[Logo: UNICAMP] Clarice Weis Arns (PhD, Professor) [Logo: IB Instuto de Biologia]
Virology Laboratory
Instituto de Biologia/Universidade Estadual de campinas-UNICAMP
CEP:13083-862 Campinas-SP-Brazil
TEL: (19)3521-6258 Email: arns@unicamp.br

Cidade Universitária, 16th December 2020

Report VIRUCIDE TEST

1) Product:
Bacoban DL
Long-lasting sanitizer – up to 10 days
Product for surfaces
Active ingredient: Benzalkonium Chloride

2) Viruses tested: Coronavirus (strain MHV-03)

Virus	**Cell Strains**
Coronavirus MHV-3	Cell L929 NCTC clone 929 [L cell, L-929, derivative of Strain L] (ATCC® CCL-1™)

3) Experimental procedure:

a) The trials were performed in an NB-2 (Biosafety Level 2) laboratory, following the ANVISA Recommendations Art. 1 and Art. 3 of IN 04/13 and IN 12/16 and using methodologies described in the Standards (EN14476:2019, ASTM E1053 - 11 and of the Robert Koch Institute – RKI) and following "Good Laboratory Practices" (GLP).
The culture medium for the viruses and cell lines was Dulbecco's Modified Eagle Medium (DMEM) containing 2% to 10% bovine foetal serum.

b) The virus titrations were performed according to the $TCID_{50}$ method (Median Tissue Culture Infectious Dose 50%). Sequential dilutions of the virus at the base 10 were performed in quadruplicate, on sterile, 96-well microplates. The respective cell (item 2) was then added, with a concentration of 1×10^5 cells per well.

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After 48 hours, the cytopathic effect (CPE), characteristic of viral infection, was verified and compared to the cellular control and viral control.

c) Initially, the product **Bacoban DL** was tested in the cell line for "Determination of Maximum Non-Toxic Dose (MNTD)" to define the concentration that does not cause cell toxicity.

d) **Bacoban DL** sample was mixed with the virus and then subjected to different contact times (**Long Lasting**: 24h, 48h, 72h, 7 days and 10 days), and then inoculated onto the permissive cells.

e) The microplates with the **product** (different times), virus and cell systems were incubated at 37ºC in an oven with 5% CO_2 for 48 hours.

f) After 48 hours of incubation, the plates were read on an Inverted Microscope to search for characteristic Cytopathic Effect (or absence) of the virus and the titres were calculated based on the Reed and Muench method, 1938. The results are expressed as a percentage of viral inactivation (Table 1) compared to the untreated viral control (virus titre).

Summary/Controls:

- Negative: Cellular control ($2x10^5$cells/ml) in DMEM, without viruses and without test sample
- Virus control: Titration of the virus (10^1 to 10^{12}) and cell culture in DMEM
- Positive test: presence of virus, **Bacoban DL** and cell strain in DMEM.

Table 1 – The results are expressed as a percentage of viral inactivation compared to the untreated viral control:

Reduction Log	Reduction factor	Percentage Inactivation/Reduction
1	10	90%
2	100	99%
3	1000	99.99%
4	**10,000**	**99.999%**
5	**100,000**	**99.999%**
6	**1,000,000**	**99.9999%**

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

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4) Results:

Table 2 – Results for the product "**Bacoban DL**" tested at different contact times with Coronavirus MHV-3

Product	**Times**	**Results as a percentage (Table 1) Coronavirus MHV-3**
Bacoban DL	24 hours	99.99% inactivation
	48 hours	99.99% inactivation
	72 hours	99.999% inactivation
	7 days	99.999% inactivation
	10 days	99.99% inactivation

5) Conclusions

* Considering that there was inactivation of up to 99.999% and reduction of viral ineffectiveness of ≥ log 5, it can be concluded that the product "**Bacoban DL**" was effective for up to 10 days of contact. Therefore, we recommend its use in the combat of the Coronavirus group, as well as COVID-19.

[Signature]

Prof. Dr Clarice Weic-Arns (Lattes ID: 8635038112182716)

(Person Responsible for the Report)

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