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FINAL STUDY REPORT

HUMAN INFLUENZA TYPE A (H1N1) - VIRUCIDAL PROPERTIES OF A DISINFECTANT PRODUCT "L44 Sanitiser" Using a Hard Surface Carrier Test

Conducted for:
Kevin Joyce
Aussan Laboratories
4/185 Briens Rd Northmead NSW 2152
Australia

Conducted by:
8 Rachael Close
Silverwater NSW 2128
Australia

Authors:
Lutfun Khan, MSc
Imtiaz Ahmed

Final Report
Ref. No 1205518 Flu A-H1N1
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SUMMARY

This study was designed to evaluate the virucidal properties of disinfectant "L44 Sanitiser" at 5% concentration against Human Influenza Virus A (H1N1). L44 Sanitiser disinfectant reduced the virus titre by 4.0 log after 10 minutes and 30 minutes exposure period at 5% concentration. This product meet the acceptance criteria to support virucidal claim for Influenza A virus, in accordance with the Australian Therapeutic Goods Order No 54/54A.

1. INTRODUCTION

A study was required by Aussan Laboratories Pty Ltd to evaluate a disinfectant product for its virucidal properties against Human Influenza Type A (H1N1) virus using a surface carrier testing protocol (ASTM-E1053-97).

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

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2. OBJECTIVE

To determine whether the disinfectant, L44 Sanitiser, was virucidal against Human Influenza A (H1N1) virus in a hard surface carrier test, using acceptance criteria of a minimum of 3 log reduction for making virucidal claims.

3. REFERENCE

ASTM- E1053-97: Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces.

4. MATERIALS AND METHODS

4.1 VIRUS STRAIN

The test virus used was Human Influenza Virus Type A, strain H1N1. The isolate was obtained from the WHO Collaborative Centre for Reference and Research on Influenza, North Melbourne, Victoria.

4.2 CELL SUBSTRATE

The Madin Darby Canine Kidney (MDCK) cells were stored in liquid nitrogen prior to use. MDCK cells were obtained from CSL Bioscience.
Cells were thawed and sub-cultured in EMEM cell growth medium.

4.3 TEST PRODUCT

The product is L44 Sanitiser, Batch No. 04/11/2011. The sample was assigned the laboratory reference number 1205518. The product was tested at a 5% concentration.

4.4 EXPERIMENTAL DESIGN

The design can be summarized as consisting of application and drying of test virus onto an inanimate surface, followed by standardized application of the test product.

Any surviving virus in test or control conditions was assayed using MDCK cells and the production of viral haemagglutinin detected by the use of chicken red blood cells.

4.5 REAGENTS AND SUPPLIERS

4.5 1 Phosphate Buffered Saline (PBS) was used for titrating haemagglutinating activity. It was supplied as pre-formulated tablets by Oxoid Australia Pty Ltd and made up as per manufacturer's instructions. 0.5% FBS was added before use.

4.5 2 Chicken Red Blood Cells were supplied by IMVS Veterinary services in Alsever's Solution prepared by ams labs. They were washed three times in PBS and adjusted to 0.8% v/v before use.

4.5 3 Medium EMEM plus all the supplements needed to prepare maintenance medium were supplied by Lonza.

4.6 PREPARATION OF CELL SUBSTRATE

All preparation was carried out in a biohazard cabinet. The MDCK cell cultures were grown in EMEM growth medium. The content of the MDCK flask was decanted into a 500mL discard jar, and using aseptic technique, approximately 2mL of TEDTA was introduced into the MDCK flasks. The flasks were gently rotated to ensure that all the surface of the monolayer was covered with the TEDTA. The flasks were incubated at 37°C for approximately 30 minutes, with the flasks checked every few minutes to see whether the cells were lifting off the plastic. Progress was checked using the invert microscope. When all cells were detached, 40mL of MDCK growth medium was added and the flask was shaken gently to suspend the cells in the medium. Equal volumes of the cell suspension were transferred into two sterile McCartney bottles. 100µL of MDCK was dispensed into each well, working from right to left across the 96 well microtitre plate and using the multichannel pipettor. When all wells were filled, the microtitre plates were incubated in the CO_2 incubator with an atmosphere of 5% CO_2 in air at a temperature of 37°C ± 2°C for 24 hours.

4.7 CONDUCT OF THE VIRUS / DISINFECTANT TEST

4.7.1. The Human Influenza Virus Type A, strain H1N1 was removed from the liquid N_2 and thawed.

4.7.2. 0.2mL of virus suspension with 5% FBS (organic soil) was pipetted into each of two petri dishes and labelled as virus control and Virus/disinfectant control. A uniform distribution of the virus suspension was achieved by using a spreader. The virus was then air dried for 30 minutes in a biohazard cabinet.

4.7.3. A 5% L44 Sanitizer sample was applied to two of the hard surface carriers.

4.7.4. The virus was exposed to the disinfectant for 10 minutes and 30 minutes. The virus control was not exposed to disinfectant.

4.7.5. At 10 minutes and 30 minutes, 0.2mL of the virus-disinfectant mixture was taken out and added to 1.8mL maintenance medium. This material was diluted further then assayed for virus.

4.8 PREPARATION OF VIRUCIDAL ASSAY CONTROLS

4.8.1. For the Neutralization Control, 0.1mL of 10^{-2} dilution of positive virus control was spiked into 0.9mL of 10^{-2} and 10^{-3} of the test product. This material was then assayed for virus.

4.8.2. For the Cytotoxicity Control, 0.2mL of the maintenance medium was pipetted onto each hard surface carrier. This was treated in the same manner as the test product, diluted and assayed for cytotoxicity.

