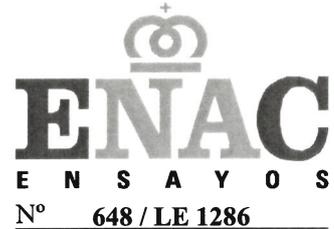


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Test with the certificate of GLPs
(Good Laboratory Practices)
No. 2/19-C.VAL. General Directorate of
Pharmacy and Medical Devices of the Health
Department of the Valencian Region. Spain

Virucidal test with the product “ECO DEGREASER” against Vaccinia Poxvirus (NF EN 14476: 2013 + A2: 2019 Standard)

Report

Registration No.: D/20/984

- 1. **Laboratory identification** Instituto Valenciano de Microbiología.
- 2. **Client identification** INTERFLON/ Pieter-Dave Boekel.
Address Postbus, 1070, 4700, BB ROOSENDAAL
HOLLAND.
- 3. **Sample identification** (information provided by the customer)
 - Product name..... ECO DEGREASER.
 - Batch number..... 090720-M.
 - Expiration date..... 2021/06/01.
 - Manufacturer (supplier)..... INTERFLON.
 - Date of manufacturer..... Not indicated.
 - Storing conditions Keep away from heat sources and direct
sunlight. Store at room temperature.
 - Active(s) Substance(s) and its
concentration (s)..... Does not contain active substances.
 - Conditions of use..... Surfaces.
 - Concentrations ordered for the assay.... 100%.

IVAMI is not responsible for customer-supplied information.



4. Information about sample reception.

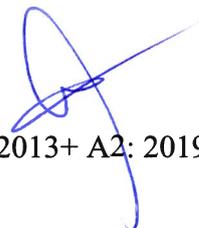
- Date of reception of order with test conditions 2020/06/04.
- Date of reception of the product..... 2020/06/02.
- Aspect of the received product..... Transparent liquid in commercial packaging.

5. Testing method

Procedure **DESIN-1078 (NF EN 14476: 2013 + A2: 2019 guideline).**

6. Experimental conditions

- Assay period..... July 7 to July 24, 2020.
- Assay temperature..... 37°C ± 1°C.
- Titration method TCID₅₀ (Tissue Culture Infective Dose 50%).
- Product concentrations for the assay.... 80%, 50% and 0.1%.
- Contact time..... 15 minutes.
- Contact temperature..... 20°C ± 1°C.
- Procedure to stop product cytotoxicity.. Molecular sieving.
- Procedure to stop product activity Cooling with ice.
- Solvent of the product used in the assay..... Sterile distilled water.
- Aspect of the dilutions of the product... Transparent dilutions.
- Stability of the mixture (interfering substance and product diluted in sterile distilled water)..... Stable.
- Interfering substance:
 - Clean conditions in the presence of bovine serum albumin 0.3 g/L.
- Identification of the origin of viral strains and number of passes..... Vaccinia Poxvirus (ATCC VR-1508), aliquot: 2018/01/22, passage 2.
- Cell lines (name, origin, number of passes and culture medium)..... BHK-21, ref: FTBH, working aliquot 3, passage 19; working aliquot 4, passages 11 and 14.



7. Validation of assay results

Vaccinia Poxvirus (ATCC VR-1508)

Titre of the viral suspension for the virus control (15 minutes):

- Clean conditions..... log 10^{-6.66}
- Cytotoxicity level (80%)..... log 10^{-0.50}

Maximum level of virus inactivation detectable (difference between the titre of the viral suspension and the cytotoxicity level):

- Clean conditions.....log 10^{-6.16}

Reference test (formaldehyde 1.4%)

Cytotoxicity level of formaldehyde 0.7%..... log 10^{-0.50}

Viral quantification in the reference test (formaldehyde) after 15 minutes and with Vaccinia Poxviruslog10^{-3.82}

Confidence interval

Titre of virus with 95% confidence interval with Vaccinia Poxvirus (15 minutes)

- Clean conditionslog 10^{-6.66 ± 0.36}

Reduction with the confidence interval of 95 %See table 1.

