

STATE INSTITUTION MARZIEIEV INSTITUTE FOR PUBLIC HEALTH OF  
THE NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE (SI  
IPH NAMSU)

Laboratory of Sanitary Microbiology and Disinfectology

Certificate of accreditation National Accreditation Agency of Ukraine No. 201480 date July 11, 2023.

Director

State institution "SI IPH NAMSU"

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*01.11.*

2024



Report

«Hydrogen peroxide 30-40%»

Mycobactericidal/tuberculocidal activity.

EN 14348:2014.

(Agreement № 7, august 1, 2024)

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and Disinfectology,

Doctor of Medicine, Professor

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Test description

Product name:	«Hydrogen peroxide 30-40%»
Sample number:	162
Batch number:	
Manufacturer:	Sanayi A.Ş., Turkiye
Manufacturer date:	
Date of delivery:	
Storage conditions:	Room temperature
Date of order:	
Test date:	October 01, 2024- October 23, 2024
Basis:	EN 14348:2014 – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants (phase 2, step 1)
Test organisms:	<i>Mycobacterium terrae</i> ATCC 15755 <i>Mycobacterium avium</i> ATCC 15769
Test solution:	9 %, 6 %, 0,3 %
Odour:	
Appearance:	clear, slightly turbid liquid
Appearance of test solutions:	colourless, turbid solutions
Active ingredients in 100 g:	Hydrogen peroxide 30-40 g
Neutralizer:	3 % Tween 80 + 3 % Saponin + 0.1 % Histidin + 0.1 % Cystein (Neutralizer II)
Interfering substance:	0.03 % albumin (clean conditions)
Test temperature:	20±1°C
Incubation temperature:	36 ± 1 °C

## Test Method

Testing is based on the European Standards EN 14348. Validation and control procedures are therefore carried out in accordance with those standards, too.

For the test, to a sample of the product «Hydrogen peroxide 30-40%» (diluted with hard water if necessary) is added to a suspension of test organisms in a solution of the interfering. The mixture is maintained at  $20 \pm 1$  °C for the required contact time. At the end of the contact time, an aliquot of 1 ml is taken; the microbicidal activity in this portion is immediately neutralized. Two 1 ml samples (per dilution step) of the resulting suspension are spread on at least 2 plates each. The number of surviving test organisms in the test mixture is calculated for each sample and the reduction is determined with respect to the corresponding test suspension  $N_0$ .

The experimental conditions (control A), the non-toxicity of the neutralizer (control B) and the dilution neutralization method (control C) are validated in accordance with the EN 14348. The test is performed under clean conditions (0.03 % albumin) using *Mycobacterium terrae* ATCC 15755, *Mycobacterium avium* ATCC 15769 as test-organisms. Results are presented in tables 1 - 2.

## Results

According to the EN 14348 the product «Hydrogen peroxide 30-40%» applied at a concentration / contact time - relation of at least 6 % - 30 min; 6 % - 60 min possesses bactericidal efficacy ( $\log R > 4$ ) at 20 °C under clean conditions for reference strains *Mycobacterium terrae* ATCC 15755, *Mycobacterium avium* ATCC 15769 (Tab. 1 - 2).

Results are validated in accordance with the requirements of the EN 14348:2014.



**Table 1. Results of the quantitative suspension test according to EN 14348**

Date:	October 01, 2024	Order number:	7
Product:	«Hydrogen peroxide 30-40%»	Sample number:	162
Test organism:	<i>Mycobacterium terrae</i> ATCC 15755	Lot number:	
Interfering substance:	0.03 % albumin		
Incubation temperature:	36 ± 1 °C	Neutralizer:	II
Test suspension (N <sub>0</sub> ):	5.15*10 <sup>8</sup> cfu / ml (8.71 log)	Incubation time:	21 days
Validation Suspension (N <sub>v</sub> ):	4.11*10 <sup>2</sup> cfu/ml (2.61 log)	Test temperature:	20± 1 °C

**contact time: 30 min**

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v <sub>c1</sub>	v <sub>c2</sub>	log Na	log R
9%	1 ml (10 <sup>0</sup> )	0	0	0	0	<14	<14	<2.15	>6.56
	1 ml (10 <sup>-1</sup> )	1	0	0	0	<14	<14		
6%	1 ml (10 <sup>0</sup> )	2	1	0	1	<14	<14	<2.15	>6.56
	1 ml (10 <sup>-1</sup> )	2	0	1	2	<14	<14		
0,3%	1 ml (10 <sup>0</sup> )	>330	>330	>330	>330	>660	>660		
	1 ml (10 <sup>-1</sup> )	>330	>330	>330	>330	>660	>660	>4.82	<3.89

**contact time: 60 min**

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v <sub>c1</sub>	v <sub>c2</sub>	log Na	log R
9%	1 ml (10 <sup>0</sup> )	0	0	0	0	<14	<14	<2.15	>6.56
	1 ml (10 <sup>-1</sup> )	0	0	0	0	<14	<14		
6%	1 ml (10 <sup>0</sup> )	0	0	0	0	<14	<14	<2.15	>6.56
	1 ml (10 <sup>-1</sup> )	1	0	0	1	<14	<14		
0,3%	1 ml (10 <sup>0</sup> )	>330	>330	>330	>330	>660	>660		
	1 ml (10 <sup>-1</sup> )	>330	>330	>330	>330	>660	>660	>4.82	<3.89

