

Test Report: EN 14476 2013 Chemical disinfectants and antiseptics - Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine - Test method and requirements (phase 2/step 1)

Test Laboratory

BluTest Laboratories Ltd

Robertson Incubator (Level 4)

Robertson Building

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Identification of sample

Name of the product

Clinell Universal Wipes

Batch number

N/A

Client

GAMA Healthcare Ltd. Unit 2,
The Exchange, Brent Cross Gardens,
London, NW43RJ

Project Code

BT-GAM-09-03

Date of Delivery

29 Jan 2015

Storage conditions

Ambient /Darkness

Active substances

None Given

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.

Neutraliser

Dilution-neutralisation/gel filtration; MEM + 10% v/v foetal bovine serum at 4°C

Antibody staining

Experimental Conditions

Period of analysis

01-Jun-15 to 12-Jun-15

Product diluent used

Sterile water

Product test concentrations

80.00%(v/v) 50.00%(v/v) 5.00%(v/v)

Appearance product dilutions

N/A

Contact time (mins)

30 seconds ± 10s 1 minutes ± 10s; 2 minutes ± 10s
20°C ± 1°C

Test temperature

Interfering substance

3.0g/l bovine albumin + 3.0ml/l erythrocytes

Stability of mixture

6 months

Temperature of incubation

37°C ± 1°C + 5% CO₂

Identification of strains

Feline immunodeficiency virus ATCC
VR-1312 / CRFK cells

PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of disinfectant and 30 second, 1 minute and 2 minute contact times. Virus is exposed to disinfectant in 24-well plates, then neutralized, serially diluted and virus titred in 96-well tissue culture plates to determine the tissue culture infectious dose₅₀ (TCID₅₀) of surviving virus. TCID₅₀ is determined by the method of Karber¹.

Cytotoxicity control

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

Interference control

The end point titration of the virus is exposed to three different sub-lethal concentrations of neutralized disinfectant to measure the effect of sub-lethal concentrations of disinfectant on virus infectivity in relation to the titre achieved on untreated cells.

Disinfectant suppression control

Virus is added to the highest concentration of disinfectant and then the mixture immediately removed and neutralized. The neutralized virus titre is then determined to assess the efficiency of the neutralization procedure.

Virus recovery control

Virus titre is determined for virus in contact with sterile hard water at t=0, t = 2 and at t =60. The virus titre after 2 minutes is then compared to the recovery of disinfectant-treated virus to measure the log reduction in virus titre. The virus titre at 60 minutes is compared to the reference virus inactivation control.

Reference virus inactivation control

Virus is exposed to 0.07% W/V formaldehyde and the recovery of virus determined by TCID₅₀ after 5, 15, 30 and 60 minutes, in order to assess that the test virus has retained reproducible biocide resistance. In addition, the formaldehyde cytotoxicity of neutralized formaldehyde is determined, to measure assay sensitivity.

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

Feline Immunodeficiency virus (HIV surrogate) ATCC VR-1312.

SOP 10000 V02 EN14476 Suspension test results for the efficacy of Clinnel Universal Wipes , BT-GAM-09-03 from GAMA Healthcare Limited against FIV

| Exposure Time | Virus Recovery 0 min | | Virus Recovery 2 min | | Cytotoxicity | | Disinfectant Suppression | | 5.00% (v/v) | | 50.00% (v/v) | | 80.00% (v/v) | |
|----------------|-------------------------|-----------------------|-------------------------|-----------------------|--------------|-----------------------|-----------------------------|-----------------------|-------------|-----------------------|--------------|-----------------------|--------------|-----------------------|
| | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml |
| | 5.33 | 6.76E+06 | 5.33 | 6.76E+06 | 1.00 | 3.16E+02 | 4.50 | 1.00E+06 | | | | | | |
| log | | 6.76E+06 | | 6.76E+06 | | 3.16E+02 | | 1.00E+06 | | | | | | |
| log difference | | 6.83 | | 6.83 | | 2.50 | | 6.00 | | | | | | |
| | | | | | | | | 0.83 | | | | | | |

