ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 78638/20/ROBCH

A) IDENTYFICATION OF THE SAMPLE	
Name of the product/Details about the product	**GEL MAINI DEZINFECTANT** Lot: 1/11.2020 Expiration date: 2022/11 Sampling quantity: 1 x 400 ml Manufacturer(supplier): CASA LEBADA Keeping conditions – Dry, without sun, 5-25^{0}C
Active(s) Substance(s) and its concentration(s)	Ethyl Alcohol, CAS 64-17-5, CE 200-578-6; (70 g active substance in 100 g of product)
Concentration ordered for the assay	80%
B) TEST METHOD	
Performed in accredited subcontracted partner laboratory: Scope of Accreditation Nr. 648/LE1286 Report D/20/1829 Virucidal test with the product **GEL MAINI DEZINFECTANT** against Vaccinia virus strain modified Vaccinia Ankara (MVA) (NF EN 14476:2013+A2:2019 Guideline)	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/Step1).AFNOR
Testing method	Procedure DESIN-1078 (NF EN 14476:2013+ A2:2019 guideline)
C) INFORMATION ABOUT SAMPLE RECEPTION	
Date of reception of order with test conditions	21.12.2020
Date of reception of the product	21.12.2020
Aspect of the received product	Colourless transparent liquid in commercial container
D) EXPERIMENTAL CONDITIONS	
Assay period	From 29.12.2020 to 15.01.2021
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	60 seconds
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
Stability of the mixture (interfering substance and product diluted in sterile hard water/distilled water)	Stable
Interfering substance	Clean conditions in the presence of bovine serum albumin 0.3 g/L.
Identification of the origin of viral stains and number of passes	Vaccinia virus strain modified Vaccinia Ankara (MVA) (ATCC VR-1508), aliquot 22/01/2018 passage 2
Cell lines (name, origin, number of passes)	BHK-21, ref: FTBH, working aliquot 9, passages 17 and 19, working aliquot 10 passage 10

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7. Validation of assay results

Vaccinia virus strain modified Vaccinia Ankara (MVA) (ATCC VR-1508)

Titre of the viral suspension for the virus control (at the requested test time):

- Clean conditions…….... $\log 10^{-6.07}$

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 $10^{-5.57}$

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 virus strain modified Vaccinia Ankara (MVA) ……..... $\log 10^{-2.49}$

Confidence interval

Titre of virus with 95% confidence interval with Vaccinia virus strain modified Vaccinia Ankara (MVA) (at the requested test time):

- Clean conditions …….. $\log 10^{-6.07\pm 0.38}$

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

Sensitivity of cells to virus

- Viral quantification of Vaccinia virus strain modified Vaccinia Ankara (MVA) with cells not treated by the test solution with the test product ……..…..log $10^{-6.33}$
- Viral quantification of Vaccinia virus strain modified Vaccinia Ankara (MVA) with cells treated by the test solution with the test product ……..…..log $10^{-6.07}$

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 $\log_{10}$.

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Control of the effectivity of the disinfectant detection activity

- Viral quantification of Vaccinia virus strain modified Vaccinia Ankara (MVA) after 30 minutes on bath ice without exposing the virus to the test product ……...$log10^{-6.15}$
- Viral quantification of Vaccinia virus strain modified Vaccinia Ankara (MVA) exposing the virus to the test product and incubated 30 minutes on ice bath.....$log10^{-5.74}$

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

Virus of assay	Test concentrations, reduction obtained with the confidence interval of 95 % and virucidal activity		
	80%	50%	0.1%
Virus Vaccinia Ankara (MVA)	≥5.57 ± 0.38 $TCID_{50}$ Shows	≥5.57 ± 0.38 $TCID_{50}$ Shows	0.16 ± 0.53 $TCID_{50}$ Does not show

Virucidal activity exists when the titre of virus shows a reduction ≥ 4 log.
$TCID_{50}$: Tissue Culture Infectious Dose 50%.

9.2 Tables of results and graphics

See tables 1 and 2 and figure 1.

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10. Conclusion

The disinfectant product **"GEL MAINI DEZINFECTANT"**, batch 1/11.2020, under clean conditions (bovine serum albumin 0.3 g/L), diluted at **80%**, requested by the customer, and during 60 seconds of exposure and 20ºC temperature, **shows** virucidal activity against Vaccinia virus strain modified Vaccinia Ankara (MVA) (ATCC VR-1508) when the activity is assayed according with the NF EN 14476: 2013 + A2: 2019 guideline.

Virucidal activity of the disinfectant **"GEL MAINI DEZINFECTANT "**, batch 1/11.2020, against Vaccinia virus strain modified Vaccinia Ankara (MVA) (ATCC VR-1508), **does not mean that the product has general virucidal activity, but only that the product shows 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 indicated".

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.

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.

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Table 1. Results of activity of the product test product with Vaccinia virus strain modified Vaccinia Ankara (MVA) (ATCC VR-1508) under test conditions requested by the customer.

