

**ENCLOSURE No. 2 TO REPORT OF ANALYSIS NO. 115112/19/CGDA**

<b>A) IDENTIFICATION OF THE SAMPLE:</b>	
Name of the product	<b>DISINFECTANT</b> Batch number: BN022
The active substance	Not indicated
Aspect of the dilutions of the product	Liquid
<b>B) TEST METHOD :</b>	
Performed in accredited subcontracted partner laboratory: Scope of Accreditation Nr 648/LE1286	<b>UNE- EN-14476:2014+A1:2015- Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine. Test method and requirements (phase 2, step 1) AENOR</b>
<b>C) EXPERIMENTAL CONDITIONS:</b>	
Product test concentrations (%V/V)	80%, 50%, 0,1%
Assay period	18/03/2019 – 11/04/2019
Contact time	5 minutes.
Titration method	TCID <sub>50</sub> (Tissue culture infective dose 50%)
Solvent of the product used in the assay	Sterile distilled water
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 (0,3g/L bovine serum albumin)
Identification of the origin of viral strains and number of passes	Norovirus aliquot: 18/05/17 passage 2
Cell lines (name, origin, number of passes and culture medium)	Raw 264.7, Public health England, working aliquot 13, passage 9 and 11.

Date: 16-04-2019

Authorized by: Agnieszka Erber, Expert Analyst, Microbiology Laboratory

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

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**ENCLOSURE No. 2 TO REPORT OF ANALYSIS NO. 115112/19/CGDA****Validation of assay results****Murine norovirus (strain S99 Berlin)**

Titre of the viral suspension for the virus control (5 minutes):

- Clean conditions.....log 10<sup>-6.91</sup>
- Cytotoxicity level (80%).....log 10<sup>-0.5</sup>

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

- Clean conditions.....log 10<sup>-6.41</sup>

**Reference test (formaldehyde 1.4%)**Cytotoxicity level of formaldehyde 0.7%..... log 10<sup>-0.5</sup>Viral quantification in the reference test (formaldehyde) after 60 minutes and with Murine Norovirus .....log 10<sup>-0.91</sup>**Confidence interval**

Title of virus with 95% confidence interval with Murine Norovirus (5 minutes)

- Clean conditions .....log 10<sup>-6.91 ± 0.30</sup>

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

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**ENCLOSURE No. 2 TO REPORT OF ANALYSIS NO. 115112/19/CGDA****Sensitivity of cells to virus**

- Viral quantification of Murine Norovirus with cells not treated with "disinfectant" .....log10<sup>-5.24</sup>
- Viral quantification of Murine Norovirus with cells treated with the "Hygiene of Sweden disinfectant" .....log10<sup>-4.74</sup>

**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 <1log<sub>10</sub>.

**Control of the effectivity of the disinfectant detection activity**

- Viral quantification of Murine Norovirus after 30 minutes on bath ice without exposing the virus to the "disinfectant" .....log10<sup>-5.32</sup>
- Viral quantification of Murine Norovirus exposing the virus to "disinfectant" and incubated 30 minutes on ice bath.....log10<sup>-4.91</sup>

**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. 2 TO REPORT OF ANALYSIS NO. 115112/19/CGDA****Assay results****Description**

The disinfectant product **disinfectant**, batch **BN022**, under clean conditions, diluted at 80% and 50% and during 5 minutes of exposure, **shows** virucidal activity against Murine Norovirus, with a reduction  $\geq 6.41 \pm 0.30$  TCID<sub>50</sub> diluted at 80% and a reduction  $5.59 \pm 0.47$  TCID<sub>50</sub> diluted at 50%, when the activity is assayed according with the UNE-EN 14476:2014 + A1:2015 guideline.

The disinfectant product **disinfectant**, batch **BN022**, under clean conditions, diluted at 0.1% and during 5 minutes of exposure, **does not show** virucidal activity against Murine Norovirus, with a reduction  $0.17 \pm 0.45$  TCID<sub>50</sub>, when the activity is assayed according with the UNE-EN 14476:2014 + A1:2015 guideline.

**Tables of results and graphics**

See tables 1 and 2 and figure 1.

