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Comparison study of hygienic properties of conveyor belt materials

Assignment

Comparison of the cleansing of stainless steel conveyor belt with plastic conveyor belts including a literature study on EU legislation, regulations and test methods

This report is an update of the Research Report VTT-S-05419-10 containing additional videometer and SEM pictures as well as a literature study on EU legislation and regulations on Food Contact Materials (FCM) in food processing equipment including methods used for verification of compliance with regulations.

Samples

The customer delivered the surfaces to be tested to VTT in April 2010. Three different conveyor belt materials were:
- Stainless steel conveyor belt (AISI 301),
- Plastic conveyor belt, solid and
- Plastic conveyor belt, lamel

Each material was studied using both new surfaces and surfaces damaged with knife. The damaging was performed by the customer before delivery of the test surfaces to VTT. Pictures of surfaces tested are shown in Table 1 (Appendix 1).

Treatment of samples

Before soiling all test surfaces were soaked in soap water containing cleaning agent meant for removing grease and the surfaces were furthermore wiped with ethanol before soiling with a mixture of *Pseudomonas fragi* E-98200T, *Candida albicans* C-85161 and *Listeria innocua* E-991340 in blood. All test strains have been taken from the VTT Culture Collection. The Gram-negative *P. fragi* is known as a strong biofilm former and has similar properties as the pathogenic *Pseudomonas aeruginosa* bacterium. *C. albicans* is an opportunic pathogenic yeast test strain. The Gram-positive *L. innocua* is behaving similarly as the pathogenic *L. monocytogenes*.

These microbes can be found in fish and meat producing facilities, when the cleaning is inaduatelty performed. Test surfaces were soiled in the above mentioned mixture for various periods (4 h, 8 h, 16 h, 24 h, 72 h, 72 h + 72 h and 72 h + 72 h + 72 h (in the two latter series cleaning was carried out between the 72 h- soilings)) simulating different situations in meat factories. The soiling procedure was performed at room temperature. The blood used was fresh frozen cow blood diluted with tap water 1:2. The microbes were refreshed in nutrient broths and mixed in blood (15 ml of each suspension into 9 l blood-water solution). 1.5 l of blood-water mixture was renewed every third day and at the same time new fresh microbes were added. The blood, water and the container for soiling were not sterile, but there was an excess of microbes inoculated which took over the growth.

Analysis

After each soiling period 2 pieces of each surface type were used to study the surfaces in dirty condition, these surfaces were rinced by dipping them in water and placed on petridish. After the surfaces were dry the dirtyness of surfaces were studied by traditional culturing, protein residue test and microscopying. One test surface of each type was swabbed with a moistured cotton-tipped swab which was transferred to test tube containing 5 ml peptone saline. Test tube was mixed and dilution series were made for culturing on plate count agar (PCA; Difco™, BD, Sparks, MD, US) and potato dextrose agar (PDA; Difco™). The PCA-dishes were incubated for 3 d at 30 °C and the PDA-dishes for 5 d at 25 °C before counting of colonies. One half of the second test surface was used for protein testing. The surface was moistened with water and wiped with a Clean Card (Orion Diagnostica Oy, Espoo, Finland), which is a rapid test showing protein residues. The protein residues left on surfaces can function as growth media for microbes. The result from the Clean Card test is obtained after 30 s by counting blue and bluish spots. The other half of the second test surface was stained for 2 min with acridine orange (Difco™) and microscoped using epifluorescence microscopy.

After each soiling period 2 pieces of each type of surfaces from blood-water-solution were also taken for cleanability studies. The cleanability of the various surfaces was studied by placing the test surfaces in racks in the open process pilot-scale cleaning simulator and using foam cleaner in combination with low pressure. Dirty surfaces were cleaned using alkaline foam cleaner (3 vol-% Diverfoam SMS HD, JohnsonDiversey, Turku, Finland) for 10 min and low pressure cleaner for rinsing (sweeping each row three times). The surfaces after the cleaning procedure were studied using similar methods as were used for the dirty surfaces.

Additionally the attachment and detachment of microbes were studied with scanning electron microscopy (SEM) on unused surfaces and surface, which had been soiled for 72 h or soiled and cleaned.

Results

Visual observation of test pieces shows that the cuts made with knife collected dirt (Tables 1 and 2, Appendix 1). The dirt was clearly seen on damaged plastic surfaces.

The amount of microbes on the surfaces was determined using cotton-tipped swabs for sampling the surfaces and performing culturing on agar plates (Table 3, Appendix 2). The amounts of microbe on new and damaged surfaces are presented as colony forming units (cfu)/cm² in Figures 1-4 (Appendix 3). The y-axis scale is logarithmic, because differences in microbial results bigger than one logarithmic unit are normally considered as meaningful differences. The microbial load on damaged stainless steel surfaces after cleaning were more than 1 log-unit smaller than the microbial load on both damaged plastic surfaces (Figures 3-4, Appendix 3). The new surfaces (Figures 1-2, Appendix 3) showed the same trends but the differences were smaller.

The results from protein tests are shown in Figures 5-11 with a summary in Table 4 (Appendix 4). The protein residue results also showed that the damaged stainless steel surfaces contained less protein residues after cleaning than the both damaged plastic surfaces. There is no reference for the accuracy of this method but this method is practical and easy to perform in field studies.

The pictures from the epifluorescence microscopying are shown in Tables 5-6 (Appendix 5). The plastic surfaces are autofluoresing and futhermore the surfaces are not smooth which makes the comparison with stainless steel surfaces difficult (some kind of grid structure is making focusing impossible). The microbes on the stainless steel surface are visible. The amount of microbes has not been counted from microscopy pictures since it is impossible to find compareable fields using same area and light settings. However from these epifluorescence pictures it can be seen that there is a major difference on microbial load between soiled and cleaned stainless steel surfaces.

The attachment and detachment of microbes were studied with SEM using both unused surfaces and surfaces which had been soiled 72 h. The SEM pictures revealed the smoothness of various surfaces (Figures 12-23, Appendix 6). The water from microbes evaporated during the SEM-sample preparation procedure and therefore the microbes are not shown in their original shape.

Conclusions

The stainless steel is more cleanable than the two different plastic surfaces tested according to the culturing results. The difference in cleanability is more significant for damaged surfaces.

Espoo, August 17, 2010

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Appendices

1. Visual observations of the three test surfaces
2. Examples of colonies formed on agars in various dilutions after 4h and 24 h
3. Culturing results of the three different surfaces tested
4. Protein residue test results of the three different surfaces tested
5. Epifluorescence microscopy results of the three different surfaces tested
6. SEM pictures of the three different surfaces tested
7. Literature study on EU legislation and regulations on Food Contact Materials (FCM) in food processing equipment including methods used for verification of compliance with regulations

Distribution

Customer	Original
Archive	Original

Table 1. Test pieces of conveyor belt materials used in comparison study

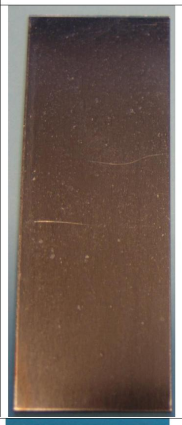
	Original new surface	New surface after 4 h soiling in blood containing microbes	Damaged surface	Damaged surface after 4 h soiling in blood containing microbes
Stainless steel conveyor belt (AISI 301)				
Plastic conveyor belt (solid)				
Plastic conveyor belt (lamel)				

Table 2. Soiled (16 h) and cleaned test surfaces

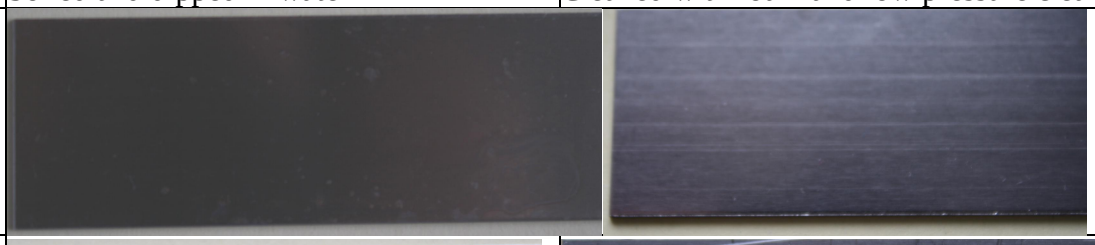
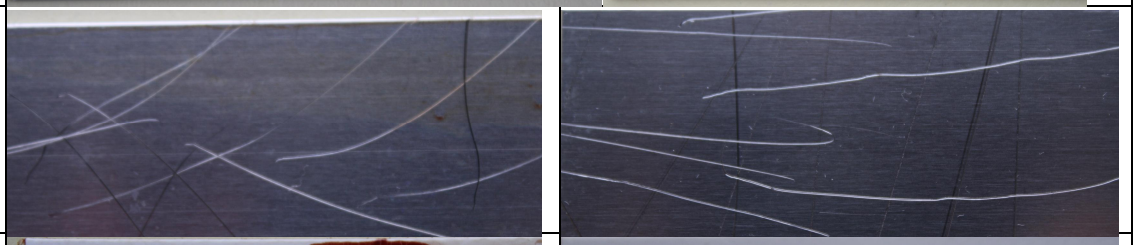
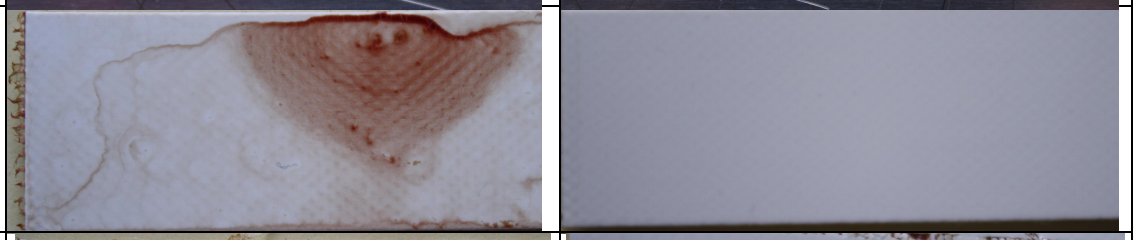
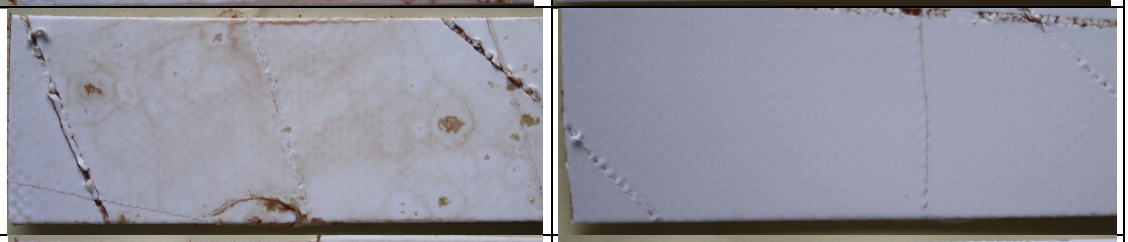
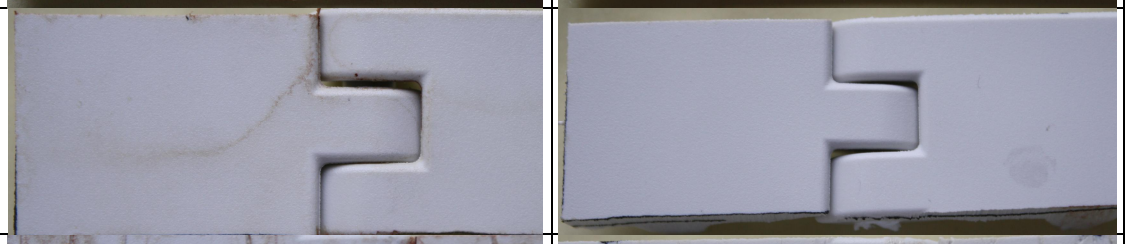
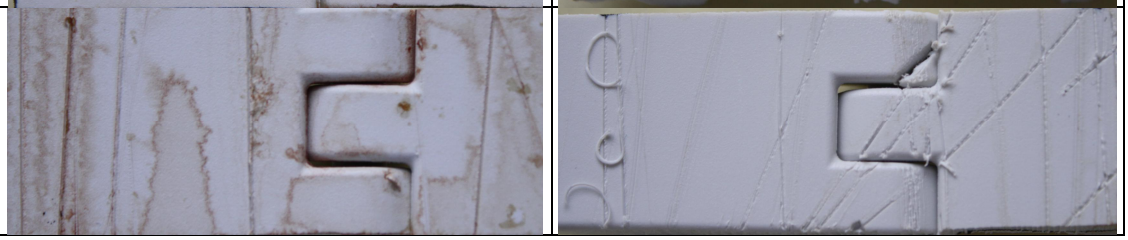
	Soiled and dipped in water	Cleaned with foam and low pressure cleaner
Stainless steel, soiled 16 h		
Damaged stainless steel, soiled 16 h		
Solid plastic, soiled 16 h		
Damaged solid plastic, soiled 16 h		
Lamell plastic, soiled 16 h		
Damaged lamell plastic, soiled 16 h		

Table 3a. Colonies on Plate Count Agar after incubation. This example is from third parallel test series after 4 h soiling. Pictures are taken with videometer.

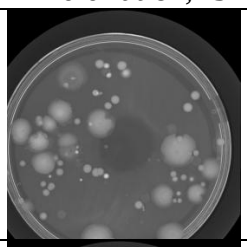
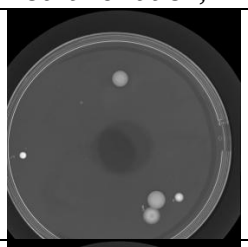
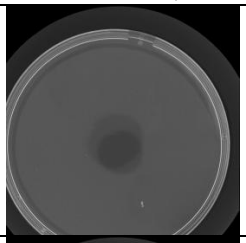
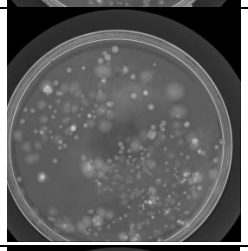
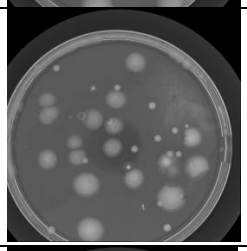
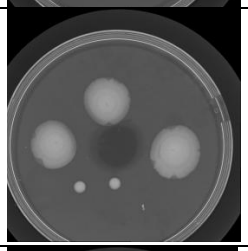
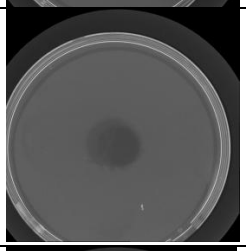
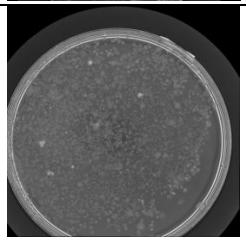
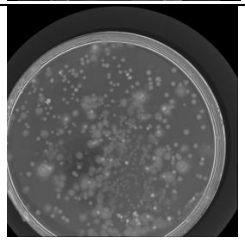
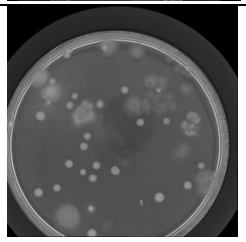
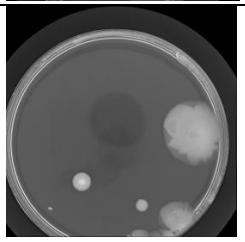
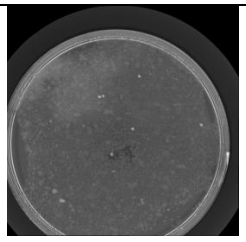
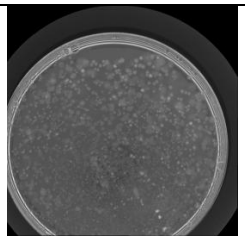
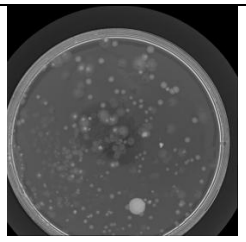
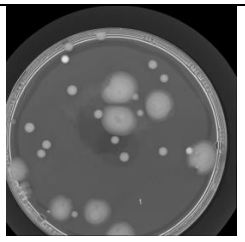
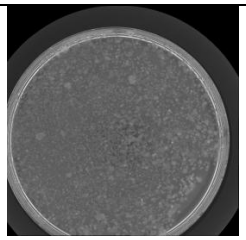
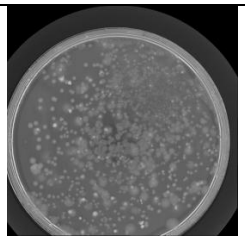
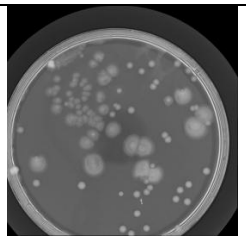
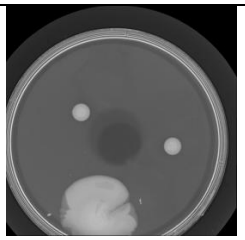
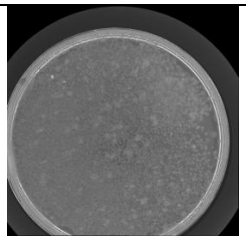
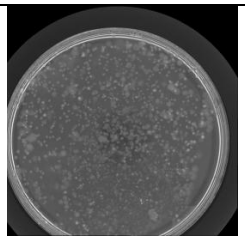
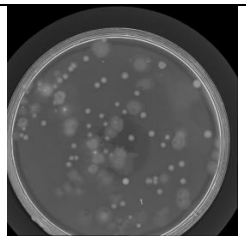
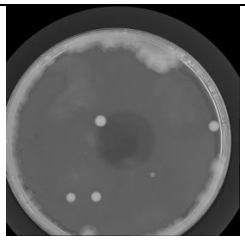
	Second dilution, -2	Third dilution, -3	Fourth dilution, -4	Fifth dilution, -5
New stainless steel, soiled 4 h				
Damaged stainless steel, soiled 4 h				
New solid plastic, soiled 4 h				
Damaged solid plastic, soiled 4 h				
New lamell plastic, soiled 4 h				
Damaged lamell plastic, soiled 4 h				

Table 3b. Colonies on Plate Count Agar after incubation. This example is from third parallel test series after 4 h soiling followed by foam and low pressure cleaning. Pictures are taken with videometer.

