J.S. Hamilton Croatia ltd. (PJ1 laboratory)
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Official food and feed laboratory
Ministry of Agriculture authorisation:
KLASA: UP/I-322-01/17-01/84; URBROJ: 525-10/0766-17-3

Official laboratory for testing of water for human consumption in purpose of acquiring usage permission for buildings
Ministry of Healthcare authorisation
KLASA: UP/I-541-02/18-03/01; URBROJ: 534-07-2-1-3/2-18-3

Zadar, 24.7.2020.

ANALYTICAL REPORT br. 554/20

V GUARD SANITIZER (MutiSan 80, MultiSan D80, Quick&Clean, V-Guard 70)

GENERAL INFORMATION

Client	**Alfa car d.o.o., 3. Travnja 58, Donja Dubrava, 40320 Donji Kraljevec**
Section	Production
Sampled and submitted by	Delivered by customer
Type of request	Order form
Delivery date	05.03.2020.
Analysis started	05.03.2020.
Temperature at sampling/delivery	Not specified °C / Not specified °C
Analysis ended	24.07.2020.

SAMPLE DESCRIPTION

Batch: Tester
Appearance: Transparent liquid,
Packaging: Original polymer packaging
Storing conditions: Room temperature
Expiration period: 5 years.
Active substances: 70% V/V Ethanol
Usage: Surfaces
Objections: No objections

CONFORMITY STATEMENT

Not requested/applicable.

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Luka Beretin, mag.chem
Quality manager

ANALYTICAL RESULTS

1. Testing method

Procedure DESIN-6225 (Based on NF EN 14476:2013+A2:2019)

2. Experimental conditions

Assay period:	26.05.2020. - 23.06.2020.
Assay temperature:	35°C ± 1°C
Titration method:	$TCID_{50}$ (Tissue Culture Infective Dose 50%)
Product concentrations for the assay:	80%, 50% and 0.1%
Contact time:	1 minute
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 distilled water):	Stabile
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 passes:	Coronavirus 229E (ATCC VR-740) aliquot: 2019/03/04 passage 2.
Cell lines (name, origin, number of passes):	MRC-5 ref. FTMR, working aliquot 5, passage 15 and working aliquot 6, passages 10 and 12.

3. Validation of experimental results

Coronavirus 229E (ATCC VR-740)

Titre of the viral suspension for the virus control (1 minute):	
Dirty conditions:	$\log 10^{-5.99}$
Internal control of dirty conditions:	$\log 10^{-5.82}$
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:	$\log 10^{-5.49}$
Internal control of dirty conditions:	$\log 10^{-5.32}$

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 Coronavirus 229E	$\log 10^{-1.91}$

Confidence interval

Titre of virus with 95% confidence interval with coronavirus 229E (1 min)	
Dirty conditions:	$\log 10^{-6.08\pm0.30}$
Internal control of dirty conditions:	$\log 10^{-5.91\pm0.35}$
Reduction with the confidence interval of 95%	See table 1.

Sensitivity of cells to virus

Viral quantification of Coronavirus 229E with cells not treated with "V Guard" disinfectant	$\log 10^{-6.00}$
Viral quantification of Coronavirus 229E with cells treated with "V Guard" disinfectant	$\log 10^{-5.57}$

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)
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 Coronavirus 229E after 30 minutes on bath ice without exposing the virus to the "V Guard" disinfectant	$\log 10^{-5.91}$
Viral quantification of Coronavirus 229E exposing the virus to "V Guard" disinfectant and incubated 30 minutes on ice bath	$\log 10^{-5.74}$

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

4. 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

5. Assay results

5.1. Description

The disinfectant product, **V Guard**, batch Tester, under dirty conditions, diluted at 80% and 50% and during 1 minute of exposure, **shows viricidal activity** against Coronavirus 229E (ATCC VR-740), with a reduction ≥ 5.58 ± 0.30 $TCID_{50}$ for both concentrations, when the activity is assayed according with the internal procedure DESIN-6255 based on the NF EN 14476:2013+A2:2019 guideline.

The disinfectant product, V Guard, batch Tester, under dirty conditions, diluted at 0.1% and during 30 seconds of exposure, does not show viricidal activity against Coronavirus 229E (ATCC VR-740), with a reduction 0.42 ± 0.43 TCID50, when the activity is assayed according with the internal procedure DESIN-6255 based on the NF EN 14476:2013+A2:2019 guideline.

5.2. Tables of results and graphics
- See annexes below

6. Conclusion

The disinfectant product **V Guard**, batch Tester, under dirty conditions (bovine serum albumin 3 g/L plus 3 mL erythrocytes 3 mL/L), diluted at 80%, requested by the customer, and during 1 minute of exposure, **shows viricidal activity against Coronavirus 229E (ATCC VR-740)**, when the activity is assayed according with the internal procedure DESIN-6255 based on the NF EN 14476:2013+A2:2019 guideline.

Tests performed only with Coronavirus strain 229E, does not allow to conclude that the product tested shows a general viricidal activity, but only that it shows activity against Coronaviruses.

Reference:
NF EN 14476:2013+A2:2019 Guideline. Viricidal 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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Vanja Baljak, mag.sanit.ing.
Laboratory manager

End of analytical report

ANNEX

Table 1. Results of activity of the product V **Guard Professional,** batch TESTER with Coronavirus 229E (ATCC VR-740) under dirty conditions.

Product	Concen-tration	Interfering sub-stance	Cytotoxicity level	$\log_{10}$ $TCID_{50}$ after:				Reduction with the confidence interval of 95 % after 1 minute
				0 min	1 min	5 min	15 min	
V Guard	80%	3 g/L BSA + 3 mL/L erythrocytes	0,5		0.50		-	≥ 5.58 ± 0.30
	50%		0,5		0.50		-	≥ 5.58 ± 0.30
	0,1%		0,5		5.66		-	≥ 0.42 ± 0.43
V Guard	80%	3 g/L BSA	0,5		0.50		-	≥ 5.41 ± 0.35
	50%		0,5		0.50	-		≥ 5.41 ± 0.35
	0.1%		0,5		5.50		-	0.41 ± 0.51
Virus control	NA	3 g/L BSA + 3 mL/L erythrocytes	NA	6.08	5.99	-		NA
Virus control	NA	3 g/L BSA	NA	5.91	5.82		-	NA
Formaldehyde	0.7% (w:v)	NA	0,5	-	-	3.16	1.91	NA
Virus control Formaldehyde	0.7% (w:v)	NA	0,5	5.83	-	-	5.66	NA

Control of sensitivity of cells to virus
(difference between decimal logarithm of titre using treated and untreated cells)……log10$^{-0.43}$

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.