IRM
INSTITUT DE RECHERCHE MICROBIOLOGIQUE

TEST REPORT

N°RE-1012/0118-1

ISSUED TO: **HYPRED SAS**
55, Boulevard Jules Verge
35800 Dinard

PRODUCT: **AGAKOK 2.5**

TEST REQUEST DATE: 17 January 2018

PRODUCT REFERENCE: P103/0118

TEST: **In accordance with the procedures of the European Standard NF EN 1656 (March 2010): chemical disinfectants and antiseptics – quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary areas.**

Test method and requirements (phase 2, step 1).

General use product

Dilution-neutralization method

This report contains 5 pages

This report applies only to the sample submitted to test

Issue date: 19 February 2018

Amandine CARRÉ
Microbiologist
Trial Manager

Philippe STROHL
Doctor of veterinary medicine
Scientific Director

I. SAMPLE IDENTIFICATION

Product name: **AGAKOK 2.5**

- Lot number: 17110901
- Manufacturer: **ANTI-GERM International GmbH**
  Memmingen site : Oberbruhlstrabe 16-18
  D- 87 700 Memmingen
- Expiration date: November 2020, as indicated in the sample file dated 15 January 2018 transmitted by the client
- Recommended product diluent: tap water
- Aspect of product and its dilutions: clear red fluid
- Active substances and concentrations: not indicated

Reception date: 17 January 2018

Storage conditions: at room temperature with possible exposure to light

II. EXPERIMENTAL CONDITIONS

Analysis period: from 23 January to 02 February 2018

Identification of obligatory conditions bacterial strains:

Enterococcus hirae DSM 3320	Incubation 48 hours at 37 ± 1°C
Proteus vulgaris DSM 30118	Incubation 48 hours at 37 ± 1°C
Pseudomonas aeruginosa DSM 939	Incubation 48 hours at 37 ± 1°C
Staphylococcus aureus DSM 799	Incubation 48 hours at 37 ± 1°C

Number of test repetitions per microorganism: 1

Obligatory test temperature: 10°C ± 1°C

Obligatory product-inoculum contact time: 30 min ± 10 sec

Interfering substance: low level soiling conditions
Composition: final concentration of 3.0 g/L of bovine albumin during the test

Product diluent used during the test: hard water at 375 mg/kg of $CaCO_3$.

Stability of product-diluent mix: formation of a precipitate at concentrations of 2% and 1% (v/v)
Opinion on test results: Chapter 5.4.2 of the standard states that counting microorganisms in a precipitate or flocculate is difficult and unreliable.

Count method: plating in agar medium

III. PROCEDURE FOR PRELIMINARY TESTS

Neutralizer:

Composition: potassium dihydrogen phosphate sterile solution comprising 10% (w/v) tween 80 - 6% (w/v) saponin - 2% (w/v) lecithin - 0.5% (w/v) sodium thiosulphate - 0.2% (w/v) L-Cysteine and 0.2% (w/v) L-Histidine.

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. VALIDATION TEST RESULTS

Test strain	Tested concentration m% (v/v)	Test bacterial suspension	Validation tests			
			Bacterial suspension	Experimental conditions	Non-toxicity of neutralizer	Inactivation by dilution-neutralization
Enterococcus hirae DSM 3320	2	10^{-6}: 237 241 10^{-7}: 19 24 N = 2.4×10^{8} N_0 = 2.4×10^{7}	32 52 Nv = 420 Nv_0 = 42	34 35 A = 34.5	40 50 B = 45	39 55 C = 47
Proteus vulgaris DSM 30118	2	10^{-6}: 244 245 10^{-7}: 17 21 N = 2.4×10^{8} N_0 = 2.4×10^{7}	52 54 Nv = 530 Nv_0 = 53	46 53 A = 49.5	57 64 B = 60.5	44 54 C = 49
Pseudomonas aeruginosa DSM 939	2	10^{-6}: >330 >330 10^{-7}: 33 40 N = 3.7×10^{8} N_0 = 3.7×10^{7}	102 115 Nv = 1085 Nv_0 = 108.5	87 99 A = 93	104 124 B = 114	106 87 C = 96.5
Staphylococcus aureus DSM 799	2	10^{-6}: 256 265 10^{-7}: 23 30 N = 2.6×10^{8} N_0 = 2.6×10^{7}	68 70 Nv = 690 Nv_0 = 69	47 67 A = 57	64 64 B = 64	62 74 C = 68

N : number of CFU/ml in the bacterial suspension
N_0: N/10
Nv: number of CFU/ml in the bacterial suspension
Nv_0: Nv/10
A: number of CFU/ml in the experimental conditions validation test
B: number of CFU/ml in the neutralizer non-toxicity validation test
C: number of CFU/ml in the inactivation by dilution-neutralization validation test
CFU: Colony Forming Unit

The method is validated if:

- N is between 1.5×10^{8} CFU/ml and 5×10^{8} CFU/ml
- N_0 is between 1.5×10^{7} CFU/ml and 5×10^{7} CFU/ml
- $N_{.0}$ is between 30 CFU/ml and 160 CFU/ml, i.e., Nv 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
- A, B and C are equal to or greater than $0.5 \times NV_0$

In the described conditions, the neutralization method is validated on the tested strains for a concentration of product **AGAKOK 2.5** of 2% (v/v).

V. TEST RESULTS

Test strain	Test bacterial suspension		Results at concentrations m% (v/v)		
			m = 2	m = 1	m = 0.5
Enterococcus hirae DSM 3320	10^{-6} : 237 241 10^{-7} : 19 24 N = 2.4×10^8 N_0 = 2.4×10^7	Vc = Na = R = Log R =	0 0 < 140 $> 1.7 \times 10^5$ **> 5.23**	0 0 < 140 $> 1.7 \times 10^5$ **> 5.23**	0 0 < 140 $> 1.7 \times 10^5$ **> 5.23**
			m = 2	m = 1	m = 0.5
Proteus vulgaris DSM 30118	10^{-6} : 244 245 10^{-7} : 17 21 N = 2.4×10^8 N_0 = 2.4×10^7	Vc = Na = R = Log R =	0 0 < 140 $> 1.7 \times 10^5$ **> 5.23**	0 0 < 140 $> 1.7 \times 10^5$ **> 5.23**	0 0 < 140 $> 1.7 \times 10^5$ **> 5.23**
			m = 2	m = 1	m = 0.5
Pseudomonas aeruginosa DSM 939	10^{-6} : >330 >330 10^{-7} : 33 40 N = 3.7×10^8 N_0 = 3.7×10^7	Vc = Na = R = Log R =	0 0 < 140 $> 2.6 \times 10^5$ **> 5.42**	0 0 < 140 $> 2.6 \times 10^5$ **> 5.42**	0 0 < 140 $> 2.6 \times 10^5$ **> 5.42**
			m = 2	m = 1	m = 0.5
Staphylococcus aureus DSM 799	10^{-6} : 256 265 10^{-7} : 23 30 N = 2.6×10^8 N_0 = 2.6×10^7	Vc Na R = Log R =	0 0 < 140 $> 1.9 \times 10^5$ **> 5.27**	0 0 < 140 $> 1.9 \times 10^5$ **> 5.27**	0 0 < 140 $> 1.9 \times 10^5$ **> 5.27**

N: number of CFU/ml in the test suspension
N_0: 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
CFU: Colony Forming Unit

Concentrations resulting in a decimal log reduction of at least 5 are bactericidal

VI. CONCLUSION

The product **AGAKOK 2.5** is bactericidal on the reference strains *Enterococcus hirae* DSM 3320, *Proteus vulgaris* DSM 30118, *Pseudomonas aeruginosa* DSM 939 and *Staphylococcus aureus* DSM 799 at concentrations of 2%, 1% and 0.5% (v/v), for an obligatory time of 30 minutes at an obligatory temperature of 10°C, in accordance with the procedures of the European Standard NF EN 1656 (March 2010), in the presence of 3.0 g/L of bovine albumin in final during the test (low level soiling conditions).

END OF TEST REPORT

Study number : 012B02-2018-01
Sponsor : HYPRED
TEST REPORT

DETERMINATION OF BACTERICIDAL ACTIVITY OF THE PRODUCT « AGAKOK 2.5 » ACCORDING TO THE STANDARD NF EN 14349

For: **HYPRED SAS**
55 boulevard Jules VERGER
35 800 DINARD

Date of the request: 01/18/2018

Study number: n°012B02-2018-01

BACTERICIDAL TESTS:

According to the methodology of the standard NF EN 14349 (december 2012) – chemical disinfectants and antiseptics – Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on nonporous surfaces without mechanical action.

Assay on the 4 references strains: *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus hirae, Proteus hauseri.*

This test report included 10 pages.

Study completion date : 04/04/2018

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professor Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
4, rue des Grandes Pièces
25770 SERRE LES SAPINS
FRANCE

2 IDENTIFICATION OF THE SAMPLE

Sample Name	AGAKOK 2.5
References	Sample EXT-25-49-1
Batch number	17110901
Expiration date	11/01/2020
Sponsor	HYPRED
Manufacturing date	Unknown
Storage conditions	Room temperature and obscurity
Active substances	Chlorocresol
Appearance of the product	Liquid, red
Diluent preconized by the sponsor	Tap water
Date of delivery of the product	01/19/2018
Date of study	From 03/27/2018 to 04/04/2018

3 EXPERIMENTAL CONDITIONS

Concentration tested in this test	2%-1%-0,5%
Appearance of the product and his dilution	Clear
Method used	Dilution-neutralization
Contact time	30 min
Test temperature	10°C
Organic soil load	low dirty conditions : albumin bovine 30g/L (final concentration)
Diluent used in this test	Hard water
Diluent of the test suspension	Tryptone-salt solution
Strain used	➢ *Staphylococcus aureus* subsp. *aureus* CIP 4.83 batch 15713-1d (ATCC 6538)-Institut Pasteur, ➢ *Proteus hauseri, 30118, batch may 06 - DSMZ* ➢ *Pseudomonas aeruginosa* DSM 939 batch 0413 (ATCC 15442) - DSMZ ➢ *Enterococcus hirae* DSM 3320 batch 0511 (ATCC 10541) - DSMZ.
Culture conditions	On TSA Agar for 24h00-48h00 at 37°C
Product stability with organic soil load	stable

4 VALIDATION OF THE METHOD

See results sheets.

<u>The product demonstrated a bactericidal activity if the reduction is ≥ 4 log for viable bacterial cells.</u>
<u>Results in low dirty conditions (averages), for an exposure time of 30 min :</u>

- For *P. aeruginosa*, R = 4,33 from 1% concentration
- For *E. hirae*, R = 4,19 from 1% concentration
- For *P. hauseri*, R = 4,52 from 1% concentration
- For *S. aureus*, R = 4,40 from 1% concentration

5 CONCLUSIONS

According to the NF EN 14349 standard (December 2012), the product « AGAKOK 2.5 », Batch n° 17110901:

- **Has a bactericidal activity from the 1% concentration on the 4 bacterial strains *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus hauseri* and *Enterococcus hirae*, in low dirty conditions at 10°C, for an exposure time of 30 min.**

6 RESULTS SHEETS

ASSAY

Staphylococcus aureus

Test suspension			Validation A			Validation B			Water control Nc			Assay		1,0%	Assay		0,5%	Assay		2,0%
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2
$1,0.10^{-7}$	244	248	$1,0.10^{-4}$	222	218	$1,0.10^{-4}$	199	203	$1,0.10^{-4}$	130	125	$1,0.10^{0}$	50	51	$1,0.10^{0}$	144	133	$1,0.10^{0}$	40	33
$1,0.10^{-8}$	28	25	$1,0.10^{-5}$	21	21	$1,0.10^{-5}$	20	20	$1,0.10^{-5}$	15	14	$1,0.10^{-1}$	5	5	$1,0.10^{-1}$	12	10	$1,0.10^{1}$	3	3
N	7,79		A	7,34		B	7,30		Nc	7,11		Nd	2,70		Nd	3,14		Nd	2,56	
			N-A	0,45		A-B	0,04		N-Nc	0,68		R	4,41		R	3,97		R	4,55	

Enterococcus hirae

Test suspension			Validation A			Validation B			Water control Nc			Assay		1,0%	Assay		0,5%	Assay		2,0%
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2
$1,0.10^{-7}$	208	219	$1,0.10^{-4}$	200	189	$1,0.10^{-4}$	211	215	$1,0.10^{-4}$	100	109	$1,0.10^{0}$	70	68	$1,0.10^{0}$	110	115	$1,0.10^{0}$	40	41
$1,0.10^{-8}$	20	22	$1,0.10^{-5}$	20	19	$1,0.10^{-5}$	21	21	$1,0.10^{-5}$	10	11	$1,0.10^{-1}$	7	7	$1,0.10^{-1}$	11	12	$1,0.10^{-1}$	4	4
N	7,73		A	7,29		B	7,33		Nc	7,02		Nd	2,84		Nd	3,05		Nd	2,61	
			N-A	0,44		A-B	-0,04		N-Nc	0,71		R	4,18		R	3,97		R	4,41	

Proteus hauserii

Test suspension			Validation A			Validation B			Water control Nc			Assay		1,0%	Assay		0,5%	Assay		2,0%
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2
$1,0.10^{-7}$	230	228	$1,0.10^{-4}$	177	170	$1,0.10^{-4}$	133	140	$1,0.10^{-4}$	114	120	$1,0.10^{0}$	44	45	$1,0.10^{0}$	133	128	$1,0.10^{0}$	18	15
$1,0.10^{-8}$	24	23	$1,0.10^{-5}$	18	17	$1,0.10^{-5}$	13	14	$1,0.10^{-5}$	12	16	$1,0.10^{-1}$	4	4	$1,0.10^{-1}$	13	13	$1,0.10^{1}$	2	2
N	7,76		A	7,24		B	7,14		Nc	7,08		Nd	2,65		Nd	3,12		Nd	2,22	
			N-A	0,52		A-B	0,10		N-Nc	0,68		R	4,43		R	3,96		R	4,86	

Pseudomonas aeruginosa

Test suspension			Validation A			Validation B			Water control Nc			Assay			1,0%	Assay			0,5%	Assay		2,0%	
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2			VC1	VC2			VC1	VC2
$1,0.10^{-7}$	240	237	$1,0.10^{-4}$	150	159	$1,0.10^{-4}$	140	148	$1,0.10^{-4}$	103	106	$1,0.10^{0}$	50	51		$1,0.10^{0}$	112	110		$1,0.10^{0}$	20	18
$1,0.10^{-8}$	25	24	$1,0.10^{-5}$	18	17	$1,0.10^{-5}$	15	15	$1,0.10^{-5}$	13	11	$1,0.10^{-1}$	5	5		$1,0.10^{-1}$	13	11		$1,0.10^{-1}$	2	3
N	7,78		A	7,19		B	7,16		Nc	7,02		Nd	2,70			Nd	3,05			Nd	2,28	
			N-A	0,58		A-B	0,03		N-Nc	0,76		R	4,32			R	3,97			R	4,74	

