

LAB TESTS

INSTITUT PASTEUR IN FRANCE

STUDY REPORT N° 96.01.157 B

Version II -A

TITLE:

<<STUDY OF THE BACTERICIDAL ACTIVITY OF A PURIFIER
OF THE WATER CONTAINED IN CISTERNS AND RESERVOIRS:
SILVERDYN A.G. 3.6.>>

Document of 7 pages

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INFORMATIONS ON THE STUDY

1.1 TITLE OF THE STUDY

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OF THE WATER CONTAINED IN CISTERNS AND RESERVOIRS:
SILVERDYN A.G. 3.6.>>

1.2 STUDY NUMBER

96.01.157 B (Version II -A-)

1.3 IDENTITY OF CUSTOMER

Laboratorios Silverdyn S.A. de C.V. , hereafter called <<the
Customer>>

Maimonides 530

Colonia Polanco

C.P. 11570 MEXICO D.F.

(Mexico)

Person in charge : Mr CHARLES VEACH

1.4 IDENTITY OF PERSONS IN CHARGE OF THE STUDY

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Director of the study:

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Pharmacien Biologiste

Responsible for the Laboratory of Bacteriology - CBMS

Technicians:

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Sylvie BREMONT

Quality insurance:

Pascal PERNOT

Quality Engineer

1.5 REALISATION SCHEDULE

Reception's date of samples and supplies 01/30/97 and 02/04/97

Beginning of the study 03/05/97

End of the study 03/21/97

1.6 STUDY FILE

A study file is opened under the following study number: 96.01.157

B (Version II -A-).

It encompasses all the study relevant documents : correspondence, study procedure, price estimation, uncorrected data, amendments, study report...

The records will be kept in our offices for 15 years.

1.7 MAILING

Two copies are sent to the Customer for attribution..

The Customer will send the copy marked << 1INSTITUT PASTEUR
- CBMS >> back with his signature.

2. INTRODUCTION

We were asked by Laboratorios SILVERDYN S.A. to study the bactericidal activity of the SILVERDYN A.G. 3.6. on contaminated water stored in containers whose walls were previously treated with the product.

3. MATERIALS AND METHODS

3.1. PRODUCT

SILVERDYN A.G. 3.6., batch n° SSB1, used at the concentration of 40 ml/m²

3.2. MATERIALS

3.2.1 Eight 100 ml earthenware, non glazed, jars were treated as follows:

- 4 with 0.4 ml of product (40ml/m²) put with a cotton swab, drying for 1 hour at room temperature, then rinsing 10 times with sterile distilled water,
- 4 without treatment.

3.2.2 The following test strains were used:

- Escherichia coli CIP 54. 127
- Pseudomonas aeruginosa CIP A 22
- Salmonella Typhi murium CIP 6062
- Vibrio cholerae CIP 6342

3.2.3 Diluant and culture mediums:

Numbering gelose

- Desiccated yeast abstract 2.5 g
- Caseine Peptone tryptic 5.0 g

- Glucose 1.0 g
- Agar-agar in powder 15.0 g
- Water for 1 ,000ml

Diluant

- Sterile distilled water for E. coil, P. aeruginosa, S. typhi murium
- 0.9% NaCl for V. cholerae.

3.3 METHODS

3.3.1 Preparation of bacterial suspensions:

For each bacterial strain, a suspension of 10^8 bacteria per ml was prepared in the diluant.

3.3.2 Numeration of bacterial suspensions:

For each strain and from the 10^8 bacteria per ml suspension, dilutions at 10^4 and 10^6 are made in the diluant. Then twice 1 ml of each of the dilutions were taken and placed in sterile Petri dishes. 20 ml numbering gelose in surfusion at 45°C were added. After homogeneisation and solidification of the gelose, the dishes were incubated for 48 hours at 37°C.

In each dish, the colonies were counted, and to obtain the number of bacteria in the suspension, the average was calculated.

3.3.3 Study of the bactericidal activity of the product:

For each strain, the suspension was placed in both a treated jar and in a non treated jar.

After each contact time : 30, 60 and 180 mn at room temperature, the content was homogeneised and a sample was taken.

For each of the samples, a double numeration of the surviving germs was made as in 3.3.2.

The bactericidal activity of the SILVERDYN A.G.3.6.was appreciated for each bacterial strain by comparing the number of surviving bacteria in the treated jar to the number of surviving

bacteria in the non treated control jar.

4. RESULTS

4.1 NUMERATION OF BACTERIAL SUSPENSIONS

Strains	Numeration
<i>Escherichia coli</i>	$0,27 \cdot 10^8$ /ml
<i>Pseudomonas aeruginosa</i>	$0,52 \cdot 10^8$ /ml
<i>Salmonella Typhi murium</i>	$0,51 \cdot 10^8$ /ml
<i>Vibrio cholerae</i>	$0,28 \cdot 10^7$ /ml

4.2 BACTERICIDAL ACTIVITY OF THE PRODUCT

Strains	Control jars			Treated jars		
	Contact times			Contact times		
	30 minutes	60 minutes	180 minutes	30 minutes	60 minutes	180 minutes
<i>Escherichia coli</i>	$0,99 \cdot 10^7$ /ml	$0,68 \cdot 10^7$ /ml	$0,26 \cdot 10^7$ /ml	< 1/ml	< 1/ml	< 1/ml
<i>Pseudomonas aeruginosa</i>	$0,38 \cdot 10^7$ /ml	$0,15 \cdot 10^7$ /ml	$0,7 \cdot 10^7$ /ml	< 1/ml	< 1/ml	< 1/ml
<i>Salmonella Typhi murium</i>	$0,94 \cdot 10^7$ /ml	$0,27 \cdot 10^7$ /ml	$0,10 \cdot 10^7$ /ml	< 1/ml	< 1/ml	< 1/ml
<i>Vibrio cholerae</i>	$0,37 \cdot 10^7$ /ml	< 1/ml	< 1/ml	< 1/ml	< 1/ml	< 1/ml

5. CONCLUSION:

In the study's conditions, SILVERDYN A.G. 3.6., at the concentration of 40 µm² shows a bactericidal activity, on the four tested strains, after 30 minutes of contact time, with a reduction of 6 logarithms of the tested bacterial suspensions (99.9999%). It was impossible to observe the reduction of the *V. cholerae* suspension at 60 and 180 minutes because of the non survival of the bacterias in NaCl.

6. SIGNATURES

Director of the study:
Mrs Ana Maria BURGUIERE
Pharmacien Biologiste
Responsible for the Laboratory of Bacteriology - CBMS.



Assurance Quality Responsible:
Mr Pascal PERNOT
Quality Engineer



Director of C.B.M.S.:
Professeur Agrégé Guy GARRIGUE



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Accepted on