

State Institution "Institute of Microbiology and Immunology"

Mechnikov Institute of Microbiology and Immunology

61057 str. Pushkinskaya, Kharkiv, Ukraine

Phone: +380 57 731 31 51

E-mail: imiamn @lkr.net

www.imiamn.org.ua

REPORT

on the study of antimicrobial action of silver solutions

Under the Agreement №18 / 17 dated 22.12.2017 "On scientific and practical cooperation of the State Institution "Institute of Microbiology and Immunology" Mechnikov Institute and National Technical University "KhPI"

Head of the research stage from SI IMIAMN
Head of the Department of Biochemistry and
Biotechnology, Leading researcher T.P. Osolodchenko



Executor of the research stage is a graduate
student of the department TE of NTU "KhPI"





Підпис *Осолодченко Т.П.*
ЗАСВІДЧУЮ:
Начальник відділу кадрів
ДУ "ІМІ НАМН"

ВІДДІЛ
КАДРІВ
№02012208

Н.А. Белякова
Н.А. Белякова

Kharkiv 2019

1.1 Laboratory and strains

The research was conducted from March 27, 2019 to April 26, 2019 in the Laboratory of Biochemistry and Biotechnology of the State Institution "Institute of Microbiology and Immunology" Mechnikov Institute (Certificate of qualification №100-081/2015, valid from April 30, 2015 to April 29, 2019).

1.2 Strain.

Used strain from American Type Culture Collection (ATCC®) Escherichia coli ATCC® 25922™

2. Materials and reagents

2.1 Test-strain preparation.

Test-strain suspensions were prepared according to the recommendations of the ATCC, which are listed in the Product Sheet of selected test-strain (paragraph 1.2). The test-strain suspension was prepared with a concentration of not less than 1×10^9 CFU / ml and diluted by dechlorinated water from the Kharkiv city water supply system with a 37 °C temperature up to the initial dose (paragraph 3.2).

2.2 The nutrient medium of the test strain.

For the Escherichia coli ATCC® 25922™ test-strain used nutrient medium - nutrient agar "ATCC® Medium 3: Nutrient agar".

2.3 Dishes for breeding and sowing.

Used disposable sterile polypropylene cups to dilute the suspension of the test strain (paragraph 2.1) to the initial dose (paragraph 3.2). Petri dishes from glass with nutrient medium, which were pre-sterilized by autoclaving were used for seeding.

2.4 Water for preparation an antibacterial solution

The composition of water for the preparation of antibacterial solution is shown in the table 1:

Table 1

Ca+	$\leq 50 \text{ mg / dm}^3$	Cl-	$\leq 50 \text{ mg / dm}^3$
Na+, K+	$20\text{-}150 \text{ mg / dm}^3$	SO ₄ ²⁻	$\leq 50 \text{ mg / dm}^3$
Mg ²⁺	$\leq 50 \text{ mg / dm}^3$	Total mineralization 200-500 mg / dm ³	
pH = 6.8			

2.5 Surface for infection.

Used non-sterile latex gloves as a surface for infection. Gloves were worn on the hands, washed hands for 60 seconds with soap to remove mechanical and / or bacterial contaminants from surfaces, dried gloves with a disposable paper towel and applied 1 ml of the initial dose (paragraph 2.1) of the test strain (paragraph 3.2) with a pipette. After application, the suspension of the test strain was evenly distributed on both hands for 60 seconds.

3. Research methodology.

3.1 The antibacterial agent was applied according to En1500 HYGIENIC HANDRUB METHOD.

3.2 Initial test strain dose

The initial dose for all test strains was at non less than 5×10^4 CFU / ml.

3.3 Preparation of antimicrobial solution.

Antimicrobial solution was prepared from drinking water (table 1) using prototypes Dew Pocket v 9.0.1 CU v 2.0.1 Black and Dew Pocket v 9.0.1 CU v 2.0.1 White (identical prototypes).

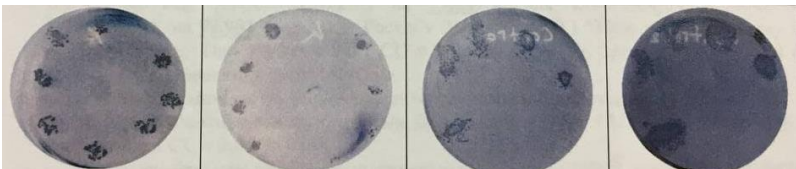
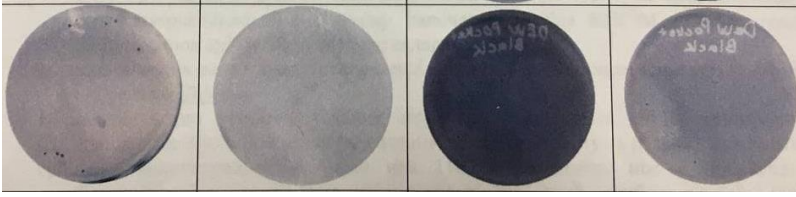
3.4 Applying antimicrobial solutions to the infected surface.

The antimicrobial agent was applied to the infected surface in the form of an aerosol from a distance of 9-10 cm. The antibacterial agent was applied evenly over the entire surface for 60 seconds, reproduced each hands movement recommended by EN1500 (paragraph 3.1), for at least 7 times. After application, the antibacterial agent was kept in contact with the infected surface for 60 seconds.

