RAS
materials + technologies

RAS AG | An der Irler Höhe 3a | D 93055 Regensburg

| Fon +49(0)941 60717-300
| Fax +49(0)941 60717-399
| www.ras-ag.com
| office@ras-ag.com

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Testing of virucide-coated germ carriers in a practical virucidal carrier test based on the RKI guideline (1995) and ISO 21702: 2019 for bovine coronavirus (BoCV; Stamm: S379 Riems) - Screening test S1 from June 17th, 2020 Brief report on the screening test S1

Translation and explanation by RAS AG, source: test Report:
Malteser - Screeningtest ISO 21702 vs. dem Bovinen Coronavirus - Kurzbericht / S1 vom 27.06.2020

Products:

- Test area: glass carrier, cut to the dimensions 1,6 cm x 6 cm
- 1. product: Surface without any active substance (reference/ negative control)
- 2. – 4. product: Test area, coated with the active substance on one side (sample)

Test parameters:

- Exposure conditions: T = 25°C, 90 % relative humidity
- Protein load: without further load;
  the virus (cell culture supernatant) was used unmodified
- Volume/Surface ratio: 25 µl/cm^2
- Virus suspension was applied to an area of 1,2 x 5 cm and subsequently covered with foil to prevent dehydration (LDPE, 110 µm) shaped into the dimensions of 1,2 x 5 cm (6 cm^2)
- All products were incubated for 1, 8 and 24 h in a climate cabinet (Incubator, Model: Klimaschrank KBF 115, Company: Binder)

Test system:

- Bovine Coronavirus (BoCv); Strain: S379 Riems
  (Origin: Friedrich-Löffler-Institut (Insel Riems) der Universität Greifswald, Greifswald)
- HRT-18 cells (Human rectal carcinoma cells), a human cell-line that serves as host for the virus
  (Origin: Inst. f. Hygiene und Infektionskrankheiten der Tiere der Universität Giessen, Giessen)

Test procedure:

- All experiments were performed based on RKI-guidelines (1995) and based on ISO 21702:2019 with a quantitative virucide carrier test at T=25°C and 90 % relative humidity in a climate cabinet (Incubator, Model: Klimaschrank KBF 115, Company: Binder)

- All experiments were performed without further load

Table 1: Tested samples (tested as received)

Sample	Product	Storage[1]
#1	Carrier / uncoated (reference/ negative control)	RT
#2	Carrier/ coated with surface coating containing nanosilver	RT
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

1) Restricted access to employees of Eurovir, RT = room temperatur

Results:
Observations:

- The test area of the samples was wetable to different degrees, but a more or less uniform liquid film could be created by covering the samples with PE foil to
- By covering the liquid film with foil, the virus supernatant was stable during the whole experiment and the liquid fil didn´t dry during the whole experiment

Table 2.1: Virus control (Determination of virus titer via endpoint titration)

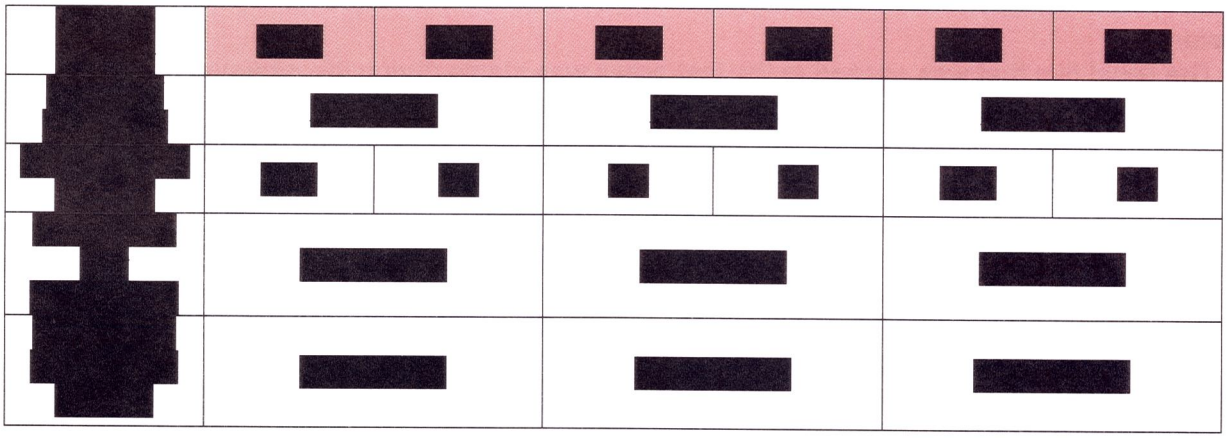
Sample number	VK-1a	VK-1b	VK-2a	VK-2b	VK-3a	VK-3b
Approach	1 h		8 h		24 h	
Virusamount /Testvol. (lg ID_{50})	5,1	4,95	4,8	4,95	3,9	3,9
Mean virus amount ± K (95%) [1]	**5,03 ± 0,29 / 100 µl**		**4,88 ± 0,27 / 100 µL**		**3,90 ± 0,38 / 100 µL**	

