AGRICULTURAL RESEARCH COUNCIL

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STUDY REPORT: DISINFECTANT EFFICACY TEST

GENERAL STUDY INFORMATION

STUDY TITLE: Test for efficacy against African swine fever virus

Client Pieter vd Westhuizen
Citrofresh

Test facility OVI:EDD

TEST SUBSTANCE IDENTITY:
Test substance name: Citrofresh

OBJECTIVE:
The objective of this study was to determine the effectiveness of the disinfectant to inactivate African swine fever virus at concentrations of 2% and 4%. The contact time was 30 minutes at 4°C.

Efficacy is demonstrated by a reduction of ASF virus titre by at least 4 $\log_{10.}$

STUDY MATERIALS

Test organism	Isolate	Growth medium	Cell line
ASFvirus	Spec 57	Earles Saline	Primary cultures of swine macrophages

Cultures used:
Primary swine macrophages grown in 96 well plates with Earles medium with 12.5 % swine serum as growth medium, in which red blood cells are present.

Reagents:
Organic Soil load description: 5% foetal calf serum.
WHO hard water (342 ppm hardness): 0.305 anhydrous calcium chloride and 0.139g magnesium dexahydrate dissolved in distilled water, 50ml foetal calf serum added. Mixture diluted to a final volume of 1L.
Phosphate buffered saline pH 7.4 + 1% normal bovine serum.

TEST METHOD:

Preparation of test organism:
African swine fever virus isolate Spec 57 was utilised from a stock stored at -70 °C.

Preparation of test substance:
Citrofresh was tested at 2% and 4% concentrations. The product was made up to 20% and 40% stocks

Exposure conditions:
0.5ml of the test organism (in PBS + 1% normal bovine serum) and 0.5ml of test substance was added to 4ml of sterile WHO hard water (containing 5% foetal calf serum).

Test system recovery:
Following the completion of the exposure period, ten serial tenfold dilutions of the test substance mixtures and untreated control were prepared in PBS + 1% bovine serum. These were inoculated into swine macrophage cultures.

Incubation and Observation:
The plates were held at 37°C for six days and examined daily for haemadsorption.

RESULTS:

Control results

Test organism:	Spec 57	Titre $10^{7.2}HAD_{50}$/ml

Test results

Test sample	Sample dilution	Reduction of titre ($Log_{10}HAD_{50}$/ml)
Citrofresh	2%	4.31
Citrofresh	4%	5.06

Conclusions:

Citrofresh demonstrated a >4 logs reduction of African swine fever virus at a dilution of 2% following incubation period of 30 minutes at 4 °C in the presence of 5% foetal calf serum in WHO hard water

Citrofresh demonstrated a >4 logs reduction of African swine fever virus at a dilution of 4% following incubation period of 30 minutes at 4 °C in the presence of 5% foetal calf serum in WHO hard water

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