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Eurovir® Hygiene-Labor
Antivirale Validierung & Rabies

# Testing the virucidal activity of test specimens equipped with an antimicrobial surface

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
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|---|---|
| Test period: | in March 2020 |
| Principal: | Munditia Technologies GmbH<br>Heegstrauchweg 54<br>D-35394 Gießen, Germany |

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***Principal:*** Munditia Technologies GmbH
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***Products:***

- Test surfaces: frosted glass carrier (according to RKI; with the dimensions of 1,6 cm x 6 cm) coated with the product(s) applied by the principal
- 1. test item: test surfaces coated on one side with the product *Mundex L*
- 2. test item: test surfaces coated on one side with the product *Mundex W*
- 3. test item: test surfaces uncoated or coated w/o the active component(s) (control samples)

***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 was not covered with foil
- 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 the virucidal active component(s) *Mundex L* (test sample) | at RT |
| #2 | Test item / coated with the virucidal active component(s) *Mundex W* (test sample) | at RT |
| #3 | 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. With the product *Mundex W* the virus material seemed to be more evenly distributed on the test items.
- During the resuspension procedure (using glass spatulas) a partial detachment of the coating was observed. It should be noted that the smooth side of the glass carriers was coated with the product).

***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 $ID_{50}$) | 4,65 | 4,5 | 4,5 | 4,35 | 4,05 | 3,6 |
| **av. virus titer ± K (95%) [1]** | **5,58 ± 0,26 / 1 mL** | | **5,43 ± 0,29 / 1 mL** | | **4,83 ± 0,25 / 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 $ID_{50}$) | 3,15 | 3,3 | ≤ 0,30 | ≤ 0,30 | ≤ 0,30 | ≤ 0,30 |
| av. virus titer ± K (95%) [1] | 4,23 ± 0,25 / mL | | ≤ 1,30 / mL | | ≤ 1,30 / mL | |
| **Reduction [2] (lg $ID_{50}$ ± K [95%])** | **1,35 ± 0,36** | | **≥ 4,13 ± 0,29** | | **≥ 3,53 ± 0,25** | |

| Sample | In-4a | In-4b | In-5a | In-5b | In-6a | In-6b |
|---|---|---|---|---|---|---|
| | Inactivation / 1 h | | Inactivation / 8 h | | Inactivation / 24 h | |
| Titer/Test vol. (lg $ID_{50}$) | 4,8 | 4,8 | 2,1 | 2,55 | ≤ 0,30 | ≤ 0,30 |
| av. virus titer ± K (95%) [1] | 5,80 ± 0,33 / mL | | 3,33 ± 0,27 / mL | | ≤ 1,30 / mL | |
| **Reduction [2] (lg $ID_{50}$ ± K [95%])** | **-0,22 ± 0,42** | | **2,10 ± 0,40** | | **≥ 3,53 ± 0,25** | |

[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:*** *(cf. Tab. 2)*

- Without coverage of the virus material by a LDPE foil (as it is recommended by ISO 21702), the amount of infectious virus was reduced to a comparatively low amount during the observation period (after 8 h: by approx. 0,15 Log and after 24 h: by approx. 0,75 Log ).
- This result is particularly interesting for the 24-hour value, since a loss of the infectious virus by about 2 log was observed after 24 hours in a test carried out in parallel using the above mentioned LDPE foil covering.
- 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 with the product *Mundex L* amounted to RF = 1,35 ± 0,36 after 1 h, to RF ≥ 4,13 ± 0,29 after 8 h and to RF ≥ 3,53 ± 0,25 after 24 h (cf. Tab. 2.2). It should be noted that even after 8 h (and after 24 h as well) no residual test virus was detectable.
- With the product *Mundex W* the virus reduction factor amounted to RF = -0,22 ± 0,42 after 1 h, to RF = 2,10 ± 0,40 after 8 h and to RF ≥ 3,53 ± 0,25 after 24 h (cf. Tab. 2.2). After 24 h no residual test virus was detectable.

***Conclusions:***

- The virus film applied on the test items 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.
- With the product *Mundex L* a very high level of virus inactivation was evident with the TGEV-Coronavirus (corresponding to 99,99 % of inactivation) after 8 h.
- Also with the product *Mundex W* a high level of virus inactivation could be demonstrated with the TGEV-Coronavierus after 24 h (corresponding to 99,97 % of inactivation).
- 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 two different virucidal coatings 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 the previous test S1 (25 vs. 21 ° C).
- With the test samples in which no residual test virus remained detectable with the limiting dilution titration method applied an improved statement can possibly be obtained with the *Large Volume Plating (LVP)* as the virus titration method.

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