

TEST REPORT**DETERMINATION OF THE VIRUCIDAL ACTIVITY OF THE BLUE
PRODUCT ACCORDING TO THE EN 17122:2019 STANDARD**Delivered to : **M OURSEL**For : **WeCleen
PEPINIERE CLEANTECH
AVENUE L. PHILIBERT
13 100 AIX-EN-PROVENCE**

Date of request: 03/03/2022

Study number: n°060D22-2022

VIRUCIDAL TESTS:

According to the NF EN 17122 (November 2019) standard – chemical antiseptics and disinfectants – quantitative non-porous surface test for the evaluation of virucidal activity of chemical disinfectants used in the veterinary area (phase 2, test 2).

Tests using the BLUE product, against the *canine parvovirus*.

This test report included 13 pages.



Study completion date: 04/07/2022

Stephanie MOROT - BIZOT
PhD in Microbiology

Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director

SUMMARY

1	PERFORMING LABORATORY	3
2	PRODUCT IDENTITY	3
3	EXPERIMENTAL CONDITIONS	3
4	VIRAL TITRE:	3
5	VALIDATION	4
6	VIRUCIDAL ASSAYS	5
7	VALIDATION OF THE METHODOLOGY	6
8	CONCLUSION	6
9	TECHNICAL APPENDIX 1	7
10	TECHNICAL APPENDIX 2	8

Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
 ESPACE INDUSTRIEL DE BEAUPRE
 3 RUE DE LA TERRE ROUGE
 25 220 ROCHE LEZ BEAUPRE
 FRANCE

2 PRODUCT IDENTITY

SAMPLE	BATCH
BLUE	NC

Expiration date: non communicated

Manufacturer: WeCleen

Date of manufacture: non communicated

Storage conditions: according to the sponsor recommendations

Active substances: non communicated

Appearance of the product: liquid, colorless

Product diluent: none, ready-to-use-product

Date of delivery of the product: 03/07/2022

Date of tests: from 03/07/2022 to 04/07/2022

3 EXPERIMENTAL CONDITIONS

Temperature used during the assays: 25°C ± 1°C

Titration units: log TCID₅₀ for canine parvovirus

Exposure time: 1 min – 2 min

Diluent used for the product: distilled water

Final concentrations tested: 100% - 80%

Viral strain: canine parvovirus ATCC VR-943, grown on fibroblast cells, at 37°C, under 5% CO₂ atmosphere

Organic soil load: 3 g/L bovine serum albumin (clean conditions), 3 g/L BSA + 3 mL/L sheep erythrocytes (dirty conditions)



Product stability: good

Stop solution: cold shock

4 VIRAL TITRE:

Viral titers are expressed in log TCID₅₀:

- For canine parvovirus, titer = 7,250 log TCID₅₀

Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

5 VALIDATION

a) CYTOTOXICITY

The cell toxicity was observed until to the dilution 10^{-2} .

b) SENSIBILITY ASSAYS

The viruses were titrated on cell cultures untreated with the product (indicator cell line) and titrated on cell cultures treated with the product. According to the European standard EN 17122, the BLUE product used at the dilution of 10^{-3} does not have an effect on the viruses titration method (the difference between viral titers must be $< 1,0 \log$):

CLEAN CONDITIONS

CANINE PARVOVIRUS		Viral titer (log TCID ₅₀)		
Dilution		Untreated cell cultures	Treated cell cultures	Viral titer (log TCID ₅₀)
BLUE	10^{-3}	7,250	6,750	0,500

DIRTY CONDITIONS

CANINE PARVOVIRUS		Viral titer (log TCID ₅₀)		
Dilution		Untreated cell cultures	Treated cell cultures	Viral titer (log TCID ₅₀)
BLUE	10^{-3}	7,250	6,500	0,750

c) VIRAL SUSPENSION AND WATER CONTROL

CLEAN CONDITIONS

Product	Organic soil load	Viral titer (log TCID ₅₀)	Viral titer after drying time (log TCID ₅₀)	Drying time (min)	Difference
BLUE	3 g/L BSA	TRIAL 1: 7,250	TRIAL 1: 6,750	50 min	0,500
		TRIAL 2: 7,250	TRIAL 2: 6,625	54 min	0,625



