

# In Vitro Effectiveness of Acid & Alcohol Based Ground And Surface Disinfectants Against Various Microorganisms

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## Abstract

In this study, in order to define how effective one alcohol based ground and surface disinfectant with three –acid characters is to which concentration against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 23853), *Candida albicans* (ATCC 10231) strains used for Test Microorganisms, final microorganisms was disinfectant into the different concentrations of the disinfectant to be tested by being prepared as  $2-5 \times 10^9$  CFU/ml. according to the McFarland 5 cloudiness of Test disinfectant. After disinfectants were activated with microorganisms at previously experimented 1.5, 15 and 30 minute-periods, colony counts at 1 ml. levels were performed by way of cast-cultural plaque method. Consequently, it was determined that 1% concentration of alcohol based ground and surface disinfectant influenced *Staphylococcus aureus* strain at the rate of 0.5% in 1 min., and other concentrations over 1% influenced other test microorganisms in 1 minute. It was also determined that 0.1% and over percent concentrations of the disinfectant containing peracetic acid were effective against *Staphylococcus aureus* in 1 minute, while other test microorganisms were effective in 1 minute at 0.5% and over percent concentrations.

**Key words:** Acid, Alcohol, Disinfectant, Microorganism

## INTRODUCTION

Disinfection, is an activity that is needed in all healthcare organizations of all sizes in our country. Hospital environments are important surfaces with high risk of infection of disease. This category is consists of several surfaces which are normally not in contact with patients. Environment surfaces can be divided into two main categories

a) Surfaces of medical equipments (Ex: control knobs or levers of hemodialysis machines, roentgenography machines, apparatus carriages or dental units that are frequently touched.)

b) Surfaces of inner spaces (ground, wall, tabletop, window frame .. etc...) [1]

*Staphylococcus aureus*, is the most prevailing pathogen that can cause systemic complications such as skin and mucosa infections, abscess or pneumonia, gastroenteritis, osteomyelitis and septicemia [2, 3]. Hydrogen peroxide is active as biosid in wide range against virus, bacteria, yeast, bacteria spors in disinfection and in antisepsi [4, 5, 6].

Ethyl and isopropyl alcohol are used as tuberculocide, fungicide and virucide. But the spores of bacteria are not affected [7].

Our study is aim at the research of activity of favorite acid and alcohol basis disinfectants that are used in hospitals against different microorganisms.

## MATERIAL and METHOD

The alcohol basis disinfectant that is belonging to A Company (10 g 2-Propanol, 50 g Ethanol, 0.05 g Didecylmethyl-oxethylammoniumpropionate combination), the acid basis tree disinfectants that are belonging to B Company (3.5% peracetic acid combination), C Company (5-10 g sulfamide acid, 10-15 g acilbensulfonate, 40-60 g potassiummono sulfide, 5-10s citric acid combination) and D Company (3.5% peracetic acid, hydrogen peroxide, acetic acid combination) are ground and surface disinfectants that are used in this study and they are obtained from medical stores.

### Test Microorganisms That Are Used In Trials

Test microorganisms that are used in this study such as *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 23853), *Candida albicans* ATCC (10231) strains are obtained from culture collection of our laboratory. When counting colonies of these microorganisms, for *Staphylococcus aureus*, the *Staphylococcus* medium 110 (Oxoid), for *Escherichia coli*, Violet Red Bile Agar (Oxoid), for *Pseudomonas aeruginosa*, *Pseudomonas* selective medium (Oxoid) and for *Candida albicans*, Sabouraud-dextrose agar (Oxoid) are used. According to the McFarland 5 blurriness, the final concentration of each strain which are used in this trial will be  $2-5 \times 10^9$  CFU/ml and they are prepared correspondent with this concentration [8].

### Preparation of Neutralizateur That are used in trials

After the activation of microorganisms of test with disinfectants, for inactivate them, 3% Tween80 + 3% saponin + 0.1%

Histidin + 0.1% Sistein combination is used as neutralizateur in the study [9, 10, 11].

