



# MedCu

## Laboratory Report

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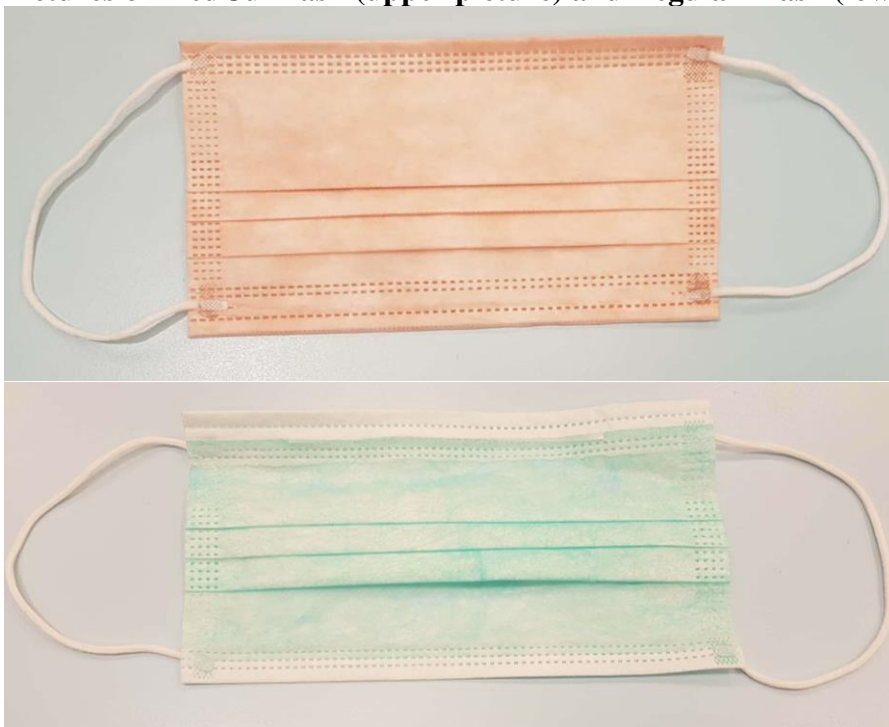
**Antimicrobial Efficacy – MedCu Mask following 7 subsequent inoculation of *Klebsiella pneumoniae***


Date: 19.7.2020

### Determination of Antimicrobial Efficacy of MedCu Masks following 7 sequential inoculations of *Klebsiella pneumoniae*

<b>Purpose:</b>	Determination of antimicrobial efficacy of MedCu Masks following subsequent multiple inoculations of bacteria.
<b>Test article details:</b>	MedCu Mask with Copper Oxide with two external nonwoven layers impregnated with copper oxide particles. As control, regular blue face masks without copper were used.
<b>Test bacteria:</b>	<i>Klebsiella pneumoniae</i> ATCC 4352.
<b>Equipment:</b>	37°C Incubator, Stomager (Bag Mixer), Pall filtration device; a 0.45 µm pore size membrane (Millipore catalogue number EZHAWG474); petri dishes.
<b>Exposure time:</b>	7 consecutive days.
<b>Sample size:</b>	Duplicate samples.
<b>Target Inoculum level per sample per inoculation:</b>	(1-2) x 10 <sup>5</sup> Colony Forming Unit (CFU) per inoculation per day.
<b>Inoculum volume:</b>	0.5 ml.
<b>Neutralizer:</b>	DeyEngley (D/E) Broth (LAB187, Lab M Limited, UK).
<b>Neutralizer volume:</b>	100 ml.
<b>Growth selective media:</b>	CHROMagar™ Orientation, CHROMagar, France.
<b>Additional Media:</b>	Nutrient broth (NB), Tryptone Soya Broth (TSB), Sterile phosphate buffered saline (PBS) and sterile 0.85% saline.
<b>Incubation Temperature:</b>	37±2°C.
<b>Protocol:</b>	<ol style="list-style-type: none"><li>1. A fresh transplant from a stock culture of <i>Klebsiella pneumoniae</i> was taken and grown overnight at 37±2°C in TSB.</li><li>2. A standard plate count was performed and the bacterial population titer was determined.</li><li>3. The microorganism population was adjusted to 1-2x10<sup>7</sup> CFU per ml in 5% NB and 0.85% saline.</li><li>4. 10 ml of the overnight culture was added to a 90 ml saline containing 5% NB.</li><li>5. 500±10 µl of the above solution was added to each mask sample, making sure that all liquid was completely absorbed into the samples.</li></ol>

