

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"



Nadiya POLKA

01.11

2024



Report

«Hydrogen peroxide 30-40%»

Bactericidal, fungicidal and yeasticidal activity.

EN 13727:2012+A2:2015, EN 13624:2022 (EN 13624:2021 IDT).

(Agreement № 9, august 29, 2024)

Chief laboratories of sanitary microbiology
and Disinfectology,

Doctor of Medicine, Professor



Olena SURMASHEVA

Test description

Product name:	«Hydrogen peroxide 30-40%»
Sample number:	162
Batch number:	
Manufacturer:	Sanayi A.Ş., Türkiye
Manufacturer date:	
Date of delivery:	
Storage conditions:	Room temperature
Date of order:	
Test date:	October 09, 2024- October 15, 2024
Basis:	EN 13727:2012+A2:2015: Chemical disinfectants and antiseptics - quantitative suspension test for the evaluation of bactericidal activity in the medical area - test method and requirements (phase 2, step 1) EN 13624:2022 (EN 13624:2021 IDT): Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal and yeasticidal activity in the medical area - test method and requirements (phase 2, step 1)
Test organisms:	<i>Pseudomonas aeruginosa</i> ATCC15442 <i>Enterococcus hirae</i> ATCC 10541 <i>Staphylococcus aureus</i> ATCC 6538 <i>Escherichia coli</i> K 12 NCTC 10538 <i>Candida albicans</i> ATCC 10231 <i>Aspergillus brasiliensis</i> ATCC 16404
Test solution:	5 %, 3 %, 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 (<i>C. albicans</i> , <i>A. brasiliensis</i> - 30 ± 1 °C)

Test Method

Testing is based on the European Standards EN 13727 and EN 13624. 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 ± 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 No.

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 13727 or EN 13624, respectively. The test is performed under clean conditions (0.03 % albumin) using *Pseudomonas aeruginosa* ATCC15442, *Enterococcus hirae* ATCC 10541, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* K 12 NCTC 10538, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404 as test-organisms. Results are presented in tables 1 - 6.

Results

According to the EN 13727 the product «Hydrogen peroxide 30-40%» applied at a concentration / contact time - relation of at least 3 % - 30 min; 3 % - 5 min possesses bactericidal efficacy ($\log_{10} R > 5$) at 20 °C under clean conditions for reference strains *Pseudomonas aeruginosa* ATCC15442, *Enterococcus hirae* ATCC 10541, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* K 12 NCTC 10538, (Tab. 1. - 4).

According to the EN 13624, the product «Hydrogen peroxide 30-40%» applied at a concentration/contact time - relation of at least 3 % - 30 min; 3 % - 15 min possesses yeasticidal efficacy ($\log_{10} R > 4$) at 20 °C under clean conditions for reference strain *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404 (Tab. 5-6).

Results are validated in accordance with the requirements of the EN 13727:2012+A2:2015 and the EN 13624:2022 (EN 13624:2021 IDT), respectively. As outlined above, results thereby also comply with EN 13727:2015 requirements.

Table 1. Results of the quantitative suspension test according to EN 13727

Date:	October 09, 2024	Order number:	7
Product:	«Hydrogen peroxide 30-40%»	Sample number:	162
Test organism:	<i>Staphylococcus aureus</i> ATCC 6538	Lot number:	
Interfering substance:	0.03 % albumin	Neutralizer:	II
Incubation temperature:	36 ± 1 °C	Incubation time:	24 h - 48 h
Test suspension (N _o): 4.30*10 ⁷ cfu /ml (7.63 log)		Test temperature:	20± 1 °C
Validation Suspension (N _v): 5.35*10 ² cfu/ml (2.73 log)			

contact time: 5 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v _{c1}	v _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>5.49
	1 ml (10 ⁻¹)	3	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	3	1	0	1	<14	<14	<2.15	>5.49
	1 ml (10 ⁻¹)	5	5	0	2	<14	<14		
0,3%	1 ml (10 ⁰)	>330	>330	>330	>330	>660	>660		
	1 ml (10 ⁻¹)	>330	>330	>330	>330	>660	>660	>4.82	<2.81

contact time: 30 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v _{c1}	v _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>5.49
	1 ml (10 ⁻¹)	0	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	1	0	0	0	<14	<14	<2.15	>5.49
	1 ml (10 ⁻¹)	1	2	0	1	<14	<14		
0,3%	1 ml (10 ⁰)	>330	>330	>330	>330	>660	>660		
	1 ml (10 ⁻¹)	>330	>330	>330	>330	>660	>660	>4.82	<2.81

