Instituto Valenciano de Microbiología

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ENAC
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Nº 648 / LE 1286

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 "EFERCLOR" against Vaccinia Poxvirus (NF EN 14476: 2013 + A2: 2019 Guideline)

Report
Registration No.: D/20/1143

1. **Laboratory identification** ..... Instituto Valenciano de Microbiología.

2. **Client identification** ..... QUIMICAS QUIMXEL, S.L.
   Address ..... Parc Industrial Ciutat de Carlet. C/Garbí, Nº 20, 46240, Carlet

3. **Sample identification** (information provided by the customer)

• Product name.....	**EFERCLOR**.
• Batch number.....	202038
• Expiration date.....	2023/06
• Manufacturer (supplier).....	QUIMICAS QUIMXEL, S.L.
• Date of manufacturer.....	Not indicated.
• Storing conditions	Keep out of direct sunlight, on a cool and dry place
• Conditions of use.....	Surfaces.
• Diluent recommended by the manufacturer	Water
• Active(s) Substance(s) and its concentration (s).....	Sodium dichloroisocyanurate dihydrate, 84% w / w
• Concentrations ordered for the assay....	3 tablets / 10 liters of water (equivalent to 450 ppm of dihydrated sodium dichloroisocyanurate).

IVAMI is not responsible for customer-supplied information.

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4. Information about sample reception.

- Date of reception of the product……. 2020/07/03.
- Date of reception of order with test conditions ...…….. 2020/07/03.
- Aspect of the received product……... White tablets in commercial packaging.

5. Testing method

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

6. Experimental conditions

- Assay period…….. 2020/08/18 to 2020/09/04.
- Assay temperature……. 37ºC ± 1ºC.
- Titration method …….. $TCID_{50}$ (Tissue Culture Infective Dose 50%).
- Product concentrations for the assay…. 3 tablets/5 L; 3 tablets/10 L; Dil 1:100 of 3 tablets/10 L
- Contact time……... 5 minutes.
- Contact temperature……... 20ºC ± 1ºC.
- Procedure to stop product cytotoxicity.. Molecular sieving.
- Procedure to stop product activity Cooling with ice.
- Solvent of the product used in the assay…….. Hard water.
- Aspect of the dilutions of the product… Transparent.
- Stability of the mixture……. Stable.
- Interfering substance:
    - Clean conditions in the presence of bovine serum albumin 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 5, passages 14, 17 and 20.

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7. Validation of assay results

Vaccinia Poxvirus (ATCC VR-1508)

Titre of the viral suspension for the virus control (5 minutes):

- Clean conditions.....log $10^{-6.08}$

Cytotoxicity level (3 tablets/5 L).....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 $10^{-5.58}$

Reference test (formaldehyde 1.4%)

Cytotoxicity level of formaldehyde 0.7%..... log $10^{-0.5}$

Viral quantification in the reference test (formaldehyde) after 15 minutes and with Vaccinia Poxvirus .....log$10^{-2.82}$

Confidence interval

Titre of virus with 95% confidence interval with Vaccinia Poxvirus (5 minutes)

- Clean conditions .....log $10^{-6.08 \pm 0.47}$

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 "EFERCLOR" disinfectant .....log$10^{-6.16}$
- Viral quantification of Vaccinia Poxvirus with cells treated with the "EFERCLOR" disinfectant.....log$10^{-5.83}$

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 title of the virus <1 $\log_{10}$.

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 "EFERCLOR" disinfectant .....$log10^{-6.08}$
- Viral quantification of Vaccinia Poxvirus exposing the virus to "EFERCLOR" disinfectant and incubated 30 minutes on ice bath.....$log10^{-5.82}$

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 the concentrations of, 3 tablets/5 L, 3 tablets/10 L, Dil1:100 3tablets/10 L.

- 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, **"EFERCLOR",** batch 202038, under clean conditions, diluted at 3 tablets/5 L, and 3 tablets/10 L, and during 5 minutes of exposure, **shows** virucidal activity against Vaccinia Poxvirus (ATCC VR-1508), with a reduction ≥5.58 ± 0.47 $TCID_{50}$, for both concentrations, when the activity is assayed according with the NF EN 14476 : 2013 + A2 : 2019 guideline.

The disinfectant product, **"EFERCLOR",** batch 202038, under clean conditions, diluted at Dil 1:100 of 3 tablets/10L and during 5 minutes of exposure, **does not show** virucidal activity against Vaccinia Poxvirus (ATCC VR-1508), with a reduction 0.26± 0.64 $TCID_{50}$, 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.

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10. Conclusion

The disinfectant product **"EFERCLOR",** batch 202038 under clean conditions (bovine serum albumin 3 g/L), diluted at **3 tablets/10 L**, requested by the customer, and during 5 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 **"EFERCLOR",** batch 202038**,** 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**, 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), September 7, 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)

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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 **"EFERCLOR"**, batch 202038 with Vaccinia Poxvirus (ATCC VR-1508) under clean conditions.

