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Final Report
No.004401.18-R2
(suspension volume correction)

Aminolab Number
91209.17-M

Study Title

Determination of Antimicrobial Efficacy
of MedCu Antimicrobial Wound Dressings with Copper Oxide

Test Facility

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Abbreviations

CFU	Colony Forming Unit
D/E	DeyEngley
ISO	International Standards Organisation
Log	Logarithm
MF	Membrane Filtration
N/A	Not Applicable
NB	Nutrient Broth
SOP	Standard Operating Procedure
Temp.	Temperature
TSA	Tryptic Soy Agar

1. Objective

1.1 To evaluate the degree of antibacterial activity of the non-aged Test Article (Test Article 1) and conditioned Test Article (Test Article 2, see additional description in section 6.3) following a contact period of 3 hours, using a quantitative assay.

2. Scope

2.1 The study applies to the MedCu Antimicrobial Wound Dressings with Copper Oxide, produced by MedCu Technologies.

3. Study site

3.1 The microbiological study was conducted at the Microbiology lab at Aminolab Ltd., Ness Ziona, Israel.

4. Background

4.1 The present study is based on the AATCC TM 100:2012 - Antibacterial Finishes on Textile Materials: Assessment of.

4.2 MedCu Antimicrobial Wound Dressings with Copper Oxide are non-adhesive sterile, soft, single use wound dressings composed of an internal absorbent layer containing -0.8% weight/weight (w/w) copper oxide particles and one external nonwoven layer impregnated with 3% (w/w) copper oxide particles. The external layer cover is intended to be in contact with the wound. The wound dressing size is of 10 cm x 10 cm.

4.3 The wound dressings are submitted sterile in individual sterilization pouches after they have been sterilized two times with ethylene oxide (EtO) and following shipping simulation.

4.4 The supply of D/E Broth, served as a neutralizer, was under the sponsor's responsibility.

5 References

5.3 AATCC TM 100:2012 - Antibacterial Finishes on Textile Materials: Assessment of.

5.4 Current USP <1072> Disinfectants and antiseptic.

5.5 ANSI/AAMI/ISO 11737-1:2006/(R) 2011 & ISO 11737-1:2018: Sterilization of

medical device - Microbiological methods - Part 1: Estimation of population microorganisms on products.

5.6 Aminolab SOP No. 50.WI.106/ Version 6: Bioburden Estimation.

5.7 Aminolab SOP No. 50.WI.107/ Version 7: Validation of Microbiological Techniques Employed in Bioburden.

5.8 Aminolab SOP NO. 50. WI.132/ Version 7: Stock culture maintenance.

6 Test Articles

6.1 Test Item: MedCu Antimicrobial Wound Dressings with Copper Oxide, Ref # 2C-1010-01, Lot # 170303.

6.2 Control Items: Life 3M Wound Dressings without copper or any antimicrobial agent with similar construction as the Test Item, Ref: Life, by 3M Israel, 91 Medinat Hayehudim St. Herzelia.

6.3 From each Test Item and Control Item, swatches of 3.3 cm x 3.3 cm were aseptically cut. The following test groups and time points were tested in the present study:

Test Group	Test Group Description	Time Point tested
Test Article 1	20 non-aged individual units of MedCu Antimicrobial Wound Dressings with Copper Oxide	Time "0"
		Time- "3 hours"
Test Article 2	20 individual units of 2-year accelerated aged MedCu Antimicrobial Wound Dressings with Copper Oxide, and two times EtO sterilized	Time "0"
		Time- "3 hours"
Control Article	20 individual units	Time "0"
		Time- "3 hours"

7 Test Microorganism

7.1 The following microorganisms were used for the study:

7.1.1 Methicillin-resistant *Staphylococcus aureus* - (MRSA) (ATCC 33591) (MicroBiologics®, Lot: 496-72, Expiry Date: 31.01.2019)

7.1.2 *Staphylococcus epidermidis* (ATCC 12228) (MicroBiologics®, Lot: 371-128, Expiry Date: 31.08.2018)

7.1.3 *Enterococcus faecalis* (ATCC 51575)-Vancomycin resistant enterococci - (VRE) (MicroBiologics®, Lot: 1089-09, Expiry Date: 30.09.2018)

7.1.4 *Pseudomonas aeruginosa* (ATCC 9028) (MicroBiologics®, Lot: 484-854, Expiry Date: 31.05.2019)

7.1.5 *Escherichia coli* (ATCC 8739) (MicroBiologics®, Lot: 483-662, Expiry Date: 31.05.2019)

