



Universidad de la República
School of Sciences
Virology Section



Technical assistance report for the evaluation of virucidal activity of XTERIDES 100, XTERICOLD, XTERIVET and HD+.

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Virucidal activity: it is defined as the capacity a compound has to inactivate or degrade viral particles on surfaces or the environment, totally or partially reducing its infectivity. It is measured by the reduction in viral particles or viral titer compared to a control treatment.

1. Objective

The object of this technical assistance was to test virucidal activity of the XTERIDES 100, XTERICOLD, XTERIVET and HD+ products, manufactured by EMARLAN S.A., with the equipment by **HyperDes Watertechnology GmbH**, from Germany, with the following models: HD VA 003, HD VA 20, HD VA 240, HD VA 75, HD VA 150, HD VA MED 20, HD HA 20, HD HA 40, on two types of surfaces, against four different virus families: **Coronavirus** (Bovine Coronavirus - BCoV), **Adenovirus**, **Orthomyxovirus** (H1N1 Influenza Virus) and **Paramyxovirus** (Human Respiratory Syncytial Virus).

2. Essay Description

a. Virus y Células

3. . CoV-Bov. Genus Betacoronavirus-wild field strain.
4. . Adenovirus 7- (ATCC #VR-7D).
5. . Virus Influenza H1N1- wild field strain.
6. . VRSh. Cepa Long (ATCC #VR-26).
7. . A549 (ATCC #CCL-185).
8. . MDCK (ATCC #CCL-34).
- . HRT-18 (ATCC #CCL-244).

a. Cytotoxicity

Cytotoxicity of the products in cell cultures was determined by calculating 50% Cytotoxic Concentration (CC50%) using a cell counting method in a Neubauer chamber and colorimetric analysis with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, USA). Protocols were based on what is established by the Environmental Protection Agency of United States, USEPA Initial Virucidal Effectiveness Test¹. Cytotoxicity was tested on cell lines that were then used for the virucidal activity essays: A549 (Adenovirus and hRSV), MDCK (H1N1 influenza) and HRT-18 (BCoV). For these assays a product concentration of 100% was assumed, and the tested concentrations were 90%, 80%, 70%, 60%, 50% and 40%.

b. Virucidal Activity

Virucidal activity was implemented strictly following protocols established by the United States Protection Agency, US-EPA Initial Virucidal Effectiveness Test. Product efficacy was calculated by *in vitro* viral titer reduction method according to the protocol specifications. Virucidal capacity was assessed on metal and slab surfaces for each virus with a 5-minute exposure time. Essays for each virus were triplicated, with two viral concentrations: 10^5 and 10^3 , 50% tissue culture infective dose (TCID50%). As treatment control (virus without treatment) phosphate buffered saline (PBS) solution was used.

9. Results

a. Cytotoxicity

XTERIDES 100, XTERICOLD, XTERIVET and HD+ proved to have a 75% CC50% on all tested cell lines. Therefore, virucidal activity of the product over both surfaces and for all the tested viruses was implemented with 75% product concentration.

b. Virucidal activity

XTERIDES 100, XTERICOLD, XTERIVET and HD+ showed virucidal activity against all the tested virus types, for both concentrations of the virus and in both surfaces after a 5-minute exposure. The virucidal effect was larger in those viruses with envelope (BCoV, H1N1 Influenza and hRSV) than in those viruses without envelope (Adenovirus), for both viral concentrations (Table 1). For envelope viruses a

reduction in the viral titer was observed between 2.2 and 3.5 log (about 100 and 3500 times). In both studied surfaces, metal and slab, the reduction levels were the same.

Table 1. Viral titer reduction by virucidal activity of XTERIDES 100 compared to untreated control, for two virus concentrations.

Titer reduction - log10*

Virus	103 TCID50%	105 TCID50%
BCov	2.5	2.4
Adenovirus	2.4	2.2
H1N1 Influenza	3.5	3.5
hRSV	2.5	2.2

*Mean of 3 independent replicas

10. Conclusions

The *in vitro* strategy developed, based on international standard manual from the USE-EPA, was designed to simulate real conditions for using XTERIDES 100, XTERICOLD, XTERIVET and HD+. The tested products reduced cytotoxicity *in vitro* for all the studied cell lines.

The maximum tested concentration of the product was 75%, and showed, after a 5-minute exposure, a very high virucidal efficiency on metal and slab against all viruses tested in this essay. The largest virucidal effect was seen on viruses members of the Coronavirus, Orthomyxovirus and Paramyxovirus families.

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Santiago Mirazo, PhD

Virology Laboratory

Universidad de la República

Montevideo, Uruguay

1.://www.epa.gov/sites/production/files/201509/documents/fc
v1_initial_surf_pcol. pdf