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.

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

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## 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 |
| *Mycobacterium terrae* CIP 104 321 | 80% (v/v) | $10^{-6}$:(>660+>660);(>660+>660)<br>$10^{-7}$:(24+21);(19+20)<br>(N = 4,2 x $10^{8}$) | 124 ; 139<br>(Nv = 1315) | 120 ; 113<br>(A = 116,5) | 106 ; 124<br>(B = 115) | 98 ; 115<br>(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 $1.5x10^{8}$ CFU/ml and $5x10^{8}$ CFU/ml;
- $N_v$ is between $6x10^{2}$ CFU/ml and $3x10^{3}$ 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 |
| *Mycobacterium terrae* CIP 104 321 | $10^{-6}$:(>660+>660);(>660+>660)<br>$10^{-7}$:(24+21);(19+20)<br>(N = 4,2 x $10^{8}$) | Vc $10^{0}$<br>Vc $10^{-1}$<br>Na<br>R<br>Log R | 3 ; 1<br>0 ; 0<br>< 140<br>> 3,0 x $10^{5}$<br>**> 5,48** | >165 ; >165<br>>165 ; >165<br>> 1 650<br><2,6 x $10^{3}$<br><3,41 | >165 ; >165<br>>165 ; >165<br>> 1 650<br><2,6 x $10^{3}$<br><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