7.1.6 *Candida albicans (ATCC 10231)* MicroBiologics®, Lot: 443-735, Expiry Date: 30.04.2019)

8 Equipment

8.1 An incubator for temp. 35±2°C – AL-1036

8.2 A refrigerator for temp. 2-8°C – AL-640

8.3 An incubator for temp. 20-25°C – AL-910

8.4 A Laminar Flow Hood – AL-81/2

8.5 A manifold and vacuum pump for membrane filtration – AL-087/3 & AL-768

8.6 A vortex – AL-759

8.7 A colony counter – AL-755

8.8 A water bath – AL-1243

8.9 A timer – AL-1207

8.10 Pipetors – PM-233, PM-239

8.11 A stomacher – AL-70/1

9 Growth Media and Material

9.1 D/E Broth, supplied by the sponsor;

9.2 0.9% Saline with 0.1% Tween, Laboratory expiry dates: 09.02.2017, 15.02.2018, 19.02.2018,21.02.2018, 27.02.2018, 04.03.2018, 05.03.2018, 11.03.2018;

9.3 5% N/B in 0.9% Saline with 0.1% Tween, Laboratory expiry dates: 13.02.2018;

9.4 Baird Parker Agar:

Batch 1/2, Oxoid, Lot# 2137638, Laboratory expiry date: 05.02.2018;

Hy-Labs, Lot# 278295, Laboratory expiry date: 12.12.2017;

9.5 m-Enterococcus Agar:

Batch 3/21, Acumedia, Lot # 108628, Laboratory expiry date: 28.11.2017;

Batch 3/22, Acumedia, Lot # 108628, Laboratory expiry date: 04.12.2017;

9.6 m-PA-C Agar:

Batch 1/20, BD, Lot# 298153, Laboratory expiry date: 05.12.2017;

Batch 2/1, BD, Lot# 6183572, Laboratory expiry date: 25.12.2017;

9.7 m-FC Agar:

BD, Lot# 278002, Expiry date: 30.01.2018;

Hy-Labs, Lot# 278150, Expiry date: 04.02.2018;

9.8 Sabouroud Dextrose Agar:

BD, Lot#6258587, Expiry date: 05.02.2018

9.9 Tween 80, Sigma, Lot# BCBT8094, Laboratory expiry date: 23.10.2018.

10 Glassware, Plastic Ware and Lab Tools

10.1 Sterile test tubes with appropriate rack.

10.2 Petri dishes.

10.3 Class A pipettes.

10.4 Membrane filters 0.45µm.

10.5 Sterile tweezers and scissors.

10.6 Sterile containers and bags.

11 Inoculum preparation

11.1 For the study purposes all microorganisms were freshly prepared from recently revived stock cultures of the microorganisms. All prepared suspensions contained about 10^5-10^6 CFU/mL.

11.2 Serial dilutions from each suspension were prepared and 1 mL from the appropriate dilution was filtered through a 0.45µm membrane filter in duplicates, to determine the size of inoculum. The filters were placed onto appropriate selective agar plate.

11.3 Sabouroud Dextrose Agar were incubated at 20-25ºC for 4 days.All plates were incubated at 35±2ºC for 2-3 days.

11.4 The colonies of microorganisms were counted and recorded.

11.5 The results of the inoculum count for the test microorganisms are summarized in section 13.10.

12 Neutralization Validation

Note: A validation was performed in order to demonstrate the suitability of the test method and absence of toxicity of the neutralizer (D/E Broth) for microorganisms. This test was performed prior to the antimicrobial efficacy study and was performed separately for each microorganism.

12.1 Three (3) non-inoculated swatches from the Test Article 1 group were placed into sterile bags, containing 100 mL of D/E Broth and stomached for 2 minutes.

12.2 Each extract was filtered through 0.45µm filter membranes.

12.3 100 mL of 5% NB in saline with 0.1% Tween 80, containing about 100 CFU of each of the test microorganism suspension were filtered through 0.45µm filter membranes and used for viability control (in triplicates).

12.4 All membranes, described in sections 12.2 and 12.3 were rinsed with 100mL of saline with 0.1% Tween 80 twice.

12.5 The membranes, described in section 12.2 were rinsed with additional 100 mL of saline with 0.1% Tween 80, containing about 100 CFU of each of the test microorganism suspension

12.6 Each one of the membranes was transferred onto appropriate selective media plate and incubated at 35±2°C for 2-3 days.