DIRTY CONDITIONS

Product	Organic soil load	Viral titer (log TCID ₅₀)	Viral titer after drying time (log TCID ₅₀)	Drying time (min)	Difference
BLUE	3 g/L BSA + 3 mL/L SE	TRIAL 1: 7,250	TRIAL 1: 6,875	51 min	0,375
		TRIAL 2: 7,250	TRIAL 2: 6,500	49 min	0,750

d) VALIDATIONS OF THE COLD SHOCK METHOD

CLEAN CONDITIONS

Product	Organic soil load	Viral titer (log TCID ₅₀)	Difference with the viral suspension
BLUE	3 g/L BSA	TRIAL 1: 7,250	0,000
		TRIAL 2: 7,250	0,000

Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

DIRTY CONDITIONS

Product	Organic soil load	Viral titer (log TCID ₅₀)	Difference with the viral suspension
BLUE	3 g/L BSA + 3 mL/L SE	TRIAL 1: 7,250	0,000
		TRIAL 2: 7,250	0,000

6 VIRUCIDAL ASSAYS

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is $\geq 3,0$ log.

CLEAN CONDITIONS

TRIAL 1 – The viral suspension (water control) was titrated at 6,750 log TCID₅₀.

Drying time: 50 min

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID ₅₀)	Viral titer reduction
BLUE	100%	1 min	25°C	2,750	4,000
	80%	2 min		3,250	3,500
		1 min		4,125	2,625

TRIAL 2 - The viral suspension (water control) was titrated at 6,625 log TCID₅₀.

Drying time: 54 min

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID ₅₀)	Viral titer reduction
BLUE	100%	1 min	25°C	2,375	4,250
	80%	2 min		2,750	3,875
		1 min		4,125	2,500

DIRTY CONDITIONS

TRIAL 1 – The viral suspension (water control) was titrated at 6,875 log TCID₅₀.



Drying time: 51 min

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID ₅₀)	Viral titer reduction
BLUE	100%	2 min	25°C	3,000	3,875
		1 min		4,125	2,750
	80%	2 min		4,625	2,250

TRIAL 2 - The viral suspension (water control) was titrated at 6,500 log TCID₅₀.

Drying time: 49 min

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID ₅₀)	Viral titer reduction
BLUE	100%	2 min	25°C	2,500	4,000
		1 min		3,750	2,750
	80%	2 min		4,500	2,000

Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

7 VALIDATION OF THE METHODOLOGY



The assays were validated as required by the European standard EN 17122:

- The viral titer of the suspension tests were sufficient in order to observe a reduction of 3 log after time exposure with the product:
 - 7,250 log TCID₅₀ for canine parvovirus
- The BLUE product does not have a cytotoxic effect on the fibroblast cells.
- The BLUE product does not affect the infectious capacity of the canine parvovirus:
 - In clean conditions, the differences in viral titers between the virus inoculated on fibroblast cells and the virus inoculated on the fibroblast cells treated with the product was $\leq 1,0$ log (0,500 log).
 - In dirty conditions, the differences in viral titers between the virus inoculated on fibroblast cells and the virus inoculated on the fibroblast cells treated with the product was $\leq 1,0$ log (0,750 log).

8 CONCLUSION

The assays performed with the BLUE product:

- **Demonstrated a virucidal activity on the canine parvovirus from the concentration 100%**, following a **1 min** exposure period, and **from the concentration 80%**, following a **2 min** exposure period as required by the European standard EN 17122, at 25°C, in low-dirty conditions.
- **Demonstrated a virucidal activity on the canine parvovirus from the concentration 100%**, following a **2 min** exposure period, as required by the European standard EN 17122, at 25°C, in high-dirty conditions.

Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

9 TECHNICAL APPENDIX 1

Cell line for canine parvovirus: fibroblast cells (ATCC, ref. CRL-1542)

Viral strain: canine parvovirus, ATCC VR-943

Buffers and media:

- PBS buffer: sodium chloride, Panreac, ref. 141659.1211, batch n° 0000204679; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch n° 1019K01021V
- MEM media, Sigma Aldrich, ref. 0268, batch n° 040M8301
- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB9336
- Fetal calf sera, Sigma Aldrich, F7524, batch n° 098K3397



Reagents:

- Albumine bovine sera, Sigma Aldrich, ref. 05479, batch n° STBB7838V
- Sheep erythrocytes, Analytic Lab, ref. 08449, batch n° bcbj3984V

Inactivation solution:

- formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

STAINLESS STEEL SURFACES – discs of 2 cm diameter in stainless steel 1.4301, 2B (ATELCO Technologies).

Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

10 TECHNICAL APPENDIX 2

Table A1 – Canine parvovirus titer, by Spaerman-Kärber method:

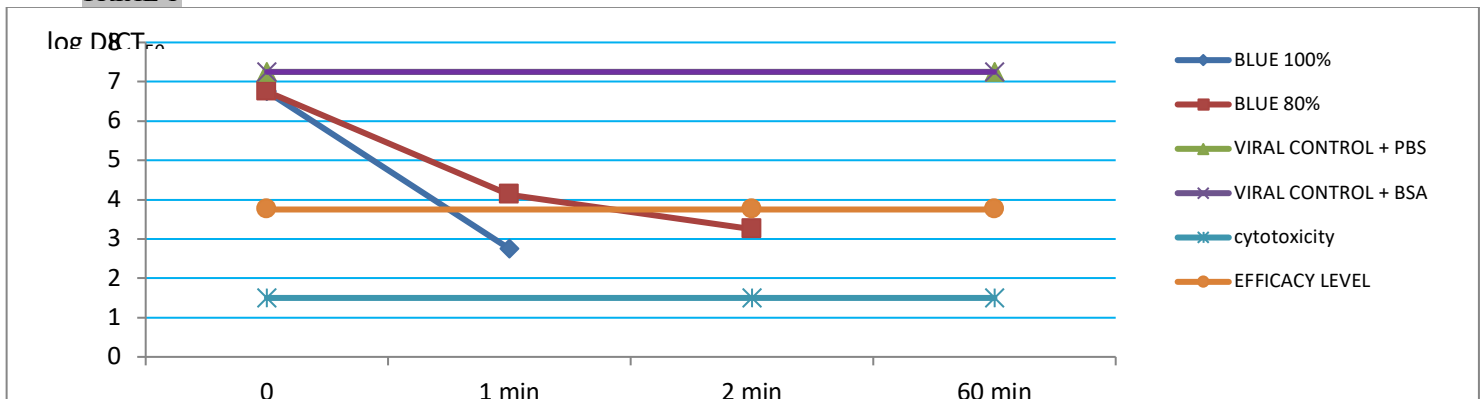
Log DICT₅₀ = 7,250

Dilution (- log)	Result	% positive results
-3	44444444	100
-4	44444444	100
-5	44444444	100
-6	44444444	100
-7	44444400	75
-8	00000000	0
-9	00000000	0
-10	00000000	0
Sum of the % of positives cultures		475

