

Determination of antiviral activity of textile products

SILVERGUARD EARTH®

SILVERGUARD JUPITER®

SILVERGUARD VEGA®

Screening test according to ISO 18184:2019

Report written by dott.ssa LUCIA SELLI

Test performed at: Lab. of Virological Diagnostic
SCT3 Department "Animal Health Diagnostic"
Institute "Zooprofilattico Sperimentale delle Venezie (IZSve)" - Italy

Date: october/november 2020

Principal: KC Wearable Technologies
Via Isonzo 1-3
25017 Lonato del Garda · Italy

Principal: KC Wearable Technologies
Via Isonzo 1-3
25017 Lonato del Garda · Italy

Samples:

- Control fabric (Cf) Cf1) textile product Earth® (w/o the active component)
Cf2) textile product Jupiter® (w/o the active component)
Cf3) textile product Vega® (w/o the active component))
- Control test (Ct) Ct1) textile product Silverguard Earth® (with active component)
Ct2) textile product Silverguard Jupiter® (with active component)
Ct3) textile product Silverguard Vega® (with active component)

All the samples were supplied by the principal who provided them as ready-to-use material. At IZSve – SCT3 – Lab.of Virologia Diagnostica, round sample disks (test disks) with $d = 2$ cm. were cut out of this sample material and stored at room temperature until time of testing.

Test system:

The test was performed following ISO 18184:2019.

Test principle: quantitative virucidal carrier test at $T = 25$ °C (in a climate chamber); this is a screening test, with the omission of validity checks, therefore does not correspond to a complete product validation according to ISO 18184.

Viral strain used:

- Bovines Coronavirus (Beta-Coronavirus); origin: strain isolated by a bovine nasal swab and characterized by biomolecular procedures (at least 10^7 TCID₅₀/ml).

Cell line used for viral titration with method of Spearman and Karber in TCID₅₀:

- HRT-18 cells (Human Rectal Tumor); origin: Biobanking of Veterinary Resources (BVR) Inst.Zooprofilattico Sperimentale della Lombardia e Emilia – Brescia - Italy

Test parameter:

- Test conditions: $T = 25$ °C (according to ISO 18184) and 90 % r.LF
- Protein load: no additional protein load; the virus material (cell culture supernatant) was spread onto the surface(s) w/o any further manipulation/alteration
- Volume to square ratio: 200 μ L distributed on 3,14 cm² ca. (discs, with $d = 2$ cm)
- Volume to mass ratio: 200 μ L distributed on $0,08 \pm 0.005$ gr.
- Incubation: 5', 15', 30', 1h., 3h. e 6h. in a climate chamber. The test discs were placed into the wells of a 12-well microplate. Incubation was carried out with the lid closed and inside a humid box.
Recovery of the virus material was performed in the same wells as the incubation. After 5 mL of cell culture medium had been added to the well, the sample disk was rinsed repeatedly (5x) with $V = 3$ mL of medium using a pipette, obtaining a 1:100 dilution of the original virus material.

Performing the test

- The test samples were not sterilized by autoclaving. The test material consists of a synthetic fiber, which does not survive the autoclaving.
- The samples of textile material (Cf and Ct) , consisting on several round disks with $D = 2$ cm ca. and with mass of $0,08 \pm 0.005$ gr. were transferred, each in a single well , to a 12-well microplate where the testing (inoculation with 200 μ L of viral suspension, incubation at the established time, virus material collection and disk rinse) was then carried out. The titration of the viral material obtained by every sample at the end of each incubation time,

was performed using the Spearman-Kärber TCID₅₀ method on 96-well cell culture microplates coated with HRT-18 cell line, incubated at 37±2°C in a climate chamber with an atmosphere of 5% CO₂ for 4-5 days.

- Virus titration (expressed with TCID₅₀) of the viral suspension was also performed immediately after the inoculation (Time Zero titration) on each textile samples Cf and Ct (see table 1).
- Every sample, at each different incubation time, was tested in quadruplicate (see tables 2, 3 and 4).
- Every sample, at each different incubation time, was also tested for cytotoxicity effects by inoculation with cell culture medium (w/o viral material) and subsequent transfer on 96-well cell culture microplates coated with HRT-18 cell line, incubated at 37±2°C in a climate chamber with an atmosphere of 5% CO₂ for 4-5 days.