4.9 VIRUS ASSAY

4.9.1. Confluent MDCK monolayers in 96 well plates were obtained by decanting the culture supernatant into the microtitre plate media discard tray.

4.9.2. The plates were washed once with PBS. Commencing with the highest dilution of the test samples, 100µL of each dilution were dispensed into the designated four wells. After one hour incubation at 37 ± 2°C in humidified CO_2 incubator, plates were washed once with PBS and 200µL of the maintenance medium was added to each well. This plating procedure was followed for all test and control materials and all assay controls. When all wells were filled, the lid of the 96 well microtitre plate was replaced and the plate incubated in the 5% CO_2 humidified incubator at 37 ± 2°C for 6 days.

4.9.3. Any surviving virus in test was assayed using haemagglutinin assay.

4.9.4. To examine for haemagglutinin activity, a further 0.1 mL of 0.8% washed chicken red blood cells were added to each well containing 0.1 mL test supernatant. The plates were gently agitated to mix the red blood cells and left to stand at ambient room temperature for 45 minutes.

4.9.5. Each well was then scored for absence of haemagglutination, by observation of a "button" of red blood cells on the bottom of the well (-) or presence, by observation of a uniformly distributed layer of red cells over the bottom of the plate (+) and (C) as sample product showed evidence of cytotoxic to MDCK cells, therefore no viral growth.

4.9.6. Presence of haemagglutination was taken as evidence of virus replication in the host and recorded accordingly.

4.9.7. The positive and negative wells at each dilution were recorded on the work sheet.

4.10 CALCULATION OF THE VIRUS TITRE

The Reed Muench $TCID_{50}$ method was used for determining the virus titre endpoint after haemagglutination.

5RESULTS

5.1. The untreated human influenza A (H1N1) virus control had a $\log_{10}$ titre of 6.5 (see Table 5.1).

5.2. The virus used in the present study was completely inactivated by the test product at the contact time of 10 minutes and 30 minutes at room temperature (see Table 5.2 and 5.3).

5.3. Sample showed cytotoxic at the 10^{-2} dilution (Table 5.4).

5.4. Product showed signs of complete neutralisation at the 10^{-3} dilution (Table 5.5).

5.5. The virus reduction in the present study is shown in Table 5.6.

TABLE 5.1 Virus Control Results

Virus Dilution	Number Inoculated	Individual Responses			
10^{-2}	4	+	+	+	+
10^{-3}	4	+	+	+	+
10^{-4}	4	+	+	+	+
10^{-5}	4	+	+	+	+
10^{-6}	4	+	+	+	+
10^{-7}	4	-	-	-	-
Total hosts:	24				

Calculated virus titre = $10^{6.5}TCID_{50}$ ($6.5\log_{10}$)

Note:
+ represents infected hosts showing haemagglutination.
- represents infected hosts showing no haemagglutination.

TABLE 5.2 Results For Virus Treated With Product

Virus Dilution	Contact time 10 Minutes				
	Number Inoculated	Individual Responses			
10^{-2}	4	C	C	C	C
10^{-3}	4	-	-	-	-
10^{-4}	4	-	-	-	-
10^{-5}	4	-	-	-	-
10^{-6}	4	-	-	-	-
Total hosts:	20				

Calculated virus titre = $10^{2.5}TCID_{50}$ ($2.5\ \log_{10}$)

TABLE 5.3 Results for Virus Treated With Product

Virus Dilution	Contact time 30 Minutes				
	Number Inoculated	Individual Responses			
10^{-2}	4	C	C	C	C
10^{-3}	4	-	-	-	-
10^{-4}	4	-	-	-	-
10^{-5}	4	-	-	-	-
10^{-6}	4	-	-	-	-
Total hosts:	20				

Calculated virus titre = $10^{2.5}TCID_{50}$ ($2.5\ \log_{10}$)

TABLE 5.4 Results for Cytotoxicity Check

Virus Dilution	Number Inoculated	Individual Responses			
10^{-2}	4	C	C	C	C
10^{-3}	4	-	-	-	-
Total Hosts	8				

Calculated virus titre= $10^{2.5}TCID_{50}$ (2.5 $\log_{10}$)

Note: Presence of virus in each response is recorded as "+"
Absence virus in each response is recorded as "-"
Cytotoxic response is recorded as "C"

TABLE 5.5 Results for Product Neutralization

Virus Dilution	Number Inoculated	Individual Responses			
10^{-2}	4	C	C	C	C
10^{-3}	4	+	+	+	+
Total Hosts	8				

TABLE 5.6 Log_{10} Reduction of Virus after Treatment

Treatment	Titre (Log_{10})	Reduction (Log_{10})
Virus Control	6.5	-
Cytotoxicity Control	2.5	-
10 mins Treatment	2.5	4.0
30 mins Treatment	2.5	4.0

6 CONCLUSIONS

The results obtained in this study clearly demonstrate that the test product, **"L44 Sanitizer"**, was able to kill Human Influenza A (H1N1) virus at room temperature with contact time of 10 minutes in a surface carrier test model (Table 5.6). Evidence of complete viral neutralisation after 10 minutes exposure period with 4.0 log (99.99%) in viral titre, complies with the efficacy requirement for disinfectant as specified in TGO 54/54A.

Compiled By: Lutfun Khan Date: 30.4.12
Lutfun Khan MSc

Checked By: Imtiaz Ahmed Date: 30.04.2012
Imtiaz Ahmed
Sterile Products Testing Manager
ams Laboratories Pty. Ltd