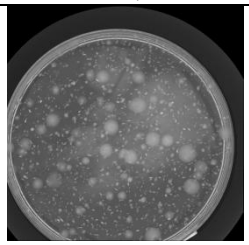
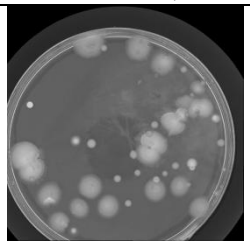
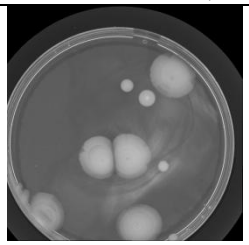
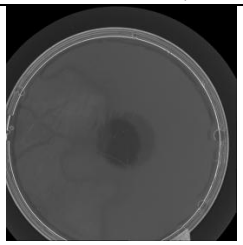
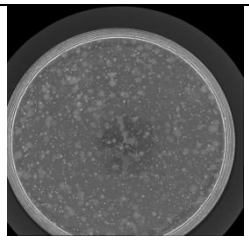
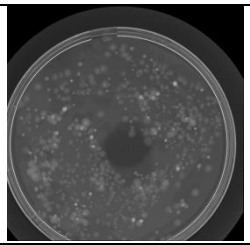
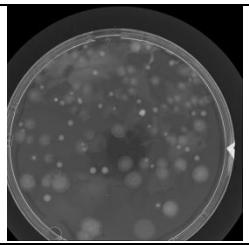
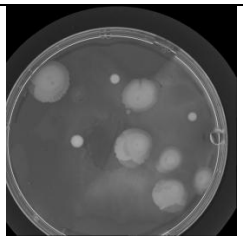
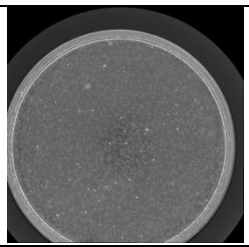
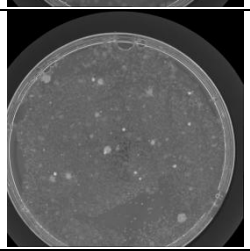
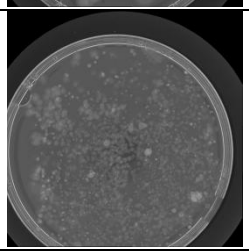
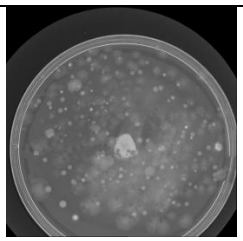
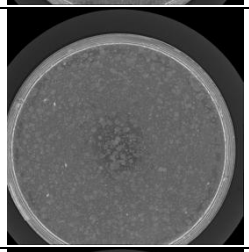
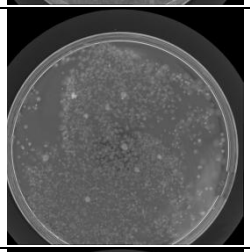
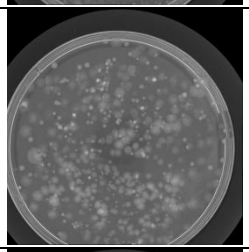
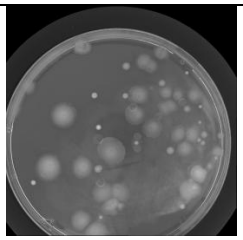
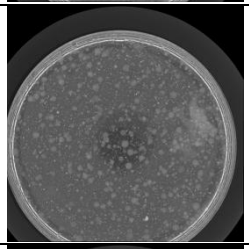
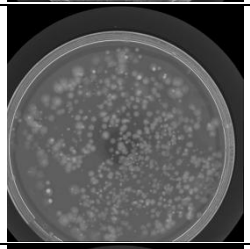
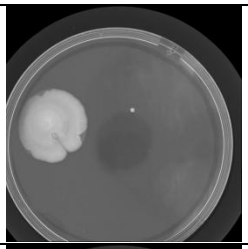
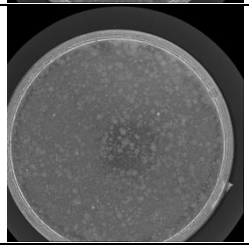
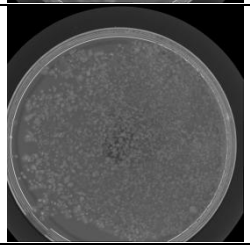
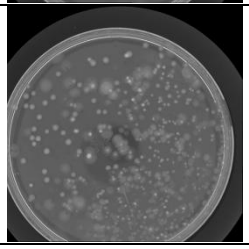
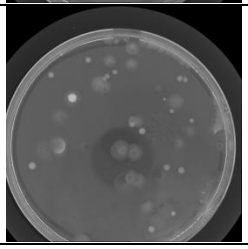
	Undiluted, 0	First dilution, -1	Second dilution, -2	Third dilution, -3
New stainless steel, soiled 4 h and cleaned				
Damaged stainless steel, soiled 4 h and cleaned				
New solid plastic, soiled 4 h and cleaned				
Damaged solid plastic, soiled 4 h and cleaned				
New lamell plastic, soiled 4 h and cleaned				
Damaged lamell plastic, soiled 4 h and cleaned				

Table 3c. Colonies on Plate Count Agar after incubation. This example is from third parallel test series after 24 h soiling. Pictures are taken with videometer.

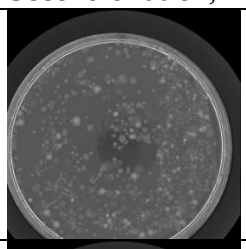
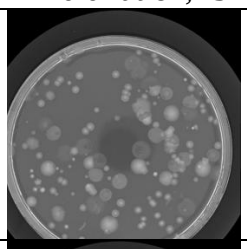
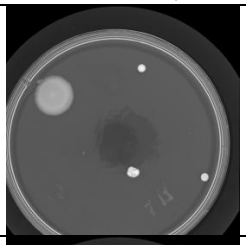
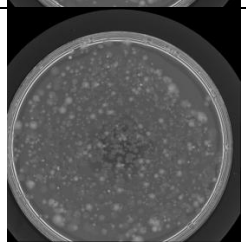
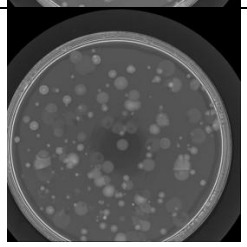
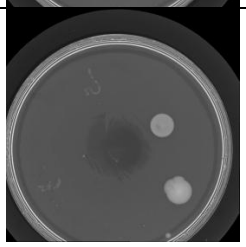
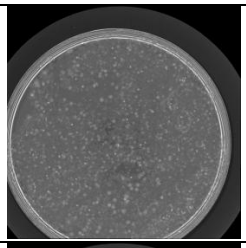
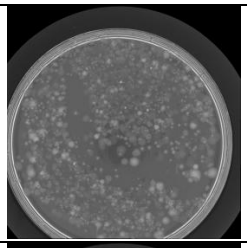
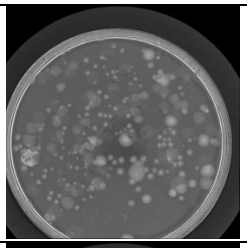
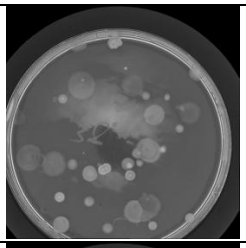
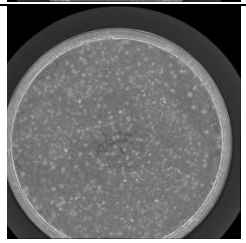
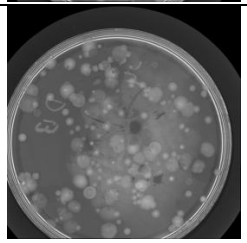
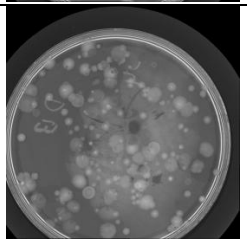
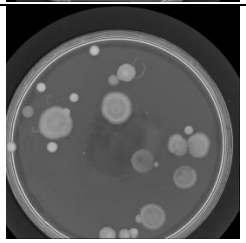
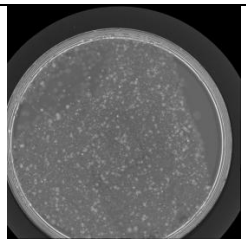
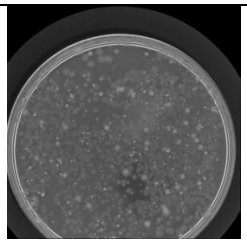
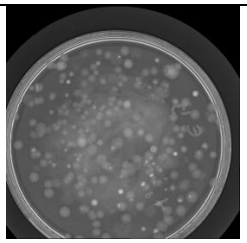
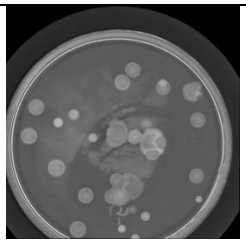
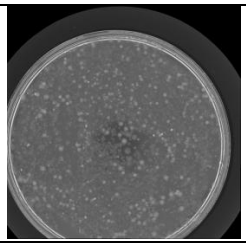
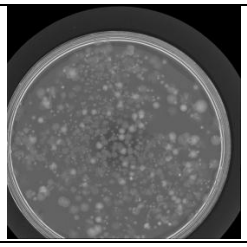
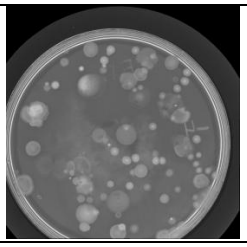
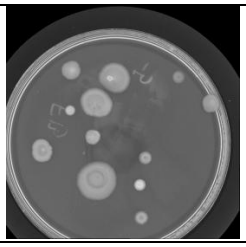
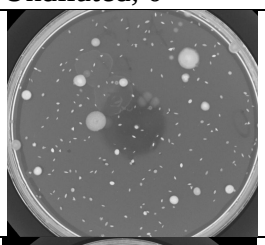
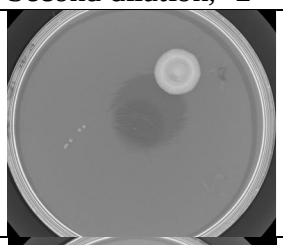
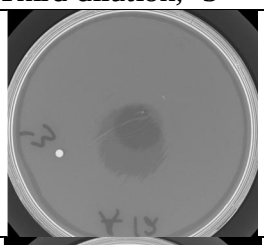
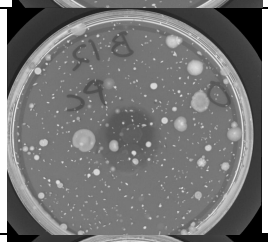
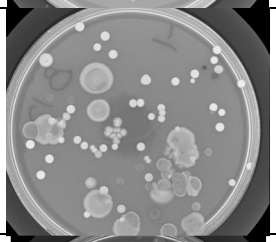
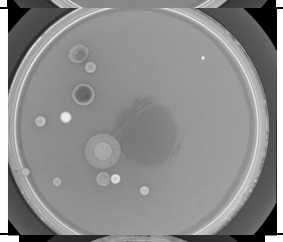
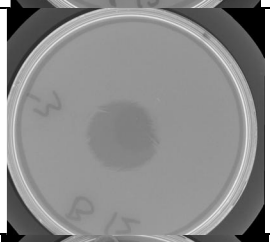
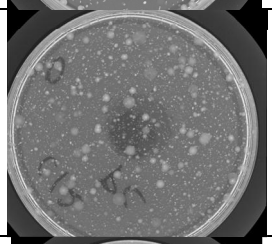
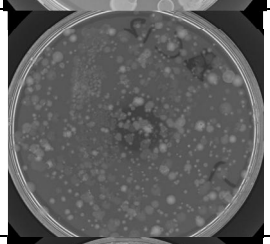
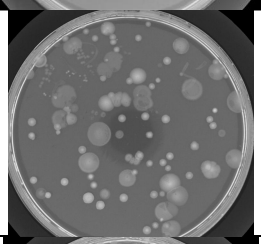
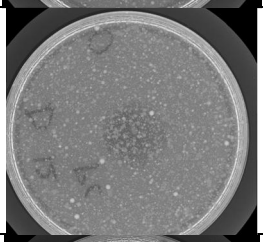
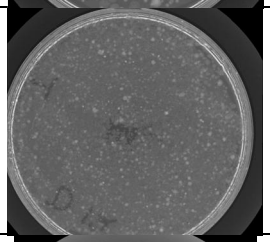
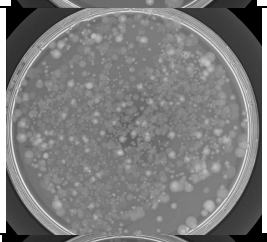
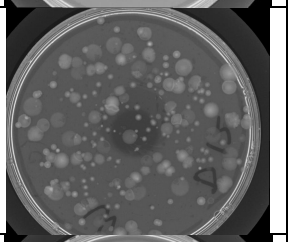
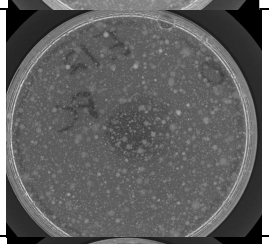
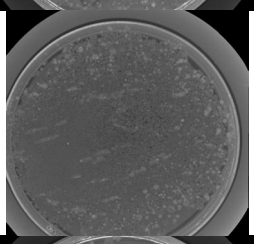
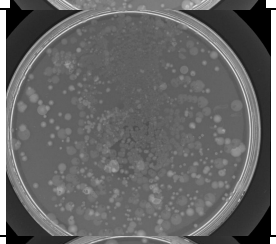
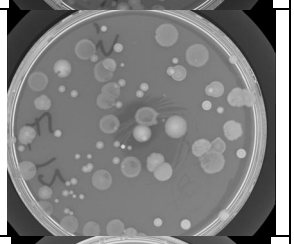
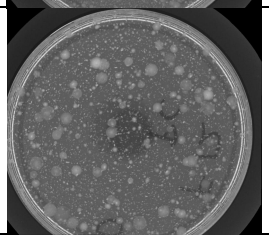
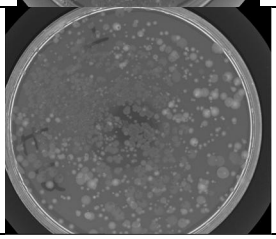
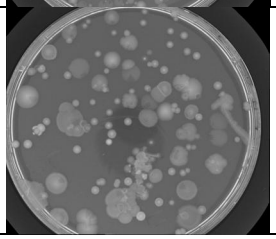
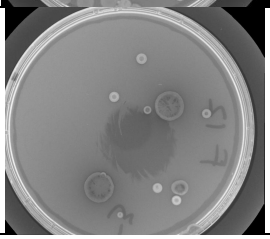
	Second dilution, -2	Third dilution, -3	Fourth dilution, -4	Fifth dilution, -5
New stainless steel, soiled 24 h				
Damaged stainless steel, soiled 24 h				
New solid plastic, soiled 24 h				
Damaged solid plastic, soiled 24 h				
New lamell plastic, soiled 24 h				
Damaged lamell plastic, soiled 24 h				

Table 3d. Colonies on Plate Count Agar after incubation. This example is from third replicate test series after 24 h soiling followed by foam and low pressure cleaning. The pictures are taken with videometer.

	Undiluted, 0	First dilution, -1	Second dilution, -2	Third dilution, -3
New stainless steel, soiled 24 h and cleaned				
Damaged stainless steel, soiled 24 h and cleaned				
New solid plastic, soiled 24 h and cleaned				
Damaged solid plastic, soiled 24 h and cleaned				
New lamell plastic, soiled 24 h and cleaned				
Damaged lamell plastic, soiled 24 h and cleaned				

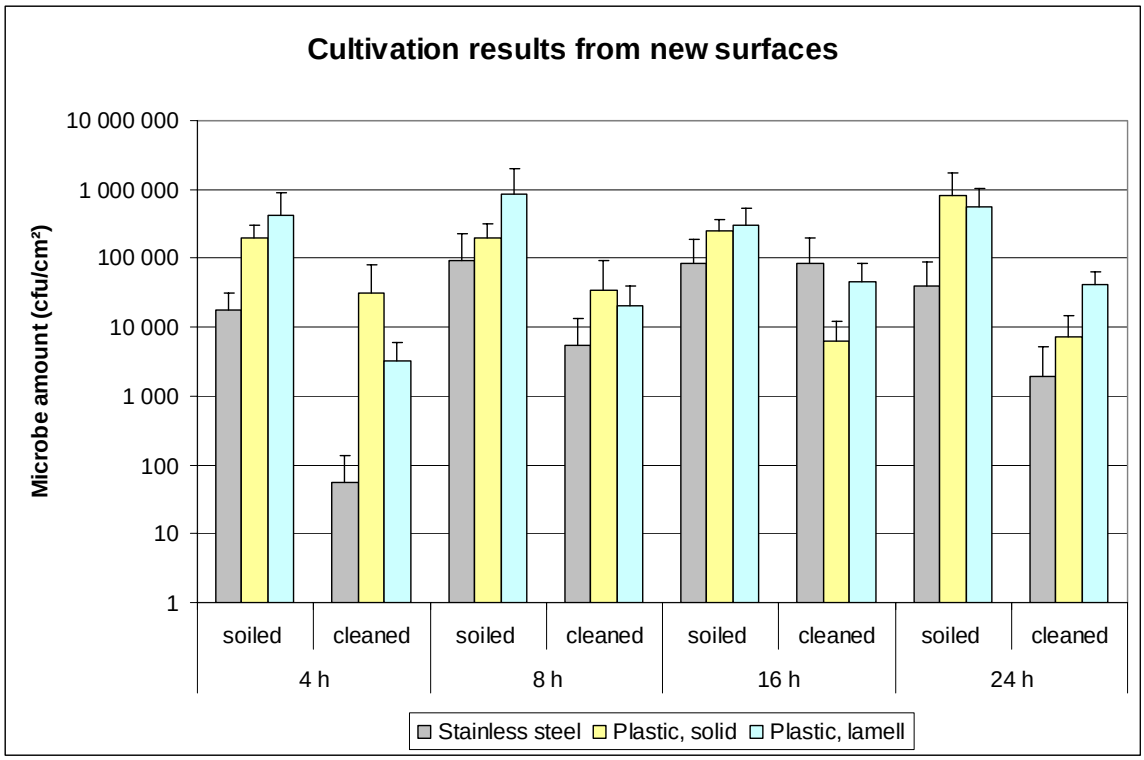


Figure 1. Culturing results from new surfaces after 4 h, 8 h, 16 h and 24 h soiling as such (=soiled) and after cleaning procedure (cleaned) including standard deviation lines.

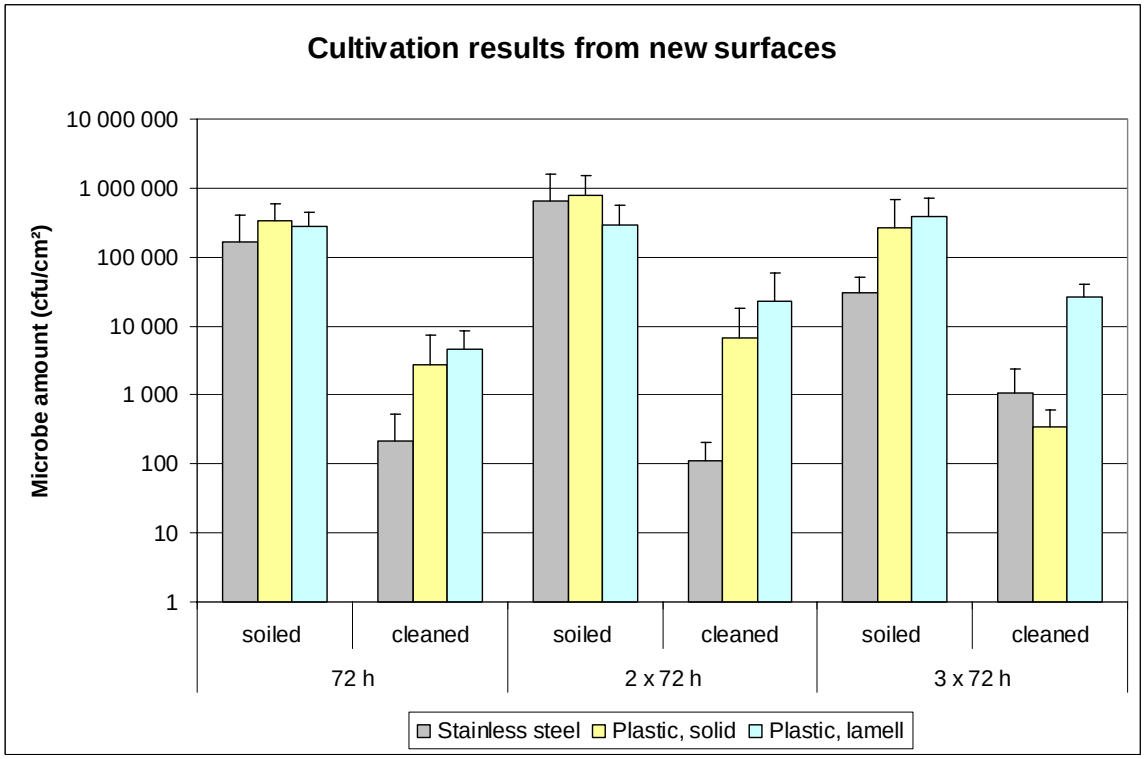


Figure 2. Culturing results from new surfaces after 72 h, 2 x 72 h and 3 x 72 h soiling as such (=soiled) and after cleaning procedure (cleaned) including standard deviation lines.

