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Eurovir® Hygiene-Labor
Antivirale Validierung & Rabies

# Testing the virucidal activity of the product Shieldex® Kiel-SK-96

Examination using a praxis-near carrier test system following ISO 21702:2019 against the *Transmissible Gastroenteritis Virus (TGEV-Coronavirus)* - Test run S1 dated 09.04.2020

Short report: screening test S1

by
PD Dr. Olaf Thraenhart and Dr. Christian Jursch

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|---|---|
| Test period: | in April 2020 |
| Principal: | STATEX Produktions- und Vertriebs GmbH<br>Kleiner Ort 11<br>D-28357 Bremen, Germany |

***Principal:*** STATEX Produktions- und Vertriebs GmbH
Kleiner Ort 11
D-28357 Bremen, Germany

***Products:***

- Test item with active component: Shieldex® Kiel-SK-96 Copper Plated Non-Woven + adhesive
- Test item w/o the active component: Shieldex® PBN II Raw material 1,5 Oz
- All test items were applied by the principal

***Test parameter:***

- Test conditions: T = 25 °C (according to ISO 18184) 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: 200 µL distributed to 3,14/cm² (discs, with d = 2 cm)
- Incubation: 1h, 2h and 4h 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 ISO 21702:2019. Test principle: quantitative virucidal carrier test at T = 25 °C (in a climate chamber)
- the test was performed w/o (additional) protein load

***Tab. 1: Product samples tested***

| No. | Product (s) | Storage conditions [1] |
|---|---|---|
| #1 | Shieldex® Kiel-SK-96 Copper Plated Non-Woven + adhesive (test sample) | at RT |
| #2 | Shieldex® PBN II Raw material 1,5 Oz (control sample) | at RT |

[1] = *access limited*

***Performing the test***

***Coating of the test items***

- The textile test material (active and control samples) were equipped with the antiviral coponent by the client, who provided this material as ready-to-use material.
- At Eurovir, round sample disks (test disks) with d = 2 cm were cut out of this sample material using a hole punch.

***Performing the test***

- The test samples were not sterilised by autoclaving. The test material consists of a synthetic fiber, which does not survive the autoclaving.
- From the square shaped product material round test disks were prepared using a 2 cm hole punch. Afterwards, the disks were transferred to a 12-well cell culture plate where the testing was then carried out. The incubation was performed with the lid closed.
- Resuspending of the virus material was also carried out in the 12-well cell culture plate. For virus recovery 5 mL of cell culture medium was added to the test specimen which was then rinsed repeatedly (15x) using a pipette (with V = 1 mL).

***Test results:***

***Observations:***

- With the "Virus control" sample disks (VK; sample material without equipment), not all of the virus suspension was absorbed by the textile specimen. Consequently, the test disk was partially immersed in the virus suspension.
- When the test disks (coated with product) were inoculated, the virus material initially remained as small droplets on the surface. As the time progressed, the material was absorbed completely.
- The test disks (coated with product) were provided with a self-adhesive material on the back (including a protective film). As a consequence, these test disks were liquid-tight towards the bottom - in contrast to the control material (VK).
- Up to 4h of incubation no drying of the material was observed.
- Resuspending of the virus material was performed apparently unremarkable.
- No further observations / unforeseen events were recorded

***Virustitrations***

***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 / 2 h | | Virus control / 4 h | |
| Titer/Test vol. ($lg\ ID_{50}$) | 3,9 | 4,2 | 3,9 | 3,6 | 2,85 | 3,45 |
| **av. virus titer ± K (95%)** [1] | **4,05 ± 0,38 / 100 µL** | | **3,75 ± 0,33 / 100 µL** | | **3,15 ± 0,33 / 100 µL** | |

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

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***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 / 2 h | | Inactivation / 4 h | |
| Titer/Test vol. (lg $ID_{50}$) | ≤ 0,30 | ≤ 0,30 | 1,35 | ≤ 0,30 | ≤ 0,90 | ≤ 0,90 |
| av. virus titer ± K (95%) [1] | ≤ 0,30 | | ≤ 0,38 ± 0,15 | | ≤ 0,90 | |
| **Reduction [2] (lg $ID_{50}$ ± K [95%])** | **≥ 3,75 ± 0,38** | | **≥ 3,37 ± 0,36** | | **≥ 2,25 ± 0,33** | |

[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)*

- Even without the virucidal component the initial amount of virus was slightly reduced with time. After 4h of incubation a virus reduction of approx. 0,9 Log was recorded.
- It was expected in general that the amount of virus would be different with the 3 different time points. To take that into account the amount of virus was determined at each exposure time point separately (virus control [s] at the respective time). Thus the amount of virus at the respective time point (cf. Table 2.1) represents the reference point for determining the product-associated virus inactivation (virus reduction; cf. Table 2.2)..
- Even after t = 1 hour, no residual virus was detectable in both test samples. After t = 2 hours, residual virus was detected in one out of the two test samples whereas the second sample was virus negative. After t = 4 hours the sample material became slightly cytotoxic ($lgTD_{50}$ = 0.9). No residual virus was detectable in the next following dilution.
- After the exposure time was due (1 hour, 2 hours and 4 hours) and under the described test conditions the following product-associated reduction factors were determined: after 1 hour RF ≥ 3,75 ± 0,38, after 2 hours RF ≥ 3,37 ± 0,36 and after 4 hours RF ≥ 2,25 ± 0,33.

***Conclusions:***

- Even after t = 1 hour a significant virus reduction was recorded. With no residual test virus detected virus reduction was calculated to RF ≥ 3,75 (corresponding to a reduction of 99,98%).
- It can be concluded from the data obtained that under the test conditions and after 1 hour of incubation a high-level virus-inactivating effect vs. the TGEV-coronavirus was given. This high-level virus-inactivating effect can be attributed to the antimicrobial equipment.

Luckenwalde, 16th of April 2020

Dr. Ch. Jursch
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