Technical director : Mlle CANTREL Emilie

Laboratory technician

REPETITION

Staphylococcus aureus

Test suspension			Validation A			Validation B			Water control Nc			Assay			1,0%	Assay			0,5%	Assay			2,0%
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2			VC1	VC2		VC1	VC2		VC1	VC2
$1,0.10^{-7}$	244	248	$1,0.10^{-4}$	222	218	$1,0.10^{-4}$	199	203	$1,0.10^{-4}$	120	117	$1,0.10^{0}$	49	50		$1,0.10^{0}$	140	141		$1,0.10^{0}$	37	35	
$1,0.10^{-8}$	28	25	$1,0.10^{-5}$	21	21	$1,0.10^{-5}$	20	20	$1,0.10^{-5}$	12	12	$1,0.10^{-1}$	4	4		$1,0.10^{-1}$	12	14		$1,0.10^{-1}$	4	3	
N	7,79		A	7,34		B	7,30		Nc	7,07		Nd	2,69			Nd	3,15			Nd	2,56		
			N-A	0,45		A-B	0,04		N-Nc	0,72		R	4,38			R	3,92			R	4,51		

Enterococcus hirae

Test suspension			Validation A			Validation B			Water control Nc			Assay			1,0%	Assay			0,5%	Assay			2,0%
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2			VC1	VC2		VC1	VC2		VC1	VC2
$1,0.10^{-7}$	208	219	$1,0.10^{-4}$	200	189	$1,0.10^{-4}$	211	215	$1,0.10^{-4}$	95	99	$1,0.10^{0}$	60	63		$1,0.10^{0}$	100	127		$1,0.10^{0}$	47	42	
$1,0.10^{-8}$	20	22	$1,0.10^{-5}$	20	19	$1,0.10^{-5}$	21	21	$1,0.10^{-5}$	10	10	$1,0.10^{-1}$	9	6		$1,0.10^{-1}$	15	13		$1,0.10^{-1}$	6	4	
N	7,73		A	7,29		B	7,33		Nc	6,99		Nd	2,79			Nd	3,06			Nd	2,65		
			N-A	0,44		A-B	-0,04		N-Nc	0,74		R	4,20			R	3,93			R	4,34		

Proteus hauserii

Test suspension			Validation A			Validation B			Water control Nc			Assay			1,0%	Assay			0,5%	Assay			2,0%
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2			VC1	VC2		VC1	VC2		VC1	VC2
$1,0.10^{-7}$	230	228	$1,0.10^{-4}$	177	170	$1,0.10^{-4}$	133	140	$1,0.10^{-4}$	108	115	$1,0.10^{0}$	28	29		$1,0.10^{0}$	128	130		$1,0.10^{0}$	17	15	
$1,0.10^{-8}$	24	23	$1,0.10^{-5}$	18	17	$1,0.10^{-5}$	13	14	$1,0.10^{-5}$	13	12	$1,0.10^{-1}$	3	3		$1,0.10^{-1}$	13	13		$1,0.10^{-1}$	2	2	
N	7,76		A	7,24		B	7,14		Nc	7,05		Nd	2,45			Nd	3,11			Nd	2,20		
			N-A	0,52		A-B	0,10		N-Nc	0,71		R	4,60			R	3,94			R	4,85		

Study number : 012B02-2018-01
Sponsor : HYPRED

Pseudomonas aeruginosa

Test suspension			Validation A			Validation B			Water control Nc			Assay		1,0%	Assay		0,5%	Assay		2,0%
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2
$1,0.10^{7}$	240	237	$1,0.10^{-4}$	150	159	$1,0.10^{-4}$	140	148	$1,0.10^{-4}$	100	99	$1,0.10^{0}$	48	43	$1,0.10^{0}$	100	109	$1,0.10^{0}$	20	20
$1,0.10^{8}$	25	24	$1,0.10^{-5}$	18	17	$1,0.10^{-5}$	15	15	$1,0.10^{-5}$	11	10	$1,0.10^{1}$	4	4	$1,0.10^{1}$	12	12	$1,0.10^{1}$	2	2
N	7,78		A	7,19		B	7,16		Nc	7,00		Nd	2,66		Nd	3,02		Nd	2,30	
			N-A	0,58		A-B	0,03		N-Nc	0,78		R	4,34		R	3,98		R	4,70	

Technical director : Mlle CANTREL Emilie

Laboratory technician

7 TECHNICAL APPENDIX

CULTURE MEDIA :

TSA (Trypto Caseine SOY), Dominique Dutscher, ref 777410, batch 709191

ORGANIC SOIL LOAD :

Serum Albumin Bovine in powder, Fraction V, Dominique dutscher, ref. 871001, Batch D1304039
Filtered sterilized

DILUENT

Tryptone-Sel (TS) solution
Ingredients in grammes per liter :

Tryptone, Dominique Dutscher, ref. 777472, batch 020437	1,00 g/L
Sodium chloride, GROSSERON, ref 9020401, batch FR08 085 793	8,50 g/L

NEUTRALIZER

Ingredients:

Polysorbate 80, Dominique dutscher, ref. 777519, batch 17048301	30 g/L
Histidine, Alfa Aesar, ref. A17627, batch 10114426	1 g/L
Sodium thiosulfate, Sigma Aldrich, ref. 72049, batch BCBD0584V	5 g/L
Na2HPO4, Sigma Aldrich, ref. S5136, batch BCBC7067V	20 g/L
Egg yolk	3 g/L

HARD WATER

Solution A: - $MgCl_2$ anhydre, ref. M8266, batch 108K0068, SIGMA ALDRICH
- $CaCl_2$ anhydre, ref. C1016, batch 059K0030, SIGMA ALDRICH

Solution B: - $NaHCO_3$, ref. S6014, lot n°059K0052, SIGMA ALDRICH

pH: 7,0 ± 0,2 at 25°C.

STAINLESS STEEL CARRIERS – carriers of 2 cm weight, stainless steel 1.4301, nuance 2B (MECAPOL).

IRM
INSTITUT DE RECHERCHE MICROBIOLOGIQUE

TEST REPORT

N°RE-1013/0118-2

ISSUED TO: **HYPRED SAS**
55, Boulevard Jules Verge
35800 Dinard

PRODUCT: **AGAKOK 2.5**

TEST REQUEST DATE: 17 January 2018

PRODUCT REFERENCE: P103/0118

TEST: **In accordance with the procedures of the European Standard NF EN 1657 (May 2016): chemical disinfectants and antiseptics – quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in veterinary areas.**

Test method and requirements (phase 2, step 1).

General use product

Dilution-neutralization method.

Test on *Candida albicans* DSM 1386

This report contains 4 pages

This report applies only to the sample submitted to test

Issue date: 27 March 2018

Amandine CARRÉ
Microbiologist
Trial Manager

Philippe STROHL
Doctor of veterinary medicine
Scientific Director

I. SAMPLE IDENTIFICATION

Product name: **AGAKOK 2.5**

- Lot number: 17110901
- Manufacturer: **ANTI-GERM International GmbH**
  Memmingen site : Oberbruhlstrabe 16-18
  D- 87 700 Memmingen
- Expiration date: November 2020, as indicated in the sample file dated 15 January 2018 transmitted by the client
- Recommended product diluent: tap water
- Aspect of product and its dilutions: clear red fluid
- Active substances and concentrations: not indicated

Reception date: 17 January 2018

Storage conditions: at room temperature with possible exposure to light

II. EXPERIMENTAL CONDITIONS

Analysis period: from 23 to 26 January 2018

Identification of obligatory fungal strain:
Candida albicans DSM 1386 Incubation 48 hours at 30 ± 1°C

Number of test repetitions per microorganism: 1

Obligatory test temperature: 10°C ± 1°C

Obligatory product-inoculum contact time: 30 min ± 10 sec

Interfering substance: low level soiling conditions
Composition: final concentration of 3.0 g/L of bovine albumin during the test

Product diluent used during the test: hard water at 375 mg/kg of $CaCO_3$.

Stability of product-diluent mix: formation of a precipitate at a concentration of 2% and 1% (v/v)
Opinion on test results: Chapter 5.4.2 of the standard states that counting microorganisms in a precipitate or flocculate is difficult and unreliable.

Count method: plating in agar medium

III. PROCEDURE FOR PRELIMINARY TESTS

Neutralizer:

Composition: potassium dihydrogen phosphate sterile solution comprising 10% (w/v) tween 80 - 6% (w/v) saponin - 2% (w/v) lecithin - 0.5% (w/v) sodium thiosulphate - 0.2% (w/v) L-Cysteine and 0.2% (w/v) L-Histidine.

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. VALIDATION TEST RESULTS

Test strain	Tested concentration m% (v/v)	Test suspension	Validation tests			
			Validation suspension	Experimental conditions	Non-toxicity of neutralizer	Inactivation by dilution-neutralization
Candida albicans DSM 1386	3.0	10^{-5} : > 330 > 330 10^{-6} : 38 34 N = 3.6×10^7 N_0 = 3.6×10^6	94 106 Nv = 1000 Nv_0 = 100	80 90 A = 85	97 107 B = 102	106 96 C = 101

N : number of CFU/ml in the test suspension
N_0: N/10
Nv: number of CFU/ml in the validation suspension
Nv_0: Nv/10
A: number of CFU/ml in the experimental conditions validation test
B: number of CFU/ml in the neutralizer non-toxicity validation test
C: number of CFU/ml in the inactivation by dilution-neutralization validation test
CFU: Colony Forming Unit

Observation of the presence of at least 75% spiny spores of *Aspergillus brasiliensis* in the test suspension, absence of germinated spores and mycelial fragments

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
- Nv_0 is between 30 CFU/ml and 160 CFU/ml, i.e., Nv 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
- A, B and C are equal to or greater than $0.5 \times NV_0$

In the described conditions, the neutralization method is validated on the tested strain for a concentration of product **AGAKOK 2.5** of 3.0% (v/v).

V. TEST RESULTS

Test strain	Test suspension		Results at concentrations m% (v/v)		
			m = 3.0	m = 2.0	m = 1.0
Candida albicans DSM 1386	10^{-5} : > 330 > 330 10^{-6} : 38 34 N = 3.6×10^7 N_0 = 3.6×10^6	Vc = Na = R = Log R =	0 0 < 140 > 2.6×10^4 > 4.41	0 0 < 140 > 2.6×10^4 > 4.41	0 0 < 140 > 2.6×10^4 > 4.41

N: number of CFU/ml in the test suspension
N_0: 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
CFU: Colony Forming Unit

Particular remarks:
All the controls and the method validation mix presented values within base limits.
At least one product concentration resulted in a log reduction of less than 4 log.

Concentrations resulting in a decimal log reduction of at least 4 are yeasticidal

VI. CONCLUSION

The product **AGAKOK 2.5** is yeasticidal on the reference strain *Candida albicans* DSM 1386 at concentrations of 3.0%, 2.0% and 1.0% (v/v) for an obligatory contact time of 30 minutes, at an obligatory temperature of 10°C, in accordance with the procedures of the European standard NF EN 1657 (May 2016), in the presence of 3.0 g/L of bovine albumin in final during the test (low level soiling conditions).

END OF TEST REPORT

ACTALIA

HYPRED
Mme Isabelle LE DREAU
Isabelle.LEDREAU@lcbfoodsafety.com

Report

EN 1657, Fungicidal activity

ACTALIA

I. CONTEXT

The HYPRED Company requested ACTALIA to validate the fungicidal activity of a disinfectant according to the NF EN 1657 standard.

II. PRODUCT DESCRIPTION

Product denomination: AGAKOK 2.5
Sample n°: EXT 25-55-1
Manufacturer: ANTI-GERM
Batch n°: 17110901
Expiry date: 11/2020
Provider: HYPRED
Storage conditions: ambiant temperature, out of direct sunlight
Product appearance: liquid, non-turbid and then pale rose after dilution in hard water
Active substance and concentration: not indicated

III. TRIAL PERIOD

Delivery date: 19/02/2018
Trial date: 15/03/2018

IV. EXPERIMENTAL CONDITIONS

Product diluent: hard water
Test concentrations: 0.5, 1 and 1.5%
Mandatory conditions:
- Test microorganism: *Aspergillus brasiliensis* ATCC16404, grown at 30°C
- Test temperature: 10°C
- Contact duration: 30 min
- Interfering substance: 3.0 g/l bovine albumin (= low level dirty condition)

Remarks regarding results:
All control conditions and validation mixtures provided results comprised within the allowed range.
No precipitate formation has been observed during tests (mixtures remained homogeneous).

V. TEST RESULTS: see attached datasheet

VI. CONCLUSION

For the product AGAKOK 2.5 (batch n°17110901), the fungicidal concentration determined according to the EN 1657 standard (mandatory conditions) in low level dirty conditions is 1% (w/w) (the mean log reduction obtained from the 3 replicates with *A. brasiliensis* after a 30 minutes contact is higher than 4.41 log).

For ACTALIA
Saint-Lô, the 21/032018
Aurélie HANIN
Project manager in microbiology

ACTALIA

Trial results (quantitative suspension test for the evaluation of fungicidal activity)

Procedure: EN 1657 Product denomination: AGAKOK 2.5 Batch n°: 17110901

Dilution-neutralization method – Pour plate method for enumeration
Neutralizing agent: Universal neutralizer (Biomérieux, ref. AEB610753)

Test temperature: 10°C [Min = 10°C – Max = 10.9°C]
Interfering substance: 3.0g/l bovine albumin
Test microorganism: *Aspergillus brasiliensis* ATCC16404
Growing temperature: 30°C

Internal lab n°: SMI 2018.045.1/BC n°161883
Trial date: 15/03/2018

Diluent for the preparation of test product solutions: hard water
Appearance of test product solutions: pale rose (milky)

Validation tests and controls

Concentration of rough *A. brasiliensis* spores >75% in the conidiospores suspension: YES – ~~NO~~
Concentration of spore suspension: 3.8×10^7 CFU/ml (comprised between 1.5 and 5×10^7 CFU/ml: COMPLIANT)

Validation suspension (N_v)			Control of environmental conditions (A)			Neutralization control (B)			Method validation (C) – concentration: 1.5% (w/w)		
Vc1	77	$\bar{x}$ = 76	Vc1	108	$\bar{x}$ = 115	Vc1	97	$\bar{x}$ = 99	Vc1	105	$\bar{x}$ = 111.5
Vc2	75		Vc2	122		Vc2	101		Vc2	118	
$30 \leq \bar{x}$ of $N_v \leq 160$ YES - ~~NO~~			$\bar{x}$ of A is ≥ to 0,5 x $\bar{x}$ of N_v YES - ~~NO~~			$\bar{x}$ of B is ≥ to 0,5 x $\bar{x}$ of N_v YES - ~~NO~~			$\bar{x}$ of C is ≥ to 0,5 x $\bar{x}$ of N_v YES - ~~NO~~		

Test suspension and test

Test suspensions (N and N_0)	N	Vc1	Vc2	
	10^{-5}	>165	>165	$\bar{x} = 38 \times 10^5$; logN = 7,58 N_0 = N/10 ; **logN_0 = 6,58** 6,17 ≤ logN_0 ≤ 6,70 YES - ~~NO~~
	10^{-6}	39	37	

Product concentration (% w/w)	Vc1	Vc2	Vc3	Na = $\bar{x}$ x10	Log Na	LogR	Contact duration
0.5	110 ; 157	116 ; 124	>165 ; >165	>1390	>3.14	<3,44	30 min
1	18 ; <14	<14 ; <14	<14 ; <14	<147	<2,17	>4,41	30 min
1.5	<14 ; <14	<14 ; <14	<14 ; <14	<140	<2,15	>4,43	30 min

Explanations:
Vc = numeration per ml (one plate or more)
$\bar{x}$ = mean of Vc1, Vc2 and Vc3
R = reduction (log R = log N_0 – log Na)

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REPORT
OFFER N°SMI.2018.150.1 / BC N°161964
Date: 20/04/18

HYPRED

Mme Isabelle LE DREAU

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Report

NF EN 16438, Yeasticidal activity

I. CONTEXT

The HYPRED Company requested ACTALIA to validate the yeasticidal activity of a disinfectant according to the NF EN 16438 standard.

