COMPARISON OF TWO SURFACE TEST METHODS, EN 14349 AND OECD, FOR THE EVALUATION OF THE BACTERICIDAL ACTIVITY OF DISINFECTANTS FOR VETERINARY PURPOSES

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SUMMARY

The aim of this study was to compare the surface test method EN 14349 – "Quantitative surface test on non-porous surfaces without mechanical action" with the "Guidelines for evaluating microbicides on surfaces" discussed at the OECD. Both methods were evaluated with regard to their effects on the test results and on the recommendations for practical use.
The results obtained with the test organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa* and the disinfectant glutaraldehyde indicate that the CEN methods are more representative for practical conditions than the proposed OECD testing. The latter results in a recommendation of too low use concentrations and exposure times.

Keywords: disinfectant testing, CEN, OECD, surface test, bactericidal activity, veterinary area

OBJECTIVES

The surface test method EN 14349 – "Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary field on non-porous surfaces without mechanical action" is a harmonized standard, which shall be applied in all European countries for assessing disinfectant efficacy on surfaces.

Following an "OECD Efficacy Workshop on Certain Antimicrobial Biocides" in April 2002 in Arlington, Virginia, USA, with the final objective to identify regulatory and scientific areas where harmonization was needed, there was a discussion about developing a surface test method based on the QCT-2 test method published by Springthorpe and Sattar (2005). The objectives of this study were to compare the two surface test methods regarding the effect of the different methods on the test results and on the recommendations for practical use. The tests were performed at the Institut für Umwelt- und Tierhygiene at the University of Hohenheim.

MATERIALS AND METHODS

EN 14349 describes a surface test method using stainless steel discs with 2 cm diameter as test surfaces. The test was carried out under simulated high level soiling conditions with a mixture of 10g/l yeast extract + 10 g/l bovine albumin serving as interfering substances.

Two min prior to the actual test 1 ml of the bacterial test suspension containing 1.5–5 x 10^9 cfu/ml was added to 1 ml of the interfering substance and mixed. The test surfaces were placed in

an open Petri dish ensuring that the stainless steel discs were in horizontal position. Then they were inoculated with 0.05 ml of the test suspension and dried in an incubator at 37°C for 45–55 min "until they were visibly dry". After drying the temperature of the surface was adjusted to room temperature. Then the inoculum was covered with 0.1 ml of the product test solution, or for the water control with water of standardized hardness instead of the product. After the chosen exposure times the surfaces were transferred into separate flasks containing 10 ml of an appropriate neutralizer and glass beads. After a neutralization time of 5 min a series of tenfold dilutions were prepared in tryptone-NaCl solution. The number of surviving test organisms was determined quantitatively using the spread plate technique by spreading 1 ml of the neutralized mixture and each dilution step on an appropriate number of surface dried Tryptone Soy Agar (TSA) plates. The viable counts were determined after 24–48 hrs of incubation at 37°C. In parallel tests for validation of the dilution neutralization and water control were carried out. For each test organism, product test concentration, and exposure time, the reduction in viability in comparison to the water control was calculated.

The draft surface test method discussed on OECD level is not yet described in detail. Based on the QCT-2 test method stainless steel discs 1 cm in diameter serve as test surfaces and the test is performed in a closed system. For preparation of the inoculum 340 µl of the bacterial test suspension containing > 10^9 cfu/ml (*Staphylococcus aureus*) or > 10^8 cfu/ml (*Pseudomonas aeruginosa*), 25 µl bovine albumin, 100 µl mucin, and 35 µl of a tryptone stock solution were mixed. The stainless steel discs were inoculated with 10 µl of this test suspension dried by keeping them for two hours at room temperature in a desiccator under vacuum. The dried discs were picked up and placed on the inside bottom surface of a vial. The inoculum on each test carrier was covered with 50 µl of the product, the vials were closed and kept at room temperature for the chosen exposure times. For the water controls 50 µl of sterile saline were pipetted onto the test surface instead of the product. At the end of the chosen contact times 9.95 ml of sterile saline – T (0.85% NaCl + 0.1% Tween 80®) were added to each vial and mixed for 45–60 s to recover surviving test organisms. For the determination of the viable counts the content of each vial and the rinsing liquid of 3 washing steps with a total of approximately 40 ml saline were filtrated in a membrane filtration equipment using membranes with a pore size of 0.45 µm. In parallel spread plate technique was used for the determination of viable counts. All plates were incubated at the 37°C for 48 hours.

For each test organism, product test concentration, and exposure time the reduction in viability in comparison to the water control was calculated.

Staphylococcus aureus and *Pseudomonas aeruginosa* were used as test organisms, and glutaraldehyde with exposure times of 5, 30 and 60 min was used for disinfection.

The product was deemed to have passed the surface test if it demonstrated a 4 lg reduction within the chosen contact times at 20°C or room temperature.

RESULTS

If *Pseudomonas aeruginosa* was used as test organism according the OECD draft after drying the discs for 2 hrs in the desiccator at room temperature under vacuum, maximum numbers of 1.89 x 10^4 cfu/ml were recovered from the test surfaces. So the amount of cells remaining on the test surface after drying was too low to determine the required 4 lg reduction (figure 1). Tests were

carried out to determine the loss of this gram negative test organism during drying and to fix an appropriate drying time (figure 2).