Test results

The results were shown on the tables 1, 2, 3 and 4

Table legend:

Sample ID Cf: viral suspension obtained after contact with Control fabric products

Sample ID Ct: viral suspension obtained after contact with Control test products "Silverguard"

Virus Titer: virus titration in Log₁₀ TCID₅₀

Mean virus titer: in Log₁₀ TCID₅₀

Log₁₀ reduction: reduction of Ct virus titer compared with Cf virus titer in Log₁₀ TCID₅₀

Percent reduction: reduction of Ct virus titer compared with Cf virus titer in percentage

N.R.: no reduction observed

TEST VIRUS	Contact time	tessuto	Sample ID	Virus Titer*
Bovine Coronavirus	0'	Earth®	Cf	4.25
			Ct	4
		Jupiter®	Cf	3.75
			Ct	4.25
		Vega®	Cf	3.75
			Ct	4

Tab. 1- Virus titration results at Time 0 (T₀)

*expressed as in Log₁₀ TCID₅₀/50 µl.

N.B. As described above, whilst being collected from the wells containing the textile disks, the virus suspension was diluted 1:100, with a 2 Log₁₀ TCID₅₀ reduction of the initial viral titer of the strain used.

TEXTILE PRODUCT EARTH® e SILVERGUARD EARTH®

TEST VIRUS	Contact time	Sample ID	Virus Titer	Mean virus titer	Log ₁₀ reduction	Percent reduction
Bovine Coronavirus	5'	Cf	4	4.25	N.R.	N.R.
			4			
			4.25			
			4.75			
		Ct	4.25	4.31		
			4.50			
			4.25			
			4.25			
	15'	Cf	4.25	4.25	0.25	43.76
			4.50			
			4			
			4.50			
		Ct	4	4		
			3.75			
			4			
			4.25			
	30'	Cf	4.25	4.06	0,31	51,02
			4			
			4			
			4			
		Ct	3.75	3.75		
			3.75			
			3.75			
			3.75			
	1 h	Cf	4.25	4.25	0.5	68,38
			4			
			4			
			4.75			
		Ct	3.75	3.75		
			4			
			3.75			
			3.50			
	3 h	Cf	4.75	4.50	1,88	98,68
			4.50			
			4.25			
			4.50			
Ct		2.25	2.62			
		2.75				
		3				
		2.50				
6 h	Cf	4.25	4.06	2	98.99	
		4				
		4				
		4				
	Ct	2	2.06			
		2				
		2.25				
		2				

Tab. 2 - Virus titration results with textile product EARTH®

TEXTILE PRODUCT JUPITER® e SILVERGUARD JUPITER®

TEST VIRUS	Contact time	Sample ID	Virus Titer	Mean virus titer	Log ₁₀ reduction	Percent reduction
Bovine Coronavirus	5'	Cf	3.25	3.50	0.50	68.38
			3.50			
			3.75			
			3.50			
		Ct	3.75	4.00		
			4			
	4					
	15'	Cf	4	3.75	1.19	93.08
			3.50			
			3.75			
			3.75			
		Ct	2.50	2.56		
			2.75			
			2.50			
			2.50			
	30'	Cf	4.25	4.17	2.67	99.78
			4			
			4.25			
			4.25			
		Ct	1.5	1.5		
			1			
	1.75					
	1 h	Cf	4	3.94	≥3.69	≥99.98
			3.75			
			4			
			4			
		Ct	≤0.25	≤0.25		
			≤0.25			
			0.25			
			≤0.25			
	3 h	Cf	3.75	4.19	≥3.94	≥99.99
			4.50			
			4.25			
			4.25			
		Ct	≤0.25	≤0.25		
			≤0.25			
≤0.25						
≤0.25						
6 h	Cf	4.25	4.19	≥3.94	≥99.99	
		4				
		4.25				
		4.25				
	Ct	≤0.25	≤0.25			
		≤0.25				
		≤0.25				
		≤0.25				

