Testing the efficacy of a disinfectant against African Swine Fever Virus

ZOONO MICROBE SHIELD-Z71

Report: ZOONO MICROBE SHIELD-Z71
Project: 1600001970

This study was organized and financed by Hoseo Toxicology Research Center

Michiel Kroese, Wageningen Bioveterinary Research (WBVR), December 2019

WAGENINGEN
UNIVERSITY & RESEARCH

General information

Study title:	Testing the efficacy of disinfectants against African Swine Fever Virus
Disinfectant(s):	ZOONO MICROBE SHIELD-Z71, Batch B56700
Manufacturer:	Zoono Group Limited, 31 Hannigan Drive, St Johns, Auckland 1072, NEW ZEALAND
Study organized by:	Sang-Hee Jeong (Professor, DVM, PhD) Biomedical Science Research Institute RIC building 312, Hoseo University 20 Hoseo-ro, 79 beon-gil, Baebang, Asan-si, Chungcheongnam-do, 31499, Republic of Korea
Test directed by:	Mr. Michiel Kroese Wageningen Bioveterinary Research P.O. Box 65 8200 AB Lelystad The Netherlands
Sponsor:	DONG BANG COSMETIC 39, Gajaeul-ro 32beon-gil, Seo-gu Incheon, 22829, Republic of Korea
Test facility:	Wageningen Bioveterinary Research Animal Biosafety Level 4 Laboratory Facilities P.O. Box 65 8200 AB Lelystad The Netherlands

Introduction

Disinfection of objects, materials and environmental surfaces in animal handling operations threatened with African Swine Fever Virus (ASFV) is essential in the process of prevention and control of ASFV outbreaks. Before routine use, efficacy testing against ASFV in a quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants is recommended.

This study was performed to examine the efficacy of various disinfectants against ASFV. Additionally, disinfectant dilution studies were performed to explore the effective range of disinfection potency. A method designed by the ISO 9001 accredited facilities at Wageningen Bioveterinary Research was used. The method is based on the European Standard EN 14675: chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (Phase 2, step 1), 2015.

In the current study, one product manufactured by Zoono Group Limited, New Zealand, was tested: ZOONO MICROBE SHIELD-Z71.

Notes

AEC – 3-Amino-9-EthylCarbazole

ASFV - African Swine Fever Virus

FCS - Fetal Calf Serum

HIS - HIStidine

HRPO - HorseRadish PerOxidase

IPMA - Immune Peroxidase Monolayer Assay

NEN EN - NEderlandse Norm ENglish

PAMs - Porcine Alveolar Macrophages

RPMI - Roswell Park Memorial Institute

$TCID_{50}$ - The TCID50 (Median Tissue Culture Infectious Dose) is one of the methods used when verifying viral titres. TCID50 signifies the concentration at which 50% of the cells are infected when a test tube or well plate upon which cells have been cultured is inoculated with a diluted solution of viral fluid.

Materials and methods

Materials (as described in the test protocol)

1) Test virus: The Netherlands ’86 ASFV isolate grown on Porcine Alveolar Macrophages (PAMs)
2) Test cell: PAMs
3) Test medium for cell culture: RPMI supplemented with 5% FCS and 1% antibiotics
4) The diluent for disinfectant and virus: hard water according to NEN-EN 14675 containing 5% FCS
5) Medium for neutralizing disinfectant: RPMI 1640 supplemented with 10% FCS and 1% antibiotics

Methods (as described in the test protocol)

1) Preparation of virus
   a. The virus titer used was about 10^7 $TCID_{50}$/ml (+/- 0.5 log) being able to determine a 4 log_{10} reduction. The virus was diluted as follows: 1 ml ASFV + 19 ml hard water containing 5% FCS
2) Preparation of the disinfectant dilution
   a. Prepared the disinfectant to the requested dilution rates that need to be tested
   b. The disinfectant was diluted with hard water containing 5% FCS and were kept at 4°C. The concentrations of the disinfectant including the various test conditions used, were summarized in Table 1
   c. Cytotoxic effects were evaluated in the IPMA assay

Temp	4°C						10°C					
Soiling	none		low (5% FCS)		high		none		low (5% FCS)		high	
Time	5'	30'	5'	30'	5'	30'	5'	30'	5'	30'	5'	30'
Controls												
Water			x	x								
1% NaOH			x	x								
2% NaOH			x	x								
Disinfectant(s)												
ZOONO MICROBE SHIELD-Z71 X1.25			x	x								
ZOONO MICROBE SHIELD-Z71 X2.50			x	x								
ZOONO MICROBE SHIELD-Z71 X3.75			x	x								