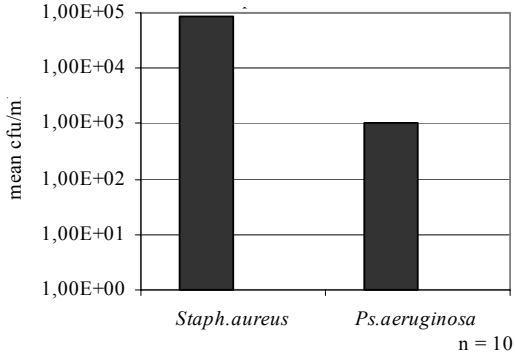

Figure 1. Recovery rates of the test organisms in the water control after drying the inoculated discs for 2 hrs in a desiccator at room temperature under vacuum

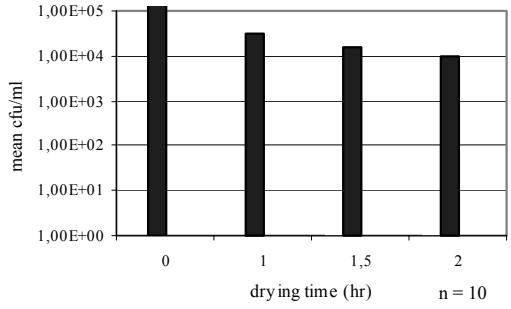

Figure 2. Mean cfu/ml of *P. aeruginosa* after different drying times in a desiccator at room temperature under vacuum

The results showed that after drying for 1 hr a loss of approximately 1.2 lg, after 1.5 hrs a mean log reduction of 1.5, and after 2 hrs drying a log reduction of 1.4 to 2.6 lg occurred (figure 3). For further testing a drying time of 1 hour at room temperature under vacuum was chosen.

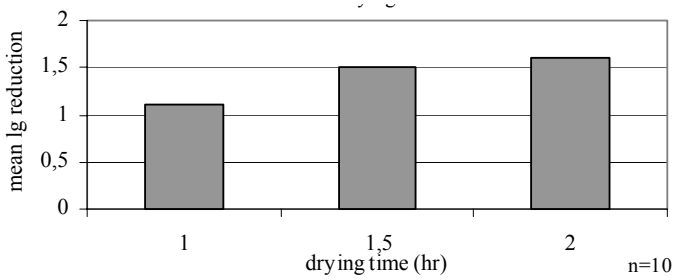

Figure 3. Mean lg reduction of *Pseudomonas aeruginosa* at different drying times at room temperature under vacuum

The results of trials where the different interfering substances according CEN and OECD were tested in the OECD method indicate that they are comparable and may both be used as interfering substances for high level soiling in the surface test, however the statistical evaluation is pending.

Due to the experience that membrane filtration method, which is recommended in the OECD draft for the determination of viable counts may lead to different results than when viable counts are determined using the spread plate technique, the OECD test was performed as described but determination of surviving test organisms was carried out using membrane filtration and spread plate technique in parallel. The results indicate that higher numbers of colony forming units were detected when using spread plate technique compared to the membrane filtration method. This might be due to added stress for already damaged test organisms or incomplete removal of some disinfectants during the membrane filtration procedure so that there is a remaining inhibiting effect on the growth of the test organisms.

Differences between the test results were also observed when the OECD method was performed in closed and open vials. As expected the lg reductions of the test organisms were higher when performing the test in closed vials. One explanation for these findings is that the disinfectant cannot evaporate during the exposure times and the aerosolized disinfectant still has an effect on the test organisms. Though this condition does not correspond to real life conditions.

Regarding the concentrations which passed the tests it could be shown that lower concentrations were sufficient to achieve the required 4 lg reduction in the OECD test than in the CEN test (figure 4).

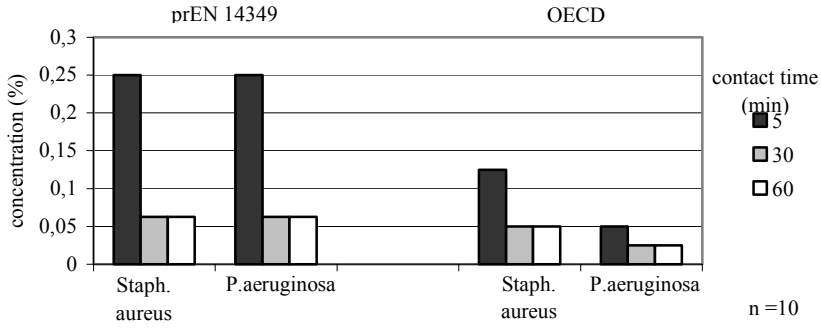

Figure 4. Concentrations which passed the test, prEN 14349 or OECD

Final results of the statistical evaluation of the test results are pending.

CONCLUSIONS

The analysis of the results obtained so far indicates that the CEN test methods are more representative for practical conditions than the proposed OECD testing. This may lead to big differences in the recommended concentrations and contact times depending on the method used. The latter results in a recommendation of too low use concentrations and exposure times.

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