Validation and Controls

Validation - Suspension (N _{vo})				Experimental condition control (A)				Neutralizer control (B)				Method validation (C); Product concentration: 5 %						
cfu / plate 1 & 2		V _c	X	cfu /plate 1 & 2		V _c	X	cfu /plate 1 & 2		V _c	X	cfu/ plate 1 & 2		V _c	X			
V _{c1}	24	27	51	53.5	V _{c1}	56	52	108	106	V _{c1}	62	61	123	V _{c2}	31	25	56	
V _{c2}	30	26	56		V _{c2}	49	55	104		V _{c2}	59	66	125		V _{c2}	33	35	68
30 ≤ $\bar{x}N_{vo}$ ≤ 160?					$\bar{x}A \geq 0,5 \bar{x}N_{vo}$?					$\bar{x}B \geq 0,5 \bar{x}N_{vo}$?					$\bar{x}C \geq 0,5 \bar{x}N_{vo}$?			
☒ yes ☐ no					☒ yes ☐ no					☒ yes ☐ no					☒ yes ☐ no			

Table 2. Results of the quantitative suspension test according to EN 13727

Date:	October 09, 2024	Order number:	7
Product:	«Hydrogen peroxide 30-40%»	Sample number:	162
Test organism:	<i>Enterococcus hirae</i> ATCC 10541	Lot number:	
Interfering substance:	0.03 % albumin		
Incubation temperature:	36 ± 1 °C	Neutralizer:	II
Test suspension (N ₀):	6.60*10 ⁷ cfu /ml (7.82 log)	Incubation time:	24 h - 48 h
Validation Suspension (N _v):	1.35*10 ³ cfu/ml (3.13 log)	Test temperature:	20± 1 °C

contact time: 5 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V _{c1}	V _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>5.67
	1 ml (10 ⁻¹)	0	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	2	1	1	2	<14	<14	<2.15	>5.67
	1 ml (10 ⁻¹)	1	3	1	2	<14	<14		
0,3%	1 ml (10 ⁰)	>330	>330	>330	>330	>660	>660		
	1 ml (10 ⁻¹)	>330	>330	>330	>330	>660	>660	>4.82	<3.00

contact time: 30 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V _{c1}	V _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>5.67
	1 ml (10 ⁻¹)	0	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>5.67
	1 ml (10 ⁻¹)	1	1	1	2	<14	<14		
0,3%	1 ml (10 ⁰)	>330	>330	>330	>330	>660	>660		
	1 ml (10 ⁻¹)	>330	>330	>330	>330	>660	>660	>4.82	<3.00

Validation and Controls

Validation - Suspension (N _{v0})				Experimental condition control (A)				Neutralizer control (B)				Method validation (C); Product concentration: 5 %							
cfu / plate 1 & 2			V _c	X	cfu / plate 1 & 2			V _c	X	cfu / plate 1 & 2			V _c	X	cfu / plate 1 & 2			V _c	X
V _{c1}	34	32	66		V _{c1}	53	55	108		V _{c1}	54	51	105		V _{c2}	42	39	81	
V _{c2}	30	38	68	67	V _{c2}	52	50	102	105	V _{c2}	59	57	116	110,5	V _{c2}	44	45	89	85
30 ≤ $\bar{x}$ N _{v0} ≤ 160?					$\bar{x}$ A ≥ 0,5 $\bar{x}$ N _{v0} ?					$\bar{x}$ B ≥ 0,5 $\bar{x}$ N _{v0} ?					$\bar{x}$ C ≥ 0,5 $\bar{x}$ N _{v0} ?				
☒ yes		☐ no		☒ yes		☐ no		☒ yes		☐ no		☒ yes		☐ no					

Table 3. Results of the quantitative suspension test according to EN 13727

Date:	October 09, 2024	Order number:	7
Product:	«Hydrogen peroxide 30-40%»	Sample number:	162
Test organism:	<i>Escherichia coli</i> K 12 NCTC 10538	Lot number:	
Interfering substance:	0.03 % albumin		
Incubation temperature:	36 ± 1 °C	Neutralizer:	II
Test suspension (N ₀):	1.60*10 ⁷ cfu /ml (7.20 log)	Incubation time:	24 h - 48 h
Validation Suspension (N ₀):	2.90*10 ² cfu/ml (2.46 log)	Test temperature:	20± 1 °C

contact time: 5 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v _{c1}	v _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>5.06
	1 ml (10 ⁻¹)	0	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	1	0	0	1	<14	<14	<2.15	>5.06
	1 ml (10 ⁻¹)	1	2	0	0	<14	<14		
0,3%	1 ml (10 ⁰)	>330	>330	>330	>330	>660	>660		
	1 ml (10 ⁻¹)	>330	>330	>330	>330	>660	>660	>4.82	<2.38

contact time: 30 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v _{c1}	v _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>5.06
	1 ml (10 ⁻¹)	0	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>5.06
	1 ml (10 ⁻¹)	1	0	0	1	<14	<14		
0,3%	1 ml (10 ⁰)	>330	>330	>330	>330	>660	>660		
	1 ml (10 ⁻¹)	>330	>330	>330	>330	>660	>660	>4.82	<2.38

