Testing the efficacy of a disinfectant against African Swine Fever Virus

D7

Report: D7
Project: 1600002127

This study was organized and financed by Decon7 Systems, LLC

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General information

Study title:	Testing the efficacy of disinfectants against African Swine Fever Virus
Disinfectant(s):	D7, batch part 1: 04-02-19-01, batch part 2: 04-02-19-01, batch part 3: 04-01-19-01. Manufacturer: Decon7 Systems, LLC
Study organized by:	Decon7 Systems, LLC 8541 East Anderson Drive Suite 106 Scottsdale Arizona 85255 USA
Test directed by:	Mr. Michiel Kroese Wageningen Bioveterinary Research P.O. Box 65 8200 AB Lelystad The Netherlands
Sponsor:	Decon7 Systems, LLC
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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, D7, a product of Decon7 Systems, LLC was tested.

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 disinfectants and virus: hard water according to NEN-EN 14675
5) Soiling solutions: according to NEN-EN 14675
6) 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 is about 10^7 $TCID_{50}$/ml (+/- 0.5 log) being able to determine a 4 log_{10} reduction
2) Preparation of the disinfectants dilution
   a. Prepare the disinfectant to 1.25 concentrated dilutions of the requested dilution rates that need to be tested
   b. The disinfectants shall be diluted with hard water, and the concentrations of the individual disinfectants including the various test conditions used, are summarized in Table 1
   c. Cytotoxic effects will be evaluated in the IPMA assay

Temp	10°C/50F								
Soiling	none			low			high		
Time	1'	10'	30'	1'	10'	30'	1'	10'	30'
Controls									
Water				x	x	x			
1% NaOH				x	x	x			
2% NaOH				x	x	x			
Disinfectant(s)									
D7 undiluted				x	x	x			
D7 diluted 1:5				x	x	x			
D7 diluted 1:10				x	x	x			

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

3) Preparation of cells for IPMA assay
   a. Primary cells are prepared from porcine lungs and stored in liquid nitrogen. When needed, cells are thawed and seeded in 96-well plates. These plates are used in the IPMA assay
4) Test procedure
   a. A sample of the product diluted with hard water is added to a test suspension of virus: one part of virus suspension is mixed with one part of hard water containing soiling agent. Eight parts of disinfectant dilution (1.25x the requested dilution to give the correct final concentration) is added and is placed in a water bath and the incubation time is started. The mixture is maintained in a water bath at 10°C/50F ± 1°C/1.8F for 1, 10, and 30 min. ± 10s (Table 1)
   b. At the end of the contact time, part of the mixture is taken and diluted tenfold in ice-cold medium to overcome the virucidal activity. These samples are

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 are tested immediately or stored at -70°C

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

Test Evaluation

1) To test the titre of the virus used, a hard water control is included, which means that hard water is used instead of a disinfectant.
2) Two positive controls as disinfectants, NaOH 1% and 2%, are included in the test. The reduction after 30 minutes of the positive controls should be within +/- 3 sd 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, is 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 at 10°C/50F at low soiling conditions showed ASFV titres satisfactory for the test at all time points. The reduction on ASFV titre 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	10°C/50F								
Soiling	none			low			high		
Time	1'	10'	30'	1'	10'	30'	1'	10'	30'
Controls									
Water				7.50	7.38	7.38			
1% NaOH				4.88	3.13	3.38			
2% NaOH				3.00	2.88	2.88			

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

Disinfectant(s)

The result of the effect of the disinfectant D7 on ASFV at 10°C/50F at low soiling conditions is displayed in Table 3. All dilutions of D7 were able to reduce ASFV titre with a minimum of 4 $\log_{10}$ reduction. Cytotoxicity was observed in PAMs with D7, see the raw data in the appendix.

Temp	10°C/50F								
Soiling	none			low			high		
Time	1'	10'	30'	1'	10'	30'	1'	10'	30'
Disinfectant(s)									
D7 undiluted				2.75*	≤2.50*	≤2.50*			
D7 diluted 1:5				2.75*	≤2.50*	≤2.50*			
D7 diluted 1:10				≤2.50*	≤2.50*	≤2.50*			

* Cytotoxicity

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/50F (obligatory test conditions NEN EN 14675 norm).