**Determination of Disinfectants Effects**

In order to determine until which concentration the disinfectant is active, the disinfectant material with different concentration (1%, 0.5%, 0.1%, 0.05%, 0.01%, 0.005%) is distributed into tubes 9 ml. by 9 ml. in each. Then by taking 1 ml of beginning microorganism suspension for each tubes they are added to test tubes which includes disinfectants with different concentrations (1 ml + 9 ml). Microorganisms are kept waiting in test tubes that includes disinfectant materials, during designed period (1, 5, 15 and 30 minutes). At the end of these contact periods 1 ml are taken from each test tube and added on to neutralizateur mateials of 9 ml which are in different test tubes. In 1-5 minutes 0,2 ml of example are taken from each tube and are placed into plaques which includes appropriate medium. After a incubation period of 48 hours at 37 °C, colonies that are reproduced in appropriate mediums are counted and bacteria numbers in 1 ml. are calculated. At the end of the first minute, the concentration of the disinfectant that cause a decline 5 log and above (the reduction factor is 5 log and above) in the number of microorganism according to the number of microorganism that are treated with disinfectant materials is accepted as effective concentration. Besides, it is confirmed that the neutralizateur material don't have a deterrent effect on the reproduction of microorganisms and don't cause decline in the number of microorganisms. And also is is confirmed that it inactivate the effect of disinfectant material by the experiments [9, 10, 11].

**RESULTS and DISCUSSION**

The results of disinfectant A against test microorganisms are given in table 1, 2, 3, 4.

**Table 1.** S.aureus's number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with A disinfectant's solutions.

Cons. (%)	Effect duration (minute)							
	1mn	RF 5	mn R	F	15mn R	F	30mn R	F
10 -								
5	-	-	-	-	-	-	-	-
2,5	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
0,5	5.5x10 <sup>2</sup>	5.65						
0,1	3.3x10 <sup>3</sup>	4.88						
0,05 1	.7x10 <sup>3</sup>	3.16 3	x10 <sup>4</sup>	3.92 5	.5x10 <sup>2</sup>	5.65 2	.2x10 <sup>3</sup>	5.05

Microorganism do not multiply. Initial suspension: \*2.5 x 10<sup>7</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 2.5 x 10<sup>8</sup> CFU/mL (8,39 log CFU/ml)

**Table 2.** E. coli's number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with A disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1mn	RF 5	mn R	F	15mn R	F	30mn R	F
10 -								
5	-	-	-	-	-	-	-	-
2,5	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
0,5								
0,1	>10 <sup>6</sup> <	2.2	>10 <sup>6</sup> <	2.2	1.6x10 <sup>3</sup> 3	1	4x10 <sup>3</sup> 3	.06
0,05 >	10 <sup>6</sup> <	2.2	>10 <sup>6</sup> <	2.2	>10 <sup>6</sup> <	2.2	>10 <sup>6</sup> <	2.2

Microorganism do not multiply. Initial suspension: 1.6 x 10<sup>9</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 1.6 x 10<sup>8</sup> CFU/mL (8,20 log CFU/ml)

**Table 3.** S.aureus's number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with A disinfectant's solutions.

Cons. (%)	Effect duration (minute)							
	1mn R	F	5mn	RF 1	5mn	RF 3	0mn	RF
10 -								
5	-	-	-	-	-	-	-	-
2,5	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
0,5	-	-	-	-	-	-	-	-
0,1	>10 <sup>6</sup> <	2.17 >	10 <sup>6</sup> <	2.17 >	10 <sup>6</sup> <	2.17 >	10 <sup>6</sup> <	2.17
0,05 >	10 <sup>6</sup> <	2.17 >	10 <sup>6</sup> <	2.17 >	10 <sup>6</sup> <	2.17 >	10 <sup>6</sup> <	2.17

Microorganism do not multiply. Initial suspension: 1.5 x 10<sup>8</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 1.5 x 10<sup>8</sup> CFU/mL (8,17 log CFU/ml)

**Table 4.** C. albicans' number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with A disinfectant's solutions.

Cons. (%)	Effect duration (minute)							
	1mn	RF 5	mn R	F	15mn R	F	30mn R	F
10 -								
5	-	-	-	-	-	-	-	-
2,5	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
0,5	-	-	-	-	-	-	-	-
0,1	1,1x10 <sup>5</sup>	2,43 2	.2x10 <sup>4</sup>	3,13 1	.1x10 <sup>5</sup>	2,43 4	.1x10 <sup>4</sup>	2,86
0,05 >	10 <sup>5</sup>	<2,47	>10 <sup>2</sup>	<2,47	>10 <sup>5</sup>	<2,47	>10 <sup>5</sup>	<2,47

Microorganism do not multiply. Initial suspension: 3 x 10<sup>8</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 3 x 10<sup>7</sup> CFU/mL (7,47 log CFU/ml)

A disinfection's solutions have been observed against to the tested microorganism in 20 minutes.

B disinfection's gave result of against the test microorganism in the table 5, 6, 7, 8.

