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FINAL STUDY REPORT

VIRUCIDAL PROPERTIES OF A DISINFECTANT PRODUCT "NCG Xtreme" AGAINST CANINE PARVOVIRUS USING A SUSPENSION TEST

Conducted for:

New Clean Generation Pty Ltd

8 Charles Street, Tingira Heights, NSW 2290

Australia

Conducted by:

Eurofins I ams Laboratories
8 Rachael Close, Silverwater NSW 2128
Australia

Authors:

Lutfun Khan, M.Sc.
Imtiaz Ahmed, M.Phil. MASM

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A Eurofins BioPharma Product Testing Laboratory

ABN: 47 075 467 757

8 Rachael Close, Silverwater
NSW 2128, Australia

2/120 Bluestone Circuit, Seventeen Mile Rocks
QLD 4073, Australia

T | +61 2 9704 2300
www.amslabs.com.au

SUMMARY

This study was designed to evaluate the virucidal properties of disinfectant, namely "NCG Xtreme" against Canine parvovirus. In this test, the sample "NCG Xtreme" reduced the virus titre by 3.0 log after 10 minutes exposure period in a suspension test. This result meets the acceptance criteria to support for making a virucidal claim in accordance with the Australian Therapeutic Goods Order Number 54/54A.

INTRODUCTION

A study was required by New Clean Generation Pty Ltd to evaluate a disinfectant product for its virucidal properties against Canine parvovirus using a suspension testing protocol (ASTM E1052-97).

The experimental work was conducted at Eurofins I ams Laboratories Pty Ltd, 8 Rachael Close, Silverwater, NSW Australia 2128.

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OBJECTIVE

To determine whether a disinfectant product "NCG Xtreme" was tested against Canine parvovirus in a suspension test, using accepted criteria for making virucidal claims.

REFERENCES

- Therapeutic Goods Order No. 54, 54A & 54B Guidelines (2009)
- Eurofins I ams Labs. - SOP TMCV-005-03 – Testing of Virucidal Activity of Disinfectants by Direct Inoculation Test Method.
- ASTM E1052-97 Standard Test Method for Efficacy of Virucidal Agents Against Viruses in Suspensions.

MATERIALS AND METHODS

A. VIRUS STRAIN

The test virus used in this study was Canine parvovirus which was obtained from ATCC. The virus was stored in liquid nitrogen prior to use.

B. CELL SUBSTRATE

The host cells used in this study were A72 cells, which were obtained from ATCC. The cells were stored in liquid nitrogen prior to use. Cells were thawed and sub-cultured in DMEM cell growth medium.

C. TEST PRODUCT

NCG Xtreme Batch No: 401

Test Concentration: 1:20 as prepared by the client

D. REAGENTS AND SUPPLIERS

1. Phosphate Buffer Solution (PBS), supplied by Eurofins I ams Labs.
2. DMEM medium plus all the supplements needed to prepare maintenance medium were supplied by Lonza.

E. TEST CONDITIONS

Test Commencement Date	**01-11-2016**
Test Virus	**Canine parvovirus**
Test Surface	**Direct Inoculation in product solution**
Test Soil	**Clean condition**
Test Disinfectant	**NCG Xtreme, 1:20**
Contact time and test temperature	**10 Minutes at Room Temperature**

F. PREPARATION OF CELL SUBSTRATE

1. All work was carried out in a class 2 biosafety cabinet.
2. Growth media was prepared by combining the following reagents in DMEM in their specified proportions; FBS (10%), L-glutamine (1%), NEAA (1%), Antibiotic-Antimycotic (1%) and Hepes Buffer (1%).
3. A 25 cm^2 flask was prepared containing a monolayer of A72 cells.
4. Once the monolayer reached confluency the growth media was discarded and the cell monolayer washed twice with 2 ml PBS, with the remaining PBS being discarded.
5. A volume of 0.5 ml Trypsin was then added to the flask, which was subsequently incubated at 37°C for approximately 5 minutes until cells were visibly lifting from the flask. Progress was checked using an inverted microscope.
6. When all the cells were detached, 5ml of growth media was added and the flask was shaken gently to resuspend the cells.

7. A volume of 1ml of the cell suspension was removed and diluted in 10ml of fresh growth media. Using a Multichannel pipette, 100 µl of this cell suspension was dispensed into each well of a 96 well microtiter plate.
8. The plates were incubated in the CO2 incubator with an atmosphere of 5% CO2 in air at a temperature of 37°C ± 1°C for 24 hours.