II. PRODUCT DESCRIPTION

Product denomination: AGAKOK 2.5 Batch n°: 17110901
Sample n°: EXT 25-55-1 Expiry date: 11/2020
Manufacturer: Anti-germ Provider: HYPRED
Storage conditions: ambient temperature, out of direct sunlight
Product appearance: liquid, non-turbid and then pale rose after dilution in hard water
Active substance and concentration: Chlorocresol CAS n° 59-50-7 : 25%

III. TRIAL PERIOD

Delivery date: 19/02/2018
Trial date: 17/04/2018

IV. EXPERIMENTAL CONDITIONS

Product diluent: hard water
Test concentrations: 1, 2 and 3%
Mandatory conditions:

- Test microorganism: C*andida albicans* DSM1386, grown at 30°C
- Test temperature: 10±1°C
- Contact duration: 30 min
- Interfering substance: 3.0 g/l bovine albumin (= low level dirty conditions)

Remarks regarding results:
All control conditions and validation mixtures provided results comprised within the allowed range.
No precipitate formation has been observed during tests (mixtures remained homogeneous).

V. TEST RESULTS: see attached datasheet

VI. CONCLUSION

For the product AGAKOK 2.5 (batch n° 17110901), the yeasticidal concentration determined according to the NF EN 16438 standard (at 10°C with a contact duration of 30 minutes) in low level dirty conditions is 1% (w/w). The mean log reduction measured from the 4 replicates with *Candida albicans* is higher than 3 log whatever the tested concentration (1, 2 or 3%).

Remarks: all tested concentrations gave log reductions higher than 3 log but it is necessary to include in the study at least one inefficient concentration.

For ACTALIA
Saint-Lô, the 20/04/2018
Aurélie HANIN
Project manager in microbiology

Trial results (quantitative surface test for the evaluation of yeasticidal activity)

Procedure: NF EN 16438 Product denomination: AGAKOK 2.5 Batch n°: 17110901

Dilution-neutralization method – Pour plate method for enumeration (2 plates per dilution)
Neutralizing agent: Universal neutralizer (Biomérieux, ref. AEB610753)
Test temperature: 10°C [Min = 10°C – Max = 10.9°C]
Interfering substance: 3.0g/l bovine albumin
Test microorganisms: *Candida albicans* DSM1386
Growing temperature: 30°C
Drying time on the surface: 60 minutes
Diluent for the preparation of test product solutions: hard water
Appearance of test product solutions: pale rose

Internal lab n°: SMI 2018.150.1/BC n°161964
Trial date: 17/04/2018

Validation tests and controls

Test suspension (N)			Neutralizing agent toxicity control (B)			Method validation (C) – concentration: 6% (w/w)		
	V_{C1}	V_{C2}		V_{C1}	V_{C2}		V_{C1}	V_{C2}
10^{-6}	nd	Nd	10^{-3}	100	72	10^{-3}	77	56
10^{-7}	17	17	10^{-4}	nd	nd	10^{-4}	<14	<14
$\bar{x}$ of N = 4.25×10^6 = 6.63 log			$\bar{x}$ = 8.60×10^5 = 5.93 log			$\bar{x}$ = 6.65×10^5 = 5.82 log		
$6.57 \leq lgN \leq 7.10$ YES - ~~NO~~			$\bar{x}$ of B is ≥ to 0,5 x $\bar{x}$ of N_w YES - ~~NO~~			$\bar{x}$ of C is ≥ to 0,5 x $\bar{x}$ of N_w YES - ~~NO~~		

Water control:

Water control (N_w)	N_w	V_{C1}	V_{C2}	$\bar{x}$ of N_w = 5.15×10^5 lg N_w = 5.71 lg N_w ≥ lg 5.27 : YES - ~~NO~~
	10^{-3}	54 ; 61	56 ; 35	
	10^{-4}	<14 ; <14	<14 ; <14	
	10^{-5}	<14 ; <14	<14 ; <14	

Test

Product concentration (% w/w)	Dilution	V_{C1}	V_{C2}	V_{C3}	V_{C4}	Na = $\bar{x}$	Log Na = log $\bar{x}$	R	Contact duration
1	10^0	0^a ; 0^a	0^a ; 0^a	1^a ; 0^a	1^a ; 0^a	<140	<2.15	>3.56	30 min
	10^{-1}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				
	10^{-2}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				
2	10^0	4^a ; 0^a	1^a ; 0^a	1^a ; 2^a	0^a ; 0^a	<140	<2.15	>3.56	30 min
	10^{-1}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				
	10^{-2}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				
3	10^0	7^a ; 8^a	1^a ; 1^a	0^a ; 0^a	0^a ; 0^a	<140	<2.15	>3.56	30 min
	10^{-1}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				
	10^{-2}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				

Explanations:
a = used for calculations
Vc = numeration per ml (one plate or more)
$\bar{x}$ = mean of V_{C1}, V_{C2}, V_{C3} and V_{C4}
R = reduction (log R = log N_0 – log Na)

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REPORT
OFFER N°SMI.2018.157.1 / BC N°161964
Date: 03/05/18

HYPRED

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Report

NF EN 16438, Fungicidal activity

I. CONTEXT

The HYPRED Company requested ACTALIA to validate the fungicidal activity of a disinfectant according to the NF EN 16438 standard.

II. PRODUCT DESCRIPTION

Product denomination: AGAKOK 2.5 Batch n°: 17110901
Sample n°: EXT 25-55-1 Expiry date: 11/2020
Manufacturer: Anti-germ Provider: HYPRED
Storage conditions: ambient temperature, out of direct sunlight
Product appearance: liquid, non-turbid and then pale rose after dilution in hard water
Active substance and concentration: Chlorocresol n°CAS 59-50-7 at 25%

III. TRIAL PERIOD

Delivery date: 19/02/2018
Trial date: 26/04/2018

IV. EXPERIMENTAL CONDITIONS

Product diluent: hard water
Test concentrations: 1, 2 and 3%
Mandatory conditions:

- Test microorganism: *Aspergillus brasiliensis* DSM1988, grown at 30°C
- Test temperature: 10±1°C
- Contact duration: 30 min
- Interfering substance: 3.0 g/l bovine albumin (= low level dirty conditions)

Remarks regarding results:
No precipitate formation has been observed during tests (mixtures remained homogeneous).

V. TEST RESULTS: see attached datasheet

VI. CONCLUSION

For the product AGAKOK 2.5 (batch n° 17110901), the fungicidal concentration determined according to the NF EN 16438 standard (at 10°C with a contact duration of 30 minutes) in low level dirty conditions is 1% (w/w). The mean log reduction measured from the 4 replicates with *Aspergillus brasiliensis* is higher than 3 log whatever the tested concentration (1, 2 or 3%).

Remarks: all tested concentrations gave log reductions higher than 3 log but it is necessary to include in the study at least one inefficient concentration.

For ACTALIA
Saint-Lô, the 03/05/2018
Aurélie HANIN
Project manager in microbiology

ACTALIA
SÉCURITÉ DES ALIMENTS

Trial results (quantitative surface test for the evaluation of fungicidal activity)

Procedure: NF EN 16438 Product denomination: AGAKOK 2.5 Batch n°: 17110901

Dilution-neutralization method – Pour plate method for enumeration (2 plates per dilution)
Neutralizing agent: Universal neutralizer (Biomérieux, ref. AEB610753)
Test temperature: 10°C [Min = 10°C – Max = 10.9°C]
Interfering substance: 3.0g/l bovine albumin
Test microorganisms: *Aspergillus brasiliensis* DSM1988
Growing temperature: 30°C
Drying time on the surface: 60 minutes
Diluent for the preparation of test product solutions: hard water
Appearance of test product solutions: pale rose

Internal lab n°: SMI 2018.157.1/BC n°161964
Trial date: 26/04/2018

Validation tests and controls

Test suspension (N)			Neutralizing agent toxicity control (B)			Method validation (C) – concentration: 6% (w/w)		
	V_{C1}	V_{C2}		V_{C1}	V_{C2}		V_{C1}	V_{C2}
10^{-6}	96	98	10^{-3}	>165	>165	10^{-3}	150	>165
10^{-7}	<14	<14	10^{-4}	<14	22	10^{-4}	19	29
$\bar{x}$ of N = 2.43×10^6 = 6.38 log			$\bar{x}$ = 2.20×10^6 = 6.34 log			$\bar{x}$ = 2.10×10^6 = 6.32 log		
$6.57 \leq lgN \leq 7.10$ ~~YES~~ - NO			$\bar{x}$ of B is ≥ to 0,5 x $\bar{x}$ of N_w YES - ~~NO~~			$\bar{x}$ of C is ≥ to 0,5 x $\bar{x}$ of N_w YES - ~~NO~~		

Water control:

Water control (N_w)	N_w	V_{C1}	V_{C2}	$\bar{x}$ of $N_w = 2.17 \times 10^6$ lg N_w = 6.34 lg N_w ≥ lg 5.27 : YES - ~~NO~~
	10^{-3}	>165 ; >165	137 ; >165	
	10^{-4}	27 ; 20	23 ; 25	
	10^{-5}	<14 ; <14	<14 ; <14	

Test

Product concentration (% w/w)	Dilution	V_{C1}	V_{C2}	V_{C3}	V_{C4}	Na = $\bar{x}$	Log Na = log $\bar{x}$	R	Contact duration
1	10^0	1^a ; 1^a	2^a ; 1^a	1^a ; 0^a	1^a ; 2^a	<140	<2.15	>4.19	30 min
	10^{-1}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				
	10^{-2}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				
2	10^0	4^a ; 0^a	1^a ; 0^a	0^a ; 0^a	0^a ; 0^a	<140	<2.15	>4.19	30 min
	10^{-1}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				
	10^{-2}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				
3	10^0	0^a ; 0^a	0^a ; 0^a	1^a ; 0^a	0^a ; 0^a	<140	<2.15	>4.19	30 min
	10^{-1}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				
	10^{-2}	0 ; 0	0 ; 0	0 ; 0	0 ; 0				

Explanations:
a = used for calculations
Vc = numeration per ml (one plate or more)
$\bar{x}$ = mean of V_{C1}, V_{C2}, V_{C3} and V_{C4}
R = reduction (log R = log N_0 – log Na)

Rapport d'essai quantitatif pour l'évaluation de l'activité fongicide et /ou levuricide d'un désinfectant liquide utilisé dans le domaine vétérinaire selon la norme NF EN 1657-mai 2016 (phase 2, étape 1)

Report on the quantitative test for the evaluation of fungicidal and /or yeasticidal activity of liquid disinfectant product used in veterinary area according to the NF EN 1657-May 2016 (phase 2, step 1)

Rap n°2018/69

Date : 12/12/2018

DEMANDEUR : **HYPRED S.A.S**
Applicant

DONNÉES RELATIVES A L'ESSAI :
Test data

Essai n° : **CMK-452**
Assay n°

Date de l'essai : **10/10/2018**
Assay date :

Essai réalisé par : **LCB Food Safety (*)**
Assay performed by: **P.A.E. Actiparc- Rue des acacias – 01190 BOZ (France)**

(*) système de management de la qualité certifié par AFNOR-AFAQ selon la norme ISO 9001 :2015
quality management system certified by AFNOR-AFAQ in compliance with standard ISO 9001: 2015

IDENTIFICATION DU PRODUIT TESTÉ :
Identification of the tested disinfectant

Nom du désinfectant : **AGAKOK 2.5**
Disinfectant Name

Numéro de lot : **1711901**
Batch number

Concentrations testées du liquide désinfectant : **2%-1%-0,5% (v/v)**
Tested concentrations of the liquid disinfectant

Diluant utilisé : **Eau dure**
Diluent used *Hard water*

Aspect des dilutions d'essai du produit : **Opaques , de couleur rose , homogènes .**
Appearance of product dilutions *Opaque , pink and homogeneous*

SOUCHE D'ESSAI : ***Aspergillus fumigatus* DSM 819**
Test strain

MÉTHODE UTILISÉE :
Method used

o Par dilution – neutralisation : **Oui/yes** ☒ Non/no ☐
Dilution neutralization method

Neutralisant utilisé *:* ***Tween 80: 3% (p/v) ; Lécithine d'oeufs : 0,3 % (p/v)***
Neutralizer used ***Histidine : 0,1% (p/v) ; Thiosulfate de sodium : 0,5%(p/v)****;*
Tween 80: 3% (w/v) ***;*** *eggs lecithin : 0,3 %(w/v);histidine :0,1% (w/v)*
Thiosulphate sodium : 0,5% (w/v)

o Par filtration sur membrane : Oui/yes ☐ **Non/no** ☒
Membrane filtration method

CONDITIONS EXPÉRIMENTALES :
Experimental conditions

1/Temps de contact /Température du produit testé:
Contact time /temperature of the product to be tested

	Temps de contact *Contact time*	**Température d'essai** *Test temperature*
Conditions d'essais obligatoires *Obligatory test conditions*	**30 minutes**	**10 °C**
	Oui/yes ☒ Non/no ☐	**Oui/yes** ☒ Non/no ☐
Conditions d'essais additionnelles *Additional test conditions*	5 ou/*or* 60 ou/*or* 120 minutes	4 ou/*or* 20 ou/*or* 40 **°C**
	Oui/yes ☐ **Non/no** ☒	Oui/yes ☐ **Non/no** ☒

2/Substances interférentes :
Interfering substances

Bas niveau de saleté (Albumine 3g/L) *Low dirtiness level (albumin 3g/L)*	**Oui/yes** ☒ Non/no ☐
Haut niveau de saleté (Albumine 10g/L + extrait de levure 10g/L) *High dirtiness level (albumin 10 g /L and yeast extract 10g/L)*	Oui/yes ☐ **Non/no** ☒

RÉSULTATS DES ESSAIS :
Test results

1/ Essais de validation :
validation test

Souche, collection d'origine et numéro dans la collection *Strain collection of origin and reference number*	**Suspension d'essai : N (UFC/ml)** *Test suspension counting: N (cfu/ml)*	**Suspension d'essai pour les essais de validation : $N_{v0}=N_v/10$ (UFC/ml)** *bacterial suspension counting for validation test: N_{v0} (cfu/ml)*	**Non toxicité du neutralisant : B (UFC/ml)** *non-toxicity of the neutralizer : B (cfu/ml)*	**Témoin des conditions expérimentales : A (UFC/ml) Bas niveau de saleté** *Control test of Experimental conditions : A (cfu/ml) Low dirtiness level*
***Aspergillus fumigatus* DSM 819**	**$3,95.10^7$**	**123**	**143**	**154,5**

