The efficacy of Virkon® S against 5 Salmonella serovars, *Campylobacter jejuni* and *Staphylococcus aureus* (MRSA)

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Test report Virkon® S carried out according to BS.EN 1656:2000

Test laboratory:

Central Veterinary Institute of Wageningen UR
Division Food Quality, Antibiotic Resistance and Zoonosis
PO BOX 65
NL 8200 AB Lelystad
The Netherlands.

Sample:Virkon® S
Batch 0907BA0116
Manufacturer:

Antec International Ltd.
Windham Road
Chilton industrial Estate Sudbury
Suffolk CO10 2XD
UK

Date of delivery: July 30, 2009
Storage conditions: Room temperature at laboratory

Active substances: Not indicated

Test method and its validation
Method Dilution Neutralization

Neutralizer:
Universal Neutralizer for Virkon® S:

Sodium thiosulphate: 5 g/l
Phosphate buffer: 0.25N (34g KH_2PO_4/l distilled water): 10 ml
Tween 80: 30 ml
L-Histidin: 1g
Lecithin: 3g
Dilute to 1 liter with distilled water.

Experimental conditions
Period of analysis: 2009-08-10 to 2009-08-22

Appearance of product Virkon® S:

Original: pink crystals
Dilutions: pink clear solutions

Virkon® S *test concentrations:*

0.5% (5g/l of standardized hard water)
1.0% (10 g/l of standardized hard water)

Test temperature:

10°C ± 1°C

Contact time:

30 min ± 10 s

Interfering substance

A: low: 3 g/l bovine albumin
B: high: 10 g/l yeast extract + 10 g/l bovine albumin

Product diluent:
Standardised hard water:

Distilled water: 986 ml
6 ml [(19,84 g anhydrous $MgCl_2$/l) + (46,24 g anhydrous $CaCl_2$/l)]
8 ml (35.02 g $NaHCO_3$/l)

Incubation temperature for all bacteria tested :

37°C ± 1°C

Bacterial counting procedure:

Spread plate technique

- Salmonella spp. on Brilliant Green Agar, incubation at 37°C for 24 h
- *Campylobacter jejuni* on Charcoal Cefoperazone Deoxycholate Agar, incubated microaerobically at 37°C for 48 h,
- *Staphylococcus aureus* (MRSA isolated from pigs) on Heart Infusion Agar with 5% sheep blood at 37°C for 24 h

Confirmation:

- Salmonella spp.: biochemically and serologically of randomly selected colonies
- *Campylobacter jejuni*: typical motility and Latex test (Oxoid)
- *Staphylococcus aureus* (MRSA): microscopical morphology

Bacterial strain used:

- *Salmonella enteritidis* (CVI-WUR-Lelystad collection, typed by National Salmonella Centre Bilthoven, the Netherlands)
- *Salmonella hadar* (CVI-WUR-Lelystad collection, typed by National Salmonella Centre Bilthoven, the Netherlands)
- *Salmonella infantis* (CVI-WUR-Lelystad collection, typed by National Salmonella Centre Bilthoven, the Netherlands),
- *Salmonella typhimurium* (CVI-WUR-Lelystad collection, typed by National Salmonella Centre Bilthoven, the Netherlands)
- *Salmonella virchow* (CVI-WUR-Lelystad collection, typed by National Salmonella Centre Bilthoven, the Netherlands)
- *Campylobacter jejuni* (CVI-WUR-Lelystad collection; type 365, typed by PCR and AFLP at CVI-WUR)
- *Staphylococcus aureus MRSA* (Isolated from pigs and typed by the Dutch Public Health Lab in Bilthoven)

Test results:

Verification of the methodology showed no unacceptable deviations from those indicated in the BS EN 1656:2000. As is indicated in table 1, the test suspension (Nv) we used, contained the appropriate number of bacteria: between 2.10^3 and 7.10^4 cfu/ml,.
Results of validation of neither experimental conditions (A) , nor Dilution neutralisation (C), were influenced by the type of interfering fluid; high or low. Neutralisation itself did not interfere with none of the test bacteria during the test (B).

