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Eurovir® Hygiene-Labor
Antivirale Validierung & Rabies

Testing the virucidal properties of biocidal test surfaces

Examination of test surfaces equipped with a virucidal active coating using a practical virucidal carrier test following the Robert Koch Institute Guideline (1995) as well as ISO 21702:2019 against the bovine coronavirus (BCoV, strain: S379 Riems) - Screening test S2, dated 29./30.07.2020

Short report: Screening test S2

by
PD Dr. Olaf Thraenhart and Dr. Christian Jursch

Test Period:	July - August 2020
Principal:	ALANOD GmbH & Co. KG Egerstraße 12 D-58256 Ennepetal, Germany

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Products:

- Tested surfaces: various metal sheets, single-side coated, cut to size 1.6 cm x 6 cm (refer table 1).

Test parameter:

- Test conditions: T = 25° C and 90 % RH
- Protein load: no additional protein added; cell culture supernatant was applied onto the surface without any further manipulation or alteration
- Volume to area ratio: 25 µL/cm^2
- Test virus suspension culture applied (150 µL) on a surface of 1.2 x 5 cm, subsequently covered with LDPE foil, 50 µm, cut to size of 1.2 cm x 5 cm (6 cm^2)
- Incubation period: 1h, 4h and 24h in BINDER Constant Climate Chamber, model KBF 115.

Testing System:

- Bovine coronavirus (BCoV; Genus: betacoronavirus), strain: S379 Riems
- Origin: Friedrich Löffler Institute (Insel Riems), University Greifswald, D-17493 Greifswald, Germany
- HRT-18 cells (human rectal carcinoma cells)
- Origin: Institute of Hygiene and Infectious Diseases of Animals, University Giessen, D-35392 Giessen, Germany

Test Procedure:

- All virucidal quantative carrier tests were carried out at a temperature of 25° C and 90 % RH (in a constant climate chamber) and follow the Robert Koch Institute Guidelines (1995) and ISO 21702:2019.
- All tests were undertaken without additional protein load.

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

No.	Product (s)	Labelling	Storage [1]
#1	Reference material (stainless steel)	P0	Room Temperature
#2	MIRO Cu 53	P4	Room Temperature
#3	MIRO Cu 711	P5	Room Temperature

[1] *=access limited to Eurovir staff*

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Test results:

Observations:

- All tested surfaces were generally wettable. By covering the wetted surfaces with LDPE foil, a more or less even liquid film could be formed. This liquid film remained stable during the testing period.
- A crystalline precipitate formed on the surfaces of samples P4 and P5; this was the case especially after 24 hours, recognisable by the cytotoxicity that occurs. After 4 hours and even after 1 hour this became apparent

Tab. 2.1: Virus control - reference material (P0) *(Virus titration by limiting dilution)*

Samples	VK-1a	VK-1a	VK-2a	VK-2b	VK-3a	VK-3a
	Virus control / 1 h		Virus control / 4 h		Virus control / 24 h	
Titer/Test vol. (lg ID_{50})	4,35	4,95	3,15	4,2	≤ 0,3	≤ 0,3
Average virus titre ± K (95%) [1]	**5,65 ± 0,30/mL**		**4,68 ± 0,35/mL**		**≤ 1,3/mL**	
Reduction [2] (lg ID_{50} ± K [95%])	-		0,97 ± 0,46 *(vs t = 1 hr)*		≥ 4,35 ± 0,30 *(vs t = 1 hr)*	

[1] = Determination of virus titre and its 95% confidence interval according to German DVV/RKI Guideline

Tab. 3.1: Virus inactivation - **MIRO Cu 53 (P4)** *(Virus titration by limiting dilution)*

Samples	In-7a	In-7b	In-8a	In-8b	In-9a	In-9b	T-7	T-8	T-9
	Inact. / 1 h		Inact. / 4 h		Inact. / 24 h		Tox /1 h	Tox /4 h	Tox/24 h
Titer/Test vol. (lg ID_{50})	≤ 0,3	3,45	≤ 0,3	≤ 0,3	≤ 1,5	≤ 1,5	≤ 0,3	≤ 0,3	1,5
Average virus titre ± K (95%) [1]	≤ 2,88 ± 0,53/mL		≤ 1,3/mL		≤ 2,5/mL		-	-	-
Reduction [2] (lg ID_{50} ± K [95%])	**≥ 2,77 ± 0,61**		**≥ 4,35 ± 0,30** *(vs VK/ t = 1 hr)*		**-1,20 ± 0,0**		-	-	-

[1] = Determination of virus titre and its 95% confidence interval according to German DVV/RKI Guideline
[2] = Virus reduction: lg ID_{50} of virus input (virus control) minus lg ID_{50} of sample (at given point in time)

Tab. 3.2: Virus inactivation - **MIRO Cu 711 (P5)** *(Virus titration by limiting dilution)*

