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SCIENTIFIC REPORT ON THE LONG-TERM ANTIMICROBIAL ACTIVITY OF HALOS-BASED COATING ON HARD SURFACES

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Project Principal Investigator: Prof. Roberta Censi, PharmD, PhD

Testing period: December 2021

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AIM of the study
Assessment of the long-term antimicrobial efficacy of Halos-coated leather surfaces.

Materials and Methods

Preparation of the antimicrobial surfaces

Polyester-coated leather surfaces of 50 x 50 mm dimensions and 2 mm thickness, provided by Viraschutz, were taken as standard surfaces, representing non-porous and commonly used materials in indoor domestic, public, and industrial environments. Each surface was first primed with ABLATOR, subsequently coated with 1 ml HALOS solution and 1 ml NOVATOR.
Upon a drying period of two hours, the excess of unbound HALOS was removed, and the prepared surfaces (ten replicates for control and treated surfaces) were stored at 54°C for 11 days, according to Cipac MT 46.3 guidelines for accelerated stability testing. Subsequently, the surfaces were tested for their antimicrobial activity.

Antimicrobial properties assessment:

The antimicrobial properties of the surfaces were assessed according to ISO 22196: 2011 method. Non-coated surfaces were taken as negative control.

Test strains:

- Staphylococcus aureus ATCC 6538
- Escherichia coli ATCC 8739

- Testing conditions:

- Contact time: 24 h ± 1 h

- Diluent: Nutrient Broth 1/500 for strains preparation
  Phosphate-buffered physiological saline: 0.85% of sodium chloride

- Neutralizer: SCDLP broth
  17,0 g of casein peptone, 3,0 g of soybean peptone, 5,0 g of sodium chloride, 2,5 g of disodium hydrogen phosphate, 2,5 g of glucose and 1,0 g of lecithin in 1 000 ml of distilled or deionized water.

- Incubation medium: Tryptic Soy Agar (TSA)

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- Incubation of the inoculated test specimens: 35°C ± 1°C, 90% relative humidity for 24 h ± 1 h, damp condition

- Incubation of the plates for the viable bacteria count: 35°C ± 1°C, 90% relative humidity for 48 h

Procedure for evaluating the antibacterial activity on non-porous surfaces according to ISO 22196:

A bacterial suspension was prepared at a concentration (ufc/ml) > 1,0 x 10^6 for E. coli and for S. aureus.

0.4 ml of bacterial cell suspension (200 µl *Escherichia Coli* and 200 µl *Staphylococcus Aureus*, bacterial concentration about 10^6 ufc/ml) was pipetted on the surface of test specimen placed into a separate sterile Petri dish for each bacterial strain, with the test surface uppermost.

The test inoculum was covered with a piece of inert film (polycarbonate film), as described in ISO 22196:2011 and then the lid replaced on the Petri dish (Figure 1).
Petri dishes containing the inoculated test specimens were incubated at a temperature of 35 ± 1°C and at a relative humidity of 90% for 24 ± 1 h. Next, 10 ml of neutralizer is added to the petri dish. A 10-fold serial dilution is performed on the neutralizer. The count of the viable bacteria recovered was performed, placing 1 ml of the recovered SCDLP from the test specimen and of each dilution into separate sterile Petri dishes. 15 ml of plate count agar were poured into each Petri dish. All petri dishes were incubated at a temperature of 35 ± 1°C and at a relative humidity of 90% for 48 h.
Immediately after inoculation three untreated test specimens were processed in the same way.
The value obtained from this bacterial count was used to determine the recovery rate of the bacteria from the test specimens under investigation.
After incubation, for each test specimen, the number of viable bacteria recovered (cfu/surface) was determined and the antibacterial activity was calculated using Equation:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

Where:

R is the antibacterial activity;
U_0 is the average of the common logarithm of the number of viable bacteria, in cells/cm^2, recovered from the untreated test specimens immediately after inoculation;
U_t is the average of the common logarithm of the number of viable bacteria, in cells/cm^2, recovered from the untreated test specimens after 24 h;
A_t is the average of the common logarithm of the number of viable bacteria, in cells/cm^2, recovered from the treated test specimens after 24 h.

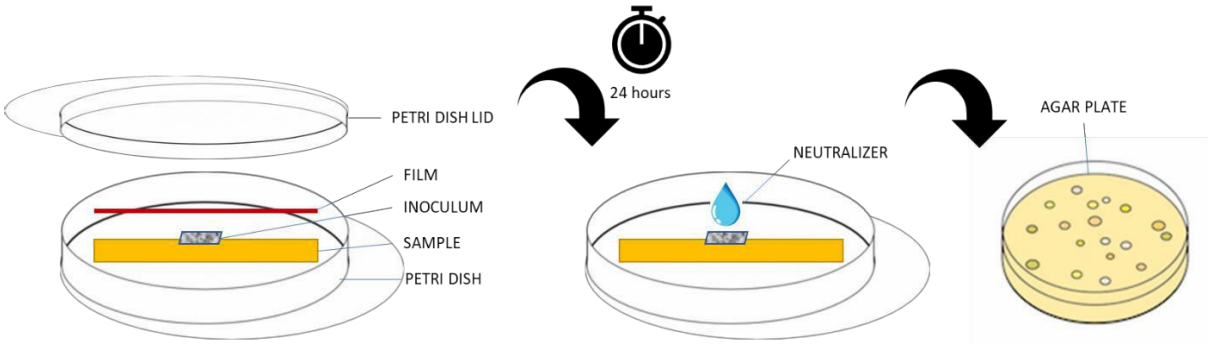

Figure 1. Schematic representation of the ISO 22196:2011 procedure

7) Results and conclusions:

Table 1: Summarizing table of the recovery after 24 h from test samples and calculation of their antimicrobial activity

Sample	A_t (cells/cm^2)	log A_t	R Antibacterial activity $U_t - A_t$	Reduction of microbial load (%)
Leather	9300	4.0	2.3	99.5

Table 2: Summarizing table of the recovery immediately after inoculation and after 24 h from uncoated surfaces

U_0: Average of the common logarithm of the number of viable bacteria, in cells/cm^2, recovered from the untreated test specimens immediately after inoculation	U_t = average of the common logarithm of the number of viable bacteria, in cells/cm^2, recovered from the untreated test specimens after 24 h
720,000	1,900,000
log U_0 = 5.9	log U_t = 6.3

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On the basis of data collected and shown in tables 1 and 2, the polyester-coated leather surfaces treated with ABLATOR, HALOS and NOVATOR show good antibacterial properties against strains of Staphylococcus Aureus ATCC 6538 and Escherichia Coli ATCC 8739, after 11 days of accelerated stability at 54°C, corresponding to 90 days at room temperature, according to the Cipac MT 46.3 guidelines.

Camerino, 25th January 2022

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