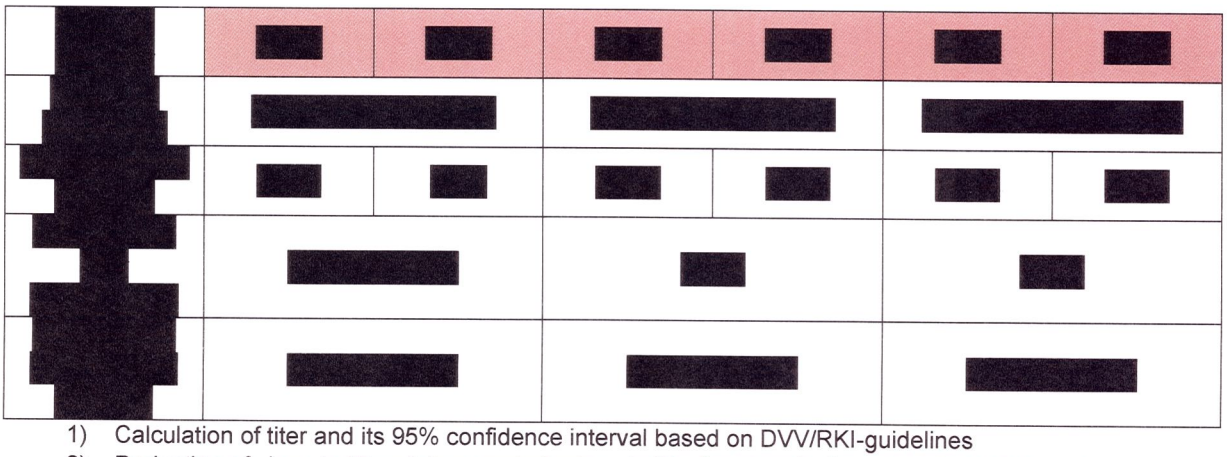
1) Calculation of titer (amount) and its 95% confidence interval based on DVV/RKI-guidelines

The Amount of virus is detected via molecular biological methods. Numbers are given as decadic logarithm, e.g. lg $10^3 = 3$

Table 3.2: Virus inactivation (Determination of virus titer via endpoint titration)

Sample number	In-1a	In-1b	In-2a	In-2b	In-3a	In-3b
Product/ Approach	surface coating containing nanosilver / 1 h		surface coating containing nanosilver / 8 h		surface coating containing nanosilver / 24 h	
Virusamount /Testvol. (lg ID_{50})	3,6	3,75	≤ 0,3	0,45	≤ 0,3	≤ 0,3
Mean virus amount ± K (95%) [1]	3,68 ± 0,29		≤ 0,38 ± 0,15		≤ 0,3	
Reduction (lg ID_{50} ± K [95%])[2]	**1,35 ± 0,42**		**≥ 4,50 ± 0,31**		**≥ 3,60 ± 0,38**	





1) Calculation of titer and its 95% confidence interval based on DVV/RKI-guidelines
2) Reduction of virus: lg ID_{50} of virus control minus lg ID_{50} the sample; based on DVV/RKI-guidelines

Results:

- Due to the exposure time (here: up to 24 hours) of the virus supernatant on the test areas, the test virus can already be reduced to a certain extent without any (additional) action. This was known from former experiments and had been expected. It should be mentioned that the extent of the reduction for the current test (1.1 log) is comparatively slight in this experiment.
- To assess the virus inactivating property, a corresponding output value was determined at each exposure time (virus control(s)). The initial virus value at the respective time point thus represents the reference point for determining the product associated virus inactivation (reduction).
- At the end of the exposure time (under the above-mentioned test conditions) the following product-associated reduction factors have been determined:

Surface coating containing nanosilver:

- 1h: RF = 1,35 ± 0,42, 8h: RF ≥ 4,50 ± 0,42 and 24 Std.: RF ≥ 3,60 ± 0,38

[redacted]

Conclusion:

- The liquid film that was applied to the test areas was stable during the observation period, even at the end of the longest exposure time and the virus supernatant had not dried out. So a continual contact between the test area and the virus supernatant was ensured during the complete observation period. Therefore a distribution of the virus supernatant (e.g. by diffusion) within the liquid phase was possible.
- Any detectable virus reduction can therefore be attributed to the coating.
- ***Surface coating:*** After an exposure time of 1 h residual virus was still detectable and the reduction was RF = 1,35. After 8 h or 24 h, virtually no residual virus was detectable, and the virus reduction was more than 4.5 log.

[redacted]

Annotation:

- The observed virus-inactivating effect of the coating was raised with *Bovine Coronavirus.* This test virus is an enveloped virus, which is generally considered to be easily inactivated. So the observed virus-inactivating effect can not be transferred to other viruses - possibly also not to enveloped viruses.
- The above described data were raised in a screening test according to ISO 21702. This is a basic test, which was carried out based on the underlying rules and under omission of validity control. This test therefore does not correspond to extensive product validation according to ISO 21702, because it was just a screeningtest, which serves as basic test to check if it is worth doing an extensive product validation.

Luckenwalde, den 27.06.2020

Dr. Ch. Jursch
(GF und Laborleiter Eurovir)

Copied signature of Eurovir CEO

Hereby RAS AG confirms, that the report was explained and translated to our best knowledge:

Signed:

ppa. Gregor Schneider
Authorized Head of Business unit agpure®