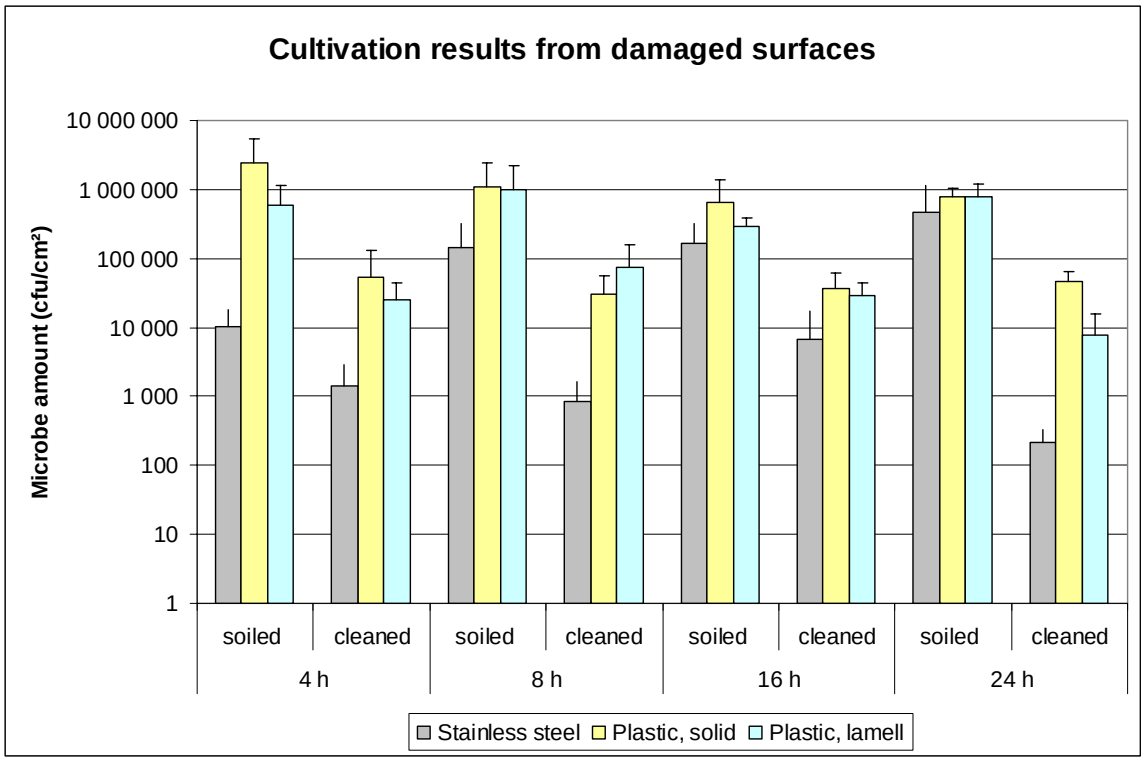


Figure 3. Culturing results from damaged surfaces after 4 h, 8 h, 16 h and 24 h soiling as such (=soiled) and after cleaning procedure (cleaned) including standard deviation lines.

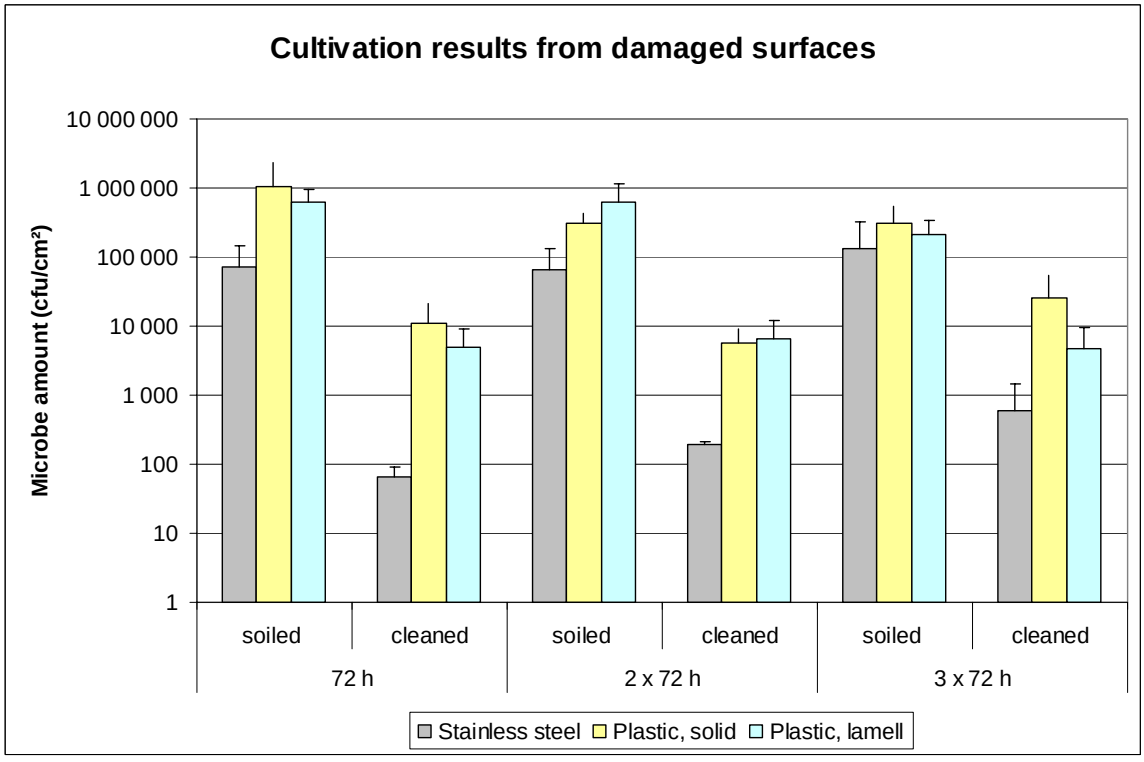


Figure 4. Culturing results from damaged surfaces after 72 h, 2 x 72 h and 3 x 72 h soiling as such (=soiled) and after cleaning procedure (cleaned) including standard deviation lines.

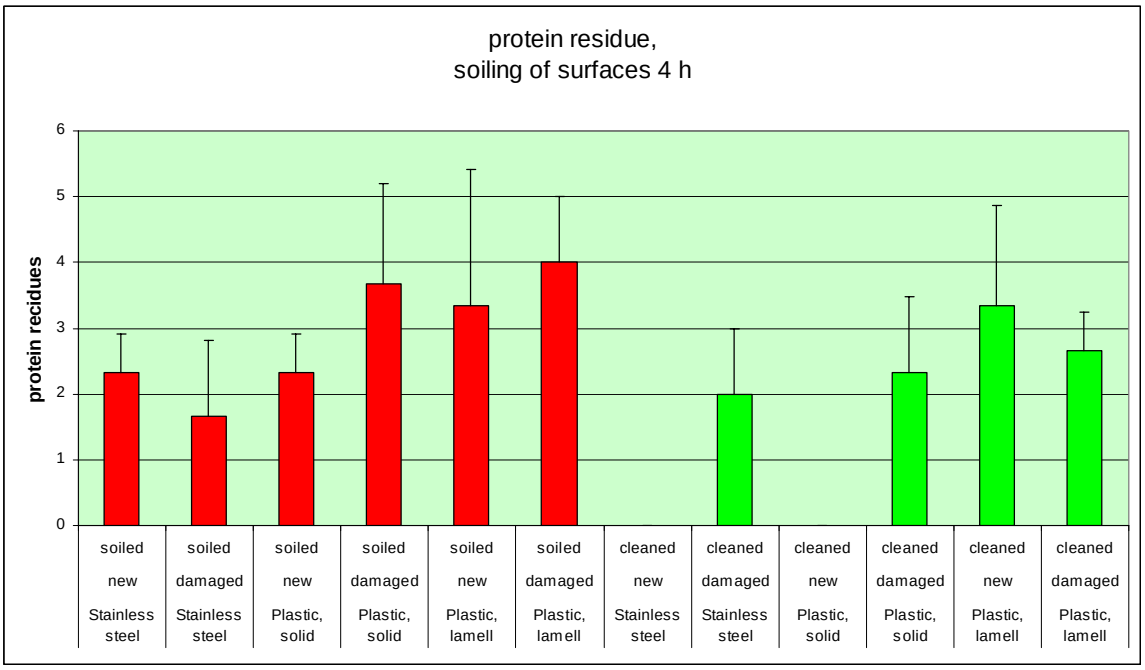


Figure 5. Protein residues on 4 h soiled surfaces before (red bars) and after (green bars) cleaning procedure including standard deviation line.

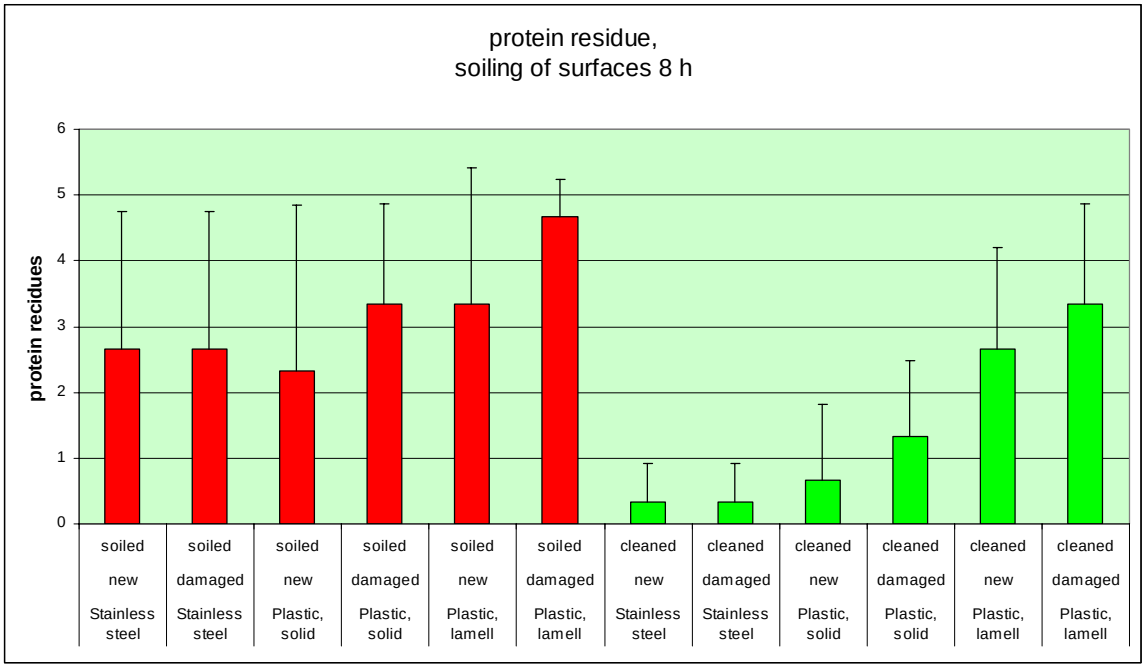


Figure 6. Protein residues on 8 h soiled surfaces before (red bars) and after (green bars) cleaning procedure including standard deviation line.

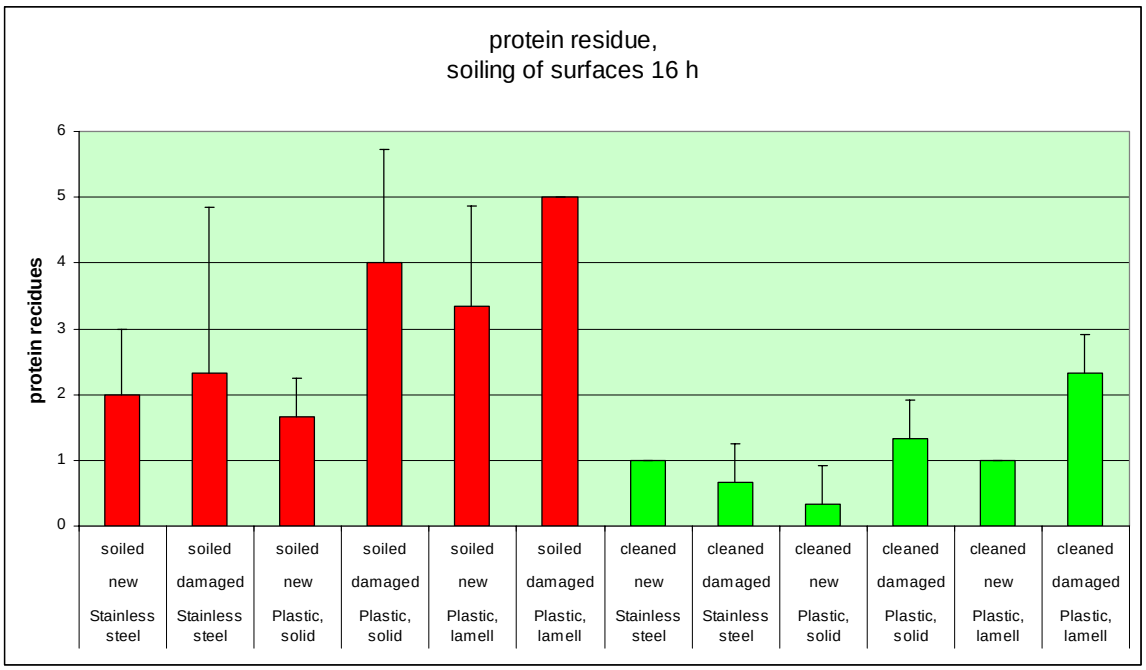


Figure 7. Protein residues on 16 h soiled surfaces before (red bars) and after (green bars) cleaning procedure including standard deviation line.

Figure 8. Protein residues on 24 h soiled surfaces before (red bars) and after (green bars) cleaning procedure including standard deviation line.

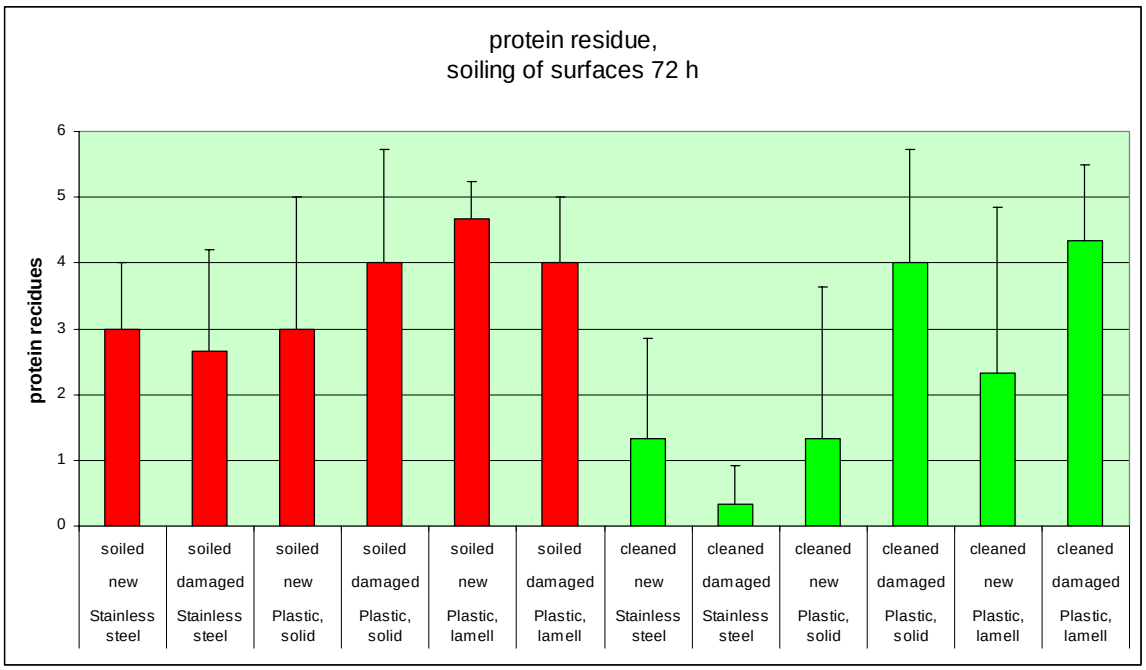


Figure 9. Protein residues on 72 h soiled surfaces before (red bars) and after (green bars) cleaning procedure including standard deviation line.

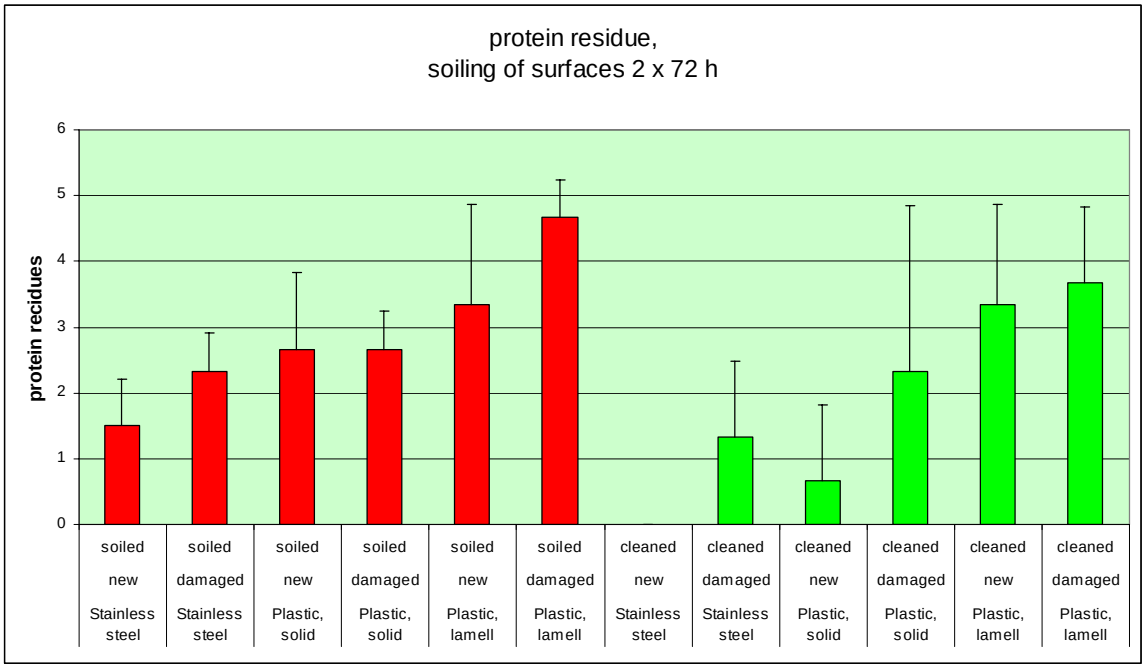


Figure 10. Protein residues on 2 x 72 h soiled surfaces before (red bars) and after (green bars) cleaning procedure including standard deviation line.

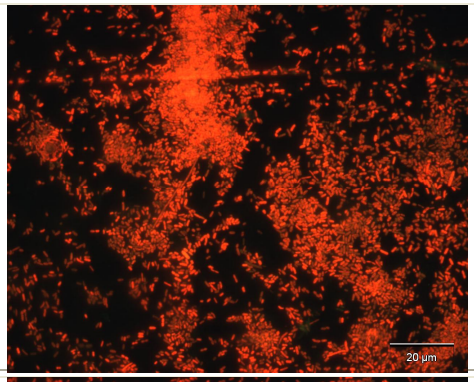
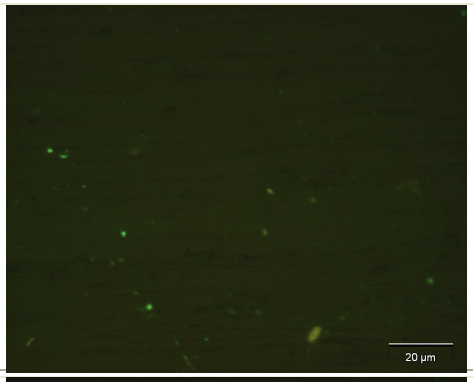
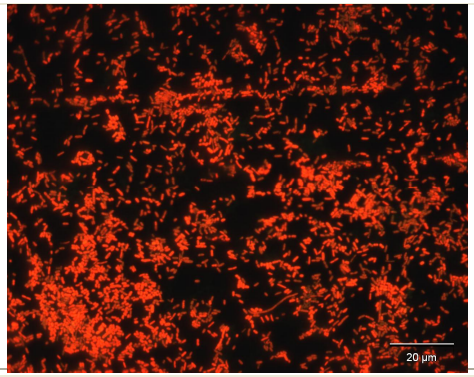
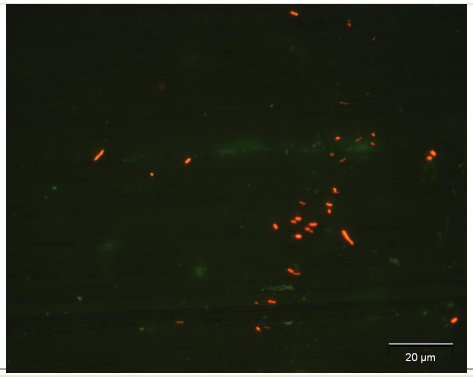
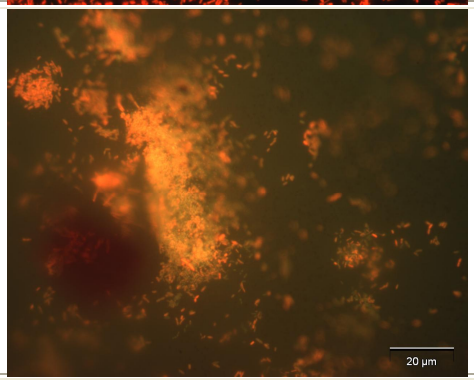
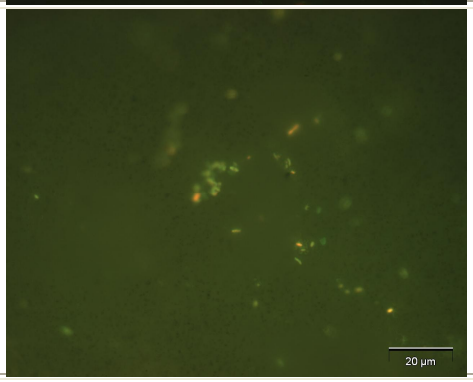
Figure 11. Protein residues on 3 x 72 h soiled surfaces before (red bars) and after (green bars) cleaning procedure including standard deviation line.