Samples	In-10a	In-10b	In-11a	In-11b	In-12a	In-12b	T-10	T-11	T-12
	Inact. / 1 h		Inact. / 4 h		Inact. / 24 h		Tox /1 h	Tox /4 h	Tox/24 h
Titer/Test vol. (lg ID_{50})	4,2	3,3	0,6	≤ 0,3	≤ 1,5	≤ 1,5	≤ 0,3	≤ 0,3	1,5
Average virus titre ± K (95%) [1]	4,75 ± 0,38/mL		≤ 1,45 ± 0,2/mL		≤ 2,5/mL		-	-	-
Reduction [2] (lg ID_{50} ± K [95%])	**0,90 ± 0,48**		**≥ 4,2 ± 0,36** *(vs VK/ t = 1 hr)*		**-1,20 ± 0,0**		-	-	-

[1] = Determination of virus titre and its 95% confidence interval according to German DVV/RKI Guideline
[2] = Virus reduction: lg ID_{50} of virus input (virus control) minus lg ID_{50} of sample (at given point in time)

Test Results: (refer tables 2 or 3)

- For an evaluation of the Cuvimetal coating virucidal efficacy, for each testing period the correlating virus initial value was established. This initial value is used as a reference value to establish the virucidal activity (inactivation/reduction of the virus) due to the surface. (refer table 2.1)
- As a reference, non-lacquered / non-coated stainless steel surfaces were tested. On this material with a slightly textured (brushed?) surface, the test virus could be reduced by quite a significant extent, even without (additional) incubation time. The virus load established after 4hours was reduced by about 1 Log compared to the value established after 1 hour exposure time. After a 24 hours exposure time, the test virus could no longer be detected. This correlates to a reduction of the viral load (in comparison to the value established after 1 hour) of RF $\geq$ 4.35.
- The determination of the virucidal properties of the product samples were complicated by the fact that the reference material also showed virucidal capacities. A negative reduction of the viral load is not synonymous with “no effect”, but means, that the virucidal effectiveness of the test material vs. BCoV was lower than that of the carrier material used as reference.
- To establish the titre of the virus load on the tested surfaces, it is advisable to compare the values established after 1 hour and 4 hours.
- ***MIRO Cu 53 (P4):*** This coating showed a significant virus reduction. Already after 1 hour, an antiviral reaction became apparent. After 4 hours, the reduction of the viral load in comparison to the reference value /t = 1 hour RF $\geq$ 4.35 (refer table 3.1). It should be noted, that with time, underneath the PE-foil, a crystalline precipitate formed. Questions pertaining to the nature of this precipitate and what effect it had on the virucidal properties of the tested surface have to remain unanswered. After 24 hours this precipitate developed cytotoxic effects ($lgTD_{50}$ = 2.5/mL).
- ***MIRO Cu 711 (P5):*** This coating also showed significant virus reducing properties. After 1 hour only a slight effect was apparent (RF = 0.9 vs. VK/t = 1h), after 4 hours the virucidal effect amounted to RF $\geq$ 4.2 (refer table 3.2). Also, in this case, a crystalline precipitate formed underneath the PE-foil which after 24 hours also developed cytotoxic effects ($lgTD_{50}$ = 2.5/mL).

Conclusions:

- The liquid film applied on the tested Cuvimetal surfaces remained stable during exposure, i.e. even at the end of the longest exposure time, the viral fluid had not completely dried out. Thus during exposure time, the biocidal surface was constantly in contact with the viral fluid. Thus, a dispersion of the viral material (i.e. per diffusion) during the liquid phase was possible.
- Above data allows the conclusion that a possible verifiable reduction of viral load can be attributed to the effect of the surface itself.
- ***MIRO Cu:*** These components proved highly effective against BCoV. Amongst these 2 surface materials, the viral reduction varied after 1 hour between 0.9 Log and more than 2,7 Log. After 4 hours, all 2 surface components showed a viral reduction rate against BCoV of more than 4 Log (corresponding to more than 99.99%).
- With time, on the PVD surfaces, crystalline precipitate formed underneath the PE-foil. The exact nature of the precipitate was not established and whether this precipitate had an effect on the virucidal efficacy of the surface remains unanswered.

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Comments:

- The virus-inactivating effect of the surface coating was tested with bovine coronavirus, BCoV (from the virus genus betacoronavirus to which SARS-Cov-2 also belongs). This test virus is an enveloped virus, which is generally considered easy to inactivate. This means that the viral activity observed cannot be directly correlated to other viruses. This might also apply to other enveloped viruses.
- All above data were collected in a so-called screening test. This test is a standard test, performed following the standard guidelines without validation control. As a result, this test does not correspond to a comprehensive product validation, carried out in accordance with ISO 21702.

Luckenwalde, 9th of September 2020

Dr. Ch. Jursch
(GF und Laborleiter Eurovir)

Disclaimer: The underlying original test report (in German) was translated into English by cmb translations, Clausthal-Zellerfelder-Str. 27, D-40595 Düsseldorf. EUROVIR is responsible for correctness of the data tables and the translator is responsible for the correct translation of the original text.