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**DETERMINATION OF THE BACTERICIDAL ACTIVITY
OF THE DISINFECTANT " ENDODIS/ENDOACT "
(OLYMPUS OPTICAL CO. EUROPE)
AT 35°C AND IN THE PRESENCE
OF SPECIFIC INTERFERING SUBSTANCES
(CLEAN CONDITIONS)
AGAINST *ENTEROCOCCUS FAECIUM* CIP 5855
ACCORDING TO THE FRENCH STANDARD
NF T 72-300⁽¹⁾**

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Marseille: 18/08/2001

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R E C H E R C H E & D E V E L O P P E M E N T**I : DESCRIPTION OF THE STUDY :**

Title :	Determination of the bactericidal activity of the disinfectant "EndoDis/EndoAct" (Olympus Optical CO. Europe) at 35°C and in the presence of specific interfering substances (mixture of albumin and hard water for clean conditions) against <i>Enterococcus faecium</i> CIP 5855 according to the French standard NF T 72-300.
Internal reference:	Study N° 033.OLY.00
Sponsor :	OLYMPUS OPTICAL CO. (EUROPA) GMBH Wendensrasse 14-16 P.O. box 104908 D-20034 HAMBURG <i>Contact : M Reinhard BLUM</i>
Test period:	10/08/2001 to 15/08/2001.
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 :

The results of a previous study (0033.OLY.00.2) shown that at 35°C within 5 minutes, the bactericidal activity of the disinfectant "EndoDis/EndoAct" in the presence of specific interfering substances was not consistent with the requirements of the French standard NF T 73-300 (reduction of the number of viable bacteria below 5 logarithmic units for *Staphylococcus aureus strain Oxford* CIP 53 154, *Enterococcus faecium* CIP 5855 and *Mycobacterium smegmatis* CIP 7326).

On the other hand, the same test performed at 20°C (0033.OLY.00.1) indicated that within 5 minutes the disinfectant " EndoDis/EndoAct " reduce by at least 10⁵ times the number of viable bacteria for the 5 tested strains.

According to the disinfectant manufacturer, the efficacy loss observed when the test temperature is increased was probably due to the way to prepare the disinfectant solution used for the test. Maintaining the activated solution at 35°C for some minutes before the test (as it is described in the standard) may cause a decrease of the active agent concentration.

So, the purpose of this study is to verify the ability of the disinfectant " EndoDis/EndoAct " (Olympus Optical CO. Europe) to reduce, within 5 minutes at 35°C and in the presence of specific interfering substances (mixture of albumin and hard water for clean conditions), by at least 10⁵ times the number of viable bacteria when the disinfectant solution is activated just before the test (less than 5 minutes before).

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

Test temperature :35°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

a) Interfering substances :**i) Hard water concentrated 2.5 times :****-Solution A**

MgCl₂ anhydrous (SIGMA M-82-66, batch n° : 128H0128):31.74 g
CaCl₂ anhydrous (Prolabo22313.294, batch n° : A07G):73.99 g
Distilled water :q.s.p. 1 litre
Sterilize by filtration.

-Solution B

NaHCO₃ (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.

Each assay are performed at least in duplicate.

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VI : RESULTS :

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) EndoAct + 1% (v/v) EndoDis] 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 ₁ ')	Neutralizing agent reference (n ₂ ')
<i>Enterococcus faecium</i> CIP 5855	279	234	213	247

For the bacterial strain 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) EndoAct + 1% (v/v) EndoDis] dilution of the tested product and for the bacterial strain used during the tests.

ii) Main test :

Table 2 : Number of viable bacteria in the reaction tube , after a 5 minutes contact time at 35°C±1°C, with a 2% (v/v) [1% (v/v) EndoAct + 1% (v/v) EndoDis] 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	Number of viable bacteria (n) (UFC/ml)				Number of viable bacteria (n) (UFC/ml) for a tested concentration of 2% (v/v) (1% A + 1% D)
	(N)	(n')	(n ₁ ')	(n ₂ ')	
<i>Enterococcus faecium</i> CIP 5855	279	234	213	247	0

N Inoculum control

n' Neutralizing test

n_1' Interfering substance reference

n_2' Neutralizing agent reference.

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Table 3 : Reduction of the number of viable bacteria in the reaction tube , after a 5 minutes contact time at 35°C±1°C, with a 2% (v/v) [1% (v/v) EndoAct + 1% (v/v) EndoDis] 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	Mean reduction of the number of viable bacteria for a 2% (v/v) tested concentration (1%A + 1%D)
<i>Enterococcus faecium</i> CIP 5855	>2.8 10 ⁶

VII : CONCLUSIONS :

When it is activated just before the test (less than 5 minutes before), a 2% (v/v) [1% (v/v) EndoAct + 1% (v/v) EndoDis] solution of the disinfectant " *EndoDis/EndoAct* " (Olympus Optical CO. Europe) (Batch n° : EndoAct : S07238/121-028, EndoDis : 4360B105) presents within 5 minutes at 35°C±1°C, a **bactericidal activity** in clean conditions (albumin : 0.3% hard water : 30 French degrees) against *Enterococcus faecium* CIP 5855 **consistent with the requirements of the French standard NF T 72-300⁽¹⁾** (November 1989).

VIII : REFERENCES :

- 1 - NF T 72 300 : November 1989, Water miscible antiseptics and disinfectants used in liquid form - Suspension test with the dilution-neutralisation method - Determination of the product efficiency on various micro-organisms in practical use conditions.
- 2 - 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.
- 3 - NF T 72-140 : August 1988, Antiseptics and disinfectants – Keeping an testing bacterial strains used for the determination of bactericidal activity.

Marseille, 19 August 2001

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