

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°4: Textile de type couleur 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. PRINCIPE

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

Name(s) ⁽¹⁾: **Processus global de lavage à froid, Générateur à ozone avec adjonction de produit lessiviels dédiés (Lavage N°4: Textile de type couleur souillé)**

Machine OTEK⁽¹⁾

Cool Star – Batch : 190422801 – Manufacture date : 29/08/2019 – 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	Essai 1: 0,1 gr / kilo de linge lavé
Renforteur dégrissant	Essai 2: 6 gr / kilo de linge lavé
	Essai 3: 6 gr / kilo de linge lavé
GARO BOOST	Essai 1: 0,1 gr / kilo de linge lavé
Renforteur alcalin séquestrant concentré	Essai 2: 6 gr / kilo de linge lavé
pour process ozone	Essai 3: 6 gr / kilo de linge lavé
GAROSIVE DETER	Essai 1: 0,1 gr / kilo de linge lavé
Détergent enzymatique pour process ozone	Essai 2: 4 gr / kilo de linge lavé
	Essai 3: 6 gr / kilo de linge lavé
GARO BOOST	Essai 1: 0,1 gr / kilo de linge lavé
Renforteur alcalin séquestrant concentré	Essai 2: 4 gr / kilo de linge lavé
pour process ozone	Essai 3: 5 gr / kilo de linge lavé
PERACID FORTE	Essai 1: 0,1 gr / kilo de linge lavé
agent de blanchiment et désinfectant concentré	Essai 2: 3 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 : **09 February 2021 to 25 February 2021**

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

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

Cells identification:

Name and source : **HeLa , CCL-2** Number of passages : **29**

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 seconds)**

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) and 5 % CO2**

IV. RESULTS

The calculation of titer reductions is based on the method of Spearman and Kärber. It is measured as the difference between the titer of the virus control and the titer of product test solution.

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	3.50E+00
Filtration on Microspin™ S-400 HR columns	1.50E+00

Comment: after dilution according to the standard protocol, the cytotoxicity of the product is **3.50E+00** log_{DICT50}. The filtration on Microspin™ 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	6.13E+00
Untreated cells B	6.13E+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	3.63E+00	2.37E+00
Formaldehyde - 60 min	0.7% (v/v)	PBS	3.38E+00	2.62E+00
Viral control formaldehyde	n.a	PBS	6.00E+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 **2.37E+00** log_{DICT50} after 30min and **2.62E+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).	6.25E+00	1.20E-01
Neutralization control	n.a		6.13E+00	

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

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°4: Textile de type couleur souillé) (1)	Essay 1	3g/l bovine albumin with 3mL/L sheep erythrocytes (dirty conditions).	5.63E+00	6.20E-01
	Essay 2		1.50E+00	4.75E+00
	Essay 3		1.50E+00	4.75E+00
Viral control T0	6.25E+00			
Viral control Tmax	6.25E+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°4: Textile de type couleur 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°4: Textile de type couleur souillé)**

