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Eurovir® Hygiene-Labor
Antivirale Validierung & Rabies

Testing the virucidal activity of *" Liquid Guard"*

Examination of test surfaces equipped with a virucidal active coating using a praxis-near carrier test system following the RKI-Richtlinie (1995) as well as ISO 21702:2019 against the *Transmissible Gastroenteritis Virus (TGEV-Coronavirus)* - Test run S1 dated 11./12.03.2020

Short report: screening test S2

by
PD Dr. Olaf Thraenhart and Dr. Christian Jursch

Test period:	in March 2020
Principal:	Nano-Care Deutschland AG Alfred Nobel-Straße 10 D-66793 Saarwellingen, Germany

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Products:

- Test surfaces: *Leneta®* foil, with the dimensions of 1,6 cm x 6 cm
- 1. test item: test surfaces coated on one side with *Liquid Guard* (containing the active component[s])
- 2. test item: uncoated test surfaces (or coated w/o the active component[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 covered with foil (LDPE, 50 µm) with the dimensions 1,2 x 5 cm (6 cm²)
- Incubation: 1h, 8h and 24h in a climate chamber KBF 115 (Fa Binder)

Test system:

- Transmissible Gastroenteritis Virus of Swine (TGEV-Coronavirus); strain: Toyama 36 [used in test as the model virus for SARS-CoV]
  (Origin: Virusbank of the Friedrich Löffler-Institute, Insel Riems, Germany)
- ST75/2 cells (foetal testis cells of swine)
  (Origin: Robert Koch-Institute, Berlin, Germany)

Test procedure:

- The test was performed following a. RKI-Richtlinie (1995) as well as b. 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**

No.	Product (s)	Storage conditions [1]
#1	Test item / coated with *Liquid Guard* (containing the virucidal active component(s) / „test sample“)	at RT
#2	Test item / uncoated (or coated w/o the virucidal active component(s) / „control sample“)	at RT

[1] = *access limited*

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 by using glass spatulas.
- 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. However, a volume reduction was recorded.

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

Sample	VK-1a	VK-1b	VK-2a	VK-2b	VK-3a	VK-3b
	Virus control / 1 h		Virus control / 8 h		Virus control / 24 h	
Titer/Test vol. ($lg\ ID_{50}$)	4,2	4,8	4,05	3,9	2,25	2,85
av. virus titer ± K (95%) [1]	**5,50 ± 0,37 / 1 mL**		**4,98 ± 0,35 / 1 mL**		**3,55 ± 0,37 / 1 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-1a	In-1b	In-2a	In-2b	In-3a	In-3b
	Inactivation / 1 h		Inactivation / 8 h		Inactivation / 24 h	
Titer/Test vol. ($lg\ ID_{50}$)	3,6	3,45	1,35	1,2	≤ 0,30	≤ 0,30
av. virus titer ± K (95%) [1]	4,53 ± 0,22 / mL		2,28 ± 0,29 / mL		≤ 1,30 / mL	
Reduction [2] ($lg\ ID_{50}$ ± K [95%])	**0,97 ± 0,43**		**2,70 ± 0,46**		**≥ 2,25 ± 0,37**	

[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 also true for the test virus used in the present testing. After presentation over 8 h and 24 h on the test surface a titer reduction of 0,5 Log was evident after 8 h and about 2 Log after 24 h (cf. tab. 2.1). It should be noted, however, that this reduction can be judged as very low when compared to 1). the general tenacity of coronaviruses and b). other viruses (even non-enveloped viruses).
- 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. With the amount of input virus at a given time point (cf. tab. 2.1) and with the correspondent amount of remaining test virus (cf. tab. 2.2) the virus reduction factor can be determined.
- After the incubation time was due and under the test conditions specified above the virus reduction factor associated with the coating containing the active component amounted to RF = 0,97 ± 0,43 after 1 h, to RF = 2,70 ± 0,46 after 8 h and to RF ≥ 2,25 ± 0,37 after 24 h (cf. Tab. 2.2). It should be noted that after 24 h no residual test virus was detectable.

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Conclusions:

- The virus film applied on the test items and covered with the LDPE-foil was stable over the entire observation period. This means that the virus film remained in the liquid state even at the end of the longest exposure time (24 h) and was not dried. 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 driven by diffusion was given.
- After t = 1 h a virus reduction of 0,97 Log was recorded (corresponding to 90 % of inactivation) and after t = 8 h the virus reduction amounted to RF = 2,7 (corresponding to 99,8 % of inactivation). Due to technical reasons demonstration of the virus reduction was limited to RF $\geq$ 2,25 after 24 h.
- The data obtained allow the conclusion that there is a virus reduction that can be attributed to the coating containing the active component(s). With the present testing a good virus inactivating activity of the virucidal coating under test was demonstrated against the TGEV-Coronavirus (as the model virus for the SARS-CoV).
- It should also be mentioned that the conditions of ISO 21702 provide for a higher incubation temperature than that used in S1 (25 vs. 21 ° C).
- The virus reduction obtained with t = 8 h suggests that at the incubation time t = 24 h a higher virus reduction is evident than could be demonstrated with the endpoint titration method. Here, virus titer determination using the *Large Volume Plating (LVP)* can possibly provide an improved statement.