Table 4. Results from protein residue test. The amount of protein residue was estimated by counting areas of test paper where the colour change had happened. 0 = no color change, 1 = one of five areas has changed colour, 2 = two of five areas have changed colour, 3 = three of five areas have changed colour, 4 = four of five areas have changed colour and 5 = five of five areas have changed colour.

soiling time	material	surface	treatment	protein residue 1. test series	protein residue 2. test series	protein residue 3. test series	protein residue average	protein residue standard deviation
4 h	Stainless steel	new	soiled	2	2	3	2,33	0,58
4 h	Stainless steel	damaged	soiled	1	1	3	1,67	1,15
4 h	Plastic, solid	new	soiled	2	2	3	2,33	0,58
4 h	Plastic, solid	damaged	soiled	2	4	5	3,67	1,53
4 h	Plastic, lamell	new	soiled	4	1	5	3,33	2,08
4 h	Plastic, lamell	damaged	soiled	4	3	5	4,00	1,00
4 h	Stainless steel	new	cleaned	0	0	0	0,00	0,00
4 h	Stainless steel	damaged	cleaned	3	1	2	2,00	1,00
4 h	Plastic, solid	new	cleaned	0	0	0	0,00	0,00
4 h	Plastic, solid	damaged	cleaned	3	1	3	2,33	1,15
4 h	Plastic, lamell	new	cleaned	3	2	5	3,33	1,53
4 h	Plastic, lamell	damaged	cleaned	3	2	3	2,67	0,58
8 h	Stainless steel	new	soiled	1	2	5	2,67	2,08
8 h	Stainless steel	damaged	soiled	1	2	5	2,67	2,08
8 h	Plastic, solid	new	soiled	0	2	5	2,33	2,52
8 h	Plastic, solid	damaged	soiled	2	3	5	3,33	1,53
8 h	Plastic, lamell	new	soiled	1	4	5	3,33	2,08
8 h	Plastic, lamell	damaged	soiled	4	5	5	4,67	0,58
8 h	Stainless steel	new	cleaned	1	0	0	0,33	0,58
8 h	Stainless steel	damaged	cleaned	1	0	0	0,33	0,58
8 h	Plastic, solid	new	cleaned	0	0	2	0,67	1,15
8 h	Plastic, solid	damaged	cleaned	0	2	2	1,33	1,15
8 h	Plastic, lamell	new	cleaned	1	3	4	2,67	1,53
8 h	Plastic, lamell	damaged	cleaned	2	3	5	3,33	1,53
16 h	Stainless steel	new	soiled	2	1	3	2,00	1,00
16 h	Stainless steel	damaged	soiled	0	2	5	2,33	2,52
16 h	Plastic, solid	new	soiled	2	1	2	1,67	0,58
16 h	Plastic, solid	damaged	soiled	2	5	5	4,00	1,73
16 h	Plastic, lamell	new	soiled	5	2	3	3,33	1,53
16 h	Plastic, lamell	damaged	soiled	5	5	5	5,00	0,00
16 h	Stainless steel	new	cleaned	1	1	-	1,00	0,00
16 h	Stainless steel	damaged	cleaned	0	1	1	0,67	0,58
16 h	Plastic, solid	new	cleaned	0	1	0	0,33	0,58
16 h	Plastic, solid	damaged	cleaned	2	1	1	1,33	0,58
16 h	Plastic, lamell	new	cleaned	1	1	1	1,00	0,00
16 h	Plastic, lamell	damaged	cleaned	2	2	3	2,33	0,58

soiling time	material	surface	treatment	protein residue 1. test series	protein residue 2. test series	protein residue 3. test series	protein residue average	protein residue standard deviation
24 h	Stainless steel	new	soiled	2	1	4	2,33	1,53
24 h	Stainless steel	damaged	soiled	2	2	5	3,00	1,73
24 h	Plastic, solid	new	soiled	1	2	5	2,67	2,08
24 h	Plastic, solid	damaged	soiled	3	4	5	4,00	1,00
24 h	Plastic, lamell	new	soiled	1	4	5	3,33	2,08
24 h	Plastic, lamell	damaged	soiled	3	5	5	4,33	1,15
24 h	Stainless steel	new	cleaned	0	0	0	0,00	0,00
24 h	Stainless steel	damaged	cleaned	4	0	2	2,00	2,00
24 h	Plastic, solid	new	cleaned	0	1	3	1,33	1,53
24 h	Plastic, solid	damaged	cleaned	3	2	4	3,00	1,00
24 h	Plastic, lamell	new	cleaned	0	3	5	2,67	2,52
24 h	Plastic, lamell	damaged	cleaned	1	3	5	3,00	2,00
72 h	Stainless steel	new	soiled	2	4	3	3,00	1,00
72 h	Stainless steel	damaged	soiled	1	3	4	2,67	1,53
72 h	Plastic, solid	new	soiled	1	3	5	3,00	2,00
72 h	Plastic, solid	damaged	soiled	2	5	5	4,00	1,73
72 h	Plastic, lamell	new	soiled	4	5	5	4,67	0,58
72 h	Plastic, lamell	damaged	soiled	3	4	5	4,00	1,00
72 h	Stainless steel	new	cleaned	1	0	3	1,33	1,53
72 h	Stainless steel	damaged	cleaned	0	0	1	0,33	0,58
72 h	Plastic, solid	new	cleaned	0	0	4	1,33	2,31
72 h	Plastic, solid	damaged	cleaned	5	2	5	4,00	1,73
72 h	Plastic, lamell	new	cleaned	0	2	5	2,33	2,52
72 h	Plastic, lamell	damaged	cleaned	5	3	5	4,33	1,15
2 x 72 h	Stainless steel	new	soiled	2	1	-	1,50	0,71
2 x 72 h	Stainless steel	damaged	soiled	2	2	3	2,33	0,58
2 x 72 h	Plastic, solid	new	soiled	2	2	4	2,67	1,15
2 x 72 h	Plastic, solid	damaged	soiled	2	3	3	2,67	0,58
2 x 72 h	Plastic, lamell	new	soiled	3	2	5	3,33	1,53
2 x 72 h	Plastic, lamell	damaged	soiled	5	4	5	4,67	0,58
2 x 72 h	Stainless steel	new	cleaned	0	0	0	0,00	0,00
2 x 72 h	Stainless steel	damaged	cleaned	2	0	2	1,33	1,15
2 x 72 h	Plastic, solid	new	cleaned	0	0	2	0,67	1,15
2 x 72 h	Plastic, solid	damaged	cleaned	2	0	5	2,33	2,52
2 x 72 h	Plastic, lamell	new	cleaned	3	2	5	3,33	1,53
2 x 72 h	Plastic, lamell	damaged	cleaned	3	3	5	3,67	1,15
3 x 72 h	Stainless steel	new	soiled	4	5	5	4,67	0,58
3 x 72 h	Stainless steel	damaged	soiled	4	5	5	4,67	0,58
3 x 72 h	Plastic, solid	new	soiled	4	5	4	4,33	0,58
3 x 72 h	Plastic, solid	damaged	soiled	-	5	5	5,00	0,00
3 x 72 h	Plastic, lamell	new	soiled	5	5	5	5,00	0,00
3 x 72 h	Plastic, lamell	damaged	soiled	4	5	5	4,67	0,58
3 x 72 h	Stainless steel	new	cleaned	1	4	5	3,33	2,08
3 x 72 h	Stainless steel	damaged	cleaned	1	4	5	3,33	2,08
3 x 72 h	Plastic, solid	new	cleaned	0	4	2	2,00	2,00
3 x 72 h	Plastic, solid	damaged	cleaned	2	5	3	3,33	1,53
3 x 72 h	Plastic, lamell	new	cleaned	4	5	5	4,67	0,58
3 x 72 h	Plastic, lamell	damaged	cleaned	4	5	5	4,67	0,58

Table 5. Epifluorescense microscopy pictures from first parallel test run. Soiled and cleaned surfaces after 8 h soiling.

	Dirty, soiled 8 h	Cleaned after 8 h soiling
New stainless steel		
Damaged stainless steel		
New solid plastic		

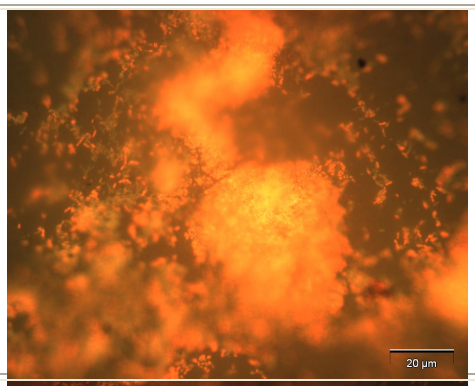
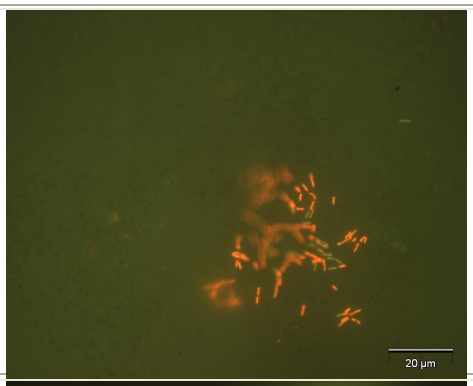
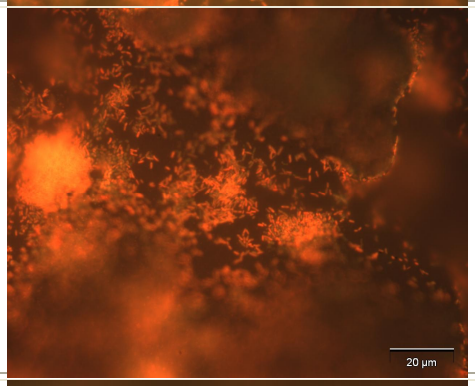
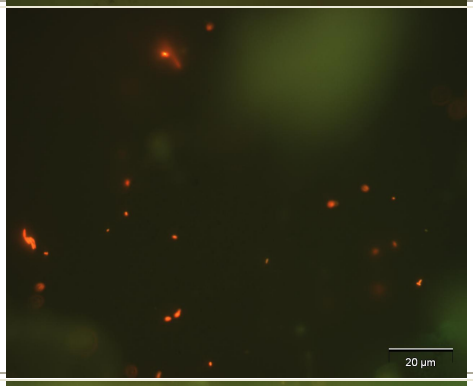
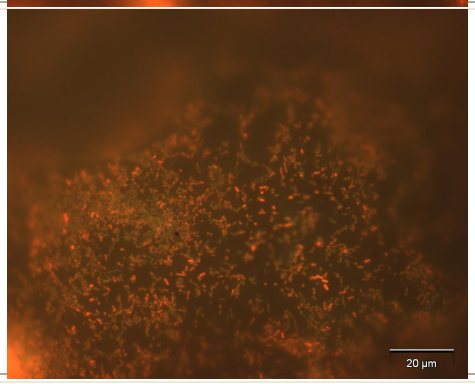
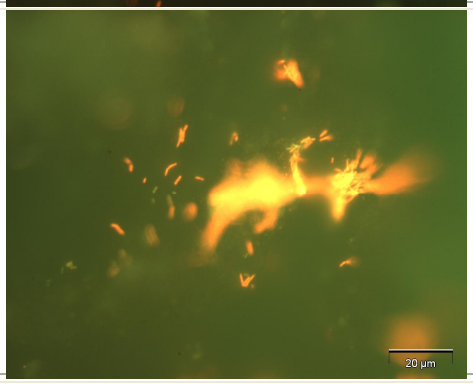
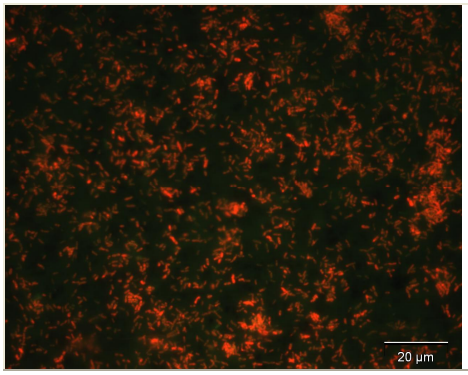
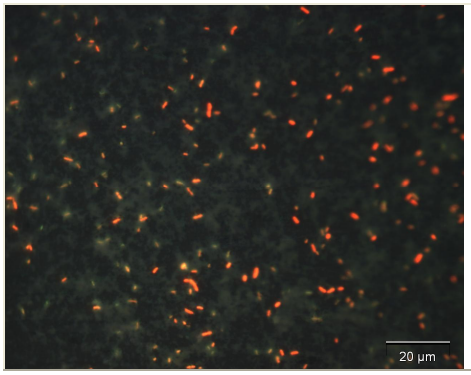
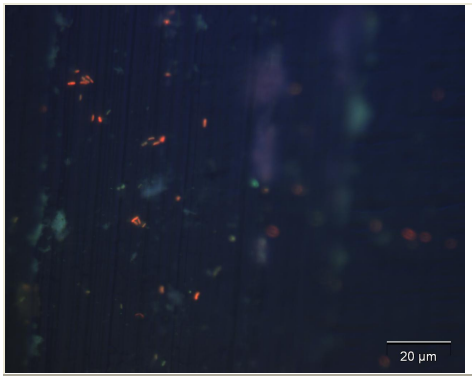
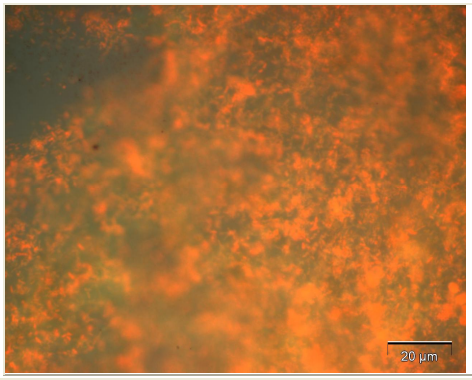
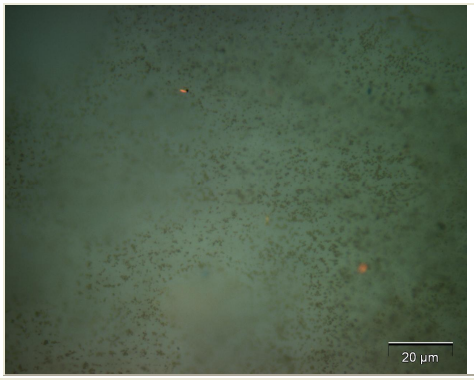
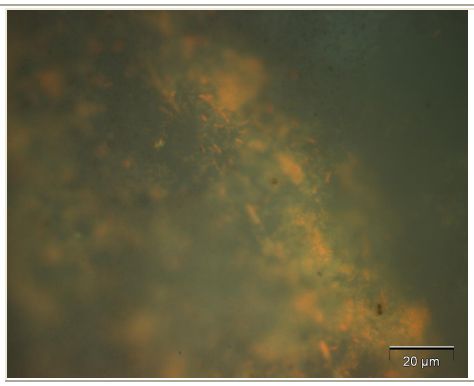
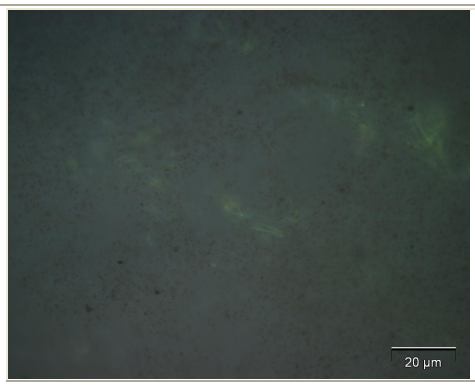
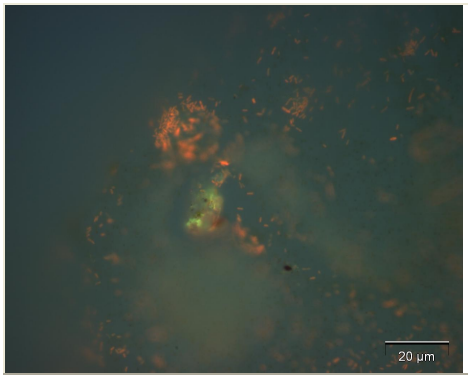
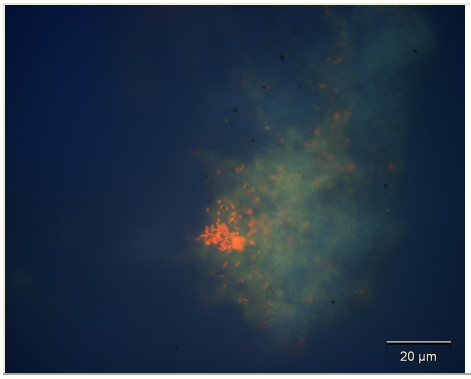
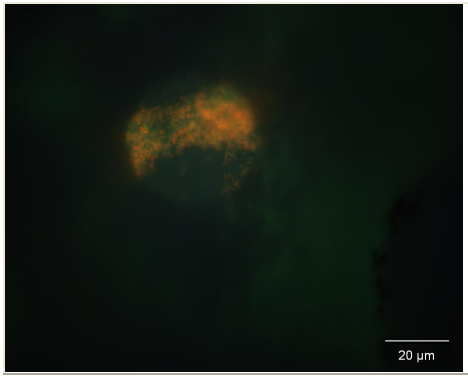
Damaged solid plastic		
New lamell plastic		
Damaged lamell plastic		

Table 6. Epifluorescense microscopy pictures from third parallel test run. Soiled and cleaned surfaces after 3 times 72 h soiling.

