Virucidal efficacy determination of a disinfectant against H1N1 swine Influenza

STUDY UPDATE 1

Update for:	GAMA Healthcare Limited
Address:	Unit 39, Hampstead House, 176 Finchley Road, London NW3 6BT
	+44 (0) 845 2011 644
Study Update Authors:	Miss Elizabeth Moane
Study Director:	Miss Elizabeth Moane
Study Carried Out at the Facilities of:	Retroscreen Virology Ltd. London Bioscience Innovation Centre 2 Royal College Street London NW1 0NH
Telephone:	+44 (0) 207 118 0727
Fax:	+44 (0) 203 070 0086
Email:	e.moane@retroscreen.com

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Virucidal Assay

360µL test article (Clinell Disinfectant 0.9% (v/v)) was added to 40µL H1N1 Swine Influenza virus. The reaction was incubated at room temperature for 30 seconds, 60 seconds, 5 minutes and 10 minutes before undergoing a ten-fold termination dilution (400µL reaction mixture added to 3.6mL Influenza infection media [MEM (Invitrogen VX10370070); 500mL, L-Glutamine (Sigma G7513); 5mL, HEPES (Sigma H0887); 5mL, Pen-Strep (Sigma P4458); 5mL, supplemented with 5625 units of Trypsin (TPCK treated)].

The terminated reaction mixture was filtered through a Sephadex column. The filtered terminated reaction was titrated from neat (111uL in first well) following a ten-fold dilution series (11uL titrated across the plate) on MDCK cells. The plates were incubated at 37°C for 1 hour. After incubation the plates were washed twice with 100µL PBS (Gibco 10010) and 100µL Influenza infection media was added to each well. The plates were incubated for a further 3 days at 37°C after which a Haemagglutination assay was carried out to determine the presence/absence of virus in each well. The qualitative HA results were used to quantify the amount of virus present in each termination mixture using the Karber Calculation.

A cytotocicity assay was also carried out in a similar manner as the virucidal assay, substituting virus with MDCK infection media. There was no observed cytotoxicity on the MDCK cell line at the 0.9% (v/v) concentration when the terminated reaction was filtered through a Sephadex column.

The virus reduction for each test article is calculated by subtracting the $TCID_{50}$ of the test article from the calculated $TCID_{50}$ of the virus control on the plate that the test article was run on. The calculated $TCID_{50}$ and resulting virus reduction for each test article is shown below;

Table 1: Reduction in virus titre of H1N1 Swine Influenza NIBRG-121 virus after treatment with Clinell Disinfectant 9% (v/v)

Incubation Time Point	Virus titre recovered (log_{10} $TCID_{50}$/ml)		Reduction in virus titre	
	Virus control	Test article	(log_{10} $TCID_{50}$/ml)	%
30 seconds	4.25	≤1.50[A]	≥2.75	≥99.82
60 seconds	4.25	≤1.50[A]	≥2.75	≥99.82
5 minutes	4.50	≤1.50[A]	≥3.00	≥99.90
10 minutes	4.50	≤1.50[A]	≥3.00	≥99.90

Summary

Clinell Disinfectant 9% (v/v) was observed to reduce the viral titre of H1N1 Swine Influenza by ≥2.75 (≥99.82) over the 30 second and 60 second time points when compared to the control virus titre.

Clinell Disinfectant 9% (v/v) was observed to reduce the viral titre of H1N1 Swine Influenza by ≥2.75 (≥99.82) over the 5 minute and 10 minute time points when compared to the control virus titre.

No virus was recovered after treatment with Clinell Disinfectant 9% (v/v) at all time points tested. The absence of virus indicated complete inactivation of H1N1 Swine Influenza Virus. Inactivation can occur as a result of destruction of the surface proteins (neuraminidase and haemagglutinin) of the virus envelope, effectively killing the H1N1 Swine Influenza virus.

[A] Limit of detection of the virucidal assay