Luckenwalde, 20th of March 2020

Dr. Ch. Jursch
(GF und Laborleiter Eurovir)

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Testing the virucidal activity of *" Liquid Guard"*

Examination of test surfaces equipped with a virucidal active coating using a praxis-near carrier test system following the RKI-Richtlinie (1995) as well as JIS Z 2801 (2010) against *Influenza A Virus (H1N1)* - Test run S1 dated 20./21.01.2020

Short report: screening test S1

by
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Test period:	in January 2020
Principal:	Nano-Care Deutschland AG Alfred Nobel-Straße 10 D-66793 Saarwellingen, Germany

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Products:

- Test surfaces: *Leneta®* foil, with the dimensions of 1,6 cm x 6 cm
- 1. test item: test surfaces coated on one side with *Liquid Guard* (containing the active component[s])
- 2. test item: uncoated test surfaces (or coated w/o the active component[s])

Test parameter:

- Test conditions: T = 21 °C and 60 % 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: 20 µL/cm²
- Virus suspension covered with foil (LDPE, 50 µm) with the dimensions 1,2 x 5 cm (6 cm²)
- Incubation: 1h, 8h and 24h in a climate chamber KBF 115 (Fa Binder)

Test system:

- Influenza A Virus; H1N1; strain: New Caledonia
  (Origin: Chiron Behring, Marburg, Germany)
- MDCK-cells (kidney cells from African green monkey [Cercopethecus aethiops])
  (Origin: Robert Koch-Institut, Berlin, Germany)

Test procedure:

- The test was performed following a. RKI-Richtlinie (1995) as well as b. JIS Z 2801 (2010)
- Test principle: quantitative virucidal carrier test at T = 21 °C and 60 % r.LF (climate chamber)
- the test was performed w/o (additional) protein load

***Tab. 1:* Product samples tested** *(as received at 13.01.2020)*

No.	Product (s)	Storage conditions [1]
#1	Test item / coated with *Liquid Guard* (containing the virucidal active component(s) / „test sample“)	at RT
#2	Test item / uncoated (or coated w/o the virucidal active component(s) / „control sample“)	at RT

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

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 by using glass spatulas.
- 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. However, a volume reduction was recorded.

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

Sample	VK-1a	VK-1b	VK-2a	VK-2b	VK-3a	VK-3b
	Virus control / 1 h		Virus control / 8 h		Virus control / 24 h	
Titer/Test vol. ($lg\ ID_{50}$)	3,15	3,3	3,45	3,15	2,7	3,15
av. virus titer ± K (95%) [1]	**3,23 ± 0,36 / 100 µL**		**3,30 ± 0,33 / 100 µL**		**2,93 ± 0,34 / 100 µL**	

[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-1a	In-1b	In-2a	In-2b	In-3a	In-3b
	Inactivation / 1 h		Inactivation / 8 h		Inactivation / 24 h	
Titer/Test vol. ($lg\ ID_{50}$)	3,45	3,15	2,4	2,4	1,2	1,2
av. virus titer ± K (95%) [1]	3,30 ± 0,32		2,40 ± 0,29		1,20 ± 0,33	
Reduction [2] ($lg\ ID_{50}$ ± K [95%])	**-0,07 ± 0,48**		**0,90 ± 0,44**		**1,73 ± 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 also true for the test virus used in the present testing. After presentation over 24 h on the test surface a titer reduction of 0,3 Log was evident (cf. tab. 2.1). It should be noted, however, that this reduction can be judged as very low when compared to 1). the general tenacity of influenza virus and b). other viruses (even non-enveloped viruses).
- 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. With the amount of input virus at a given time point (cf. tab. 2.1) and with the correspondent amount of remaining test virus (cf. tab. 2.2) the virus reduction factor can be determined.
- After the incubation time was due and under the test conditions specified above the virus reduction factor associated with the coating containing the active component amounted to RF = -0,07 ± 0,48 after 1 h, to RF = 0,90 ± 0,44 after 8 h and to RF = 1,73 ± 0,47 after 24 h (cf. Tab. 2.2).

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Conclusions:

- The virus film applied on the test items and covered with the LDPE-foil was stable over the entire observation period. This means that the virus film remained in the liquid state even at the end of the longest exposure time (24 h) and was not dried. 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 driven by diffusion was given.
- The data obtained allow the conclusion that there is a virus reduction that can be attributed to the coating containing the active component(s).
- The virus reduction rate progresses rather slowly over the observation period. No virus inactivation was detectable after a contact time of 1 hour and after 8 hours the virus reduction was approximately 1 Log (corresponding to a virus reduction of approximately 90%). After 24 hours virus reduction reached approximately 2 Log (corresponding to a reduction of approximately 99%).
- The observed virus-inactivating effect of the coating (containing the active component[s]) was determined using the *influenza A virus* as the test virus. This virus is in general considered to be inactivated easily, even when compared with other enveloped virus. This means that the observed virus inactivation capacity of the tested coating, as obtained with *influenza A virus,* cannot be transferred necessarily to other viruses. This also applies to other enveloped viruses.

Luckenwalde, 4th of March 2020

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