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July, 22th, 2020

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Virucide Report: Air Deco Pyramide Equipment,
Bipolar Ionization Technology by Bioclimatic GmbH, Germany.

1. **Product:** Air Deco Pyramide Bipolar Ionization by Bioclimatic GmbH, Germany.

2. **Applicant:**
LV Engenharia, Maquinas e Equipamentos EIRELI
Rua do Vidraceiro, 72 - Jardim Werner Plaas
Americana - SP CEP: 13478734 FONE: (19) 3601-6660
Company: Icy Cold

3. **Viruses tested:** Coronavirus strain MHV-3 genus *Betacoronavirus* (same genus and family of SARS-CoV-1, SARS-CoV-2, MERS and others).
Cellular Strain: L929, NCTC clone 929 L cell, (ATCC® CCL-1™).

4. **Experimental procedure:**

a) The tests were carried out 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 DIN EN 14476: Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements, ASTM E1053-11 and the Robert Koch Institute - RKI. The entire trial followed Good Laboratory Practice (GLP) standards.
The culture medium for viruses and cell lines used was the Minimum Essential Medium of Dulbecco (DMEM).

b) A BIOSAFETY CABIN CLASS II (AC2-4E8) was used for the experimental tests in which the Air Deco Pyramide equipment was placed inside. The coronavirus MHV-3 strain, with 100 $DICT_{50}$, was distributed by spray and 05 sterile Petri dishes (Ø 90 mm x 15 mm) with 1 ml of DMEM culture medium were distributed in different positions. The Air Deco Pyramide equipment was on for 10 minutes, 20 minutes and 30 minutes. After the lapse of time, the Petri dishes were removed and the steps of the virucidal test were performed. The negative control, 5 Petri dishes with 1 mL of DMEM medium were distributed and left with the equipment on for another 5 minutes.

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c) The 96-well microplates were inoculated with 50 µl of each sample and different times, 50 µl of virus and 100 µl cellular line (L929). The microplates were incubated at 37°C in a 5% CO_2 atmosphere and observed for cytopathic effects (CPEs) daily for up to five days. Titers were calculated by $TCID_{50}$ based on the using the Reed-Muench method (1938).
d) Samples were mixed with viruses, submitted to different contact times (10, 20 and 30 minutes) and inoculated into permissive cell (L929).
e) The microplates with samples (different times), virus and cellular line were incubated at 37°C with 5% CO_2 for 48 hours.
f) Results are expressed as **a percentage of viral inactivation (Table 3)** compared to untreated viral control (virus titer).

Summary:

- Negative: cell control ($2x10^5$ cell/mL) in Dulbecco minimal essential medium (DMEM), without virus and samples;
- Virus control: Virus titration (10^1 to 10^{12}) and cell culture in Dulbecco minimal essential medium (DMEM) containing 10% fetal bovine serum.
- Positive test: presence of virus, SAMPLES and cell lines.

5. Results:

Table 1 – Coronavirus (strain MHV-3), different contact times to the "Air Deco Pyramide Equipment"

Product	Time	Different positions of the plates in the Biosafety cabin	Coronavirus (strain MHV-3) Table 3*	Negative Control
Air Deco Pyramide	10 minutes	L1	*99,999% (virucide)	Negative (no virus)
	20 minutes	L2, L5	99,999% (virucide)	Negative (no virus)
	30 minutes	L3, L4	99,999% (virucide)	Negative (no virus)

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Table 2 – Coronavirus (MHV-3 strain) titer, Virus titer after treatment with "Air Deco Pyramide", Reduction of viral infectivity, percentage of inactivation and cellular toxicity.

Virus	Virus Titer $TCID_{50}$/mL (Log 10) (control)	Virus titer after treatment with "Air Deco Pyramide" $TCID_{50}$/mL (Log 10)	Reduction of viral infectivity $TCID_{50}$/mL (log10)	Percentage of inactivation	Cellular toxicity Cell Line L929
Coronavirus-MHV-3	8,0	3,0	5,0	99,999%	No toxic

*** Table 3 -** Results are expressed as a percentage of viral inactivation (≥ 99.99%) compared to the untreated viral control:

Log Reduction	Reduction Factor	Percent reduction/virus inactivation	
1	10	90%	
2	100	99%	
3	1000	99,9%	
4	10.000	99,99%	Virucide
5	100.000	99,999%	Virucide
6	1.000,000	99,9999%	Virucide

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

6. Conclusions:

The analyzed **Equipment** showed virucidal activity.

• Considering that virus was inactivated (99.999%), we recommend using "**Air Deco Pyramide Equipment**" as a virucidal agent for the Coronavirus group (included SARS-CoV-2) after 10 minutes of contact.
• The results to use the Air Deco Pyramide Equipment in contact with the cell line did not produce toxicity *in vitro*.

Sincerely,

Prof. Dr. Clarice Weis-Arns (ID Lattes: 8635038112182716)
Responsible for the Report

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References:

ANVISA - Ministério da Saúde/Agência Nacional de Vigilância Sanitária
INSTRUÇÃO NORMATIVA Nº 4, DE 2 DE JULHO DE 2013
http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2013/int0004_02_07_2013.html

ANVISA- INSTRUÇÃO NORMATIVA Nº 12, DE 11 DE OUTUBRO DE 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/

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

BS EN 14476:2013+A2:2019
Incorporating corrigendum August 2019
Chemical disinfectants and antiseptics -Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements (Phase 2/Step 1)

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.

Britta Becker, Lars Henningsen, Dajana Paulmann, Birte Bischoff, Daniel Todt , Eike Steinmann, Joerg Steinmann, Florian H. H. Brill and Jochen Steinmann
Evaluation of the virucidal efficacy of disinfectant wipes with a test method simulating practical conditions
Antimicrobial Resistance and Infection Control (2019) 8:121
https://doi.org/10.1186/s13756-019-0569-4

G. Kampf D., Todt, S. Pfaender , E. Steinmann
Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents
Journal of Hospital Infection 104 (2020) 246e251
https://doi.org/10.1016/j.jhin.2020.01.022 0195-6701

Rabenau HF, Schwebke I, Blumel J, Eggers M, Glebe D, Rapp I, Sauerbrei A, Steinmann E, Steinmann J, Willkommen H, Wutzler P.
Guideline of the German Association for the Control of Virus Diseases (DVV) e.V. and the **Robert Koch-Institute (RKI)** for testing chemical disinfectants for effectiveness against viruses in human medicine. Version of 1st December, 2014.
Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2015;58: 493–504

Reed LJ, Muench H.
A simple method of estimating fifty per cent endpoints. Am J Hyg. 1938; 27:493–497.