BluTest
GLOBAL MICROBIOLOGY EXPERTISE

# 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**<br>Robertson Incubator (Level 4)<br>Robertson Building<br>56 Dumbarton Road<br>Glasgow<br>UK - G11 6NU |

**Identification of sample**

| | |
|---|---|
| Name of the product | **CreBiSol x10** |
| Batch number | BT-HIP-05-01 |
| Client | Creative Biocidal Solutions-Ireland |
| Project Code | BT-HIP-05 |
| Date of Delivery | 28-Apr-15 |
| Storage conditions | Ambient Temperature, darkness |
| Active substances | Not Specified |

**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, neutralization control and a formaldehyde internal standard. |
| Neutralizer | Dilution-neutralization/gel filtration; Dulbecco's modified Eagles medium + 5% v/v foetal bovine serum at 4°C |

**Experimental Conditions**

| | |
|---|---|
| Period of analysis | 19-May-15 to 22-May-15 |
| Product diluent used | Hard Water |
| Product test concentrations | 1in20 / 1in50 |
| Appearance product dilutions | Clear |
| Contact time (mins) | 5 ± 10s |
| Test temperature | 20°C ± 1°C |
| Interfering substance | 3.0g/l bovine albumin + 3.0 ml/l sheep erythrocytes |
| Stability of mixture | 6 months |
| Temperature of incubation | 37°C ± 1°C + 5% $CO_2$ |
| Identification of strains | **Murine norovirus Berlin s99 / RAW cells** |



Robertson Incubator, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU
Email: info@blutest.com Website: www.blutest.com
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## PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of disinfectant and a 5 and 60 minute contact time. 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 dose$_{50}$ ($TCID_{50}$) of surviving virus. $TCID_{50}$ is determined by the method of Karber[1].

**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 = 5 and at t =60. 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 60 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 $TCID_{50}$ 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.



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## Murine norovirus Berlin strain s99.

**SOP 10000 V04 EN14476 Suspension test results for the efficacy of CreBiSol x10, Batch BT-HIP-05 from Creative Biocidal Solutions-Ireland against MNV**

| Exposure Time | Virus Recovery 0 min | | Virus Recovery 5 min | | Cytotoxicity | | Disinfectant Suppression | | 1in20 | | 1in50 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml |
| t = 5 | 4.33 | **6.76E+05** | 4.00 | **3.16E+05** | 1.00 | **3.16E+02** | 2.50 | **1.00E+04** | 0.00 | **3.16E+01** | 0.50 | **1.00E+02** |
| | | **6.76E+05** | | **3.16E+05** | | **3.16E+02** | | **1.00E+04** | | **3.16E+01** | | **1.00E+02** |
| log | | **5.83** | | **5.50** | | **2.50** | | **4.00** | | **1.50** | | **2.00** |
| log difference | | | | | | | | **1.50** | | **4.00** | | **3.50** |

**Table of results of virucidal activity against MNV under dirty conditions for CreBiSol x10, Batch BT-HIP-05 from Creative Biocidal Solutions-Ireland**

| Product: CreBiSol x10 | Interfering substance | Concentration | Level of cytotoxicity | lg $TCID_{50}$ | | | | | >4 lg reduction after .. Min |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 min | 5 min | 15 min | 30min | 60 min | |
| | 3.0g/l BSA + 3.0ml/l erythrocytes | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| | | 1in50 | 2.50 | 5.83 | 2.00 | n.a. | n.a. | n.a. | >5 |
| | | 1in20 | 2.50 | 5.83 | 1.50 | n.a. | n.a. | n.a. | <5 |
| | 3.0g/l BSA | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| | | 1in50 | 2.50 | 5.83 | 2.00 | n.a. | n.a. | n.a. | >5 |
| | | 1in20 | 2.50 | 5.83 | 1.50 | n.a. | n.a. | n.a. | <5 |
| Formaldehyde | PBS | 0.7% (w/v) | 3.50 | 5.83 | 5.50 | 4.83 | 4.00 | 3.50 | >60 |
| Virus Control | BSA + erythrocytes | n.a. | n.a. | 5.83 | 5.50 | n.a. | n.a. | n.a. | n.a. |
| Virus Control | BSA | n.a. | n.a. | 5.83 | 5.50 | n.a. | n.a. | n.a. | n.a. |



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## Control Data

**Control Data for:** BT-HIP-05

**Parallel control test**

| Exposure Time | Virus Recovery 0 min | | Virus Recovery 5 min | | 1in20 | | 1in50 | |
|---|---|---|---|---|---|---|---|---|
| | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml |
| t = 5 | 4.33 | **6.76E+05** | 4.00 | **3.16E+05** | 0.00 | **3.16E+01** | 0.50 | **1.00E+02** |
| | | **6.76E+05** | | **3.16E+05** | | **3.16E+01** | | **1.00E+02** |
| log | | **5.83** | | **5.50** | | **1.50** | | **2.00** |
| log difference | | | | | | **4.00** | | **3.50** |

| **Stock Virus ($TCID_{50}$)** | 5.50 | **1.00E+07** |
|---|---|---|

**Formaldehyde reference inactivation control**

| Exposure time | Virus recovery 0 min | | Virus recovery 60 min | | Cytotoxicity | | 0.7% Formaldehyde | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 5 | | 15 | | 30 | | 60 | |
| | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml | raw data | $TCID_{50}$/ml |
| 60 min | 4.33 | **6.76E+05** | 4.00 | **3.16E+05** | 2.00 | **3.16E+03** | 4.00 | **3.16E+05** | 3.33 | **6.76E+04** | 2.50 | **1.00E+04** | 2.00 | **3.16E+03** |
| | | **6.76E+05** | | **3.16E+05** | | **3.16E+03** | | **3.16E+05** | | **6.76E+04** | | **1.00E+04** | | **3.16E+03** |
| log | | **5.83** | | **5.50** | | **3.50** | | **5.50** | | **4.83** | | **4.00** | | **3.50** |
| log difference | | | | | | | | **0.00** | | **0.67** | | **1.50** | | **2.00** |

**No Column Control**

| Virus Recovery 5 min | |
|---|---|
| raw data | $TCID_{50}$/ml |
| 4.83 | **2.14E+06** |
| | **2.14E+06** |
| | **6.33** |

**Interference control**

| Virus dilution | Cytoxicity dilution | | | |
|---|---|---|---|---|
| | -1 | -2 | -3 | Mock |
| -4 | 3 | 3 | 3 | 3 |
| -5 | 3 | 3 | 3 | 3 |
| -6 | 3 | 3 | 3 | 3 |



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## 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 $\log_{10}$ reduction of the virus titre.

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 – 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 $\log_{10}$ 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 slightly elevated indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 1/20.

f) A difference of <0.5 $\log_{10}$ 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, **CreBiSol x10 POSSESSES VIRUCIDAL** activity at a concentration of **1/20** as tested after **5 MINUTES** at **20°C** under **DIRTY** conditions (3.0 g/l bovine albumin + 3.0 ml/l erythrocytes) against Murine norovirus Berlin s99 / RAW cells **.**

Signed

Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK
Date: 05 June 2015

UKAS TESTING

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Expanded Uncertainty of Measurement U = ± 0.44



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