EFFECTS OF THE DRYING CONDITIONS ON DIFFERENT GRAM NEGATIVE BACTERIA ON STAINLESS STEEL DISCS USED IN THE "QUANTITATIVE SURFACE TEST FOR THE EVALUATION OF BACTERICIDAL ACTIVITY OF CHEMICAL DISINFECTANTS" (EN 14349)

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Introduction

The European Standard EN 14349[1] specifies a surface test to establish whether a chemical disinfectant for use in the veterinary field applied onto non-porous surfaces without mechanical action has a bactericidal activity. Due to the fact that the different drying conditions and interfering substances have an effect on the recovery rate of *Pseudomonas aeruginosa*, which is defined as one test organism in EN 14349, the effects of these basic parameters on other gram negative bacteria were determined. The aim of this study was to confirm *Pseudomonas aeruginosa* as a test organism or identify a more suitable test organism.

The trials were performed using 3 different interfering substances and *Pseudomonas aeruginosa*, *Salmonella Senftenberg*, *Escherichia coli,* and *Aeromonas hydrophila* to be examined.

Material and methods

Principle

A test suspension of bacteria and interfering substance was inoculated onto the test surface and dried. After the drying time the number of surviving organisms, which could be recovered from the surface was determined quantitatively. The number of bacteria on a surface without being dried was also determined and the reduction in viable counts was calculated by the difference.

Method

According to EN 14349 stainless steel discs (2 cm diameter discs) 304 with grade 2 finish on both sides were used as test surfaces. Before use the discs were cleaned and sterilized as given in the guidelines.

Pseudomonas aeruginosa (DSM 936), *Salmonella Senftenberg* (DSM 112), *Escherichia coli* (DSM 682), and *Aeromonas hydrophila* (DSM 30020) were chosen as test

organisms. Based on EN 14349 “Low level soiling” (3,0 g/l Bovine albumin solution), “High level soiling” (Mixture of bovine albumin solution with yeast extract in a final concentration of 10 g/l yeast extract and 10 g/l bovine albumin in the test) were used as interfering substances. Additional 0,3 g/l Bovine albumin solution, representing “clean conditions” was tested.

The bacterial test suspension containing $1{,}5x10^9$ – $5{,}0x10^9$ cfu/ml) was prepared as described in EN 14349.

Just before starting the test procedure 1 ml of the bacterial suspension was added to 1 ml of the interfering substance and mixed. 0,05 ml of this microbial test suspension was inoculated on the test surface.

The inoculated test surfaces were dried under different drying conditions until they were visibly dry. The effect of drying in an incubator without fan at 37°C±1°C, with a relative humidity of 15-20% and drying in an incubator fitted with a fan at 37°C±1°C at a relative humidity of 15-20% was determined.

After the drying time the number of surviving organisms, which could be recovered from the surface, was determined quantitatively by using the spread plate technique. The number of bacteria on a surface without being dried was also determined and the reduction in viable counts calculated by difference.

10 repetitions with each test organism, each interfering substance and each drying condition were carried out.

Results

Drying time took 55 - 60 min in the incubator without fan, whereas only 25 - 30 min was needed in the incubator fitted with a fan until the surfaces were visibly dry. The longer drying time in the incubator without fan resulted in higher reductions (see figure 1).
The higher the concentration of the interfering substance the higher was the survival rate of the tested gram negative bacteria.

In the trials with *Pseudomonas aeruginosa*, depending on drying time and interfering substance, mean log reductions from 0,24 to 1,89 were determined. Comparable results were seen when *Escherichia coli* was used as test organism.

Within 25 – 30 min drying time more than 3 log reductions were found when using 0,3 g/l or 3,0 g/l bovine albumin as interfering substance and *Aeromonas hydrophila* as test organism. When 10g/l yeast extract and 10g/l bovine albumin were added a mean log reduction of 2,3 was determined.

Salmonella Senftenberg was more resistant to the different drying conditions than the other gram negative bacteria tested in this study. Depending on interfering substance and drying time mean log reductions ranged from 0,1 to 0,91.