	Dirty, soiled 3 times 72 h	Cleaned after soiling 3 times 72 h
New stainless steel		
Damaged stainless steel		
New solid plastic		

Damaged solid plastic		
New lamell plastic		
Damaged lamell plastic		

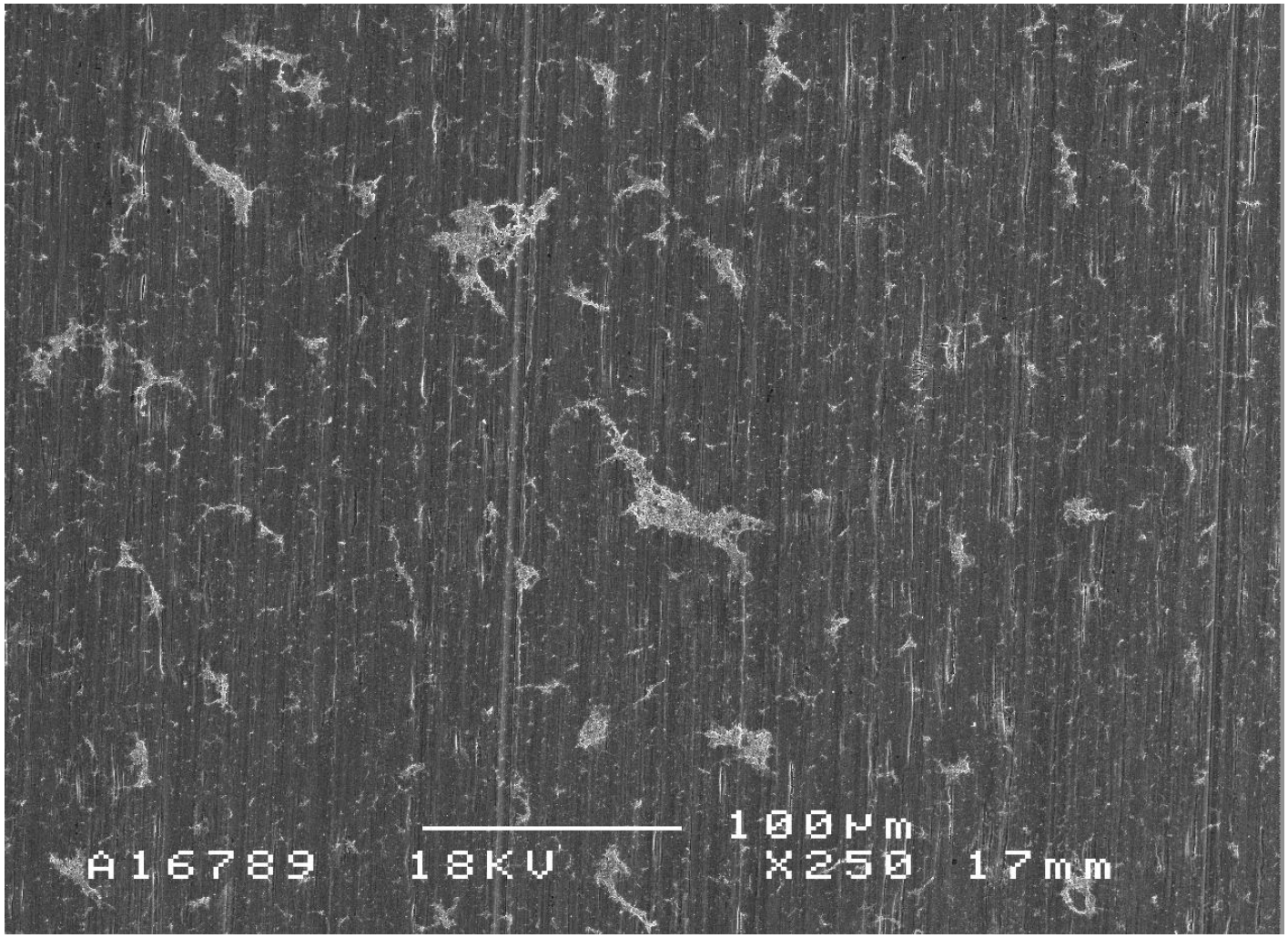


Figure 12. A scanning electron microscopy picture of stainless steel conveyor belt (AISI 301) soiled with blood containing microbes for 72 h. Scanning electron microscopy pictures are taken by Tom Gustafsson

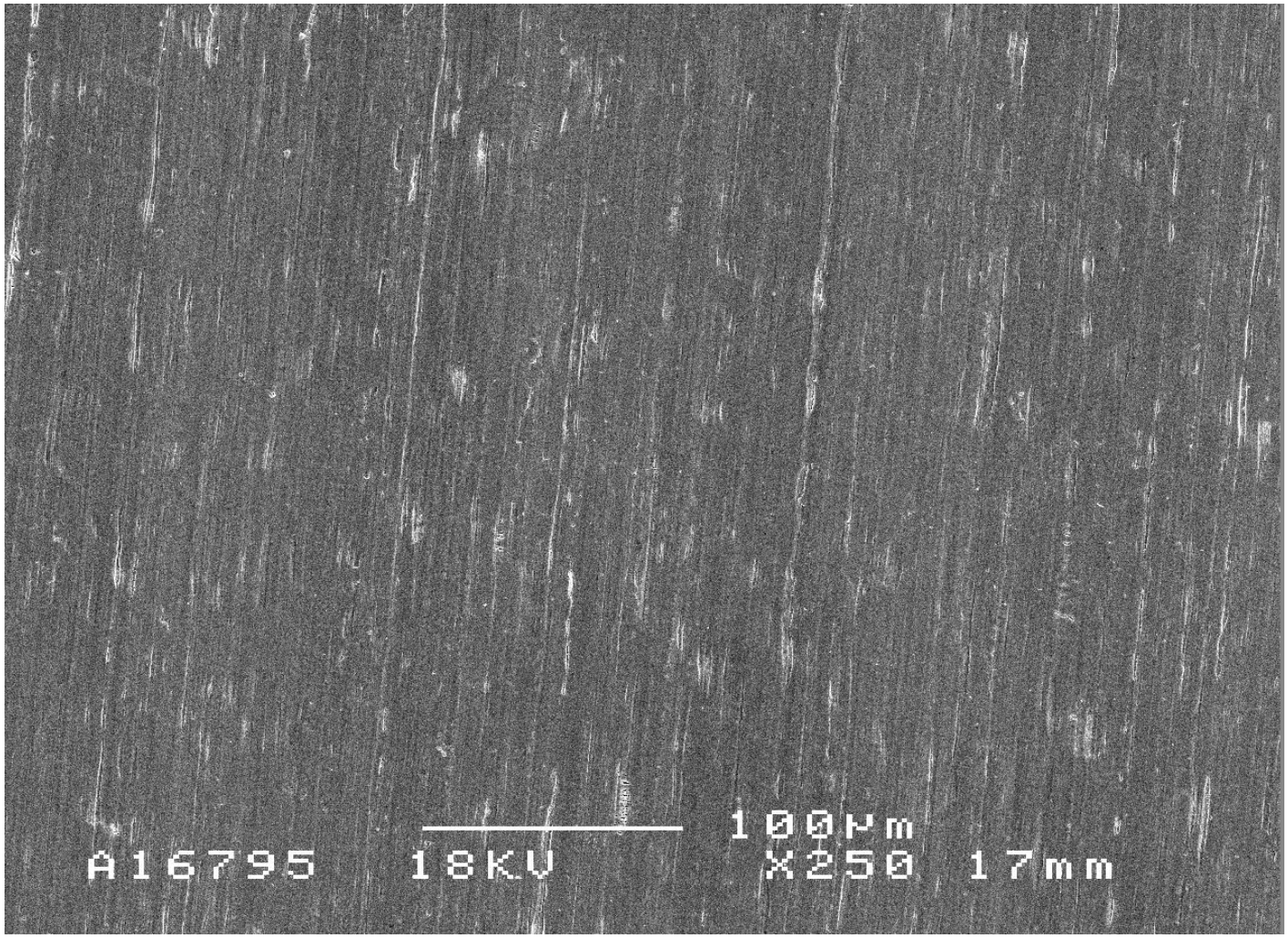


Figure 13. A scanning electron microscopy picture of stainless steel conveyor belt (AISI 301) soiled with blood containing microbes for 72 h and thereafter the surface was cleaned with foam for 10 min and rinsed with low pressure.

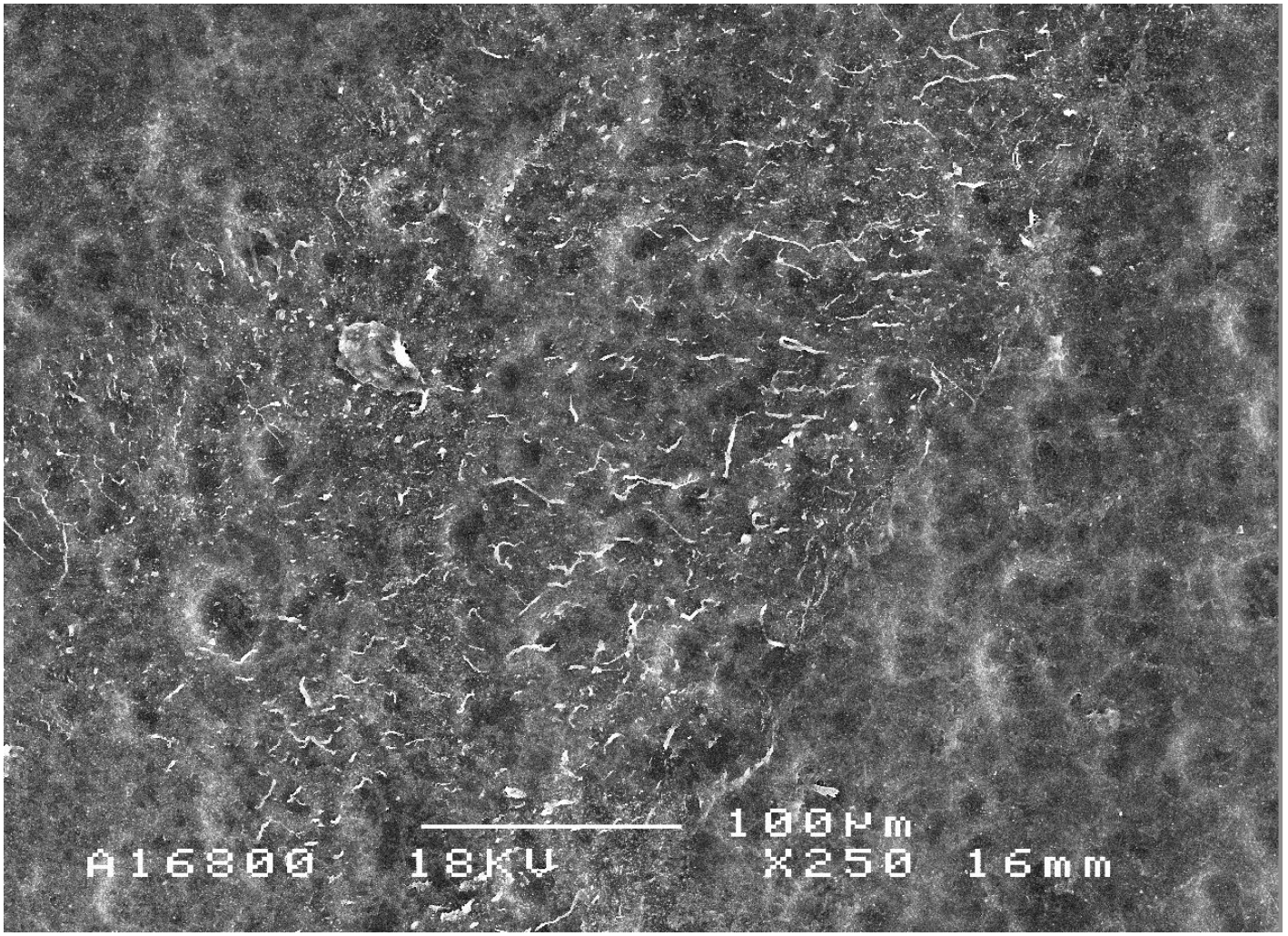


Figure 14. A scanning electron microscopy picture of plastic conveyor belt (solid) soiled with blood containing microbes for 72 h.

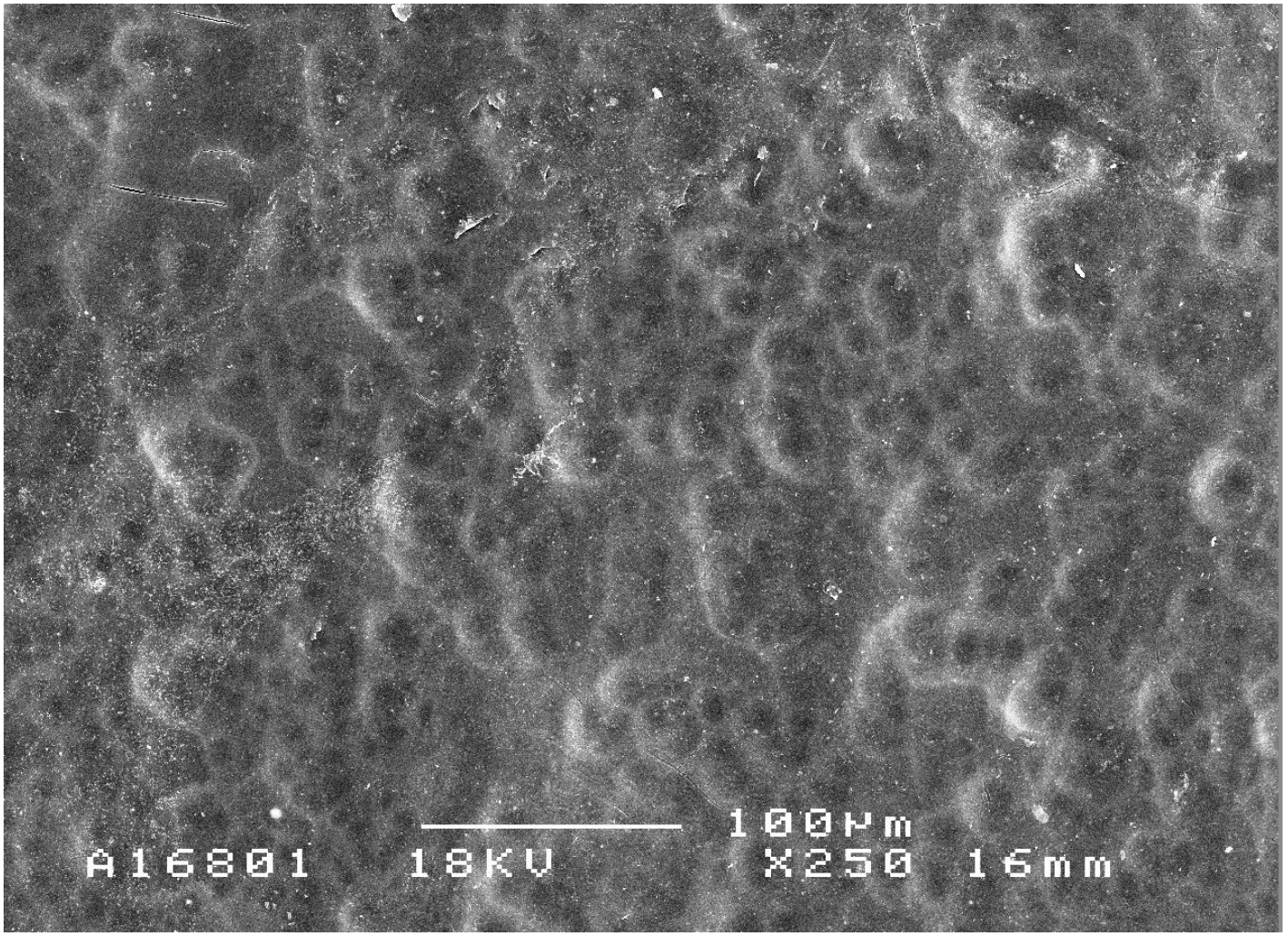


Figure15. A scanning electron microscopy picture of plastic conveyor belt (solid) soiled with blood containing microbes for 72 h and thereafter the surface was cleaned with foam for 10 min and rinsed with low pressure.

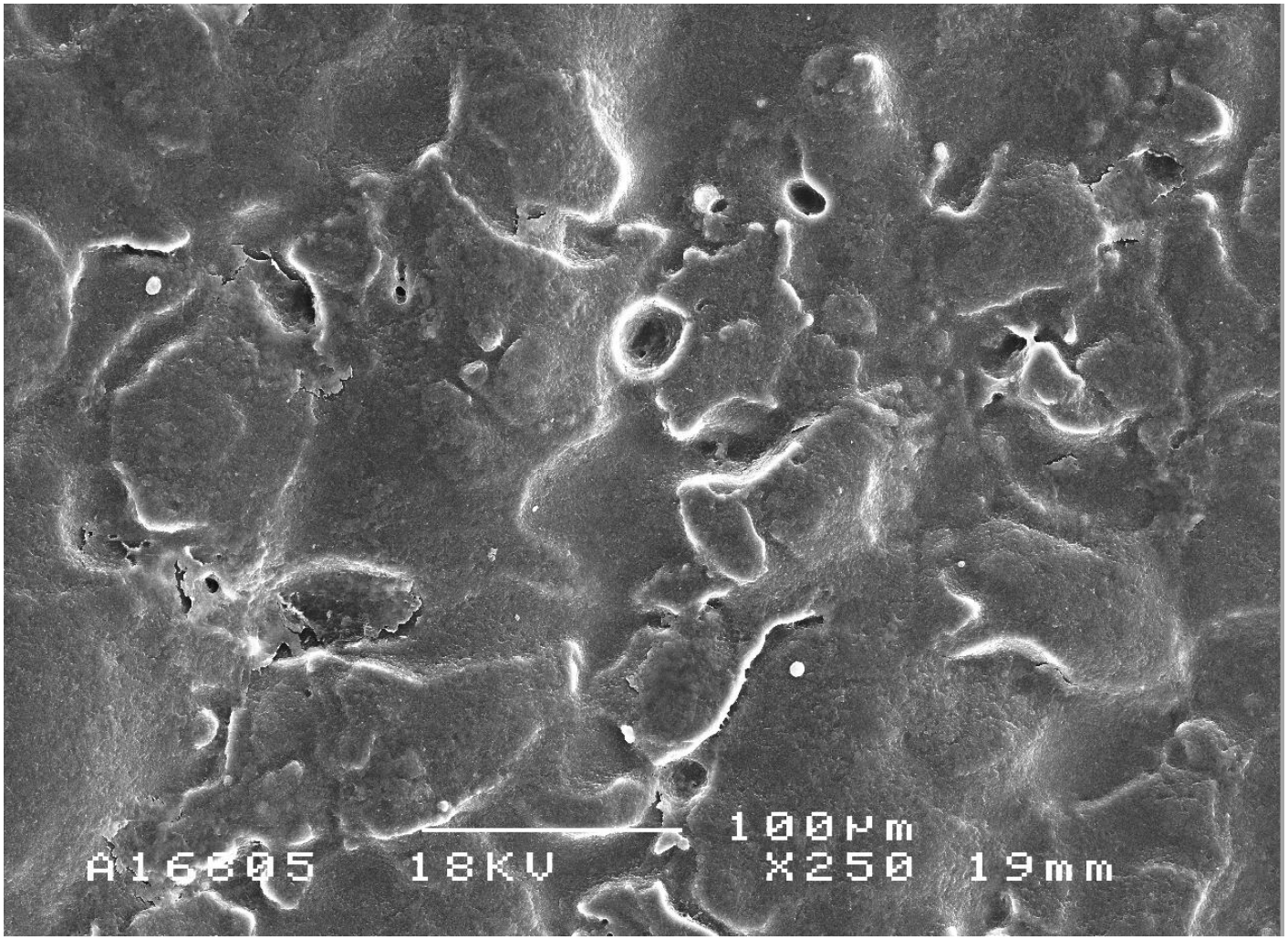


Figure 16. A scanning electron microscopy picture of plastic conveyor belt (lamel) soiled with blood containing microbes for 72 h.

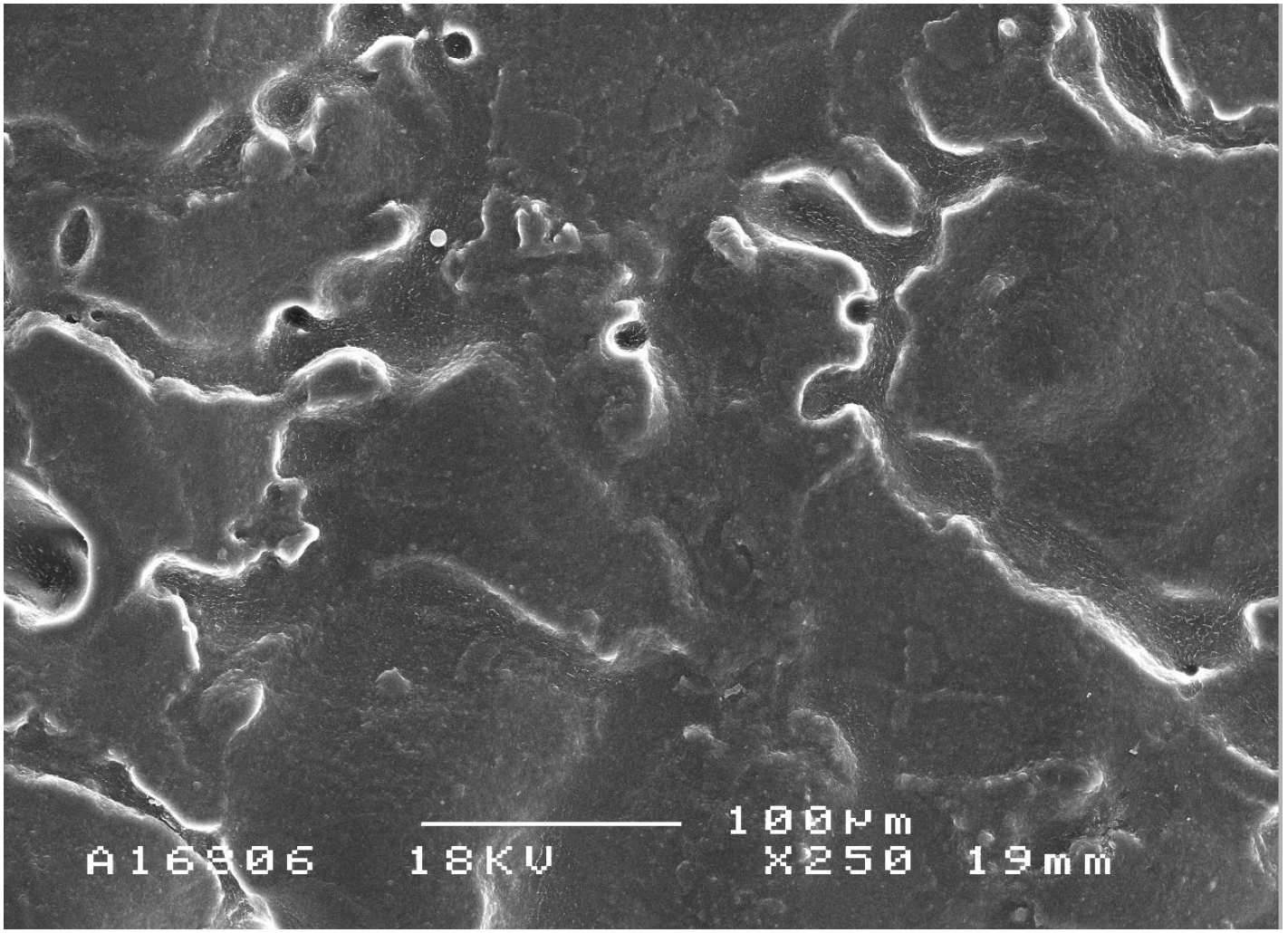


Figure 17. A scanning electron microscopy picture of plastic conveyor belt (lamel) soiled with blood containing microbes for 72 h and thereafter the surface was cleaned with foam for 10 min and rinsed with low pressure.

