

REPORT N° 2023-MER-028

NF EN 14349:2012

Quantitative surface test for the evaluation of
bactericidal activity of chemical disinfectants and
antiseptics used in the veterinary area on non-porous
surfaces without mechanical action

LOW LEVEL SOILING CONDITIONS 30MIN 10°C

PROPHYL S

Reference strains:

Salmonella enterica

DSM 5569

Operator :	Validation :
Romain GACHE Microbiologist	Cécile COFFY Chemical Engineer- Technical Manager
	

06/10/2023

HUVEPHARMA SA,
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SUMMARY

1. LABORATORY IDENTIFICATION.....	2
2. SAMPLE IDENTIFICATION	2
3. TRIAL METHOD AND VALIDATION.....	3
4. EXPERIMENTAL CONDITIONS	3
5. RESULTS.....	4
6. INTERPRETATION	5
7. CONCLUSION	5
ANNEX 1: EQUIPMENT AND REAGENTS.....	6
ANNEX 2: CERTIFICATE OF ANALYSIS.....	7

1. Laboratory Identification

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2. Sample Identification

2.1-Product name	PROPHYL S
2.2-Batch number	PBO-016243
2.3-Manufacturer :	Huvepharma SA
2.4-Receipt date	11/05/2023
2.5-Storage conditions	Ambient Temperature
2.6-Product Dilutant	Tap water
2.7-Active Substances	4-CHLORO-3-METHYLPHENOL CAS: 59-50-7 17% (m/m) GLYCOLIC ACID CAS : 79-14-1 4.9% (m/m)

3. Trial method and validation

3.1-Method	Dilution neutralization
3.2-Neutralizing solution	Lecithin 3g/L Sodium Thiosulfate 5g/L L-Histidin 1g/L Tween 80 30g/L Phosphate disodium 12H ₂ O 85g/l

4. Experimental conditions

4.1- Test period :	From 04/10/2023 until 06/10/2023
4.2-Diluent used for the product test solutions	Hard water according to the norm
4.3-Tested Concentrations of the product (% v/v)	0.2% - 0.4% - 0.6%
4.4- Appearance of the product and of its dilutions:	Slightly blue liquid
4.5- Contact Time:	30min+/- 10 s
4.6- Temperature of the test:	10°C+/-1°C
4.7-Interferring Substance :	3 g/l bovine albumin
4.8-Stability of the mixture	No sedimentation
4.9-Incubation temperature	37°C +/- 1 °C
4.10-Bacteria Strains Identification :	<i>Salmonella enterica</i> DSM 5569

5. Results

- Salmonella enterica :**

Strain (Collection number)	<i>Salmonella enterica</i> DSM 5569	
Dilution 10^{-7}	241	256
Dilution 10^{-8}	27	33
Bacteria Suspension $N = \text{Log} \frac{c * 0,025}{(n1+0,1 n2) * d}$	N = 7,80	
Validity	$7.57 \leq N \leq 8.10$	

- Validation and control:**

Water control (Nw)			Neutralization control (B)			Method Validation (C) Product Concentrations : 0,60 %		
dilutions	Vc1	Vc2	dilutions	Vc1	Vc2	dilutions	Vc1	Vc2
10^{-3}	>330	>330	10^{-3}	>330	>330	10^{-3}	>330	>330
10^{-4}	138	142	10^{-4}	127	158	10^{-4}	126	115
10^{-5}	<14	<14	10^{-5}	<14	17	10^{-5}	<14	15
Nw =	7,15		B =	7,16		C =	7,09	
Validity	Nw $\geq 6,27$		Validity	B $> 0,5$ Nw		Validity	C $> 0,5$ Nw	

- assay :**

Tested concentrations	0,20 %		0,40 %		0,60 %	
Dilution	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2
10^0	>330	>330	15	18	<14	<14
10^{-1}	>330	>330	<14	<14	<14	<14
10^{-2}	>330	>330	<14	<14	<14	<14
Nts	>100		3		0	
Na	>3,82		2,22		<2,15	
R=Nw-Na	<3,33		4,93		>5,00	

