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Kristiina Ylihonko

Kristiina Ylihonko, PhD (Biochem)
Entrepreneur, Project management for pharmaceuticals
Care4living Oy, FI-21130 Naantali
Visiting professor of Turku University (microbiology & molecular biology), Finland

Project details:

Project identifier:	C4L02820
Reference:	Service project to Immitec
Confidentiality:	Confidential, for customer
Product:	Copper Inside – Disposable Medical Face mask coated with CuO
Type:	IIR
Site of the study:	University of Turku, Department of Biochemistry, Biocity, Turku Finland
Work performance by:	Care4living, responsible person, Aleksi Nyqvist, MSc (Biochem), Laboratory manager
Purpose of the study:	Analysis for microbiological purity of the masks after usage in different conditions for comparison purpose.

Summary: Copper face masks were tested for microbiological purity and compared to a typical disposable face mask. The disposable mask showed significantly more microbial growth than the copper masks. The time for the usage does not increase microbial impurities in the masks coated with CuO.

Protocol: In-house, according to EN 14683:2019.

Samples

Four different copper face masks and one disposable face mask were tested. Three copper masks had been used for different time periods by different individuals and one was an unused mask. As a control, a typical disposable face mask worn for 5 hours was used. Samples are listed in table 1.

Table 1. Samples studied.

Sample ID	Face mask type	Time used
C1	Copper	2 weeks
C2	Copper	4 weeks
C3	Copper	6 weeks
C4	Copper	unused
D1	Disposable	5 hours

Materials & Methods

A piece from the middle of each mask was cut (specifics in table 2) and incubated in 200 ml of Tryptone Soy Broth (TSB) medium at +30°C with slight aeration (no shaking). After 24 hours, solution was concentrated and samples were taken and plated on Tryptic Soy Agar (TSA), Potato Dextrose Agar (PDA) and Sabourad agar. OD_{600} was measured from the cultures. TSA plates were incubated at +30°C for 24 hours and continued for 5 days with daily follow up. PDA and Sabourad agar plates were incubated at +26°C for 5 days at 26°C.

Table 2. The mass of the cut samples derived from masks.

Sample ID	Mass (g)
C1	1,03
C2	1,07
C3	1,07
C4	0,96
D1	1,02

Results and Discussion

Bacterial growth was measured first by OD_{600} -values, which indicates generally the microbial growth. See Table 3 for the values of undiluted samples,

Table 3. The absorbance at 600 nm of the undiluted samples after 24 hours of incubation.

Sample ID	Absorbance (600 nm)
C1	2,54
C2	2,88
C3	2,13
C4	2,49
D1	4,47

According to the detected OD_{600} -values, the uncoated mask gives the highest value suggesting that there are more microbes than in the samples derived from CuO coated masks. However, no remarkable difference is seen between unused (C4) or used (C1-C3) Copper Inside masks.

For TVAC (Total Viable Aerobic Count), the samples were cultivated on TSA plates. Yeast and mold (TYMC) are growing on PDA and Sabourad agar plates. Since no antibiotic was used for yeast and mold agar, there are probably also bacterial colonies. The results are shown in Table 4.

Table 4. Number of colonies on agar plates and calculated microbiological purity per gram of mask.

Sample ID	TSA, CFU*	SDA, CFU*	Microbiological purity CFU/g	PDA, CFU*	Notes
C1	28	3	30	37	Mask used 2 weeks by public health nurse, 8 h/ d
C2	42	52	88	69	Mask daily used 4 weeks at office
C3	18	2	19	22	Mask used 6 weeks by manicurist 6-8 h/d
C4	27	4	32	45	Unused Cu-mask (non-sterile)
D1	110	374	475	422	Control, usual IIR 5 h in use

* Average value

Conclusion

The study shows and supports well the antimicrobial properties of Copper Inside masks. The slices of masks were incubated in TSB solution for 24 h. This will allow growth of microbes, but we considered it important to extract all microbes from masks to the liquid for analysis. This incubation was considered in the calculation together with dilutions. The results obtained with different agar plates and based on turbidity are concordant confirming the analytical results.

There are minor differences between used masks and only C2 differs from the general level. The release specification for unused type IIR masks is ≤30 CFU/g and as is shown in Table 4, the specification has been met even after 6 weeks usage. As the study was made mainly for comparison purpose to see how much the usage of the Cu-masks affects microbial purity, we can conclude that even a long-time usage does not decrease the antimicrobial properties of CuO in the masks.

Compared with a normal IIR type disposable mask, there is a significant difference in microbiological purity to the benefit of Cu-masks. Cleanness of Cu-masks is ten times higher than with a control (D1). Exception is the sample C2, which is not as clean as others for an unknown reason. However, it gives also more than 5 times higher microbiological purity than a typical IIR-type mask.

Despite the results to support re-useability of Copper Inside masks, they are CE-marked Class 1 medical devices, which limits the marketing claims in the EU.