



Univerza v Mariboru

Fakulteta za kmetijstvo
in biosistemske vede

Pivola 10
2311 Hoče, Slovenija

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POROČILO testiranja "Antimicrobial plastic film Clean-Cu"

Antimikrobno plastično folijo "Clean-Cu" smo testirali glede antimikrobne učinkovitosti. Učinkovitost smo testirali z uporabo dveh metod:

- Preko testiranja direktno na vratnih kljukah v realnih pogojih
- Preko metode ISO 22196:2011

Testiranja na vratnih kljukah v realnih pogojih

METODA:

V delovni organizaciji smo izbrali dva ročaja vrat iz nerjavečega jekla: pisarna in WC (stranišče). Antimikrobna folija Clean-Cu je bila nanešena na eno polovico ročaja, druga polovica pa je ostala nepokrita. Osebu za čiščenje objekta je bilo naročeno, da očistijo ročaje na enak način kot prej. Z uporabo tega sistema se je vsaka oseba, ki se je dotaknila ročaja, z roko dotaknila obeh površin kljuke, torej nepokrite in pokrite s folijo. Vsakodnevno smo pred čiščenjem s pomočjo sterilnih bombažnih palčk odvzeli mikrobiološke brise površin z obeh strani ročaja. Palčke z brisi smo prenesli v 0,5 mL fiziološke raztopine, pustili 10 minut in 0,2 mL raztopine nanесли na mikrobiološke plošče s splošnim agarim gojiščem PCA (splošni agar, "plate count"). Kolonije (CFU) smo šteli po 48 h inkubacije pri 30 °C, kar je predstavljalo relativen rezultat mikrobne onesnaženosti površine.

REZULTATI:

Grafikona 1 in 2 prikazujeta gibanje CFU v odvzetih mikrobioloških brisih z vratnih kljuk po dnevih od montaže folije (kontrola – nepokrit del in pokriti del s folijo Clean-Cu).

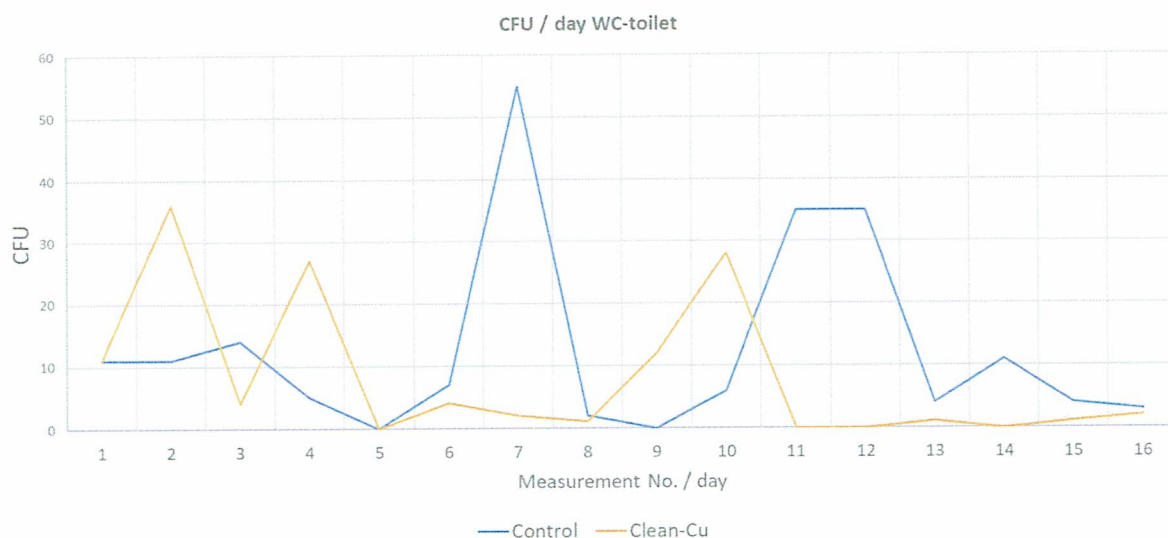


Figure 1. Microbiological load on the door handle surfaces in the selected toilet (control: part of the door handle without the film; Clean-Cu: part of the handle covered with the Clean-Cu foil). The foil was applied on day 1, when the measurements began as well.