**Validation and Controls**

Validation - Suspension (N <sub>vo</sub> )				Experimental condition control (A)				Neutralizer control (B)				Method validation (C); Product concentration: 9 %						
	cfu / plate 1 & 2	V <sub>c</sub>	X		cfu / plate 1 & 2	V <sub>c</sub>	X		cfu / plate 1 & 2	V <sub>c</sub>	X		cfu / plate 1 & 2	V <sub>c</sub>	X			
V <sub>c1</sub>	48	55	103	105	V <sub>c1</sub>	52	54	106	107	V <sub>c1</sub>	58	61	119	124	V <sub>c2</sub>	51	45	96
V <sub>c2</sub>	52	54	106		V <sub>c2</sub>	53	55	108		V <sub>c2</sub>	62	66	128		V <sub>c2</sub>	45	47	92
30 ≤ $\bar{x}$ N <sub>vo</sub> ≤ 160?				$\bar{x}$ A ≥ 0,5 $\bar{x}$ N <sub>vo</sub> ?				$\bar{x}$ B ≥ 0,5 $\bar{x}$ N <sub>vo</sub> ?				$\bar{x}$ C ≥ 0,5 $\bar{x}$ N <sub>vo</sub> ?						
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no						



Table 2. Results of the quantitative suspension test according to EN 14348

Date:	October 01, 2024	Order number:	7
Product:	«Hydrogen peroxide 30-40%»	Sample number:	162
Test organism:	<i>Mycobacterium avium</i> ATCC 15769	Lot number:	
Interfering substance:	0.03 % albumin		
Incubation temperature:	36 ± 1 °C	Neutralizer:	II
Test suspension (N <sub>0</sub> ):	4.60*10 <sup>8</sup> cfu /ml (8.66 log)	Incubation time:	21 days
Validation Suspension (N <sub>v</sub> ):	1.05*10 <sup>3</sup> cfu/ml (3.02 log)	Test temperature:	20± 1 °C

contact time: 30 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v <sub>c1</sub>	v <sub>c2</sub>	log Na	log R
9%	1 ml (10 <sup>0</sup> )	0	0	0	0	<14	<14	<2.15	>6.51
	1 ml (10 <sup>-1</sup> )	0	0	0	0	<14	<14		
6%	1 ml (10 <sup>0</sup> )	0	1	1	1	<14	<14	<2.15	>6.51
	1 ml (10 <sup>-1</sup> )	1	1	1	1	<14	<14		
0,3%	1 ml (10 <sup>0</sup> )	>330	>330	>330	>330	>660	>660		
	1 ml (10 <sup>-1</sup> )	>330	>330	>330	>330	>660	>660	>4.82	<3.84

contact time: 60 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v <sub>c1</sub>	v <sub>c2</sub>	log Na	log R
9%	1 ml (10 <sup>0</sup> )	0	0	0	0	<14	<14	<2.15	>6.51
	1 ml (10 <sup>-1</sup> )	0	0	0	0	<14	<14		
6%	1 ml (10 <sup>0</sup> )	0	0	0	0	<14	<14	<2.15	>6.51
	1 ml (10 <sup>-1</sup> )	1	1	1	2	<14	<14		
0,3%	1 ml (10 <sup>0</sup> )	>330	>330	>330	>330	>660	>660		
	1 ml (10 <sup>-1</sup> )	>330	>330	>330	>330	>660	>660	>4.82	<3.84

**Validation and Controls**

Validation - Suspension (N <sub>vo</sub> )				Experimental condition control (A)				Neutralizer control (B)				Method validation (C); Product concentration: 9 %						
	cfu / plate 1 & 2	V <sub>c</sub>	X		cfu / plate 1 & 2	V <sub>c</sub>	X		cfu / plate 1 & 2	V <sub>c</sub>	X		cfu / plate 1 & 2	V <sub>c</sub>	X			
V <sub>c1</sub>	57	53	110	109	V <sub>c1</sub>	61	52	113	112	V <sub>c1</sub>	55	61	111	107	V <sub>c2</sub>	52	54	106
V <sub>c2</sub>	52	56	108		V <sub>c2</sub>	54	57	111		V <sub>c2</sub>	50	53	103		V <sub>c2</sub>	50	52	102
30 ≤ $\bar{x}$ N <sub>vo</sub> ≤ 160?				$\bar{x}$ A ≥ 0,5 $\bar{x}$ N <sub>vo</sub> ?				$\bar{x}$ B ≥ 0,5 $\bar{x}$ N <sub>vo</sub> ?				$\bar{x}$ C ≥ 0,5 $\bar{x}$ N <sub>vo</sub> ?						
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no						

В звіті пронумеровано та проінцуровано

6 аркушів

Завідувач лабораторії санітарної мікробіології  
та дезінфектології С.В. Сурмашева О.В.

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