6. All samples were put in a disposable vessel and closed hermetically to prevent any evaporation.
7. The samples were then incubated at  $37\pm 2^{\circ}\text{C}$  overnight.
8. Steps 1-7 were repeated every 24 hours for 6 additional consecutive times (total 7 days).
9. At Day 8, the test items were put in stomacher bags containing 100 ml of D/E broth and stomached vigorously for 2 minutes with a Stomager (Bag mixer).
10.  $10\pm 1\ \mu\text{l}$ ;  $100\pm 10\ \mu\text{l}$ ,  $1\pm 0.1\ \text{ml}$  aliquots of the liquid from each bag were added to  $50\pm 5\ \text{ml}$  D/E broth and passed through a  $0.45\ \mu\text{m}$  pore size membranes using a Pall filtration device.
11. The filters containing the bacteria were then rinsed twice with  $100\pm 5\ \text{ml}$  of D/E broth.
12. The membranes with bacteria were then placed on Petri dish containing CHROMagar Orientation agar and incubated at  $37\pm 2^{\circ}\text{C}$  for 24 hr.
13. The colony forming units (CFU) were then determined.
14. The percent reduction was determined using the following formula:  $100 - ((B/A) \times 100)$ , where B is the final CFU recovered from the masks on Day 7 and A is the initial inoculum added onto the masks on Day 7.

**Pictures of MedCu Mask (upper picture) and Regular Mask (lower picture) tested:**

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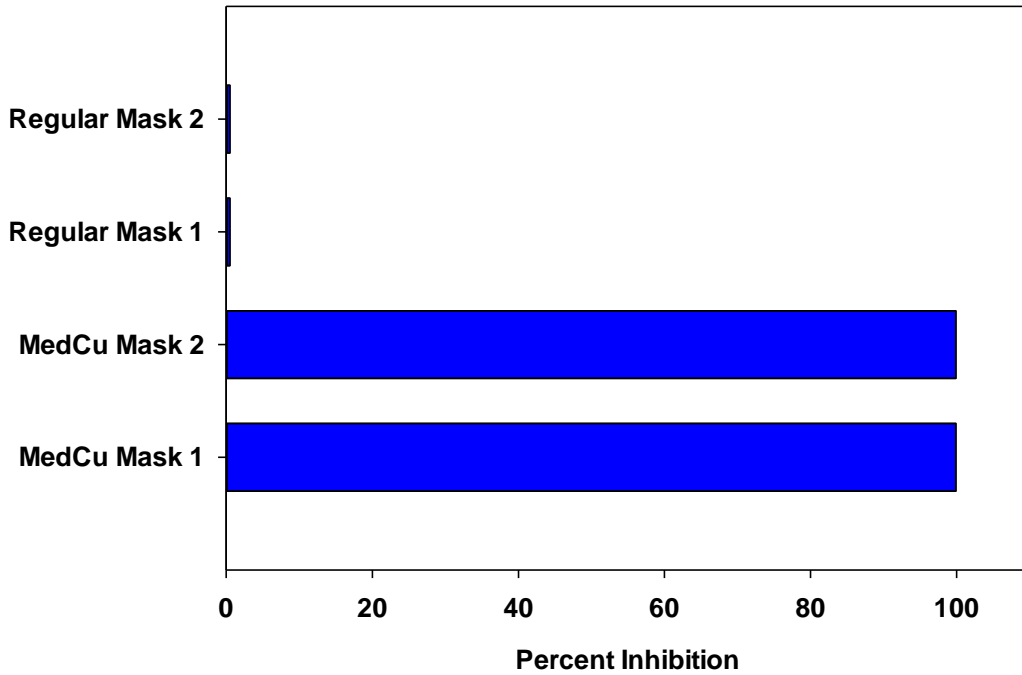
**Results**

Initial Inoculum on Day 7 (A): 300,000 CFU (colony forming units)

Sample	Replicate	Final Titer Recovered on Day 7 (B)	Percent Reduction of inoculum on Day 7
MedCu Mask	1	200	>99.9
	2	100*	>99.9
Control Mask	1	920,000	None
	2	1,100,000	None



\*Lower limit of detection

Inhibition of Bacterial Growth after 7 Sequential Inoculations



**Conclusion:**

MedCu Masks showed very good antimicrobial efficacy, achieving > 99.9% antimicrobial efficacy even after 7 repeated inoculations of high titers of bacteria.

	Position	Full Name	Signature	Date
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<b>Reviewed and Approved by</b>	CEO	Danny Lustiger		19/7/20