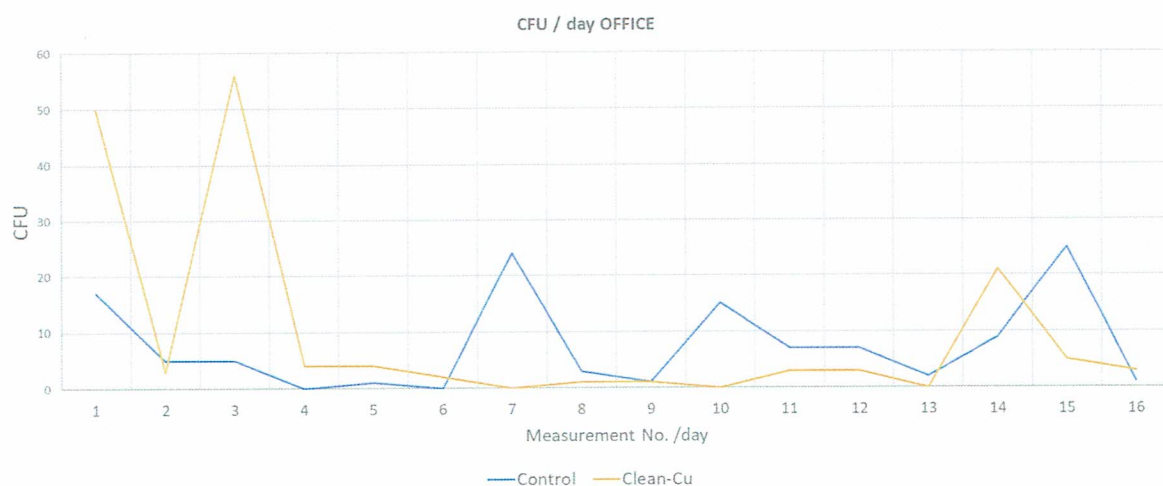


Figure 2. Microbiological load on the door handle surfaces in the selected office (control: part of the door handle without the film; Clean-Cu: part of the handle covered with the Clean-Cu foil). The foil was applied on day 1, when the measurements began as well.

The results of the microbial load on each tested door handle were statistically evaluated with the statistical package V22 (SPSS, IBM). To determine the antimicrobial activity of the film, we used the McNemar test for data processing, where the test statistics is distributed according to the chi-square distribution. When entering the results in the test statistics, we changed the values for control and Clean-Cu film to "yes" if the plastic film was effective on the selected

day and "no" if it was not. Answer "yes" we denoted by 1, "no" by 0. The null hypothesis says that the variables are not related, and the alternative hypothesis says that they are related. Since the calculated significance parameter $p = 0.3865$ was higher than the significance level $\alpha = 0.05$, the null hypothesis was not rejected. Therefore, at a 5% significance level we can claim that the two variables are not related. **The microbial load on the Clean-Cu plastic film was statistically not the same as on the part of the uncovered handle.**

Method ISO 22196:2011

METHOD:

ISO 22196 specifies a method of evaluating the antibacterial activity of plastics enhanced with antibacterial properties and other non-porous surfaces of products (including intermediate products). We generally followed the method, except for the test microorganisms. As gram-negative microorganisms *Salmonella enterica* serovar Typhimurium (ATCC 14028) was applied instead of *Escherichia coli*. *Staphylococcus aureus* (ATCC 29213) was used as a representative of gram-positive bacteria.

Briefly, samples of Clean-Cu plastic film were cut into 50 mm × 50 mm squares, disinfected and placed in separate sterile petri dishes. Test surfaces were inoculated with 200 µl test inoculum (4×10^5 CFU/ml *Salmonella enterica* ser. Typhimurium / *Staphylococcus aureus*). In parallel to the Clean-Cu plastic, usual PE plastic film was used as a control. The test inoculum was covered with a PE film measuring 40 mm x 40 mm and the petri dish closed with a lid. Petri dishes were incubated for 4 and 24 h at 37 °C. Next, 10 ml of neutralizer was added to the petri dish and dilutions were plated on PCA agar plates. The plates were incubated at 37°C for 24h, and colonies were counted. All experiments were performed in triplicates.