**Conclusion**

The disinfectant product **disinfectant**, batch **BN022**, under clean conditions (0.3 g/L BSA), diluted at **80%** and **50%** and during 5 minutes of exposure, **shows** virucidal activity against Murine Norovirus when the activity is assayed according with the UNE-EN 14476: 2014+ A1: 2015 guideline, with deviations; due that the test has been performed not strictly following the guideline recommendations with respect to the viruses assayed.

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".

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**ENCLOSURE No. 2 TO REPORT OF ANALYSIS NO. 115112/19/CGDA**
**Table 1.** Results of activity of the **disinfectant, batch BN022, with Murine Norovirus, strain S99 Berlin, under clean conditions:**

Product	Concentration*	Interfering substance	Cytotoxicity level	log <sub>10</sub> TCID <sub>50</sub> after.....				Reduction with the confidence interval of 95 % after 5 minutes
				0 min	5 min	30 min	60 min	
Hygiene of Sweden disinfectant	80%	0.3 g/L BSA	0.5	-	0.50	-	-	≥ 6.41 ± 0.30
	50%		0.5	-	1.32	-	-	5.59 ± 0.47
	0.1%		0.5	-	6.74	-	-	0.17 ± 0.45
Formaldehyde	0.7% (p.v)	PBS	0.5	-	-	2.41	0.91	NA
Virus control	NA	3 g/L BSA	NA	7.00	6.91	NR	NR	NA
Virus control Formaldehyde	0.7% (p.v)	PBS	0.5	5.08	NR	NR	5.50	NA
Control of sensitivity of cells to virus (difference between decimal logarithm of titre using treated and untreated cells) .....log <sub>10</sub> <sup>-0.41</sup>								
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 <sub>10</sub> <sup>-0.33</sup>								
NA: not applicable; NR: not realized Times recommended by Guideline for surfaces: maximum 5 or 60 minute Times recommended by Guideline for instruments: maximum 60 minute 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 titer 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 **disinfectant, batch BN022**, with Murine Norovirus strain S99 Berlin (Assay of titration with 12 wells), under clean conditions:

Product	Concentration *	Interfering substance	Time of contact (min)	Dilutions (log10) <sup>a,b</sup>								
				1	2	3	4	5	6	7	8	
Hygiene of Sweden disinfectant	80%	0.3 g/L BSA	5	0000	0000	0000	0000	0000	0000	0000	0000	0000
				0000	0000	0000	0000	0000	0000	0000	0000	0000
				0000	0000	0000	0000	0000	0000	0000	0000	0000
	50%		5	0033	2001	0000	0000	0000	0000	0000	0000	
				3333	0000	0000	0000	0000	0000	0000	0000	
				3030	0000	0000	0000	0000	0000	0000	0000	
	0.1%		5	4444	4444	4444	3333	0323	0000	0000	0000	
				4444	4444	4444	3334	3333	2202	0000	0000	
				4444	4444	4444	3222	3333	0100	0000	0000	
Cytotoxicity	80%	NA	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	4444	4444	4444	4444	4444	4444	0032	0000	
				4444	4444	4444	4444	4444	4444	0220	0000	
				4444	4444	4444	4444	4444	4444	2200	0000	
			5	4444	4444	4444	4444	4444	4444	0003	0000	
				4444	4444	4444	4444	4444	4444	3030	0000	
				4444	4444	4444	4444	4444	4444	3300	0000	
Formaldehyde	0.7 (p/v)	PBS	30	4444	0322	0000	0000	0000	0000	0000	0000	
				4444	2222	0000	0000	0000	0000	0000	0000	
				4444	2222	0000	0000	0000	0000	0000	0000	
			60	0032	0000	0000	0000	0000	0000	0000	0000	
				0202	0000	0000	0000	0000	0000	0000	0000	
				0200	0000	0000	0000	0000	0000	0000	0000	
Control of folmaldehyde cytotoxicity	0.7 (p/v)	PBS	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 folmaldehyde	0.7 (p/v)	PBS	0	4444	4444	4444	4444	0330	0000	0000	0000	
				4444	4444	4444	4444	3333	2202	0000	0000	
				4444	4444	4444	4444	3333	0000	0000	0000	
			60	4444	4444	4444	4444	0220	0000	0000	0000	
				4444	4444	4444	4444	3333	2012	0000	0000	
				4444	4444	4444	4444	3303	0000	0000	0000	