Tab. 3 - Virus titration results with textile product JUPITER®

TEXTILE PRODUCT VEGA® e SILVERGUARD VEGA®

TEST VIRUS	Contact time	Sample ID	Virus Titer	Mean virus titer	Log ₁₀ reduction	Percent reduction
Bovine Coronavirus	5'	Cf	3.25	3.56	N.R.	N.R.
			3.50			
			3.75			
			3.75			
		Ct	3.50	3.62		
			3.75			
			3.75			
			3.50			
	15'	Cf	3.25	3.56	0.37	57.34
			3.50			
			3.75			
			3.75			
		Ct	3	3.19		
			3			
			3.50			
			3.25			
	30'	Cf	3.50	3.62	0.81	84.51
			3.75			
			3.75			
			3.50			
		Ct	3	2.81		
			2.75			
			2.75			
			2.75			
	1 h	Cf	3.50	3.37	1.37	95.73
			3.25			
			3.25			
			3.50			
Ct		2	2			
		1.75				
		2				
		2.25				
3 h	Cf	3.25	3.19	2.57	99.73	
		3.25				
		3.25				
		3				
	Ct	0.5	0.62			
		0.5				
		0.75				
		0.75				
6 h	Cf	3.25	3.19	≥3.02	≥99.88	
		3				
		3.25				
		3.25				
	Ct	≤0.17	≤0.17			
		≤0.17				
		≤0.17				
		≤0.17				

Tab. 4 – Virus titration results with textile product VEGA®

Observations:

- Up to 6 hours of incubation no drying of the material was observed.
- Resuspending of the virus material was performed apparently unremarkable.
- Citotoxicity tests, performed on all textile samples (Cf1, 2 and 3; Ct1, 2 and 3), for each incubation times, showed negative results, i.e. there was no evidence either of toxic effect on host cell monolayers or any difference in cell morphology and quantity versus the cell monolayers used as control.
- Virus amount able to replicate, within the test time (up to six hours), was reduced at negligible levels (see comparison with virus titer obtained by control textile samples Cf 1, 2 and 3 at time =0 and at time = 6 hrs).
- It was expected that the amount of virus would be different at each sampling times; therefore, to evaluate dynamics of virus inactivation for every textile test samples (Ct), at each incubation times, the virus titration results have been compared with those obtained from control samples (Cf) at the same conditions.
- No further observations / unforeseen events were recorded.

Conclusions

- Textile Silverguard Earth®:
 - After 15', 30' and 1hour incubation, partial reductions of viral infectivity vs the control fabric sample (Cf) have been observed.
 - After 3 hours incubation a significant reduction of the viral infectivity vs the control fabric sample has been observed, which has also been confirmed after 6 hours incubation, with viral titer reductions corresponding to inactivation percentages of 98,68% and 98,99% respectively.
- Textile Silverguard Jupiter®:
 - Just after 5 mins incubation a viral infectivity reduction vs the control fabric sample (Cf) has been observed.
 - After 15, 30 mins and 1 hours incubation time, the viral infectivity had significantly reduced vs the control fabric sample (Cf) with viral titer reductions corresponding to inactivation percentages of 93,08%, 99,78% and 99,98% respectively.
 - After 3 and 6 hours incubation, no residual viral infectivity was present, with a virus titer reduction corresponding to a inactivation percentage $\geq 99,99\%$.
- Textile Silverguard Vega®:
 - Just after 15 mins incubation a viral infectivity reduction vs the control fabric sample (Cf) has been observed.
 - After 30 mins, 1 and 3 hours incubation a significant reduction of the viral infectivity vs the control fabric sample (Cf) has been observed, with viral titer reductions corresponding to inactivation percentages of 84,51%, 95,73% and 99,73% respectively.
 - After 6 hours incubation no residual viral infectivity was present, with a reduction of viral titer corresponding to an inactivation percentage $\geq 99,88\%$.

It can be concluded, from the data obtained, that under the test conditions a high level of virus-inactivating effect against the *bovine Coronavirus* was obtained, especially with textile products Silverguard Jupiter® and Silverguard Vega®. This high-level of antiviral activity can be attributed to the antimicrobial property of "Silverguard®" treated textiles.

The observed virus-inactivating effect was determined using the *bovine Coronavirus* as the test virus. This virus belongs to the enveloped viruses which are generally considered relatively easy to be inactivated. This means that the observed virus inactivation cannot be transferred necessarily to other viruses. This may also apply to other enveloped viruses.

Legnaro (PD) 24.11.2020



Dott.ssa Lucia Selli
DVM
Lab. of Virological Diagnostic
Dip. SCT3 "Animal Health Diagnostic"
Institute Zooprofilattico Sperimentale delle Venezie