FONdation pour le DEveloppement de la REcherche PHARmaceutique

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STUDY 17-2197

DETERMINATION OF VIRICIDAL ACTIVITY OF CHEMICAL ANTISEPTICS AND DISINFECTANTS USED IN HUMAN MEDECINE AS STANDARD NF EN 14476+A1 (OCTOBER 2015) CLEAN CONDITIONS

Promotor Study

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DETERMINATION OF VIRICIDAL ACTIVITY OF CHEMICAL ANTISEPTICS AND DISINFECTANTS USED IN HUMAN MEDECINE AS STANDARD NF EN 14476+A1 (OCTOBER 2015) CLEAN CONDITIONS

I - IDENTIFICATION OF TESTING LABORATORY

FONDEREPHAR
Faculté des Sciences Pharmaceutiques
35 Chemin des Maraîchers
31062 Toulouse cedex 09

II - SAMPLE IDENTIFICATION

Product name	NOCOLYSE
Batch number	041017N
Expiry date	10/2019
Packaging	1 L
Receipt date	October/05/2017
Internal code	17-2197-1
Active substance	Hydrogen Peroxyde (H_2O_2)
Supplier	OXYPHARM
Period of testing	October-November 2017

Storage conditions during period of testing: Ambient temperature

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III - TEST METHODE

III-1 Virus test

POLIOVIRUS TYPE 1

Origin	ANSM
Batch number ANSM	NIBSC 01/528
Authorization number	S-2632FON12007-4
Internal batch number	SS-4-060114 (passage N°4) and SS-5-280213 (passage N°5)

ADENOVIRUS TYPE 5

Origin:	ATCC
Reference:	VR-5
Supplier Batch number:	58486654
Internal Batch number :	SS-1-181212 (Passage N°1)

MURINE NOROVIRUS

Origin:	Freidrich-Loeffler-Institut
Reference:	FLI-RVB-651
Batch number	4/200409/220409
Internal batch number:	SS-1-200217 (Passage N°1)

III-2 Recipient cells

VERO cells (POLIOVIRUS TYPE 1)

Origin:	ATCC
Reference ATCC:	CCL-81
Batch number ATCC:	3372621
Interrnal batch number:	WCB-251013 (Passages N°29, 31, and 54)

HELA cells (ADENOVIRUS TYPE 5)

Origin:	ATCC
Reference ATCC:	CCL-2
Batch number ATCC:	4440136
Interrnal batch number:	WCB-010713 (Passages N°37, N°40 and 44)

RAW 264-7 cells (MURINE NOROVIRUS)

Origin:	ATCC
Reference ATCC:	TIB-71
Batch number ATCC:	5822175
Interrnal batch number:	WCB-230916 (Passages N° 40, 41 and 47)

IV -EXPERIMENTAL CONDITIONS

IV-1 Analysis conditions

- **Concentrations test (V/V):** 80% (V/V); 60% (V/V); and 0.5% (V/V)
- **Contact time:** 60min ± 10s
- **Test temperature:** 20°C ± 1°C
- **Incubation temperature:** 36°C ± 1°C
- **Interfering substance:** 0.3g/L to BSA (Batch N°185)
- **Reference substance:** Formaldehyde (Batch N°7109)
- **Product diluent:** Water (Batch N° 19KL09GA)
- **Culture medium (Poliovirus type 1 and Adenovirus Type 5):** EMEM (Batch N°1949)
- **Culture medium (Murine Norovirus):** DMEM (Batches N°1953 and 1958)
- **Molecular sieve filter:** Sephadex LH20 (Batches N°7492 and 7510)
- **PBS :** Batch N°S15615L0615

Stability and appearance of the test mixture during the test: Preparation homogeneous during the test

IV-2 Methods

IV-2-1 Virus suspension title

The assay was performed by the microplate method of suspension cells. The cytopathic effect was determined at least 4 days of culture.
The number of infectious units is estimated by the SPEARMAN-KÄRBER method by calculating the negative logarithm of the 50% limit point ($lgDICT_{50}$) using the following formula:
$lgDICT_{50}$ = Negative logarithm of the highest concentration of virus used - [(Sum of % assigned to each dilution/100 - 0.5) X (lg of dilution)]

IV-2-2 Cytotoxicity of product solutions

IV-2-2-1 Direct cytotoxicity

This test determines the concentration of disinfectant that does not induce signs of cytotoxicity to the cell line for the demonstration of the effect of virus.

