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Determination of Antimicrobial Efficacy MedCu Antimicrobial Wound Dressing 2C-1010-01 using Test Method AATCC 100


Purpose:	Determination of antimicrobial efficacy of the wound dressing (WD) 2C-1010-01 non-aged (Test Article 1) and after accelerated 2-year aging (Test Article 2), against <i>Klebsiella pneumoniae</i> .
Test article details:	MedCu Antimicrobial Wound Dressings with Copper Oxide Test Article Ref: 2C-1010-01 Test Article Lot #: 170303 Test Article Description: MedCu Antimicrobial Wound Dressings with Copper Oxide are nonadhesive 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.
Control article:	Name: Life 3M Wound Dressings Control Article Ref: Life, by 3M Israel, 91 Medinat Hayehudim St. Herzelia. Control Article Description: The Life 3M Wound Dressings are wound dressings without copper or any antimicrobial agent with similar construction as the Test Item. They are composed of an internal absorbent layer and an external non adherent nonwoven layer. The wound dressing size is of 10 cm x 10 cm. The wound dressings are sterile in individual sterilization pouches.
Treatments:	Test Article 1 and 2 placed in individual sterilization pouches, have been shipping and handling simulated. Test Item 2 was sterilized two times with ethylene oxide (EtO), and then was 2-years aged using accelerated aging simulation. The Control articles were EtO sterilized.
Test organism:	<i>Klebsiella pneumoniae</i> ATCC 4352.
Equipment:	37°C Incubator, Stomager (Bag Mixer), Pall filtration device; a 0.45 µm pore size membrane (Millipore catalogue number EZHAWG474); petri dishes.
Exposure time:	0 and 3 hours.
Sample size:	Triplicate samples per test item per time point.
Number of layers:	One.
Target Inoculum level per sample:	(1-2) x 10 ⁶ Colony Forming Unit (CFU).

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Inoculum volume:	1.0 ml.
Inoculum carrier:	Nutrient Broth (NB, Sigma-Aldrich - catalog number 03856 media).
Neutralizer:	DeyEngley (D/E) Broth (LAB187, Lab M Limited, UK).
Neutralizer volume:	100 ml.
Growth selective media:	CHROMagar™ Orientation, CHROMagar, France.
Additional Media:	Tryptone Soya Broth (TSB), sterile 0.85% saline/0.1% Tween 80 (ST).
Incubation Temperature:	37±2°C.
Neutralization Validation	<ol style="list-style-type: none"> Three 3.3 cm x 3.3 cm swatches of each of the Test Article 1 or Test Article 2 were cut and each one placed in individual sterile bags containing 100 mL D/E Broth. The samples were stomached for 2 minutes in a Stomacher. The entire 100 mL of D/E Broth containing the extract from the samples were passed through 0.45 µm filter membranes by using a Pall filtration device and the filters were rinsed two times with 100 mL ST. The filters were then rinsed a third time with another 100 mL aliquot of ST containing ~100 CFU of the bacteria being tested. The filtered membranes were placed on petri dishes containing Chromagar, and incubated at 37±2°C for 3 days. In parallel, for viability control, three separate aliquots of 100 mL of 5% NB in 0.85% saline containing ~100 CFU of the tested bacteria were passed through 0.45 µm filter membranes and then two times rinsed with 100 mL ST. The filtered membranes were placed on petri dishes containing Chromagar and incubated at 37±2°C for 3 days. The CFU were counted after 3 days of incubation. The percent recovery was calculated according to the following formula: Percent recovery = % Recovery = (Average of triplicate test article / Average of viability control) x 100 Acceptance criteria: > 80% recovery.
Antimicrobial determination:	<ol style="list-style-type: none"> A fresh transplant from a stock culture of <i>Klebsiella pneumoniae</i> was taken and grown overnight at 37±2°C in TBS. A standard plate count was performed and the bacterial population titer was determined. The microorganism population was adjusted to 1-2x10⁷ CFU per ml in 5% NB and ST.

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	<ol style="list-style-type: none"> 4. 10 ml of the overnight culture was added to a 90 ml ST containing 5% NB. 5. 1000±10 µl of the above solution was added to each test sample, making sure that all liquid was completely absorbed into the control and test samples. 6. All samples (with the exemption of the 0 time controls) were put in a disposable vessel and closed hermetically to prevent any evaporation. 7. The samples were then incubate at 37±2°C for 3 hours. 8. The “0 minutes” test samples were added immediately to bags containing 100±5 ml of D/E broth. 9. The “0 minutes” and “3 hours” test samples bags were stomached vigorously immediately or after the 3 hr of incubation, accordingly, for 2 minutes with a Stomager (Bag mixer). 10. 10±1 µl; 100±10 µl, 1±0.1 ml and 10 ml ±0.3 ml aliquots of the liquid from each bag were added to 50±5 ml D/E broth and passed through a 0.45 µm pore size membranes using a Pall filtration device. 11. The filters containing the bacteria were then rinsed twice with 100±5 ml of ST. 12. The membranes with bacteria were then placed on Petri dish containing Chromagar and incubated at 37±2°C for 3 days. 13. The CFU were then determined. 14. The log reduction was determined using the following formula: $\log A - \log B = \log \text{reduction}$, where A is the colony forming units of the initial inoculum, and B is the log of the remaining bacteria in the test sample. The mean of triplicates is used for the calculations. 15. Acceptance Criteria: The "3 hours" samples should demonstrate 4 log reduction as compared to the Time "0" samples.
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Results

Table 1: Neutralization Validation

Sample	Replicate	CFU	Average	% Recovery	Acceptance criteria
Viability Control	1	105	100.6	N/A	N/A
	2	100			
	3	97			
Test Item 1	1	93	91.6	91.05	Pass
	2	87			
	3	95			
Test Item 2	1	83	94.3	93.73	Pass
	2	98			
	3	102			

Table 2: Antimicrobial Efficacy

Initial Inoculum (A): 1,550,000 CFU (log=6.19)



WD	Exposure Time (hr)	Replicate	Final Titer			Log Reduction compared to initial titer	Log Reduction compared to “time 0”
			CFU	Log ₁₀	Mean Log ₁₀ ± SD		
Control	0	1	1,080,000	6.03	6.02 ± 0.01	0.17	N/A
		2	1,080,000	6.03			
		3	1,020,000	6.01			
	3	1	2,000,000	6.3	6.27 ± 0.04	-0.08	-0.15
		2	1,720,000	6.23			
		3	1,900,000	6.27			
Test Item 1	0	1	1,070,000	6.03	6.03 ± 0.05	0.16	N/A
		2	1,250,000	6.09			
		3	970,000	5.98			
	3	1	<100	<1.00	1.00 ± 0.0	>5.19	>5.03
		2	<100	<1.00			
		3	<100	<1.00			
Test Item 2	0	1	1,020,000	6.01	6.11 ± 0.12	0.08	N/A
		2	1,060,000	6.25			
		3	1,150,000	6.06			
	18	1	200	1.00	1.49 ± 0.50	4.7	4.62
		2	<100	<2.00			
		3	100	1.48			

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Conclusion:

The non-aged and the two years aged MedCu Antimicrobial Wound Dressing 2C-1010-01 achieved at least a 4 log reduction of the *Klebsiella pneumoniae* initial titer load within 3 hours of incubation and met the study acceptance criteria.

The control test item showed no antimicrobial activity as anticipated.

	Position	Full Name	Signature	Date
Written by:	Chief Scientist	Dr. Gadi Borkow		1/2/18
Reviewed and Approved by	CEO	Danny Lustiger		4/2/18