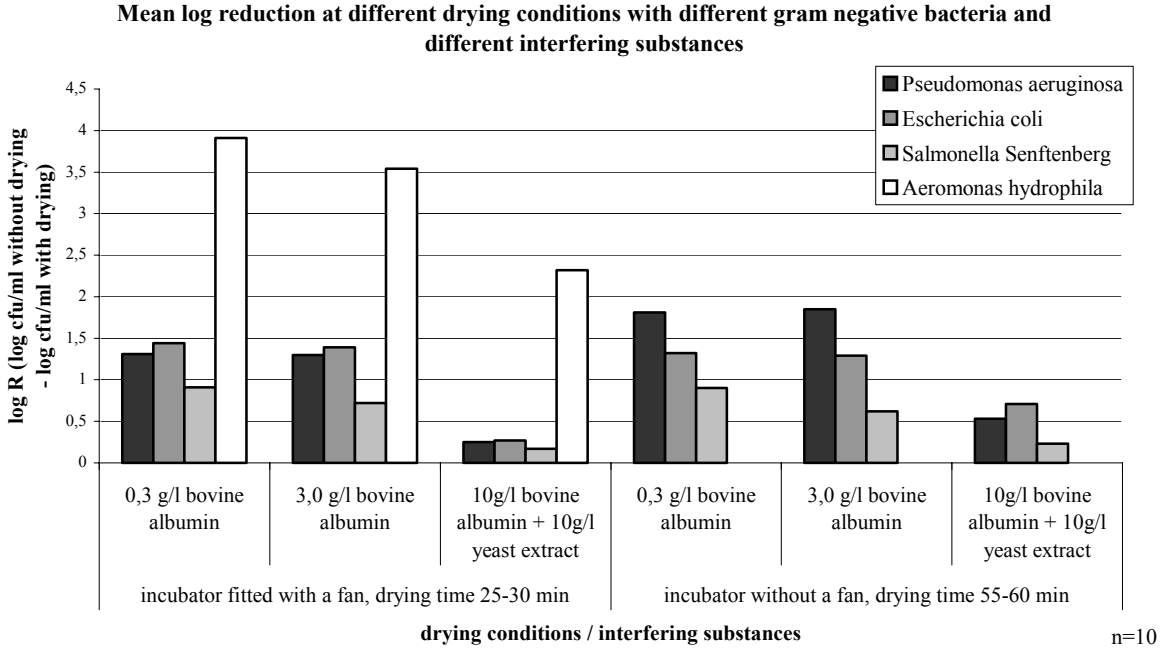

Figure 1: Mean log reductions

Discussion and conclusions

The drying time and the choice of interfering substance have a significant effect on the survival rate of all tested gram negative bacteria.

Consequently he drying time should be clearly defined in EN 14349 and should not exceed 60 min. The higher the concentration of the interfering substance, the higher was the survival rate of the tested microorganisms due to the fact that the protecting effect increased.

Due to the fact that *Aeromonas hydrophila* was found the most susceptible test organism to drying it is not suitable to replace *Pseudomonas aeruginosa* in EN 14349 by this test organism. *Pseudomonas aeruginosa* and *Escherichia coli* showed comparable results. *Salmonella Senftenberg* is more resistant to the different drying conditions than the other tested gram negative bacteria.

The results show that

- It is not suitable to replace *Pseudomonas aeruginosa* by *Escherichia coli* or *Aeromonas hydrophila*
- *Salmonella Senftenberg* is more resistant to the different drying conditions than *Pseudomonas aeruginosa*, but in further testing the resistance against the disinfectant activity has to be determined
- The survival rate of *Pseudomonas aeruginosa* can be increased by defined drying conditions, so that *Pseudomonas aeruginosa* can be confirmed as test organism

References

1 *CEN, European Committee for Standardization, Comité Européen de Normalisation, Europäisches Komitee für Normung (2004)*
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CEN, Central Secretariat: rue de Stassart 36, 1050 Brussels, Belgium