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Evaluation of the virucidal activity according to the NF EN 14476 + A2 : 2019 standard

Product(1) : Global cold washing process, ozone generator OTEK with the addition of dedicated detergents (Lavage n°5: Lavage de type blanc souillé)
Tested virus : *Human coronavirus 229E (HCOV-229E)*
Test temperature : 10°C

Batch(1) : /

On request of(1) :

GACHES CHIMIE SPECIALITES
ACTIVITE ENTRETIEN TEXTILE
2 BIS CHEMIN DE LA SCIERIE
FR 64800 OS MARSILLON

Loos, the 25 February 2021

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Lab manager

The test report includes : **9** pages

I. PRINCIPLE

The virucidal activity was determined according to the protocol of the NF EN 14476+A2 standard: "Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (phase 2, step 1)." - July 2019.

II. IDENTIFICATION OF SAMPLE

Product[(1)]: **Processus global de lavage à froid, Générateur à ozone avec adjonction de produits lessiviels dédiés (Lavage n°5: Lavage de type blanc souillé).**

Machine OTEK[(1)]

Cool Star – Batch : 190422801 – Manufacture date : 29/08/2019 – Expiry date : /

Hypochlorite de sodium 12.5% (47/55° chlorométrique) – Batch : 4200401 – Manufacture date : / – Expiry date : /

Garosive deter – Batch: 200080001 – Manufacture date : 24/02/2020 – Expiry date : /

Garo boost – Batch : 200101601 – Manufacture date : 05/03/2020 – Expiry date : /

Peracid forte – Batch : 200015501 – Manufacture date : 15/01/2020 – Expiry date : /

Type d'adjonction de produit lessiviel	Dosage gr de produit par kilo de linge lavé * 1kg de linge = 4 litres d'eau par pas de lavage
COOL STAR Renforçateur dégressant	Essai 1: 0,1 gr / kilo de linge lavé Essai 2: 5 gr / kilo de linge lavé Essai 3: 6 gr / kilo de linge lavé
JAVEL 12,5% Détachant , désinfectant, oxydant, décolorant	Essai 1: 0,1 gr / kilo de linge lavé Essai 2: 12 gr / kilo de linge lavé Essai 3: 20 gr / kilo de linge lavé
GAROSIVE DETER Détérgent enzymatique pour process ozone	Essai 1: 0,1 gr / kilo de linge lavé Essai 2: 4 gr / kilo de linge lavé Essai 3: 6 gr / kilo de linge lavé
GARO BOOST Renforçateur alcalin séquestrant concentré pour process ozone	Essai 1: 0,1 gr / kilo de linge lavé Essai 2: 4 gr / kilo de linge lavé Essai 3: 5 gr / kilo de linge lavé
PERACID FORTE gent de blanchiment et désinfectant concentr	Essai 1: 0,1 gr / kilo de linge lavé Essai 2: 4 gr / kilo de linge lavé Essai 3: 6 gr / kilo de linge lavé

(Mix of products made by the MIDAC Laboratory with the addition of products in the vertical direction from top to bottom)

Society: **GACHES CHIMIE SPECIALITES**

Received at the laboratory: **17 June 2020**

Storage conditions at the laboratory: **Room temperature, in the darkness.**

Appearance of the product: **Garosive deter is green liquid and others products are colorless liquid.**

Product diluent recommended by the manufacturer: **Undiluted.**

III. EXPERIMENTAL CONDITIONS

Period of analysis : **18 January 2021** to **29 January 2021**

Product test concentrations : **see table(s) on next page(s).**

Virus identification: **Human Coronavirus 229E (HCOV-229E), P1**

Name and source : **HeLa , CCL-2** Number of passages : **16**

Cell culture medium : **MEM 10%SVF, 1% AANE, 1%ATB, 1%L-Glu**

Product diluents used : **Distilled water.**

Appearance product dilutions :

Essay 1 : Colorless homogeneous liquid

Essay 2 : Whitish homogeneous liquid

Essay 3 : Whitish homogeneous liquid

Stability of mixture: **None precipitate observed during the test**

Test temperature : **10°C (± 1°C).**

Contact times : **7 minutes (+/- 10 secondes)**

Interfering substances : **3g/l bovine albumin with 3mL/L sheep erythrocytes (dirty conditions)**

Method used for product neutralization : **Microfiltration method** - Columns: **Microspin S-400 HR**

Incubation temperature : **37°C (± 1°C) et 5 % CO2**

IV. RESULTS

The calculation of titer reductions is based on the method of Spearman and Kärber.