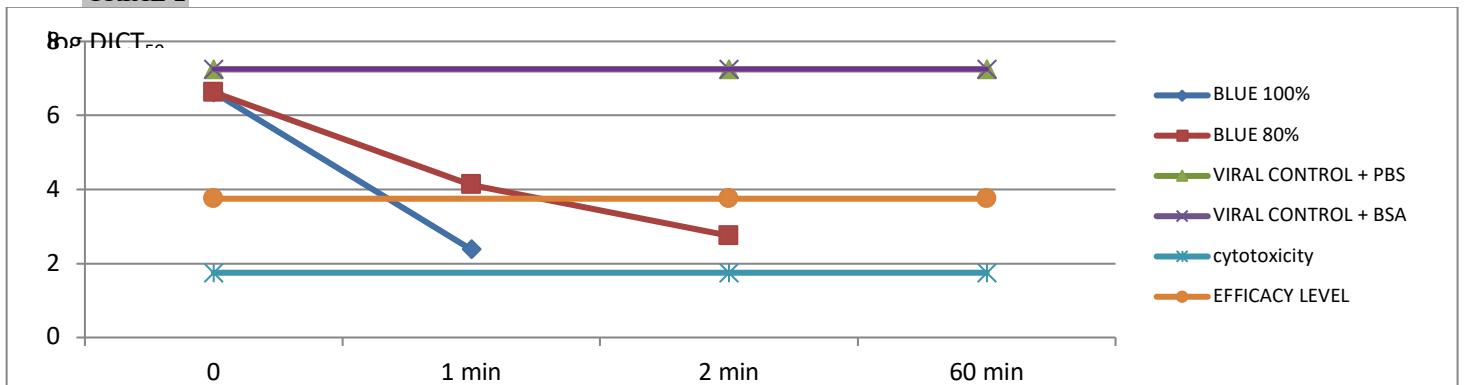
In clean conditions

Chart 1 – Trials on canine parvovirus:

TRIAL 1



TRIAL 2





Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

Table A2 - Cells sensitivity to the virus :

Product	Dilution	Organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	
BLUE	10 ⁻³	3 g/L BSA	Untreated cells	4444	4444	4444	4444	4444	4444	4444	0000	0000
			Treated cells	4444	4444	4444	4444	4444	4400	0000	0000	0000

Table A3 — Results on canine parvovirus in low-dirty conditions

Product	Concentration	ORGANIC SOIL LOAD	CYTOTOXICITY LEVEL	Lg TCID ₅₀				Reduction
				0	1 min	2 min	60 min	
BLUE TRIAL 1	100,00%	3 g/L BSA	1,500	6,750	2,750	N.T.	N.T.	1 min LOG R = 4,000
	80,00%		N.T.		4,125	3,250	N.T.	2 min LOG R = 3,500
BLUE TRIAL 2	100,00%	3 g/L BSA	1,750	6,625	2,375	N.T.	N.T.	1 min LOG R = 4,250
	80%%		N.T.		4,125	2,750	N.T.	2 min LOG R = 3,875
VIRAL CONTROL OF INFECTIVITY TRIAL 1	N.A.	PBS	N.A.	7,250	N.T.	N.T.	7,250	
VIRAL CONTROL OF INFECTIVITY TRIAL 1	N.A.	3 g/L BSA	N.A.	7,250	N.T.	N.T.	7,250	
VIRAL CONTROL OF INFECTIVITY TRIAL 2	N.A.	PBS	N.A.	7,250	N.T.	N.T.	7,250	
VIRAL CONTROL OF INFECTIVITY TRIAL 2	N.A.	3 g/L BSA	N.A.	7,250	N.T.	N.T.	7,250	
CELL SENSITIVITY TO THE VIRUS	10 ⁻²	N.A.	Untreated cells	7,250	N.T.	N.T.	7,250	
		N.A.	Treated cells	6,750	N.T.	N.T.	6,750	





Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

Table A4 — Raw results

TRIAL 1	Concentration	ORGANIC SOIL LOAD	EXPOSURE TIME	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	
BLUE TRIAL 1	100,00%	3 g/L BSA	1 min	4444	4444	4400	0000	0000	0000	0000	0000	0000	0000
	4444			4444	0000	0000	0000	0000	0000	0000	0000	0000	
	80,00%		2 min	4444	4444	4444	0000	0000	0000	0000	0000	0000	0000
				4444	4444	4400	0000	0000	0000	0000	0000	0000	0000
	1 min	4444	4444	4444	4444	0000	0000	0000	0000	0000	0000		
		4444	4444	4444	4000	0000	0000	0000	0000	0000	0000		
WATER CONTROL				4444	4444	4444	4444	4444	4444	4444	4400	0000	0000
				4444	4444	4444	4444	4444	4444	4444	0000	0000	0000
BLUE cytotoxicity	100,00%	3 g/L BSA	N.A.	4444	0000	0000	0000	0000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000	0000
VIRAL CONTROL OF INFECTIVITY	N.A.	PBS	0	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4444	4400	0000	0000
60 min				4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4444	4400	0000	0000
VIRAL CONTROL OF INFECTIVITY	N.A.	3 g/L BSA	0	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4444	4400	0000	0000
60 min				4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4444	4400	0000	0000

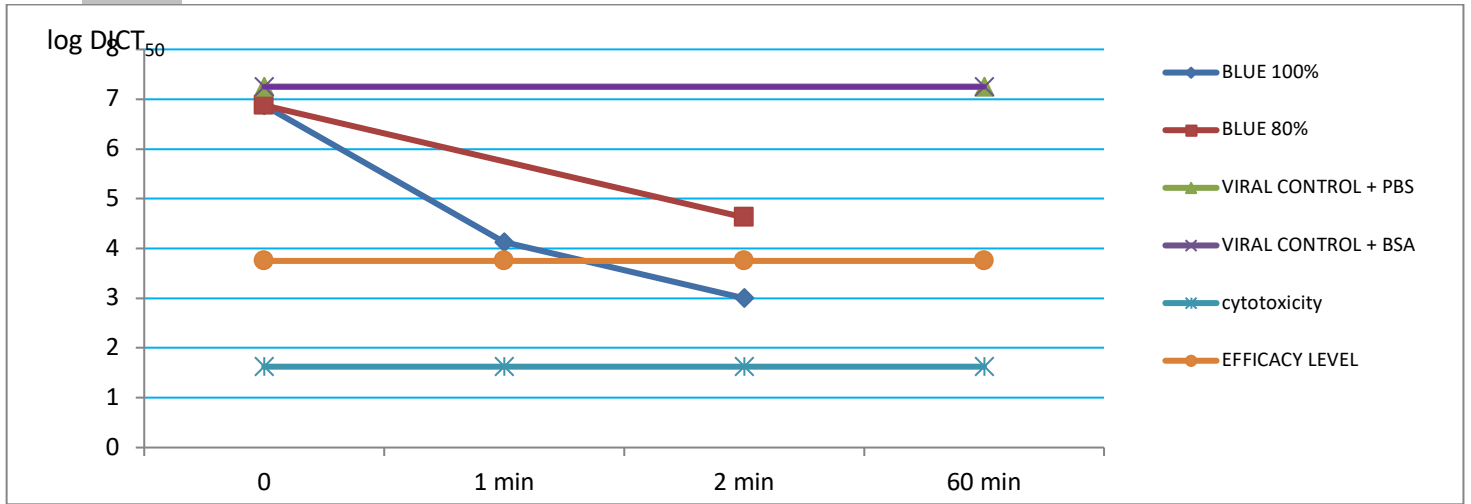
TRIAL 2	Concentration	ORGANIC SOIL LOAD	EXPOSURE TIME	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	
BLUE TRIAL 2	100,00%	3 g/L BSA	1 min	4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
	4444			4440	0000	0000	0000	0000	0000	0000	0000	0000	
	80,00%		2 min	4444	4444	4400	0000	0000	0000	0000	0000	0000	0000
				4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
	1 min	4444	4444	4444	4444	0000	0000	0000	0000	0000	0000		
		4444	4444	4444	4000	0000	0000	0000	0000	0000	0000		
WATER CONTROL				4444	4444	4444	4444	4444	4444	4444	4000	0000	0000
				4444	4444	4444	4444	4444	4444	4444	0000	0000	0000
BLUE cytotoxicity	100,00%	3 g/L BSA	N.A.	4444	4400	0000	0000	0000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000	0000
VIRAL CONTROL OF INFECTIVITY	N.A.	PBS	0	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4444	4400	0000	0000
60 min				4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4444	4400	0000	0000
VIRAL CONTROL OF INFECTIVITY	N.A.	3 g/L BSA	0	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4444	4400	0000	0000
60 min				4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4444	4400	0000	0000

Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

In dirty conditions

Chart 2 – Trials on canine parvovirus:

TRIAL 1



TRIAL 2

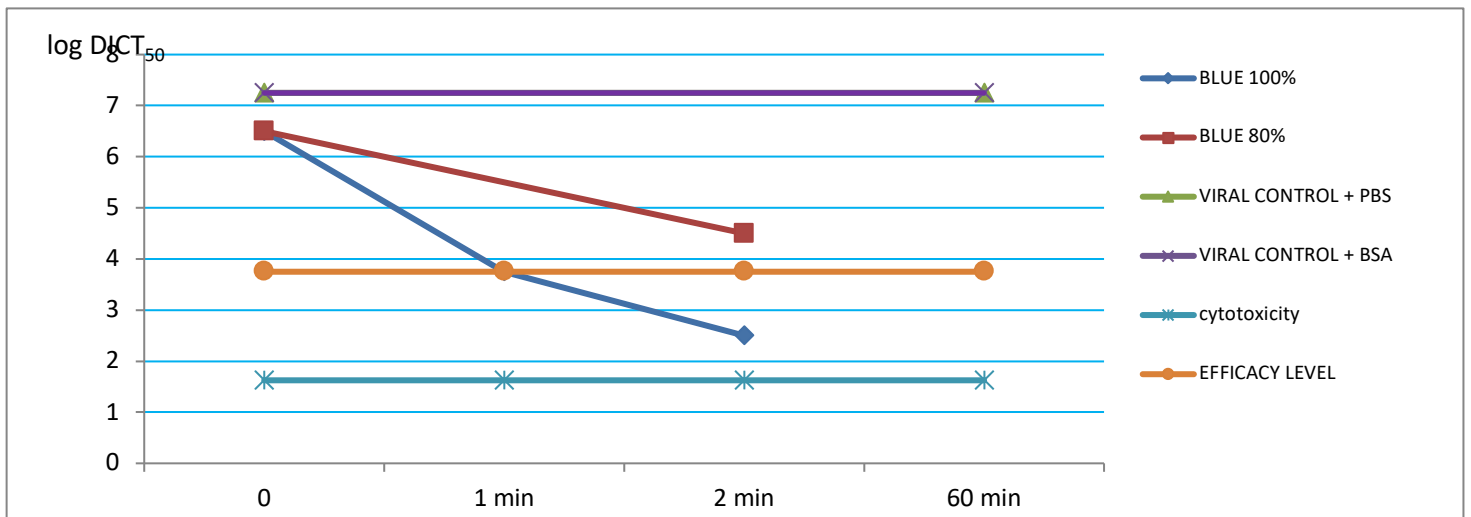


Table A5 - Cells sensitivity to the virus :

Product	Dilution	Organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	
BLUE	10 ⁻³	3 g/L BSA + 3 mL/L SE	Untreated cells	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4400	0000	0000	
			Treated cells	4444	4444	4444	4444	4444	0000	0000	0000	
				4444	4444	4444	4444	4444	0000	0000	0000	



Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

Table A6 — Results on canine parvovirus in high-dirty conditions

Product	Concentration	ORGANIC SOIL LOAD	CYTOTOXICITY LEVEL	Lg TCID ₅₀				Reduction
				0	1 min	2 min	60 min	
BLUE TRIAL 1	100,00%	3 g/L BSA + 3 mL/L SE	1,625	6,875	4,125	3,000	N.T.	2 min LOG R = 3,875
	80,00%		N.T.		N.T.	4,625	N.T.	2 min LOG R = 2,250
BLUE TRIAL 2	100,00%	3 g/L BSA + 3 mL/L SE	1,625	6,500	3,750	2,500	N.T.	2 min LOG R = 4,000
	80%%		N.T.		N.T.	4,500	N.T.	2 min LOG R = 2,000
VIRAL CONTROL OF INFECTIVITY TRIAL 1	N.A.	PBS	N.A.	7,250	N.T.	N.T.	7,250	
VIRAL CONTROL OF INFECTIVITY TRIAL 1	N.A.	3 g/L BSA + 3 mL/L SE	N.A.	7,250	N.T.	N.T.	7,250	
VIRAL CONTROL OF INFECTIVITY TRIAL 2	N.A.	PBS	N.A.	7,250	N.T.	N.T.	7,250	
VIRAL CONTROL OF INFECTIVITY TRIAL 2	N.A.	3 g/L BSA + 3 mL/L SE	N.A.	7,250	N.T.	N.T.	7,250	
CELL SENSITIVITY TO THE VIRUS	10 ⁻²	N.A.	Untreated cells	7,250	N.T.	N.T.	7,250	
		N.A.	Treated cells	7,250	N.T.	N.T.	6,500	



Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

Table A7 — Raw results

TRIAL 1	Concentration	ORGANIC SOIL LOAD	EXPOSURE TIME	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	
BLUE TRIAL 1	100,00%	3 g/L BSA + 3 mL/L SE	2 min	4444	4444	4444	0000	0000	0000	0000	0000	0000	0000
				4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
	80,00%		1 min	4444	4444	4444	4444	0000	0000	0000	0000	0000	0000
				4444	4444	4444	4000	0000	0000	0000	0000	0000	0000
WATER CONTROL	4444	4444	4444	4444	4444	4000	0000	0000	0000	0000	0000		
	4444	4444	4444	4444	4444	4444	4444	4440	0000	0000	0000		
BLUE cytotoxicity	100,00%	3 g/L BSA + 3 mL/L SE	N.A.	4444	4000	0000	0000	0000	0000	0000	0000	0000	
VIRAL CONTROL OF INFECTIVITY	N.A.	PBS	0	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4400	0000	0000	0000
60 min	4444	4444	4444	4444	4444	4444	4444	4444	4444	4400	0000	0000	
	4444	4444	4444	4444	4444	4444	4444	4400	0000	0000	0000		
VIRAL CONTROL OF INFECTIVITY	N.A.	3 g/L BSA + 3 mL/L SE	0	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4400	0000	0000	0000
60 min	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	4400	0000	
	4444	4444	4444	4444	4444	4444	4444	4400	0000	0000	0000		

TRIAL 2	Concentration	ORGANIC SOIL LOAD	EXPOSURE TIME	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	
BLUE TRIAL 2	100,00%	3 g/L BSA + 3 mL/L SE	2 min	4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
	80,00%		1 min	4444	4444	4444	4400	0000	0000	0000	0000	0000	0000
				4444	4444	4444	0000	0000	0000	0000	0000	0000	0000
WATER CONTROL	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000	0000		
	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000	0000		
BLUE cytotoxicity	100,00%	3 g/L BSA + 3 mL/L SE	N.A.	4444	4000	0000	0000	0000	0000	0000	0000	0000	
VIRAL CONTROL OF INFECTIVITY	N.A.	PBS	0	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4400	0000	0000	0000
60 min	4444	4444	4444	4444	4444	4444	4444	4444	4444	4400	0000	0000	
	4444	4444	4444	4444	4444	4444	4444	4400	0000	0000	0000		
VIRAL CONTROL OF INFECTIVITY	N.A.	3 g/L BSA + 3 mL/L SE	0	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4400	0000	0000	0000
60 min	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	4400	0000	
	4444	4444	4444	4444	4444	4444	4444	4400	0000	0000	0000		

Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