Concentration du désinfectant liquide testé *Concentration of the liquid disinfectant to be tested*	**Essai de validation de la méthode utilisée : C (UFC/ml) Bas niveau de saleté** *Validation assay of the method used Low dirtiness level (cfu/ml)*
2%	**140**

UFC /ml : Unité Formant Colonie par ml
cfu/ml : colony –forming unit(s) per milliliter

Conditions expérimentales de validité de l'essai :
Experimental conditions of validity of the test

$1,5 \times 10^7 \leq N \leq 5 \times 10^7$ UFC/ml : $(1,5 \times 10^6 \leq N_0 \leq 5 \times 10^6$ UFC/ml)	**Oui/yes**	☒	Non/no	☐
$3 \times 10^1 \leq N_{v0} \leq 1,6 \times 10^2$ UFC/ml : $(3 \times 10^2 \leq Nv \leq 1,6 \times 10^3$ UFC/ml)	**Oui/yes**	☒	Non/no	☐
A, B, C $\geq 0,5\ N_{v0}$	**Oui/yes**	☒	Non/no	☐
Pour *Aspergillus brasiliensis* ATCC 16404 *Pourcentage minimum de spores échinulées>à 75%* *Minimal percentage of echinulated spores required : 75%*	Oui/yes	☐	Non/no	☐

2/ Résultats d'essais :

Test results

Souche, collection d'origine et numéro dans la collection *Strain collection of origin and reference number*	**Essai en conditions de bas niveau de saleté** *Assay in low soiling level conditions*		
	Concentrations testées *Tested concentrations*	**Nombre de cellules viables après exposition dans le mélange d'essai : Na (UFC/ml)** *Counting of viable microorganisms after exposure to the assay mixture: Na (cfu/ml)*	**Taux de réduction : TR = lg N0-lg Na** *Reduction rate (lg):TR*
***Aspergillus fumigatus* DSM 819**	**2%**	**<140**	**>4,45**
	1%	**<140**	**>4,45**
	0,5%	**>1,65.10³**	**<3,38**

Conclusion
Conclusion

Selon la norme **NF EN 1657**-mai 2016, en **conditions de bas niveau de saleté**, à la température de **10°C** et avec un temps de contact de **30 minutes**, le désinfectant liquide **AGAKOK 2.5** présente une **activité fongicide à la concentration de 1%** sur la souche ***Aspergillus fumigatus* DSM 819**.

*According to the standard **NF EN 1657**-may 2016, in **low soiling level conditions**, at the temperature **10°C** and with a **30 minutes** contact time, the liquid disinfectant **AGAKOK 2.5** is **fungicide at the concentration 1%** on the strain **Aspergillus fumigatus* DSM 819.**

Isabelle Le Dréau
Microbiologiste
Microbiologist

Patrick Arnould
Responsable Réglementation & Laboratoire
Regulatory & Laboratory Manager

ANNEXE
APPENDIX

Dénombrement des témoins de validations :
Counting of validation tests control

Souche testée *Tested strain*	**Suspension de validation** N_{v0} **(=**N_v**/10)** *Validation suspension*		**Témoins des conditions expérimentales : A** **Bas niveau de saleté** *Control test of Experimental conditions: A* *Low dirtiness level*		**Témoin de non toxicité du neutralisant : B** *Control test of non-toxicity of the neutralizer :* B		**Validation de la méthode : C** **Concentration du produit :2%** **Bas niveau de saleté** *Method validation : C* *Product concentration: 2%* *Low dirtiness level*	
Aspergillus fumigatus **DSM 819**	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2
	126	**120**	**153**	**156**	**153**	**133**	**135**	**145**
	$\bar{x}$: 123		**$\bar{x}$: 154,5**		**$\bar{x}$: 144**		**$\bar{x}$: 140**	

Dénombrement de la suspension d'essai et de l'essai :
Counting of assay suspension and of the assay

Souche testée *Tested strain*	**Suspension d'essai N** *Test suspension counting:* N		
Aspergillus fumigatus **DSM 819**	dilution	**Vc1**	**Vc2**
	10^{-5}	**>165**	**>165**
	10^{-6}	**38**	**41**
	N($\bar{x}$)= 3,95.10⁷ **lg(N)=7,60 lg(N₀=N/10)=6,60**		

Souche testée *Tested strain*	**Concentrations testées du produit** *Product tested concentrations*	**Dénombrement des essais (UFC/ml)** *Counting of assays (cfu/ml)*			**lg (Na)**	**TR** = lg(N₀) -lg(Na)
		Vc1	**Vc2**	**Na =** **$\bar{x}$ x 10**		
Aspergillus fumigatus **DSM 819**	**2%**	**0**	**0**	**<140**	**<2,15**	**>4,45**
	1%	**0**	**0**	**<140**	**<2,15**	**>4,45**
	0,5%	**>165**	**>165**	**>1,65.10³**	**>3,22**	**<3,38**

Vc= Nombre d'UFC dénombrées par échantillon de 1ml .
Cfu counted per 1 ml- sample

Rapport d'essai quantitatif pour l'évaluation de l'activité fongicide et /ou levuricide d'un désinfectant liquide utilisé dans le domaine vétérinaire selon la norme NF EN 16438-Mars 2014 (phase 2, étape 2)

Report on the quantitative test for the evaluation of fungicidal and/or yeasticidal activity of liquid disinfectant product used in veterinary area according to the NF EN 16438-March 2014 (phase 2, step 2)

Rap n°2018/70

Date : 13/12/2018

DEMANDEUR : **HYPRED S.A.S**
Applicant

DONNÉES RELATIVES A L'ESSAI :
Test data

Essai n° : **CMK-453**
Assay n°

Date de l'essai : **11/10/2018**
Assay date :

Essai réalisé par : **LCB Food Safety (*)**
Assay performed by: **P.A.E. Actiparc- Rue des acacias – 01190 BOZ (France)**

(*) système de management de la qualité certifié par AFNOR-AFAQ selon la norme ISO 9001 :2015
quality management system certified by AFNOR-AFAQ in compliance with standard ISO 9001: 2015

IDENTIFICATION DU PRODUIT TESTÉ :
Identification of the tested disinfectant

Nom du désinfectant : **AGAKOK 2.5**
Disinfectant Name

Numéro de lot : **1711901**
Batch number

Concentrations testées du liquide désinfectant : **1,5%-1%-0,5% (v/v)**
Tested concentrations of the liquid disinfectant

Diluant utilisé : **Eau dure**
Diluent used *Hard water*

Aspect des dilutions d'essai du produit : **Opaques , de couleur rose , homogènes** .
Appearance of product dilutions *Opaque, pink, homogeneous*

SOUCHE D'ESSAI : ***Aspergillus fumigatus* DSM 819**
Test strain

MÉTHODE UTILISÉE :
Method used

Par dilution – neutralisation : **Oui / *yes*** ☒ Non / *no* ☐
Dilution neutralization method

Neutralisant utilisé *:* ***Tween 80: 3% (p/v) ; Lécithine d'oeufs : 0,3 % (p/v)***
Neutralizer used ***Histidine : 0,1% (p/v) ; Thiosulfate de sodium : 0,5%(p/v)****;*
Tween 80: 3% (w/v) ***;*** *eggs lecithin : 0,3 %(w/v);histidine :0,1% (w/v)*
Thiosulphate sodium : 0,5% (w/v)

CONDITIONS EXPÉRIMENTALES :
Experimental conditions

1/Temps de contact /Température du produit testé:
Contact time /temperature of the product to be tested

	Temps de contact *Contact time*	**Température d'essai** *Test temperature*
Conditions d'essais obligatoires	60 minutes	**10 °C**
Obligatory test conditions	Oui/yes ☐ Non/no ☒	**Oui/yes** ☒ Non/no ☐
Conditions d'essais additionnelles	5 ou/*or* **30** ou/*or* 120 **minutes**	4 ou/*or* 20 ou/*or* 40 **°C**
Additional test conditions	**Oui/yes** ☒ Non/no ☐	Oui/yes ☐ Non/no ☒

2/Conditions de saleté :
Dirtiness conditions

Bas niveau de saleté (Albumine 3g/L) *Low dirtiness level (albumin 3g/L)*	**Oui/yes** ☒ Non/no ☐
Haut niveau de saleté (Albumine 10g/L + extrait de levure 10g/L) *High dirtiness level (albumin 10 g /L and yeast extract 10g/L)*	Oui/yes ☐ Non/no ☐

RÉSULTATS DES ESSAIS :
Test results

1/ Essais de validation :
validation test

Souche, collection d'origine et numéro dans la collection *Strain collection of origin and reference number*	**Suspension d'essai : N (UFC/0.025 ml)** *Test suspension counting: N (cfu/0.025ml)*	**Non toxicité du neutralisant : B (UFC/unité de surface)** *non-toxicity of the neutralizer:* B *(cfu/unit surface)*
***Aspergillus fumigatus* DSM 819**	**$9{,}13.10^6$**	**$4{,}65.10^6$**

Concentration du désinfectant liquide testé *Concentration of the liquid disinfectant to be tested*	**Essai de validation de la méthode utilisée : C (UFC/unité de surface) Bas niveau de saleté** *Validation assay of the method used: C (cfu/unit surface)* *Low dirtiness level*
1,5%	**$3{,}35.10^6$**

UFC : Unité Formant Colonies
cfu–forming unit(s) per surface unit

Conditions expérimentales de validité de l'essai :
Experimental conditions of validity of the test

$3{,}75 \times 10^6 \leq N \leq 1{,}25 \times 10^7$ UFC/ml : $(6{,}57 \leq \lg(N) \leq 7{,}10)$	**Oui / *yes*** ☒	Non / *no* ☐
$\lg(Nw) \geq 5{,}27$:	**Oui / *yes*** ☒	Non / *no* ☐
$B, C \geq 0{,}5\ Nw$:	**Oui / *yes*** ☒	Non / *no* ☐
Pour *Aspergillus brasiliensis* ATCC 16404 *pourcentage de spores échinulées*>à 75% *Minimal percentage of echinulated spores required : 75%*	Oui / *yes* ☐	Non / *no* ☐

2/ Résultats d'essais :
Test results

Souche, collection d'origine et numéro dans la collection *Strain collection of origin and reference number*	Témoin eau Nw (UFC/unité de surface) *Water control (cfu/unit surface)*	Essai en conditions de bas niveau de saleté *Assay in low soiling level conditions*		
		Concentrations testées *Tested concentrations*	Nombre de cellules viables après exposition dans le mélange d'essai : Na (UFC/unité de surface) *Counting of viable microorganisms after exposure to the assay mixture: Na (cfu/unit surface)*	Taux de réduction : R = lg Nw – lg Na *Reduction rate : R*
***Aspergillus fumigatus* DSM 819**	**$2{,}55.10^6$**	**1,5%**	**<140**	**>4,26**
		1%	**<140**	**3,65**
		0,5%	**$>1{,}65.10^5$**	**<1,19**

Conclusion
Conclusion

Selon la norme **NF EN 16438**-mars 2014, en **conditions de bas niveau de saleté**, à la température de **10°C** et avec un temps de contact de **30 minutes**, le désinfectant liquide **AGAKOK 2.5** présente une **activité fongicide à la concentration de 1%** sur la souche ***Aspergillus fumigatus* DSM 819**.

*According to the standard **NF EN 16438**-march 2014, in **low soiling level conditions**, at the temperature **10°C** and with a **30 minutes** contact time, the liquid disinfectant **AGAKOK 2.5** is **fungicide at the concentration 1%** on the strain **Aspergillus fumigatus DSM 819**.*

Isabelle Le Dréau
Microbiologiste
Microbiologist

Patrick Arnould
Responsable Réglementation & Laboratoire
Regulatory & Laboratory Manager

ANNEXE
APPENDIX

Dénombrement de la suspension d'essai et des témoins de validations :
Counting of the assay suspension and validation control

Souche testée *Tested strain*	**Suspension d'essai N (UFC/0.025 ml)** *Test suspension N (cfu/0.025ml)*			**Témoin de non toxicité du neutralisant : B** *Control test of non-toxicity of the neutralizer :* B			**Validation de la méthode : C** **Concentration du produit :1,5%** **Bas niveau de saleté** *Method validation : C* *Product concentration: 1,5%* *Low dirtiness level*		
***Aspergillus fumigatus* DSM 819**	dilution	Vc1	Vc2	dilution	Vc1	Vc2	dilution	Vc1	Vc2
	10^{-6}	**>165**	**>165**	10^{-2}	**>165**	**>165**	10^{-2}	**>165**	**>165**
				10^{-3}	**>165**	**>165**	10^{-3}	**>165**	**>165**
	10^{-7}	**36**	**39**	10^{-4}	**42**	**51**	10^{-4}	**32**	**35**
				10^{-5}	**6**	**5**	10^{-5}	**2**	**7**
	N($\bar{x}$ x 0.025/d)=9,13.10^6 Lg(N)=6,96			B ($\bar{x}$ x 10/d)=4,65.10^6 Lg(B)= 6,67			C ($\bar{x}$ x 10/d)= 3,35.10^6 Lg(C)= 6,53		

Dénombrement du témoin eau et des essais :
Counting of water control and of the assays

Souche testée *Tested strain*	**Témoin eau Nw** *Water control Nw*		
***Aspergillus fumigatus* DSM 819**	dilution	Vc1	Vc2
	10^{-2}	**>165**	**>165**
	10^{-3}	**>165**	**>165**
	10^{-4}	**28**	**23**
	10^{-5}	**2**	**2**
	Nw($\bar{x}$ x 10/d)= 2,55.10^6 Lg(Nw)= 6,41		

Souche testée *Tested strain*	**Concentrations testées du produit** *Product tested concentrations*	**Dénombrement des essais (UFC/ml)** *Counting of assays (cfu/ml)*				**lg Na**	**R= lg Nw- lg Na**
		dilution	**Vc1**	**Vc2**	**Na = $\bar{x}$ x 10/d**		
***Aspergillus fumigatus* DSM 819**	**1,5%**	10^{-0}	**0**	**0**	**<140**	**<2,15**	**>4,26**
		10^{-1}	**0**	**0**			
		10^{-2}	**0**	**0**			
	1%	10^{-0}	**51**	**64**	**575**	**2,76**	**3,65**
		10^{-1}	**10**	**5**			
		10^{-2}	**3**	**0**			
	0,5%	10^{-0}	**>165**	**>165**	**>1,65.10**	**>5,22**	**<1,19**
		10^{-1}	**>165**	**>165**			
		10^{-2}	**>165**	**>165**			

Vc= Nombre d'UFC dénombrées par échantillon de 1ml .
Cfu counted per 1 ml- sample

TEST REPORT

VIRUCIDAL ACTIVITY OF THE AGAKOK 2.5 PRODUCT AGAINST THE ENTERIC CYTOPATHOGENIC BOVINE ORPHAN VIRUS (ECBO)

For: **HYPRED SAS**
55 boulevard Jules VERGER
35 800 DINARD

Study number: n°012V01-2018-02

VIRUCIDAL TESTS:

According to the NF EN 14675 standard (May 2015) – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in veterinary area.

