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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 "FIN CLEAN ALL2" against Vaccinia Poxvirus (NF EN 14476: 2013 + A2: 2019 Guideline)

Report
Registration No.: D/20/983

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..... FIN CLEAN ALL2.
- Batch number..... 070520-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.... 20%.

IVAMI is not responsible for customer-supplied information.

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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 package.

5. Testing method

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

6. Experimental conditions

- Assay period..... July 6 to July 23, 2020.
- Assay temperature..... 37ºC ± 1ºC.
- Titration method ..... $TCID_{50}$ (Tissue Culture Infective Dose 50%).
- Product concentrations for the assay.... 80%, 20% and 0.1%.
- Contact time..... 15 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 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 21; working aliquot 4, passages 10 and 14.

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7. Validation of assay results

Vaccinia Poxvirus (ATCC VR-1508)

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

- Clean conditions..... log $10^{-6.58}$

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 $10^{-6.08}$

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 Poxvirus .....log$10^{-3.82}$

Confidence interval

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

- Clean conditions .....log $10^{-6.58 \pm 0.39}$

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 "FIN CLEAN ALL2" disinfectant .....log $10^{-6.66}$
- Viral quantification of Vaccinia Poxvirus with cells treated with the "FIN CLEAN ALL2" disinfectant.....log $10^{-6.57}$

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 "FIN CLEAN ALL2" disinfectant ……..…log10$^{-6.50}$
- Viral quantification of Vaccinia Poxvirus exposing the virus to "FIN CLEAN ALL2" disinfectant and incubated 30 minutes on ice bath…..log10$^{-6.23}$

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%; 20% 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, **"FIN CLEAN ALL2",** batch 070520-M, under clean conditions, diluted at 80% and 20% and during 15 minutes of exposure, **shows** virucidal activity against Vaccinia Poxvirus (ATCC VR-1508), with a reduction 5.17 ± 0.52 $TCID_{50}$ when tested at 80%, and with a reduction 4.67 ± 0.51 $TCID_{50}$ when tested at 20%, when the activity is assayed according with the **NF EN 14476: 2013 + A2: 2019** guideline.

The disinfectant product, **"FIN CLEAN ALL2",** batch 070520-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 ± 0.55 $TCID_{50}$, 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.

10. Conclusion

The disinfectant product **"FIN CLEAN ALL2",** batch 070520-M, under clean conditions (bovine serum albumin 0.3 g/L), diluted at **20%**, 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 **"FIN CLEAN ALL2",** batch 070520-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 27, 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 **"FIN CLEAN ALL2"**, batch 070520-M, 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 15 minutes
				0 min	60 seg	5 min	15 min	
FIN CLEAN ALL2	80%	0.3 g/L BSA	0.5	-	-	-	1.41	5.17 ± 0.52
	20%		0.5	-	-	-	1.91	4.67 ± 0.51
	0.1%		0.5	-	-	-	6.41	0.17 ± 0.55
Virus control	NA	0.3 g/L BSA	NA	6.66	-	-	6.58	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

Control of sensitivity of cells to virus (difference between decimal logarithm of titre using treated and untreated cells) ……$log10^{-0.09}$
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)……$log10^{-0.27}$