Product	Concen-tration*	Interfering substance	Cytoto-xicity level	log_{10} $TCID_{50}$ after…..			Reduction with the confidence interval of 95 % after 5 minutes
				0 min	5 min	15 min	
EFERCLOR	3 tablets/5L	3 g/L BSA	0,5	-	0,50	-	≥ 5,58 ± 0,47
	3 tablets/10L		0,5	-	0,50	-	≥ 5,58 ± 0,47
	Dil 1:100 3 tablets/10L		0,5	-	5,82	-	0,26 ± 0,64
Virus control	NA	3 g/L BSA	NA	6,16	6,08	-	NA
Formaldehyde	0.7% (w:v)	NA	0,5	NR	3,74	2,82	NA
Virus control Formaldehyde	0.7% (w:v)	NA	NA	6,41	NR	6,24	NA

Control of sensitivity of cells to virus (difference between decimal logarithm of titre using treated and untreated cells) ……..…….$log10^{-0.33}$

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)……..……..$log10^{-0.26}$

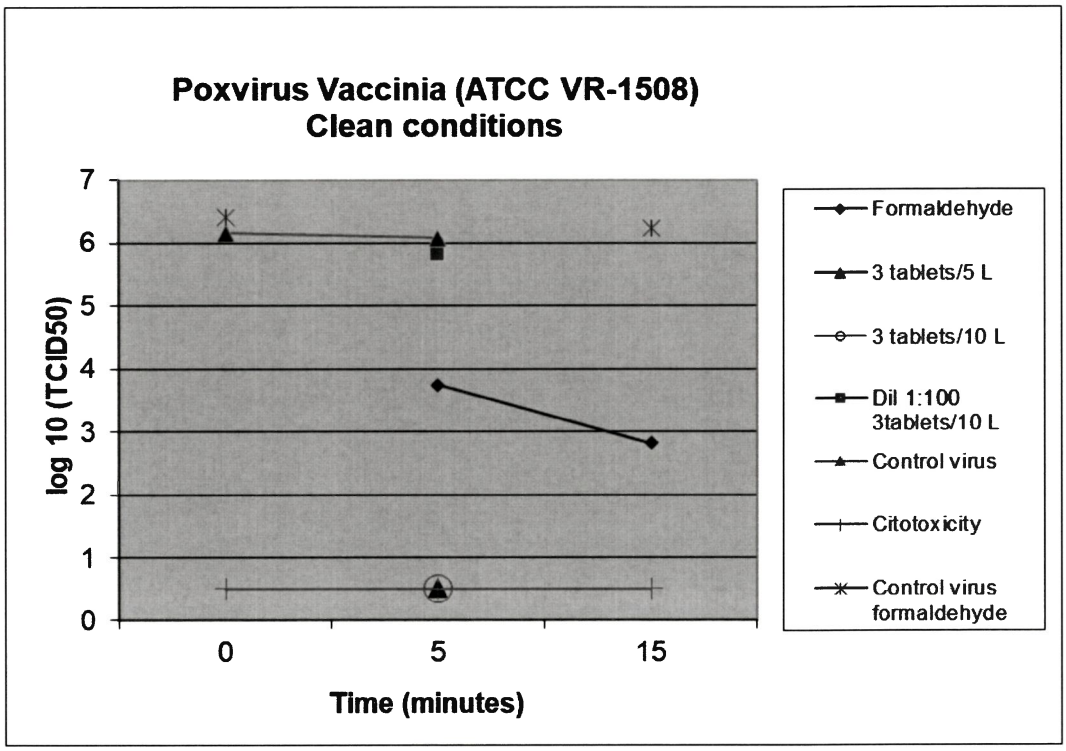
NA: not applicable; NR: not realized
Times recommended by Guideline for surfaces: maximum 5 or 60 minutes
Times recommended by Guideline for instruments: maximum 60 minutes
Times recommended by Guideline for Hygienic treatment of hands by friction and hygienic handwashing: between 30 or 120 seconds.
PBS: phosphate buffered saline; BSA: bovine serum albumin.
Virucidal activity exists when the titre of virus shows a reduction ≥4 log.
*: see Special remarks to understand the values of these concentrations.

Table 2. Results of the activity of the product **"EFERCLOR"**, batch 202038, with Vaccinia Poxvirus (ATCC VR-1508) (Assay of titration with 12 wells), under clean conditions.

Product	Concen-tration *	Interfering substance	Time of contact (min)	Dilutions (log10)[a,b]							
				1	2	3	4	5	6	7	8
EFERCLOR	3tablets/5L	3 g/L BSA	5	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	3tablets/10L		5	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	Dil 1:100 3tablets/10L		5	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	2033 2442 3320	0020 1100 2000	0000 0011 0000	NR
Cytotoxicity	3tablets/5L	3 g/L BSA	NA	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
Virus control	NA	3 g/L BSA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0200 3302 0030	0000 0010 1100	NR
			5	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3023 3023 1220	0201 2210 2200	0000 0001 0101	NR
Formaldehyde	0.7 (w/v)	NA	5	4444 4444 4444	4444 4444 4444	3223 0343 3242	0022 0010 0200	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
			15	4444 4444 4444	2340 4224 3324	2010 0020 0220	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
Control of formaldehyde cytotoxicity	0.7 (w/v)	3 g/L BSA	NA	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
Virus control formaldehyde	0.7 (w/v)	NA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3203 0332 0203	0020 0000 1002	0000 0000 0000
			15	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0302 2032 2030	0000 1000 1000	0000 0000 0000
Sensitivity control of cells to virus	NA	NA	Cells not treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	C0C0 CCC0 0000	000C 000C 0C00	NR
			Cells treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC C0CC C0CC	0C0C CC0C 0C00	0000 0000 0000	NR
Effectiveness control of the disinfectant detection activity	NA	3 g/L BSA	Without PRODUCT	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0C00 CC00 C000	0000 00C0 0CC0	NR
			With PRODUCT	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	C0CC CCCC CCCC	0C00 CC0C 0C00	0000 0000 0000	NR

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 **"EFERCLOR",** batch 202038, 3tablets/5L, 3tablets/10L, Dil:100 3tablets/10L, concentrations 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*.