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Sensitivity control of cells to virus	NA	NA	Cells not treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	00C0 CCCC 0CC0	0000 00C0 0C00	0000 0000 0000	0000 0000 0000
			Cells treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0CCC CCCC CCCC	0000 CCCC C000	0000 0000 0000	0000 0000 0000	
Effectiveness control of the disinfectant detection activity	NA	0.3 g/L BSA	Without Hygiene of Sweden disinfectant	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0C0C CCCC C0C0	0000 00C0 0C00	0000 0000 0000	0000 0000 0000
			With Hygiene of Sweden disinfectant	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	000C C0C0 C000	0000 0000 0000	0000 0000 0000	

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: minute

\*: see Special remarks to understand the values of these concentrations.

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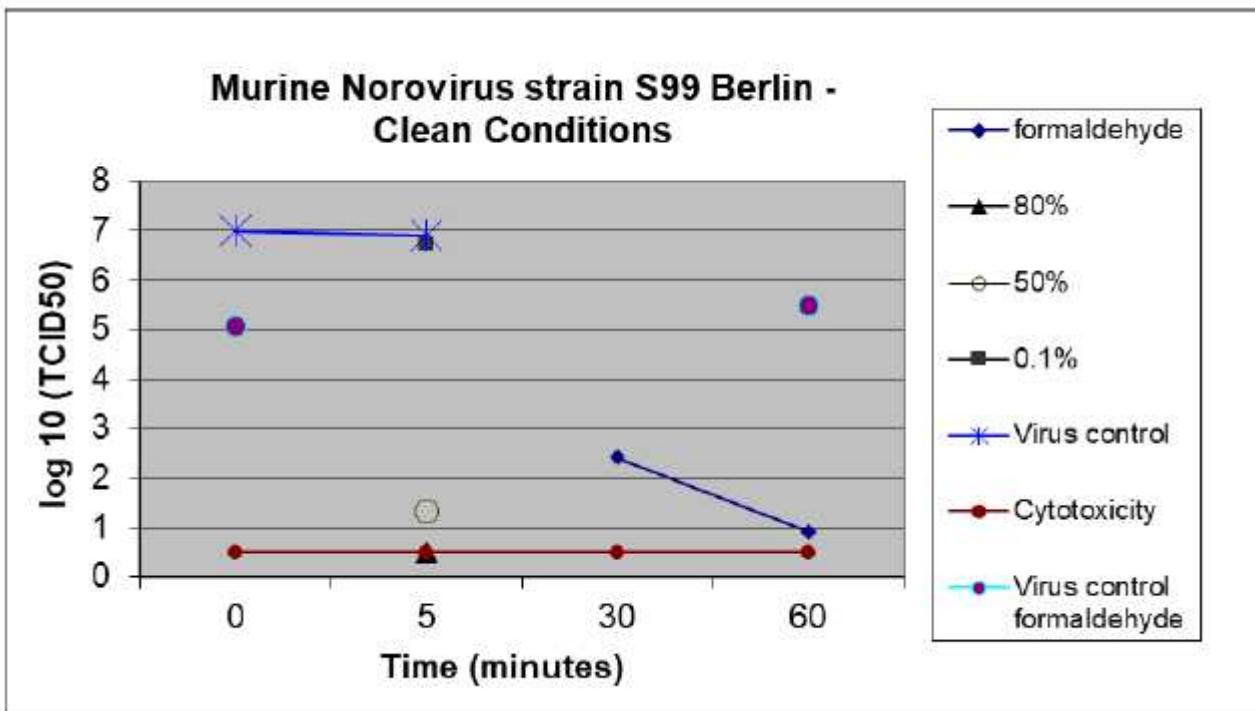
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**Figure 1.** Results of the activity of the product **disinfectant**, batch **BN022**, at 80%, 50% and 0.1% concentration under clean conditions with Murine Norovirus strain S99 Berlin.



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