Sensitivity of cells to virus

- Viral quantification of Vaccinia Poxvirus with cells not treated with “ECO DEGREASER” disinfectantlog 10^{-6.32}
- Viral quantification of Vaccinia Poxvirus with cells treated with the “ECO DEGREASER” disinfectant.....log 10^{-5.82}

Note: only can be used to determine the infectivity of cells, those dilutions which: a) show a low degree of cellular destruction (< 25% of cell monolayer) and b) produce a reduction of the titre of the virus <1 log₁₀.



Control of the effectivity of the disinfectant detection activity

- Viral quantification of Vaccinia Poxvirus after 30 minutes on bath ice without exposing the virus to the “ECO DEGREASER” disinfectantlog10^{-6.75}
- Viral quantification of Vaccinia Poxvirus exposing the virus to “ECO DEGREASER” disinfectant and incubated 30 minutes on ice bath.....log10^{-6.49}

Note: The difference between decimal logarithm of titre without exposing the virus to the product and of the test suspension should be ≤ 0.5

8. Special remarks

The product is tested at 80%; 50% and 0.1%. The highest concentration that can be tested in the test is 80%, because of the mixtures made during the test.

All controls and validation were between the basic limits.

One concentration at least showed a log reduction less than 4 log.

One concentration at least showed a log reduction higher than ≥ 4 log.

9. Assay results

9.1 Description

The disinfectant product, “ECO DEGREASER”, batch 090720-M, under clean conditions, diluted at 80% and 50% and during 15 minutes of exposure, **shows** virucidal activity against Vaccinia Poxvirus (ATCC VR-1508), with a reduction 5.17 ± 0.48 TCID₅₀ when tested at 80%, and with a reduction 4.59 ± 0.52 TCID₅₀ when tested at 50%, when the activity is assayed according with the **NF EN 14476: 2013 + A2: 2019** guideline.

The disinfectant product, “ECO DEGREASER”, batch 090720-M, under clean conditions, diluted at 0.1% and during 15 minutes of exposure, **does not show** virucidal activity against Vaccinia Poxvirus (ATCC VR-1508), with a reduction 0.17 ± 0.54 TCID₅₀, when the activity is assayed according with the **NF EN 14476: 2013 + A2: 2019** guideline.

9.2 Tables of results and graphics

See tables 1 and 2 and figure 1.



10. Conclusion

The disinfectant product “**ECO DEGREASER**”, batch 090720-M, under clean conditions (bovine serum albumin 0.3 g/L), diluted at **80%**, requested by the customer, and during 15 minutes of exposure, **shows** virucidal activity against Vaccinia Poxvirus (ATCC VR-1508) when the activity is assayed according with the **NF EN 14476: 2013 + A2: 2019** guideline.

The activity of the disinfectant “**ECO DEGREASER**”, batch 090720-M, against Poxvirus Vaccinia (ATCC VR-1508), **does not means that the product has general virucidal activity, but only that the product shows activity against Poxvirus Vaccinia, thereby showing virucidal activity against the enveloped virus presented in annex A** for surfaces, when tested according to **NF EN 14476: 2013 + A2: 2019** guideline.

Note 1: The results obtained correspond to the product received in this laboratory.

Note 2: The information that depend on the information received from the client and are not facilitated by the same one, shown as "not provided".

Bétera (Valencia), July 29, 2020

Signed. Ruth Novella
Responsible Technician
(Investigator)

Quality Assurance Review:

The assay development and the results obtained have been supervised by the Director of the study.

The Quality Assurance Director has inspected the development of the assay, proving that has been realized following the proper procedure and using the adequate media, materials and reagents, following as well the Good Laboratory Practices (GLPs) and the final report contains the primary data obtained.

