

ENCLOSURE No. 1 TO REPORT OF ANALYSIS NO. 245948/20/JSHB

A) IDENTIFICATION OF THE SAMPLE:	
Name of the product	Non alcoholic disinfectant Batch number: Not indicated
Expiration date	2020.05.11
The active substance	Alkyl (C12-16) dimethylbenzylamonium chloride, CAS No. 68424-85-1; 0.4g/100g (0.4%)
B) TEST METHOD :	
Performed in accredited subcontracted partner laboratory: Scope of Accreditation Nr 648/LE1286	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.
Testing method	Procedure DESIN-1078
C) EXPERIMENTAL CONDITIONS:	
Assay period	07/07/2020 – 24/07/2020
Product test concentrations (%V/V)	80%, 50%, 0,1%
Contact time	10 minutes
Assay temperature	37°C ± 1°C
Titration method	TCID50 (Tissue Culture Infective Dose 50%)
Solvent of the product used in the assay	Sterile distilled water
Aspect of the dilutions of the product	Transparent
Contact temperature	20°C ± 1°C
Procedure to stop product cytotoxicity	Molecular sieving
Procedure to stop product activity	Cooling with ice
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 20 and working aliquot 4, passages 11 and 14

Date: 07.08.2020

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 Approved by: Hanna Wachowska, Laboratory Director (*Approved with qualified electronic signature*)

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ENCLOSURE No. 1 TO REPORT OF ANALYSIS NO. 245948/20/JSHB**Validation of assay results****Vaccinia Poxvirus (ATCC VR-1508)**

Titre of the viral suspension for the virus control (10 minutes):

- Clean conditions..... log 10^{-6.25}
- Cytotoxicity level (80%)..... log 10
- ^{-0.5}

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

- Clean conditions.....log 10^{-5.75}

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 Poxviruslog10^{-3.82}**Confidence interval**

Titre of virus with 95% confidence interval with Vaccinia Poxvirus (10 minutes)

- Clean conditionslog 10^{-6.25 ± 0.26}

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

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ENCLOSURE No. 1 TO REPORT OF ANALYSIS NO. 245948/20/JS HB**Sensitivity of cells to virus**

- Viral quantification of Vaccinia Poxvirus with cells not treated with “Non alcoholic disinfectant” disinfectantlog10^{-6.08}
- Viral quantification of Vaccinia Poxvirus with cells treated with the “Non alcoholic disinfectant” disinfectant.....log10^{-5.91}

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 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 “Non alcoholic disinfectant” disinfectantlog10^{-6.08}
- Viral quantification of Vaccinia Poxvirus exposing the virus to “Non alcoholic disinfectant” disinfectant and incubated 30 minutes on ice bath.....log10^{-5.91}

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

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.

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ENCLOSURE No. 1 TO REPORT OF ANALYSIS NO. 245948/20/JS HB**Assay results****Description**

The disinfectant product, “**Non alcoholic disinfectant**”, batch n/a, under clean conditions, diluted at 80% and 50% and during 10 minutes of exposure, **shows** virucidal activity against Vaccinia Poxvirus (ATCC VR-1508), with a reduction $\geq 5.75 \pm 0.26$ TCID₅₀ for both concentrations, when the activity is assayed according with the **NF EN 14476: 2013 + A2: 2019** guideline.

The disinfectant product, “**Non alcoholic disinfectant**”, batch n/a, under clean conditions, diluted at 0.1% and during 10 minutes of exposure, **does not show** virucidal activity against Vaccinia Poxvirus (ATCC VR-1508), with a reduction 0.18 ± 0.45 TCID₅₀, when the activity is assayed according with the **NF EN 14476: 2013 + A2: 2019** guideline.

Tables of results and graphics

See tables 1 and 2 and figure 1.

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ENCLOSURE No. 1 TO REPORT OF ANALYSIS NO. 245948/20/JSHB**Conclusion**

The disinfectant product “**Non alcoholic disinfectant**”, batch n/a under clean conditions (bovine serum albumin 0.3 g/L), diluted at **80%**, requested by the customer, and during 10 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 “**Non alcoholic disinfectant**”, batch n/a, 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.

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Table 1. Results of activity of the product “Non alcoholic disinfectant”, batch n/a 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 10 minutes
				0 min	5 min	10 min	15 min	
Non alcoholic disinfectant	80%	0.3 g/L BSA	0.5	-	-	0.50	-	≥ 5.75 ± 0.26
	50%		0.5	-	-	0.50	-	≥ 5.75 ± 0.26
	0.1%		0.5	-	-	6.07	-	0.18 ± 0.45
Virus control	NA	0.3 g/L BSA	NA	6.32	-	6.25	-	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 ^{-0.42} 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 ^{-0.17}								
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.								

