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CONFIDENTIAL FINAL REPORT

SPONSOR: ENVIROCLEANSE

SPONSOR'S REPRESENTATIVE: Scott Mack

STUDY TITLE: VIRUCIDAL HARD-SURFACE EFFICACY TEST – Avian Influenza A Virus (H5N1)
Non-GLP

STUDY IDENTIFICATION: Microbac Project No. 668-144 (refer to signed Protocol No.668.V.25.001)

Test Substance Name	Lot No.	Active Ingredient(s)	Date Received	DS No.
Envirocleanse A	020525	HOCl	02/05/25	Q029

CHALLENGE ORGANISM: Avian Influenza A Virus (H5N1); Strain: NIBRG-14; Source: Charles River Laboratories/CDC #2006719965

HOST CELL LINE: MDCK cells; Source: ATCC CCL-34

DILUTION MEDIUM: Minimum Essential Medium (MEM) + 1.0 µg/mL Trypsin

NEUTRALIZER: MEM + 1% Newborn Calf Serum (NCS) + 0.5% Polysorbate 80 + 0.5% $Na_2S_2O_3$

CONTACT TIME: 2 minutes

CONTACT TEMPERATURE: Room Temperature (20±2°C) (actual: 22°C)

NUMBER OF REPLICATES: 1 replicate (four wells per dilution)

INCUBATION TEMPERATURE: 36±2°C with 5±3% CO_2

INCUBATION TIME: 4 - 6 days (actual: 6 days)

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DILUTION: Ready to use

ORGANIC LOAD: 5.0% Fetal Bovine Serum (FBS) in vial inoculum

CARRIER INOCULATION AND DRY TIME: Glass Petri dishes were inoculated with 0.4 mL of the challenge organism and dried for 30 minutes at 22°C with 40-41% Relative Humidity (RH).

TEST APPLICATION:

The average volume of spray produced was calculated and used to determine the volume of neutralizer required for the test and control samples. An average volume of 2.0 mL (two pump sprays) was produced during the mock sprays.

The average volume (mean) from the mock spray runs was used as the volume of the DM applied to the dried virus and neutralizer dispensed for the PRC run.

The test substance was then sprayed (two pump sprays) onto the virus carrier in a horizontal position until thoroughly wet from a distance of 6" - 8". The treated carrier was then held for the contact time of 2 minutes at 22°C with 40% RH.

TITER CALCULATION:

The 50% Tissue Culture Infectious Dose per mL ($TCID_{50}$/mL) was determined using the Spearman-Karber method using the following formula:

$$m = x_k + \left(\frac{d}{2}\right) - d\sum p_i$$

where: m = the logarithm of the dilution at which half of the wells are infected relative to the test volume
x_k = the logarithm of the smallest dosage which induces infection in all cultures
d = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i
$\sum p_i$ = the sum of p_i (starting with the highest dilution producing 100% infection)

The values were converted to $TCID_{50}$/mL using a sample inoculum of 1.0 mL.

TITER CALCULATION (continued):

Viral Load Calculation:

Virus Load (Log_{10} $TCID_{50}$) = Virus Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [volume per sample (mL)]

Viral Reduction Calculation:

Log_{10} Reduction Factor = Initial Viral Load (Log_{10} $TCID_{50}$*) – Output Viral Load (Log_{10} $TCID_{50}$*)

* per assayed volume and per carrier

The fold of dilution for the NE/VI control was calculated in the following manner:

$D = C * (A / B)$

Where,

A = Units of virus per mL in the stock virus (in natural number, not logarithm number)
B = The fold of dilution
C = Volume (mL) of the diluted virus added per NE/VI dilution
D = Units of virus spiked into each NE/VI dilution *(must be ≤ 5,000 units)*

RESULTS:

Results are presented in Tables 1 – 5.

Key (for all tables):

T/y = Cytotoxicity observed in y wells inoculated; viral cytopathic effect (CPE) could not be determined
X/y = X wells out of y wells inoculated exhibited positive viral cytopathic effect
0/y = 0 out of y wells inoculated exhibited positive viral CPE; no cytotoxicity or bacterial contamination was observed in any of the wells inoculated

RESULTS (continued):

Table 1
Plate Recovery Controls (PRC)

Dilution*	PRC
10^{-3}	4/4
10^{-4}	4/4
10^{-5}	4/4
10^{-6}	2/4
10^{-7}	0/4
10^{-8}	0/4
Titer (Log_{10} $TCID_{50}$/mL)	6.00
Load (Log_{10} $TCID_{50}$)**	5.60

*Dilution refers to the fold of dilution from the virus inoculum.
**Per carrier (0.40 mL of Undilute [10^0])

Table 2
Test Substance

Dilution*	Envirocleanse A Lot No. 020525
10^{-2}	T/4
10^{-3}	0/4
10^{-4}	0/4
10^{-5}	0/4
10^{-6}	0/4
10^{-7}	0/4
Titer (Log_{10} $TCID_{50}$/mL)	≤ 2.50
Load (Log_{10} $TCID_{50}$)**	≤ 2.10
Log_{10} Reduction per carrier and per mL	≥ 3.50

*Dilution refers to the fold of dilution from the virus inoculum.
**0.40 mL of Undilute [10^0]

RESULTS (continued):

Table 3
Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls

Dilution*	Envirocleanse A Lot No. 020525	
	NE/VI	CT
10^{-2}	T/4	T/4
10^{-3}	4/4	0/4
10^{-4}	4/4	0/4

*Dilution refers to the fold of dilution from the mock inoculum.

Table 4
Virus Stock Titer Control

Dilution*	Virus Stock Titer Control
10^{-4}	4/4
10^{-5}	4/4
10^{-6}	4/4
10^{-7}	2/4
10^{-8}	0/4
10^{-9}	0/4
Titer (Log_{10} $TCID_{50}$/mL)	7.00

*Dilution refers to the fold of dilution from the virus inoculum.

Table 5
Viability Control Results

Cell Viability Control
0/4
Cells were viable; media was sterile

CONCLUSION:

According to the US Environmental Protection Agency and Health Canada, the test substance passes the test if the following criteria are met:

- The product must demonstrate a ≥ 3 Log_{10} reduction on each surface in the presence or absence of cytotoxicity, taking into account the level of neutralization when the minimum recoverable viral titer is ≥ 4.80 Log_{10} per carrier.
- If cytotoxicity is present, the virus control titer should be increased to demonstrate a ≥ 3 Log_{10} reduction in viral titer on each surface beyond the cytotoxic level and taking into account the level of neutralization.

When tested as described, Envirocleanse A, Lot No. 020525, passed the Virucidal Hard-Surface Efficacy Test with log a reduction of ≥ 3.50 when Avian Influenza A Virus (H5N1), containing 5.0% Fetal Bovine Serum, was exposed to the test substance for 2 minutes at 22°C and 40% RH.

All controls met the criteria for a valid test. These conclusions are based on observed data

Study Director: Semhar Fanuel _____ 03/31/25

Semhar Fanuel Date