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Short description of the test methodology to test the effect of ageing on the antimicrobial efficiency of high-touch surfaces intended for e.g. indoor hygiene applications.

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Method

Ageing (oxidation/weathering) sample surfaces

1. Test coupons (approximately 10x10 cm) exposed in a climate chamber on a 24 h cycle - 4 h 90% RH,2 h 0% RH, 16 h 90% RH, 2 h 0% humidity.
   Temperature held constant at 25 °C.

2. Number of cycles: 1, 7, 14, 28 (1 cycle=24 h) depending on desired ageing of the surface

3. Test material exposed in climate chamber with and without deposition of artificial sweat (ASW)
   ASW deposited with an airbrush forming small droplets on the sample surface (approximately 7 mg ASW/cm^2)
   ASW composition according to EN1811
   - NaCl – 5g/L
   - Urea – 1 g/L
   - Lactic acid 940 µL/L
   - pH adjusted to 6.5±0.05 using NaOH

Bacterial testing

1. Bacteria culture (e.g. *Escherichia coli-E-coli)* grown in Nutrient Broth (NB) medium overnight (~20 h) at 37 ºC

2. Centrifuge the culture at 2500 rpm for 5 min and collect the bacteria cell pellet

3. Discard the supernatant and add ASW (filter sterilized) to suspend the bacteria cells followed by centrifugation at 2500 rpm for 5 min

4. Discard the supernatant and suspended the cells in ASW

5. Measure the Optical Density (OD) at a wavelength of 600 nm and dilute the bacteria cell culture to get an OD =0.1

6. Deposit the bacterial culture (3 drops of 1 µL) in droplets on the test coupons (covering most of the sample surface – not complete water layer, quasi-dry method)

7. Incubate the test coupons with deposited bacteria at a fixed period of time at room temperature by placing them in a sterile petri plate covered with lid

8. Microscopic glass slides, no ageing but sterilized by autoclave, are used as control following the same procedure as described above.

9. After incubation, the samples are placed in a tube containing ASW that is vortexed for 1 min with high speed to release loosely attached bacterial cells from the surfaces

10. A volume of 100 µL is plated on nutrient agar plate and incubated at 37 °C for 24 h

11. A serial dilution of the vortexed solution is prepared and plated on nutrient agar plate and incubated at 37 °C for 24 h

12. Another set of the test sample is imprinted onto nutrient agar plate to observe the growth of live bacteria remaining on the surfaces

13. The growth of the live bacteria was observed and calculated in the colony forming units (CFU)

14. A schematic of the protocol is given in figure 1

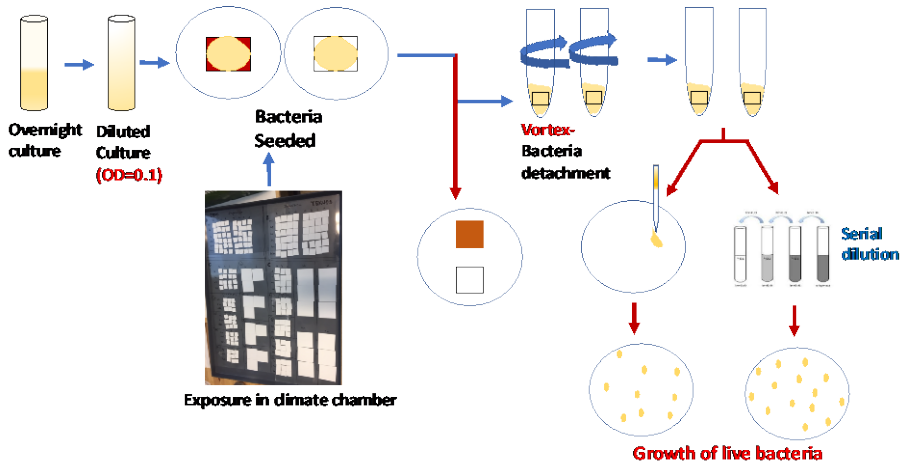

Figure 1. Overview of the protocol