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Testing the virucidal activity of test specimens equipped with a biocidal surface

Examination of test surfaces equipped with a virucidal active coating using a praxis-near carrier test system following the ISO 21702:2019 against the *Bovine Coronavirus (BoCV; strain: S379 Riems)* - Screening test S8 dated 09.02.2021

Short report: screening test S8

by
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Test period:	in February 2021
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Products:

- Test surfaces: cut from product with the dimensions of a. 1,6 cm x 6 cm or b. 5 cm x 5 cm
- test surfaces w/o the active component(s) (control samples) and
- test surfaces coated with coatings (test samples); with or w/o a aging test

Test parameter:

- Test conditions: T = 25 °C 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: 25 µL/cm²
- Virus suspension 150 µL (6 cm²) or 400 µL (16 cm²) distributed on a test square 1,2 cm x 5 cm [6 cm²] or 4 cm x 4 cm [16 cm²]; covered with foil (PP, 110 µm) with the dimensions 1,2 cm x 5 cm [6 cm²] or 4 cm x 4 cm [16 cm²]
- Incubation: all test samples with t = 30 min. in a climate chamber KBF 115 (Fa Binder)
- Resuspension of the virus material in 5,0 mL (6 cm²) or 10,0 mL (16 cm²) of medium

Test system:

- Bovine Coronavirus (BoCV [a Beta-Coronavirus]); strain: S379 Riems
  (Origin: Friedrich Löffler-Institut (Insel Riems) of the University Greifswald, Germany)
- HRT-18 Zellen (human rectal carcinoma cells)
  (Origin: Inst. f. Hygiene und Infektionskrankheiten der Tiere of the University Giessen, Germany)

Screening test procedure:

- The test was performed as a basis (screening) test following the methodology of ISO 21702:2019
- Test principle: quantitative virucidal carrier test at T = 25 °C and 90 % r.LF (climate chamber)
- the test was performed w/o (additional) protein load

Tab. 1: ***Product samples tested*** *(as received)*

No.	Product (s)	Storage [1]
#1	Test item - TT / w/o the virucidal active component(s)	at RT
#2	Test item - TT / coated with *THAT's it GOLD*	at RT
#3	Test item - TT / coated with *THAT's it GOLD + aging procedure / 0,5a*	at RT
#4	Test item - TT / coated with *THAT's it GOLD + aging procedure / 1a*	at RT
#5	Test item - TT / coated with *THAT's it GOLD + aging procedure / 2a*	at RT

[1] = *access limited to the personnel of Eurovir*

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Test results:

Observations:

- The test surfaces were largely wetable by the aqueous virus suspension; thus, a more or less uniform liquid film could be produced on the test squares. After covering the virus with the PP-foil, the virus material remained stable as a film over the entire observation period and did not dry out.
- With the test samples no cytotoxicity could be detected (all test samples: lg $TD_{50} \leq$ 1,30/mL)

Tab. 2.1: Virus control *(Virus titration by limiting dilution)*

Sample	VK-1a	VK-1b	Ø	VK-2a	VK-2b	Ø	VK-3a	VK-3b	Ø
	VK / Set #1 / 30 Min.			VK / Set #2 / 30 Min.			VK / Set #3 / 30 Min.		
Titer/Test vol. (lg ID_{50})	4,2	5,25	**4,73**	5,1	4,95	**5,03**	4,95	4,35	**4,65**
av. virus titer ± K (95%) [1]	**5,73 ± 0,37/mL**			**6,03 ± 0,34/mL**			**5,65 ± 0,30/mL**		

[1] = Calculation of the virus titer and its 95% confidence interval according to EN14476

Tab. 2.2: Virus inactivation / Set #2 - THAT's it GOLD *(Virus titration by limiting dilution)*

Sample	In-5a	In-5b	Ø	In-6a	In-6b	Ø	In-7a	In-7b	Ø	In-8a	In-8b	Ø
	w/o aging			aging 0,5 years			aging 1 year			aging 2 years		
Titer/Test vol. (lg ID_{50})	4,2	3,9	**4,0**	4,2	3,9	**4,0**	4,0	4,2	**4,1**	4,2	4,5	**4,3**
av. virus titer ± K (95%) [1]	5,05 ± 0,35/mL			5,05 ± 0,37/mL			5,13 ± 0,27/mL			5,35 ± 0,33/mL		
Reduction [2] **(lg ID_{50} ± K [95%])**	**0,98 ± 0,48** (90% / 30 min)			**0,98 ± 0,50** (90% / 30 min)			**0,90 ± 0,43** (87,5% / 30 min)			**0,68 ± 0,47** (79% / 30 min)		

[1] = Calculation of the virus titer and its 95% confidence interval according to EN14476
[2] = Virus reduction: lg ID_{50} of virus input (virus control) minus lg ID_{50} of sample (at the given time point)

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Virus inactivation and conclusions: *(cf. Tab. 2)*

- The virus film applied on the test items covered with the PP-foil was stable over the entire observation period and was not dried at the end of the exposure. Thus, a continuous contact between the virus material and the surface of the test carrier was ensured all over the observation period and a distribution of the virus material in the liquid phase (e.g. driven by diffusion) was given. In the case that a virucidal activity is present that virus reduction can be attributed to the coating containing the active component(s).
- In order to assess the virus inactivating capacity of the coating under test as a single factor an individual virus input control was analysed with the time point tested (virus control). This virus input control represents the reference point for determining the virus reduction (cf. Tab. 2.1)
- With the amount of input virus at a given time point and with the correspondent amount of remaining test virus (cf. Tab. 2.2 ff) the virus reduction factor was determined.
- *THAT's it GOLD* - with no aging procedure applied a minor virus reduction was observed (RF = 0,98 ± 0,48). With the aging procedures applied a weak tendency to lower the virus reduction may be evident (RF = 0,98 ± 0,50 [0,5 a], RF = 0,90 ± 0,43 [1 a] and RF = 0,68 ± 0,47 [2 a]). It should be noted that the differences between the reduction factors obtained in this test series are in the non-significant range (RF $\leq$ 0,3 Log).

General annotation:

- The observed virus-inactivating effect of the coating was determined using the *bovine coronavirus* as the test virus *(from the virus-genus Beta-Coronavirus to which the SARS-CoV-2 also belongs).* This virus belongs to the enveloped viruses which are in general considered to be inactivated comparable easily. This means that the observed virus inactivation cannot be transferred necessarily to other viruses. This may also apply to other enveloped viruses.
- The data described above were collected in a so-called screening test. This test is a basic test, carried out based on the underlying set of rules and with the omission of validity checks. This test therefore does not correspond to a complete product validation according to ISO 21702.

Luckenwalde, 7th of August 2021

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