IV-2-2-2 Indirect Cytotoxicity: molecular sievfilter

Elimination of cytotoxicity test product
The activity of the product is stopped by the molecular sieving method using a sieve filter, i.e. Sephadex LH 20.
The neutralization of the formaldehyde was validated by passing it through Sephadex at a dilution 1/10.
The neutralization of the product was validated by passing it through Sephadex at a dilution 1/20 (VERO cells) and 1/50 (HELA cells and Raw 264-7 cells).

IV-2-3 Sensitivity of cells to viruses

Comparative assays were performed on cells treated or not with the disinfectant to ensure that the disinfectant subcytotoxic concentration does not change the behavior of the virus to receive cell.

IV-2-4 Verification of effectiveness of stopping the disinfectant activity at T0

Immediately after preparation of the test mixture at time 0, the reaction was stopped in ice for 30 min ± 10 s and then infectious virus titre was determined.

IV-2-5 Reference virus inactivation

Virucidal effect of formaldehyde is tested to control the uniformity of the behavior of virus to chemical agents over time (30 min and 60 min).

V RESULTS OF POLIOVIRUS TYPE 1

V-1 Validations

V-1-1 Title of virus suspension

lg $DICT_{50}$ = 8.8

V-1-2 Cytotoxicity of product solutions

V-1-2-1 Direct cytotoxicity

No-cytotoxic concentration from 1.22 10^{-3}% (V/V)

VI-1-2-2 Indirect cytotoxicity

No-cytotoxic concentration from 0.125% (V/V)

V-1-3 Sensitivity of cell to virus

S1 (in presence of product): lg $DICT_{50}$ = 8.3
S2 (in absence of product): lg $DICT_{50}$ = 8.2
S2 - S1 = 0.1
Sensitivity valid (limit S2 - S1 < 1 lg)

V-2 Assay

V-2-1 Title of virus suspension

lg $DICT_{50}$ = 9.1

V-2-2 Verification of control virus suspension assay in presence or absence of Sephadex

- Interfering substance BSA 0.3g/l:

Title of virus assay suspension
- Before Sephadex
lg $DICT_{50}$ = 5.7
- After Sephadex
lg $DICT_{50}$ = 6.0
Difference ≤ 0.5 lg (verification valid)

V-2-3 Verification of effectiveness of stopping the disinfectant activity at T0

I1 (In presence of product): lg $DICT_{50}$ = 5.6
I2 (In absence of product): lg $DICT_{50}$ = 5.6
Difference ≤ 0.5 lg (verification valid)

V-2-4 Assay of inactivation of virus reference

V-2-4-1 Direct cytotoxicity

No-cytotoxic concentration from 1.71 10^{-4}% (V/V)

V-2-4-2 Indirect cytotoxicity

No-cytotoxic concentration from 1.1 10^{-3}% (V/V)

V-2-4-3 Control virus suspension assay PBS

Before Sephadex
lg $DICT_{50}$ PBS = 5.9
After Sephadex
lg $DICT_{50}$ PBS = 5.7
Difference ≤ 0.5 lg (verification valid)

Formaldehyde concentration: 1.3%

V-2-4-4 Title result of formaldehyde

Contact time	30 min	60 min
lg $DICT_{50}$	3.9	2.9
Logarithmic reduction	1.8	2.8

V-2-4-5 Results of assay product

POLIOVIRUS TYPE 1 - 60 min ± 10s - 20°C ± 1°C								
	In presence of dilutions of test product						Control	
-log Dilution	Degree of cytopathogenic effect			% positive wells			Degree of cytopathogenic effect *	% positive wells
	80%	60%	0.5%	80%	60%	0.5%		
0,6	00000000	00000000	44444444	0%	0%	100%	44444444	100%
1,2	00000000	00000000	44444444	0%	0%	100%	44444444	100%
1,8	00000000	00000000	44444444	0%	0%	100%	44444444	100%
2,4	00000000	00000000	44444444	0%	0%	100%	44444444	100%
3,0	00000000	00000000	44444444	0%	0%	100%	44444444	100%
3,6	00000000	00000000	44444444	0%	0%	100%	44444444	100%
4,2	00000000	00000000	44444444	0%	0%	100%	44444444	100%
4,8	00000000	00000000	21440420	0%	0%	75%	44444444	100%
5,4	00000000	00000000	20010222	0%	0%	62.5%	02443012	75%
6,0	00000000	00000000	00000000	0%	0%	0%	10011121	75%
6,6	00000000	00000000	00000000	0%	0%	0%	00000000	0%
7,2	00000000	00000000	00000000	0%	0%	0%	00000000	0%
lg $DICT_{50}$				≤0.3	≤0.3	5.3		6.0