Signed. Noelia Ros
Responsible for the Laboratory Area
(Study Director)



Signed. Encarna Esteban
Technical Director
(Quality Assurance Director)

Reference:

- **NF EN 14476: 2013 + A2: 2019** Guideline. Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine. Test method and requirements (Phase 2/Step 1). AFNOR.

Table 1. Results of activity of the product “ECO DEGREASER”, batch 090720-M, with Vaccinia Poxvirus (ATCC VR-1508) under clean conditions.

Product	Concentration*	Interfering substance	Cytotoxicity level	log ₁₀ TCID ₅₀ after.....				Reduction with the confidence interval of 95 % after 15 minutes
				0 min	60 seg	5 min	15 min	
ECO DEGREASER	80%	0.3 g/L BSA	0.5	-	-	-	1.49	5.17 ± 0.48
	50%		0.5	-	-	-	2.07	4.59 ± 0.52
	0.1%		0.5	-	-	-	6.49	0.17 ± 0.54
Virus control	NA	0.3 g/L BSA	NA	6.74	-	-	6.66	NA
Formaldehyde	0.7% (w:v)	NA	0.5	-	-	4.66	3.82	NA
Virus control Formaldehyde	0.7% (w:v)	NA	0.5	6.66	-	-	6.58	NA
<p>Control of sensitivity of cells to virus (difference between decimal logarithm of titre using treated and untreated cells)log₁₀^{-0.50}</p> <p>Control of the effectiveness of the disinfectant detection activity (difference between decimal logarithm of titre without exposing the virus to the product and of the test suspension).....log₁₀^{-0.26}</p>								
<p>NA: not applicable; NR: not realized</p> <p>Times recommended by Guideline for surfaces: maximum 5 or 60 minutes</p> <p>Times recommended by Guideline for instruments: maximum 60 minutes</p> <p>Times recommended by Guideline for Hygienic treatment of hands by friction and hygienic handwashing: between 30 or 120 seconds.</p> <p>PBS: phosphate buffered saline; BSA: bovine serum albumin.</p> <p>Virucidal activity exists when the titre of virus shows a reduction ≥4 log.</p> <p>*: see Special remarks to understand the values of these concentrations.</p>								

Table 2. Results of the activity of the product “**ECO DEGREASER**”, batch 090720-M, with Vaccinia Poxvirus (ATCC VR-1508) (Assay of titration with 12 wells), under clean conditions.