| t = 30 secs | TOD ₅₀ /ml | | TOD ₅₀ /ml | | raw data | | TOD ₅₀ /ml | | raw data | | TOD ₅₀ /ml | | raw data | | TOD ₅₀ /ml | |
|----------------|-----------------------|-----------------------|-----------------------|-----------------------|----------|-----------------------|-----------------------|-----------------------|----------|-----------------------|-----------------------|-----------------------|----------|-----------------------|-----------------------|-----------------------|
| | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml |
| | 5.33 | 6.76E+06 | 5.33 | 6.76E+06 | | | 2.00 | 3.16E+03 | 1.00 | 3.16E+02 | 1.00 | 3.16E+02 | 1.00 | 3.16E+02 | 1.00 | 3.16E+02 |
| log | | 6.76E+06 | | 6.76E+06 | | | | 3.16E+03 | | 3.16E+02 | | 3.16E+02 | | 3.16E+02 | | 3.16E+02 |
| log difference | | 6.83 | | 6.83 | | | | 3.50 | | 2.50 | | 2.50 | | 2.50 | | 2.50 |
| | | | | | | | | 3.33 | | 4.33 | | 4.33 | | 4.33 | | 4.33 |

| t = 1 | TOD ₅₀ /ml | | TOD ₅₀ /ml | | raw data | | TOD ₅₀ /ml | | raw data | | TOD ₅₀ /ml | | raw data | | TOD ₅₀ /ml | |
|----------------|-----------------------|-----------------------|-----------------------|-----------------------|----------|-----------------------|-----------------------|-----------------------|----------|-----------------------|-----------------------|-----------------------|----------|-----------------------|-----------------------|-----------------------|
| | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml |
| | 5.33 | 6.76E+06 | 5.33 | 6.76E+06 | | | 1.83 | 2.14E+03 | 1.00 | 3.16E+02 | 1.00 | 3.16E+02 | 1.00 | 3.16E+02 | 1.00 | 3.16E+02 |
| log | | 6.76E+06 | | 6.76E+06 | | | | 2.14E+03 | | 3.16E+02 | | 3.16E+02 | | 3.16E+02 | | 3.16E+02 |
| log difference | | 6.83 | | 6.83 | | | | 3.33 | | 2.50 | | 2.50 | | 2.50 | | 2.50 |
| | | | | | | | | 3.50 | | 4.33 | | 4.33 | | 4.33 | | 4.33 |

| t = 2 | TOD ₅₀ /ml | | TOD ₅₀ /ml | | raw data | | TOD ₅₀ /ml | | raw data | | TOD ₅₀ /ml | | raw data | | TOD ₅₀ /ml | |
|----------------|-----------------------|-----------------------|-----------------------|-----------------------|----------|-----------------------|-----------------------|-----------------------|----------|-----------------------|-----------------------|-----------------------|----------|-----------------------|-----------------------|-----------------------|
| | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml | raw data | TOD ₅₀ /ml |
| | 5.33 | 6.76E+06 | 5.33 | 6.76E+06 | | | 1.50 | 1.00E+03 | 1.17 | 4.68E+02 | 1.17 | 4.68E+02 | 1.00 | 3.16E+02 | 1.00 | 3.16E+02 |
| log | | 6.76E+06 | | 6.76E+06 | | | | 1.00E+03 | | 4.68E+02 | | 4.68E+02 | | 3.16E+02 | | 3.16E+02 |
| log difference | | 6.83 | | 6.83 | | | | 3.00 | | 2.67 | | 2.67 | | 2.50 | | 2.50 |
| | | | | | | | | 3.83 | | 4.16 | | 4.16 | | 4.33 | | 4.33 |