1. Method validation

Assays were validated by:

- a cytotoxicity control
- a sensitivity control of untreated cells
- a formaldehyde internal standard control
- a product neutralization control

a) Product cytotoxicity

	Product cytotoxicity (log $_{DICT50}$)
Dilution method	**4.50E+00**
Filtration on Microspin ^{TM} S-400 HR columns	**1.50E+00**

Comment: *after dilution according to the standard protocol, the cytotoxicity of the product is* **4.50E+00** *log $_{DICT50}$. The filtration on Microspin TM S-400 HR columns leads to the reduction of the toxicity at* **1.50E+00** *log $_{DICT50}$.*

b) Cells sensitivity

	Sensitivity (log $_{DICT50}$)
Treated cells **A**	**5.38E+00**
Untreated cells **B**	**5.50E+00**

Comment: *The difference between the virus titer of treated cells* ***(A)*** *and untreated cells* ***(B)*** *must be less than 1 log.*

c) Formaldehyde internal standard control

Product	**Test concentration**	**Interfering substance**	**Viral titer in the test** (log $_{DICT50}$)	**Reduction** (log $_{DICT50}$)
Formaldehyde – 30 min	0.7% (v/v)	PBS	**3.13E+00**	**2.00E+00**
Formaldehyde - 60 min	0.7% (v/v)	PBS	**2.75E+00**	**2.38E+00**
Viral control formaldehyde	n.a	PBS	**5.13E+00**	

Comment: *The difference between the titer of the viral control, expressed as a logarithm, and the titer of the virus in the formaldehyde internal standard control is* **2.00E+00** *log $_{DICT50}$ after 30 min and* **2.38E+00** *log $_{DICT50}$ after 60min.*

d) Neutralization control

Product	Test concentration	Interfering substance	Viral titer in the test (log DICT50)	Reduction (log DICT50)
Neutralization of the product	**Essay 3**	**3g/l bovine albumin with 3mL/L sheep erythrocytes (dirty conditions).**	**5.63E+00**	**0.00E+00**
Neutralization control	n.a		**5.63E+00**	

Comment: *Checking the efficiency of the neutralization of product activity, the difference of titer with the test suspension must be ≤ 0.5log.*

2. Test results: Evaluation of virucidal activity

Product	Test concentration	Interfering substance	Viral titer in the test (log DICT50)	Reduction (log DICT50)
Global cold washing process, ozone generator OTEK with the addition of dedicated detergents Lavage n°5: Lavage de type blanc souillé: /	**Essay 1**	**3g/l bovine albumin with 3mL/L sheep erythrocytes (dirty conditions).**	**3.88E+00**	**1.75E+00**
	Essay 2		**1.50E+00**	**4.13E+00**
	Essay 3		**1.50E+00**	**4.13E+00**
Viral control T0			**5.63E+00**	
Viral control Tmax			**5.63E+00**	

Comment: *At least one concentration per test must show a reduction of 4log or more, and at least one concentration must show a log reduction of less than 4.*

V. CONCLUSION

According to **NF EN 14476+A2: 2019 standard**, The product

Global cold washing process, ozone generator OTEK with the addition of dedicated detergents (**Lavage n°5: Lavage de type blanc souillé) (1)**
batch(1) : /

possesses a virucidal activity tested at **Essay 2** after **7 minutes (+/- 10 seconds)** at **10°C (± 1°C)** with **3g/l bovine albumin with 3mL/L sheep erythrocytes (dirty conditions)** against **Human Coronavirus 229E, (HCOV-229E**)**.**

VI. REMARKS

The COFRAC accreditation attests laboratories are competent for the only tests covered by the program.

Copy of this test report is authorized only in its entirety.

This report concerns only the tested product.

(1) Data provided by the customer.

