Institut de
Recherche
Microbiologique

TEST REPORT

N°408/1010-2/M-1

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

PRODUCT: **NOCOLYSE ONE SHOT**

TEST REQUEST DATE: 11 October 2010

ANALYSIS REFERENCE: 408/1010

TEST: **EUROPEAN STANDARD NF EN 1650 (October 2008)**: Chemical disinfectants and antiseptics - quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. Test method and requirements (phase 2. step 1).

Partial test on the strain *Aspergillus niger* ATCC 16404 with an additional contact time of 30 minutes

Dilution-neutralization method.

This report contains 5 pages.

This report applies only to the product submitted to test.

Issue date: 18 October 2010

Re-Issue date: 16 December 2010

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

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I - COMPLETE SAMPLE IDENTIFICATION

Product name: **NOCOLYSE ONE SHOT**

* batch number: 280910OS

* manufacturer: **AIREL**
917. rue Marcel Paul
94500 CHAMPIGNY-sur-MARNE

* expiration date: not indicated

I.R.M. reception date: 11 October 2010

Storage conditions: laboratory counter top. ambient temperature

Analysis period: from 11 to 14 October 2010

Active substance(s) and concentration(s): not indicated

Aspect of product and its dilutions: clear, colourless solution

II - EXPERIMENTAL CONDITIONS

Additionnal contact time: 30 min ± 10 sec
The obligatory contact-time required by the standard is 15 minutes

Test temperature: 20°C ± 1°C

Product diluent used during the tests: sterile distilled water

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

Test repetitions per microorganism: 1

Test strain:

Aspergillus niger	ATCC 16404	incubation 72 hrs at 30°C ± 1°C

Interfering substance: clean conditions

* Composition: final 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: by inclusion in agar medium

III - PROCEDURE FOR PRELIMINARY TESTS

Neutralizer:

* Composition: Trypton soya broth 5% (w/v) tween 80, 1% (w/v) lecithin, 0.025% (w/v) catalase
* The catalase is added in aseptic conditions after autoclave sterilization and before use
* preparation mode: heat dissolution of ingredients and autoclave sterilization, 122°C/15 minutes

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

Other additions to count medium: none

Particular count media: none

IV - NEUTRALIZATION METHOD VALIDATION

Microorganism strain	Tested concentration of product	Test fungal suspension	Validation test			
			Fungal suspension	Experimental conditions	Non-toxicity of neutralizer	Inactivation by dilution-neutralization
Aspergillus niger ATCC 16404	80% (v/v)	10^{-5} : >165 ; >165 10^{-6} : 28 ; 28 ($N = 2.8 \times 10^7$) ($N_0 = 2.8 \times 10^6$)	54 ; 62 ($Nv = 580$) ($Nv_0 = 58$)	50 ; 62 ($A = 56$)	41 ; 49 ($B = 45$)	58 ; 50 ($C = 54$)

N = number of CFU/ml of the test fungal suspension

$No = \frac{N}{10}$

Nv = number of CFU/ml of the fungal suspension

$Nvo = \frac{Nv}{10}$

A = number of CFU/ml in experimental conditions validation test

B = number of CFU/ml in neutralizer non-toxicity validation test

C = number of CFU/ml in inactivation by dilution-neutralization validation test

The method is validated if:

- N is between 1.5×10^7 CFU/ml and 5×10^7 CFU/ml;
- N_0 is between 1.5×10^6 CFU/ml and 5×10^6 CFU/ml;
- N_{V0} is between 30 CFU/ml and 160 CFU/ml;
  (N_v is between 3.0×10^2 CFU/ml and 1.6×10^3 CFU/ml);
- the quotient of counts obtained by weighted mean is between 5.0 and 15.0.

The neutralization method is validated if A, B and C are equal to or greater than $0.5 \times N_{V0}$.

In the described conditions. the neutralization method is validated for the strain *Aspergillus niger* ATCC 16404 when a concentration of 80% (v/v) of the product **NOCOLYSE ONE SHOT** is used.

V - TEST RESULTS

Microorganism strain	Test fungal suspension	Results at concentration m% (v/v)			
			m = 80	m = 40	m = 1
Aspergillus niger ATCC 16404	10^{-5} : >165 ; >165 10^{-6} : 28 ; 28 (N = 2.8 x 10^7) (N_0 = 2.8 x 10^6)	Vc Na R Log R	0 ; 0 <140 >2.0 x 10^4 **>4.3**	0 ; 0 <140 >2.0 x 10^4 **>4.3**	>165 ; >165 >1 650 >1.7 x 10^3 <3.2

N = number of CFU/ml of the test fungal suspension
No = $\frac{N}{10}$
Vc = number of colonies counted in the dishes
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

Concentrations resulting in a decimal logarithmic reduction of at least 4 are fungicidal.

VI - CONCLUSION

The partial test demonstrates that the product **NOCOLYSE ONE SHOT** is fungicidal on the strain *Aspergillus niger* ATCC 16404 at the concentration of 40% (v/v) in accordance with the European standard NF EN 1650 (October 2008), with a contact of 30 minutes (additional contact time) at 20°C in the presence of a final concentration of 0.3 g/l of bovine albumin during the test (clean conditions)

END OF TEST REPORT