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%»

Sporicidal activity.

EN 13704:2018.

(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., Turkiye
Manufacturer date:	
Date of delivery:	
Storage conditions:	Room temperature
Date of order:	
Test date:	October 09, 2024- October 15, 2024
Basis:	EN 13704:2018: Chemical disinfectants - Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)
Test organisms:	*Bacillus subtilis* ATCC 6633
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 13704. 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 sporicidal 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 13704. The test is performed under clean conditions (0.03 % albumin) using *Bacillus subtilis* ATCC 6633.

Results are presented in tables 1.

Results

According to the EN 13704 the product «Hydrogen peroxide 30-40%» applied at a concentration / contact time - relation of at least 6 % - 30 min; 6 % - 60 min possesses sporicidal efficacy ($\log_{10}$ R > 4) at 20 °C under clean conditions for reference strains *Bacillus subtilis* ATCC 6633 (Tab. 1).

Results are validated in accordance with the requirements of the EN 13704:2018.

Table 1. Results of the quantitative suspension test according to EN 13704

ate:	October 09, 2024	Order number:	7
roduct:	«Hydrogen peroxide 30-40%»	Sample number:	162
Test organism:	*Bacillus subtilis*	Lot number:	
Interfering substance:	0.03 % albumin		
Incubation temperature:	36 ± 1 °C	Neutralizer:	II
Test suspension (N_0): $2.35*10^7$cfu /ml (7.37 log)		Incubation time:	24 h - 48 h
Validation Suspension (N_v): $6.31*10^2$cfu/ml (2.80 log)		Test temperature:	20± 1 °C

contact time: 60 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	v_{c1}	v_{c2}	log Na	log R
9%	1 ml (10^0)	0	0	0	0	<14	<14	<2.15	**>5.22**
	1 ml (10^{-1})	0	0	0	0	<14	<14		
6%	1 ml (10^0)	0	1	0	1	<14	<14	<2.15	**>5.22**
	1 ml (10^{-1})	1	2	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	**<2.55**

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
9%	1 ml (10^0)	0	0	0	0	<14	<14	<2.15	**>5.22**
	1 ml (10^{-1})	0	0	0	0	<14	<14		
6%	1 ml (10^0)	1	2	2	3	<14	<14	<2.15	**>5.22**
	1 ml (10^{-1})	1	2	2	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	**<2.55**

Validation and Controls

Validation - Suspension (N_{vo})				**Experimental condition control (A)**				**Neutralizer control (B)**				**Method validation (C); Product concentration: 9 %**			
	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}	55	41	96	100	v_{c1}	52	58	110	111	V_{c1}	59	61	120	115	V_{c2}	50	46	96	102
V_{c2}	48	56	104		v_{c2}	57	55	112		V_{c2}	58	52	110		V_{c2}	53	55	108	
$30 \leq \bar{x}Nvo \leq 160$?					$\bar{x}$ A $\geq 0{,}5\,\bar{x}$Nvo?					$\bar{x}$ B $\geq 0{,}5\,\bar{x}$ Nvo?					$\bar{x}$C $\geq 0{,}5\,\bar{x}$ Nvo?				
☒ yes ☐ no					☒ yes ☐ no					☒ yes ☐ no					☒ yes ☐ no				

В звіті пронумеровано та прошнуровано
4 аркушів

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

«01» 11 2024 р.