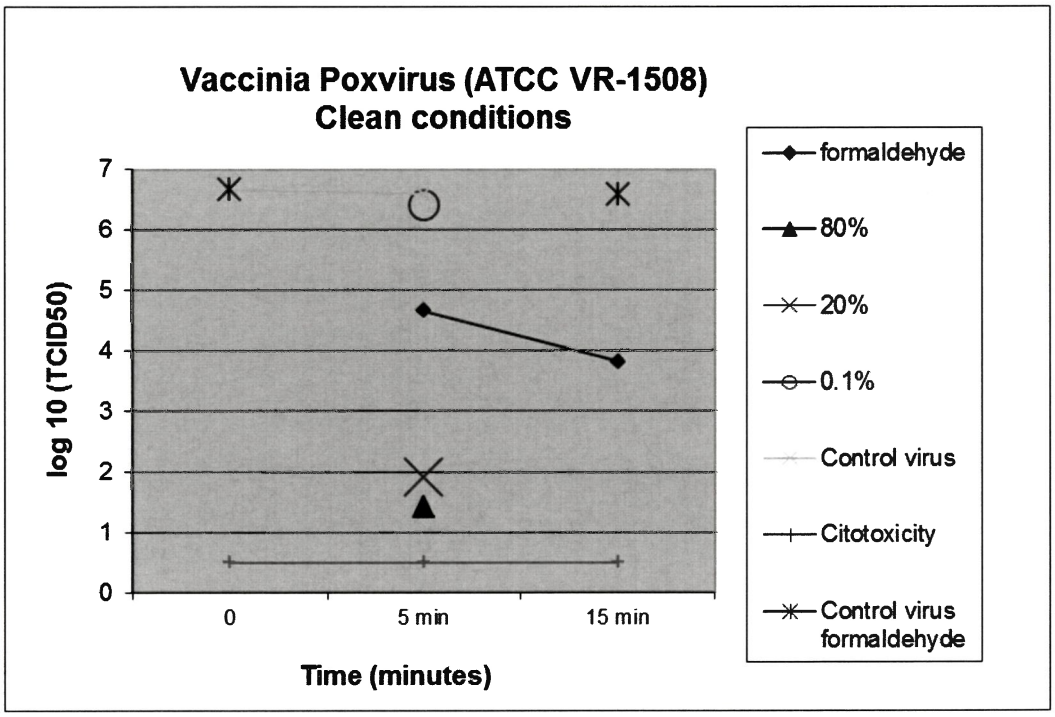
NA: not applicable; NR: not realized
Times recommended by Guideline for surfaces: maximum 5 or 60 minutes
Times recommended by Guideline for instruments: maximum 60 minutes
Times recommended by Guideline for Hygienic treatment of hands by friction and hygienic handwashing: between 30 or 120 seconds.
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 **"FIN CLEAN ALL2"**, batch 070520-M, with Vaccinia Poxvirus (ATCC VR-1508) (Assay of titration with 12 wells), under clean conditions.

Product	Concen-tration *	Interfering substance	Time of contact (sec/min)	Dilutions (log10)[a,b]							
				1	2	3	4	5	6	7	8
FIN CLEAN ALL2	80%	0.3 g/L BSA	15 min	0203 4402 4311	1000 0001 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	20%		15 min	3241 4234 4332	0201 0001 2000	0000 0100 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	0.1%		15 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0304 4330 4023	0010 0220 0000	NR
Cytotoxicity	80%	0.3 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	0000 0000 0000
Virus control	NA	0.3 g/L BSA	0 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3024 2330 1242	0012 0210 0000	NR
			15 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	2032 2044 3023	0001 0210 1000	NR0
Formaldehyde	0.7 (w/v)	NA	5 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	3232 0223 2202	1200 1001 0000	0000 0000 0000	0000 0000 0000	NR
			15 min	4444 4444 4444	4444 4444 4444	2234 4320 4224	0200 2102 0002	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
Control of formaldehyde cytotoxicity	0.7 (w/v)	0.3 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 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3203 3022 3320	2010 0012 1000	0000 0000 0000
			15 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	2332 0323 0230	0101 2000 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	C0CC CCC0 CCCC	00C0 CC00 C000	NR
			Cells treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	C0CC CCC0 CC00	00C0 CC00 C0C0	NR
Effectiveness control of the disinfectant detection activity	NA	0.3 g/L BSA	Without PRODUCT	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	C0CC 0CCC C0CC	0000 C00C C000	NR
			With PRODUCT	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	C0CC CCCC CCCC	CC0C C0C0 C0CC	00C0 0C00 0000	NR

a): 1 to 4, virus present and grade of cytophatic 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 **"FIN CLEAN ALL2",** batch 070520-M, at 80%, 20% 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*.