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Test report regarding the

Microbiological effectiveness of "ProfilGate®-aqua" on rollers of underfloor vehicles (trolleys) and on soles of working boots (challenge testing)

Total pages: 10

Test-No.: P07-19

Order by: Christian Löwe, HEUTE Maschinenfabrik GmbH & Co. KG

Origin of samples/
Execution: Fa. HEUTE Maschinenfabrik GmbH & Co. KG, Werk 2

Test design: Christian Löwe, Prof. Dr. Johannes Krämer, Prof. Dr. Dr. Alexander Prange

Test execution
(microbiological test): Prof. Dr. Dr. Alexander Prange
Prof. Dr. Johannes Krämer

Test execution
(Set up test stand /
desinfectand): Employees Fa. HEUTE Maschinenfabrik GmbH & Co. KG

Test date: January 17th, 2019

Intake of sample: 17.01.2019

Transportation: In Cooler

Intake temperature +10 °C

Storage: none

Start date of the test: 17.01.2019

End date of the test: 05.02.2019

Die Prüfergebnisse beziehen sich ausschließlich auf den Prüfgegenstand und den Umfang der Untersuchungen. Die Veröffentlichung und Vervielfältigung unserer Prüfberichte und Gutachten -auch auszugsweise- bedarf der schriftlichen Genehmigung der IBELIN GmbH & Co. KG.

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1 Objective of the test:

Determination of the microbiological effectiveness of "ProfilGate®-aqua" on rollers of underfloor vehicles (single approach)

2 Test objects:

Rollers of polyamide trolleys (PA rollers)



Fig. 1: Used trolley (new trolley on HEUTE's test stand).

3 Test Setup

A trolley with standard polyamide rollers (PA rollers) (150mm diameter, 50 mm width) was pushed at walking speed across a ProfilGate®-aqua field (virgin material) of approx. 2m length. The stainless steel tubs of the cleaning field contained a 2% disinfectant solution (P3-topax 990, Ecolab). HEUTE's specifications are a simulated standard process (as it is common practice).

The rolls were disinfected with a surface disinfectant (Milliseptol®) prior to (each) transfer and then they were successively contaminated with a suspension of gram-negative bacteria (*Escherichia coli*), gram-positive bacteria (*Enterococcus faecium*) and mould spores (*Aspergillus niger*).

Directly after the contamination and additionally after 15 minutes drying time a microbiological sample (O-value) was taken (swab sample and impression with RODAC plates; swab method and RODAC impression according to DIN 10113-1-3 - both methods in their execution: semi-quantitative due to the given test materials; roller boot soles - 25cm² could not always be swabbed). Then the trolley was driven over the above mentioned cleaning field ProfilGate®-aqua. In order to check the microbiological reduction effect, the rolls were finally microbiologically sampled again after the transfer and compared with the initial concentration of the microorganisms.

Schematic test procedure:

a) Preparation

- Fill 2 ProfilGate®-aqua fields with 2% disinfectant solution (P3-topax 990) - clean and wash rolls
Dry rollers (with blower)
- Select test areas on the roles
- Apply test organisms in high concentration (10⁶ to 10⁷ KbE/ml test suspension) to the marked test area (spray bottle).
- Dry for at least 15 minutes

Microbiological sampling (O-value)

- Abklatschtest with RODAC plates, Direct evaluation (counting of colonies on the surface of the RODAC plates - 25 cm² after incubation, subsequently in the laboratory)
- Swab with sterile swab from the marked test area of the rolls (approx. 5 x 5 cm - approx. 25 cm²) - Place swab in 1 ml buffer, mix and plate 0,1 ml of the suspension onto the appropriate nutrient agar plate, count colonies after incubation of plates in the laboratory

b) Test

Driving over the ProfilGate®-aqua fields with the trolley (without stop) at walking speed (3-4 km/h)



Fig. 2 Crossing the test area



Fig. 3 Abklatschtest with RODAC plate

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Fig. 4 Swab sample

4 Selection of disinfectant solutions

The stainless steel tubs of the cleaning field contained 15l water and 310ml of the disinfectant solution. Accordingly, the concentration of the mixture was 2%; employees of HEUTE had prepared the cleaning area and the mixture. As the disinfectant solution P3-topax 990 from Ecolab was applied.



Fig. 5 Original container disinfectant (Ecolab P3-topax 990).



Fig. 6 The concentration (2%) of P3-topax-990 was checked with indicator paper specially provided by Ecolab (at the starting point, *At half way and at the end of the test*).

5 Test organisms

A broad variety of microorganisms can cause food poisoning. This is very frequently the case with gram-negative bacteria such as salmonella and pathogenic *E. coli* strains and gram-positive bacteria such as listeria.

Examples of these pathogens or their surrogates were therefore used as challenge organisms:

a) *Escherichia coli*

Escherichia coli was selected as test organism for gram-negative rod-shaped bacteria.