The antimicrobial efficacy of the Clean-Cu film was calculated under selected conditions as: $[\text{CFU (control)} - \text{CFU (Clean-Cu)}] / [\text{CFU (control)}]$. Mann-Whitney U test was used to calculate the effectiveness of the Clean-Cu film relative to the control.

RESULTS:

Table 1: Antimicrobial properties of Clean-Cu film according to the standard method ISO 22196:2011 and statistical significance relative to the control.

	Antimicrobial effectivity of Clean-Cu film (%); statistical significance relative to the control (Mann-Whitney U test)	
	4 h	24 h
<i>Staphylococcus aureus</i>	95.6%; $p < 0.01$	>99%, $p < 0.01$
<i>Salmonella enterica</i> ser. Typhimurium	>60%; N.D.	>99%, $p < 0.01$

N.D. not determined

Figures 1-4 show representative results of antimicrobial properties of Clean-Cu film in petri-dishes with PCA agar.

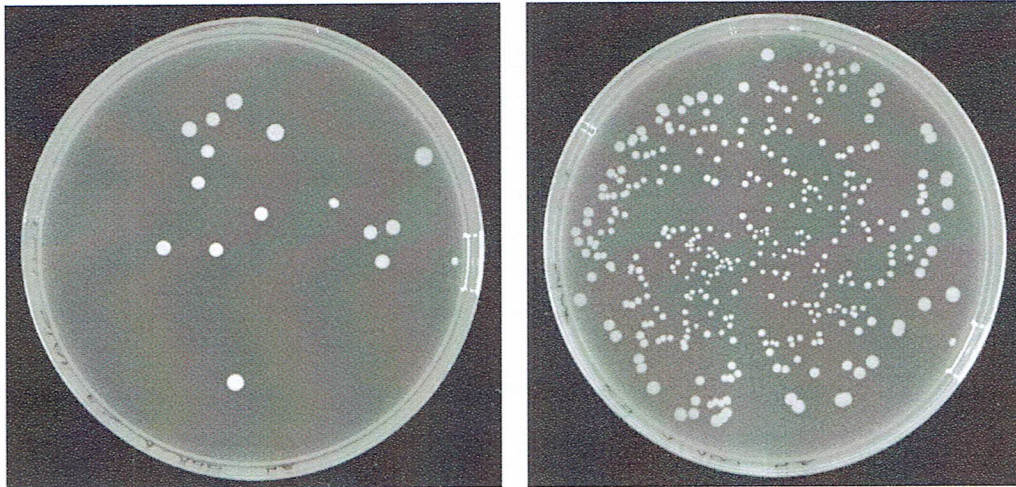


Figure 1: *Staphylococcus aureus*, left: Clean-Cu film, right: control, contact time 4 h.

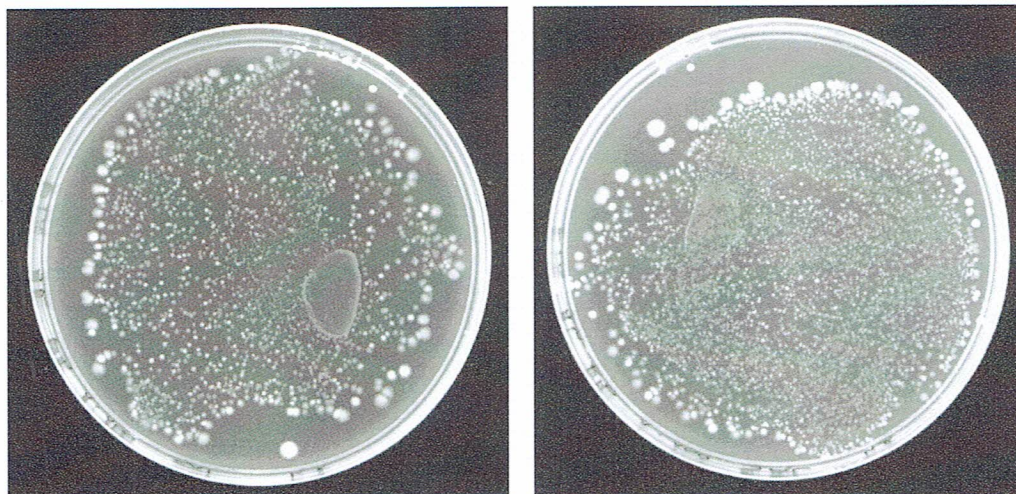


Figure 2: *Salmonella enterica* ser. Typhimurium, left: Clean-Cu film, right: control, contact time 4 h.