G. PREPARATION OF VIRUS/DISINFECTANT TEST

1. A vial of CPV was removed from liquid nitrogen storage and thawed in a 37°C water bath for approximately 5 mins. A volume of 0.2 ml of virus suspension was directly inoculated into 1.8 ml of sample solution. The disinfectant-virus mixture was allowed to have an exposure time of 10 min at RT in the biosafety cabinet.

2. The test solution was prepared by the client.

3. After 10 minutes contact time, 0.6 ml of the disinfectant-virus mixture was then transferred into a 3cc Sephadex gel column and was filtered. The surviving virus particles were then assayed by serial dilution and plating out onto a cell monolayer.

H. PREPARATION OF POSITIVE VIRUS CONTROL

The positive control was prepared by mixing 1.8 ml of DMEM maintenance media and the virus suspension. Following the 10 minutes contact time, 0.6 ml of the mixture was then immediately passed through an individual Sephadex column (3 cc) using the syringe plunger. The filtrate was serially diluted and assayed for infectivity.

I. PREPARATION OF CYTOTOXICITY CONTROL

An aliquot of 0.2 ml of maintenance medium was mixed with 1.8 ml of sample and subjected to the same treatment as test mixture. At the end of the contact time, 0.6 ml of the mixture was then immediately filtered through 3cc Sephadex gel using a syringe plunger. The filtrate was serially diluted to 10^{-4}. Infectivity was assayed by plating out an aliquot of 100 µL of each dilution into quadruplicate wells.

J. PREPARATION OF NEUTRALISATION CONTROL

Dilutions of neutralised sample from (10^{-2} to 10^{-4}) were spiked with 0.1ml of low titer of virus suspension to determine the dilution(s) at which the disinfectant virucidal activity is completely neutralised. A volume of 0.1ml of spiked neutralisation dilutions were plated out in quadruplicate.

K. METHOD FOR VIRAL ASSAY

1. Maintenance medium was prepared by combining the following reagents in DMEM in their specified proportions; FBS (7.5%), L-glutamine (1%), NEAA (1%), Antibiotic-Antimycotic (1%) and Hepes Buffer (1%).
2. The virus was diluted in series from 10^{-2} to 10^{-6}.

L. INOCULATION OF THE TEST SAMPLES AND CONTROLS

1. Commencing with the highest dilution of the test samples, 100 µl from each dilution was dispensed into quadruplicate wells.
2. When all wells were filled, the plate was incubated at 37°C ± 1°C in a humidified atmosphere of 5% CO_2.

M. CALCULATION OF VIRUS TITRE

The Reed & Muench LD50 Method was used for determining the virus titre endpoint.

RESULTS:

The titre of the test CPV control was 5.5 $\log_{10}$. There was no virus infectivity observed in the disinfectant-virus test mixture assayed after the 10 minutes contact time.

The neutralisation control shows no viral inhibition at 10^{-3} dilution, which means the sample was neutralised at 2.5 log10. All cell substrate controls were normal.

Considering the cytotoxicity and neutralisation test results, the sample "NCG Xtreme" has shown virucidal efficacy against Canine parvovirus by achieving a greater than 3.0 log reduction in virus concentration after 10 minutes exposure period.

TABLE 1: Final Test Results :

Virus Dilution	Virus Control	Cytotoxicity	Neutralisation	NCG Xtreme 10 minutes
10^{-2}	$4^{+}/4$	CT	CT	CT
10^{-3}	$4^{+}/4$	$0^{+}/4$	$4^{+}/4$	$0^{+}/4$
10^{-4}	$4^{+}/4$	$0^{+}/4$	$4^{+}/4$	$0^{+}/4$
10^{-5}	4+/4	-	-	$0^{+}/4$
10^{-6}	$0^{+}/4$	-	-	$0^{+}/4$
Log_{10}	**5.5**	**2.5**	**2.5**	**2.5**
Log_{10} Reduction of virus titre after Treatment				**>3.0**

Note: Presence of virus in each response is recorded as "+"
Absence of virus in each response is recorded as "0"
Presence of cytotoxicity in each response is recorded as "CT"

CONCLUSION:

The sample "NCG Xtreme" has demonstrated virucidal efficacy against Canine parvovirus, after 10 minutes contact time. The sample has achieved a greater than 3.0 log reduction in virus titre and hence meets the acceptance criteria for making virucidal claim against canine parvovirus according to the Australian Therapeutic Goods Order No 54/54A.

Signed:

Imtiaz Ahmed

Sterile Products Testing Manager