Reason: Gram-negative pathogens include pathogenic *E. coli* strains (e.g. STEC, EHEC) and salmonella. It can be generally assumed that the environmental behaviour of salmonella is the same as it is the case with the selected test organism *E. coli*.

b) Test of a surrogate for all vegetative gram-positive pathogenic bacteria strains:

***Enterococcus faecium* ATCC 8459** (NRRL B-2354) - Biosafety Level: 1- International strain of the US American Strain Cultures Collection.

Reason: Gram-positive bacteria (e.g. *Listeria monocytogenes*) are more resistant to environmental influences than gram-negative bacteria. Therefore, the gram-positive *Enterococcus faecium* ATCC 8459 (NRRL B-2354) - Biosafety Level: 1 shall be applied. This gram-positive *Enterococcus strain* is considered non-pathogenic and is used internationally as a surrogate for the validation of industrial processes also within industrial companies, because it has a very high environmental resistance compared to other gram-positive microorganisms.

c) Mould fungus *Aspergillus niger*

A mixture of conidia (spores) and vegetative hyphae of the mould fungus *Aspergillus niger* shall be used.

Reason: the mould fungus *Aspergillus niger* is widespread in nature and acts a food spoilage agent and is generally used as an international test strain.

6 Concentration of the microorganisms used in the challenge test

106 - 107 KbE/ml (Mc Farland standard), cultural control of the strains.

7 Results: Influence of Profil IG ate®-aqua on the loading of the contaminated rollers of trolleys with *Escherichia coli*

| <i>E. coli</i> | Wheels without preparation | Wheels after disinfection | Wheels according to the order of MO | Wheels after spraying on and drying on | Wheels after crossing of the areas | Reduction |
|----------------|----------------------------|---------------------------|-------------------------------------|--|------------------------------------|--|
| RODAC | 0 KbE/ 25 cm ² | 0 KbE/ 25 cm ² | Rasen*/ 25 cm ² | Rasen*/ 25 cm ² | 141 KbE/ 25 cm ² | Significant Reduction |
| Swab | 0 KbE/ml | 0 KbE/ml | appr. 10 ⁶ KbE/ ml ** | appr. 10 ⁵ KbE/ml ** | appr. 10 ³ KbE/ ml ** | 2log ₁₀ - Steps = 99% Reduction |

* Lawn: Overgrowth of the nutrient agar surface by microorganisms (> 1,000 germs per plate)

** approx.: this is a semi-quantitative method

Result: One crossing of the ProfilGate®-aqua (2 fields) filled with disinfectant (see above) significantly reduced the microbiological load with *Escherichia coli* by a factor of 2log₁₀, i.e. by approx. 99%.

8 Influence of ProfilGate®-aqua on the loading of rollers of trolleys with *Enterococcus faecium*

| <i>E. faecium</i> | Wheels without preparation | Wheels after disinfection | Wheels according to the order of MO | Wheels after spraying on and drying on | Wheels after crossing of the areas | Reduction |
|-------------------|----------------------------|---------------------------|-------------------------------------|--|------------------------------------|--|
| RODAC | 0 KbE/ 25 cm ² | 0 KbE/ 25 cm ² | Rasen*/ 25 cm ² | Rasen*/ 25 cm ² | 46 KbE/ 25 cm ² | Significant Reduction |
| Swab | 0 KbE/ml | 0 KbE/ml | appr. 10 ⁶ KbE/ml | appr. 10 ⁶ KbE/ ml ** | appr. 10 ⁴ KbE/ ml ** | 2log ₁₀ - steps = 99% Reduction |

* Lawn: Overgrowth of the nutrient agar surface by microorganisms (> 1,000 germs per plate)

** approx.: this is a semi-quantitative method

Result: One crossing of ProfilGate®-aqua (2 fields) filled with disinfectant (see above) significantly reduced the microbiological load with *Enterococcus faecium* by a factor of 2log₁₀, i.e. by approx. 99%.

9 Effect of ProfilGate®-aqua on the loading of rollers of trolleys with spores of *Aspergillus niger*

| <i>A. niger</i> | Wheels without preparation | Wheels after disinfection | Wheels according to the order of MO | Wheels after spraying on and drying on | Wheels after crossing of the areas | Reduction |
|-----------------|----------------------------|---------------------------|-------------------------------------|--|------------------------------------|--|
| RODAC | 0 KbE/ 25 cm2 | 0 KbE/ 25 cm2 | Lawn*/ 25 cm2 | Lawn*/ 25 cm2 | Lawn*/ 25 cm2 | No discernible reduction |
| Swab | 0 KbE/ml | 0 KbE/ml | appr. 10 ⁴ KbE/ ml ** | appr. 10 ⁴ KbE/ml ** | appr. 10 ³ KbE/ml ** | 1log ₁₀ - Steps = 90% Reduction |

* Lawn: Overgrowth of the nutrient agar surface by microorganisms (> 1,000 germs per plate)

** This is an approximate value. A semi-quantitative method has been applied.

Result: One crossing of the ProfilGate®- aqua (2 fields) filled with disinfectant (see above) reduced the microbiological load with *Aspergillus niger* spores by a factor of 2log₁₀, i.e. by approx. 90% (semi-quantitative swab method).