Product	Concentration *	Interfering substance	Time of contact (sec/min)	Dilutions (log10) ^{a,b}										
				1	2	3	4	5	6	7	8			
ECO DEGREASER	80%	0.3 g/L BSA	15 min	2303	0020	0000	0000	0000	0000	0000	0000	0000	NR	
				4430	0000	0000	0000	0000	0000	0000	0000			
				2230	0200	0000	0000	0000	0000	0000	0000			
	50%		15 min	2443	0302	0000	0000	0000	0000	0000	0000	0000	0000	NR
				3443	2030	0000	0000	0000	0000	0000	0000	0000		
				2344	0200	0000	0000	0000	0000	0000	0000	0000		
	0.1%		15 min	4444	4444	4444	4444	4444	4444	0203	0020	0020	NR	
				4444	4444	4444	4444	4444	4444	2020	0203	0000		
				4444	4444	4444	4444	4444	4444	3443	2000	0000		
Cytotoxicity	80%	0.3 g/L BSA	NA	0000	0000	0000	0000	0000	0000	0000	0000	0000		
Virus control	NA	0.3 g/L BSA	0 min	4444	4444	4444	4444	4444	3220	2002	0000	0000		
				4444	4444	4444	4444	4444	3440	2003	0000			
				4444	4444	4444	4444	4444	0322	2000	0020			
			15 min	4444	4444	4444	4444	4444	2320	0002	0000	0000		
				4444	4444	4444	4444	4444	2344	3200	0000			
				4444	4444	4444	4444	4444	3202	0020	0000			
Formaldehyde	0.7 (w/v)	NA	5 min	4444	4444	4444	3232	1200	0000	0000	NR			
				4444	4444	4444	0223	1001	0000	0000				
				4444	4444	4444	2202	0000	0000	0000				
			15 min	4444	4444	2234	0200	0000	0000	0000	NR			
				4444	4444	4320	2102	0000	0000	0000				
				4444	4444	4224	0002	0000	0000	0000				
Control of formaldehyde cytotoxicity	0.7 (w/v)	0.3 g/L BSA	NA	0000	0000	0000	0000	0000	0000	0000	0000	NR		
Virus control formaldehyde	0.7 (w/v)	NA	0 min	4444	4444	4444	4444	4444	3203	2010	0000	0000		
				4444	4444	4444	4444	4444	3022	0012	0000			
				4444	4444	4444	4444	4444	3320	1000	0000			
			15 min	4444	4444	4444	4444	4444	2332	0101	0000	0000		
				4444	4444	4444	4444	4444	0323	2000	0000			
				4444	4444	4444	4444	4444	0230	1000	0000			
Sensitivity control of cells to virus	NA	NA	Cells not treated	CCCC	CCCC	CCCC	CCCC	CCCC	C0CC	0000	0000	0000		
				CCCC	CCCC	CCCC	CCCC	CCCC	C0C0	0C00	0000			
				CCCC	CCCC	CCCC	CCCC	CCCC	C0CC	0C00	0000			
			Cells treated	CCCC	CCCC	CCCC	CCCC	CCCC	00C0	000C	0000	0000		
				CCCC	CCCC	CCCC	CCCC	CCCC	C00C	0000	0000			
				CCCC	CCCC	CCCC	CCCC	CCCC	C00C	0000	0000			
Effectiveness control of the disinfectant detection activity	NA	0.3 g/L BSA	Without PRODUCT	CCCC	CCCC	CCCC	CCCC	CCCC	CCCC	00CC	0000	0000		
				CCCC	CCCC	CCCC	CCCC	CCCC	CCCC	CCCC	0000			
				CCCC	CCCC	CCCC	CCCC	CCCC	CCCC	C000	0000			
			With PRODUCT	CCCC	CCCC	CCCC	CCCC	CCCC	CCCC	CCCC	0C00	0000	0000	
				CCCC	CCCC	CCCC	CCCC	CCCC	CCCC	00C0	CC0C			
				CCCC	CCCC	CCCC	CCCC	CCCC	CCCC	C0CC	0000			

a): 1 to 4, virus present and grade of cytopathic effect in 12 units of cellular culture, or grade of cellular lesions in the cytotoxicity assay.

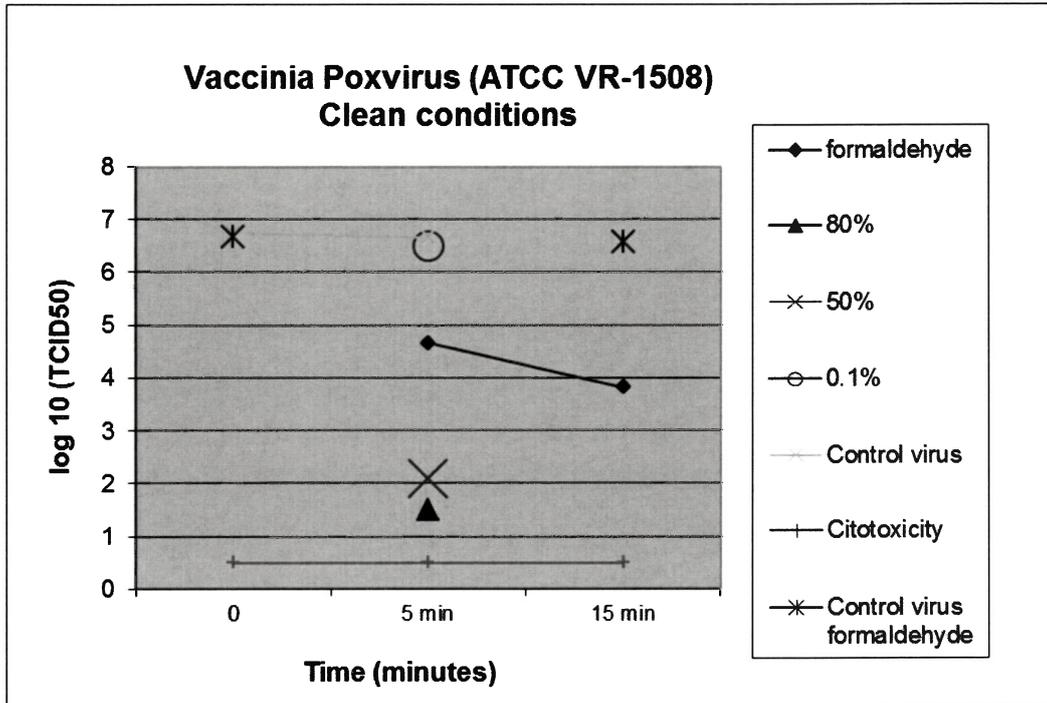
C = cytopathic effect with presence of virus (in this case and according to guideline does not take into account the degree of cytopathic effect only, the presence or absence of the same).