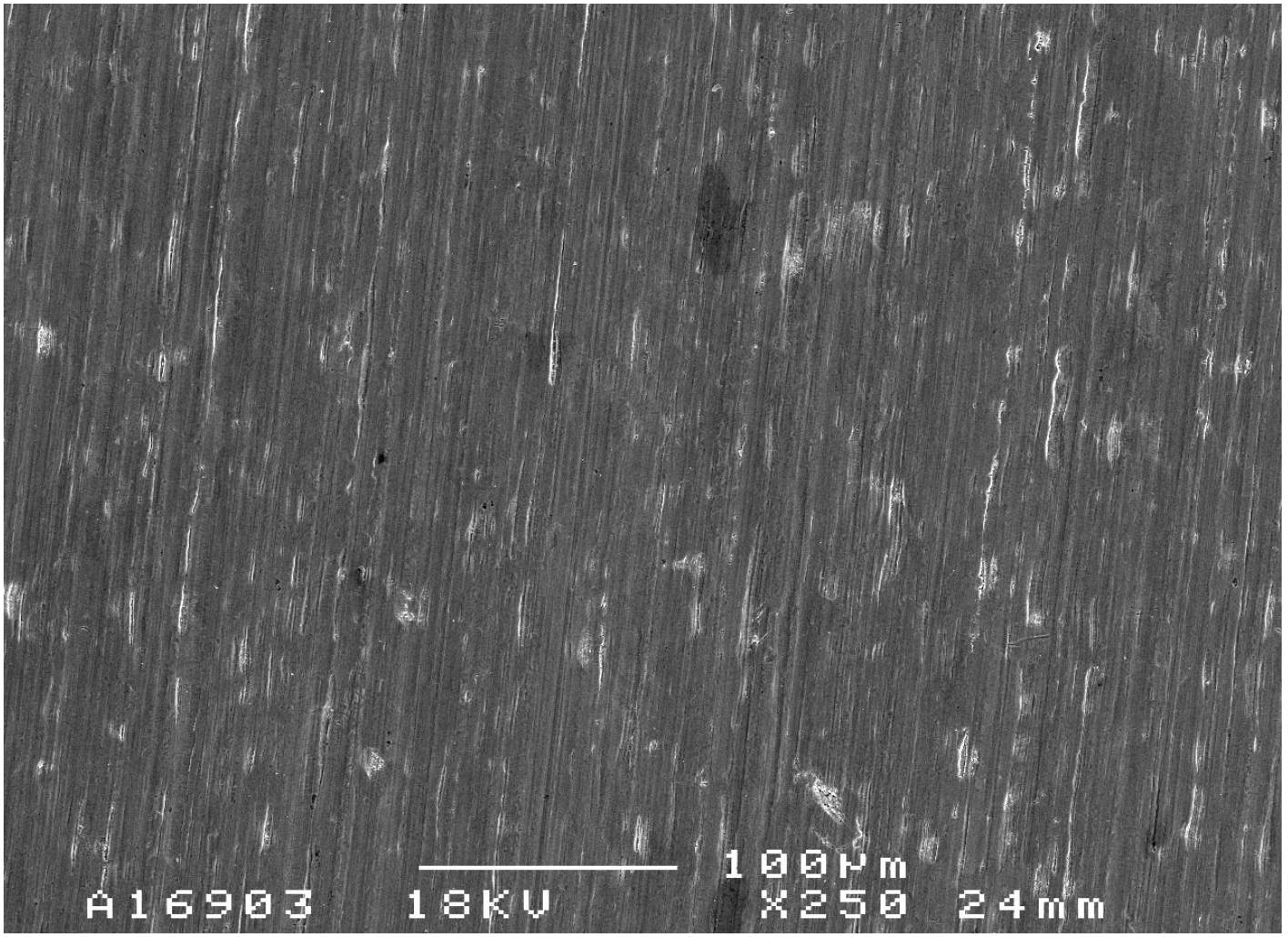


Figure 18. A scanning electron microscopy picture of unused stainless steel conveyor belt (AISI 301).

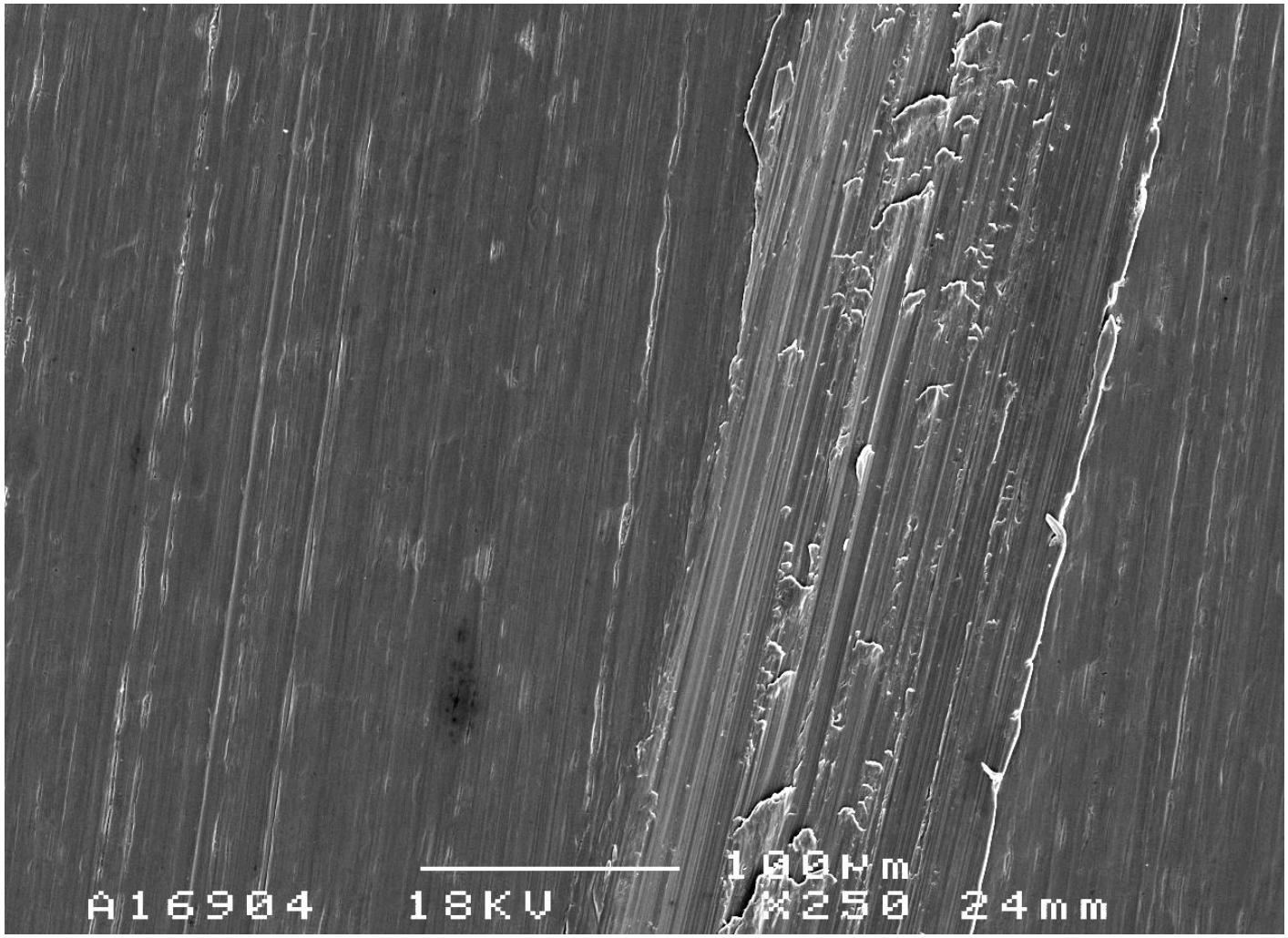


Figure 19. A scanning electron microscopy picture of unused stainless steel conveyor belt (AISI 301) which is damaged with knife.

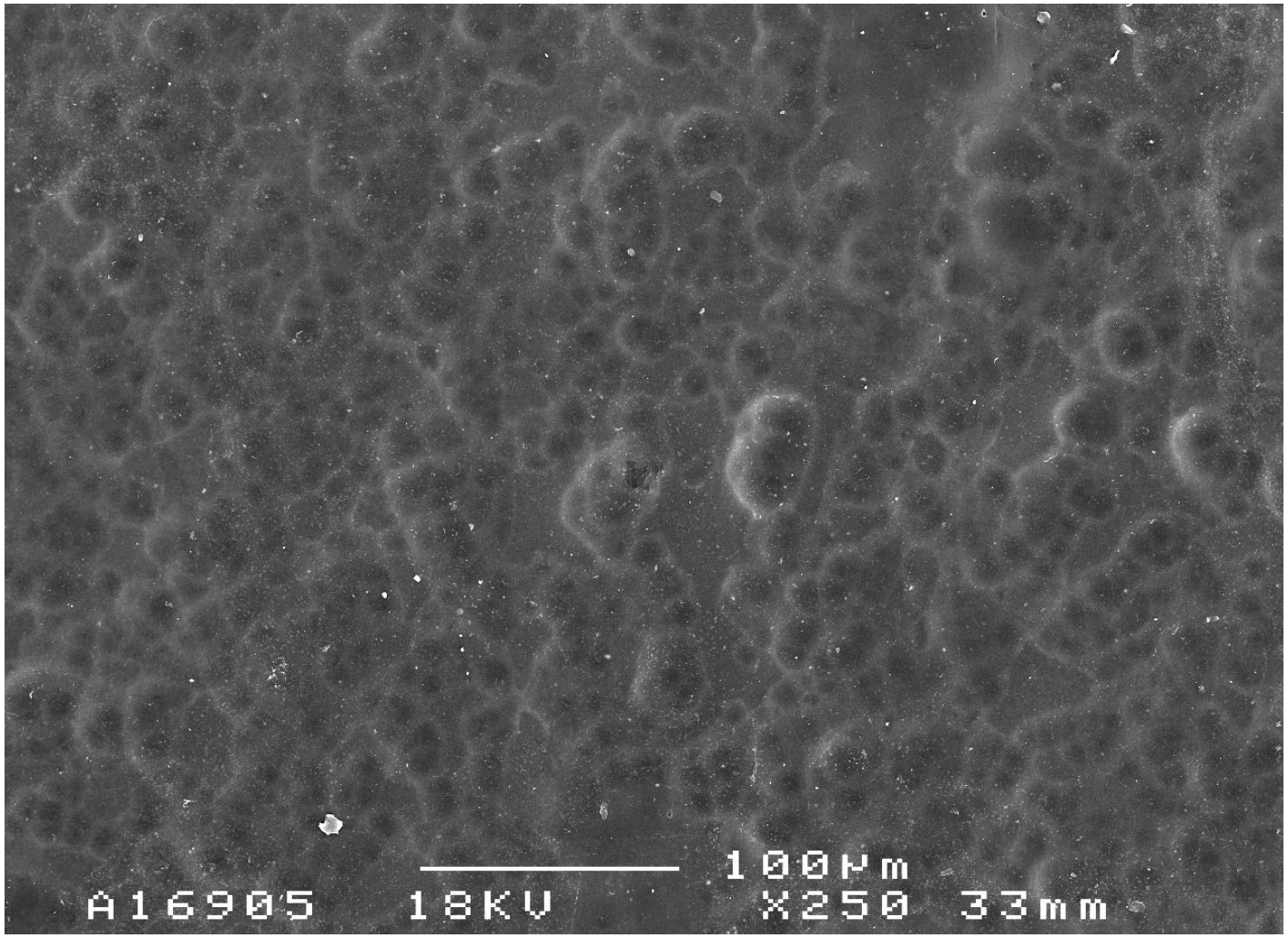


Figure 20. A scanning electron microscopy picture of unused plastic conveyor belt (solid).

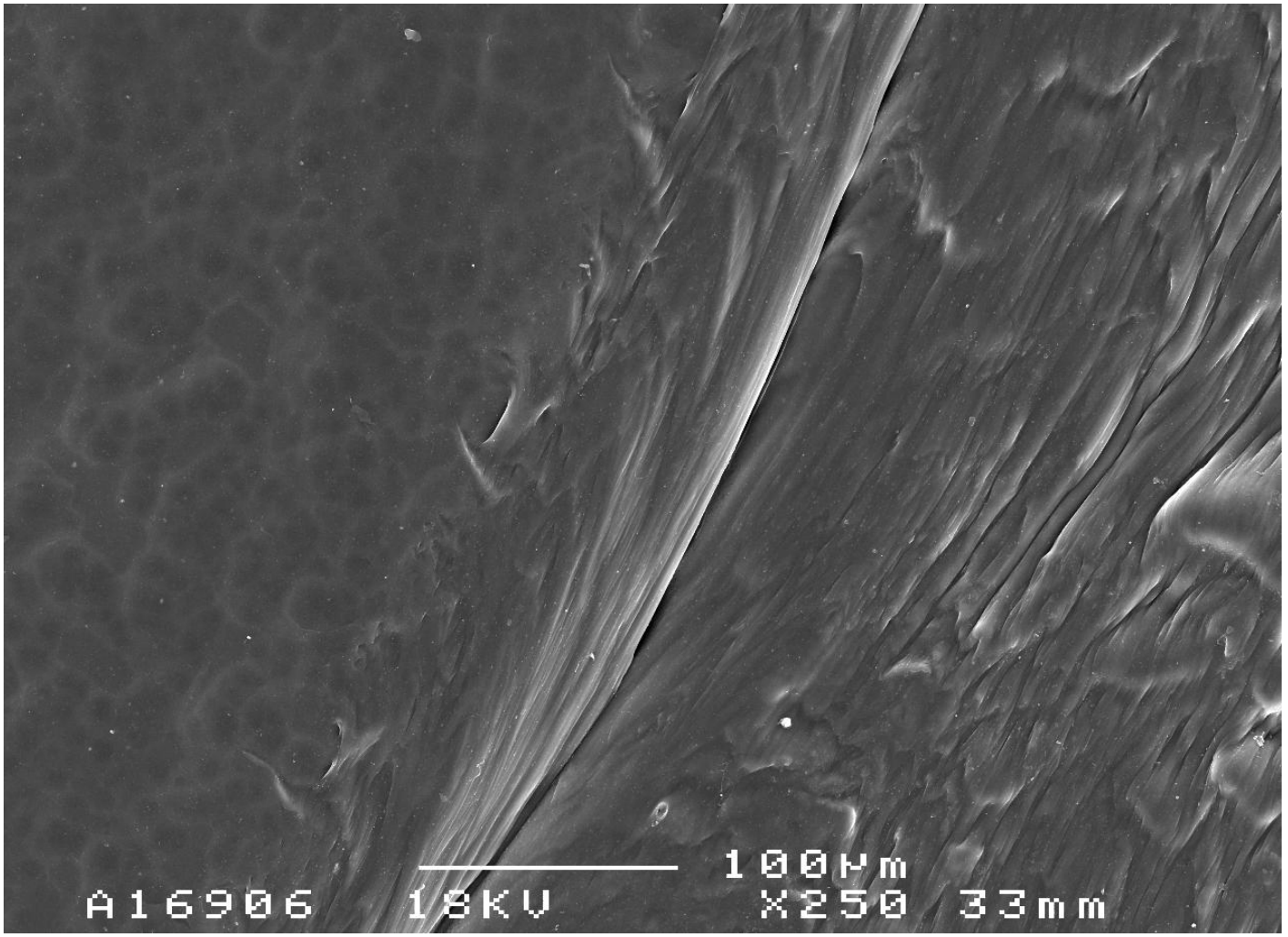


Figure 21. A scanning electron microscopy picture of unused plastic conveyor belt (solid) which is damaged with knife.

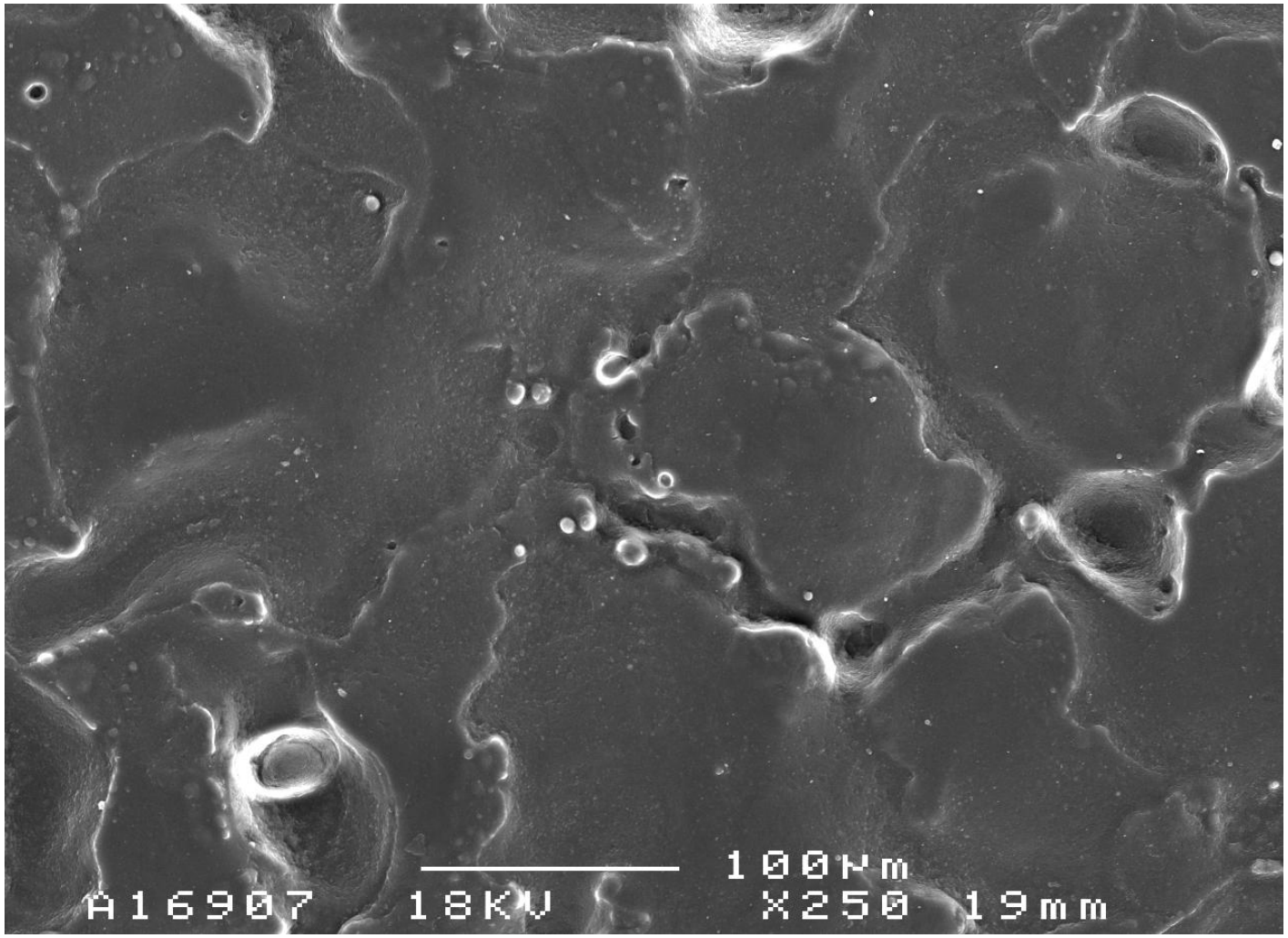


Figure 22. A scanning electron microscopy picture of unused plastic conveyor belt (lamel).