Table 1: Concentrations of disinfectant(s) and corresponding test conditions

3) Preparation of cells for IPMA assay
   a. Primary cells were prepared from porcine lungs and stored in liquid nitrogen. When needed, cells were thawed and seeded in 96-well plates. These plates were used in the IPMA assay
4) Test procedure
   a. A sample of the product diluted with hard water containing 5% FCS was added to a test suspension of virus: 2.5 ml of virus suspension was mixed with 2.5 ml of disinfectant at the requested dilution, and is maintained in a water bath at 4°C ± 1°C for 30 min ± 10 s. During the latter incubation time every 10 min the mixture is mixed.
   b. At the end of the contact time, part of the mixture was taken and diluted tenfold in ice-cold medium to overcome the virucidal activity. These samples were directly diluted in six serial tenfold dilutions in cold medium (so the 6 dilutions to be tested are 10E-2 up to 10E-7). The dilutions were tested immediately or stored at -70°C.
5) IPMA assay (end point titration)

a. (After thawing) 100 µl of each dilution was inoculated (in 8-fold) into separate wells of a 96-well plate and 100µl PAM cells were added. The plates were incubated at 37°C in a humidified incubator with 5% CO_2 for four days. After four days of incubation, the plates were washed, dried and frozen. Subsequently, the cells were fixed, plates were washed again and stained using ASF-HIS, Mouse-anti-Swine IgG/HRPO conjugate and AEC (IPMA protocol).
b. Plates were read microscopically and judged for the presence of virus. Titers were calculated according to Spearman-Kärber.

Test Evaluation

1) To test the titre of the virus used, a hard water control was included, which means that hard water was used instead of a disinfectant.
2) Two positive controls as disinfectants, NaOH 1% and 2%, were included in the test. The reduction after 30 minutes of the positive controls should be within +/- 3sd of the mean valid for these controls for a valid test. Our passed experience showed that formaldehyde 0.7% was toxic for PAMs and was therefore replaced by NaOH 1% and 2%.
3) The reduction in ASFV titre, induced by each dilution of the disinfectant, was calculated by subtracting the ASF virus titre, measured in the mix with disinfectant, from the titre measured in the water control.
4) A minimum of a 4 $\log_{10}$ reduction reduction after 30 minutes at 10°C is needed for a disinfectant to pass the test. In the current study, these conditions were not included.

Test validation

1) According to the NEN-EN 14675 norm, the validation of the used method regarding the control of efficiency for suppression of disinfectant activity require that the difference with the viral suspension assay does not exceed 0.5 $\log_{10}$. It is impracticable to test as there is no suspension test available for ASFV either with or without primary macrophages.

Note: this effectivity test is built upon a biological system containing living cells and challenging virus. The outcome of the test is therefore dependent on the effect of the disinfectant(s) on the virus as well as on the cells. The difference between the viral titer obtained from cells exposed to the disinfectant at a non-cytotoxic concentration and the viral titer obtained from cells non-exposed to the disinfectant should be lower than 1 $\log_{10}$ according to the NEN EN 14675 norm. It is our view that a treatment of cells at a non-cytotoxic concentration of the disinfectant should by definition yield the same titer as at non-exposed cells, otherwise it is toxic. Therefore, it does not make any sense scientifically to test this issue. Unquestionably, we are aware of the possible effect of the disinfectant on either cells or virus. In case the cells are affected by the disinfectants tested, no conclusive data can be generated relating to the effect of the disinfectant on the virus applied according to the NEN-EN 14675 norm. The NEN EN 14675 norm does not define a differentiation between these two effects.

Results

Controls

The hard water controls with 5% FCS as soiling agent at 4°C for 5 and 30 minutes showed titres with values satisfactory for the test. The reduction observed with the two positive reduction controls were within reach of validity of the test performed. See table 2 for the $\log_{10}$ values of all controls included.

Temp	4°C						10°C					
Soiling	none		low (5% FCS)		high		none		low (5% FCS)		high	
Time	5'	30'	5'	30'	5'	30'	5'	30'	5'	30'	5'	30'
Controls												
Water			7.50	7.00								
1% NaOH			3.38	2.75								
2% NaOH			2.88	2.75								

Table 2: $\log_{10}$ values of controls

Disinfectant(s)

The result of the effect of the disinfectant ZOONO MICROBE SHIELD-Z71 on ASFV in low soiling conditions is displayed in Table 3. No cytotoxicity was observed in PAMs using ZOONO MICROBE SHIELD-Z71, see also appendix 1: raw data.

Temp	4°C						10°C					
Soiling	none		low (5% FCS)		high		none		low (5% FCS)		high	
Time	5'	30'	5'	30'	5'	30'	5'	30'	5'	30'	5'	30'
Disinfectant(s)												
ZOONO MICROBE SHIELD-Z71 X1.25			≤2.50	≤2.50								
ZOONO MICROBE SHIELD-Z71 X2.50			2.75	≤2.50								
ZOONO MICROBE SHIELD-Z71 X3.75			≤2.50	≤2.50								

Table 3: $\log_{10}$ values of disinfectant(s)

Conclusions

In order to pass the test, a disinfectant should show a minimum of a 4 $\log_{10}$ reduction in titre after 30 min at 10°C (obligatory test conditions NEN EN 14675 norm).32

The disinfectant ZOONO MICROBE SHIELD-Z71 showed a strong effect on the titre of ASFV in low soiling conditions at 4°C during 5 and 30 min. incubation. The maximum reduction was ≥5.00 $\log_{10}$, see table 4.

