



Institut de
Recherche
Microbiologique

TEST REPORT

N°479/1211-1/M-1

ISSUED TO: OXYPHARM / AIREL
917, rue Marcel Paul
94500 CHAMPIGNY-sur-MARNE

PRODUCT : NOCOLYSE

TEST REQUEST DATE: 01 december 2011

ANALYSIS REFERENCE: 479/1211

TEST: Quantitative suspension test for the evaluation of tuberculocidal activity according to standards **NF EN 13727 (July 2004)** and **NF EN 14348 (June 2005)**.

Membrane filtration method.

Test on *Mycobacterium terrae* CIP 104 321

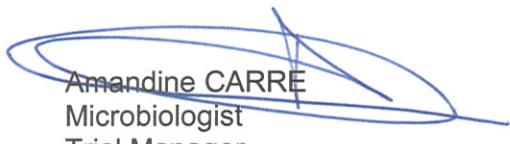
This report contains 5 pages.

This report applies only to the product submitted to test.

Issue date: 16 april 2012

Re-issue date: 16 april 2012

Seule la version française fait foi / Only the French version is valid


Amandine CARRE
Microbiologist
Trial Manager


Philippe STROHL
Doctor of veterinary medicine
Scientific Director

I - COMPLETE SAMPLE IDENTIFICATION

Product name: **NOCOLYSE**

- batch number: 011211N
- manufacturer: **OXYPHARM / AIREL**
917, rue Marcel Paul
94500 CHAMPIGNY-sur-MARNE
- expiration date: not indicated

I.R.M. reception date: 01 december 2011

Storage conditions: laboratory counter top, ambient temperature

Analysis period: from 06 december 2011 to 05 april 2012

Active substance and concentration: not indicated

Aspect of product and its dilutions: clear, colourless solution

II - EXPERIMENTAL CONDITIONS

Contact time: 60 min ± 10 sec

Test temperature: 20°C ± 1°C

Product diluent used during the test: sterile distilled water.

Product diluent recommended by manufacturer: none (ready-to-use product).

Tested strain:

Mycobacterium terrae CIP 104 321 incubation 21 days at 37°C ± 1°C
Test strain of the NF EN 14348 (June 2005)

Interfering substance: clean conditions (NF EN 14348 (June 2005))

- composition: Final test concentration of 0.3 g/l of bovine albumin during the test

Stability of product / interfering substance mix: no precipitate during the test

Count method: spread plate for inoculum and membrane filtration for validation and main tests.

III - PROCEDURE FOR PRELIMINARY TESTS

Rinsing liquid for membranes:

- composition: distilled water containing 0.5% (w/v) tween 80
- preparation mode: heat dissolution of ingredients and sterilization at 122°C for 15 minutes

Rinsing method for membranes:

- number of rinses: 3
- volume for each rinse: 50 ml

Neutralizer(s) added to count medium and concentration(s): none

Other additions to count medium: none

Particular count media: Middlebrook 7H10 agar medium with 10% OADC enrichment (standard NF EN 14348 (June 2005))

IV - VALIDATION OF THE MEMBRANE FILTRATION METHOD

Microorganism strain	Tested concentration of product	Test bacterial suspension	Validation test				
			Bacterial suspension	Experimental conditions	Filtration control	Inactivation by filtration	
<i>Mycobacterium terrae</i> CIP 104 321	80% (v/v)	10^6 :(>660+>660);(>660+>660) 10^7 :(24+21);(19+20) (N = $4,2 \times 10^8$)	124 ; 139 (Nv = 1315)	120 ; 113 (A = 116,5)	106 ; 124 (B = 115)	98 ; 115 (C = 106,5)	
N =	number of CFU / ml in the test bacterial suspension						
Nv =	number of CFU / ml in the bacterial suspension						
A =	number of CFU / ml in the experimental conditions validation test						
B =	number of CFU / ml in the membrane filtration control						
C =	number of CFU / ml in the membrane filtration validation test						

The membrane filtration method is validated if:

- N is between 1.5×10^8 CFU/ml and 5×10^8 CFU/ml;
- N_v is between 6×10^2 CFU/ml and 3×10^3 CFU/ml;
- A and B are equal to or greater than $0.05.N_v$;
- C is equal to or greater than $0.5.B$.

In the described conditions, the membrane filtration method is validated on the tested strain with a concentration of the product **NOCOLYSE** of 80% (v/v).

V - TEST RESULTS

Microorganism strain	Test suspension N	Results at concentration m% (v/v)			
			m = 80	m = 40	m = 20
<i>Mycobacterium</i> <i>terrae</i> CIP 104 321	10^{-6} :(>660+>660);(>660+>660) 10^{-7} :(24+21);(19+20) (N = $4,2 \times 10^8$)	Vc 10^0 Vc 10^{-1} Na R Log R	3 ; 1 0 ; 0 < 140 $> 3,0 \times 10^5$ <u>> 5,48</u>	>165 ; >165 >165 ; >165 > 1 650 $<2,6 \times 10^3$ <3,41	>165 ; >165 >165 ; >165 > 1 650 $<2,6 \times 10^3$ <3,41

N = number of CFU / ml in the test bacterial suspension

Vc 10^x = number of colonies counted in the dishes for a given dilution

Na = number of CFU / ml in the test mix

R = reduction in number of viable cells

Log R = log reduction in number of viable cells

⇒ According to the criteria of standard NF EN 14348 (June 2005), concentrations resulting in a reduction of vial titre equal to or greater 4.0 log are tuberculocidal

VI - CONCLUSION

According to the methodologies of standards NF EN 13727 (July 2004) and NF EN 14348 (June 2005), product NOCOLYSE is tuberculocidal on *Mycobacterium terrae* CIP 104 321 at a concentration of 80% (v/v) for a contact time of 60 minutes at 20°C in the presence of 0.3 g/l of bovine albumin during the test (clean conditions).

END OF TEST REPORT
