



**FEDERAL UNIVERSITY OF RIO DE JANEIRO  
PRO-RECTOR OF EDUCATION**

Rua Miguel de Frias, 9, Icarai, Niteroi,  
Rio de Janeiro - Brazil Postal Code: 24220-  
**PHONE: (21)** Email: [proex@proex.uff.br](mailto:proex@proex.uff.br)

Niteroi, October 27, 2020

**Supplier Details:**

Contracting company: Incocryl Industria e Comercio de Tintas Ltda – ME  
Tax ID: 56.848.476/0001-31 State Reg.: 115.292.059.115  
Address: Rua Jacinto Goncalves, 254 Jd Sao Jorge – Sao Paulo, SP – Postal Code  
05568-240 Contact: Mariana Amorim - (11) 97238-2461 – [mariana@incocryl.com.br](mailto:mariana@incocryl.com.br)

**REF.: VIRUCIDAL REPORT REVITARE NANO IS-47 Chikungunya**

**1. Product:**

**REVITARE NANO IS-47**

Lot: EXP082001 Man.; 8/25/2020 (exp.: 12 months)

**2. Virus tested: Chikungunya, sequence deposited at GenBank under access number MK910739 (Cirne-Santos et al. 2019).**

<b>Viruses</b>	<b>Cell Lineage</b>
Chikungunya	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), ORDINANCE No. 2,349, SEPTEMBER 14, 2017, which Approves the Risk Classification of Biological Agents prepared in 2017, by the Health Biosafety Commission (CBS), of 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 of Robert Koch Institute – RKI).



**FEDERAL UNIVERSITY OF RIO DE JANEIRO  
PRO-RECTOR OF EDUCATION**

Rua Miguel de Frias, 9, Icarai, Niteroi,  
Rio de Janeiro - Brazil Postal Code: 24220-  
**PHONE: (21)** Email: proex@proex.uff.br

Niteroi, October 27, 2020

- c) This method is effective for assessing virucidal efficacy. These methods consist of a vial containing the fractionated product being inoculated with the selected virus. Virucidal activity methods are quantitative, which means that percentage and logarithmic reductions are calculated by determining the TCID<sub>50</sub> (50% infectious tissue culture dose) before and after treatment with the disinfectant. The disinfectant must demonstrate complete virus inactivation up to the detection limit of the assay or (if cytotoxicity is observed) a reduction  $\geq 3.00 \log_{10}$  (99.9%).
- d) The titration of the Chikungunya virus was performed according to the DICT<sub>50</sub> method (50% Tissue Culture Infectious Doses). Sequential dilutions of the virus were performed in triplicate, in 96-well flat-bottomed sterile microplates. The plates were evaluated every 24 hours and in 72 hours the cytopathic effect (ECP) of the viral infection is verified, in comparison with cell control and viral control. The viral titer was calculated by counting plaque-forming units (Cooper PD. The plaque assay of animal viruses. Adv. Virus Res. 1961).

**Controls:**

- Negative: Vero cells (2x10<sup>5</sup> 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<sup>8</sup>).
- Positive test: Samples with total cell lysis were characterized as positive tests.

**4. Results:**

**Table 1** - Results of tests with Chikungunya and different times of contact with the samples of REVITARE NANO IS-47

Sample	Evaluation Time	Virucidal activity [5 $\mu$ L/50 $\mu$ L]
REVITARE NANO IS-47	2 hours	92% / 98%
	1 hour	91% / 99%
	30 minutes	92% / 99%



**FEDERAL UNIVERSITY OF RIO DE JANEIRO  
PRO-RECTOR OF EDUCATION**

Rua Miguel de Frias, 9, Icarai, Niteroi,  
Rio de Janeiro - Brazil Postal Code: 24220-  
**PHONE: (21)** Email: proex@proex.uff.br

Niteroi, October 27, 2020

**5. Conclusions:**

- Considering that there was over 90% inhibition of the tested chikungunya virus, 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 the chikungunya virus.

**6. Consulted Bibliography:**

OFFICIAL GAZETTE Published on: 9/22/2017 | Issue: 183 | Section: 1 |Page:51 Body: Ministry of Health / ORDINANCE No. 2,349, OF SEPTEMBER 14, 2017.

ANVISA - Ministry of Health / National Health Surveillance Agency NORMATIVE INSTRUCTION No. 4 OF JULY 2, 2013  
[http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2013/int0004\\_02\\_07\\_2013.html](http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2013/int0004_02_07_2013.html)

ANVISA - NORMATIVE INSTRUCTION No. 12, OF OCTOBER 11, 2016 – ANVISA.  
<https://alimentusconsultoria.com.br/instrucao-normativa-no-12-2016-anvisa/>  
<https://alimentusconsultoria.com.br/instrucao-normativa-in-no-50-de-3-de-dezembro-de-2019-anvisa/>

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

**BS EN 16777:2018:** *Chemical disinfectants and antiseptics. Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area*

**DIN EN 14476:2015.** Chemical disinfectants and antiseptics. Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine. Test method and requirements [phase 2, step 1]. Brussels 2015, CEN-Comité Européen de Normalisation.

SIDDHARTA, Anindya et al. Virucidal activity of World Health Organization–recommended formulations against enveloped viruses, including zika, ebola, and emerging coronaviruses. **The Journal of infectious diseases**, v. 215, n. 6, p. 902-906, 2017.

ARTIKA, I. Made; MA’ROEF, Chairin Nisa. Laboratory biosafety for handling emerging viruses. **Asian Pacific Journal of Tropical Biomedicine**, v. 7, n. 5, p. 483-491, 2017.

Cirne-Santos CC, Barros CdS, Azevedo RC, Nogueira C, Yamamoto K, Meira G, Vasconcelos Z, Raticliffe N, Teixeira VL, Schmidt-Chanasit J (2019) Inhibition by marine algae of chikungunya virus isolated from patients in a recent disease outbreak in Rio de Janeiro. *Frontiers in microbiology* 10:2426