

## Test Report: BS 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)

### Test Laboratory

### BluTest Laboratories Ltd

5 Robroyston Oval, Nova Business Park, Glasgow, G33 1AP

### Identification of sample

Name of the product  
Batch number  
Client  
Client Address

Alcohol Free Sanitiser  
F2620  
Healthguard Hygiene LTD  
Briary Barn, First Floor Pury Hill Business Park, Towcester NN12 7LS

Project Code  
Date of Delivery  
Storage conditions  
Active substances  
Appearance  
Condition upon receipt

BT-HGH-02  
28 August 2020  
Ambient  
Didecyldimethyl Ammonium Chloride, Benzalkonium Chloride  
Liquid  
Undamaged

### Test Method and its validation

Method

1 part interfering substance + 1 part virus suspension + 8 parts biocide were mixed and incubated at the indicated contact temperature for the indicated contact times. Assays were validated by a cytotoxicity control, interference control, neutralisation control and a formaldehyde internal standard.

Neutralisation

Dilution-neutralisation/gel filtration  
Eagles Minimum Essential Medium + 5.0% v/v foetal bovine serum at 4°C

### Experimental Conditions

Period of analysis  
Product diluents used  
Product test concentrations  
Appearance product dilutions  
Appearance in test mixture  
Contact times (minutes)  
Test temperature  
Interfering substances  
Temperature of incubation  
Identification and passage (P) of virus  
Identification and passage (P) of cells

04 September 2020 to 09 September 2020  
Sterile distilled water  
0.50% v/v; 0.66% v/v; 1.00% v/v  
No changes noted- stable  
No changes noted- stable  
5 ± 10s  
20°C ± 1°C  
0.3g/l bovine albumin  
37°C ± 1°C + 5% CO<sub>2</sub>  
**Vaccinia virus VR-1549 Elstree strain (P06)**  
Vero Cells (P 49)

## PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of test product solution and a 5-minute contact time. Virus is exposed to disinfectant in 24-well plates, then neutralised, serially diluted and virus titred in 96-well tissue culture plates to determine the tissue culture infectious dose<sub>50</sub> (TCID<sub>50</sub>) of surviving virus. *Vaccinia virus* VR-1549 Elstree strain / Vero cells are assayed in parallel in each test. TCID<sub>50</sub> is determined by the method of Karber<sup>1</sup>.

### **Cytotoxicity control**

The test product solution is measured for its effects on the host cells used to propagate the virus, to determine the sensitivity of the assay.

### **Interference control**

The effect of the cells after treatment of the test product solution are verified to ensure the cells can show susceptibility for virus infection. This is compared against cells that have not been treated with test product.

### **Disinfectant suppression control VS1**

Virus is added to the highest concentration of test product solution and then the mixture immediately removed and neutralised. The neutralised virus titre is then determined to assess the efficiency of the neutralisation procedure.

### **Disinfectant suppression control VS2**

Internal control which adds virus to neutralised test product solution to assess the efficiency of the neutralisation procedure.

### **No column Control**

Internal control on the highest contact time to assess any impact of the Microspin™ S 400 HR columns.

### **Virus recovery control**

Virus titre is determined for virus in contact with sterile distilled water at t=0, t = 5 and at t =15. The virus titre after 5 minutes is then compared to the recovery of disinfectant-treated virus to measure the log reduction in virus titre. The virus titre at 15 minutes is compared to the reference virus inactivation control.

### **Reference virus inactivation control**

Virus is exposed to 0.7% W/V formaldehyde and the recovery of virus determined by TCID<sub>50</sub> after 5 and 15 minutes, in order to assess that the test virus has retained reproducible biocide resistance. In addition, the formaldehyde cytotoxicity of neutralised formaldehyde is determined, to measure assay sensitivity.

1Kärber, G.: Beitrag zur Kollektiven Behandlung Pharmakologischer Reihenversuche. Arch. Exp. Path. Pharmak. 162 (1931): 480-487.

**CONCLUSION****Verification of the methodology**

A test is only valid if the following criteria are fulfilled:

- a) The titre of the test suspension of at least  $10^8$  TCID<sub>50</sub> /ml is sufficiently high to at least enable a titre reduction of 4 lg to verify the method.
- b) Detectable titre reduction is at least  $4 \log_{10}$ .
- c) Difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test is between:
  - Between 0.75 and 3.5 after 5 min and between 2.0 and 4.0 after 15 min for Vaccinia virus
- d) Cytotoxicity of the product solution does not affect cell morphology and growth or susceptibility for the test virus in the dilutions of the test mixtures which are necessary to demonstrate a  $4 \log_{10}$  reduction of the virus.
- e) The interference control result does not show a difference of  $> 1.0 \log_{10}$  of virus titre for test product treated cells in comparison to the non-treated cells.
- f) Neutralisation validation. This is called the disinfectant suppression test in this protocol. The disinfectant was neutralised by column chromatography through an Illustra Microspin S-400 HR column to achieve the best possible neutralisation available for this test. The difference for virus is greater than  $0.5 \log_{10}$  indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 80.0% v/v for VS1. This neutralisation validation has been verified by VS2, which shows the product has been successfully neutralised.

According to EN 14476:2013 + A2:2019, **Alcohol Free Sanitiser POSSESSES VIRUCIDAL** activity at a concentration of **1.00% v/v** as tested after **5 MINUTES** at **20°C** under **CLEAN** conditions (0.3 g/l bovine albumin) against *Vaccinia virus* VR-1549 Elstree strain / Vero cells.

**This product therefore is effective against all enveloped viruses as defined in EN 14476:2013 + A2:2019 Annex A\*. This therefore includes all coronaviruses and SARS-CoV-2.**

Authorised signatory



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Glasgow, UK.

Date: 18 SEPTEMBER 2020

**DISCLAIMER**

The results in this test report only pertain to the sample supplied.

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**\*EN 14476 2013 + A2 2019 Annex A (informative – Enveloped viruses)**

Poxviridae  
Herpesviridae  
Filoviridae (e.g. Ebola, Marburg)  
Flavivirus  
Hepatitis C Virus (HCV)  
Hepatitis Delta Virus (HDV)  
Influenza Virus  
Paramyxoviridae  
Rubella Virus  
Measles Virus  
Rabies Virus  
Coronavirus (e.g. SARS, MERS)  
Human Immunodeficiency Virus (HIV)  
Human T Cell Leukemia Virus (HTLV)  
Hepatitis B virus (HBV)

Reference: Van Regenmortel MHV et al.,Eds.: Virus Taxonomy, Classification and Nomenclature of Viruses, seventh report of the international committee on taxonomy of viruses. Academic Press, San Diego, 2000