Summary table of results of virucidal activity against FIV under dirty conditions for Clinell Universal Wipes, BT-GAM-09-03 from GAMA Healthcare Limited

| Product: | Interfering substance | Concentration | Level of cytotoxicity | lg TCID ₅₀ | | | | | >4 lg reduction after ... Min |
|-------------------------|-----------------------------------|---------------|-----------------------|-----------------------|-------|-------|------|--------|-------------------------------|
| | | | | 0 min | 2 min | 1 min | 30 s | 60 min | |
| Clinell Universal Wipes | 3.0g/l BSA + 3.0ml/l erythrocytes | 80.00% (v/v) | 2.50 | 6.83 | 2.50 | 2.50 | 2.50 | 2.50 | 30s |
| | | 50.00% (v/v) | 2.50 | 6.83 | 2.67 | 2.50 | 2.50 | 2.50 | 30s |
| | | 5.00% (v/v) | 2.50 | 6.83 | 3.00 | 3.33 | 3.50 | 3.50 | >2 |
| | 3.0g/l BSA | 80.00% (v/v) | 2.50 | 6.83 | 2.50 | 2.50 | 2.50 | 2.50 | 30s |
| | | 50.00% (v/v) | 2.50 | 6.83 | 2.50 | 2.67 | 2.50 | 2.50 | 30s |
| Formaldehyde | PBS | 5.00% (v/v) | 2.50 | 6.83 | 3.00 | 3.50 | 3.50 | 3.50 | >2 |
| | | 0.7% (w/v) | 2.50 | 6.83 | 4.83 | 3.67 | 2.83 | 2.83 | 30 |
| Virus Control | BSA + erythrocytes | n.a. | n.a. | 6.83 | n.a. | n.a. | n.a. | n.a. | n.a. |
| Virus Control | BSA | n.a. | n.a. | 6.83 | n.a. | n.a. | n.a. | 7.00 | n.a. |

Control Data

Control Data for: BT-GAM-09-03

Parallel control test

| Exposure Time | Virus Recovery 0 min | | Virus Recovery 2 min | | 5.00% (v/v) | | 50.00% (v/v) | | 80.00% (v/v) | |
|----------------|-------------------------|------------------------|-------------------------|------------------------|-------------|------------------------|--------------|------------------------|--------------|------------------------|
| | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml |
| | 5.33 | 6.76E+06 | 5.33 | 6.76E+06 | | 3.16E+01 | | 3.16E+01 | | 3.16E+01 |
| log | | 6.76E+06 | | 6.76E+06 | | 3.16E+01 | | 3.16E+01 | | 3.16E+01 |
| log difference | | 6.83 | | 6.83 | | 1.50 | | 1.50 | | 1.50 |
| | | | | | | 5.33 | | 5.33 | | 5.33 |
| t = 30 secs | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml |
| | 5.33 | 6.76E+06 | 5.33 | 6.76E+06 | 2.00 | 3.16E+03 | 1.17 | 4.68E+02 | 1.00 | 3.16E+02 |
| log | | 6.76E+06 | | 6.76E+06 | | 3.16E+03 | | 4.68E+02 | | 3.16E+02 |
| log difference | | 6.83 | | 6.83 | | 3.50 | | 2.67 | | 2.50 |
| | | | | | | 3.33 | | 4.16 | | 4.33 |
| t = 1 | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml |
| | 5.33 | 6.76E+06 | 5.33 | 6.76E+06 | 1.50 | 1.00E+03 | 1.00 | 3.16E+02 | 1.00 | 3.16E+02 |
| log | | 6.76E+06 | | 6.76E+06 | | 1.00E+03 | | 3.16E+02 | | 3.16E+02 |
| log difference | | 6.83 | | 6.83 | | 3.00 | | 2.50 | | 2.50 |
| | | | | | | 3.83 | | 4.33 | | 4.33 |
| t = 2 | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml | raw data | TOD ₅₀₂ /ml |
| | 5.33 | 6.76E+06 | 5.33 | 6.76E+06 | 1.17 | 4.68E+02 | 1.00 | 3.16E+02 | 1.00 | 3.16E+02 |
| log | | 6.76E+06 | | 6.76E+06 | | 4.68E+02 | | 3.16E+02 | | 3.16E+02 |
| log difference | | 6.83 | | 6.83 | | 2.67 | | 2.50 | | 2.50 |
| | | | | | | 4.16 | | 4.33 | | 4.33 |