Laboratoire MIDAC assumes no responsibility for the information provided by the customer

VII. REVISION

Date	Revision description	Version
n.a	**n.a**	**n.a**

Annex 1 : Raw Data

Product[1] : **Global cold washing process, ozone generator OTEK with the addition of dedicated detergents (Lavage n°5: Lavage de type blanc souillé)**

Batch[1] :/

Product	**Concentration**	**Interfering substance**	**Contact Time**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Global cold washing process, ozone generator OTEK with the addition of dedicated detergents (Lavage n°5: Lavage de type blanc souillé) Cytotoxicity	**Essay 3**		n.a	TTTT TTTT	TTTT TTTT	TTTT TTTT	TTTT TTTT	0000 0000	0000 0000	0000 0000	0000 0000
Global cold washing process, ozone generator OTEK with the addition of dedicated detergents (Lavage n°5: Lavage de type blanc souillé) Cytotoxicity after Microspin	**Essay 3**	**3g/l bovine albumin with 3mL/L sheep erythrocytes (dirty conditions)**	n.a	TTTT TTTT	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Sensitivity of treated cells	**Essay 3**		60min	3333 3333	2222 2222	1111 1111	1110 1111	1101 1101	1010 0000	0000 0000	0000 0000
Sensitivity of untreated cells	n.a		60min	4444 4444	2222 2222	2122 2223	3122 2222	1001 1133	1010 0000	0000 0000	0000 0000
Formaldehyde 30min	0,7 % (v/v)		30min	4444 4444	4444 4444	1100 1034	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Formaldehyde 60min	0,7 % (v/v)	PBS	60min	4444 4444	4444 4444	0001 0001	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Formaldehyde control	n.a		60min	3333 3333	3333 3333	1111 1233	0111 0133	1100 0033	0100 0011	0000 0000	0000 0000
Formaldehyde Cytotoxicity	0.7%(v/v)		n.a	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Viral control	n.a		0	2222 2222	1111 1111	1111 1123	0010 1112	1111 1111	1100 0011	0000 0000	0000 0000
Viral control	n.a		7 minutes (+/- 10 seconds)	2222 2221	1111 1111	1111 1111	1111 1102	1111 1001	1001 1001	0000 0000	0000 0000
Global cold washing process, ozone generator OTEK with the addition of dedicated detergents (Lavage n°5: Lavage de type blanc souillé)	**Essay 1**	**3g/l bovine albumin with 3mL/L sheep erythrocytes (dirty conditions)**	7 minutes (+/- 10 seconds)	1111 1111	1011 1111	1111 1110	1001 1101	0000 0000	0000 0000	0000 0000	0000 0000
	Essay 2			4444 4444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	Essay 3			4444 4444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Inactivation	**Essay 3**		30min	4444 4444	4444 4444	2222 2222	2111 1111	1101 1111	0100 0100	0000 0000	0000 0000
Inactivation control	n.a		30min	2222 2222	1111 1111	1111 1111	1111 1111	1110 0111	0110 0010	0000 0000	0000 0000