In Table 2 the test results are presented of decontamination trials with two concentrations of Virkon® S, during respectively 30 minutes at 10°C. The bacterial suspensions (N) of the different bacterial species contained $4,95.10^7$ and $1.60.10^8$ cfu/ml, according to the BS EN 1656:2000. The results are given as cfu/ml (Na).
From these results at low dose (0,5%) of Virkon® S, the lethal effect at high concentrations of interfering substance is more than 3 log for all Salmonella serotypes, and even more than 6 log for *Campylobacter jejuni*. *Staphylococcus aureus* (MSRA) showed more than 2 log reduction, but there was no effect of concentrations of interfering substance.
At low concentrations of interfering substance there was a reduction below $1.5.10^2$ cfu/ml for both *Campylobacter jejuni* and all *Salmonella serotypes*.
At high dose of Virkon® S (1%), both at high and low concentrations of interfering substance there was a reduction below $1.5.10^2$ cfu/ml for both *Campylobacter jejuni* and all Salmonella serotypes.

Staphylococcus aureus (MRSA) at low concentrations of interfering substance was reduced for more than 3 log, whereas at high concentrations of interfering substance the cfu number was below $1.5.10^2$ cfu/ml, so more than 5 log reduction.

In conclusion, reduction in viability of Salmonella spp., *Campylobacter jejuni* as indicated in table 4 shows that exposure time of 30 minutes at both 0.5% and 1% of Virkon® S at with low level of interfering substance, and 30 minutes with high level of interfering substance at 1% of Virkon® S is sufficiently effective to pass the requirements of EN 1656:2000. In Table 4 the shaded results indicate that the EN 1656:2000 requirements could not be obtained.

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Table 1: Verification of methodology and validation of dilution neutralisation for concentrations 0.5% and 1% Virkon® S.

Test organism	Colony counts per plate (average number of cfu)				
	Test suspension	Validation experimental conditions (A)	Neutraliser toxicity control (B)	Dilution neutralisation control (C)	
Salmonella enteritidis	Validation A 10^{-2}: 154;440 10^{-3}: 9;11 (Nv=$2.97.10^4$) Validation B 10^{-2}: 154;94 10^{-3}: 9;11 (Nv=$1.22.10^4$) Validation C 10^{-2}: 52;48;137 10^{-3}: 4;5;18 (Nv= Virkon® S $7.05.10^4$)	low interfering substance 10^{-2}: 116; 286 10^{-3}: 38; 20 (A=$2.09.10^4$) high interfering substance 10^{-2}: >250; 204 10^{-3}: 61; 24 (A=$2.45.10^4$)	For Virkon® S 10^{-2}: 97; 133 10^{-3}: 11; 16 (B=$1.17.10^4$)	0.5% Virkon® S. low interfering substance 10^{-2}: 188; 132 10^{-3}: 25; 25 (C=$1.68.10^4$) high interfering substance 10^{-2}: 197; 219 10^{-3}: 21; 22 (C=$2.09.10^4$)	1.0% Virkon® S. low interfering substance 10^{-2}: 440; 416 10^{-3}: 28; 31 (C=$4.16.10^4$) high interfering substance 10^{-2}: 392; 324 10^{-3}: 30; 37 (C=$3.56.10^4$)
Salmonella hadar	Validation A 10^{-2}: 53;144 10^{-3}: 3;8 (Nv=$9.45.10^3$) Validation B 10^{-2}: 53;40 10^{-3}: 3;3 (Nv=$4.50.10^3$) Validation C 10^{-2}: 102;71;58 10^{-3}: 15;2;2 (Nv= Virkon® S $5.32.10^3$)	low interfering substance 10^{-2}: 33; 123 10^{-3}: 4; 8 (A= $7.64.10^3$) high interfering substance 10^{-2}: 36; 141 10^{-3}: 2; 17 (A=$2.13.10^3$)	For Virkon® S 10^{-2}: 63, 80 10^{-3}: 8, 3 (B=$7.05.10^3$)	0.5% Virkon® S. low interfering substance 10^{-2}: 192; 132 10^{-3}: 7; 12 (C=$1.87.10^4$) high interfering substance 10^{-2}: 131; 180 10^{-3}: 11; 12 (C=$1.52.10^4$)	1.0% Virkon® S low interfering substance 10^{-2}: 164; 156 10^{-3}: 19; 8 (C=$1.58.10^4$) high interfering substance 10^{-2}: 148; 180 10^{-3}: 19; 10 (C=$1.62.10^4$)