Stock Virus (TCID₅₀)

| | |
|------|----------|
| 6.50 | 1.00E+08 |
|------|----------|

Formaldehyde reference inactivation control

| Exposure time | Virus recovery 0 min | | Virus recovery 60 min | | Cytotoxicity | | 0.07% Formaldehyde | | | | | | | |
|----------------|----------------------|------------------------|-----------------------|------------------------|--------------|------------------------|--------------------|----------|------|----------|------|----------|------|----------|
| | raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml | raw data | TCID ₅₀ /ml | 5 | | 15 | | 30 | | 60 | |
| 60 min | 5.33 | 6.76E+06 | 5.50 | 1.00E+07 | 1.00 | 3.16E+02 | 3.33 | 6.76E+04 | 2.17 | 4.68E+03 | 1.33 | 6.76E+02 | 1.33 | 6.76E+02 |
| log difference | | 6.76E+06 | | 1.00E+07 | | 3.16E+02 | | 6.76E+04 | | 4.68E+03 | | 6.76E+02 | | 6.76E+02 |
| | | 6.83 | | 7.00 | | 2.50 | | 4.83 | | 3.67 | | 2.83 | | 2.83 |
| | | | | | | | | 2.17 | | 3.33 | | 4.17 | | 4.17 |

No Column Control

| Virus Recovery | |
|----------------|------------------------|
| 30 min | TCID ₅₀ /ml |
| raw data | 2.14E+06 |
| 4.83 | 2.14E+06 |
| | 2.14E+06 |
| | 6.33 |

Interference control

| Virus dilution | Cytotoxicity dilution | | |
|----------------|-----------------------|----|------|
| | -1 | -2 | -3 |
| -5 | na | 3 | na |
| -6 | na | 2 | na |
| -7 | na | 1 | na |
| | | | Mock |
| | | | 3 |
| | | | 3 |
| | | | 2 |

CONCLUSION

Verification of the methodology

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

- a) Test virus suspension has at least a concentration which allows the determination of a 4 log₁₀ reduction of the virus titre.
- b) Detectable titre reduction is at least 4 log₁₀.
- 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 - 0.5 and - 2.5 after 30 min and between - 2 and - 4.5 after 60 min for 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 reduction of the virus.
- e) The interference control result does not show a difference of < 1.0 log₁₀ of virus titre in comparison to the virus recovery control; dilutions of disinfectant to sub-acute levels did not interfere in the generation of viral cytopathic effect.
- e) Neutralisation validation. This is called the disinfectant suppression test in this protocol. The difference for virus is not greater than 1.0 log₁₀ indicating effective neutralization of the virucidal activity of the disinfectant by dilution at a concentration of 80.00 % v/v.
- f) A difference of <0.5 log₁₀ is not observed between virus recovered directly from the virus recovery control at 60 minutes and virus from the same control recovered through an Illustra Microspin S-400 HR column

According to EN 14476 2013, Clinell Universal Wipes **POSSESSES VIRUCIDAL** activity at a concentration of **80.00% % v/v** of the working concentration as tested after **30 seconds** at **20°C** under **DIRTY** conditions (3.0 g/l bovine albumin + 3.0 ml/l erythrocytes) against Feline immunodeficiency virus ATCC VR-1312 / CRFK cells.

Signed



Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK
Date: 06 July 2015

DISCLAIMER

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