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**Supplier Details:**

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Tintas Ltda  
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**Ref.: VIRUCIDAL VARNISH REPORT**

**1. Product:**

**REVITARE NANO IS-47** Satin 18 L Ref: IS47  
Smart Protective Varnish  
Nano Silver (colloidal silver dispersion)  
Lot: 082006B Manufacturing: 8/25/2020

**2. Virus tested: MHV coronavirus strain, Genus *Betacoronavirus*** (same genus and family of SARS-CoV1, SARS-CoV-2/Covid19, MERS).

Viruses	Cell Lineage
MHV3 coronavirus strain	Cell: L929 NCTC clone 929 [L cell, L-929, derivative of Strain L] (ATCC® CCL-1™)

**3. Methodology:**

- a) The tests were performed in laboratory NB-2 (Biosafety Level 2) following the Recommendations of ANVISA Art. 1 and Art. 3 of IN 04/13 and IN 12/16 and methodologies described in the standards: INTERNATIONAL STANDARD ISO- BS ISO 21702:2019 (First edition 2019-05-27): "Measurement of antiviral activity on plastics and other non-porous surfaces" and by the Robert Koch Institute - RKI) and following Good Laboratory Practices (GLP).  
The culture medium for viruses and cell lines was used the Dulbecco Minimum Essential Medium (DMEM) containing 2% to 10% fetal bovine serum.
- b) Coronavirus titration (MHV strain) was performed according to the DICT50 method (Infectious Doses of Tissue Cultures 50%). Sequential dilutions of the virus at base 10 were performed in quadruplicate, in 96-well sterile microplate. Then L929 cells with a concentration of  $2 \times 10^5$  cells/well were added.  
After 48 hours, the cytopathic effect (ECP) of the viral infection is verified, in comparison with cell control and viral control. Titles were calculated based on the method of Reed and Muench, 1938.
- c) The first stage of the tests was to perform the "Determination of the Non-toxic Maximum Concentration (CMNT)" in the different cells tested, to determine the concentration that



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causes toxicity to the cells. Because the test substance must be active only against the virus and not against cells.

- d) **REVITARE NANO IS-47** Ink Sample was diluted in 5% water and prepared in 4 repetitions in sterile Petri dishes that received virus (100DICT50) covering the inoculum. After the sample was inoculated, the Petri dishes were closed with the lid and incubated under gentle agitation (including untreated test samples), incubated at  $(25 \pm 1)^\circ\text{C}$  with relative humidity not less than 90% for 24 hours.
- e) After the 24-hour incubation period, samples were collected and treated.
- Each suspension (Virus + Different samples and different contact times) was pipetted 100  $\mu\text{L}$  in microplates, homogenized and diluted.
  - Then 100  $\mu\text{L}$  of the cell (L929) was pipetted onto the suspension and incubated at  $37^\circ\text{C}$  in a greenhouse with 5%  $\text{CO}_2$  for 48 hours (see item b).
- f) Titles were calculated based on the method of Reed and Muench, 1938. The results are expressed as a percentage of viral inactivation (**Table 1**) compared to untreated viral control (virus titer).

#### Summary/Controls:

- Negative: cell control ( $2 \times 10^5$  cells/mL) in DMEM medium, without virus and without test samples.
- Virus control: Virus titration ( $10_1$  to  $10_{12}$ ) and cell culture in DMEM medium
- Positive test: presence of virus, each test sample and cell line in DMEM.

**\*Table 1** - Results are expressed as a percentage of viral inactivation compared to untreated viral control

Reduction Log	Reduction Factor	Inactivity Percentage/Activity Reduction
1	10	90%
2	100	99%
3	1000	99.9%
4	10,000	99.99% virucidal
5	100,000	99.999% virucidal
6	1,000,000	99.9999% virucidal

<https://microchemlab.com/information/log-and-percent-reductions-microbiology-and-antimicrobial-testing>

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#### 4. Results

**Table 1** - Results of tests with Coronavirus (MHV strain) and different times of contact with the samples of REVITARE NANO IS-47

Products/features	Contact time	Inactivation result in Percent* (table 1) Coronavirus	Cell Cytotoxicity <i>in vitro</i> Cell lineage L929
REVITARE NANO IS-47	1 minute	(99.9%)	No toxicity
Satin 18 L_	5 minutes	Virucidal (99.99%)	No toxicity
Smart Nano Silver Protective	10 minutes	Virucidal (99.99%)	Low toxicity
Varnish (colloidal silver dispersion)	24 hours	Virucidal (99.99%)	Low toxicity

#### 5. Conclusions:

- Considering that there was 99.99% inhibition in relation to the tested Coronavirus, it can be concluded that:
- The **REVITARE NANO IS-47** product was effective for inactivating viral particles, and therefore we recommend using it as a potential virucidal agent for Coronavirus similar to COVID-19.
- The result of the *in vitro* cytotoxicity evaluation showed that the product was not toxic to the cells at 1 and 5 minutes.



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### **INTERNATIONAL STANDARD ISO- BS ISO 21702:2019 (First edition 2019-05-27): “Measurement of antiviral activity on plastics and other non-porous surfaces”**

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