Assay	Concen-tration*	Interfering substance	Cytoto-xicity level	$\log_{10}$ TCID$_{50}$ after…..				Reduction with the confidence interval of 95 %
				0 min	60 sec	5 min	15 min	
Test product	80%	0.3 g/L BSA	0.5	-	0.50	-	-	≥5.57 ± 0.38
	50%		0.5	-	0.50	-	-	≥5.57 ± 0.38
	0.1%		0.5	-	5.91	-	-	0.16 ± 0.53
Virus control	NA	0.3 g/L BSA	NA	6.16	6.07	-	-	NA
Formaldehyde	0.7% (w:v)	NA	0.5	NR	NR	3.66	2.49	NA
Virus control Formaldehyde	0.7% (w:v)	NA	0.5	6.50	NR	NR	6.32	NA

Control of sensitivity of cells to virus (difference between decimal logarithm of titre using treated and untreated cells) ……..….$\log 10^{-0.26}$

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)…….....$\log 10^{-0.41}$

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 test product, with Vaccinia virus strain modified Vaccinia Ankara (MVA) (ATCC VR-1508) (Assay of titration with 12 wells), under test conditions requested by the customer.

Assay	Concentration *	Interfering substance	Time of contact (sec/min)	Dilutions (log10)[a,b] 1	2	3	4	5	6	7	8
Test product	80%	0.3 g/L BSA	60 sec	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	50%		60 sec	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	0.1%		60 sec	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3023 3204 3342	0102 1101 0210	0000 0000 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	NR
Virus control	NA	0.3 g/L BSA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3303 3200 4322	0000 2001 1000	NR
			60 sec	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0303 4200 3222	0000 0002 1000	NR
Formaldehyde	0.7 (w/v)	NA	5 min	4444 4444 4444	4444 4444 4444	3333 0442 0232	0002 1000 2100	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
			15 min	4444 4444 4444	3330 4222 0322	0000 0000 0000	0000 0000 0000	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	3303 3200 4322	0000 2001 1000	NR
			15 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0303 4200 3222	0000 0002 1000	NR
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 CCC0 0CC0	00C0 00CC 0000	NR
			Cells Treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0CCC CCCC 0CCC	C00C CC0C 0CCC	00C0 0000 0000	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 0CCC CCCC	0C0C CC0C C0CC	0000 000C 0000	NR
			With product	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0CCC 0CCC CCCC	C00C 00C0 C00C	0000 0000 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.

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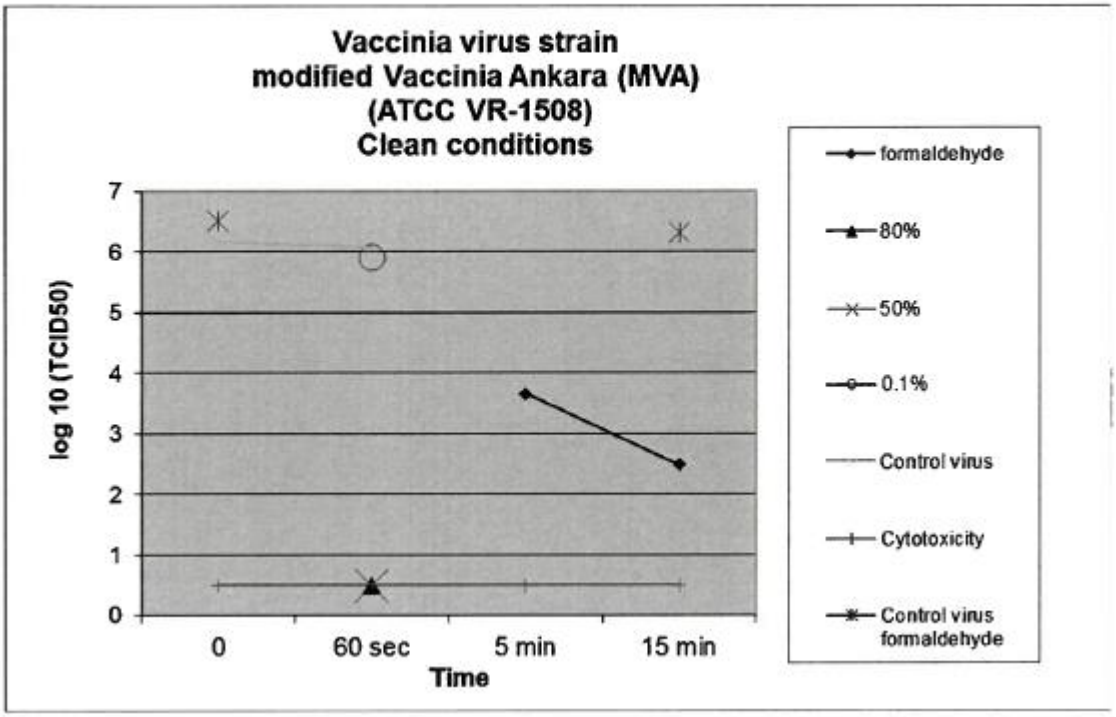
Figure 1. Results of the activity of the test product under test conditions requested by the customer with Vaccinia virus strain modified Vaccinia Ankara (MVA) (ATCC VR-1508).



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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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