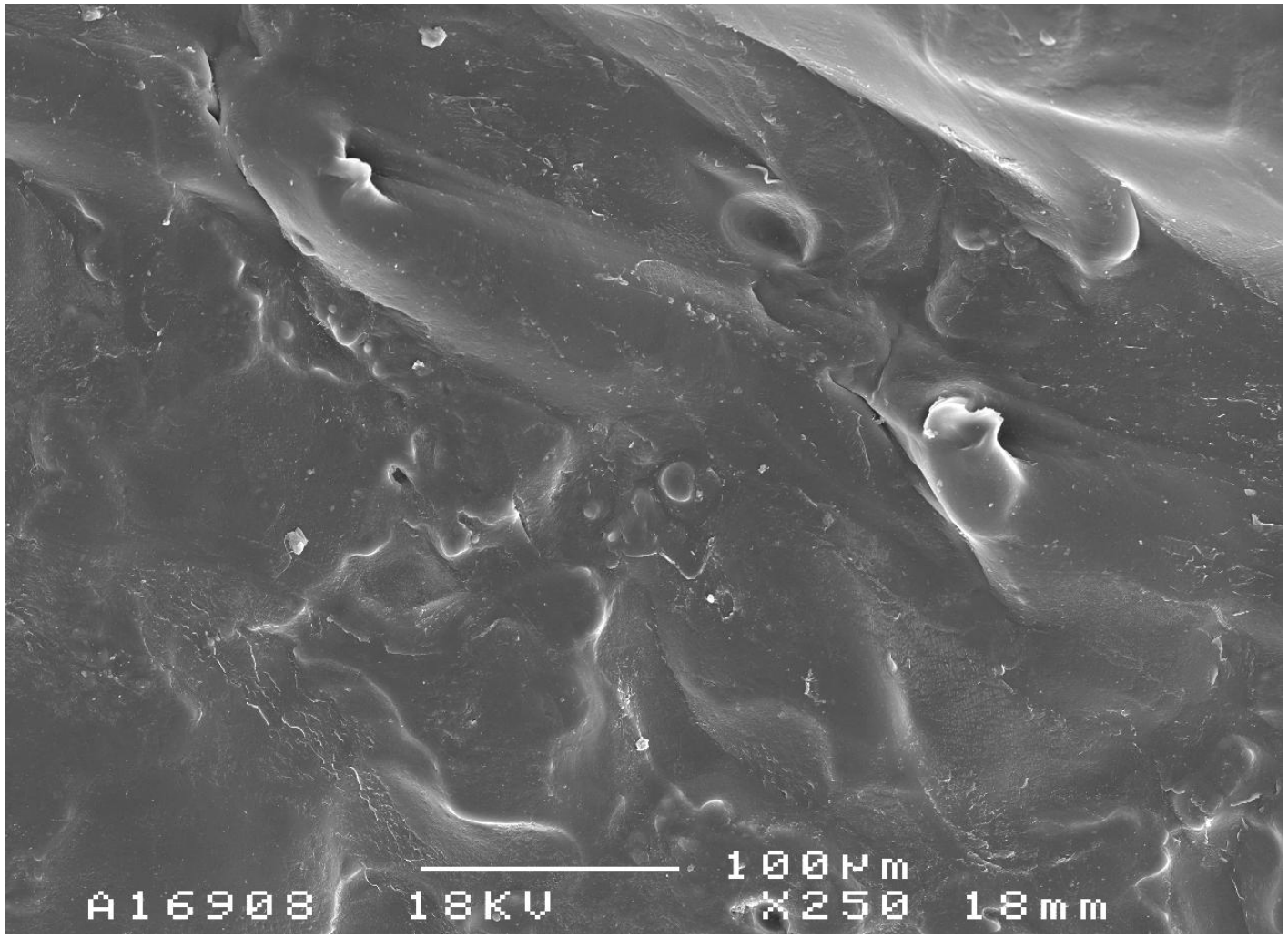


Figure 23. A scanning electron microscopy picture of unused plastic conveyor belt (lamel) which is damaged with knife.

EU legislation and regulations on food contact materials in food processing equipment including testing methods used for verification of compliance with regulations

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Contents

Part I. Legislation on materials and articles intended for contact food

1. Introduction

A range of EU legislation is regulating the quality and properties of any material or article intended to be in contact with food (EC, 2010). The regulations cover all the steps throughout the entire food chain from the primary production to the consumers. In the food processing industries, in particular, the quality of all food contact materials is strictly regulated. Materials used have to comply with the requirements of the regulations and this has to be verified to the food control authorities. Similarly, a number of EU regulations stipulate the manufacturers of food contact materials. The aim of Part I of this report is to provide an overview on the key legislation regulating the stakeholders operating in food and contact material production and processing activities. In part II (p. 10), the report provides a summary of 10 case studies focusing on surface hygiene.

2. Definition of food contact materials (FCM)

Food contact materials (FCM) are defined as all products that are brought into contact with food: materials used in food processing equipment, working surfaces, utensils, pipelines (excluding fixed water supply equipment) and packaging for wrapping and eating. The assortment of food contact materials is thus wide and includes e.g. active and intelligent materials and articles, adhesives, ceramics, glass, enamel, metal alloys, cork, wood, textile, paper board, regenerated cellulose film, plastics, varnish coating, printing inks, silicone, wax, rubber, and ion exchange resin. The materials are used either as such or in combinations e.g. in complex multilayer materials.

3. Legislative requirements for materials contact with foods

3.1 General requirements in food and hygiene legislation

General hygiene requirements for establishments preparing foodstuffs are stated in the general hygiene regulation No. 852/2004 (EC, 2004a). In accordance with the requirement in Appendix II, chapter I the layout, design, construction, placement and size of food premises are:

- to permit adequate maintenance, cleaning and/or disinfection, to avoid or to minimise airborne contamination,
- to provide adequate working space to allow for the hygienic performance of all operations,
- to protect against the accumulation of dirt, contact with toxic materials, the shedding of particles into food and the formation of condensation or undesirable mould on surfaces and
- to permit good food hygiene practices, including protection against contamination and, in particular, pest control.

Furthermore, it is required that the surfaces (including surfaces of equipment) in areas where foods are handled and in particular those in contact with food are to be maintained in a sound condition and be easy to clean and, where necessary, to disinfect. This will require the use of smooth, washable corrosion-resistant and non-toxic materials, unless food business operators can satisfy the competent authority that other materials used are appropriate. The Regulation No 853/2004 (EC, 2004b) provides additional hygiene requirements for establishments handling products of animal origin. In accordance with the official control Regulation No 882/2004 (EC, 2004c), the control of materials and articles intended to come into contact with foods is one objective of the official control of food and feeds.

3.2 Legislation on food contact materials

3.2.1 EC regulation 1935/2004 (Framework regulation)

According to the EC regulation 1935/2004 (EC, 2004d) any material or article intended to come into contact directly or indirectly with food must be sufficiently inert to preclude substances from being transferred to food in quantities large enough to endanger human health or to bring about an unacceptable change in the composition of the food or a deterioration in its sensory properties. In addition to this regulation, a number of specific product and substance based regulations are provided, in particular to plastics. Furthermore, a range of national regulations are available besides the EC regulations (EC, 2009a).

The regulation 1935/2004 covers also active and intelligent food contact materials and articles. Active food contact materials and articles are designed to deliberately incorporate 'active' components intended to be released into the food or to absorb substances from the food. However, the regulation states that active food contact materials and articles may change the composition or the sensory properties of the food only if the changes comply with the Community provisions applicable to food, such as the provisions of Directive 89/107/EEC (EC, 1988) on food additives. In particular, substances such as food additives deliberately incorporated into certain active food contact materials and articles for release into packaged foods or the environment surrounding such foods, should be authorised under the relevant Community provisions applicable to food and also be subject to other rules which will be established in a specific measure.

3.2.2 Specific legislation and guidelines

During the last decades, surface materials with different antimicrobial coatings have intensively been studied. Particularly interest has been focussed on materials applicable for food packaging (Appendini and Hotchkiss, 2002; Cha and Chinnan, 2004). Currently there is no specific legislation for these surface materials. In food processing environments the requirements for antimicrobial coatings are the same as for active food contact materials.

In addition to general legislation, EU has specific legislation on plastic (plenty of specific regulations), ceramics and regenerated cellulose. Furthermore, as there is still today a range of national regulations (EC, 2009a), the legislation on food contact materials in EU is currently under harmonization. All the EC legislation is currently compiled at the DG Sancon Food Contact materials website of EC. Guidelines and resolutions on food contact materials are also available for metals and alloys (TD, 2002), paper and board (Resolution AP, 2002), printing inks (Resolution AP, 2005) and coatings (Policy statement, 2004). These guidelines are not legally binding but are considered as reference documents by experts on food contact materials in Council of Europe Committee member states. The requirements mentioned are only requirements related to legislation on FCM. Attention must be paid to other requirements e.g. in the legislation in the environmental area. Furthermore, the food business operators should consider whether FCM contains components e.g. nickel or ingredients listed in EU directive no. 2000/13, which might cause allergic reactions. However, regulations or methods to ensure the technical properties of FCM like permeability of oxygen, strength or water absorbance are not included. The method of ISO 62:2008 can be applied for testing moisture absorbance of plastic coatings (ISO, 2008).

3.3 Legislation on cleanability and disinfectability of food contact surfaces

Today, with regard to cleanability and disinfectability no common regulations are available in EU for the FCM surfaces. In the general hygiene regulation EC 852/2004 (EC, 2004a) the requirements are general and control authorities do not require certificates providing evidence for the compliance with the regulation. In general, ideal would be a surface easy to clean and disinfect, i.e. a non-porous surface not susceptible to adhesion of soil particles, such as stainless steel but not wood. Appropriate material should be washable, resistant to cleaning agents and disinfectants, and resist mechanical damages.

In general, the rougher the surface the more readily soil particles will adhere to it resulting in adverse effects on the cleanability of the surface. Minor defects in surface finishing may also enhance the contamination risks. According to the recommendations of European Hygienic Engineering & Design Group (EHEDG) the R_a value describing the roughness of the surface should not exceed 0.8 µm (EHEDG, 1993). However, R_a value alone can be misleading indicator of the susceptibility of surface to accumulate contamination. The method used in surface finishing (turning, milling, mechanical polishing, impact treatment, etc.) is often more important (EHEDG, 2007).

3.4 Requirements for manufacturers of food contact materials

The EC regulation 2023/2006 (EC, 2006) on good manufacturing practise (GMP) in manufacturing of FCM became effective on August 1, 2008. It requires that manufacturers have established an in-house control system for verifying the compliance of the products with the regulatory requirements.

Nordic food control authorities have prepared in 2008 (NCM, 2008) a guideline for minimum requirements for in –house control documentation needed by operators in industry and trade.

3.5 Traceability

The traceability of materials and articles shall be ensured at all stages in order to facilitate control, recall of defective products, consumer information and attribution of responsibility. Food business operators shall have in-place-systems and procedures to allow identification of materials, articles, substances or products, which are covered by the EU Regulation no. 1935/2004. The information shall be made available to the competent authorities on demand. The basic principle for traceability in the food production is that the food business operator should be able to trace one step backwards and one step forward. Each link in this chain has responsibility for the compliance with the legal requirements.

3.6 Legislation on coatings, printing inks, adhesives and varnishes

Similarly as for all FCM, the general FCM regulations 1935/2004 (EC, 2004d) and 2023/2006 (EC, 2006) are valid also for coatings, adhesives, varnishes, printing inks etc. Specific legislation is not available for these FCMs but directives for plastic materials are largely applicable. Regulations for plastic materials are currently under revision and in the future they will most likely cover composite materials as well (see 3.2.2 on pages 3-4).

3.7 Legislation on plastic materials

During the recent years, EU legislation on plastic materials has been amended repeatedly. Currently plastics are regulated by the new Commission Directive 2002/72 (EC, 2002), which establishes that:

- An overall migration limit of 60 mg (of substances)/ kg (of foodstuff or food simulants) for all substances migrating from a material into foodstuffs;
- A positive list of authorised monomers and other starting substances, with restrictions on their use (such as specific migration limits) where applicable. Some monomers remain provisionally authorised at national level pending a re-evaluation by the European Food Safety Authority (EFSA);

- A list of authorised additives and for some of them, restrictions on their use e.g. specific migration limits. In addition there exist also national lists of authorised additives;
- The procedures for adapting, revising and/or completing the lists of authorised substances.

A range of amendments has been made to the plastic directive 2002/72 since its establishment. An amendment Directive 2004/19 (EC, 2004e) lays down that the list of authorised additives will become a positive list. To this end the following have been set:

- The additive must be permitted in one or more of the Member States no later than 31 December 2006
- Commission will establish a provisional list of additives which may continue to be used subject to national law until EFSA has evaluated them.

Directive 2004/19 (EC, 2004e) lays down that stricter limit applies for migration of food contact material additives, which also are permitted as direct food additives. These additives shall not have a technological function in the final foodstuffs.

By the amendment Directive 2007/19 (EC, 2007) both of the Directive 2002/72 (EC, 2002a) and the Council Directive 85/572 (EU, 1985) were amended. As result, new regulations were given for simulants used in testing of migration from plastic based materials intended to come into contact with foods.

Furthermore, Directive 2002/72 (EC, 2002) has been amended by Directive 2008/39 (EC, 2008). This amendment establishes that the Community list of additives became a positive list on 1 January 2010, meaning that after this date only those additives listed will be permitted for the manufacture of plastics. Substances on the provisional list may continue to be used until a decision is taken on their possible inclusion in the positive list of additives. This amendment also clarifies the criteria for removal of an additive from the provisional list and updates the list of authorised substances used for the manufacture of plastic materials and articles intended to come into contact with food.

EC Regulation 450/2009 (EC, 2009b) sets down additional requirements to Regulation No 1935/2004 (EC, 2004d) for active and intelligent materials and articles to ensure their safe use and introduces an authorisation scheme for substances used for active and intelligent functions in food contact materials.

Today, legislation on plastic materials does not cover composite materials. However, as regulations for composite materials are still missing, legislation on plastic is widely applied to plastic coated steel structures.

4. Standards and guidelines

A technical report of CEN, CEN/TR 15623, provides guidance to machinery manufacturers for the selection of suitable materials for machinery intended for production of foods (CEN, 2008).

A range of European standard methods for testing and analysing food contact materials and articles are available from The European Committee for Standardization (CEN). (p. 22-24).

In USA, material related standards are available e.g. from following organisations:
- American National Standards Institute (ANSI, http://www.ansi.org/)
- American Society for Testing and Materials (ASTM, http://www.astm.org/)
- National Institute of Standards and Technology (NIST, http://www.nist.gov/)
- National Sanitation Foundation (NSF, http://www.nsf.org/)
- Society of Plastics Engineers (SPE, http://www.4spe.org/)
- Society of Plastics Industry (SPI, http://www.plasticsindustry.org/)
- 3-A Sanitary standards (3-A Sanitary Standards, Inc, 3-A Sanitary Standards, Incorporated
- International organization for standardization (ISO, http://www.iso.org/)

Standards are also available from Food and Drug Administration (FDA, http://www.fda.gov/).

OECD provides a comprehensive information package on currently available testing methods for assessment of antimicrobial activity of different kind of treated materials and articles (OECD, 2008).

5. Overview on testing methods used for verification compliance with regulations

5.1 Background

Declaration on compliance with regulations is based on EC Regulation 1935/2004 (EC, 2004d) and material related special regulations.

Evaluation of compliance with the legal requirements will include evaluation of
- raw materials and chemicals used (chemical composition of the material)
- migration of chemicals from the FCM to the food
- unacceptable changes in the sensory properties to food

The evaluation of potential or actual migration can be done in different ways, like analytical testing, calculations based on knowledge of the recipes of the materials or calculations based on mathematical modeling for plastic monolayer materials.

Analytical testing is most often used. In the testing instead of real foodstuffs food simulants are used for migration properties under strictly standardized testing

conditions. The test should be performed in high quality manners and according to standard methods if available.

If a company does not have special resources or competence in the area of FCM and the evaluation of compliance with the legislation in the field of EC or different national legislation, the company should turn to a competent consultant or external laboratory. The laboratory must know the composition/recipe for the material, purpose of use and processing conditions in order to perform the necessary tests and analyses.

5.2 Migration characteristics of FCM

For determination of overall migration several standard methods are available, e.g. EN 1186-1…15, 2003 (EN, 2003) for plastics. Testing conditions should correspond as well as possible to the conditions of intended use. The corresponding standard method for coatings on metal substrates is EN/TS 14235, 2002 (EN, 2002) and for polymeric coating on paper and board standard method is EN/TS 14234, 2002 (EN, 2002/2).

Besides overall migration, specific migration has to be determined, if any component in the composition would require it. For this purpose CEN standard EN 13130-1…28, 2004, is available (EN, 2004) for plastics.

5.3 Effects of FCM on the sensory quality of foodstuffs

In accordance with EC Regulations for FCM, FCM should not cause any changes in the sensory quality of food. Sensory analysis is a practical tool especially in combination with analysis of migration properties of packaging materials. For this purpose ISO standards are available, for example standard ISO 13302 (2003) for assessing modifications to the flavour of foodstuffs due to packaging (ISO, 2003). Also for paper and board EN standards EN 1230-1…2 are available (EN, 2010).

5.4 Cleanability of surfaces

Official standard methods for testing of cleanability are obviously not available. However, ASTM International standard C 757-87 “Standard Test Method for Cleanability of Surface Finishes”can be used to compare cleanability of different kind of surfaces (ASTM, 2006).

5.5 Disinfectability of surfaces

Official standard methods are not available for testing disinfectability of different surfaces. However, a range of surface testing methods are currently available for assessing the efficacy of disinfectants applicable in food industry. Among them the CEN standard EN 13697, for example, provides a method for the evaluation of efficacy of disinfectants used in food industry. Although the method as such is not intended for comparing different kind of surfaces, it could easily with certain modifications be applied for comparison of disinfectability of different surfaces as well (EN, 2001).

5.6 Fungal growth on surfaces

In the OECD report entitled ”Analysis and assessment of current protocols to develop harmonized test methods and relevant performance standards for the efficacy testing of treated articles/treated materials” (OECD, 2007), several standard methods applicable for evaluating their ability to inhibit or promote fungal growth are introduced. Among them, the method ISO 846:1997(Plastics. Evaluation of the action of microorganisms) can be used for evaluation of fungal growth on the surface of plastic materials under different kind of nutritional conditions (ISO, 1997). Another standard applicable for evaluating antibacterial properties of plastic surfaces is provided by ISO 22196:2007 (ISO, 2007).

Part II. An overview of 10 case studies on the characteristics of FCM affecting their hygienic properties e.g. cleanability, sensitivity to microbial adhesion and biofilm formation**.**

A. Effect of surface characteristics on cleanability

Verran, J., Packer, A., Kelly, P. and Whitehead, K.A. The retention of bacteria on hygienic surfaces presenting scratches of microbial dimensions**, Letters in Applied Microbiology** 50 (2010) 258–263

Objectives: To study the effect of microtopography of surfaces on the cleanability of worn and new stainless steel surfaces

Materials and methods: Surfaces were fabricated with parallel linear features of 30 μm ("worn" surface stimulant) or of microbial dimensions (1.02 and 0.59 μm width) feature dimensions ("new" surface simulants). Topographical continuity of surfaces was checked by atomic force microscopy. The surfaces were contaminated by two test organisms, *Listeria monocytogenes* (rod) and *Staphylococcus sciuri* (coccus). After 1h incubation the surfaces were rinsed. Any retained cells were let to dry in laminar hood. Dried surface samples were thereafter prepared for SEM.

