MINISTERIO
DE CIENCIA E
INNOVACIÓN

INIA
Instituto Nacional de Investigación
y Tecnología Agraria y Alimentaria

SUBDIRECCIÓN GENERAL DE
INVESTIGACIÓN Y TECNOLOGÍA
CENTRO DE INVESTIGACIÓN
EN SANIDAD ANIMAL

Dear Dr. Adelina Francois:

Please find enclosed the report: "In Vitro" Evaluation of Virocid Disinfectant (CID LINES Innovative Hygiene Solutions) Efficiency against African Swine Fever Virus.

Best Regards,

Dr. Carmina Gallardo
Researcher Laboratory Coordinator at ASF-EURL

INSTITUTO NACIONAL DE INVESTIGACIÓN
Y TECNOLOGÍA AGRARIA Y ALIMENTARIA
CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL
SALIDA
18 JUL. 2011
NÚM. REGISTRO 979

CORREO ELECTRÓNICO:

Ctra. de Algete a El Casar, s/n
28130 Valdeolmos
MADRID
TEL: 91 620 23 00
FAX: 91 620 22 47

MINISTERIO
DE CIENCIA E
INNOVACIÓN

INIA
Instituto Nacional de Investigación
y Tecnología Agraria y Alimentaria

GOBIERNO
DE ESPAÑA

MINISTERIO
DE CIENCIA
E INNOVACIÓN

INIA
Instituto Nacional de Investigación
y Tecnología Agraria y Alimentaria

"*In vitro*" Evaluation of The Virocid Disinfectant (CID LINES, Innovative Hygiene Solutions) Efficiency Against African Swine Fever Virus

Performed by the EUROPEAN UNION REFERENCE LABORATORY FOR AFRICAN SWINE FEVER (URL)

URL for ASF, Centro de Investigación en Sanidad Animal
CISA-INIA, Valdeolmos 28130, Madrid, Spain. E-mail: arias@inia.es; gallardo@inia.es;; mfolgar@inia.es

CONTENTS

1. Objectives.

The aim of this study was to test "*in vitro*" the Virocid disinfectant *(CID LINES, Innovative Hygiene Solutions)* in order to evaluate the virucidal activity "*in vitro*" against African Swine Fever Virus (ASFV), in presence and in absence of interfering substance solution to simulate the organic soiling that can reduce the virucidal activity of disinfectants.

2. Critical points to be evaluated.

1. ***Cytotoxicity control*** of the disinfectant against Vero culture cells to detect any possible structural alteration of cells caused by the Virocid (not by the virus).

2. ***Virucidal activity against ASFV*** to determine the highest dilution effective of the Virocid disinfectant to inactivate or to reduce the virus activity, in presence or absence of interfering substance solution (inactivated Serum Fetal Bovine, iSFB).

3. ***Establishment of the minimum action time*** of the Virocid selected dilution, against ASFV, in presence or absence of interfering substance solution (inactivated Serum Fetal Bovine, iSFB).

3. Materials and Methods.

3.1.*Viral strain and cell cultures.* Vero (African green monkey kidney) cells were obtained from the American Type Culture Collection (ATCC) and grown in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% serum fetal bovine (SFB). The Vero cell culture-adapted ASFV Spanish strain BA71V belonging to p72-genotype I was used in the study. The virus was previously propagated and titrated by limit dilution on Vero cells.

3.2.*Cytotoxicity control of the disinfectant against Vero culture cells.* To determine the cytotoxicity, ten-fold dilutions of the disinfectant (from 10^{-1} to10^{-21}) were prepared in cell culture medium supplemented with 2% of iSFB, in a final volume of 500µl and added per duplicate on Vero cells monolayers in 48 cell culture plates. The

plates. The inoculated cells were fixed at 96 hours post-inoculation with 70% of Methanol and 30% of Acetone cold solution and subjected to Immunoperoxidase Technique (IPT), an immune-cytochemistry technique on fixed cells to determine the antibody-antigen complex through the action of the peroxidase enzyme. Briefly, the infected cells were incubated with a positive ASF reference serum sample diluted at 1/80 and the HRPO secondary antibody (1/5000) was used to detect the complexes antigen-antibody formed in the cytoplasm of Vero cells. The reaction was developed by means the substrate solution (3 aminoetilcarbazol-dimetilformamide diluted in Acetate buffer). The virucidal capacity was finally estimated by counting the number of the red stained cells against viral control wells.

3.3.3. Virucidal activity of Virocid disinfectant against ASFV using the effective working dilution of 0.5% (1:200) recommended by manufactures.

- Viral suspension dilutions. Two-fold dilutions from 1/20 up to 1/1280 of ASFV Ba71V isolate with an initial titer of $2{,}1x10^{12}$ $TCDI_{50}$/ml were prepared in cell culture medium supplemented with 2% of iSFB.
- The suspension test was performed in a volume of 10ml. It consisted of 1ml of sterile H_2O or 1ml of interfering substance (iSFB), 1ml of each viral suspension dilution and 8 ml of Virocid diluted 1/200 in medium supplemented with 2% iSFB. After mixing all the components, the tubes were incubated for 30 min at 10°C ± 1°C.
- Ten fold dilutions of suspension test were performed before adding the mixture onto Vero cells monolayers in 48 culture plates. The dilutions made in a range from 10^{-1} to 10^{-9} included the "not cytotoxic" Virocid dilution previously established.
- Finally, 200µl of each suspension test was inoculated by duplicate in Vero cells monolayers in 48 culture plates. Viral controls wells, without disinfectant, and cell control wells, without virus, were included in the assay.
- The cytopathic effect or structural changes in Vero cells were observed in the microscope against the control cells, from 24 to 96 hours post inoculation.
- To check the cytopathic effect due to the ASFV (not to the disinfectant), after 96 hours post inoculation, Vero cells were fixed with 70% of Methanol and 30% of Acetone cold solution and subjected to IPT according to the protocol established in 3.3.2. section. The specific cytopathic effect was estimated by the presence of red stained cells.

5. Conclusion.

The *"in vitro"* evaluation of the **Virocid disinfectant at the recommended dilution (0.5% or 1:200) by manufactures was able to reduce in a 100% the specific cytopathic effect** on Vero cells induced by the ASFV Ba71V isolate. As a final conclusion we can establish that the *"in vitro"* evaluation of the **Virocid disinfectant at the recommended dilution (0.5% or 1:200) was able to avoid the ASFV replication on Vero cells in absence and in presence of interfering substance solution (iSFB).**

Report Performed by: Drs. Raquel Martín, Carmina Gallardo and Marisa Arias.

Dr. Carmina Gallardo
Researcher Laboratory Coordinator at ASF-EURL

Dr. Marisa Arias
Technical Director of CISA-INIA,
contract manager conducting the ASF-URL