0* 1 to 4: Presence of virus, with cytopathogenic effect observed in cell wells
0: Absence of virus, with absence of cytopathogenic effect

VI- RESULTS OF ADENOVIRUS TYPE 5

VI-1 Validations

VI-1-1 Title of virus suspension

lg $DICT_{50}$ = 8.4

VI-1-2 Cytotoxicity of product solutions

VI-1-2-1 Direct cytotoxicity

No-cytotoxic concentration from 0.020% (V/V)

VI-1-2-2 Indirect cytotoxicity

No-cytotoxic concentration from 0.125% (V/V)

VI-1-3 Sensitivity of cell to virus

S1 (in presence of product): lg $DICT_{50}$ = 7.7
S2 (in absence of product): lg $DICT_{50}$ = 7.6
S2 - S1 = 0.1
Sensitivity valid (limit S2 - S1 < 1 lg)

VI-2 Assay

VI-2-1 Title of virus suspension

lg $DICT_{50}$ = 8.9

VI-2-2 Verification of control virus suspension assay in presence or absence of Sephadex

- Interfering substance BSA 0.3g/l:

Title of virus assay suspension
- Before Sephadex
lg $DICT_{50}$ = 6.3
- After Sephadex
lg $DICT_{50}$ = 6.3
Difference ≤ 0.5 lg (verification valid)

VI-2-3 Verification of effectiveness of stopping the disinfectant activity at TO

I1 (In presence of product): lg $DICT_{50}$ = 6.3
I2 (In absence of product): lg $DICT_{50}$ = 6.5
Difference ≤ 0.5 lg (verification valid)

VI-2-4 Assay of inactivation of virus reference

VI-2-4-1 Direct cytotoxicity

No-cytotoxic concentration from 1.7 10^{-4}% (V/V)

VI-2-4-2 Indirect cytotoxicity

No-cytotoxic concentration from 1.1 10^{-3}% (V/V)

VI-2-4-3 Control virus suspension assay PBS

Before Sephadex
lg $DICT_{50}$ PBS = 6.1
After Sephadex
lg $DICT_{50}$ PBS = 5.9
Difference ≤ 0.5 lg (verification valid)

Formaldehyde concentration: 1.3%

VI-2-4-4 Title result of formaldehyde

Contact time	30 min	60 min
lg $DICT_{50}$	4.0	2.5
Logarithmic reduction	1.9	3.4

VI-2-4-5 Results of assay product

ADENOVIRUS TYPE 5 - 60 min ± 10s - 20°C ± 1°C								
	In presence of dilutions of test product						Control	
-log Dilution	Degree of cytopathogenic effect			% positive wells			Degree of cytopathogenic effect *	% positive wells
	80%	60%	0.5%	80%	60%	0.5%		
0,6	00000000	00000000	44444444	0%	0%	100%	44444444	100%
1,2	00000000	00000000	44444444	0%	0%	100%	44444444	100%
1,8	00000000	00000000	44444444	0%	0%	100%	44444444	100%
2,4	00000000	00000000	44444444	0%	0%	100%	44444444	100%
3,0	00000000	00000000	44444444	0%	0%	100%	44444444	100%
3,6	00000000	00000000	22222222	0%	0%	100%	44444444	100%
4,2	00000000	00000000	12220111	0%	0%	87.5%	33333333	100%
4,8	00000000	00000000	10111101	0%	0%	75%	22222222	100%
5,4	00000000	00000000	111110100	0%	0%	62.5%	11211021	87.5%
6,0	00000000	00000000	00000000	0%	0%	0%	01110010	50%
6,6	00000000	00000000	00000000	0%	0%	0%	10101110	62.5%
7,2							00000000	0%
lg $DICT_{50}$				≤0.3	≤0.3	5.3		6.3

* 1 to 4: Presence of virus, with cytopathogenic effect observed in cell wells
0: Absence of virus, with absence of cytopathogenic effect

VII- RESULTS OF MURINE NOROVIRUS

VII-1 Validations

VII-1-1 Title of virus suspension

lg $DICT_{50}$ = 8.4

VII-1-2 Cytotoxicity of product solutions

VII-1-2-1 Direct cytotoxicity

No-cytotoxic concentration from 0.02% (V/V)