0 = no virus present or absence of cellular lesions in the cytotoxicity assay; NA: not applicable; NR: not realized; BSA: Bovine serum albumin; PBS: phosphate buffered saline.

sec: seconds; min: minutes.

*: see Special remarks to understand the values of these concentrations.

Figure 1. Results of the activity of the product “ECO DEGREASER”, batch 090720-M, at 80%, 50% and 0.1% concentration under clean conditions with Vaccinia Poxvirus (ATCC VR-1508).



Annex A of the guideline NF EN 14476: 2013 + A2: 2019: Examples of viruses that can contaminate medical instruments, hands or surfaces (Note 1: this list is not exhaustive; Note 2: Enveloped viruses are in bold).

Blood:

Enterovirus, *Filoviridae*, *Flavivirus*, *Herpesviridae*, Hepatitis A virus (HAV), **Hepatitis B virus (HBV)**, **Hepatitis C virus (HCV)**, **Hepatitis Delta virus (HDV)**, **Human Immunodeficiency virus (HIV)**, **Human T-cell lymphotropic virus (HTLV)**, *Parvovirus B19*.

Respiratory tract:

Adenovirus, *Coronavirus*, *Enterovirus*, *Herpesviridae*, *Influenza virus*, *Paramyxoviridae*, *Rhinovirus*, **Rubella virus**.

Nervous system, ears & nose, eyes:

Adenovirus, *Enterovirus*, *Herpesviridae*, **Measles virus**, **Human Immunodeficiency virus (HIV)**, *Polyomavirus*, **Rabies virus**, **Rubella virus**.

Gastrointestinal tract:

Adenovirus, *Caliciviridae*, *Coronavirus*, *Astrovirus*, *Enterovirus*, Hepatitis A virus (HAV), Hepatitis E virus (HEV), *Rotavirus*.

Skin, Breast, maternal milk:

Enterovirus, *Herpeviridae*, **Human Immunodeficiency virus (HIV)**, **Human T-cell lymphotropic virus (HTLV)**, *Papillomavirus*, *Poxviridae*.

Spleen and lymph nodes:

Human T-cell lymphotropic virus (HTLV), **Human Immunodeficiency virus (HIV)**.

Dental procedures:

Adenovirus, *Enterovirus*, *Herpesviridae*, **Hepatitis B virus (HBV)**, **Hepatitis C virus (HCV)**, **Hepatitis D virus (HDV)**, **Human Immunodeficiency virus (HIV)**.

Urogenital tract:

Hepatitis B virus (HBV), *Herpesviridae*, **Human Immunodeficiency virus (HIV)**, **Human T-cell lymphotropic virus (HTLV)**, *Papillomavirus*, *Polyomavirus*.