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Table 2. Results of the activity of the product “Non alcoholic disinfectant”, batch n/a, with Vaccinia Poxvirus (ATCC VR-1508) (Assay of titration with 12 wells), under clean conditions.

Product	Concentration *	Interfering substance	Time of contact (min)	Dilutions (log10) ^{a,b}							
				1	2	3	4	5	6	7	8
Non alcoholic disinfectant	80%	0.3 g/L BSA	10	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	50%		10	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	0.1%		10	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3202 0003 0030	0000 0200 0200	0000 0000 0000
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
Virus control	NA	0.3 g/L BSA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	2330 0230 2302	0000 2000 0200	0000 0020 0000
			10	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3210 1210 0232	0000 0000 0000	0000 0000 0000
Formaldehyde	0.7 (w/v)	NA	5	4444 4444 4444	4444 4444 4444	4444 4444 4444	3232 0223 2202	1200 1001 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000
			15	4444 4444 4444	4444 4444 4444	2234 4320 4224	0200 2102 0002	0000 0000 0000	0000 0000 0000	0000 0000 0000	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 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 3022 3320	2010 0012 1000	0000 0000 0000
			15	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	0C0C CC0C 0CCC	0000 0000 0000	0000 0000 0000
			Cells treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0CCC CCCC CCCC	C000 CC00 0C00	0000 0000 0000	0000 0000 0000
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	0C0C CC00 CC0C	0000 0000 0000	0000 0000 0000
			With PRODUCT	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0C0C C00C 00C0	0000 0000 0000	0000 0000 0000

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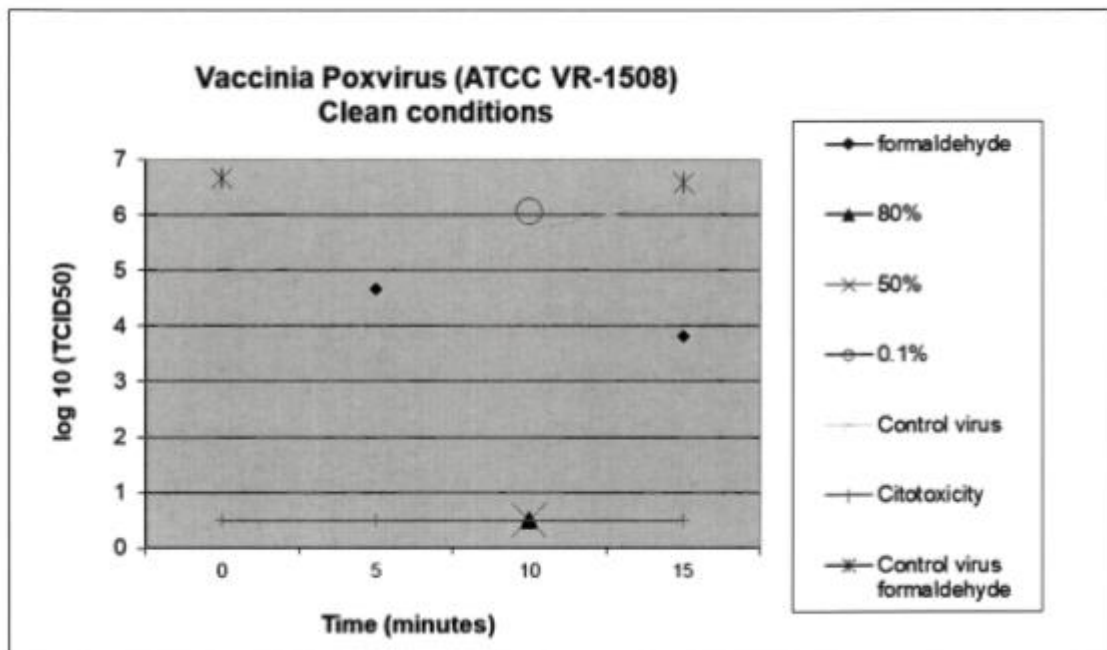
a): 1 to 4, virus present and grade of cytopathic effect in 12 units of cellular culture, or grade of cellular lesions in the cytotoxicity assay.

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.

Figura 1. Results of the activity of the product “Non alcoholic disinfectant”, batch n/a, at 80%, 50% and 0.1% concentration under clean conditions with Vaccinia Poxvirus (ATCC VR-1508).



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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.

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