Tests using the AGAKOK 2.5 product batch n° 17110901 against the enteric cytopathogenic bovine orphan virus (ECBO).

This test report included 13 pages.

Study completion date: 04/04/2018

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professeur Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
4, rue des Grandes Pièces
25770 SERRE LES SAPINS
FRANCE

2 PRODUCT IDENTITY

Reference	Batch n°
AGAKOK 2.5	17110901

- Expiration date: 11/01/2020
- Manufacturer: ANTI GERM Memmingen, Germany
- Manufacturing date: non communicated
- Storage conditions: room temperature
- Active compounds: chlorocresol
- Appearance of the product: clear, red
- Product diluent recommended by the manufacturer for use: tap water
- Date of delivery of the product: 01/19/2018
- Date of tests: from 02/12/2018 to 03/15/2018

3 EXPERIMENTAL CONDITIONS

- Temperature used during the assays: 10°C ± 1°C
- Titration unit: log $TCID_{50}$
- Exposure Time: 5 minutes
- Tested concentration: 2.0%, 3.0% and 4.0%
- Diluent used for the product: hard water
- Viral strain: Enteric cytopathogenic bovine orphan virus ATCC VR-248 (ECBO), grown on VERO cells, at 37°C, under 5% CO2 atmosphere
- Organic soil load: BSA 3g/L (low dirty conditions)
- Product stability: stable
- Stop solution: cold shock

Viral titer:

Viral titers are expressed in $TCID_{50}$, according to the Spearman-Kärber method = 6,875 log $TCID_{50}$.

4 VALIDATIONS

a) Cytotoxicity

The AGAKOK 2.5 product has been tested at 4% on the VERO cells and no toxicity was observed.

b) Cells sensitivity to the virus

For each viral suspension, comparative titers of the virus were performed on cells treated or untreated with the product.

	Viral titer (log $TCID_{50}$)		
Product dilution	Viral suspension on untreated cells	Viral suspension on treated cells	**Viral titer difference (log $TCID_{50}$)**
AGAKOK 2.5 10^{-1}	6,875	6,375	**0,500**

The AGAKOK 2.5 product do not affect the infectious capacity of the virus: the differences in viral titers between the virus inoculated on VERO cells and the virus inoculated on the VERO cells treated with the AGAKOK 2.5 product was ≤ 1,0 log (0,500 log).

c) Inactivation of the virus

	Viral titer (log $TCID_{50}$)	**Viral titer reduction (log $TCID_{50}$)**
Viral suspension	6,875	
Formaldehyde 0,7%		
Inactivation after 5 min	6,625	
Inactivation after 15 min	6,250	
Inactivation after 30 min	5,750	**1,125**

The virus is inactivated with the control solution of 0,7 % formaldehyde after 30 min of exposure if the reduction is comprised between |-0,5| and |-2,5| log. The reduction observed was of 1,125 log for the ECBO virus.

5 VIRUCIDAL ASSAYS

TRIAL 1

The viral suspension control was titered at 6,875 log $TCID_{50}$.

PRODUCT	Final concentration (v/v)	Exposure time	Temperature	Titer after trial (log $TCID_{50}$)	Viral titer reduction
AGAKOK 2.5	**4.0%**	5 min	10°C	2,125	**4,750**
	3.0%			2,500	**4,375**
	2.0%			4,500	2,375

TRIAL 2

The viral suspension control was titered at 6,875 log $TCID_{50}$.

PRODUCT	Final concentration (v/v)	Exposure time	Temperature	Titer after trial (log $TCID_{50}$)	Viral titer reduction
AGAKOK 2.5	**4.0%**	5 min	10°C	2,000	**4,875**
	3.0%			2,625	**4,250**
	2.0%			4,375	2,500

The product has a virucidal effect if the viral titer reduction is ≥ 4,0 log.

6 VALIDATION OF THE METHODOLOGY

The assays were validated as required by the European standard EN 14675:2015:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product. The viral titer of the ECBO virus was 6,875 log $TCID_{50}$.
- The virus was inactivated with the control solution of 0,7 % formaldehyde after 30 min of exposure : the reduction observed was of 1,125 log for the ECBO virus.
- The AGAKOK 2.5 product has a weak cytotoxic effect on the VERO cells.
- The AGAKOK 2.5 product do not affect the infectious capacity of the virus: the differences in viral titers between the virus inoculated on VERO cells and the virus inoculated on the VERO cells treated with the AGAKOK 2.5 product was ≤ 1,0 log (0,500 log).

7 CONCLUSION

The assays performed with the AGAKOK 2.5 batch n° 17110901product demonstrated that:

- **The product AGAKOK 2.5 demonstrated a virucidal activity against the ECBO virus from the concentration 3.0%, as required by the European standard EN 14675:2015, following a 5 minutes exposure period, at 10°C, in low dirty conditions.**

TECHNICAL APPENDIX 1

Cell line for ECBO virus: VERO cells (R&D Biotech, ref. 84009, batch n°110118-110V)

Viral strain: Enteric cytopathogenic bovine orphan virus strain (ECBO), ATCC VR-248 (batch n° 58087749)

Buffer and reagents:

- Buffer PBS: sodium chloride, Dominique DUTSCHER, ref. 836751; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch n° 1019K01021V
- MEM media, PAN Biotech, ref. P04-03590, batch n°9420112
- Horse serum, Sigma Aldrich, ref. H1270, batch n° 12J395

Organic soil load:

- Bovine Sera Albumin, Sigma Aldrich, ref. A5479, batch n° STBB7838V

Inactivation solution:

- Formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

TECHNICAL APPENDIX 2

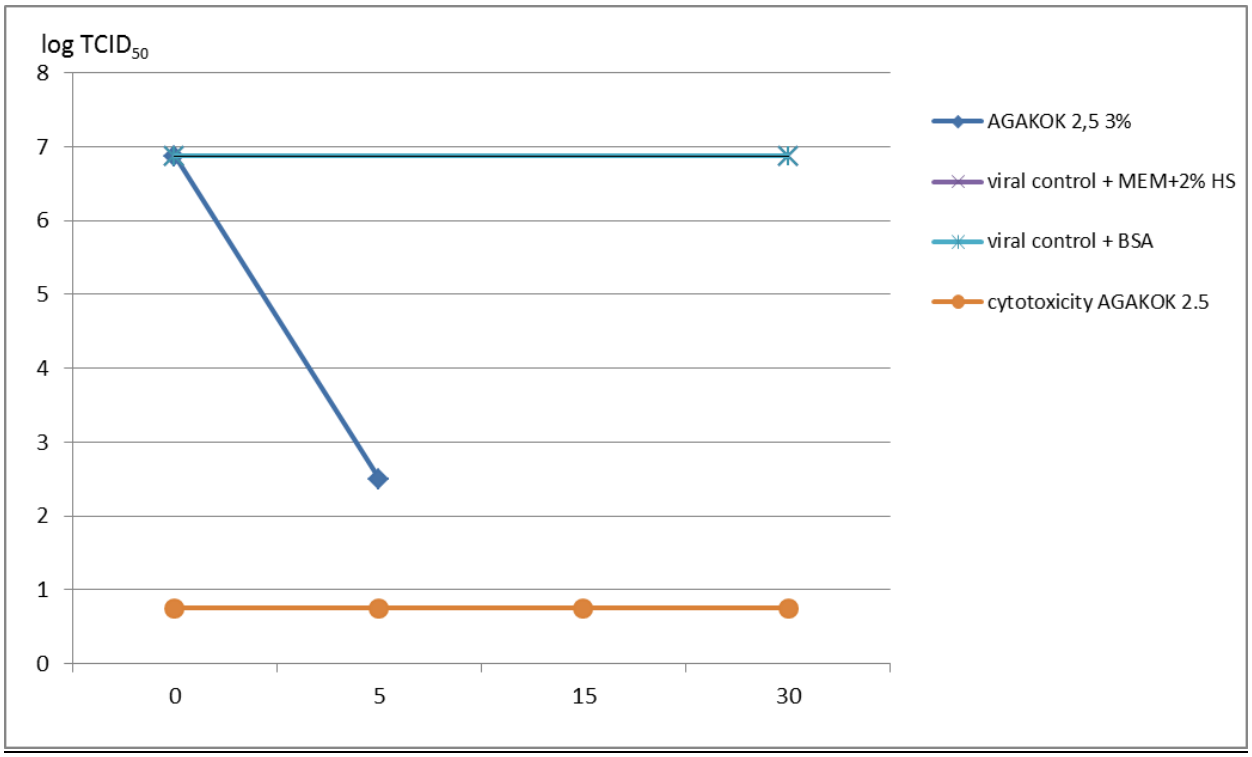
Table A1 – Viral titer of ECBO virus calculated with the Spaerman-Kärber (cytopathic effect method):

log $TCID_{50}$ = 6,875

Dilution (- log)	Result	% positive results
-3	44444444	100
-4	44444444	100
-5	44444444	100
-6	11111111	100
-7	11000001	37,5
-8	00000000	0
-9	00000000	0
Sum of the % of the positive wells		437,5

Chart 1 –graphical chart of the results:

Trial 1



Trial 2

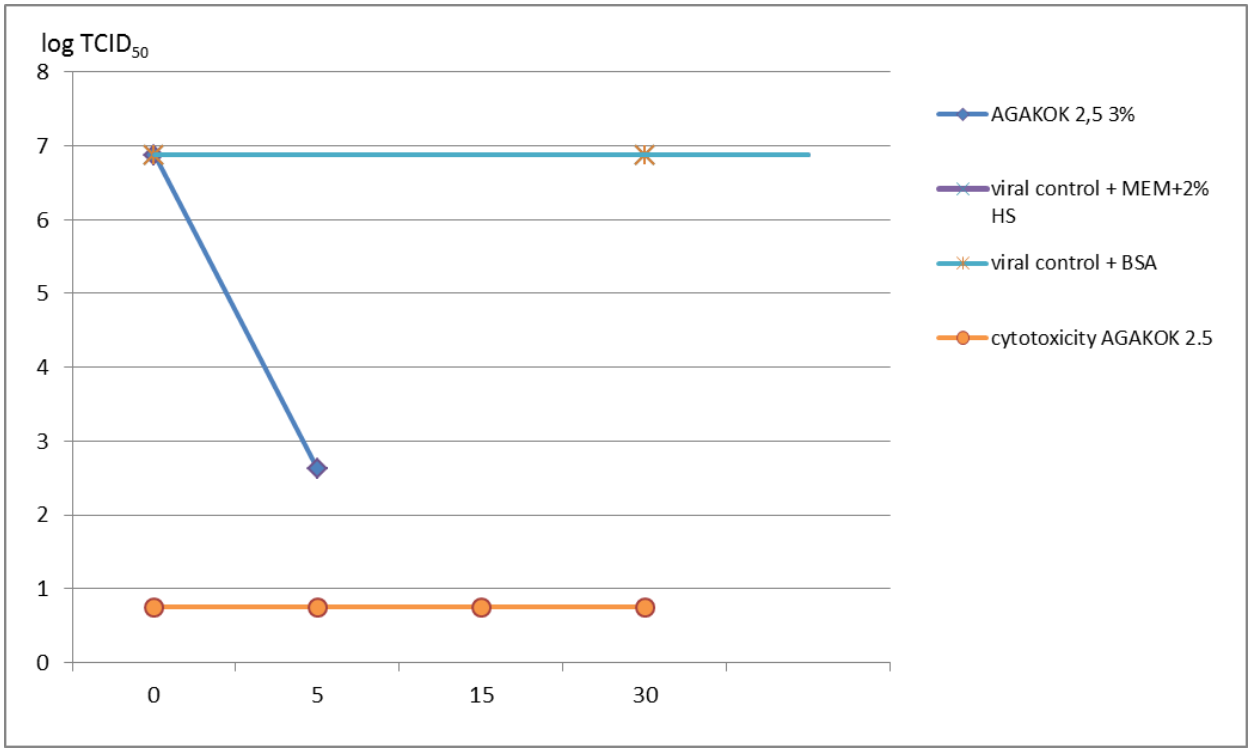


Table A2 — results with the AGAKOK 2.5 product against the ECBO virus in low dirty conditions

Product	Concentration	Organic soil load	cytotoxicity level	Lg $DICT_{50}$				Reduction
				0	5 min	15 min	30 min	
AGAKOK 2.5 TRIAL 1	3.0%	3 g/L BSA	0,75	6,875	2,5	N.T.	N.T.	R=
AGAKOK 2.5 TRIAL 2	3.0%		0,75	6,875	2,625	N.T.	N.T.	4,375 R=
Formaldehyde	0,70%	MEM+2% HS	2,5	6,875	6,625	6,25	5,75	4,25
infectivity control TRIAL 1	N.A.	MEM+2% HS	N.A	6,875	N.T.	N.T.	6,875	
	N.A.	3 g/L BSA	N.A	6,875	N.T.	N.T.	6,875	
infectivity control TRIAL 2	N.A.	MEM+2% HS	N.A	6,875	N.T.	N.T.	6,875	
	N.A.	3 g/L BSA	N.A	6,875	N.T.	N.T.	6,875	
Cells sensitivity	AGAKOK 2.5	N.A.	Untreated cells	N.T.	N.T.	N.T.	6,875	
	10^{-1}	N.A.	Treated cells	N.T.	N.T.	N.T.	6,375	

HS : Horse Serum

Other concentrations tested :

Trial 1	Concentration	organic soil load	exposure time	Log $DICT_{50}$		Reduction	
				0	30 min		
AGAKOK 2.5	4.0%	3 g/l BSA	5 min	6,875	2,125	4,75	active
	2.0%			6,875	4,5	2,375	inactive

Trial 2	Concentration	organic soil load	exposure time	Log $DICT_{50}$		Reduction	
				0	30 min		
AGAKOK 2.5	4.0%	3 g/l BSA	5 min	6,875	2	4,875	active
	2.0%			6,875	4,375	2,5	inactive

Table A3 — Raw data for the AGAKOK 2.5 product against the ECBO virus, in low dirty conditions (cytopathic effect; 8 wells)

Trial 1

	Concentration	organic soil load	exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5 trial 1	3.0%	3 g/L BSA	5 min	4444	1111	0000	0000	0000	0000	0000	0000	0000	0000
				4444	1111	0000	0000	0000	0000	0000	0000	0000	0000
			viral	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
			control	4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
AGAKOK 2.5 trial 1 cytotoxicity	3.0%	3 g/L BSA	N.A.	1100	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
				0000	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
viral control of infectivity	N.A.	MEM+2% HS	0	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
			30	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
	N.A.	3 g/L BSA	0	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
			30	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
Formaldehyde	0,70%	MEM+2% HS	5	4444	4444	4444	4444	1111	1111	1000	0000	0000	0000
				4444	4444	4444	4444	1111	1111	0000	0000	0000	0000
			15	4444	4444	4444	1111	1111	1111	0000	0000	0000	0000
				4444	4444	4433	1111	1111	1100	0000	0000	0000	0000
			30	4444	4444	4444	1111	1111	1100	0000	0000	0000	0000
				4444	4444	4444	1111	1111	0000	0000	0000	0000	0000
Formaldehyde (cytotoxicity)	0,70%	MEM+2% HS	N.A.	4444	1111	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	1111	0000	0000						