10 Microbiological effectiveness of "ProfilGate®-aqua" on soles of working boots

10.1 Object

Determination of the microbiological effectiveness of "ProfilGate®-aqua" on the bacteriological infestation of shoe soles (rubber boots) (single approach)

10.2 Test objects:

Soles of rubber boots



Fig. 7 Rubber boots which were used

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10.3 Test procedure

By means of bacterial microorganisms (*Escherichia coli* and *Enterococcus faecium* ATCC 8459) a test on the microbiological effectiveness of ProfilGate®-aqua on soles of boots was performed. The boots were first disinfected and then contaminated with bacterial suspensions (see above). One sample was taken immediately after contamination and another one after 15 minutes of drying time. After crossing the ProfilGate®-aqua fields more samples were taken.

Schematic test procedure:

- a) Preparation
- Fill 2 ProfilGate®-aqua fields with 2% disinfectant solution (P3-topax 990)
 - Clean, wash and disinfect soles
 - Dry soles - 15 minutes
 - Apply test organisms in high concentration (10⁶ to 10⁷ KbE /ml test suspension) to the soles.
 - Dry for 15 minutes

 - Microbiological sampling (O-value)
 - - Abklatschtest with RODAC plates, direct evaluation (counting of colonies on the surface of the RODAC plates - 25 cm² in the laboratory)
 - - Swab with sterile swab from the marked test area of the rolls (approx. 50 x 50 mm - 25 cm²) - Place swab in 1 ml buffer, mix and plate 1 ml of suspension onto the appropriate nutrient agar plate, count colonies after incubation of plates.
- b) Test
- Crossing the ProfilGate®-aqua fields (without stop) at walking speed (3-4 km/h)



Abb.8: Crossing of the cleaning areas.

10.4 Test organisms (see above)

- a) *Escherichia coli*
b) *Enterococcus faecium* ATCC 8459

10.5 Concentration of the microorganisms used in the challenge test

10⁶ -10⁷ KbE/ml (McFarland Standard), cultural control of the strains.

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10.6 Influence of Profi IG ate®-a qua on the load on the rubber boot soles of *Escherichia coli*

| <i>E. coli</i> | Sole, disinfected and dried | Soles after application of <i>E. coli</i> and drying | Soles after Crossing of testing areas | Reduction |
|----------------|-----------------------------|--|---------------------------------------|---|
| RODAC | 0 KbE/ 25 cm ² | Lawn* / 25 cm ² | Lawn* / 25 cm ² | No discernible reduction** |
| Swab | 0 KbE/ml | appr. 10 ⁵ KbE/ ml** | appr. 10 ³ KbE/ ml" | 2log ₁₀ -steps = 99% reduction |

* Lawn: Overgrowth of the nutrient agar surface by microorganisms (> 1,000 germs per plate)

** This is an approximate value. A semi-quantitative method has been applied.

** For reduction values in this area, the squeeze method often does not prove to be sensitive enough.

Result: One crossing of the disinfectant-filled ProfilGate®-aqua (2 fields) significantly reduced the microbiological infestation of *Escherichia coli* on the boot soles by a factor of 2log₁₀, i.e. by approx. 99% (semi-quantitative swab method).

10.7 Influence of ProfilGate®-aqua on the infestation on the rubber boot soles of *Enterococcus faecium*

| <i>E. faecium</i> | Sole, disinfected and dried | Soles after application of <i>E. faecium</i> and drying | Soles after Crossing of testing areas | Reduction |
|-------------------|-----------------------------|---|---------------------------------------|--|
| RODAC | 0 KbE/ 25 cm ² | Lawn* / 25 cm ² | 60 KbE / 25 cm ² | Significant Reduction |
| Swab | 0 KbE/ml | appr. 10 ⁵ KbE/ml" | appr. 10 ³ KbE/ ml" | 2log ₁₀ steps = 99% reduction |

* Lawn: Overgrowth of the nutrient agar surface by microorganisms (> 1,000 germs per plate)

* - approx.: it is a semi-quantitative process

Result: One crossing of the disinfectant-filled ProfilGate®-aqua (2 fields) significantly reduced the microbiological load *Escherichia coli* on the soles of the boots by a factor of 2log₁₀, i.e. by approx. 99% (semi-quantitative swab method).

11 Summary “Trolley”

Considering the above-mentioned test conditions: When crossing a ProfilGate®-aqua (2m length) field filled with disinfectant (trolley) the amount of microorganisms was significantly reduced (approx. 99% (bacteria) and 90% (moulds)).

12 Summary “Soles of working boots”

Considering the above-mentioned test conditions: When crossing a ProfilGate®-aqua (2m length) field filled with disinfectant (boots) the amount of microorganisms was significantly reduced (approx. 99% bacteria).

Bonn/Brilon, dated February 10th, 2019



Prof. Dr. Johannes Krämer



Prof. Dr. Dr. Alexander Prange