6. Interpretation

- Vc : Number of colonies counted on Petri dish
 N : Number of CFU/ml in bacteria suspension of trial
 N0 : Number of CFU/ml in trial mixture
 Nv0 : Number of CFU/ml in A / B / C mixtures
 Na : Number of survivors per milliliter in trial mixture
 Lg R : Logarithmic reduction of viable cells : $Lg N_0 - Lg N_a$
 A : Number of CFU/ml in the validation test of experimental conditions
 B : Number of CFU/ml in the validation test of non-toxicity of neutralizing solution/filtration
 C : Number of CFU/ml in the validation test of the inactivation by dilution-neutralization/filtration

Validation:

N is between 1.5 and 5.0×10^8	$A \geq 0.5 N_{v0}$
Nv0 is between 30 and 160	$B \geq 0.5 N_{v0}$
Lg N0 is between 7.17 and 7.70	$C \geq 0.5 N_{v0}$

Bactericidal concentrations = $Lg R \geq 4$

7. Conclusion

According to the standard **NF EN 14349:2012**,

Product **PROPHYL S**, batch **PBO-016243** when used at a concentration:

-0.4 % in hard water shows a bactericidal activity on non-porous surfaces in **30min** at **10°C** in **low level soiling conditions**.

against following strain:

Salmonella enterica

Saint Etienne on **06/10/2023**

Romain Gache

Microbiologist Technician



ANNEX 1: Equipment and reagents

1. EQUIPMENT and GLASSWARE

- Autoclave GETINGE AVOR 60
- Ventilated Incubator Fisherbrand (30 °C +/- 1 °C)
- Freezer Liebbherr GS 1423-25
- Refrigerator FKS 1800
- Laminar vertical flow hood Jouan, biohazard classe 2
- Analytic weighing scale DENVER P2002
- Stirrer Bioblock
- pH meter CRISON GL P21
- Spectrophotometer PRIM LIGHT
- Colonies counter STUART SC6
- quartz Chronometer, Fisher
- Sterile Pipettes 1ml, STERILIN
- Sterile Pipettes 2 ml STERILIN
- Sterile Pipettes 5 ml STERILIN
- Sterile Pipettes 10 ml STERILIN
- Petri dishes (diameter 90) GOSSELIN
- Sterilizable glass Flasks, FISHERBRAND
- Glass test tubes borosilicated and sterilizable
- Glass marbles (diameter 4), VWR, ref 00680043

2. REAGENTS

- Distilled water
- NaCl, VWR International ref 27810295
- Anhydrous CaCl₂, VWR International ref 22319294
- Agar-Agar, VWR International ref 20768235
- Soya Peptone, VWR international, ref 1072120500
- Anhydrous MgCl₂, SIGMA, ref 63063
- Tryptone Casein, Sigma, ref 95039
- Bovine Albumin, Sigma, ref A 2153
- Malt extract, Sigma, ref 70167
- Lecithin, Sigma, ref 61755
- Tween 80, Sigma, ref 63161
- NaHCO₃, VWR ref 27778293

3. DILUENT and GROWING MEDIUM

- Trypton-salt Solution prepared in accordance with Standard
- Agar TSA prepared in accordance with Standard

4. INTERFERRING SUBSTANCE

- 3 g/L bovine albumin

ANNEX 2 : Certificate of Analysis

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**- CERTIFICAT D'ANALYSE / CERTIFICATE OF ANALYSIS**

Produit / Product : **PROPHYL S**

Fabrication / Manufacturing : **05/2023**

Lot / Batch n° : **PBO-016243**

Péremption / Validity : **05/2026**

Criteria / Active Substance	Spécification limits		Résultats/ Result
	Minimum	Maximum	
pH	2.0	3.0	2.4
Density	1.06	1.08	1.07
Color	Blue		Blue
Chloro-4-Methyl-3-Phenol	15.98%	18.02%	16.91%
Glycolic Acid	4.41%	5.39%	4.90%

« Les analyses réalisées sur ce produit sont conformes aux spécifications établies. Le produit est sûr et fabriqué selon les normes de sécurité »

« Analysis made on products is in conformity with the specifications. The products is safe and produced according to safety standard »

Saint Etienne, 11/05/2023

Romain GACHE
Contrôle Qualité / Quality Control

