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Niterói, November 9, 2020

Supplier Data:
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REFERENCE: REPORT VIRUCIDA REVITARE NANO IS-47 Zika VIRUS

1. Product:

REVITARE NANO IS-47
lot: 082006B Fab; 08/25/2020 (val: 18 months)

2. Virus tested:

Virus	Cell line
Zika virus ZIKV (ATCC® VR-1839 ™)	Cell: VERO cells (African green monkey kidney) VERO-ATCC CCL 81

3. Methodology:

a) The tests were carried out in laboratory NB-2 (Biosafety Level 2) 2,349, OF SEPTEMBER 14, 2017 Approving the Risk Classification of Biological Agents elaborated in 2017, by the Health Biosafety Commission (CBS), from the Ministry of Health.

b) The entire methodology was carried out 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 the Robert Koch Institute - RKI).

c) This method is effective for assessing virucidal efficacy. These methods consist of a vial containing the fractionated product being inoculated with the virus selected. Virucidal activity methods are quantitative, which means that percentage and logarithmic reductions are calculated by determining the $TCID_{50}$ (50% infectious dose of tissue culture) before and after treatment with the disinfectant. The disinfectant must demonstrate complete inactivation of the virus up to the detection limit of the assay or (if cytotoxicity is observed) a reduction ≥ 3.00 log10 (99.9%).

d) Zika virus titration was performed according to the $DICT_{50}$ method (Infectious Doses Tissue Crops 50%). Sequential dilutions of the virus were performed in triplicate, in 96-well flat-

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bottomed sterile microplates. The Plates were evaluated at each 24 hours and in 72 hours the cytopathic effect (ECP) of the viral infection is verified, in comparison with cell control and viral control. Titles were calculated by plaque forming unit count (Cooper PD. The plaque assay of animal viruses. Adv. Virus Res. 1961).

Controls:

• Negative: Vero cells (2x10 $_5$ cells / mL) in DMEM medium supplemented with 5% fetal bovine serum, without virus and without test samples.

• Virus control: The virus aliquots used had a viral titer in (10^8).

• Positive test: It was characterized as a positive test the samples that presented total cell lysis.

4. Results

Table 1 - Results of tests with Zika virus and different contact times with REVITARE NANO IS-47 sample

Sample	**Evaluation Tim**	**Virucidal Activity [5µL / 50 µL]**
REVITARE NANO IS-47	2 hours	90% / 96%
	1 hour	90% / 98%
	30 minutes	98% / 99%

5. Conclusions:

•Considering that there was over 90% inhibition of the Zika virus tested, it can be concluded what:

•The **REVITARE NANO IS-47 product** was effective for inactivating viral particles, and, therefore, we recommend using it as a potential virucidal agent for the Zika virus.

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