treatment with the product test solution,
- The difference between BN and BT must be ≤0.50 to validate the inactivation method, and
- The reduction of the virus in the reference test after 5 and 15 minutes, C5 and C15. No passing criteria were given for coronavirus

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TEST RESULTS

For each product concentration and contact time, the log reduction (lg R) is calculated using the formula lg R =N– Na, in which:

- N is the lg TCID50 per mL of the virus control at the end of the contact time, and
- Na is the lg TCID50 per mL of the test mixture at the end of the contact time.

Test organism: Human coronavirus (HCoV-229E) ATCC VR-740

Virus control, N	N1: 6.50 ± 0.00 N2: 6.50 ± 0.00
Cytotoxicity effect, CE	CE1: 2.50 ± 0.00 CE2: 2.50 ± 0.00

Concentration / Contact Time	Test, Na	Reduction, lg R = N – Na	Average Reduction,lg R
100%* / 5 minutes	Na1: ≤2.50 ± 0.00 Na2: ≤2.50 ± 0.00	lg R1: ≥4.00 ± 0.00 lg R2: ≥4.00 ± 0.00	lg R: ≥4.00 ± 0.00 %R: ≥99.990 %

CONCLUSION

The test item achieved a reduction of ≥4.00 log against the test organism Human coronavirus 229E (HCoV┐ 229E) ATCC VR-740 under the tested conditions. Therefore, test sample has demonstrated a virucidal activity against human coronavirus according to EN 14476:2013+A2:2019 under the following conditions:

Concentration	Contact Time	Test Temperature	Soiling
100%*	5 minute	30 °C	Clean condition

Everlyn Shanthi A/P Pushparaja
B.Sc.(Hons)
Senior Microbiologist
MJMM0993

THE ABOVE ANALYSIS IS BASED SOLELY ON THE SAMPLES SUBMITTED BY THE CUSTOMER