The disinfectant D7 at all dilutions (undiluted, 1:5, and 1:10) at 10°C/50F during 1, 10, and 30 minutes incubation showed a clear reduction in ASFV titre being at least 4 $\log_{10}$. The disinfectant D7 at all dilutions passed the NEN EN 14675 norm.

Appendix 1: Raw data

Order: 2019 ASFV Decon7 Systems-1													
Water							**water**						
Temp: 10°C							Temp: 10°C						
Soiling: low							Soiling: low						
Time (min): 1							Time (min): 10						
TCID50/ml: 7.50							TCID50/ml: 7.38						
	1	2	3	4	5	6	7	8	9	10	11	12	
A	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos	neg	A
B	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos	pos	neg	B
C	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos	neg	C
D	pos	pos	pos	pos	neg	neg	pos	pos	pos	pos	pos	neg	D
E	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos	neg	E
F	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos	neg	F
G	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	neg	G
H	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos	neg	H

Order: 2019 ASFV Decon7 Systems-1													
Water													
Temp: 10°C							Temp:						
Soiling: low							Soiling:						
Time (min): 30							Time (min):						
TCID50/ml: 7.38							TCID50/ml:						
	1	2	3	4	5	6	7	8	9	10	11	12	
A	pos	pos	pos	pos	neg	neg							A
B	pos	pos	pos	pos	pos	neg							B
C	pos	pos	pos	pos	pos	neg							C
D	pos	pos	pos	pos	pos	neg							D
E	pos	pos	pos	pos	neg	neg							E
F	pos	pos	pos	pos	pos	neg							F
G	pos	pos	pos	pos	pos	pos							G
H	pos	pos	pos	pos	pos	neg							H

Order: 2019 ASFV Decon7 Systems-1													
1% NaOH							**1% NaOH**						
Temp: 10°C							Temp: 10°C						
Soiling: low							Soiling: low						
Time (min): 1							Time (min): 10						
TCID50/ml: 4.88							TCID50/ml: 3.13						
	1	2	3	4	5	6	7	8	9	10	11	12	
A	pos	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	A
B	pos	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	B
C	pos	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	C
D	pos	pos	pos	neg	neg	neg	pos	neg	neg	neg	neg	neg	D
E	pos	pos	pos	neg	neg	neg	pos	pos	neg	neg	neg	neg	E
F	pos	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	F
G	pos	pos	pos	neg	neg	neg	pos	neg	neg	neg	neg	neg	G
H	pos	pos	neg	neg	neg	neg	pos	neg	neg	neg	neg	neg	H

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Order: 2019 ASFV Decon7 Systems-1

1% NaOH													
Temp: 10°C							Temp:						
Soiling: low							Soiling:						
Time (min): 30							Time (min):						
TCID50/ml: 3.38							TCID50/ml:						
	1	2	3	4	5	6	7	8	9	10	11	12	
A	neg	neg	pos	neg	neg	neg							A
B	neg	neg	neg	neg	neg	neg							B
C	neg	neg	neg	neg	neg	neg							C
D	neg	neg	neg	neg	neg	neg							D
E	neg	neg	neg	neg	neg	neg							E
F	pos	pos	neg	neg	neg	neg							F
G	pos	pos	neg	neg	neg	neg							G
H	pos	neg	pos	neg	neg	neg							H

Order: 2019 ASFV Decon7 Systems-1

2% NaOH							**2% NaOH**						
Temp: 10°C							Temp: 10°C						
Soiling: low							Soiling: low						
Time (min): 1							Time (min): 10						
TCID50/ml: 3.00							TCID50/ml: 2.88						
	1	2	3	4	5	6	7	8	9	10	11	12	
A	neg	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	pos	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	C
D	neg	neg	neg	neg	neg	neg	neg	pos	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	pos	neg	neg	neg	neg	F
G	neg	pos	neg	neg	neg	neg	pos	neg	neg	neg	neg	neg	G
H	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	H