Other concentrations tested :

	Concentration	organic soil load	exposure time	dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5	4.0%	3 g/L BSA	5 min	4444	1111	0000	0000	00000	0000	0000	0000	0000	0000
				4444	1000	0000	0000	0000	0000	0000	0000	0000	0000
	2.0%			4444	4444	4444	4444	0000	0000	0000	0000	0000	0000
				4444	4444	4444	4444	0000	0000	0000	0000	0000	0000

Trial 2

	Concentration	organic soil load	exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5 trial 1	3.0%	3 g/L BSA	5 min	4444	3311	4000	0000	0000	0000	0000	0000	0000	0000
				4444	1111	0000	0000	0000	0000	0000	0000	0000	0000
			viral	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
			control	4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
AGAKOK 2.5 trial 1 cytotoxicity	3.0%	3 g/L BSA	N.A.	1100	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
				0000	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
viral control of infectivity	N.A.	MEM+2% HS	0	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
			30	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
	N.A.	3 g/L BSA	0	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
			30	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
Formaldehyde	0,70%	MEM+2% HS	5	4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
			15	4444	4444	4444	1111	1111	1111	0000	0000	0000	0000
				4444	4444	4444	1111	1111	0000	0000	0000	0000	0000
			30	4444	4444	4444	1111	1111	0000	0000	0000	0000	0000
				4444	4444	4444	1111	1111	0000	0000	0000	0000	0000
Formaldehyde (cytotoxicity)	0,70%	MEM+2% HS	N.A.	4444	4411	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	1111	0000	0000						

Other concentrations tested :

	Concentration	organic soil load	exposure time	dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5	4.0%	3 g/L BSA	5 min	4444	1111	0000	0000	00000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000	0000
	2.0%			4444	4444	4444	4444	0000	0000	0000	0000	0000	0000
				4444	4444	4444	4440	0000	0000	0000	0000	0000	0000

Sensitivity of the cells to the virus

Trial 1

Product	dilution	organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5	10^{-1}	3 g/l BSA	Untreated cells	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	1111	0000	0000	0000	0000
			Treated cells	4444	4444	4444	4 444	1111	0000	0000	0000	0000
				4444	4444	4444	4444	1110	0000	0000	0000	0000

Trial 2

Product	dilution	organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5	10^{-1}	3 g/l BSA	Untreated cells	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	1111	0000	0000	0000	0000
			Treated cells	4444	4444	4444	4 444	1111	0000	0000	0000	0000
				4444	4444	4444	4444	1110	0000	0000	0000	0000

Validation of the stop method (microspin filtration and cold shock)

Product	dilution	organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5	10^{-1}	MEM+2% HS	Cold dilution	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	1111	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	1111	0000	0000	0000	0000

Cold dilution : viral titer = 6,875 R = 0

Viral control : viral titer = 6,875 The stop method is validated (R ≤ 0,5 log)

IRM Institut de Recherche Microbiologique

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R.C. Meaux B 351 422 183

N° TVA intracommunautaire :
FR 423 514 221 83

TEST REPORT

N°RE-1014/0118

ISSUED TO: **HYPRED S.A.S**
55, boulevard Jules Verger
35800 DINARD

PRODUCT: **AGAKOK 2.5**

TEST REQUEST DATE: 17 January 2018

PRODUCT REFERENCE: P103/0118

ESSAI : **European Standard NF EN 14204 (December 2012): chemical disinfectants and antiseptics – quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants and antiseptics used in veterinary areas.**

Test method and requirements (phase 2, step 1).

Membrane filtration method

This report contains 4 pages.

This report applies only to the product submitted to test.

Issue date: 19 March 2018

Amandine CARRÉ
Microbiologist
Trial Manager

Philippe STROHL
Doctor of veterinary medicine
Scientific Director

cofrac ESSAIS

I. SAMPLE IDENTIFICATION

Product name: **AGAKOK 2.5**

- Batch number: 17110901
- Manufacturer: **ANTI-GERM International GmbH**
  Memmingen site: Oberbruhlstrabe 16-18
  D 87 700 Memmingen
- Expiration date: November 2020, as indicated in the sample file dated 15 January 2018 transmitted by the client
- Recommended product diluent: tap water
- Aspect of product and its dilutions: clear red fluid
- Active substances and concentrations: not indicated

Reception date: 17 January 2018

Storage conditions: at room temperature with possible exposure to light.

II. EXPERIMENTAL CONDITIONS

Analysis period: from 30 January to 27 February 2018

Identification of tested strain:

Mycobaterium avium DSM 44157 incubation 28 days at 37°C ± 1°C

Obligatory test Temperature: 10°C ± 1°C

Obligatory product-inoculum contact time: 60 min ± 10 sec

Interfering substance: low level soiling conditions
Composition: final concentration of 3 g/L of bovine albumin during the test

Product diluent used during the test: hard water at 375 mg/kg of $CaCO_3$

Stability of product diluent mix: formation of a precipitate at concentrations of 2% and 1%(v/v)

Opinion on test results: counting microorganisms in a precipitate or flocculate is difficult and unreliable.

Count method: spread plate for inoculum and membrane filtration for preliminary and main tests.

III. METHODS FOR PRELIMINARY TESTS

Rinsing liquid for membranes:

- composition: distilled water containing 0.5% (w/v) tween 80
- preparation mode: heat dissolution of ingredients and sterilization at 122°C for 15 minutes

Rinsing method for membranes:

- number of rinses: 3
- volume for each rinse: 50 ml

Particular count media: Middlebrook 7H10 agar medium with 10% OADC enrichment

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

Other additions to count medium: none

IV. METHOD VALIDATION RESULTS

Microorganism strain	Tested concentration m% (v/v)	Test suspension	Validation test			
			Validation suspension	Experimental conditions	Filtration control	Inactivation by filtration
Mycobacterium avium DSM 44157	2	10^{-6} : (106+98+95+111) (101+83+110+99) 10^{-7} : (12+10+9+9) (8+9+11+9) N = 4.0×10^8 N_0 = 4.0×10^7	(35+19+19+17) (32+29+27+31) Nv = 1045 Nv_0 = 104.5	93 94 A = 93.5	83 82 B = 82.5	71 68 C = 69.5

N : number of CFU/ml in the test suspension
N_0 : N/10
Nv : number of CFU/ml in the validation suspension
Nv_0 : Nv/10
A : number of CFU/ml in the experimental conditions validation test
B : number of CFU/ml in the membrane filtration control
C : number of CFU/ml in the membrane filtration validation test for the strains count on membranes having been treated with the product
CFU : colony forming unit

The method is validated if :

- N_0 is between 3.0×10^7 and 8.0×10^7 CFU/ml, N is between 3.0×10^8 and 8.0×10^8 CFU/ml
- Nv_0 is between 30 CFU/ml and 160 CFU/ml, N_V is between 3.0×10^2 and 1.6×10^3 CFU/ml
- The quotient of counts by pondered means is between 5.0 and 15.0.
- A, B and C are greater than or equal to $0.5 \times NV_0$.

In the described conditions, the membrane filtration method is validated on tested strain with a concentration of product **AGAKOK 2.5** of 2% (v/v).

V. TEST RESULTS

Microorganism strain	Test suspension		Results at concentration m% (v/v)		
			m = 2	m = 1	m = 0.5
Mycobacterium avium DSM 44157	10^{-6} : (106+98+95+111) (101+83+110+99)	Vc 10^{0} =	0 0	0 0	> 165 > 165
	10^{-7} : (12+10+9+9) (8+9+11+9)	Vc 10^{-1} =	0 0	0 0	> 165 > 165
	N = 4.0×10^{8}	Na =	< 140	< 140	$> 1.7 \times 10^{4}$
	N_0 = 4.0×10^{7}	R =	$> 2.9 \times 10^{5}$	$> 2.9 \times 10^{5}$	$< 2.4 \times 10^{3}$
		Log R =	**> 5.46**	**> 5.46**	< 3.39

N : number of CFU / ml in the test suspension
N_0 : 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
CFU : colony forming unit

Concentrations resulting in a log reduction of at least 4 are mycobactericidal

VI. CONCLUSION

The product **AGAKOK 2.5** is mycobactericidal on the reference strain *Mycobacterium avium* DSM 44157 at a concentration of 1% (v/v), in accordance with the standard **NF EN 14204 (December 2012)** for an obligatory contact time of 60 minutes, at an obligatory temperature of 10°C, in the presence of 3 g/l of bovine albumin in final during the test (low level soiling conditions).

END OF TEST REPORT

TEST REPORT

VIRUCIDAL ACTIVITY OF THE AGAKOK 2.5 PRODUCT AGAINST THE ENTERIC CYTOPATHOGENIC BOVINE ORPHAN VIRUS (ECBO)

For: **HYPRED SAS**
55 boulevard Jules VERGER
35 800 DINARD

Study number: n° 012V01-2018-01

VIRUCIDAL TESTS:

According to the NF EN 14675 standard (May 2015) – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in veterinary area.

Tests using the AGAKOK 2.5 product batch n° 17110901 against the enteric cytopathogenic bovine orphan virus (ECBO).

This test report included 13 pages.

Study completion date: 04/04/2018

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professeur Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
4, rue des Grandes Pièces
25770 SERRE LES SAPINS
FRANCE

2 PRODUCT IDENTITY

Reference	Batch n°
AGAKOK 2.5	17110901

- Expiration date: 11/01/2020
- Manufacturer: ANTI GERM Memmingen, Germany
- Manufacturing date: non communicated
- Storage conditions: room temperature
- Active compounds: chlorocresol
- Appearance of the product: clear, red
- Product diluent recommended by the manufacturer for use: tap water
- Date of delivery of the product: 01/19/2018
- Date of tests: from 02/12/2018 to 03/15/2018

3 EXPERIMENTAL CONDITIONS

- Temperature used during the assays: 10 °C ± 1 °C
- Titration unit: log $TCID_{50}$
- Exposure Time: 30 minutes
- Tested concentration: 0.5%, 1.0% and 2.0%
- Diluent used for the product: hard water
- Viral strain: Enteric cytopathogenic bovine orphan virus ATCC VR-248 (ECBO), grown on VERO cells, at 37 °C, under 5% CO2 atmosphere
- Organic soil load: BSA 3g/L (low dirty conditions)
- Product stability: stable
- Stop solution: cold shock

Viral titer:

Viral titers are expressed in $TCID_{50}$, according to the Spearman-Kärber method = 6,875 log $TCID_{50}$.

4 VALIDATIONS

a) Cytotoxicity

The AGAKOK 2.5 product has been tested at 2% on the VERO cells and no toxicity was observed.

b) Cells sensitivity to the virus

For each viral suspension, comparative titers of the virus were performed on cells treated or untreated with the product.

	Viral titer (log $TCID_{50}$)		
Product dilution	Viral suspension on untreated cells	Viral suspension on treated cells	**Viral titer difference (log $TCID_{50}$)**
AGAKOK 2.5 10^{-1}	6,875	6,500	**0,375**

The AGAKOK 2.5 product do not affect the infectious capacity of the virus: the differences in viral titers between the virus inoculated on VERO cells and the virus inoculated on the VERO cells treated with the AGAKOK 2.5 product was ≤ 1,0 log (0,375 log).

c) Inactivation of the virus

	Viral titer (log $TCID_{50}$)	Viral titer reduction (log $TCID_{50}$)
Viral suspension	6,875	
Formaldehyde 0,7%		
Inactivation after 5 min	6,625	
Inactivation after 15 min	6,250	
Inactivation after 30 min	5,750	**1,125**

The virus is inactivated with the control solution of 0,7 % formaldehyde after 30 min of exposure if the reduction is comprised between |-0,5| and |-2,5| log. The reduction observed was of 1,125 log for the ECBO virus.

5 VIRUCIDAL ASSAYS

TRIAL 1

The viral suspension control was titered at 6,875 log $TCID_{50}$.

PRODUCT	Final concentration (v/v)	Exposure time	Temperature	Titer after trial (log $TCID_{50}$)	Viral titer reduction
AGAKOK 2.5	**2.0%**	30 min	10°C	2,000	**4,875**
	1.0%			2,375	**4,500**
	0.5%			4,000	2,875

TRIAL 2

The viral suspension control was titered at 6,875 log $TCID_{50}$.

PRODUCT	Final concentration (v/v)	Exposure time	Temperature	Titer after trial (log $TCID_{50}$)	Viral titer reduction
AGAKOK 2.5	**2.0%**	30 min	10°C	1,875	**5,000**
	1.0%			2,500	**4,375**
	0.5%			4,000	2,875

The product has a virucidal effect if the viral titer reduction is ≥ 4,0 log.

6 VALIDATION OF THE METHODOLOGY

The assays were validated as required by the European standard EN 14675:2015:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product. The viral titer of the ECBO virus was 6,875 log $TCID_{50}$.
- The virus was inactivated with the control solution of 0,7 % formaldehyde after 30 min of exposure : the reduction observed was of 1,125 log for the ECBO virus.
- The AGAKOK 2.5 product has a weak cytotoxic effect on the VERO cells.
- The AGAKOK 2.5 product do not affect the infectious capacity of the virus: the differences in viral titers between the virus inoculated on VERO cells and the virus inoculated on the VERO cells treated with the AGAKOK 2.5 product was ≤ 1,0 log (0,375 log).