**Table 5.** S.aureus's number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with B disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1mn R	F	5mn	RF 1	5mn	RF 3	0mn	RF
1	-	-	-	-	-	-	-	-
0,5								
0,1	-	-	-	-	-	-	-	-
0,05 3	.3x10 <sup>3</sup> 5	.49	-	1	x10 <sup>3</sup> 6	1	x10 <sup>3</sup> 6	
0,01 5	.5x10 <sup>4</sup> 4	.26	-	5	x10 <sup>3</sup> 5	.31	5x10 <sup>3</sup> 5	.31

Microorganism do not multiply. Initial suspension: 1 x 10<sup>10</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 1 x 10<sup>3</sup> CFU/mL (9 log CFU/ml)

**Table 6.** E. coli's number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with B disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1mn R	F	5mn	RF 1	5mn	RF 3	0mn	RF
1	-	-	-	-	-	-	-	-
0,5								
0,1	-	-	-	-	-	-	-	-
0,05 -								
0,01 1	.65x10 <sup>3</sup> 3	.83	-	-	-	-	-	-

Microorganism do not multiply. Initial suspension: 1.12 x 10<sup>9</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 1.12 x 10<sup>8</sup> CFU/mL (8.04 log CFU/ml)

B solutions of disinfectant have been determined effective against the tested microorganism as from 0.05% concentration.

**Table 8.** *Aeruginosa's* number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with B disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1mn R	F	5mn	RF 1	5mn	RF 3	0mn	RF
1	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-
0.1	-	-	-	-	-	-	-	-
0.05 -	-	-	-	-	-	-	-	-
0.01 >	10 <sup>4</sup> <	3.24 >	10 <sup>3</sup> <	3.24 >	10 <sup>3</sup> <	3.24 >	10 <sup>3</sup> <	3.24 >

Microorganism do not multiply. Initial suspension: 1.75 x 10<sup>9</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 1.75 x 10<sup>8</sup> CFU/mL (8.24 log CFU/ml)

**Table 8.** *C. albicans'* number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with B disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1mn	RF 5	mn R	F	15mn R	F	30mn R	F
1	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-
0.1	-	-	-	-	-	-	-	-
0.05 -	-	-	-	-	-	-	-	-
0.01 >	10 <sup>4</sup> <	2.57 >	10 <sup>3</sup> <	2.57 >	10 <sup>3</sup> <	2.57 >	10 <sup>3</sup> <	2.57 >

Microorganism do not multiply. Initial suspension: 3.75 x 10<sup>8</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 3.75 x 10<sup>7</sup> CFU/mL (8.24 log CFU/ml)

10%, 5%, 1% solutions of C disinfectant has been used in pre-test and there is no reproduction in any microorganism after 1 minute. According to this result to find the least concentration which is C disinfectant effected different solutions that is effective under 1% were used for another tests. In the below 9, 10, 11, 12 tables show results of C disinfectant across to test microorganism.

**Table 9.** *S.aureus's* number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with C disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1mn	RF 5	mn R	F	15mn R	F	30mn R	F
1	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-
0.1	-	-	-	-	-	-	-	-
0.05 3	.3x10 <sup>5</sup>	.49	-	1	x10 <sup>5</sup> 6	1	x10 <sup>5</sup> 6	
0.01 5	.5x10 <sup>4</sup>	4.26	-	5	x10 <sup>5</sup> 5	.31	5x10 <sup>5</sup> 5	.31

Microorganism do not multiply. Initial suspension: 1 x 10<sup>10</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 1 x 10<sup>9</sup> CFU/mL (9 log CFU/ml)

**Table 10.** *E. coli's* number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with C disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1mn R	F	5mn	RF 1	5mn	RF 3	0mn	RF
1	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-
0.1	-	-	-	-	-	-	-	-
0.05 -	-	-	-	-	-	-	-	-
0.01 1	.65x10 <sup>4</sup>	3.83 -	-	-	-	-	-	-

Microorganism do not multiply. Initial suspension: 1.12 x 10<sup>9</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 1.12 x 10<sup>8</sup> CFU/mL (8.04 log CFU/ml)

**Table 11.** *P.aeruginosa's* number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with C disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1mn R	F	5mn	RF 1	5mn	RF 3	0mn	RF
1	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-
0.1	-	-	-	-	-	-	-	-
0.05 -	-	-	-	-	-	-	-	-
0.01 >	10 <sup>4</sup> <	3.24 >	10 <sup>3</sup> <	3.24 >	10 <sup>3</sup> <	3.24 >	10 <sup>3</sup> <	3.24 >