Batch⁽¹⁾ :/

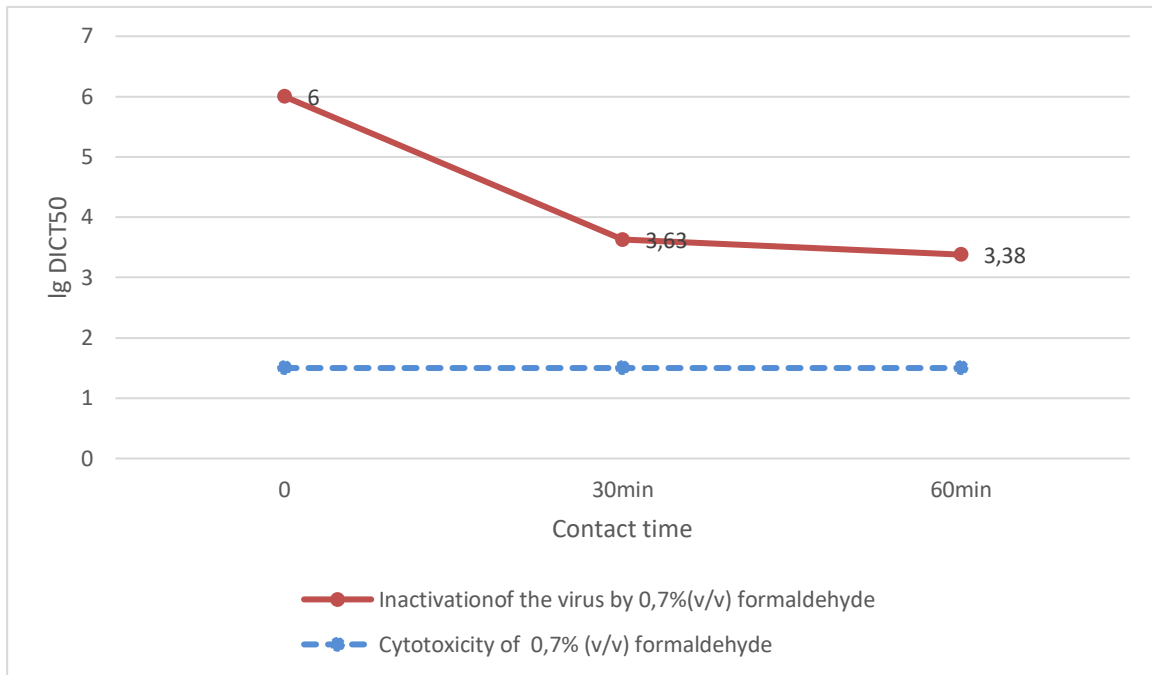
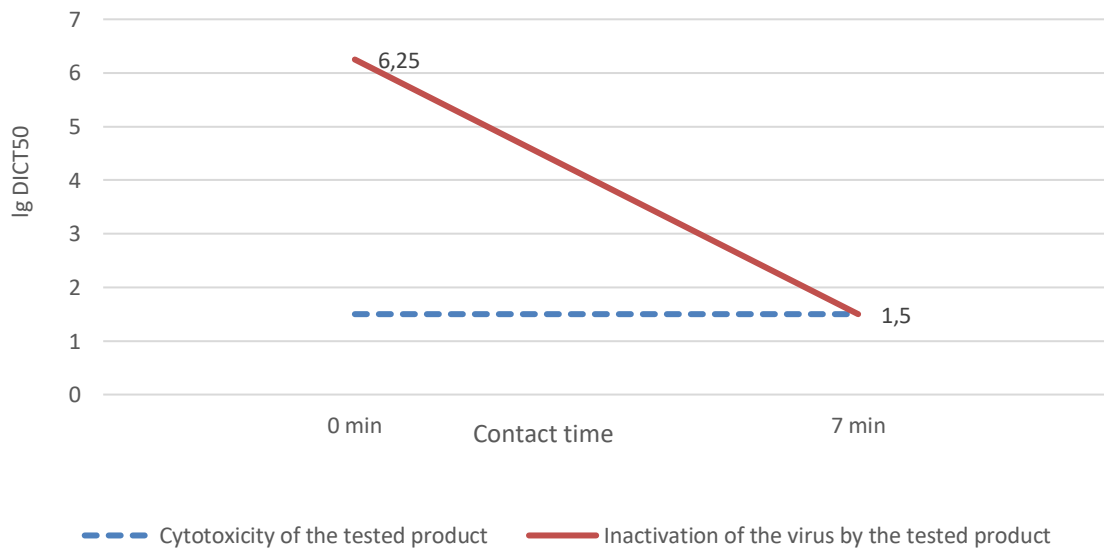
Product	Concentration	Interfering substance	Contact Time	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
Global cold washing process, ozone generator OTEK with the addition of dedicated detergents (Lavage n°4: Textile de type couleur souillé) Cytotoxicity	Essay 3		n.a	TTTT TTTT	TTTT TTTT	TTTT TTTT	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
			n.a	TTTT TTTT	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Global cold washing process, ozone generator OTEK with the addition of dedicated detergents (Lavage n°4: Textile de type couleur 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	4444 4444	4444 4444	3333 3333	2111 2122	1111 1100	1110 1011	0000 0001	0000 0000
Sensitivity of untreated cells	n.a	PBS	60min	4444 4444	3333 3333	2222 2222	2222 2222	1112 2211	0101 1011	0000 0000	0000 0000
Formaldehyde 30min	0,7 % (v/v)		30min	4444 4444	4444 4444	4444 4444	0001 0000	0000 0000	0000 0000	0000 0000	0000 0000
Formaldehyde 60min	0,7 % (v/v)		60min	4444 4444	4444 4444	4044 4444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Formaldehyde control	n.a		60min	3333 3333	3333 3333	2222 2222	1111 1111	1011 2111	1101 0101	0000 0000	0000 0000
Formaldehyde Cytotoxicity	0.7%(v/v)		n.a	TTTT TTTT	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Viral control	n.a		0	4444 4444	3333 3333	3333 3333	1111 1111	1111 1011	1110 1011	1000 0000	0000 0000
Viral control	n.a		7 minutes (+/- 10 seconds)	3333 3333	3333 3333	2222 2222	1111 1111	1111 1101	1101 0111	0000 0001	0000 0000
Global cold washing process, ozone generator OTEK with the addition of dedicated detergents (Lavage n°4: Textile de type couleur souillé)	Essay 1	3g/l bovine albumin with 3mL/L sheep erythrocytes (dirty conditions)	7 minutes (+/- 10 seconds)	3333 3333	2222 2222	1111 1111	1111 1111	1110 0101	0110 1010	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	4444 4444	3333 3333	1111 1111	1011 0011	0000 0100	0000 0000	
Inactivation control	n.a	30min	4444 4444	3333 3333	3333 3333	1111 1123	1011 1111	1011 1012	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 ≤ 0.5 log.

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.