12.7 After the incubation, the total number of colonies on each plate was counted and recorded.

12.8 The percent recovery was calculated according to the following formula:

% Recovery = (Average of triplicate test article / Average of viability control) x 100

Acceptance Criteria:

The Test Article group shall demonstrate a number of recovered CFU not less than 80% of that recovered from the viability control.

12.9 The results are summarized in the following table:

Test Microorganisms	Colony count CFU/ plate (average of triplicate)		
	Viability control	Test article	% recovery
Staphylococcus aureus (ATCC 33591)	79	72	91.0
Staphylococcus epidermidis (ATCC 12228)	29	24	82.8
Enterococcus faecalis (ATCC 51575)	98	92	93.9
Escherichia coli (ATCC 8739)	95	88	92.6
Pseudomonas aeruginosa (ATCC 9027)	51	42	82.4
Candida albicans (ATCC 10231)	27	22	81.5

Conclusion: The tests for all microorganisms, met the acceptance criteria for neutralization validation.

13 Test Articles Inoculation and Antimicrobial Efficacy Test

13.1 According to the sponsor's instructions, swatches, that were cut and labeled as described in section 6.3, were placed in sterile petri plates.

13.2 Each swatch was inoculated with 1ml of suspension from each of the tested microorganisms containing about 10^5-10^6 CFU/ml.

13.3 Subsequently, swatches from Time "0" group were immediately transferred to sterile bags, containing 100mL D/E Broth and stomached for 2 minutes each.

13.4 Serial dilutions were prepared and filtered through 0.45μm filter membranes. Those membranes were additionally rinsed with 100mL of D/E Broth twice and transferred onto appropriate selective agar plate.

13.5 All plates were incubated at 35±2°C for 2-3 days, excluding Sabouroud Dextrose Agar plates, were incubated at 20-25°C for 4 days.

13.6 Swatches from Time "3 hours" group were closed and transferred to 35±2°C immediately after inoculation. After 3 hours of incubation, steps described in sections 13.3-13.5 were repeated for this group.

13.7 After the incubation, the total number of colonies (CFU/filter) on each plate was counted and recorded.

13.8 The results are expressed as CFU (Colony Forming Unit) per swatch.

with Time "0" was calculated.

Log A - Log B = Log reduction

where

A = The number of bacteria recovered from the inoculated treated test specimen in the petri plate immediately after inoculation (at zero contact time).

B = The number of bacteria recovered from the inoculated treated test specimen swatches in the petri plate incubated over the desired contact period (3 hours).

Acceptance Criteria

The "3 hours" samples should demonstrate 4 log reduction as compared to the Time "0" samples.

13.10 The results are summarized in the following tables:

Inoculum sizes

Test date	Name of Microorganism	Inoculum size CFU/ mL
16.11.2017	*Staphylococcus aureus* (ATCC 33591)	$7.0x10^5$
20.11.2017	*Enterococcus faecalis* (ATCC 51575)	$2.1x10^7$
04.12.2017	*Escherichia coli* (ATCC 8739)	$6.7x10^6$
05.12.2017	*Staphylococcus epidermidis* (ATCC 12228)	$2.4x10^6$
13.12.2017	*Pseudomonas aeruginosa* (ATCC 9027)	$6.1x10^6$
22.112017	*Candida albicans (ATCC 10231)*	$7.9 x10^6$

***Staphylococcus aureus* (ATCC 33591)**				
Test Article 1				
Time "0"		Time- "3 hours"		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
4.60×10^4	4.66	3	0.48	4.18
5.80×10^4	4.76	5	0.70	4.06
5.40×10^4	4.73	1	0.00	4.73
Test Article 2				
Time "0"		Time- "3 hours"		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
1.58×10^5	5.20	2	0.30	4.90
8.90×10^4	4.95	4	0.60	4.35
7.10×10^4	4.85	3	0.48	4.37
Control Article				
Time "0"		Time- "3 hours"		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
6.50×10^5	5.81	4.30×10^5	5.63	0.18
5.10×10^5	5.71	4.90×10^5	5.69	0.02
6.40×10^5	5.81	5.20×10^5	5.72	0.09

Enterococcus faecalis (ATCC 51575)				
Test Article 1				
Time "0"		Time- "3 hours"		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
4.50×10^6	6.65	133	2.12	4.53
4.40×10^6	6.64	171	2.23	4.41
7.10×10^6	6.85	209	2.32	4.53
Test Article 2				
Time "0"		Time- "3 hours"		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
9.50×10^6	6.98	187	2.27	4.71
8.60×10^6	6.93	185	2.27	4.66
7.10×10^6	6.85	246	2.39	4.46
Control Article				
Time "0"		Time- "3 hours"		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
1.94×10^7	7.29	1.86×10^7	7.27	0.02
2.12×10^7	7.33	1.76×10^7	7.25	0.08
1.90×10^7	7.28	2.01×10^7	7.30	N/A