Test organism	Colony counts per plate (average number of cfu)				
	Test suspension	Validation experimental conditions (A)	Neutraliser toxicity control (B)	Dilution neutralisation control (C)	
Salmonella infantis	Validation A 10^{-2}: 71;57 10^{-3}: 4;1 (Nv=$6.05.10^3$) Validation B 10^{-2}: 71;86 10^{-3}: 4;6 (Nv=$7.59.10^3$) Validation C 10^{-2}: 43;37;39 10^{-3}: 5;0;3 (Nv= Virkon® S $2.18.10^3$)	low interfering substance 10^{-2}: 140;83 10^{-3}: 9;5 (A=$1.08.10^4$) high interfering substance 10^{-2}: 151; 66 10^{-3}: 11; 3 (A=$1.05.10^4$)	For Virkon® S 10^{-2}: 90;56 10^{-3}: 4;5 (B=$7.05.10^3$)	0.5% Virkon® S. low interfering substance 10^{-2}: 67; 61 10^{-3}: 5;4 (C=$6.09.10^3$) high interfering substance 10^{-2}: 19; 12 10^{-3}: 16; 9 (C=$2.55.10^3$)	1.0% Virkon® S. low interfering substance 10^{-2}: 106; 99 10^{-3}: 9; 3 (C=$9.68.10^3$) high interfering substance 10^{-2}: 101; 80 10^{-3}: 7; 6 (C=$1.41.10^4$)
Salmonella typhimurium	Validation A 10^{-2}: 48;62 10^{-3}: 6;8 (Nv=$7.0.10^3$) Validation B 10^{-2}: 48;117 10^{-3}: 6;5 (Nv=$8.0.10^3$) Validation C 10^{-2}: 79;46;152 10^{-3}: 4;3;5 (Nv= Virkon® S $7.14.10^3$)	low interfering substance 10^{-2}: 67; 103 10^{-3}: 6;10 (A= $8.45.10^3$) high interfering substance 10^{-2}: 76; 136 10^{-3}: 6; 8 (A=$1.03.10^4$)	For Virkon® S 10^{-2}: 54; 73 10^{-3}: 6;3 (B=$6.18.10^3$)	0.5% Virkon® S. low interfering substance 10^{-2}: 171; 150 10^{-3}: 10; 12 (C=$1.56.10^4$) high interfering substance 10^{-2}: 93; 147 10^{-3}: 22; 13 (C=$1.25.10^4$)	1.0% Virkon® S. low interfering substance 10^{-2}: 192; 178 10^{-3}: 15; 8 (C=$1.79.10^4$) high interfering substance 10^{-2}: 124; 180 10^{-3}: 8; 9 (C=$1.46.10^4$)