Microorganism do not multiply. Initial suspension: 1.75 x 10<sup>9</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 1.75 x 10<sup>8</sup> CFU/mL (8.24 log CFU/ml)

**Table 12.** *C. albicans'* number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with C disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1mn	RF 5	mn R	F	15mn R	F	30mn R	F
1	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-
0.1	-	-	-	-	-	-	-	-
0.05 -	-	-	-	-	-	-	-	-
0.01 >	10 <sup>3</sup> <	2.57 >	10 <sup>3</sup> <	2.57 >	10 <sup>3</sup> <	2.57 >	10 <sup>3</sup> <	2.57 >

Microorganism do not multiply. Initial suspension: 3.75 x 10<sup>8</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 3.75 x 10<sup>7</sup> CFU/mL (8.24 log CFU/ml)

C solution of disinfectant have been determined effective against the tested microorganism as from 0.05% concentration.

Result of D disinfectant against the test microorganism have been showed table 13, 14, 15, 16.

**Table 13.** *S.aureus's* number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with D disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1mn	RF 5	mn	RF 1	5 mn R	F	30mn R	F
10 -	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
2.5	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
0.5	5.5x10 <sup>2</sup> 5	.65	-	-	-	-	-	-
0.1	3.3x10 <sup>3</sup> 4	.88 -	-	-	-	-	-	-
0.05 1	.7x10 <sup>3</sup> 3	.16	3x10 <sup>3</sup> 3	.92	5.5x10 <sup>2</sup> 5	.65	2.2x10 <sup>5</sup> 5	.05

Microorganism do not multiply. Initial suspension: 2.5 x 10<sup>9</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 2.5 x 10<sup>8</sup> CFU/mL (8.39 log CFU/ml)

**Table 14.** *E. coli's* number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with D disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1dk	RF 5	dk	RF 1	5 dk R	F	30dk	RF
10 -	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
2.5	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-
0.1	>10 <sup>6</sup> <	2.2	>10 <sup>6</sup> <	2.2	1.6x10 <sup>2</sup> 3	1	4x10 <sup>7</sup>	3.06
0.05 >	10 <sup>6</sup> <	2.2	>10 <sup>6</sup> <	2.2	>10 <sup>6</sup>	<2.2	>10 <sup>6</sup>	<2.2

Microorganism do not multiply. Initial suspension: 1.6 x 10<sup>9</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 1.6 x 10<sup>8</sup> CFU/mL (8.20 log CFU/ml)

**Table 15.** *P.aeruginosa*'s number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with D disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1dk	RF 5	dk	RF 1	5 dk R	F	30dk R	F
10 -	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
2.5	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-
0.1	>10 <sup>6</sup> <	2.2	>10 <sup>6</sup> <	2.2	1.6x10 <sup>3</sup>	1	.4x10 <sup>3</sup>	.06
0.05>	10 <sup>6</sup> <	2.2	>10 <sup>6</sup> <	2.2	>10 <sup>6</sup> <	2.2	>10 <sup>6</sup> <	2.2

Microorganism do not multiply. Initial suspension: 1.5 x 10<sup>9</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 1.5 x 10<sup>8</sup> CFU/mL (8.17 log CFU/ml)

**Table 16.** *C. albicans*' number of colony in 1 ml after the time limit (CFU/ml) In different concentrations treated with D disinfectant's solutions.

Cons. (%)	Effect duration(minute)							
	1dk	RF 5	dk	RF 1	5 dk R	F	30dk R	F
10 -	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
2.5	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-
0.1	1.1x10 <sup>5</sup>	2.43 2	2x10 <sup>4</sup> 3	.13	1.1x10 <sup>2</sup> 2	.43	4.1x10 <sup>2</sup> 2	.86
0.05 >	10 <sup>5</sup>	<2.47	>10 <sup>5</sup> <	2.47 >	10 <sup>5</sup> <	2.47 >	10 <sup>5</sup> <	2.47

Microorganism do not multiply. Initial suspension: 3 x 10<sup>8</sup> CFU/mL RF: log reduction factor. Final cons. in the disinfectant: 3 x 10<sup>7</sup> CFU/mL (7.47 log CFU/ml)

D solution of disinfectant has been determined effective against the tested microorganism as from 0.05% concentration. They have found that Cloromin B Chloromix DT Dikotin and Savoprim are completely effective against to *S.aureus* and *P.aeruginosa*. (12). As a result it can be find easily and cheap as an area and ground disinfectant and also it does not take any effect from environment pathogen bacteria in a type of vegetative prepartate yet we thought.

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