Escherichia coli (ATCC 8739)				
Test Article 1				
Time "0"		Time- "3 hours "		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
5.60×10^6	6.75	9	0.95	5.80
7.00×10^6	6.85	11	1.04	5.81
6.10×10^6	6.79	1	0.00	6.79
Test Article 2				
Time "0"		Time- "3 hours "		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
5.40×10^6	6.73	94	1.97	4.76
6.40×10^6	6.81	82	1.91	4.91
4.60×10^6	6.66	28	1.45	5.21
Control Article				
Time "0"		Time- "3 hours "		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
6.00×10^6	6.78	1.93×10^6	6.29	1.49
5.70×10^6	6.76	1.28×10^6	6.11	0.15
3.60×10^6	6.56	1.85×10^6	6.27	0.29

***Staphylococcus epidermidis* (ATCC 12228)**				
Test Article 1				
Time "0"		Time- "3 hours "		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
1.85×10^6	6.27	109	2.04	4.23
1.69×10^6	6.23	144	2.16	4.07
1.48×10^6	6.17	108	2.03	4.14
Test Article 2				
Time "0"		Time- "3 hours "		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
1.82×10^6	6.26	41	1.61	4.65
1.55×10^6	6.19	56	1.75	4.44
1.59×10^6	6.20	81	1.91	4.29
Control Article				
Time "0"		Time- "3 hours "		
CFU/swatch	Log (A)	CFU/swatch	Log (B)	Log reduction
1.65×10^6	6.22	2.07×10^6	6.32	N/A
1.85×10^6	6.27	1.69×10^6	6.23	0.04
1.90×10^6	6.28	1.96×10^6	6.29	N/A

Pseudomonas aeruginosa (ATCC 9027)				
Test Article 1				
Time "0"		**Time- "3 hours "**		
CFU/swatch	**Log (A)**	**CFU/swatch**	**Log (B)**	**Log reduction**
3.50×10^6	6.54	76	1.88	4.66
2.90×10^6	6.46	65	1.81	4.65
3.70×10^6	6.57	91	1.96	4.61
Test Article 2				
Time "0"		**Time- "3 hours "**		
CFU/swatch	**Log (A)**	**CFU/swatch**	**Log (B)**	**Log reduction**
4.10×10^6	6.61	18	1.26	5.35
4.80×10^6	6.68	33	1.52	5.16
4.10×10^6	6.61	25	1.40	5.21
Control Article				
Time "0"		**Time- "3 hours "**		
CFU/swatch	**Log (A)**	**CFU/swatch**	**Log (B)**	**Log reduction**
4.50×10^6	6.65	2.10×10^5	5.32	1.33
5.80×10^6	6.76	2.50×10^5	5.40	1.36
5.50×10^6	6.74	2.70×10^5	5.43	1.31

Candida albicans (ATCC 10231)				
Test Article 1				
Time "0"		**Time- "3 hours "**		
CFU/swatch	**Log (A)**	**CFU/swatch**	**Log (B)**	**Log reduction**
3.50×10^6	6.54	3.70×10^5	5.57	0.97
3.80×10^6	6.58	4.50×10^5	5.65	0.93
3.00×10^6	6.48	5.10×10^5	5.71	0.77
Test Article 2				
Time "0"		**Time- "3 hours "**		
CFU/swatch	**Log (A)**	**CFU/swatch**	**Log (B)**	**Log reduction**
4.30×10^6	6.63	3.40×10^5	5.53	1.10
3.20×10^6	6.51	3.80×10^5	5.58	0.93
2.80×10^6	6.45	4.9×10^5	5.69	0.76
Control Article				
Time "0"		**Time- "3 hours "**		
CFU/swatch	**Log (A)**	**CFU/swatch**	**Log (B)**	**Log reduction**
3.90×10^6	6.59	4.60×10^6	6.66	N/A
4.20×10^6	6.62	4.80×10^6	6.68	N/A
2.90×10^6	6.46	4.10×10^6	6.61	N/A

Conclusion:

More than 4-Log reduction of all microorganisms was achieved following 3 hours in both Test Article groups with the exception of *Candida albicans*.

Examined by Naira Gortsunian, Ph.D: _____

Checked and confirmed by Helena Ashkenazi, Ph.D, Department Manager: _____

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