Validation and Controls

Validation - Suspension (N _{vo})				Experimental condition control (A)				Neutralizer control (B)				Method validation (C); Product concentration: 5 %							
	cfu / plate 1 & 2		V _c	X		cfu /plate 1 & 2		V _c	X		cfu /plate 1 & 2		V _c	X		cfu/ plate 1 & 2		V _c	X
V _{c1}	32	38	70	72	V _{c1}	44	42	86	91	V _{c1}	51	49	100	114	V _{c2}	37	35	72	78
V _{c2}	33	41	74		V _{c2}	45	50	96		V _{c2}	55	63	128		V _{c2}	45	39	84	
30 ≤ $\bar{x}$ Nvo ≤ 160?					$\bar{x}$ A ≥ 0,5 $\bar{x}$ Nvo?					$\bar{x}$ B ≥ 0,5 $\bar{x}$ Nvo?					$\bar{x}$ C ≥ 0,5 $\bar{x}$ Nvo?				
☒ yes ☐ no					☒ yes ☐ no					☒ yes ☐ no					☒ yes ☐ no				

Table 4. Results of the quantitative suspension test according to EN 13727

Date:	October 09, 2024	Order number:	7
Product:	«Hydrogen peroxide 30-40%»	Sample number:	162
Test organism:	<i>Pseudomonas aeruginosa</i> ATCC15442	Lot number:	
Interfering substance:	0.03 % albumin		
Incubation temperature:	36 ± 1 °C	Neutralizer:	II
Test suspension (N _t): 1.70*10 ⁷ cfu /ml (7.23 log)		Incubation time:	24 h - 48 h
Validation Suspension (N _v): 5.60*10 ² cfu/ml (2.75 log)		Test temperature:	20± 1 °C

contact time: 5 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V _{c1}	V _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>5.08
	1 ml (10 ⁻¹)	0	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	2	0	2	1	<14	<14	<2.15	>5.08
	1 ml (10 ⁻¹)	3	1	1	0	<14	<14		
0,3%	1 ml (10 ⁰)	>330	>330	>330	>330	>660	>660		
	1 ml (10 ⁻¹)	>330	>330	>330	>330	>660	>660	>4.82	<2.41

contact time: 30 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V _{c1}	V _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>5.08
	1 ml (10 ⁻¹)	0	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	0	0	1	0	<14	<14	<2.15	>5.08
	1 ml (10 ⁻¹)	1	0	1	1	<14	<14		
0,3%	1 ml (10 ⁰)	>330	>330	>330	>330	>660	>660		
	1 ml (10 ⁻¹)	>330	>330	>330	>330	>660	>660	>4.82	<2.75

Validation and Controls

Validation - Suspension (N _{vo})				Experimental condition control (A)				Neutralizer control (B)				Method validation (C); Product concentration: 5 %							
	cfu / plate 1 & 2		V _c	X		cfu /plate 1 & 2		V _c	X		cfu /plate 1 & 2		V _c	X		cfu/ plate 1 & 2		V _c	X
V _{c1}	34	36	70	79	V _{c1}	49	51	100	103	V _{c1}	55	63	118	111	V _{c2}	41	35	76	88
V _{c2}	42	46	88		V _{c2}	53	53	106		V _{c2}	53	51	104		V _{c2}	43	47	90	
	30 ≤ $\bar{x}N_{vo}$ ≤ 160?					$\bar{x}A \geq 0,5\bar{x}N_{vo}$?					$\bar{x}B \geq 0,5\bar{x}N_{vo}$?					$\bar{x}C \geq 0,5\bar{x}N_{vo}$?			
	☒ yes ☐ no					☒ yes ☐ no					☒ yes ☐ no					☒ yes ☐ no			

Table 5. Results of the quantitative suspension test according to EN 13624

Date:	October 09, 2024	Order number:	7
Product:	«Hydrogen peroxide 30-40%»	Sample number:	162
Test organism:	<i>Candida albicans</i> ATCC 10231	Lot number:	
Interfering substance:	0.03 % albumin		
Incubation temperature:	30 ± 1 °C	Neutralizer:	II
Test suspension (N ₀): 2.80*10 ⁶ cfu /ml (6.45 log)		Incubation time:	24 h - 48 h
Validation Suspension (N _v):2.58*10 ³ cfu/ml (3.41 log)		Test temperature:	20± 1 °C

contact time: 15 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v _{c1}	v _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>4.30
	1 ml (10 ⁻¹)	1	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	2	1	3	0	<14	<14	<2.15	>4.30
	1 ml (10 ⁻¹)	2	0	0	2	<14	<14		
0,3%	1 ml (10 ⁰)	>330	>330	>330	>330	>660	>660		
	1 ml (10 ⁻¹)	>330	>330	>330	>330	>660	>660	>4.82	<1.63

contact time: 30 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v _{c1}	v _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>4.30
	1 ml (10 ⁻¹)	0	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	1	0	0	0	<14	<14	<2.15	>4.30
	1 ml (10 ⁻¹)	0	2	0	1	<14	<14		
0,3%	1 ml (10 ⁰)	>330	>330	>330	>330	>660	>660		
	1 ml (10 ⁻¹)	>330	>330	>330	>330	>660	>660	>4.82	<1.63

