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Test Report No.: TR-23-0294

Determination of the Mycobactericidal Activity of **VITASEPT E75 GEL** according to EN 14348:2005

Test Method
EN 14348:2005
Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants – Test method and requirements (Phase 2, step 1)

Client
Goodpoint Chemicals
Urda tee 2/1
Jälgimäe 76404
Estonia

Testing Laboratory
TECOLAB Sdn. Bhd.
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Malaysia

Kuala Lumpur, 29 May 2023

Dr Marven Lee Cheng Shoou
Managing Director

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IDENTIFICATION OF TESTING LABORATORY

TECOLAB Sdn. Bhd.
J-2-6, Pusat Komersial Jalan Kuching
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IDENTIFICATION OF CLIENT

Goodpoint Chemicals
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Jälgimäe 76404
Estonia

IDENTIFICATION OF TEST ITEM

Test item name:	Vitasept E75 Gel
Lab ID:	G007-23-004
Batch no.:	23094
Expiry date:	March 2026
Manufacturer:	Goodpoint Chemicals
Receipt date:	5 April 2023
Storage conditions:	Room temperature away from sunlight
Product diluent recommended by manufacturer:	Not specified
Active substances:	80% w/w Ethanol Quaternary ammonium compounds
Product appearance:	Clear, colourless liquid

TEST METHOD & VALIDATION

Test method:	EN 14348:2005 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants – Test method and requirements (Phase 2, step 1)
Inactivation method:	Dilution-neutralization method
Inactivator:	30 g/L Tween 80 4 g/L Sodium dodecyl sulphate 3 g/L Lecithin

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EXPERIMENTAL CONDITIONS

Date of test:	5 May 2023
Product diluent:	Distilled water
Concentration / contact time:	100%* / 1 minute ± 5 seconds
Test temperature:	(20 ± 1) °C
Interfering substance:	Clean condition (0.3 g/L bovine serum albumin)
Test organism:	*Mycobacterium avium* ATCC 15769
Incubation temperature:	(37 ± 1) °C
Incubation period:	21 days
Appearance of the product dilutions:	Clear, colourless liquid
Stability and appearance of product dilutions during test:	Homogenous without any precipitate

* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

CONTROLS AND VALIDATION

Test Organism	Validation Suspension	Experimental Conditions Control	Neutralizer Control	Method Validation
M. avium ATCC 15769	N_V/10: 120.0	A: 133.0	B: 130.0	C: 124.0

The control and validation tests A, B, and C were within the basic limits:

- The number of cells per mL in the validation suspension, N_V/10, must be between 30 and 160,
- A must be equal to or greater than 0.5 x N_V/10 to verify the absence of any lethal effect in the experimental conditions,
- B must be equal to or greater than 0.5 x N_V/10 to verify the absence of neutralizer toxicity, and
- C must be equal to or greater than 0.5 x N_V/10 to validate the dilution-neutralization method.

TEST RESULTS

For each product concentration and contact time, the log reduction (lg R) is calculated using the formula lg R = lg N_0 – lg Na, in which:

- N_0 is the number of cells per mL in the test mixture at the beginning of the contact time, and
- Na is the number of cells per mL in the test mixture at the end of the contact time and before neutralization.

Test organism: *Mycobacterium avium* ATCC 15769

Test suspension, N	N_0: 4.99×10^9 lg N_0: 8.70

Concentration / Contact Time	**Test, Na**	**Reduction, lg R = lg N_0 – lg Na**
100%* / 1 minute	Na: $<1.40 \times 10^2$ lg Na: <2.15	lg R: >6.55 ± 0.11 %R: >99.99997%

* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

CONCLUSION

The test item achieved a reduction of ≥4.00 log against the test organism *Mycobacterium avium* ATCC 15769 under the tested conditions.

Therefore, **Vitasept E75 Gel** has demonstrated a mycobactericidal activity against *Mycobacterium avium* according to EN 14348:2005 under the following conditions:

Concentration	Contact Time	Test Temperature	Soiling
100%*	1 minute	20 °C	Clean condition

Kuala Lumpur, 29 May 2023

Norazzira Zulkharnain
Microbiologist

* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

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EXPERT OPINION

This expert opinion is based on the test report TR-23-0294 dated 29 May 2023. Opinions and interpretations expressed herein are outside the scope of the Laboratory Accreditation Scheme of Malaysia (SAMM).

The product **Vitasept E75 Gel** was tested according to EN 14348:2005 against *Mycobacterium avium* ATCC 15769. This organism is one of the minimum test organisms and it has been chosen as representative species for mycobacteria, taking into account its relative resistance, relevance to practical use, handling properties, and microbiological safety.

