



# BIOTECH - GERMANDE

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**H**YGIENE - **F**ORMATION - **E**VALUATION  
**R**ECHERCHE & **D**EVELOPPEMENT

**DETERMINATION OF THE  
BACTERICIDAL ACTIVITY  
OF THE DISINFECTANT "ENDODIS/ENDOACT"  
(OLYMPUS OPTICAL CO. EUROPE)  
ACCORDING TO THE FRENCH STANDARD  
NF T 72-170<sup>(1)</sup>**

Rapport written by : Dr Lionel PINEAU

Marseille: 12/05/2001

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## I : DESCRIPTION OF THE STUDY :

**Title :** Determination of the bactericidal activity of the disinfectant "EndoDis/EndoAct" (Olympus Optical CO. Europe) in the presence of specific interfering substances according to the standard NF T 72-170.

**Internal reference:** Study N° 033.OLY.00

**Sponsor :** OLYMPUS OPTICAL CO. (EUROPA) GMBH  
Wendensrasse 14-16  
P.O. box 104908  
D-20034 HAMBURG  
*Contact : M Reinhard BLUM*

**Test period:** 19/04/2001 to 26/04/2001.

**Test manager :** Dr Lionel Pineau .

**Operations manager :** Audrey Ribiollet

**Test laboratory :** Laboratoire BIOTECH-GERMANDE  
Parc Scientifique de Luminy  
163 Avenue de Luminy – Case 927  
13288 Marseille Cedex 9

## II : PURPOSE OF THE STUDY :

Determinate according to the experimental conditions described in the French standard NF T 72-170 the ability of disinfectant "EndoDis/EndoAct" (Olympus Optical CO. Europe) to reduce, in the presence of specific interfering substances within 5 minutes at 20°C, by at least 10<sup>5</sup> times the number of viable bacteria belonging to 5 determined bacterial strains.

## III : MATERIAL :

### a) Test product :

Name of the test product:....."EndoDis/EndoAct disinfectant"  
Nature of the test product:.....peracetic acid based disinfectant  
(EndoDis) + activator (EndoAct).  
Batch n°:.....EndoDis "disinfectant": 4360B105  
EndoAct "activator": S07238/121-028  
Manufacturer: .....Henkel-Ecolab  
Date of acceptance at the laboratory:.....02/04/2001  
Internal reference:.....EndoDis "disinfectant": 0033.OLY.00.150  
EndoAct "activator": 0033.OLY.00.151  
Active substance and concentration:.....Peracetic acid 4.5%  
PH of the ready to use solution 6.5 to 8.5.  
Storage conditions:.....Room temperature

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## IV : METHOD :

a) Bacterial strains:

*Pseudomonas aeruginosa* CIP A22  
*Escherichia coli* CIP 54127  
*Staphylococcus aureus* strain Oxford CIP 53 154  
*Enterococcus faecium* CIP 5855  
*Mycobacterium smegmatis* CIP 7326

The conditions of preservation and control of the bacterial strains used for the determination of the bactericidal activity are those described in the French standard NF T 72-140<sup>(2)</sup>.

b) Test method : .....Dilution - neutralisation

c) Neutralising solution :

Tween 80 (SIGMA P17-54, batch n°: 99H0100):..... 50 ml  
Sodium thiosulphate (SIGMA S85-03, batch n°:119H0216):..... 5g  
Saponin (SIGMA S79-00, batch n°: 39H1326):..... 10g  
Lecithin (SIGMA P53-94, batch n°: 128H8002):..... 10g  
Trypticase soya broth (Biomérieux 51019, batch n°: 746472501):..... q.s.p.500 ml

Stein sterilised at 121°C for 21 minutes.

d) Maintaining and counting medium :

Trypticase soya agar (Biomérieux 51044, Batch n°: 750379801)

## V : TEST CONDITIONS :

Tested concentration:.....2% (v/v) [1% (v/v) EndoDis +  
1% (v/v) EndoAct] that is to say  
450 ppm of peracetic acid

Test temperature : .....20°C ± 1°C

Contact time : .....5 minutes

Diluent of the product :

-Recommended by the manufacturer.....none

-used during tests.....sterile distilled water

Incubation temperature : .....37°C± 1°C

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a) Interfering substances :

i) Mixture of albumin and hard water for clean and dirty conditions :

Hard water concentrated 2.5 times

-Solution A

MgCl<sub>2</sub> anhydrous (SIGMA M-82-66, batch n°: 128H0128): .....31.74 g  
CaCl<sub>2</sub> anhydrous (Prolabo22313.294, batch n°: A07G): .....73.99 g  
Distilled water: .....q.s.p. 1 litre  
Sterilize by filtration.