Validation and Controls

Validation - Suspension (N _{vo})				Experimental condition control (A)				Neutralizer control (B)				Method validation (C); Product concentration: 5 %							
	cfu / plate 1 & 2		V _c	X		cfu /plate 1 & 2		V _c	X		cfu /plate 1 & 2		V _c	X		cfu/ plate 1 & 2		V _c	X
c1	55	57	112	109	v _{c1}	56	58	114	113	V _{c1}	56	60	116	121	V _{c2}	40	38	78	82
c2	50	56	106		v _{c2}	60	52	112		V _{c2}	62	64	126		V _{c2}	44	42	86	
30 ≤ $\bar{x}N_{vo}$ ≤ 160?					$\bar{x}A \geq 0,5 \bar{x}N_{vo}$?					$\bar{x}B \geq 0,5 \bar{x}N_{vo}$?					$\bar{x}C \geq 0,5 \bar{x}N_{vo}$?				
☒ yes ☐ no					☒ yes ☐ no					☒ yes ☐ no					☒ yes ☐ no				

Table 6. Results of the quantitative suspension test according to EN 13624

Date:	October 09, 2024	Order number:	7
Product:	«Hydrogen peroxide 30-40%»	Sample number:	162
Test organism:	<i>Aspergillus brasiliensis</i> ATCC 16404	Lot number:	
Interfering substance:	0.03 % albumin		
Incubation temperature:	30 ± 1 °C	Neutralizer:	II
Test suspension (N _o):	2.51*10 ⁶ cfu /ml (6.40 log)	Incubation time:	24 h - 48 h
Validation Suspension (N _v):	2.44*10 ³ cfu/ml (3.39 log)	Test temperature:	20± 1 °C

contact time: 15 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v _{c1}	v _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>4.25
	1 ml (10 ⁻¹)	1	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	0	1	1	0	<14	<14	<2.15	>4.25
	1 ml (10 ⁻¹)	0	0	0	1	<14	<14		
0,3%	1 ml (10 ⁰)	>165	>165	>165	>165	>330	>330		
	1 ml (10 ⁻¹)	>165	>165	>165	>165	>330	>330	>4.52	<1.88

contact time: 30 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v _{c1}	v _{c2}	log Na	log R
5%	1 ml (10 ⁰)	0	0	0	0	<14	<14	<2.15	>4.25
	1 ml (10 ⁻¹)	0	0	0	0	<14	<14		
3%	1 ml (10 ⁰)	0	1	1	0	<14	<14	<2.15	>4.25
	1 ml (10 ⁻¹)	0	2	0	1	<14	<14		
0,3%	1 ml (10 ⁰)	>165	>165	>165	>165	>330	>330		
	1 ml (10 ⁻¹)	>165	>165	>165	>165	>330	>330	>4.52	<1.88

Validation and Controls

Validation - Suspension (N _{vo})				Experimental condition control (A)				Neutralizer control (B)				Method validation (C); Product concentration: 5 %							
	cfu / plate 1 & 2		V _c	X		cfu /plate 1 & 2		V _c	X		cfu /plate 1 & 2		V _c	X					
V _{c1}	38	36	74	70	v _{c1}	36	32	68	68	V _{c1}	32	28	60	62	V _{c2}	27	33	60	63
V _{c2}	35	31	66		v _{c2}	30	38	68		V _{c2}	34	30	64		V _{c2}	31	35	66	
	30 ≤ $\bar{x}$ Nvo ≤ 160?					$\bar{x}$ A ≥ 0,5 $\bar{x}$ Nvo?					$\bar{x}$ B ≥ 0,5 $\bar{x}$ Nvo?					$\bar{x}$ C ≥ 0,5 $\bar{x}$ Nvo?			
	☒ yes		☐ no			☒ yes		☐ no			☒ yes		☐ no			☒ yes		☐ no	

Laboratory of Sanitary Microbiology and Disinfectology

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Bactericidal, fungicidal and yeasticidal activity.

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(Agreement № 9, august 29, 2024)

Chief laboratories of sanitary microbiology

and Disinfectology,

Doctor of Medicine, Professor

Olena SURMASHEVA

В звітї пронумеровано та прошнуровано
10 аркушів

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

«21» 11 2021 р.