1 to 4 Presence of virus. 1 = 25% CPE and 4 = 100% CPE

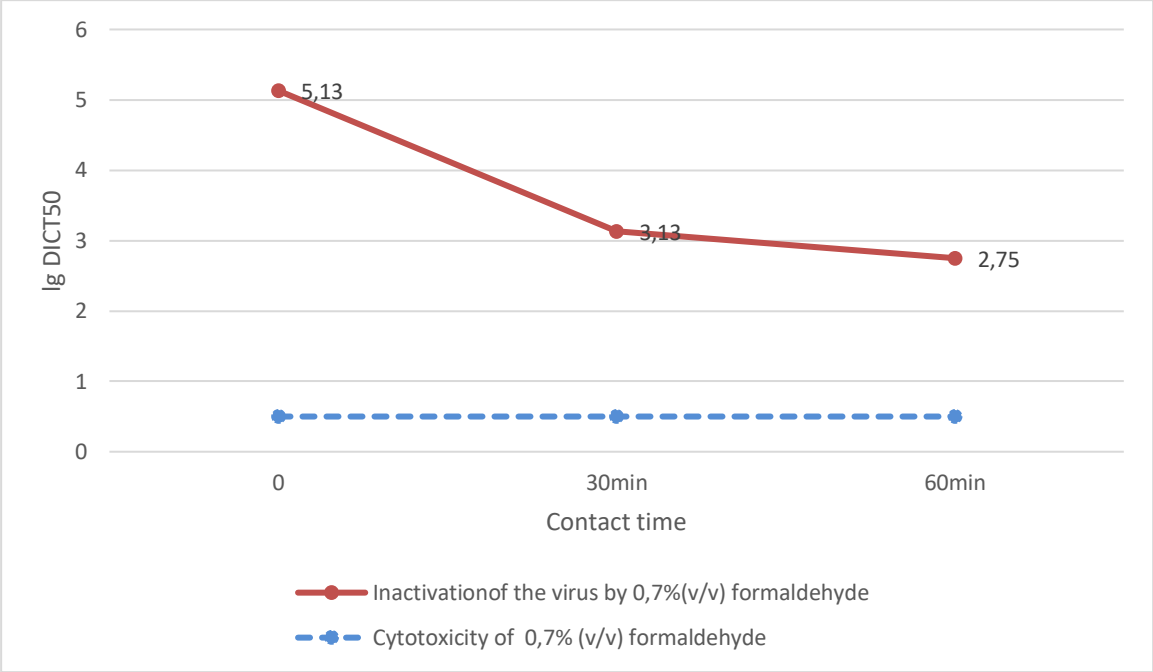
0 Absence of virus

T Toxic

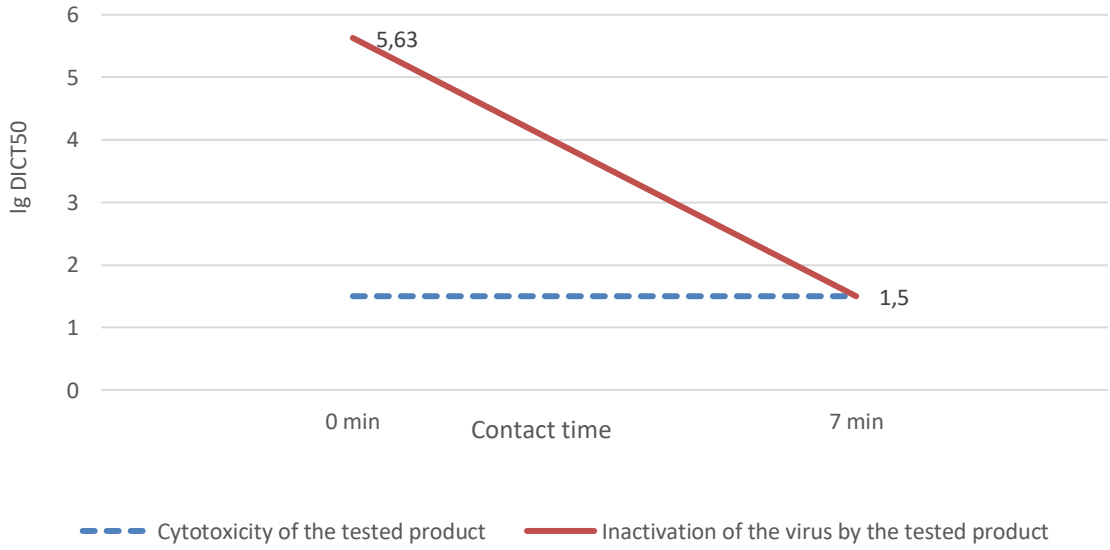
n.a non-applicable

Annex 2: Graphic presentation of the test results

a) Reference inactivation of the virus by 0.7% (v/v) formaldehyde



b) Evaluation of virucidal activity of the product



Annex 3: Methodology verification

A test is only valid if the following criteria are fulfilled:

a/ Test virus suspension has at least a concentration which allows the determination of a 4 log10 reduction of the virus titer.

b/ The difference between the titer of the viral control, expressed as a logarithm, and the titer of the virus in the inactivation reference test is

between -0,5 and -2,5 after 30min and between -2 and -4,5 after 60min for poliovirus,

between -3 and -5 after 30min and -3,5 and 5,5 after 60min for adenovirus,

between 0.0 and -2 after 30min and -0.5 and 2,5 after 60min for parvovirus,

between -0.75 and -3,5 after 5min and between -2,0 and ≥ -4 after 15min for vaccina virus,

between -1 and -3 after 30min and between -2,0 and -4,0 after 60min for murine norovirus

c/ Cytotoxicity of the product does not affect cell morphology and growth or susceptibility for the test virus in the dilutions of the test mixtures which are necessary to demonstrate a 4log reduction of the virus.

d/ Comparative titration of the virus on the treated cell cultures with dilutions of the test mixture and in parallel with PBS results in a difference <1 lg of the viral titer.

e/ When checking the efficiency of the neutralization of product activity, the difference of titer with the test suspension must be ≤ 0.5log.

f/ At least one concentration per test must show a reduction of 4log or more, and at least one concentration must show a log reduction of less than 4.