-Solution B

NaHCO<sub>3</sub> (Prolabo 27777.290, batch n°: B02G): .....56.03 g  
Distilled water: .....q.s.p. 1 litre  
Sterilize by filtration.

Put at least 50 ml of sterile distilled water into a 100 ml sterile flask, add 0.75 ml of solution A and 1 ml of solution B. Make up 100 ml with sterile distilled water.

ii) Mixture of albumin and hard water for clean conditions.

Bovine albumin (SIGMA A2153, batch n°: 118H0563): .....0.75 g  
Hard water: .....q.s.p. 100 ml  
Sterilize by filtration.

With this mixture the final concentration in the test will be 0.3% for the bovine albumin and 30 French degrees for the hardness.

iii) Mixture of albumin and hard water for dirty conditions.

Bovine Albumin (SIGMA P17-54, batch n°: 39H0092): .....2.5 g  
Hard water: .....q.s.p. 100 ml  
Sterilize by filtration.

With this mixture the final concentration in the test will be 1% for the bovine albumin and 30 French degrees for the hardness.

Each assay are performed at least in duplicate.

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a) Determination of the bactericidal activity in clean conditions :

i) Preliminary tests :

**Table 1:** Validity of the bacterial suspension and selection of neutralizing agent for a 2% (v/v) [1% (v/v) EndoDis + 1% (v/v) EndoAct] tested concentration of the disinfectant "EndoDis/EndoAct".

Strains	Number of viable bacteria (UFC/ml)			
	Inoculum control (N)	Neutralizing agent test (n')	Interfering substance reference (n <sub>1</sub> ')	Neutralizing agent reference (n <sub>2</sub> ')
<i>Pseudomonas aeruginosa</i> CIP A22	163	132	146	146
<i>Escherichia coli</i> CIP 54127	220	192	215	160
<i>Staphylococcus aureus</i> strain Oxford CIP 53 154	104	76	86	88
<i>Enterococcus faecium</i> CIP 5855	234	171	180	198
<i>Mycobacterium smegmatis</i> CIP 7326	114	81	99	91

For the 5 bacterial strains tested :

N is between 100 UFC/ml and 300 UFC/ml

 $n' \geq 0,5 \times n_2'$ n<sub>1</sub>' and n<sub>2</sub>' are equivalent to N

The neutralisation method is validated with the tested neutralizing agent for a 2% (v/v) [1% (v/v) EndoDis + 1% (v/v) EndoAct] dilution of the tested product and for the 5 bacterial strains used during the tests.

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ii) Main test :

**Table 2** : Number of viable bacteria in the reaction tube , after a 5 minutes contact time at 20°C±1°C, with a 2% (v/v) [1% (v/v) EndoDis + 1% (v/v) EndoAct] dilution of the disinfectant "EndoDis/EndoAct" (Olympus Optical CO. Europe) in the presence of specific interfering substances (mixture of albumin and hard water for clean conditions).

Strains	(N)	(n')	(n <sub>1</sub> )	(n <sub>2</sub> )	Number of viable bacteria (n) (UFC/ml) for a tested concentration of 2% (v/v)
<i>Pseudomonas aeruginosa</i> CIP A22	163	132	146	146	0
<i>Escherichia coli</i> CIP 54127	221	192	215	160	0
<i>Staphylococcus aureus</i> strain Oxford CIP 53 154	104	76	86	88	0
<i>Enterococcus faecium</i> CIP 5855	234	171	180	198	0
<i>Mycobacterium smegmatis</i> CIP 7326	114	81	99	91	1

N Inoculum control

n' Neutralizing test

n<sub>1</sub>' Interfering substance referencen<sub>2</sub>' Neutralizing agent reference.**BIOTECH-GERMANDE**SARL au capital de 122 000 Euros N° SIRET : 423 865 419 00018 R.C.S Marseille APE : 743B  
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## b) Determination of the bactericidal activity in dirty conditions:

## i) Preliminary tests :

**Table 4** : Validity of the bacterial suspension and selection of neutralizing agent for a 2% (v/v) [1% (v/v) EndoDis + 1% (v/v) EndoAct] tested concentration of the disinfectant "EndoDis/EndoAct".

