



Study Report

GENERAL STUDY INFORMATION

STUDY TITLE Test for efficacy against African swine fever virus

Study Director Stuart Williams

Project number Dis P4/02

Sponsor Antec International,
Windham Road,
Chilton Industrial Estate,
Sudbury,
Suffolk,
CO10 2XD.

Test facility Institute for Animal Health
Pirbright Laboratory
Ash Road, Pirbright,
Surrey, GU24 0NF.

TEST SUBSTANCE IDENTITY

Test Substance Name: Virkon S

Lot/Batch(es) 6191 22/5/02

STUDY DATES

Experimental Start Date: July 18, 2002

Experimental End Date: July 24, 2002

OBJECTIVE

The objective of this study was to determine the effectiveness of a disinfectant to inactivate African swine fever virus at varying dilutions. A method designed by the Institute for Animal Health and adopted by the United Kingdom's Department for Environment, Food and Rural affairs for the UK registration of disinfectants was employed. Standard operating procedures extant in the containment laboratory were followed.



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Date 18/4/12



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STUDY MATERIALS

Test Organism	Isolate	Growth Medium	Cell line
ASF virus	LIL 20/1	Earles Saline	Primary cultures of Pig bone marrow

Cultures used

Primary pig bone marrow cultures grown in glass 6cm tubes with Earles Saline with 15% porcine serum as growth medium, in which red blood cells from the pig are present.

Reagents

Organic Soil Load Description: 1% foetal calf serum.

WHO Hard Water (342ppm hardness): 0.305g anhydrous calcium chloride and 0.139g magnesium dextrahydrate dissolved in distilled water, 10 ml foetal calf serum added. Mixture diluted to a final volume of 1L.

Phosphate Buffered Saline (PBS) pH 7.2 + 1% bovine serum.

TEST METHOD

Preparation of Test Organisms

African swine fever virus isolate LIL 20/1 which was isolated from a tick of the *Ornithodoros moubata* group collected in Malawi in 1983 and had been given 2 passes in pigs at Pirbright, was utilised from a stock stored at -70°C. It was used as a 1/10 suspension of an infected spleen ground up and suspended in PBS + 1% bovine serum. Primary pig bone marrow cells (PBM's) were grown in glass tubes in Earles saline + 15% porcine serum.

Preparation of Test Substance

Virkon S batch 6191 was received from the Sponsor on 22nd May 2002. The product was tested at 1:500, 1:600, 1:700 and 1:800. The product was made up to a 10% (W/V) solution and diluted to 1:50, 1:60, 1:70 and 1:80 in WHO Hard Water + 1% foetal calf serum. The test substance dilutions were homogenous as determined by visual observation.

Exposure Conditions

For each test substance dilution, 1 ml of the test organism (in PBS + 1% bovine serum) and 1 ml of the test substance was added to 8 ml of sterile WHO hard water (containing 1% foetal calf serum). Each mixture was held at 4°C for 30 min.

An untreated control was prepared by mixing 0.5 ml of the test organism with 4.5 ml of sterile WHO hard water + 1% foetal calf serum.

Test System Recovery

Following the completion of the exposure period, seven serial tenfold dilutions of the test substance mixtures and the untreated control were prepared in PBS + 1% bovine serum. These were inoculated into PBM tube cultures, dividing 1 ml of each dilution between three tubes.

An ASFV isolate from Malta, collected in 1978, of known titre was assayed in PBM cultures as positive control

Incubation and Observation

The tubes were held at 37°C for six days and examined daily for haemadsorption. Positive cultures were those in which haemadsorbing (HAD) cells are present. HAD cells are infected pig cells to which pig erythrocytes, present within the cultures, adhere.

Study Retention

Record Retention

The original raw data for this study will be archived at the Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Surrey, GU24 0NF for a minimum of seven years.

Test Substance Retention

The test substance will be retained at the Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Surrey, GU24 0NF for a minimum of six months. Antec International will be responsible for long term retention of the sample.

RESULTS

Control Results

Test Organism: ASFV LIL 20/1	Titre $10^{5.75}$ HAD ₅₀ / ml
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Test Results

Test Substance	Sample Dilution	Reduction of Titre (Log ₁₀ HAD ₅₀ / ml)
Virkon S Lot 6191	1:500	≥4.75
Virkon S Lot 6191	1:600	≥4.75
Virkon S Lot 6191	1:700	≥4.75
Virkon S Lot 6191	1:800	≥4.75

Conclusions

Virkon S (Lot 6191) demonstrated a ≥4.75 log₁₀ reduction of African swine fever virus at a dilution of 1:500 following a 30 minute exposure in the presence of 1% foetal calf serum in WHO hard water.

Virkon S (Lot 6191) demonstrated a ≥4.75 log₁₀ reduction of African swine fever virus at a dilution of 1:600 following a 30 minute exposure in the presence of 1% foetal calf serum in WHO hard water.



Virkon S (Lot 6191) demonstrated a $\geq 4.75 \log_{10}$ reduction of African swine fever virus at a dilution of 1:700 following a 30 minute exposure in the presence of 1% foetal calf serum in WHO hard water.

Virkon S (Lot 6191) demonstrated a $\geq 4.75 \log_{10}$ reduction of African swine fever virus at a dilution of 1:800 following a 30 minute exposure in the presence of 1% foetal calf serum in WHO hard water.

Produced by

G Hutchings

Reviewed by

Stuart Williams
Study Director

Date 5TH AUGUST 2002

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