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Eurovir® Hygiene-Labor
Antivirale Validierung & Rabies

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 S2 dated 12.06.2020

Short report: screening test S2

by
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Test period:	in June 2020
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Products:

- Test surfaces: cut from real product with the dimensions of 1,6 cm x 6 cm (x 1 mm)
- 1. test item: test surfaces w/o the active component(s) (control samples)
- 2. test item: test surfaces with coating and treated with a aging test, performed by 700 cycles of abrasion with aircraft-cleaner A18-S

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) distributed on a test square by 1,2 cm x 5 cm; covered with foil (LDPE, 50 µm) with the dimensions 1,2 cm x 5 cm (6 cm²)
- Incubation: all test samples with t = 30 min. in a climate chamber KBF 115 (Fa Binder)
- Resuspension of the virus material in 5 mL 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 screening test was performed as a basis test following the 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 conditions [1]
#1	Test item / w/o the virucidal active component(s) (control sample)	at RT
#4	Test item / coated with *LOGIS GRIPS® ANTIKEIMFOLIE* (test sample)	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 LDPE 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 $TD_{50} \leq$ 0,30)

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

Sample	VK-1a	VK-1b
	Virus control / 30 min.	
Titer/Test vol. (lg ID_{50})	4,35	4,8
av. virus titer ± K (95%) [1]	**5,58 ± 0,32/mL**	

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

Tab. 2.2: Virus inactivation *(Virus titration by limiting dilution)*

Sample	In-9a (#507)	In-9b (#508)
	LOGIS GRIPS® ANTIKEIMFOLIE	
Titer/Test vol. (lg ID_{50})	0,3	1,35
av. virus titer ± K (95%) [1]	1,83 ± 0,34/mL	
Reduction [2] (lg ID_{50} ± K [95%])	**3,75 ± 0,47**	

[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)

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

- When the virus material is distributed onto a surface a certain virus titer reduction could be observed with almost all viruses. This is driven by time and do also occur without any other influence. This is already known and must be accepted accordingly.
- In order to assess the virus inactivating capacity of the coating under test as a single factor an individual virus input control was analysed at each 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) the virus reduction factor was determined.
- *LOGIS GRIPS® ANTIKEIMFOLIE* - with this coating virus inactivation was the highest in this testing, amounting to RF = 3,75 ± 0,47 (equivalent to a reduction rate of 99,98%).

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Conclusions:

- The virus film applied on the test items covered with the LDPE-foil was stable over the entire observation period and was not dried at the end of the longest exposure time (24 h). 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.
- The data obtained allow the conclusion that the virus reduction can be attributed to the coating containing the active component(s).
- The observed virus-inactivating effect of the coating was determined using the *bovine coronavirus* as the test virus *(a Beta-Coronavirus).* 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.

Annotation:

- 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, 12th of October 2020

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(GF und Laborleiter Eurovir)