4. Study result

4.1 The research results of Dew Pocket v 9.0.1 CU v 2.0.1 Black

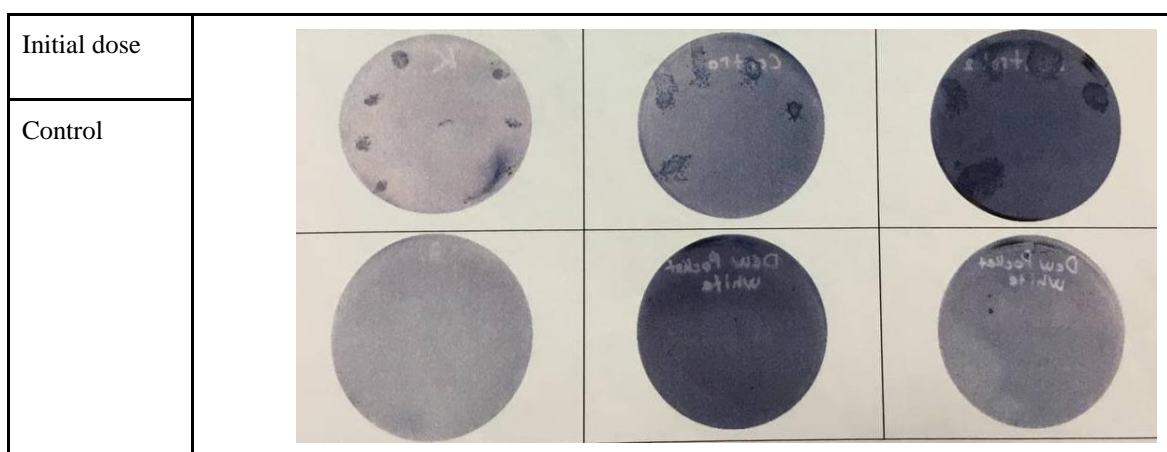
Test number	Escherichia coli ATCC® 25922™ (Gr.+Bac.)	Dew Pocket v 9.0.1 CU v 2.0.1 Black	Alcohol solution "Septol 70%" (ethyl alcohol solution)	Solution of silver nanoparticles KNC- M 20 mg/dm ³
	Initial dose	Control		
1	$\geq 5 \times 10^4$ CFU / ml	11 ($\geq 99.978\%$)	6 ($\geq 99.986\%$)	n/d
2		0 ($\geq 99.999\%$)	16 ($\geq 99.968\%$)	n/d
3		6 ($\geq 99.988\%$)	3 ($\geq 99.994\%$)	12 ($\geq 99.976\%$)
4		7 ($\geq 99.986\%$)	6 ($\geq 99.986\%$)	n/d

Initial dose				
Control				
	Test 1	Test 2	Test 3	Test 4

Test 1-4 Photo of Initial dose and Control

4.1 The research results of Dew Pocket v 9.0.1 CU v 2.0.1 White

Test number	Escherichia coli ATCC [®] 25922 TM (Gr.+Bac.)	Dew Pocket v 9.0.1 CU v 2.0.1 Black	Alcohol solution "Septol 70%" (ethyl alcohol solution)	Antibacterial solution based on silver ions DE Sept
	Initial dose	Control		
2	≥ 5x10 ⁴ CFU / ml	5 (≥99.99%)	16 (≥99.968%)	~1x10 ³ (≤98%)
3		11 (≥99.978%)	3 (≥99.994%)	n/d
4		12 (≥99.976%)	6 (≥99.986%)	n/d



Test 1-4 Photo of Initial dose and Control

Conclusions

It was found that the antimicrobial agent based on an aqueous solution of silver, synthesized by identical prototypes Dew Pocket v 9.0.1 CU v 2.0.1 Black and Dew Pocket v 9.0.1 CU v 2.0.1 White with the maximum possible concentration of the active ingredient 0.18 mg / dm³ exhibits bactericidal and bacteriostatic action against the selected gram-negative test strain.

According to the results of 7 tests, the average reduction of bacterial load of Escherichia coli ATCC[®] 25922TM: for Dew Pocket v 9.0.1 CU v 2.0.1 Black is 99.987% and Dew Pocket v 9.0.1 CU v 2.0.1 White is 99.984%. Average for prototypes Dew Pocket v 9.0.1 at an initial dose for strain not less than 5x10⁴ CFU / ml.

Compared the antibacterial effect of the test substance synthesized by the prototypes Dew Pocket v 9.0.1 CU v 2.0.1 Black and Dew Pocket v 9.0.1 CU v 2.0.1 White in accordance to the results of 7 tests:

- antibacterial action of alcohol solution "Septol 70%" (ethyl alcohol solution) - 99.983%;

- antibacterial action of solution of silver nanoparticles KNC-M 20 mg/dm³ - 99, 976%;
- antibacterial action of antibacterial solution based on silver ions DE Sept - 98%.

It was found that the synthesized antibacterial agent is not inferior to the bactericidal action of 70% solution of ethyl alcohol and a solution of silver nanoparticles with a concentration more than 110 times higher than that of the experimental antibacterial agent. It was found that the experimental antibacterial agent is more than 130 times more effective in bactericidal action than a solution of stabilized silver ions. The demonstrated level of reduction of bacterial load is sufficient for the hygienic use of the test substance.