Touch transfer assay for the evaluation of antimicrobial surfaces Version 2

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Abstract

Transmission of bacteria from inanimate surfaces in healthcare associated environments is an important source of hospital acquired infections. A number of commercially available medical devices promise to fulfill antibacterial activity to reduce environmental contamination. Under usual ambient air conditions of hospital rooms no condensation of humidity is expected on inanimate surfaces. In contrast, current standardized methods for the analysis of antibacterial activity of solid surfaces in general use mostly planktonic bacterial cells which are kept in thin liquid or agarose layers on tested surfaces. Therefore, we developed a touch transfer assay modeling fingerprint transmission to investigate the antibacterial activity of surfaces in a dry state. We suggest the newly developed touch transfer assay as a new additional tool for the assessment of potential antimicrobial surfaces prior utilization in hospital environments.

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Protocol

Preparation of the primary contaminated surface (PCS)

Step 1.

Enterococcus faecium ATCC 6057 is cultivated on Columbia Agar + 5 % sheep blood at 36 ± 2 °C under ambient conditions overnight. Bacteria are inoculated in sterile 0.85 % NaCl solution to reach the appropriate bacterial concentration (usually $5x10^7$ bacteria/mL). 200 µl of bacterial suspensions were spread homogenously on sterilized surfaces of 5 x 5 cm ceramic tiles white matt glaze (#3709PN00, Villeroy & Boch, Mettlach, Germany) and dried for 1 hour under standardized conditions in a climate chamber at 22 °C and 50% rH. This primary contaminated surface (PCS) is used for touch transfer.

Notes:

1. The number of surfaces contaminated with the bacterial inoculum influences the time needed for desiccation in the climate chamber. For experiments to be matched together for analysis similar time periods for the desiccation process should be assured by using identical experimental setups with the same number of surfaces drying together in the climate chamber.
2. Other bacterial species and/or strains can be used in the assay. For each individual strain

repeated experiments are necessary to estimate the bacterial loss during dessiccation to get standardized amounts of viable bacteria on the PCS prior touch transfer experiments.

Preparation of test surfaces

Step 2.

As control during the touch transfer assay standardized 5 x 5 cm ceramic tiles with white matt glaze (#3709PN00, Villeroy & Boch, Mettlach, Germany) can be used for quantitative control of the touch transfer. Depending on the kind of potential antimicrobial surface to be analyzed appropriate control surfaces for the without antimicrobial activity should be selected. If possible identical materials without the antimicrobial active component should be used. If no direct control is available the ceramic tiles or stainless steel UNS S30400 plates can be used as non-antimicrobial control during experiments analyzing antimicrobial activity. All surfaces must be disinfected (e. g. 70% isopropyl alcohol) prior the touch transfer.

Notes:

1. Surfaces can alternatively sterilized using different methods depending on the sensitivity of the surface against different methods.
2. The transfer rate from the PCS to the secondary contaminates surface depends in part on the surface hydrophobicity of the secondary surface. As non-antimicrobial control only surfaces displaying a similar transfer rate (see below) should be used, to allow transfer from identically prepared PCS during the touch transfer assay.

Touch transfer

Step 3.

Uptake of bacteria from the PCS is performed by the test person with the forefinger or thumb covered with moistened sterile cotton gloves warn over disinfected single use nitrile gloves. Moistening and addition of organic soil load for cotton gloves mimicing the clinical situation of having organic soil matrix as a companion with bacterial burden is performed by touching Columbia Agar + 5 % sheep blood for 10 sec without pressure. For the touch transfer of fingers with gloves are rolled like taking fingerprints with low pressure without removing the finger for 10 sec on the PCS and subsequently the fingers are rolled in the same way on the respective sterilized surfaces for 10 sec resulting in the secondary contaminated surface (SCS).

Quantitative culture of the SCS (see below) was performed immediately or after 24 h of incubation at 22 °C and 50% rH in double determination or by enumeration using Replicate Organism Detection And Counting (RODAC) agar plates containing TSA with disinhibitor plus (Oxoid, Basingstoke, UK).

Notes:

For the establishemnt of the assay a target contamination of 1000-3000 colony forming units (cfu) / 25 sqcm was used. The quantity of contamination on the SCS can be adjusted by the contamination of the PCS as the transfer rate between PCS and SCS remains stable over a wide range (for details see publication).

The hydrophobicity of the SCS influences the transfer rate. If SCS with different materials (which might have differences in their hydrophobicity) are used experiments determing the transfer rate between PCS and SCS shoulb be performed

Quantitative culture

Step 4.

For quantification of surviving bacteria surfaces are harvested from the surfaces by heavy scrubbing with a foam swab (∑-Transwab, Medical Wire, Corsham, UK) moistened with Tryptone Soya Broth (TSB) with LTHTh 373r-20p (Merck, Darmstadt, Germany). The swab is subsequently transferred into 1 ml of TSB with LTHTh. Appropriate volumes of the suspension (or appropriate dilutions) are streaked on COS agar plates in double determination. Colony numbers are counted after incubation for 24 h at 36±2 °C. Using this method the detection limit is about 10 cfu/surface. Therefore, Replicate Organism Detection And Counting (RODAC) agar plates containing Tryptone Soya Agar with disinhibitor plus (Oxoid, Basingstoke, UK) can be used for highly active antimicrobial surfaces to increase the sensitivity for surviving bacteria (detection limit 1 cfu/surface).

Notes:

- RODAC plates can only be used in case of highly active antimicrobial surfaces as quantification on these plates is difficult if surviving bacteria exceed about 100 cfu/surface.
- Alternatively to the ∑-Transwab System other swabs designed for liquid based microbiology with a high release of material back to the liquid can be used to harvest surviving bacteria from surfaces.