INDEPENDENT CHEMLAB TESTS RESULTS USING HYDROCELL ON CORONOVIRUS

HydroCell vs Coronavirus 2022

Independent lab tests using HydroCell on Human Coronavirus have shown a four-log reduction (99.99% effectiveness) within 5 minutes of exposure to the virus. (See page 6). These tests scientifically verify HydroCell's excellent virucidal activity against the Human coronavirus.

This is a significant development and relief for millions using HydroCell to prevent this dreadful virus. The fact that HealthWest HydroCell is scientifically lab-tested provides a high confidence level in its reliability.

Historically, silver has been the best antimicrobial for thousands of years, even before the introduction of antibiotics in 1940.

https://pubmed.ncbi.nlm.nih.gov/25418435/

In 2012, Rice University confirmed that silver ions were the antimicrobial agent and that the nanoparticles were practically benign in the presence of microbes. https://news2.rice.edu/2012/07/11/ions-not-particles-make-silver-toxic-to-bacteria-3/

In June 2022, the Chemlab independent test against the coronavirus proves HealthWest HydroCell effective against coronavirus: The Oxygen in HydroCell dramatically multiply the antimicrobial effect, offering a safer alternative to vaccines, which can have side effects.

HydroCell uses nature's two most powerful healing agents, Oxygen and Silver Ions, in a synergistic complex. Over two decades, no known side effects have been reported against HydroCell, a safe and effective health solution you can trust.

Moreover, HydroCell's versatility empowers you to use it in various ways. You can gargle it as a mouthwash to maintain good oral health, and it is safe to swallow.

HydroCell is declared Hypo-Allogenic and suitable for all skin types and children over five. The purity of HydroCell is guaranteed to have no particles or nanoparticles and is safe to drink regularly for optimum health.

Pour a few drops of HydroCell into the nebuliser to freshen the room and spray in fridges to eliminate odour caused by rotting food and bacteria. Spray into air

conditioners in rooms or cars to eradicate germs and experience the freshness of germ-free air in the room and vehicles.

Spray or soak some HydroCell into fungi-infected toes a few times daily or other body parts to eliminate BO. Spray on chronic wounds and experience speedy healing using HydroCell. Experienced users called HydroCell "Magic Water".

HydroCell has been the 'Best family choice' for many years, providing quick and visible results. This is great value for family with a variety of health problems. HydroCell is practical, environmentally friendly, and suitable for pet use.

Keep a bottle in your pantry; you may never know when you will need it.

To good health and change the way we look at diseases.

HealthWest Australia





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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)

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

Health West Pty Ltd Manufacturer:

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

- Test Procedure Na: Determination of Virucidal Concentrations
 - 100 µL of interfering substance was pipetted into a tube. 100 µL of virus test suspension was added to the tube and mixed.
 - 1.2 800 µL of the product test solution was added to tube. The tube was mixed and the stopwatch was started at once. The tube was placed in a water bath controlled at the chosen test temperature θ for the contact time t.
 - 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:
 - 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 MicroSpinTM S-400 HR columns. 100 μ L 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.
 - 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 1.7 '1' to '4' for approximately 25%, 50%, 75%, and 100% CPE, respectively.
 - The virus titre was calculated using the Spearman-Kärber method and expressed as lg TCID50/mL,i.e., the 50% infecting dose of 1.8 a virus suspension that induces a CPE in 50% of cell culture units.
- 2 Virus Control N
 - 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 2.1 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.
 - 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
 - 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

- 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 to Sections 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.
- Interference Control A: Verification that the Susceptibility of the Cells for the Virus Infection is Not Influenced Negatively by 4. the Treatment with the Test Product
 - 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.
 - For the test AT, 100 µL of the lowest apparently non-cytotoxic dilution (determined from the cytotoxicity effect test CE) of the 4.2 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.
 - 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.
- 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 Nausing 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 cytotoxicity of 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 μ L of the eluate was transferred into 400 μ L ice-cold maintenance medium. 50 μ L 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.
 - At the end of the 30 minutes incubation, a series of ten-fold dilutions of the inactivated mixture BT 5.4 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	N: 6.50 ± 0.00 CE:	APBS: 7.00 ± 0.38	BN: 6.50 ± 0.00 BT:	C5: ≥2.00 ± 0.00
ATCC VR-740	2.50 ± 0.00	AT: 6.75 ± 0.33	6.13 ± 0.37	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 \geq 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,	CE1: 2.50 ± 0.00
CE	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	lg R1: ≥4.00 ± 0.00 lg	lg R: ≥4.00 ± 0.00
	Na2: ≤2.50 ± 0.00	R2: ≥4.00 ± 0.00	%R: ≥99.990 %

CONCLUSION

The test item achieved a reduction of \geq 4.00 log against the test organism Human coronavirus 229E (HCoV $_1$ 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 Shanini A/P Pushparaja B.Sc.(Hons) Senior Microbiologist M.IMM0993

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