Test organism	Colony counts per plate (average number of cfu)				
	Test suspension	Validation experimental conditions (A)	Neutraliser toxicity control (B)	Dilution neutralisation control (C)	
Salmonella virchow	Validation A 10^{-2}: 39;104 10^{-3}: 3;10 (Nv=$7.09.10^3$) Validation B 10^{-2}: 39;38 10^{-3}: 3;3 (Nv=$3.77.10^3$) Validation C 10^{-2}: 96;33;42 10^{-3}: 4;3;0 (Nv= Virkon® S $4.55.10^3$)	low interfering substance 10^{-2}: 71; 109 10^{-3}: 3; 7 (A= $8.64.10^3$) high interfering substance 10^{-2}: 55, 106 10^{-3}: 2, 10 (A=$7.86.10^3$)	For Virkon® S 10^{-2}: 45; 63 10^{-3}: 1; 8 (B=$5.32.10^3$)	0.5% Virkon® S. low interfering substance 10^{-2}: 144; 94 10^{-3}: 14; 12 (C=$1.20.10^4$) high interfering substance 10^{-2}: 88; 101 10^{-3}: 16; 15 (C=$5.68.10^3$)	1.0% Virkon® S. low interfering substance 10^{-2}: 278; 155 10^{-3}: 16; 18 (C=$1.91.10^4$) high interfering substance 10^{-2}: 197; 219 10^{-3}: 21; 22 (C=$2.12.10^4$)
Campylobacter jejuni	Validation A 10^{-2}: 110;195 10^{-3}: 9;20 (Nv=$1.52.10^4$) Validation B 10^{-2}: 110;165 10^{-3}: 9;18 (Nv=$1.37.10^4$) Validation C 10^{-2}: 10^{-3}: (Nv= Virkon® S $1.11.10^4$)	low interfering substance 10^{-2}:88; 43 10^{-3}: 3; 8 (A= $6.45.10^3$) high interfering substance 10^{-2}: 78; 33 10^{-3}: 2; 2 (A=$5.23.10^3$)	For Virkon® S 10^{-2}: 84 10^{-3}: 3 (B=$3.95.10^3$)	0.5% Virkon® S. low interfering substance 10-2: 103; 111 10-3: 16; 10 (C=1.09.104) high interfering substance 10-2: 88; 76 10-3: 11; 15 (C=8.64.104)	1.0% Virkon® S. low interfering substance 10-2: 118; 89 10-3: 16; 12 (C=1.07.104) high interfering substance 10-2: 211; 148 10-3: 23; 18 (C=1.82.104)

Test organism	Colony counts per plate (average number of cfu)				
	Test suspension	Validation experimental conditions (A)	Neutraliser toxicity control (B)	Dilution neutralisation control (C)	
Staphylococcus aureus MRSA	Validation A 10^{-2}: 154;216 10^{-3}: 22;36 (Nv=$1.95.10^4$) Validation B 10^{-2}: 154;298 10^{-3}: 22;50 (Nv=$2.38.10^4$) Validation C 10^{-2}: 109;32;44 10^{-3}: 5;2;16 (Nv= Virkon® S $5.18.10^3$)	low interfering substance 10^{-2}: 180, 360 10^{-3}: 31, 25 (A= $2.71.10^4$) high interfering substance 10^{-2}: 380; 198 10^{-3}: 62, 7 (A=$2.94.10^4$)	For Virkon® S 10^{-2}: 220; 71 10^{-3}: 53; 2 (B=$1.57.10^4$)	0.5% Virkon® S. low interfering substance 10^{-2}: 75; 166 10^{-3}: 22; 32 (C=$1.34.10^4$) high interfering substance 10^{-2}: 135; 111 10^{-3}: 30; 22 (C=$1.35.10^4$)	1.0% Virkon® S. low interfering substance 10^{-2}: 132; 121 10^{-3}: 16; 13 (C=$1.28.10^4$) high interfering substance 10^{-2}: 132; 200 10^{-3}: 15; 20 (C=$8.5.10^3$)
Vc= viable counts Nv= number of cfu/ml of bacterial suspension A= number of cfu/ml of the validation of experimental conditions B= number of cfu/ml of the neutralizer toxicity validation C= number of cfu/ml of the experimental conditions control					

Table 2.Test results of two concentrations of Virkon® S(concentrations 0.5% and 1%) at 10°C ± 1°C with dilution neutralisation method.

