Instituto Valenciano de Microbiología

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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 "WELL CLEAN FELÜLETFERTŐTLENÍTŐ" against Influenza A (H1N1) pdm09, strain A/California/7/2009 (H1N1) (NF EN 14476: 2013 + A2: 2019 Guideline)

Report
Registration No.: D/20/1367

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

2. **Client identification** ..... J.S Hamilton Hungaria Kft..
   Address ..... Berlini u. 47-49 – 1045 Budapest.

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

• Product name.....	WELL CLEAN FELÜLETFERTŐTLENÍTŐ.
• Batch number.....	24.08.2020.
• Expiration date.....	2 years.
• Manufacturer (supplier).....	Well Done St. Moritz L.t.d.
• Date of manufacturer.....	Not indicated.
• Store conditions.....	Room temperature.
• Conditions of use.....	Surfaces.
• Diluent of the product recommended by the manufacturer.....	No dilution required.
• Active(s) Substance(s) and its concentration (s).....	Benzyl-alkil (C12-16)-dimetil-ammónium-kloride 4%.
• Concentrations ordered for the assay.....	80% (ready to use).

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 ..... 2020/09/09.
- Date of reception of the product..... 2020/09/09.
- Aspect of the received product..... Colourless transparent liquid in bottle of 750 mL.

5. Testing method

Procedure **DESIN-1072**. (based on **NF EN 14476: 2013 + A2: 2019** Guideline).

6. Experimental conditions

- Assay period..... 2020/10/06 to 2020/10/23.
- Assay temperature..... 37ºC ± 1ºC.
- Titration method ..... $TCID_{50}$ (Tissue culture infective dose 50%).
- Method of detection of virus Influenza..... Hemagglutination with guinea-pig erythrocytes 1%.
- Product concentrations for the assay..... 80%, 50% and 0,1%.
- Contact time..... 35 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..... Sterile distilled water.
- Aspect of the dilutions of the product..... Transparent.
- Stability of the mixture (interfering substance and product diluted in sterile distilled water).. Stable.
- Interfering substance:
    - Internal control of dirty conditions in the presence of bovine serum albumin 3 g/L.
    - Dirty conditions in the presence of bovine serum albumin 3 g/L plus 3 mL erythrocytes 3 mL/L.

- Identification of the origin of viral strains and number of passages..... Influenza A (H1N1) pdm09 aliquot: 2017/11/20, passage 4.
- Cell lines (name, origin, number of passages)..... MDCK, ref: FTMD, working aliquot 6 passages 18 and working aliquot 7 passages 11 and 12.

7. Validation of assay results

Influenza A (H1N1) pdm09, strain A/California/7/2009 (H1N1):

Titre of the viral suspension for the virus control (35 minutes):

- Dirty conditions..... log $10^{-6.07}$
- Internal control of dirty conditions..... log $10^{-6.08}$

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):

- Dirty conditions..... log $10^{-5.57}$
- Internal control of dirty 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 60 minutes..... log$10^{-1.66}$

Confidence interval

Titre of virus with 95% confidence interval with Influenza A (H1N1) pdm09 (35 minutes)

- Dirty conditions ..... log $10^{-6.07 \pm 0.37}$
- Internal control of dirty conditions ..... log $10^{-6.08 \pm 0.34}$

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

Control of interference of cell susceptibility to virus

- Viral quantification of Influenza A (H1N1) pdm09 with cells not treated with the "WELL CLEAN FELÜLETFERTŐTLENÍTŐ" disinfectant ..... log$10^{-6.41}$
- Viral quantification of Influenza A (H1N1) pdm09 with cells treated with the "WELL CLEAN FELÜLETFERTŐTLENÍTŐ" disinfectant..... log$10^{-6.08}$

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 Influenza A (H1N1) pdm09 after 30 minutes on bath ice without exposing the virus to the "WELL CLEAN FELÜLETFERTŐTLENÍTŐ" disinfectant..... $log10^{-6.32}$
- Viral quantification of Influenza A (H1N1) pdm09 exposing the virus to "WELL CLEAN FELÜLETFERTŐTLENÍTŐ" disinfectant after incubated 30 minutes on ice bath..... $log10^{-6.08}$

Note: The difference between decimal logarithm of the titre without exposing the virus to the product and the titre of the viral 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 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, **"WELL CLEAN FELÜLETFERTŐTLENÍTŐ",** batch 24.08.2020, under dirty conditions, diluted at 80% and 50%, and during 35 minutes of exposure, **shows** virucidal activity against Influenza A (H1N1) pdm09 strain A/California/7/2009 (H1N1), with a reduction ≥ 5.16 ± 0.47 log $TCID_{50}$, when diluted at 80% and with a reduction 4.83 ± 0.52 log $TCID_{50}$, when diluted at 50%, when the activity is assayed according with the internal procedure DESIN-1072 based on the NF EN 14476: 2013 + A2: 2019 Guideline.