7 CONCLUSION

The assays performed with the AGAKOK 2.5 batch n° 17110901 product demonstrated that:

- **The product AGAKOK 2.5 demonstrated a virucidal activity against the ECBO virus from the concentration 1.0%, as required by the European standard EN 14675:2015, following a 30 minutes exposure period, at 10°C, in low dirty conditions.**

TECHNICAL APPENDIX 1

Cell line for ECBO virus: VERO cells (R&D Biotech, ref. 84009, batch n° 110118-110V)

Viral strain: Enteric cytopathogenic bovine orphan virus strain (ECBO), ATCC VR-248 (batch n° 58087749)

Buffer and reagents:

- Buffer PBS: sodium chloride, Dominique DUTSCHER, ref. 836751; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch n° 1019K01021V
- MEM media, PAN Biotech, ref. P04-03590, batch n° 9420112
- Horse serum, Sigma Aldrich, ref. H1270, batch n° 12J395

Organic soil load:

- Bovine Sera Albumin, Sigma Aldrich, ref. A5479, batch n° STBB7838V

Inactivation solution:

- Formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

TECHNICAL APPENDIX 2

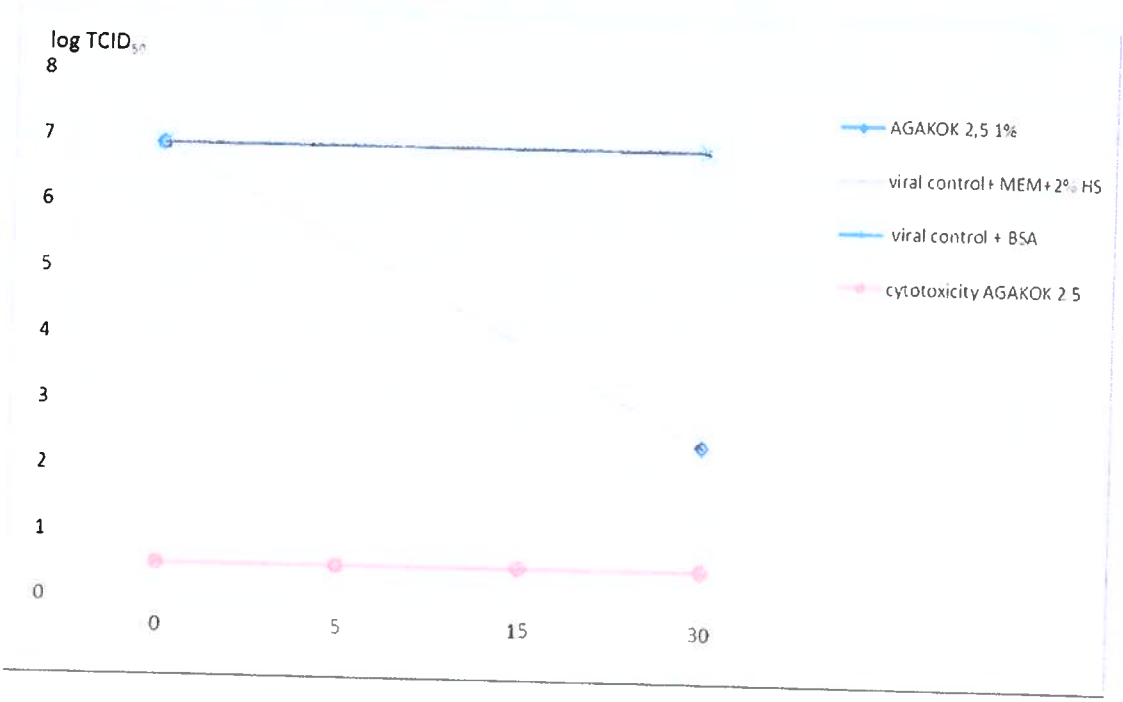
Table A1 – Viral titer of ECBO virus calculated with the Spaerman-Kärber (cytopathic effect method):

log $TCID_{50}$ = 6,875

Dilution (- log)	Result	% positive results
-3	44444444	100
-4	44444444	100
-5	44444444	100
-6	11111111	100
-7	11000001	37,5
-8	00000000	0
-9	00000000	0
Sum of the % of the positive wells		437,5

Chart 1 –graphical chart of the results :

Trial 1



Trial 2

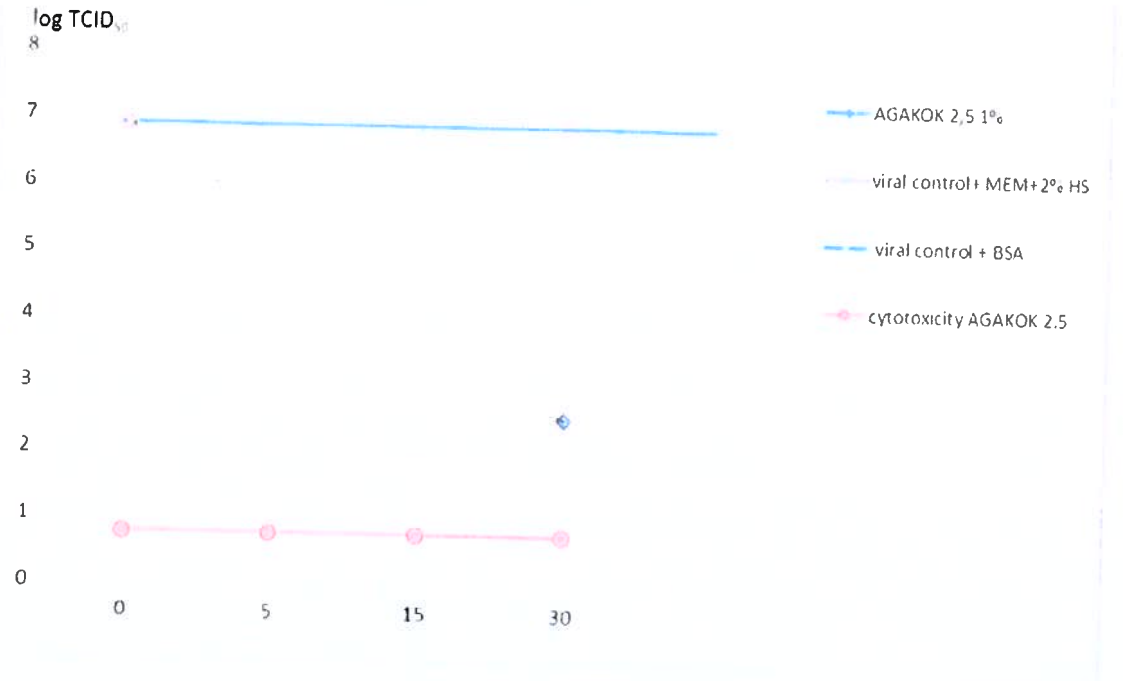


Table A2 — results with the AGAKOK 2.5 product against the ECBO virus in low dirty conditions

Product	Concentration	Organic soil load	cytotoxicity level	Lg $TCID_{50}$				Reduction
				0	5 min	15 min	30 min	
AGAKOK 2.5 TRIAL 1 AGAKOK 2.5 TRIAL 2	1.0%	3 g/L BSA	0,5	6,875	N.T.	N.T.	2,375	R= 4,5
Formaldehyde	1.0%		0,75	6,875	N.T.	N.T.	2,5	R= 4,375
infectivity control TRIAL 1	0,70%	MEM · 2% HS	2,5	6,875	6,625	6,25	5,75	
infectivity control TRIAL 2	N.A.	MEM · 2% HS	N.A	6,875	N.T.	N.T.	6,875	
	N.A.	3 g/L BSA	N.A	6,875	N.T.	N.T.	6,875	
Cells sensitivity	N.A.	MEM+2% HS	N.A	6,875	N.T.	N.T.	6,875	
	N.A.	3 g/L BSA	N.A	6,875	N.T.	N.T.	6,875	
	AGAKOK 2.5	N.A.	Untreated cells	N.T.	N.T.	N.T.	6,875	
	10^{-1}	N.A.	Treated cells	N.T.	N.T.	N.T.	6,5	

HS : Horse Serum

Other concentrations tested :

Trial 1	Concentration	organic soil load	exposure time	Log $DICT_{50}$		Reduction	
				0	30 min		
AGAKOK 2.5	2.0%	3 g/l BSA	30 min	6,875	2,000	4,875	active
	0.5%			6,875	4,000	2,875	inactive

Trial 2	Concentration	organic soil load	exposure time	Log $DICT_{50}$		Reduction	
				0	30 min		
AGAKOK 2.5	2.0%	3 g/l BSA	30 min	6,875	1,875	5,000	active
	0.5%			6,875	4,000	2,875	inactive

Table A3 - - Raw data for the AGAKOK 2.5 product against the ECBO virus, in low dirty conditions (cytopathic effect; 8 wells)

Trial 1

	Concentration	organic soil load	exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5 trial 1	1.0%	3 g l BSA	30 min	4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4444	4440	0000	0000	0000	0000	0000	0000	0000	0000
			viral	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
			control	4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
AGAKOK 2.5 trial 1 cytotoxicity	1.0%	3 g/l BSA	N.A.	0000	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
				0000	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
viral control of infectivity	N.A.	MEM+2% HS	0	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
			30	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
	N.A.	3 g l BSA	0	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
			30	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
Formaldehyde	0,70%	MEM+2% HS	5	4444	4444	4444	4444	1111	1111	1000	0000	0000	0000
				4444	4444	4444	4444	1111	1111	0000	0000	0000	0000
			15	4444	4444	4444	1111	1111	1111	0000	0000	0000	0000
				4444	4444	4433	1111	1111	1100	0000	0000	0000	0000
			30	4444	4444	4444	1111	1111	1100	0000	0000	0000	0000
				4444	4444	4444	1111	1111	0000	0000	0000	0000	0000
Formaldehyde (cytotoxicity)	0,70%	MEM+2% HS	N.A.	4444	1111	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	1111	0000	0000						

Other concentrations tested :

	Concentration	organic soil load	exposure time	dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5	2.0%	3 g l BSA	30 min	4444	1111	0000	0000	00000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000	0000
	0.5%			4444	4444	4444	4444	0000	0000	0000	0000	0000	0000
				4444	4444	4444	0000	0000	0000	0000	0000	0000	0000

Trial 2

	Concentration	organic soil load	exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5 trial 1	1.0%	3 g/l BSA	30 min	4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
			viral	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
			control	4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
AGAKOK 2.5 trial 1 cytotoxicity	1.0%	3 g l BSA	N.A.	1100	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
				0000	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
viral control of infectivity	N.A.	MEM 2% HS	0	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
			30	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
	N.A.	3 g l BSA	0	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
			30	4444	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
Formaldehyde	0,70%	MEM 2% HS	5	4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
				4444	4444	4444	4444	4444	1111	0000	0000	0000	0000
			15	4444	4444	4444	1111	1111	1111	0000	0000	0000	0000
				4444	4444	4444	1111	1111	0000	0000	0000	0000	0000
			30	4444	4444	4444	1111	1111	0000	0000	0000	0000	0000
				4444	4444	4444	1111	1111	0000	0000	0000	0000	0000
Formaldehyde (cytotoxicity)	0,70%	MEM+2% HS	N.A.	4444	4411	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	1111	0000	0000						

Other concentrations tested :

	Concentration	organic soil load	exposure time	dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5	2.0%	3 g/l BSA	30 min	4444	1110	0000	0000	00000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000	0000
	0.5%			4444	4444	4444	4444	0000	0000	0000	0000	0000	0000
				4444	4444	4444	0000	0000	0000	0000	0000	0000	0000

Sensitivity of the cells to the virus

Trial 1

Product	dilution	organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5	10^{-1}	3 g/l BSA	Untreated cells	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	1111	0000	0000	0000	0000
			Treated cells	4444	4444	4444	4 444	1111	0000	0000	0000	0000
				4444	4444	4444	4444	1111	0000	0000	0000	0000

Trial 2

Product	dilution	organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5	10^{-1}	3 g/l BSA	Untreated cells	4444	4444	4444	4444	1111	0000	0000	0000	0000
				4444	4444	4444	4444	1111	0000	0000	0000	0000
			Treated cells	4444	4444	4444	4 444	1000	0000	0000	0000	0000
				4444	4444	4444	4444	1110	0000	0000	0000	0000

Validation of the stop method (microspin filtration and cold shock)

Product	dilution	organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
AGAKOK 2.5	10^{-1}	MEM+2% HS	Cold dilution	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	1111	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	1111	1110	0000	0000	0000
				4444	4444	4444	4444	1111	0000	0000	0000	0000

Cold dilution : viral titer 6,875 R 0

Viral control : viral titer 6,875 The stop method is validated (R < 0,5 log)

EXPERT OPINION

Testing the disinfectant effect of Agakok 2.5:

Application: animal husbandry, anticoccidial effect

Batch number: 16041501

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This is an original product.

Personal expert opinion issued by:

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Basis for the expert opinion:
Decision by the DVG Disinfectant Commission to recognise in-vitro testing for *Cryptosporidium parvum* based on the SOP for the Institut für Parasitologie, Universität Leipzig (Minutes 66, Meeting of the Disinfectant Commission on 19 May 2015)

Notes:

The expert opinion is solely for presentation to the DVG's Disinfectant Committee and must be provided within one year after it is issued.
The proposals on listing made in the expert opinion are not a recommendation on application; they are to be used by the Disinfectant Committee along with the results of at least one more expert opinion to establish the values with which it is to be placed on the disinfectant list.
The expert opinion may not be used by the client for advertising purposes with a note along the lines of "tested based on DVG guidelines" until the Disinfectant Committee has listed it and thus recognised the expert opinion.

1. Introduction

The test was done based on the SOP for disinfectant testing for *Cryptosporidium parvum* of the Deutsche Veterinärmedizinische Gesellschaft (DVG; Disinfectant Committee) for listing on the "Liste geprüfter und als wirksam befundenen Desinfektionsmittel für den Tierhaltungsbereich" [List of disinfectants tested and found effective for animal husbandry] (Column 8b: antiparasitic effect/single-cell parasites).

2. Method

2.1. Test preparation

The disinfectant tested, Agakok 2.5, is a test sample from ANTI-GERM International GmbH (batch number 16041501).
The test preparation has the following properties:

- pink-coloured, clear fluid
- nothing unusual
- slightly pungent odour
- after the user solutions are applied (2.5%; 3.0%; 3.5%) milky/cloudy
- pH of the user solutions: 2.5% = pH 1.82
  3.0% = pH 1.71
  3.5% = pH 1.49

2.2. Test organism

Infectious oocysts of *Cryptosporidium (C.) parvum* about 2 months old were used to test the disinfectant effect.

2.3. Germ carrier test

The germ carrier test was conducted in accordance with the valid SOP. Various Agakok 2.5 concentrations were tested at 10°C or room temperature (18-22°C) for anticoccidial disinfectant effect and compared to positive and negative controls (see Table 1).

To do that, a suspension of *C. parvum* oocysts was placed on metal plates. After the plates were dried, disinfectant containing the relevant Agakok 2.5 concentration in standardised water hardness (WSH) to be tested was placed on the germ carrier based on the treatment group. After the 120-minute application period, the germ carrier was washed and the resulting oocyst suspension for each germ carrier was seeded in triplicate in host cell cultures (HCT8 cells). The increase in parasites in the various treatment groups was measured after 24 hours based on determining the entire cell population for each cell culture well and DNA extraction using quantitative PCR. The ability of the parasites to reproduce in the host cell culture is thus seen as a parameter for inactivation by the previous disinfection.

Table 1: Treatment groups and Agakok 2.5 test concentrations in the germ carrier test

Treatment group	*C. parvum* infection	*C. parvum* oocyst treatment (germ carrier)	application period (in hours)
Negative control germ carrier 10°C	yes	none	none
Positive control heat-inactivated germ carrier 10°C	yes	Heat (70°C for 20 minutes; physical positive control)	---
AGAKOK 2.5; 2.5% germ carrier 10°C	yes	AGAKOK 2.5; 2.5% at 10°C	2
AGAKOK 2.5; 3.0% germ carrier 10°C	yes	AGAKOK 2.5; 3.0% at 10°C	2
AGAKOK 2.5; 3.5% germ carrier 10°C	yes	AGAKOK 2.5; 3.5% at 10°C	2
Negative control germ carrier 22°C	yes	none	none
Positive control heat-inactivated germ carrier room temperature	yes	Heat (70°C for 20 minutes; physical positive control)	---
AGAKOK 2.5; 2.5% germ carrier room temperature	yes	AGAKOK 2.5; 2.5% at 22°C	2
NTC-CC	no	---	---

The negative control carried out to generate a base value for the ability of the parasite batches to reproduce was infected using untreated *C. parvum* oocysts. The physically-treated positive control — Positive control heat-inactivated germ carrier — was infected using heat-inactivated oocysts (inactivation at 70°C for 20 minutes).
The effectiveness of the various disinfectant treatments is computed based on the reduction in copies of the causative organism (determined in quantitative PCR) when compared to the negative control:

$$\%\ Reduction = 100 - \left| \frac{median\ number\ of\ copies\ sample \times 100}{median\ number\ of\ copies\ negative\ control} \right|$$

(Number of copies: number of copies of causative organism DNA measured in quantitative PCR)

Sufficient effectiveness of the disinfectant tested, Agakok 2.5, for the application concentration and temperature used was reached with at least 99% effectiveness.
It was assumed that the test was valid if the positive control reached at least the 99% threshold for effectiveness. In addition, an uninfected process control (NTC-CC) was conducted.