Order: 2019 ASFV Decon7 Systems-1

2% NaOH													
Temp: 10°C							Temp:						
Soiling: low							Soiling:						
Time (min): 30							Time (min):						
TCID50/ml: 2.88							TCID50/ml:						
	1	2	3	4	5	6	7	8	9	10	11	12	
A	neg	neg	neg	neg	neg	neg							A
B	neg	neg	neg	neg	neg	neg							B
C	neg	pos	neg	neg	neg	neg							C
D	neg	neg	neg	neg	neg	neg							D
E	neg	neg	neg	neg	neg	neg							E
F	pos	neg	neg	neg	neg	neg							F
G	pos	neg	neg	neg	neg	neg							G
H	neg	neg	neg	neg	neg	neg							H

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Order: 2019 ASFV Decon7 Systems-1													
D7 undiluted							**D7 undiluted**						
Temp: 10°C							Temp: 10°C						
Soiling: low							Soiling: low						
Time (min): 1							Time (min): 10						
TCID50/ml: 2.75							TCID50/ml: ≤2.50						
	1*	2	3	4	5	6	7*	8*	9*	10*	11*	12	
A	neg	neg	neg	neg	pos	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	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	H

Order: 2019 ASFV Decon7 Systems-1													
D7 undiluted													
Temp: 10°C							Temp:						
Soiling: low							Soiling:						
Time (min): 30							Time (min):						
TCID50/ml: ≤2.50							TCID50/ml:						
	1*	2*	3*	4*	5*	6*	7	8	9	10	11	12	
A	neg	neg	neg	neg	neg	neg							A
B	neg	neg	neg	neg	neg	neg							B
C	neg	neg	neg	neg	neg	neg							C
D	neg	neg	neg	neg	neg	neg							D
E	neg	neg	neg	neg	neg	neg							E
F	neg	neg	neg	neg	neg	neg							F
G	neg	neg	neg	neg	neg	neg							G
H	neg	neg	neg	neg	neg	neg							H

Order: 2019 ASFV Decon7 Systems-1													
D7 X5							**D7 X5**						
Temp: 10°C							Temp: 10°C						
Soiling: low							Soiling: low						
Time (min): 1							Time (min): 10						
TCID50/ml: 2.75							TCID50/ml: ≤2.50						
	1*	2	3	4	5	6	7*	8*	9*	10*	11*	12*	
A	neg	neg	pos	neg	neg	neg	neg	pos	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	pos	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

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Order: 2019 ASFV Decon7 Systems-1													
D7 X5													
Temp: 10°C							Temp:						
Soiling: low							Soiling:						
Time (min): 30							Time (min):						
TCID50/ml: ≤2.50							TCID50/ml:						
	1*	2*	3*	4*	5*	6*	7	8	9	10	11	12	
A	neg	neg	neg	neg	neg	neg							A
B	neg	neg	neg	neg	neg	neg							B
C	neg	neg	neg	neg	neg	neg							C
D	neg	neg	neg	neg	neg	neg							D
E	neg	neg	neg	neg	neg	neg							E
F	neg	neg	neg	neg	neg	neg							F
G	neg	neg	neg	neg	neg	neg							G
H	neg	neg	neg	neg	neg	neg							H

Order: 2019 ASFV Decon7 Systems-1													
D7 X10							**D7 X10**						
Temp: 10°C							Temp: 10°C						
Soiling: low							Soiling: low						
Time (min): 1							Time (min): 10						
TCID50/ml: ≤2.50							TCID50/ml: ≤2.50						
	1*	2	3	4	5	6	7*	8*	9*	10*	11*	12*	
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	pos	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	pos	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 Decon7 Systems-1													
D7 X10													
Temp: 10°C							Temp:						
Soiling: low							Soiling:						
Time (min): 30							Time (min):						
TCID50/ml: ≤2.50							TCID50/ml:						
	1*	2*	3*	4*	5*	6*	7	8	9	10	11	12	
A	neg	neg	neg	neg	neg	neg							A
B	neg	neg	neg	neg	neg	neg							B
C	neg	neg	neg	neg	neg	neg							C
D	neg	neg	neg	neg	neg	neg							D
E	neg	neg	neg	neg	neg	neg							E
F	neg	neg	neg	neg	neg	neg							F
G	neg	neg	neg	neg	neg	neg							G
H	neg	neg	neg	neg	neg	neg							H

*Cytotoxicity