Temp	4°C						10°C					
Soiling	none		low (5% FCS)		high		none		low (5% FCS)		high	
Time	5'	30'	5'	30'	5'	30'	5'	30'	5'	30'	5'	30'
Disinfectant(s)												
ZOONO MICROBE SHIELD-Z71 X1.25			≥5.00	≥4.50								
ZOONO MICROBE SHIELD-Z71 X2.50			4.75	≥4.50								
ZOONO MICROBE SHIELD-Z71 X3.75			≥5.00	≥4.50								

Table 4: reduction values of disinfectant(s)

Appendix 1: raw data

Order: 2019 ASFV Hoseo-2													
Water							**Water**						
Temp: 4°C							Temp: 4°C						
Soiling: low (5% FCS)							Soiling: low (5% FCS)						
Time (min): 5							Time (min): 30						
TCID50/ml: 7.50							TCID50/ml: 7.00						
	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	
A	pos	pos	pos	pos	neg	neg	pos	pos	pos	pos	pos	neg	A
B	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	neg	B
C	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	neg	neg	C
D	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos	neg	D
E	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	neg	neg	E
F	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos	neg	F
G	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	neg	neg	G
H	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos	neg	H

Order: 2019 ASFV Hoseo-2													
1% NaOH							**1% NaOH**						
Temp: 4°C							Temp: 4°C						
Soiling: low (5% FCS)							Soiling: low (5% FCS)						
Time (min): 5							Time (min): 30						
TCID50/ml: 3.38							TCID50/ml: 2.75						
	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	
A	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	A
B	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	B
C	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	C
D	pos	neg	neg	neg	neg	neg	pos	neg	neg	neg	neg	neg	D
E	pos	neg	neg	neg	neg	neg	pos	neg	neg	neg	neg	neg	E
F	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	F
G	pos	neg	neg	neg	neg	neg	neg	neg	pos	neg	neg	neg	G
H	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	H

Order: 2019 ASFV Hoseo-2													
2% NaOH							**2% NaOH**						
Temp: 4°C							Temp: 4°C						
Soiling: low (5% FCS)							Soiling: low (5% FCS)						
Time (min): 5							Time (min): 30						
TCID50/ml: 3.25							TCID50/ml: 3.00						
	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	
A	neg	pos	neg	pos	neg	neg	neg	neg	neg	neg	neg	neg	A
B	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	B
C	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	C
D	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	D
E	neg	pos	pos	neg	neg	neg	neg	pos	neg	neg	neg	neg	E
F	neg	neg	pos	neg	neg	neg	neg	pos	neg	neg	neg	neg	F
G	neg	pos	neg	neg	neg	neg	neg	pos	neg	neg	neg	neg	G
H	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	H

WAGENINGEN
UNIVERSITY & RESEARCH

Order: 2019 ASFV Hoseo-2													
ZOONO MICROBE SHIELD-Z71 X1.25							**ZOONO MICROBE SHIELD-Z71 X1.25**						
Temp: 4°C							Temp: 4°C						
Soiling: low (5% FCS)							Soiling: low (5% FCS)						
Time (min): 5							Time (min): 30						
TCID50/ml: ≤2.50							TCID50/ml: ≤2.50						
	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	
A	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	A
B	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	B
C	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	C
D	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	D
E	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	E
F	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	F
G	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	G
H	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	H

Order: 2019 ASFV Hoseo-2													
ZOONO MICROBE SHIELD-Z71 X2.50							**ZOONO MICROBE SHIELD-Z71 X2.50**						
Temp: 4°C							Temp: 4°C						
Soiling: low (5% FCS)							Soiling: low (5% FCS)						
Time (min): 5							Time (min): 30						
TCID50/ml: 2.75							TCID50/ml: ≤2.50						
	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	
A	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	A
B	neg	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	B
C	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	C
D	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	D
E	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	E
F	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	F
G	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	G
H	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	H

Order: 2019 ASFV Hoseo-2													
ZOONO MICROBE SHIELD-Z71 X3.75							**ZOONO MICROBE SHIELD-Z71 X3.75**						
Temp: 4°C							Temp: 4°C						
Soiling: low (5% FCS)							Soiling: low (5% FCS)						
Time (min): 5							Time (min): 30						
TCID50/ml: ≤2.50							TCID50/ml: ≤2.50						
	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	
A	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	A
B	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	B
C	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	C
D	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	D
E	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	E
F	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	F
G	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	G
H	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	H

*Cytotoxicity