2.4. Suspension test

The suspension test was conducted in accordance with the valid SOP. The oocysts to be disinfected were pelletised for the various batches (treatment groups) in different reaction vessels (1.5 ml). The oocyst pellets were then treated as positive or negative controls with the concentration of Agakok 2.5 in WSH to be tested (See Table 2).

Table 2: Treatment groups and Agakok 2.5 test concentrations in the suspension test

Treatment group	*C. parvum* infection	*C. parvum* oocyst treatment (germ carrier)	application period (in hours)
Negative control suspension 10°C	yes	none	none
Positive control heat-inactivated suspension 10°C	yes	Heat (70°C for 20 minutes; physical positive control)	---
Agakok 2.5; 2.5% Suspension 10°C	yes	AGAKOK 2.5; 2.5% at 10°C	2
Agakok 2.5; 3.0% Suspension 10°C	yes	AGAKOK 2.5; 3.0% at 10°C	2
Agakok 2.5; 3.5% Suspension 10°C	yes	AGAKOK 2.5; 3.5% at 10°C	2
Negative control suspension room temperature	yes	none	none
Positive control heat-inactivated suspension room temperature	yes	Heat (70°C for 20 minutes; physical positive control)	---
AGAKOK 2.5; 2.5% room temperature	yes	AGAKOK 2.5; 2.5% at 22°C	2
NTC-CC	no	---	---

After the 120-minute application period, the oocysts were pelletised out of the disinfection suspension by centrifugation and washing twice and were then placed in a cell culture medium. The resulting oocyst suspension from each batch was sown in triplicate on host cell cultures (HCT8 cells).
The increase in parasites in the various treatment groups was measured after 24 hours based on determining the entire cell population for each cell culture well and DNA extraction using quantitative PCR (See 2.3). The ability of the parasites to reproduce in the host cell culture is thus seen as a parameter for inactivation by the previous disinfection.
The negative control carried out to generate a base value for the ability of the parasite batches used to reproduce was made using untreated *C. parvum* oocysts to infect cells. The physically-treated positive control — Positive control heat-inactivated suspension — was infected using heat-inactivated oocysts (70°C for 20 minutes).
The effectiveness of the various disinfectant treatments is computed analogous to the germ carrier test (see 2.3) based on the reduction in copies of the causative organism (determined in quantitative PCR) when compared to the negative control.
Sufficient effectiveness of the disinfectant tested, Agakok 2.5, for the application concentration and temperature used was reached with at least 99% effectiveness. It was assumed that the test was valid if the positive control reached at least the 99% threshold for effectiveness.

3. Results and conclusions

The experiment was conducted using the SOP recognised by the DVG on disinfectant testing. No copies of *C. parvum* DNA were found in the process control. Both positive controls reached the 99.0% effectiveness threshold required in both test procedures (germ carrier and suspension test) (see Tables 3 and 4).

In the groups treated with Agakok 2.5, the disinfectant effect found at 10°C was insufficient for all the concentrations tested. At room temperature (18-22°C), the tested concentration of 2.5% was sufficiently effective for disinfection of *C. parvum* oocysts in the germ carrier and in the suspension test.

As Table 3 shows, the effectiveness of Agakok 2.5 in the germ carrier tests was 99.77% in the germ carrier test with a concentration of 2.5%. In the suspension test, Agakok 2.5 in the same 2.5% concentration had an effectiveness of 99.91% (see Table 4).

Table 3: Effectiveness of the disinfection treatments in the germ carrier test

Treatment group	proven number of copies of C. parvum DNA	average effectiveness	Standard deviation (percentage of relative effectiveness)
Negative control germ carrier 10°C	10741	-	-
Positive control heat-inactivated germ carrier 10°C	512	99.52	0.03
AGAKOK 2.5; 2.5% germ carrier 10°C	375	96.51	0.44
AGAKOK 2.5; 3.0% germ carrier 10°C	401	96.26	0.73
AGAKOK 2.5; 3.5% germ carrier 10°C	626	94.18	1.58
Negative control germ carrier room temperature	12240	-	-
Positive control heat-inactivated germ carrier room temperature	45	99.63	0.01
AGAKOK 2.5; 2.5% germ carrier room temperature	28	**99.77**	0.08

Table 4: Effectiveness of the disinfection treatments in the suspension test

Treatment group	proven number of copies of C. parvum DNA	average effectiveness	Standard deviation (percentage of relative effectiveness)
Negative control suspension 10°C	27325	-	-
Positive control non-heat-inactivated suspension 10°C	2101	92.31	0.77
Positive control heat-inactivated suspension 10°C	82	99.70	0.02
Agakok 2.5; 2.5% Suspension 10°C	1217	95.55	0.67
Agakok 2.5; 3.0% Suspension 10°C	1836	93.28	0.72
Agakok 2.5; 3.5% Suspension 10°C	1481	94.58	1.72
Negative control suspension 22°C	12427	-	-
Positive control non-heat-inactivated suspension 10°C	68	99.45	0.48
Positive control heat-inactivated suspension room temperature	125	99.00	0.04
AGAKOK 2.5; 2.5% room temperature	11	**99.91**	0.03
NTC-CC	0	-	-

The tests required for entry in the "Liste geprüfter Desinfektionsmittel für die Tierhaltung" [List of disinfectants tested for animal husbandry] (suspension test and germ carrier test for the *Cryptosporidium parvum* cell culture model) have all been conducted.
In both tests, the required effectiveness of 99% using the disinfectant Agakok 2.5 at room temperature (18-22°C) and application period of 120 minutes was significantly exceeded (99.77% in the germ carrier test and 99.91% in the suspension test). At 10°C, there was not sufficient evidence of anticoccidial effectiveness in the tested concentrations of 2.5%, 3.0% and 3.5%.

We recommend that the disinfectant AGAKOK 2.5 with a concentration of 2.5% and a minimum application period of 120 minutes at room temperature be placed on the "Liste geprüfter Desinfektionsmittel für die Tierhaltung".

Leipzig, 30 September 2016

Prof. Dr A. Daugschies
Institution Director

PD Dr B. Bangoura
Researcher

Expert opinion

Testing the disinfectant effect of AGAKOK 2.5 (Anti-Germ Deutschland GmbH) for roundworm eggs *(Ascaris suum)* in pigs

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Size: 5 pages

1. Introduction

The study was conducted based on the guidelines of the Deutsche Veterinärmedizinische Gesellschaft (DVG) for *Ascaris suum* in the current, valid version (as of September 2000).

2. Method

2.1. Test preparation

The disinfectant tested is the product AGAKOK 2.5 (UN 2920; batch number 16041501) from Anti-Germ Deutschland GmbH. The test preparation has the following properties:

- clear, red fluid
- after the user solutions are applied (5%; 2.5%), milky/cloudy, pink-coloured; slightly foamy

2.2. Test organism

To test the disinfectant effect, fresh eggs taken from roundworm *Ascaris suum* per the DVG guidelines were used.

2.3. Suspension test

The disinfectant to be tested was set in doubled concentration with standardised hardness water (WSH) immediately before the test (tests at 20°C on 26 May 2016; tests at 10°C on 28 June 2016).

- AGAKOK 2.5 as a 5% solution

For each batch, 0.5 ml of the disinfectant used and 0.5 ml of egg suspension were incubated in glass Petri dishes at room temperature (10°C ± 2°C) on a tilting table. For the test at 10°C, the application was carried out with disinfectant / egg suspension at 10°C; the batches were incubated in an incubator at 10°C on a tilting table. The application periods were 30, 60, 90, 120 and 180 minutes. The termination of disinfection, washing, incubation and ventilation for 20 days in cell culture plates, counting and evaluation of the eggs (tests at 20°C on 17 June 2016; tests at 10°C on 19 July 2016) were carried out in accordance with DVG guidelines. For each batch, 6 sets of 50 eggs were counted out and the proportion of embryonised and non-embryonised eggs was determined. Only eggs with clearly visible larvae were considered embryonised.
Two test batches were done per application period.

The percentage of embryonised roundworms (absolute embryonisation rate = abs. ER) was computed and the median of the duplicate use was determined. (See Table 1)

The relative embryonisation rate (rel. ER) was computed using the following formula (see Table 1):

rel ER [%] = abs. ER [%] x 100 /abs. ER for control [%]

The disinfectant effect was evaluated using the degree of inhibition of development (see Table 1):

Effectiveness [%] = 100- rel ER [%]

Table 1:

Absolute and relative embryonisation rates as well as effectiveness after disinfection of roundworm eggs with AGAKOK 2.5 - suspension test (at 20°C and 10°C)

Concentration used	Application period (in minutes)	Absolute embryonisation rate Abs. ER in %	Relative embryonisation Rel. ER in %	Effectiveness (in percent)
		20°C		
2.5% AGAKOK 2.5	30	8.83	8.94	91.06
2.5% AGAKOK 2.5	60	4.17	4.22	95.78
2.5% AGAKOK 2.5	90	2.83	2.87	97.13
2.5% AGAKOK 2.5	120	0.00	0.00	100.00
2.5% AGAKOK 2.5	180	0.00	0.00	100.00
Control	-	98.83	-	-
		10°C		
2.5% AGAKOK 2.5	30	21.17	21.56	78.44
2.5% AGAKOK 2.5	60	10.67	10.87	89.13
2.5% AGAKOK 2.5	90	7.83	7.98	92.02
2.5% AGAKOK 2.5	120	3.33	3.40	96.60
2.5% AGAKOK 2.5	180	0.00	0.00	100.00
Control	-	98.17	-	-

2.4. Germ carrier test

The roundworm eggs were set at a density of 100,000 eggs/ml using the McMaster chamber. The disinfectant to be tested was set in single-strength concentration with standardised hardness water (WSH) immediately before the test (tests at 20°C on 30 May 2016; tests at 10°C on 28 June 2016).

- AGAKOK 2.5 as a 2.5% solution

Five ml of egg suspension (100,000 eggs/ml) were placed on the back of mats placed in plastic sleeves and prepared per DVG guidelines. After the eggs were dried for 1 hour, the disinfectant solution was applied to it and more disinfectant solution was applied 10 minutes later. For the test at 10°C, the application was carried out with disinfectant / egg suspension or stored mats at 10°C; the batches were incubated in an incubator at 10°C. After the relevant application period (30, 60, and 90, 120 or 180 minutes) had passed, the eggs were brushed off in accordance with the DVG guidelines. The termination of disinfection, washing, incubation and ventilation for 20 days in cell culture plates, counting and evaluation of the eggs (tests at 20°C on 20 June 2016; tests at 10°C on 19 July 2016) were carried out in accordance with DVG guidelines. For each batch, 6 sets of 50 eggs were counted out and the proportion of embryonised and non-embryonised eggs was determined. Only eggs with clearly visible larvae were considered embryonised.
Two test batches were done per application period.

The percentage of embryonised roundworms (absolute embryonisation rate = abs. ER) were computed and the median of the duplicate use was determined. (See Table 2)

The relative embryonisation rate (rel. ER) was computed using the following formula (see Table 2):

rel ER [%] = abs. ER [%] x 100 /abs. ER for control [%]

The disinfectant effect was evaluated using the degree of inhibition of development (see Table 2):

Effectiveness [%] = 100- rel ER [%]

Table 2:
Absolute and relative embryonisation rates as well as effectiveness after disinfection of roundworm eggs with AGAKOK 2.5 - germ carrier test (at 20°C and 10°C)

Concentration used	Application period (in minutes)	Absolute embryonisation rate Abs. ER in %	Relative embryonisation Rel. ER in %	Effectiveness (in percent)
		20°C		
2.5% AGAKOK 2.5	30	57.00	58.36	41.64
2.5% AGAKOK 2.5	60	19.67	20.14	79.86
2.5% AGAKOK 2.5	90	6.83	7.00	93.00
2.5% AGAKOK 2.5	120	3.17	3.24	96.76
2.5% AGAKOK 2.5	180	1.17	1.19	98.81
Control	-	98.17	-	-
		10°C		
2.5% AGAKOK 2.5	30	80.00	84.51	15.49
2.5% AGAKOK 2.5	60	68.33	72.18	27.82
2.5% AGAKOK 2.5	90	54.33	57.39	42.61
2.5% AGAKOK 2.5	120	40.00	42.25	57.75
2.5% AGAKOK 2.5	180	17.00	17.96	82.04
Control	-	94.67	-	-

3. Results and conclusions

The experiment was conducted using the DVG guidelines on disinfectant testing that currently apply.

The effectiveness of the disinfectant AGAKOK 2.5 at 20°C (2.5%) in the suspension test with an application of 120 minutes was over the 98% effectiveness required in the guidelines. In the application period of 120 minutes that is relevant to listing, a development inhibition of 100% was found for roundworm eggs.
When 2.5% AGAKOK 2.5 solution was used at 10°C, the 98% required effectiveness was not reached (96.6% with 120 minutes application period). It was not until they were incubated for 180 minutes that this required effectiveness was reached (100%).

The effectiveness of the disinfectant AGAKOK 2.5 at 20°C (2.5%) in the germ carrier test with an application of 120 minutes was over the 95% effectiveness required in the guidelines. In this application period of 120 minutes that is relevant to listing, a development inhibition of 96.8% was found for roundworm eggs.
When 2.5% AGAKOK 2.5 solution was used at 10°C, the 95% effectiveness required was not reached (82.0% with 180 minutes application period).

The tests required for entry in the "Liste geprüfter Desinfektionsmittel für die Tierhaltung" [List of disinfectants tested for animal husbandry] (suspension test and germ carrier test, each done for 30, 60, 90, 120 and 180 minutes; testing at 10°C) have all been conducted. The effectiveness required, 98% in the suspension test and 95% in the germ carrier test with 120 minutes application, is reached for the tested disinfectant AGAKOK 2.5 when using a 2.5% concentration at application temperatures of 20°C. For application temperatures of 10°C, the necessary effectiveness was not reached.

We recommend that disinfectant AGAKOK 2.5 (Anti-Germ Deutschland GmbH) with a concentration of 2.5%, an application temperature of 20°C and a minimum application period of 120 minutes be placed on the "Liste geprüfter Desinfektionsmittel für die Tierhaltung" for use against roundworms.

Leipzig, 21 July 2016

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