Mycobactericidal activity is defined as a capability of a product or active substance to produce a reduction in the number of viable mycobacterial cells of relevant test organisms under defined conditions. According to EN 14348, a disinfectant is considered to possess a mycobactericidal activity if it demonstrates a reduction of ≥4.00 log against the minimum spectrum of test organisms within 60 minutes when tested at 20 °C under clean (0.3 g/L bovine serum albumin) or dirty (3.0 g/L bovine serum albumin and 3.0 mL/L sheep erythrocytes) condition. A tuberculocidal activity is demonstrated if the required log reduction is achieved against *Mycobacterium terrae* only.

When tested under the following conditions, **Vitasept E75 Gel** achieved a reduction of ≥4.00 log against *Mycobacterium avium* ATCC 15769:

Concentration	**Contact Time**	**Test Temperature**	**Soiling**
100%*	1 minute	20 °C	Clean condition

Therefore, **Vitasept E75 Gel** has demonstrated a mycobactericidal activity against *Mycobacterium avium* conforming to EN 14348:2005 under the aforementioned conditions.

Kuala Lumpur, 29 May 2023

Dr Marven Lee Cheng Shoou
Managing Director

* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

INFORMATION ON MEASUREMENT UNCERTAINTY & DECISION RULE

The statement of conformity given by EN 14348:2005 states that the test item shall be considered to have passed EN 14348 if it demonstrates ≥4.00 log reduction under the defined conditions.

The laboratory employs the simple acceptance decision rule to account for the measurement uncertainty when stating the statement of conformity. The measurement uncertainty and conformance probability are shown in the raw data and are summarized as follows:

Test Organism	Concentration / Contact Time	Log Reduction	Conformance	Conformance Probability†
M. avium ATCC 15769	100%* / 1 minute	>6.55 ± 0.11	Yes	<0.001% chance of false acceptance

* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

† The conformance probability follows a normal distribution. Therefore, the percentage of conformance can never be zero or 100% due to the asymptotic tails.

RAW DATA

Test Method:	EN 14348:2005				
Product Name:	Vitasept E75 Gel			Batch No.:	23094
Product Diluent:	Distilled water			Lab ID:	G007-23-004
Appearance of Product Dilutions:	Clear, colourless solution				
Inactivation:	Dilution-neutralization	Dilution Method:	Standard (80%)	Test Temperature (°C):	20
Neutralizer:	30 g/L Tween 80, 4 g/L Sodium dodecyl sulphate, 3 g/L Lecithin				
Interfering Substance:	0.3 g/L bovine serum albumin				
Test Organism:	Mycobacterium avium ATCC 15769			Plating Method:	Spread plate
Incubation Temperature (°C):	37	Passing Criteria (lg):	4.00	Measurement Uncertainty (±):	0.11
Testing Period:	05/05/2023	Tested By:	NII	Verified By:	CSE

Validation & Controls

Validation Suspension (N_V)	V_{C1}	V_{C2}	N_{V0} = 120.0 $N_{V0} = N_V/10$
	114	126	Limit: $30 \le N_{V0} \le 160$
Validation Suspension (N_{VB})	V_{C1}	V_{C2}	N_{V0} = $N_{V0} = N_{VB}/1000$
	-	-	Limit: $30 \le N_{V0} \le 160$

Experimental Conditions Control (A)	V_{C1}	V_{C2}	A = 133.0
	132	134	Limit: $A \ge 0.5 \times N_V/10$
Neutralizer Control (B)	V_{C1}	V_{C2}	B = 130.0
	125	135	Limit: $B \ge 0.5 \times N_V/10$ or $N_{VB}/1000$
Method Validation (C)	V_{C1}	V_{C2}	C = 124.0
Conc.: 100%	126	122	Limit: $C \ge 0.5 \times N_V/10$

Test Suspension & Procedure

Test Suspension (N)	N	V_{C1}	V_{C2}	$\bar{x}_{wm} = N = 4.99E+09$
	10^{-7}	502	494	$N_0 = N/10$ $\lg N_0 = 8.70$
	10^{-8}	53	48	Limit: 8.17 $\lg N_0 \le 8.70$

Product Concentration	Contact Time	Dilution	V_{C1}	V_{C2}	Na = $\bar{x}$ or $\bar{x}_{wm}$ x 10	lg Na	lg R = lg N_0 - lg Na	Conformance Probability
100%	1 min	10^0	<14	<14	<1.40E+02	<2.15	>6.55 ± 0.11	>99.999%
		10^{-1}	<14	<14				
		10^{-2}	<14	<14			>99.99997%	
		10^{-3}	<14	<14				
		10^0						
		10^{-1}						
		10^{-2}						
		10^{-3}						
		10^0						
		10^{-1}						
		10^{-2}						
		10^{-3}						

Raw Data of Colony Count

	N_V		N_{VB}		A		B		C		N^{-7}		N^{-8}	
V_{C1}	63	51	-	-	69	63	57	68	69	57	248	254	22	31
V_{C2}	69	57	-	-	66	68	65	70	59	63	251	243	29	19

Product Concentration	Contact Time	Na^X		Na^{X-1}		Na^{X-2}		Na^{X-3}	
		V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}
100%	1 min	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0

TEST PROCEDURE

1. Test *Na*: Determination of Mycobactericidal Concentrations

   1.1 1.0 mL of the interfering substance was pipetted into a tube. 1.0 mL of the test suspension *N* (1.5 – 5.0 x 10^9 cfu/mL) was added to the tube.