Test micro-organism	Virkon® S: 0.5%		Virkon® S: 1%		Bacterial test suspension
	30 min$_1$	30 min$_1$	30 min$_1$	30 min$_1$	
	High interfering substance	Low interfering substance	High interfering substance	Low interfering substance	
Salmonella enteritidis	Vc 10^{-2}: 180; 139 10^{-3}: 15, 9 (Na= $1.89.10^4$)	Vc 10^{-1}: 0, 0 10^{-2}: 0, 0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-6}: 23; 77 10^{-7}: 4; 5 (N=$4.95.10^7$)
Salmonella hadar	Vc 10^{-2}: 186; 246 10^{-3}: 12, 18 (Na=$1.46.10^4$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-6}: 48; 118 10^{-7}: 1; 15 (N=$8.27.10^7$)
Salmonella infantis	Vc 10^{-2}: 136; 228 10^{-3}: 9;11 (Na=$4.45.10^3$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-6}: 105; 98 10^{-7}: 15; * (N= $1.02.10^8$)
Salmonella typhimurium	Vc 10^{-2}: 266; 186 10^{-3}: 15, 8 (Na=$4.15.10^4$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 ((Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-6}: 49; 74 10^{-7}: 6; 8 (N=$6.23.10^7$)
Salmonella virchow	Vc 10^{-2}: 63; 82 10^{-3}: 6; 8 (Na=$4.18.10^4$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-6}: 113; 11 10^{-7}: 8; 3 (N= $6,14.10^7$)
Campylobacter jejuni	Vc 10^{-1}: 0;1 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 7;3 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na= $<1.510^2$)	Vc 10^{-6}: 106;180 10^{-7}: 43;22 (N=$1.60.10^8$)
Staphylococcus aureus MRSA	Vc 10^{-3}: >500;>500 (Na=>10^5)	Vc 10^{-3}: >500; >500 (Na=>10^5)	Vc 10^{-2}: 164; 106 10^{-3}: 13; 8 (Na=$3.91.10^4$)	Vc 10^{-1}: 0;0 10^{-2}: 0;0 (Na=<$1.5.10^2$)	Vc 10^{-6}: 91; 58 10^{-7}: 9; 1 (N=$7.23.10^7$)

$_1$ exposure time at 10°C
$Vc_=$ viable counts
N= number of cfu/ml in bacterial test suspension
Na= number of cfu/ml in test mixture after treatment

Table 3.Reduction in viability R of two concentrations of Virkon® S(concentrations 0.5% and 1.0%) at 10°C ± 1°C with dilution neutralisation method.

Test micro-organism	Virkon® S0.5%		Virkon® S1.0%	
	30 min_1	30 min_1	30 min_1	30 min_1
	High interfering substance	Low interfering substance	High interfering substance	Low interfering substance
Salmonella enteritidis	$R < 10^4$	$R > 10^5$	$R > 10^5$	$R > 10^5$
Salmonella hadar	$R < 10^4$	$R > 10^5$	$R > 10^5$	$R > 10^5$
Salmonella infantis	$10^4 < R < 10^5$	$R > 10^5$	$R > 10^5$	$R > 10^5$
Salmonella typhimurium	$R < 10^4$	$R > 10^5$	$R > 10^5$	$R > 10^5$
Salmonella virchow	$R < 10^4$	$R > 10^5$	$R > 10^5$	$R > 10^5$
Campylobacter jejuni	$R > 10^5$	$R > 10^5$	$R > 10^5$	$R > 10^5$
Staphylococcus aureus MRSA	$R < 10^4$	$R < 10^4$	$R < 10^4$	$R > 10^5$
$_1$ exposure time at 10°C				