The disinfectant product, **"WELL CLEAN FELÜLETFERTŐTLENÍTŐ",** batch 24.08.2020, under dirty conditions, diluted at 0.1%, and during 35 minutes of exposure, **does not show** virucidal activity against Influenza A (H1N1) pdm09 strain A/California/7/2009 (H1N1), with a reduction 0.25 ± 0.52 log $TCID_{50}$, when the activity is assayed according with the internal procedure DESIN-1072 based on 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 **"WELL CLEAN FELÜLETFERTŐTLENÍTŐ"**, batch 24.08.2020, under dirty conditions (bovine serum albumin 3 g/L plus 3 mL erythrocytes) diluted at **80%**, requested by the customer, and during 35 minutes of exposure and 20ºC, **shows** virucidal activity against Influenza A (H1N1) pdm09, strain A/California/7/2009 (H1N1) when the activity is assayed according with activity is assayed according with the internal procedure DESIN-1072 based on the **NF EN 14476: 2013 + A2: 2019** Guideline.

Tests performed only with Influenza A (H1N1) pdm09, strain A/California/7/2009 (H1N1), **do not allow to conclude that the product tested shows a general virucidal activity**, but only that it shows activity against Influenza A (H1N1).

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), October 30, 2020.

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

Signed. Encarna Esteban
Technical Director
(Quality Assurance Director)

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Reference:

- NF EN 14476: 2013 + A2: 2019. Ensayo cuantitativo de suspensión para la evaluación de la actividad virucida en Medicina. Método de ensayo y requisitos (Fase 2/Etapa 1). AFNOR.

Table 1. Results of activity of the product **"WELL CLEAN FELÜLETFERTŐTLENÍTŐ",** batch 24.08.2020, Influenza A (H1N1) pdm09, strain A/California/7/2009 (H1N1), under dirty conditions.

Product	Concen-tration*	Interfering substance	Cytoto-xicity level	log_{10} $TCID_{50}$ after…..				Reduction with the confidence interval of 95 % after 35 minutes
				0 min	30 min	35 min	60 min	
WELL CLEAN FELÜLETFER-TŐTLENÍTŐ	80%	3 g/L BSA + 3 mL/L erythrocytes	0.5	-	-	0.91	-	5.16 ± 0.47
	50%		0.5	-	-	1.24	-	4.83 ± 0.52
	0.1%		0.5	-	-	5.82	-	0.25 ± 0.52
WELL CLEAN FELÜLETFER-TŐTLENÍTŐ	80%	3 g/L BSA	0.5	-	-	0.99	-	5.09 ± 0.48
	50%		0.5	-	-	1.08	-	5.00 ± 0.49
	0.1%		0.5	-	-	5.74	-	0.34 ± 0.48
Formaldehyde	0.7% (w:v)	NA	0.5	NR	NR	2.66	1.66	NA
Virus control	NA	3 g/L BSA + 3 mL/L erythrocytes	NA	6.08	6.41	-	-	NA
Virus control	NA	3 g/L BSA	NA	6.24	6.08	-	-	NA
Virus control Formaldehyde	0.7% (w:v)	NA	0.5	6.32	NR	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)..... $log10^{-0.24}$

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 **"WELL CLEAN FELÜLETFERTŐTLENÍTŐ",** batch 24.08.2020, with Influenza A (H1N1) pdm09, strain A/California/7/2009 (H1N1) (Quantification test with 12 wells), dirty conditions.

