

## Test Report: EN 14476 Standard Test Method for Efficacy of Antimicrobial Agents against Viruses in Suspension modified for Canine Parvovirus

### Test Laboratory

### BluTest Laboratories Ltd

Robertson Incubator (Level 4)  
Robertson Building  
56 Dumbarton Road  
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### Identification of sample

Name of the product  
Manufacturer

#### SAFE 4

Safe Solutions Ltd, Wharton Green House, Bostock, Winsford,  
Cheshire, CW7 3BD

Project Code  
Date of Delivery  
Storage conditions  
Active substances

BT-SAF-04  
11 October 2010  
Room temperature and darkness  
Not Known

### Test Method and its validation

Method

SAFE 4 was diluted and 0.8 ml mixed with 0.1 ml virus suspension (DMEM + 5% FBS) and 0.1 ml interfering substance. Dilution-neutralization. *Virus CPE detected by antibody staining*  
Column chromatography

Neutralizer

### Experimental Conditions

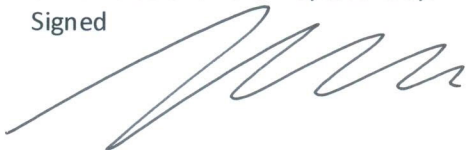
Period of analysis  
Product diluent used  
Product test concentrations  
Contact times (minutes)  
Test temperature  
Interfering substance  
Stability of mixture  
Temperature of incubation  
Identification of virus

11 to 18 October 2010  
Sterile Hard Water  
1.0 % V/V; 2.0 % V/V  
5 ± 10s; 15 ± 10s; 30 ± 10s; 60 ± 10s;  
20°C ± 1°C  
0.3 g/L BSA + 0.6 g/l foetal bovine serum  
Precipitate absent throughout the test  
37°C ± 1°C + 5% CO<sub>2</sub>  
Canine parvovirus (MDCK cells)

### Conclusion

According to a modified EN 14476, **SAFE 4** retains virucidal activity (> 4.0 log<sub>10</sub> reduction) at 5 minutes contact at 2.0 % V/V and 30 minutes contact, at 1.0 % V/V, (reduction in viral viability at 5 minutes, 1.0% V/V, is 3.33 Log<sub>10</sub>) at 20°C under **CLEAN** conditions (0.3 g/L BSA + 0.6 g/L protein as foetal bovine serum) for suspensions of canine parvovirus. **SAFE 4** also shows low cytotoxicity.

Signed



Dr Chris Woodall  
Director, BluTest Laboratories Ltd  
Glasgow, UK, 18 October 2010

## Suspension test results for the efficacy of SAFE 4 from Safe Solutions against Canine Parvovirus-1

Exposure Time	Virus Recovery 0 min		Virus Recovery t min		Cytotoxicity		Disinfectant Suppression		2% (v/v)		1% (v/v)	
	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml
t = 60	5.67	1.48E+07 1.48E+07	6.00	3.16E+07 3.16E+07	0.00	3.16E+01 3.16E+01	2.00	3.16E+03 3.16E+03	0.30	6.31E+01 6.31E+01	1.67	1.48E+03 1.48E+03
	log	7.17	7.50	1.50	3.50	5.70	4.33					
	log difference											
t = 30	5.67	1.48E+07 1.48E+07	6.00	3.16E+07 3.16E+07	0.00	3.16E+01 3.16E+01	2.00	3.16E+03 3.16E+03	0.67	1.48E+02 1.48E+02	1.67	1.48E+03 1.48E+03
	log	7.17	7.50	1.50	3.50	5.33	4.33					
	log difference											
t = 15	5.67	1.48E+07 1.48E+07	6.00	3.16E+07 3.16E+07	0.00	3.16E+01 3.16E+01	2.00	3.16E+03 3.16E+03	1.67	1.48E+03 1.48E+03	2.30	6.31E+03 6.31E+03
	log	7.17	7.50	1.50	3.50	4.33	3.70					
	log difference											
t = 5	5.67	1.48E+07 1.48E+07	6.00	3.16E+07 3.16E+07	0.00	3.16E+01 3.16E+01	2.00	3.16E+03 3.16E+03	1.67	1.48E+03 1.48E+03	2.67	1.48E+04 1.48E+04
	log	7.17	7.50	1.50	3.50	4.33	4.17					
	log difference						3.33					

**Table of results of virucidal activity of SAFE 4 from Safe Solutions against Canine Parvovirus**

Product:	Interfering substance	Concentration	Level of cytotoxicity	lg TCID50					>4 lg reduction after .. Min
				0 min	5 min	15 min	30min	60 min	
SAFE 4	0.3g/l BSA	2% (v/v)	2.50	7.17	3.17	3.17	2.17	1.80	< 5
		1% (v/v)	1.50	7.17	4.17	2.30	1.67	1.67	< 30
Formaldehyde		0.7 % (w/v)	2.17	7.17	3.50	2.80	2.50	1.80	60
Virus Control		n.a.	n.a.	7.17	n.a.	n.a.	n.a.	7.50	n.a.



## CONTROL DATA

Stock Virus (TCID<sub>50</sub>)

5.95	2.82E+07
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Formaldehyde reference inactivation control

Exposure time	Virus recovery 0 min		Virus recovery 60 min		Cytotoxicity		0.7% Formaldehyde							
							5		15		30		60	
	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml	raw data	TCID <sub>50</sub> /ml
60 min	5.67	1.48E+07	6.00	3.16E+07	0.67	1.48E+02	2.00	3.16E+03	1.30	6.31E+02	1.00	3.16E+02	0.30	6.31E+01
log		1.48E+07		3.16E+07		1.48E+02		3.16E+03		6.31E+02		3.16E+02		6.31E+01
log difference		7.17		7.50		2.17		3.50		2.80		2.50		1.80
								4.00		4.70		5.00		5.70

No Column Control

Virus Recovery t min	
raw data	TCID <sub>50</sub> /ml
6.00	3.16E+07
	3.16E+07
	7.50

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