Strains	Number of viable bacteria (UFC/ml)			
	Inoculum control (N)	Neutralizing agent test (n')	Interfering substance reference (n <sub>1</sub> ')	Neutralizing agent reference (n <sub>2</sub> ')
<i>Pseudomonas aeruginosa</i> CIP A22	112	92	91	98
<i>Escherichia coli</i> CIP 54127	217	142	185	175
<i>Staphylococcus aureus</i> strain Oxford CIP 53 154	211	148	183	157
<i>Enterococcus faecium</i> CIP 5855	191	144	165	168
<i>Mycobacterium smegmatis</i> CIP 7326	182	105	145	108

For the 5 bacterial strains tested :

N is between 100 UFC/ml and 300 UFC/ml

$n' \geq 0,5 \times n_2'$

$n_1'$  and  $n_2'$  are equivalent to N

The neutralisation method is validated with the tested neutralizing agent for a 2% (v/v) [1% (v/v) EndoDis + 1% (v/v) EndoAct] dilution of the tested product and for the 5 bacterial strains used during the tests.

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ii) Main tests :

**Table 5** : Number of viable bacteria in the reaction tube , after a 5 minutes contact time at 20°C±1°C, with a 2% (v/v) [1% (v/v) EndoDis + 1% (v/v) EndoAct] dilution of the disinfectant "EndoDis/EndoAct" (Olympus Optical CO. Europe) in the presence of specific interfering substances (mixture of albumin and hard water for clean conditions).

Strains	(N)	(n')	(n <sub>1</sub> ')	(n <sub>2</sub> ')	Number of viable bacteria (n) (UFC/ml) for a tested concentration of 2% (v/v)
<i>Pseudomonas aeruginosa</i> CIP A22	112	92	91	98	<b>0</b>
<i>Escherichia coli</i> CIP 54127	217	142	185	175	<b>8</b>
<i>Staphylococcus aureus</i> strain Oxford CIP 53 154	211	148	183	157	<b>2</b>
<i>Enterococcus faecium</i> CIP 5855	191	144	165	168	<b>11</b>
<i>Mycobacterium smegmatis</i> CIP 7326	182	105	145	108	<b>0</b>

N Inoculum control

n' Neutralizing test

n<sub>1</sub>' Interfering substance referencen<sub>2</sub>' Neutralizing agent reference.**BIOTECH-GERMANDE**

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**Table 6** : Reduction of the number of viable bacteria in the reaction tube , after a 5 minutes contact time at 20°C±1°C, with a 2% (v/v) [1% (v/v) EndoDis + 1% (v/v) EndoAct] dilution of the disinfectant "EndoDis/EndoAct" (Olympus Optical CO. Europe) in the presence of specific interfering substances (mixture of albumin and hard water for clean and dirty conditions).

Strains	Mean reduction of the number of viable bacteria for a 2% (v/v) tested concentration	
	Clean conditions	Dirty conditions
<i>Pseudomonas aeruginosa</i> CIP A22	≥ 1,5.10 <sup>6</sup>	≥ 9,8.10 <sup>5</sup>
<i>Escherichia coli</i> CIP 54127	≥ 1,6.10 <sup>6</sup>	2,2.10 <sup>5</sup>
<i>Staphylococcus aureus</i> strain Oxford CIP 53 154	≥ 8,8.10 <sup>5</sup>	7,8.10 <sup>5</sup>
<i>Enterococcus faecium</i> CIP 5855	≥ 2,0.10 <sup>6</sup>	1,5.10 <sup>5</sup>
<i>Mycobacterium smegmatis</i> CIP 7326	9,1.10 <sup>5</sup>	≥ 1,1.10 <sup>6</sup>

**VII : CONCLUSION :**

A 2% (v/v) [1% (v/v) EndoDis + 1% (v/v) EndoAct] solution of the disinfectant "EndoDis/EndoAct" (Olympus Optical CO. Europe) (Batch n° : EndoAct : S07238/121-028, EndoDis : 4360B105 ) presents within 5 minutes at 20°C±1°C, a bactericidal activity in clean conditions (albumin : 0.3% hard water : 30 French degrees) and in dirty conditions (albumin : 1% hard water : 30 French degrees) consistent with the requirements of the French standard NF T 72-170<sup>(1)</sup> (November 1988).

**VIII : REFERENCES :**

- 1 - NF T 72-170: November 1988, Water miscible antiseptics and disinfectants used in liquid form - Suspension test with the dilution-neutralisation method - Determination of the bactericidal activity in the presence of specific interfering substances.
- 2 - NF T 72-140: August 1988, Antiseptics and disinfectants – Keeping an testing bacterial strains used for the determination of bactericidal activity.

Marseille, 12 may 2001

Dr Lionel PINEAU  
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