Product	Concen-tration *	Interfering substance	Time of contact (min)	Dilutions (log10)[a,b]							
				1	2	3	4	5	6	7	8
WELL CLEAN FELÜLETFERTŐT LENÍTŐ	80%	3 g/L BSA + 3 mL/L erythrocytes	35	SSHH HSSH HSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	NR
	50%		35	SSHH HSSH HHHS	SSSS SSHS HSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	NR
	0.1%		35	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHS HHHH HHHH	SSSS HSHH SHSS	SSSS SSSS SSHS	NR
WELL CLEAN FELÜLETFERTŐT LENÍTŐ	80%	3 g/L BSA	35	SSSH HHSS HHSS	SSSS SSSS SHSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	NR
	50%		35	SSHH HSSS HHHS	SSSS SSSH SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	NR
	0.1%		35	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHS HHHH HHHH	SSSS HHHS SHSS	SSSS SSSS SSSS	NR
Cytotoxicity	80%	3 g/L BSA + 3 mL/L erythrocytes	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 + 3 mL/L erythrocytes	0	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	SSSH HHSS HHSH	SSSS SSSS SHSS	NR
			35	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	SSSS HHHS SSHH	SSHH SSSS SSSS	NR
Virus control	NA	3 g/L BSA	0	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	SHSS HHHS HHHS	SSSS SSSH HSSS	NR
			35	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	SSHH HSSH HSHS	SSSS SSSS SSHS	NR
Formaldehyde	0.7 (w/v)	NA	30	HHHH HHHH HHHH	HHHH HHHH HHHH	SSSH HSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	NR
			60	HHHS HHHH HHHH	SSSS SHHS SHSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	SSSS SSSS SSSS	NR
Control of formaldehyde cytotoxicity	0.7 (w/v)	3 g/L BSA + 3 mL/L erythrocytes	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	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	SHHH SSHH HSHS	SSSS SSSH SSHS	NR
			60	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	SHHH HHHS HHSS	SSSS SSSS SHHS	NR

Sensitivity control of cells to virus	NA	NA	Cells not treated	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHSS HHHH HHHS	SSSS SSSS HHSS	NR
			Cells treated	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	SSSH HHSH HSSH	SSSS SSSS SHSS	NR
Effectiveness control of the disinfectant detection activity	NA	3 g/L BSA + 3 mL/L erythrocytes	Without product	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHSS HHHH HHSS	SSSS SSSS HHSS	NR
			With product	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	HHHH HHHH HHHH	SSSSH HHHH SHSS	SSSS SSSS SSHS	NR

H: Hemagglutination; S: Sediment of erythrocytes, in 12 units (wells) of cellular culture in the test of Hemagglutination.
NA: not applicable; NR: not realized; BSA: Bovine serum albumin; PBS: phosphate buffered saline.

Figure 1. Results of the activity of the product **"WELL CLEAN FELÜLETFERTŐTLENÍTŐ"**, batch 24.08.2020, at different concentrations 80%, 50% and 10%, under dirty conditions with Influenza A (H1N1) pdm09, strain A/California/7/2009 (H1N1), and for the time of contact indicated.

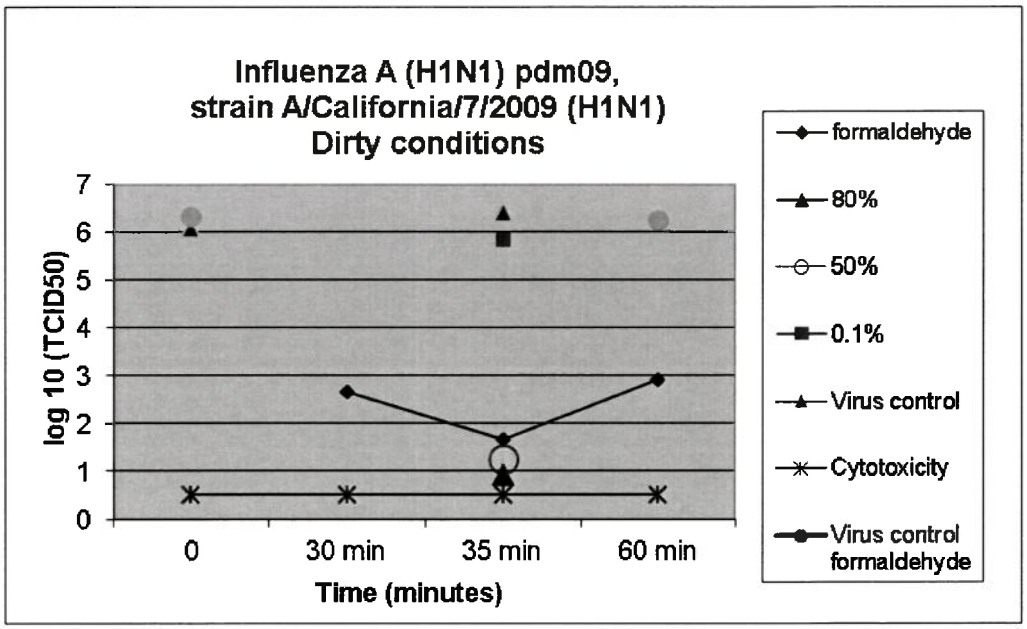

Figure 1.1. Results of the activity of the product **"WELL CLEAN FELÜLETFERTŐTLENÍTŐ"**, batch 24.08.2020, at different concentrations 80%, 50% and 10%, under internal control of dirty conditions with Influenza A (H1N1) pdm09, strain A/California/7/2009 (H1N1), and for the time of contact indicated.