   1.2 The stopwatch was started immediately and the tube was mixed and placed in a water bath controlled at test temperature θ for 2 minutes ± 10 seconds.

   1.3 At the end of the 2 minutes, 8.0 mL of the product test solution was added to the tube. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at θ for the contact time *t*. Just before the end of *t*, the tube was mixed again.

   1.4 At the end of *t*, 1.0 mL sample of the test mixture *Na* was transferred into a tube containing 8.0 mL of neutralizer and 1.0 mL of distilled water. The neutralizer tube was mixed and placed in a water bath controlled at (20 ± 1) °C.

   1.5 After a neutralization time of 5 minutes ± 10 seconds, the neutralizer tube was mixed and 1.0 mL of the neutralized test mixture *Na* (containing neutralizer, product test solution, interfering substance, and test suspension) was taken in duplicate and inoculated using the spread plate technique.

   1.6 Additionally, 0.5 mL of the neutralized test mixture *Na* was transferred into a tube containing 4.5 mL of neutralizer to obtain a 10^{-1} dilution of *Na*. The mixture was diluted accordingly in neutralizer to produce 10^{-2} and 10^{-3} dilutions of *Na*. 1.0 mL of each dilution was taken in duplicate and inoculated using the spread plate technique.

   1.7 The procedure was performed using other product test solutions at the same time.

2. Experimental Conditions Control *A*: Verification of the Absence of Any Lethal Effect in the Experimental Conditions

   2.1 1.0 mL of the interfering substance used in the test *Na* was pipetted into a tube. 1.0 mL of the validation suspension N_V (0.3 – 1.6 x 10^3 cfu/mL) was added to the tube.

   2.2 The stopwatch was started immediately and the tube was mixed and placed in a water bath controlled at test temperature θ for 2 minutes ± 10 seconds.

   2.3 At the end of the 2 minutes, 8.0 mL of hard water (distilled water for ready-to-use product) was added to the tube. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at θ for the contact time *t*. Just before the end of *t*, the tube was mixed again.

   2.4 At the end of *t*, 1.0 mL sample of the test mixture *A* was taken in duplicate and inoculated using the spread plate technique.

3. Neutralizer Control *B*: Verification of the Absence of Toxicity of the Neutralizer

   3.1 8.0 mL of the neutralizer used in the test *Na* and 1.0 mL of distilled water were pipetted into a tube. 1.0 mL of the validation suspension N_V was added to the tube.

   3.2 The stopwatch was started at the beginning of the addition and the tube was mixed and placed in a water bath controlled at (20 ± 1) °C for 5 minutes ± 10 seconds. Just before the end of this time, the tube was mixed.

3.3 At the end of the time, 1.0 mL sample of the test mixture *B* was taken in duplicate and inoculated using the spread plate technique.

4. Method Validation *C*: Validation of the Dilution-Neutralization Method

4.1 1.0 mL of the interfering substance used in the test *Na* was pipetted into a tube. 1.0 mL of diluent was added and then, starting a stopwatch, 8.0 mL of the product test solution of the highest concentration used in the test *Na* was added to the tube. The tube was mixed and placed in a water bath controlled at test temperature θ for contact time *t*. Just before the end of *t*, the tube was mixed again.

4.2 At the end of *t*, 1.0 mL of the mixture was transferred into a tube containing 8.0 mL of neutralizer used in the test *Na*. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at (20 ± 1) °C for 5 minutes ± 10 seconds.

4.3 1.0 mL of the validation suspension N_V was added. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at (20 ± 1) °C for (30 ± 1) minutes. Just before the end of this time, the tube was mixed again.

4.4 At the end of this time, 1.0 mL sample of the test mixture *C* was taken in duplicate and inoculated using the spread plate technique.

5. Incubation and Counting

5.1 The plates were incubated for 21 days. The plates were counted to determine the number of cfu. Any plates which were not countable for any reason were discarded.

5.2 For each plate, the exact number of colonies were noted but any counts higher than 330 colonies were recorded as '>330'.

5.3 All experimental data were reported as V_C values, in which a V_C value is the number of cfu counted per 1.0 mL sample inoculated.

5.4 Only V_C values within the counting limits, i.e., 14 to 330 colonies, were taken into account for further calculation, except in the case of *Na*.