VII-1-2-2 Indirect cytotoxicity

No-cytotoxic concentration from 0.13% (V/V)

VII-1-3 Sensitivity of cell to virus

S1 (in presence of product): lg $DICT_{50}$ = 7.4
S2 (in absence of product): lg $DICT_{50}$ = 7.4
S2 – S1 = 0
Sensitivity valid (limit S2 – S1 < 1 lg)

VII-2 Assay

VII-2-1 Title of virus suspension

lg $DICT_{50}$ = 8.9

VII-2-2 Verification of control virus suspension assay in presence or absence of Sephadex

- Interfering substance BSA 0.3g/l:

Title of virus assay suspension
- Before Sephadex
lg $DICT_{50}$ = 6.0
- After Sephadex
lg $DICT_{50}$ = 5.9
Difference ≤ 0.5 lg (verification valid)

VII-2-3 Verification of effectiveness of stopping the disinfectant activity at T0

I1 (In presence of product): lg $DICT_{50}$ = 5.9
I2 (In absence of product): lg $DICT_{50}$ = 5.9
Difference ≤ 0.5 lg (verification valid)

VII-2-4 Assay of inactivation of virus reference

VII-2-4-1 Direct cytotoxicity
No-cytotoxic concentration from 4.3 10^{-5}% (V/V)

VII-2-4-2 Indirect cytotoxicity
No-cytotoxic concentration from 2.7 10^{-4}% (V/V)

VII-2-4-3 Control virus suspension assay PBS

Before Sephadex
lg DICT $_{50}$ PBS = 5.8
After Sephadex
lg DICT $_{50}$ PBS = 5.5
Difference ≤ 0.5 lg (Verification valid)

Formaldehyde concentration: 1.3%

VII-2-4-4 Title result of formaldehyde

Contact time	30 min	60 min
lg $DICT_{50}$	3.2	1.9
Logarithmic reduction	2.3	3.6

VII-2-4-5 Results of assay product

MURINE NOROVIRUS - 60 min ± 10s - 20°C ± 1°C								
	In presence of dilutions of test product						Control	
-log Dilution	Degree of cytopathogenic effect			% positive wells			Degree of cytopathogenic effect *	% positive wells
	80%	60%	0.5%	80%	60%	0.5%		
0,6	01000001	01100100	44444444	25%	37.5%	100%	33333333	100%
1,2	00000000	00000000	44444444	0%	0%	100%	33333333	100%
1,8	00000000	00000000	22222222	0%	0%	100%	33333333	100%
2,4	00000000	00000000	22222222	0%	0%	100%	33333333	100%
3,0	00000000	00000000	11111111	0%	0%	100%	22222222	100%
3,6	00000000	00000000	11111111	0%	0%	100%	11111111	100%
4,2	00000000	00000000	10110100	0%	0%	50%	11111111	100%
4,8	00000000	00000000	00000010	0%	0%	12.5%	11111111	100%
5,4	00000000	00000000	00000000	0%	0%	0%	11111111	100%
6,0	00000000	00000000	00000000	0%	0%	0%	10000011	37.5%
6,6	00000000	00000000	00000000	0%	0%	0%	00000000	0%
7,2							00000000	0%
lg $DICT_{50}$				0.5	0.5	4.3		5.9

* 1 to 4: Presence of virus, with cytopathogenic effect observed in cell wells
0: Absence of virus, with absence of cytopathogenic effect

VIII REDUCTION LOGARITHMIC

VIII- 1 POLIOVIRUS TYPE 1

Concentrations	80% (V/V)	60% (V/V)	0.5% (V/V)
Logarithmic reduction	≥5.7	≥5.7	0.7

VIII- 2 ADENOVIRUS TYPE 5

Concentrations	80% (V/V)	60% (V/V)	0.5% (V/V)
Logarithmic reduction	≥6.0	≥6.0	1.0

VIII- 3 MURINE NOROVIRUS

Concentrations	80% (V/V)	60% (V/V)	0.5% (V/V)
Logarithmic reduction	5.4	5.4	1.6

IX- CONCLUSION

According to standard NF EN 14476+A1 (October 2015), the product NOCOLYSE (Batch 041017N) in clean conditions - for the contact time 60 min±10S at 20°C±1°C presents a virucidal activity against Poliovirus type 1, Adenovirus type 5 and Murine Norovirus at the concentration 80% (V/V) and 60%(V/V).

These results hold only to the product under assay.