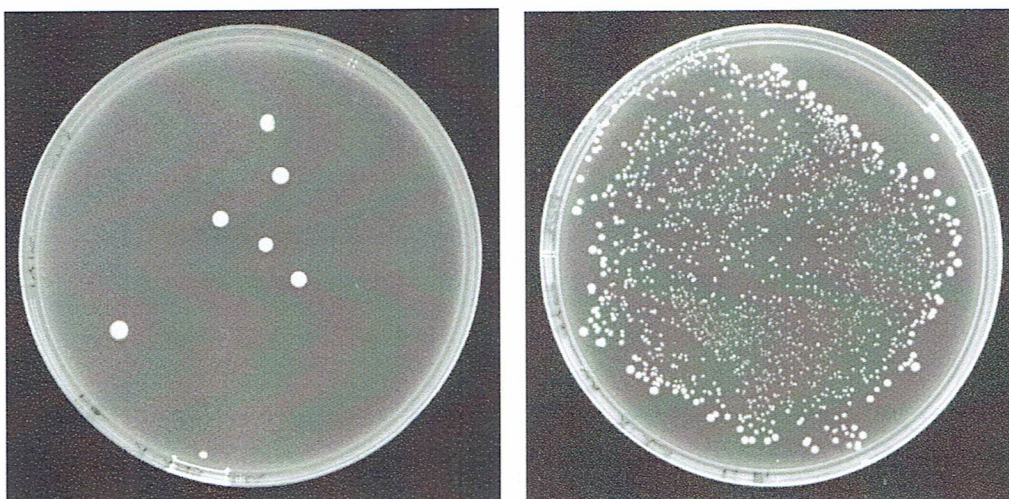


Figure 3: *Staphylococcus aureus*, left: Clean-Cu film, right: control, contact time 24 h.

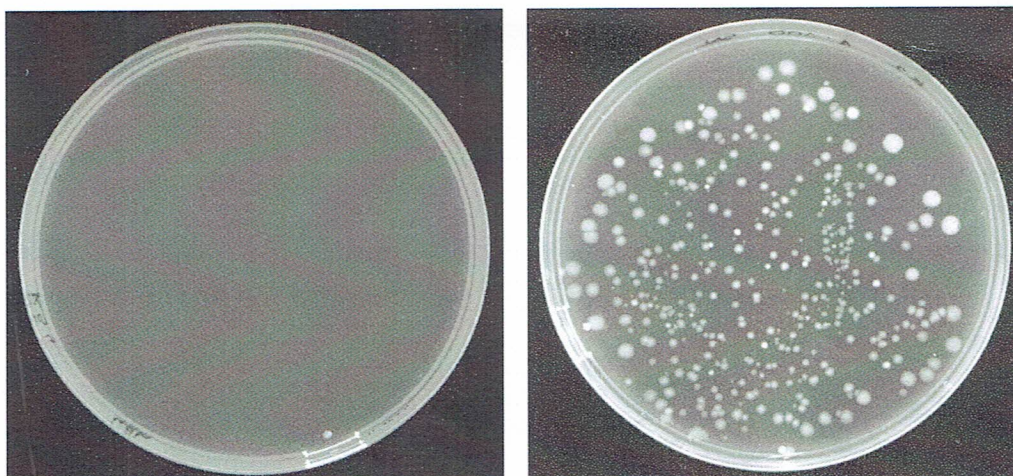


Figure 4: *Salmonella enterica* serovar Typhimurium, left: Clean-Cu film, right: control, contact time 24 h.

SUMMARY

Clean-Cu antimicrobial foil was tested using two methods, in real conditions on door handles and by the method ISO 22196: 2011. In both cases, we found a statistically significant reduction in the number of microbes on the tested foil compared to the control.

Assoc. professor dr. Tomaž Langerholc, Head of the laboratory

Department of Microbiology, Biochemistry, Molecular Biology and Biotechnology