Results: SEM images of the cells retained on the surfaces revealed different patterns of cell distribution across the surfaces that could be related to the topography. On the smooth surface, clusters of cocci, often linked to one another by extracellular fibrils, were apparent. Similarly, clusters of *L. monocytogenes* were visible on the smooth surfaces. On surfaces with features of 0.59 μm width, considerable fewer coccal cells were retained than on the other surfaces presented, and these tended to be retained individually or in pairs. On the 0.59- μm surfaces *L. monocytogenes* cells looked to be more numerous, than on the other surfaces presented. Retained cells were distributed uniformly across the smooth 30 μm featured surfaces but were retained in high numbers on microtopographies at the 'peaks' between the wide grooves. On smaller features, retention was attributed to the maximum area of contact between cells and substratum being attained, with cocci being embedded in 1.02-μm-width grooves, and rods aligned along (and across) the densely packed parallel 0.59-μm grooves. with cells lying along and across surface features. On surfaces with features of 1.02 μm width, *S. sciuri* were retained in the surface features in high numbers as single cells. If cell clusters were present, typically one cell of the cluster was retained in a surface feature. For the *L. monocytogenes*, the majority of the retained cells were trapped lengthways in the linear features, with a few cells straddling them. Lower numbers of cells appeared to be retained than on the 0.59-lm linear featured surface. Quantitative data confirmed these observations. Coccal cells were retained in significantly ($P < 0.05$) higher numbers on the 1.02- μm feature surfaces, whilst the rod-shaped cells were retained in significantly higher numbers on the 0.59- μm feature surface. Further, more *L. monocytogenes* cells were retained on the smooth surfaces than the coccal-shaped *S. sciuri* cells ($P < 0.05$).

Conclusions: The dimensions of surface features may enhance or impede cell retention. This phenomenon is also related to the size and shape of the microbial cell.

Whitehead, K.A., Smith, L.A., Verran, J. The detection and influence of food soils on microorganisms on stainless steel using scanning electron microscopy and epifluorescence microscopy, **International Journal of Food Microbiology** xxx (2010) xxx–xxx (in press).

Objectives: The objective of this work was to investigate the extent to which two microscopic methods, SEM and epifluorescence microscopy, can detect organic material and cells on hygienic food contact surfaces and to determine how the presence of organic material on the surfaces affects the pattern and quantity of cell attachment (*Escherichia coli*).

Materials and methods: A range of food soils and components (complex [meat extract, fish extract, and cottage cheese extract]; oils [cholesterol, fish oil, and mixed fatty acids]; proteins [bovine serum albumin (BSA), fish peptones, and casein]; and carbohydrates [glycogen, starch, and lactose]) were deposited onto 304 2B finish stainless steel surfaces at different concentrations (10–0.001%). Scanning electron microscopy (SEM) and epifluorescence microscopy were used to visualise the cell and food soil distribution across the surface. Epifluorescence microscopy was also used to quantify the percentage of a field covered by cells or soil.

Results: At 10% concentration, most soils, with the exception of BSA and fish peptone were easily visualised using SEM, presenting differences in gross soil morphology and distribution. When soil was stained with acridine orange and visualised by epifluorescence microscopy, the limit of detection of the method varied between soils, but some (meat, cottage cheese and glycogen) were detected at the lowest concentrations used (0.001%). The decrease in soil concentration did not always relate to the surface coverage measurement. When 10% food soil was applied to a surface with *Escherichia coli* and compared, cell attachment differed depending on the nature of the soil. The highest percentage coverage of cells was observed on surfaces with fish extract and related products (fish peptone and fish oil), followed by carbohydrates, meat extract/meat protein, cottage cheese/casein and the least to the oils (cholesterol and mixed fatty acids). Cells could not be clearly observed in the presence of some food soils using SEM. Findings demonstrate that food soils heterogeneously covered stainless steel surfaces in differing patterns. The pattern and amount of cell attachment was related to food soil type rather than to the amount of food soil detected.

Conclusions: This work demonstrates that in the study of conditioning film and cell retention on the hygienic properties of surfaces, SEM may not reveal the presence of retained conditioning film, and thus methods such as epifluorescence microscopy should also be used. This is an essential fact to the methodology design of future work carried out in our laboratories on the effectiveness of the removal of cells and conditioning films from surfaces using different cleaning regimes.

Verran, J., Packer, A., Kelly, P. and Whitehead, K.A. Titanium-coating of stainless steel as an aid to improved cleanability. **International Journal of Food Microbiology** xx(2010) xxx-xxx (in press)

Objectives: The aims of this study were: 1) to develop a rapid and simple non-destructive method for indirect characterisation of surface wear *in situ*, and characterise the shape and dimensions of surface features in the range of typical microbial dimensions. Using these data, a titanium coating was applied to a fine polished stainless steel surface with features of dimension comparable to those found on the worn surface and 2) to investigate the effect of surface chemistry on the retention of *Escherichia coli* cells in the presence and absence of a meat conditioning film.

Materials and methods: Two types of stainless steel (SS) surfaces were used in the characterization study: Type 304 fine polished stainless steel [FP] (Outokumpu stainless Ltd, Sheffield, UK) represented a new, unworn SS, while a worn stainless steel (type unknown) sample for the study was taken from a horizontal food preparation area in a canteen. Both surfaces were coated with titanium via magnetron sputtering and uniform deposition of titanium was ensured by SEM from the cross sections. The topography of the surfaces was determined from replica samples first by SEM, and then using Atomic Force Microscopy (AFM) and white light profilometry. By AFM two and three dimensional maps were imaged from the surfaces and R_a values were measured. The surface topography of the new surfaces was measured using white light profilometry. Titanium coated surfaces were checked for conformity of film deposition using SEM and energy dispersive X ray spectroscopy (EDX) characterization.

For the retention assays with *E. coli*, the test surfaces were coated with meat exudate (simulating soiled conditions) and dried under aseptic conditions. Dried surfaces were covered with *E. coli* cell suspension (8.4×10^7 cfu/ml) and let to soak without agitation for 1 h at 37°C. After rinsing the test surfaces were dried and stained with DAPI (*E. coli* cells) and Rhodamine B (meat exudate). Samples were visualized by epifluorescence microscopy.

Results: Significant differences in R_a values were observed between the new and worn surfaces ($p<0.001$). Titanium coating did not affect the topography and the R_a values of the fine polished surfaces.

Conclusions: The results obtained demonstrated that the surface chemistry may play a more important role in the retention than expected. In spite of the differences in the surface topography, coating with titanium clearly reduced the retention of *E. coli* and organic soil on the surfaces studied. Titanium coating might thus further improve the hygienic properties of stainless steel as a food contact surface.

Vickery M**.** How clean is your conveyor? **Food Processing UK** 70 (2001)1: 13

Objectives**:** The aim of the study was to compare under food production conditions the hygienic properties of two conveyor belt materials, stainless steel (SS) and polypropylene (PP).

Materials and methods: The material studied were stainless steel belt type 302 with mesh 7.26 mm x 1.57 mm (Wire Belt Co) and generic modular polypropylene flush grid belt. The belts were run for 10 h period in a production line of peeled and diced carrots and given the bacteria an incubation time for 24 h. The temperature was 15°C. Swab samples were taken before cleaning the belts with three commercial detergents. Second sampling took place after the cleaning. The samples were analysed for the total number of aerobic bacteria.

Results with conclusions: Before cleaning, on stainless steel belt microbial growth was 0.95 logs lower than on the polypropylene belt. This was explained by the different constructions of the belts: the contact area of SS belt with the product was 25% smaller than the contact area of the PP belt. At the optimal dosage of cleaning chemicals, the reduction of bacteria on SS belt was 3.8-4.1 logs while on PP belts the reduction was 3.3-3.6 logs. On the basis of the results obtained Vickery concludes that the hygienic properties of SS over those of PE, PU, PP and PVC were reaffirmed.

B. Survival of pathogenic risk organisms on the surfaces

Wilks, S.A., Michels, H. and Keevil, C.W. The survival of *Escherichia coli* O157 on a range of metal surfaces**, International Journal of Food Microbiology** 105 (2005) 445– 454

Background: *Escherichia coli* O157:H7 is a serious pathogen causing haemorrhagic colitis. It has been responsible for several large-scale outbreaks in recent years. *E. coli* O157:H7 is able to survive in a range of environments, under various conditions. The risk of infection from contaminated surfaces is recognised, especially due to the low infectious dose required.

Objectives: The aim of the study was to investigate the survival of *E. coli* O157 (NCTC 12900) on a wide range of copper-containing alloy materials that might be suitable for use as work surfaces in industrial and domestic environments. The main aim was to to assess the antibacterial properties of other metal alloys containing copper and compare their performance to pure coppers and stainless steel.

Materials and methods: A total of 22 metal alloys were tested. They were divided into six groups: coppers, brasses, copper nickels, copper nickel zinc alloys, and stainless steel. In the work a high concentration of bacterial cells was used (to represent a worst case scenario) onto each alloy and monitoring the bacterial levels over time at two temperatures: 20 °C and 4 °C, representing room and refrigeration temperature environment

Results: In this study, a high concentration (10^7 cells) of *E. coli* O157 was placed onto different metals and survival time measured. Results showed *E. coli* O157 to survive for over 28 days at both refrigeration and room temperatures on stainless steel. Copper, in contrast, has strong antibacterial properties (no bacteria can be recovered after only 90 min exposure at 20 °C, increasing to 270 min at 4 °C) but its poor corrosion resistance and durability make it unsuitable for use as a surface material. Other copper-containing alloys, such as copper nickels and copper silvers, have improved durability and anticorrosion properties and greatly reduce bacterial survival times at these two temperatures (after 120 min at 20 °C and 360 min at 4 °C, no *E. coli* could be detected on a copper nickel with a 73% copper content).

Conclusions: Use of a surface material with antibacterial properties could aid in preventing cross-contamination events in food processing and domestic environments, if standard hygiene measures fail.

Kusumaningrum, H.D., Riboldi, G., Hazeleger, W.C., Beumer , R.R. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods, **International Journal of Food Microbiology** 85 (2003) 227– 236

Background: The retention of bacteria on food contact surfaces increases the risk of cross-contamination of these microorganisms to food. The risk has been considered to be lowered when the surfaces are dry, partly because bacterial growth and survival would be reduced. However, some non-spore-forming bacteria might be able to withstand dry conditions on surfaces for an extensive period of time.

Objectives: The aim of this study was to determine the survival of the pathogenic bacteria *Salmonella enteritidis*, *Staphylococcus aureus* and *Campylobacter jejuni* on stainless steel surfaces (SS) at different initial levels. The transfer rates of these pathogens from kitchen sponges to SS and from SS to foods were also investigated.

Materials and methods: Disinfected stainless steel surfaces (AISI type 304 standard, ODS). The test surfaces were contaminated at three levels of *S. enteritidis*, *S. aureus* and *C. jejuni*: 10^7 cfu/cm^2 (high), 10^5 cfu/cm^2 (moderate) and 10^3 cfu/cm^2 (low). Samples were collected with contact plates after 100 h. In the cross contamination studies, the plates were first contaminated by kitchen sponges, and let to dry in a laminar hood. Thereafter model foods (cucumber, roasted chicken) were placed on the surfaces. The foods were exposed to the surface for 10 s. Contamination rate of the model foods was determined by a spiral plater. SEM was used as an additional tool in visualization of contamination rates.

Results: *S. aureus* was recovered from SS for at least 4 d when the contamination level was high (10^5 cfu/cm^2) or moderate (10^3 cfu/cm^2). At low levels (10 cfu/cm^2), the surviving numbers decreased below the detection limit (4 cfu/100 cm^2) within 2 d. *S. enteritidis* was recovered from surfaces for at least 4 d at high contamination levels, but at moderate level, the numbers decreased to the detection limit within 24 h and at low level within 1 h. *C. jejuni* was the most susceptible to slow-air-drying on surfaces; at high contamination levels, the numbers decreased below the detection

limit within 4 h. The test organisms were readily transmitted from the wet sponges to the stainless steel surfaces and from these surfaces to the cucumber and chicken fillet slices, with the transfer rates varying from 20% to 100%.

Conclusions: This study highlighted the fact that pathogens remain viable on dry stainless steel surfaces and present a contamination hazard for considerable periods of time, dependent on the contamination levels and type of pathogen. Systematic studies on the risks of pathogen transfer associated with surface cleaning using contaminated sponges provide quantitative data from which a model of risks assessment in domestic setting could lead.

Gundelley, R., Youm, G.W. and Kwon, Y.M. Survival of bacterial pathogens on anti-microbial conveyor belts**, Journal of Rapid Methods & Automation in Microbiology** 15(2007) 259-266.

Objectives: The aim of the study was to evaluate the antimicrobial activities of three commercial conveyor belts manufactured to contain antimicrobial additive against selected pathogenic bacteria, *Escherichia coli* 0157:H7 and *Listeria monocytogenes*.

Materials and methods: Antimicrobial conveyor belts (HabaGUARD) were made of three different materials, fabric, polyethylene (PE) and polyoxymethylene (POM). The antimicrobial additive used in the belts was approved by EPA. The same belt products without the antimicrobial were used as the respective controls. The *E. coli* 0157:H7 strain used as one of the test organisms was originally isolated from beef, whereas *L. monocytogenes* was used as a cocktail of five strains representing five different serotypes. The conveyor belt samples were cleaned with 70% ethanol and dried in a biosafety cabinet before contamination. Dried belt samples were contaminated with the test organisms, and thereafter incubated in closed, sterile plastic bags at 37 °C for 24 h. For enumeration of bacterial growth, each sample were first gently stomached with 100 ml of peptone saline for 10 min at 37 °C. Bacterial counts were determined from the peptone saline using selective media.

Results: While the initial *E. coli* count before incubation was 6.4×10^5cfu/sample, viable cell counts on the antimicrobial belt products was below detection limit ($< 10^2$ cfu/sample) after the incubation. The viable counts on the control belts without antimicrobial additive varied in a range of $5.2 \times 10^4 - 4.5 \times 10^6$ cfu/sample. While the initial *L. monocytogenes* count was 1.3×10^6 cfu/sample, only in one of the anti-microbial belt samples studied (PE) low numbers (9.7×10^2 cfu/sample) could be detected after experiment. In the control belt samples the counts varied within a range from $5.8 \times 10^4 - 7.4 \times 10^5$ cfu/sample. The results were encouraging. However, as the contact time under the experimental conditions was long (24 h) similar results hardly would be obtained under production conditions where the contaminants are not in direct contact with the conveyor belt and the contact time is much shorter.

Conclusions: The results obtained may demonstrate that antimicrobial compounds incorporated into conveyor belts might prevent biofilm formation on the surface of conveyor belts.

Tolvanen, R., Lundén, J., Korkeala, H., and Wirtanen, G. Ultrasonic cleaning of conveyor belt materials using *Listeria monocytogenes* as a model organism**, Journal of Food Protection**, 70 (2007)3: 758–761

Background: Persistent *Listeria monocytogenes* contamination of food industry equipment is a difficult problem to solve. Ultrasonic cleaning offers new possibilities for cleaning conveyors and other equipment that are not easy to clean.

Objectives: The aim of the study was to test ultrasonic cleaning on three conveyor belt materials: polypropylene, acetal, and stainless steel (cold-rolled, AISI 304).

Materials and methods: Cleaning efficiency was tested at two temperatures (30°C and 45 °C) and two cleaning times (30 s and 60 s) with two cleaning detergents (KOH, and NaOH combined with KOH). Conveyor belt materials were soiled with milk-based soil and *L. monocytogenes* strains V1, V3, and B9, and then incubated for 72 h to attach bacteria to surfaces.

Results: The cleaning treatment was considered effective if *L. monocytogenes* reduction after treatment was at least 3 log units. In three of the tested ultrasonic cleaning combinations, the logarithmic reduction of *L. monocytogenes* was less than 3 log units on polypropylene, and *L. monocytogenes* contamination was present on polypropylene after all cleaning treatments. The logarithmic reduction of *L. monocytogenes* was significantly greater in stainless steel than it was in plastic materials. Ultrasonic cleaning treatments reduced *L. monocytogenes* counts on stainless steel 4.6 to 5.9 log units; on acetal, 3.4 to 5.6 log units; and on polypropylene, 2.3 to 4.4 log units. The logarithmic reduction differences were statistically analyzed by analysis of variance. The logarithmic reduction was significantly greater in stainless steel than in plastic materials ($P < 0.001$ for polypropylene, $P = 0.023$ for acetal). Higher temperatures enhanced the cleaning efficiency in tested materials. No significant difference occurred between cleaning times. The logarithmic reduction was significantly higher ($P = 0.013$) in cleaning treatments with potassium hydroxide detergent.

Conclusions: Ultrasonic cleaning was shown to be an effective method of detaching *L. monocytogenes* from conveyor materials. Short ultrasonic washing treatment may provide a new possibility in cleaning conveyor belts that are difficult to clean with conventional methods.

C. Biofilms in food industry (reviews)

Simões, M., Simões, L.C., and Vieira, M.J. A review of current and emergent biofilm control strategies, **LWT - Food Science and Technology** 43 (2010) 573–583

Abstract: Microbial adhesion to surfaces and the consequent biofilm formation has been documented in many different environments. Biofilms constitute a protected mode of growth that allows microorganisms to survival in hostile environments, being their physiology and behaviour significantly different from their planktonic counterparts. In dairy industry, biofilms may be a source of recalcitrant contaminations, causing food spoilage and are possible sources of public health problems such as outbreaks of foodborne pathogens. Biofilms are difficult to eradicate due to their resistant phenotype. However, conventional cleaning and disinfection regimens may also contribute to inefficient biofilm control and to the dissemination of resistance. Consequently, new control strategies are constantly emerging with main incidence in the use of biosolutions (enzymes, phages, interspecies interactions and antimicrobial molecules from microbial origin). The present review will focus on describing the mechanisms involved in biofilm formation and behaviour, deleterious effects associated with their presence, and some of the current and emergent control strategies, providing new insight of concern for food industry.

Conclusions: Microbial control in food processing has the main aims of reduction/eradication of microbes and their activity, and the prevention/control of the formation of biological deposits on the process equipment. Nowadays, the most efficient practical means for limiting microbial growth includes good production hygiene, a rational running of the process line, and effective use of cleaning and disinfectant products. Due to the increased resistance of biofilms to conventional disinfection processes, novel means for their control are constantly sought through the control of environmental factors on the process line and the use of new control strategies. Much more needs to be learned about the impact of antimicrobial products on microbial biofilms and their recovery responses to damage, as microorganisms can develop resistance and subsequently survive previously effective control procedures. The discovery of new biofilm control strategies, following the specifications needed to be used in food industry, and based on the use of biological-based solutions with high antimicrobial activity and specificity seem to be a step ahead in overcoming the biofilm resistance issue.

Boulané-Petermann, L. Processes of bioadhesion on Stainless steel surfaces and Cleanability: a review with special reference to the food industry**, Biofouling**, 10 (1996) 275-300

Abstract: Biofouling of equipment surfaces in the food industry is due initially to physico-chemical adhesion processes, and subsequently to the proliferation of microbes within an extracellular polymer matrix. Two physico-chemical theories can be applied to predict simple cases of bacterial adhesion. However, these models are limited in their applicability owing to the complexity of bacterial surfaces and the surrounding medium. Various factors that can affect the bacterial adhesion process

have been listed, all directly linked to the solid substratum, the suspension liquid or the microorganism. For stainless steel surfaces, it is important to take into account the grade of steel, the type of finish, surface roughness, the cleaning procedures used and the age of the steel. Regarding the suspension fluid within which adhesion takes place, pH, ionic composition and the presence of macromolecules are important variables. In addition, the adhering microorganisms have extremely complex surfaces and many factors must be taken into account when conducting adhesion tests, such as the presence of cell appendages, the method of culture, the contact time between the microorganism and the surface, and exopolymer synthesis. Research on biofilms growing on stainless steel has confirmed results obtained with other materials, regarding resistance to disinfectants, the role of the extracellular matrix and the process by which the biofilm forms. However, it appears that the bactericidal activity of disinfectants on biofilms differs according to the type of surface on which they are growing. The main cleaners and disinfectants used in the food industry are alkaline and acid detergents, peracetic acid, quaternary ammonium chlorides and iodophors. The cleanability and disinfectability of stainless steel surfaces have been compared with those of other materials**.** According to the published research findings, stainless steel is comparable in its biological cleanability to glass, and significantly better than polymers, aluminium or copper. Moreover, microorganisms in a biofilm developing on a stainless steel surface can be killed with lower concentrations of disinfectant than those on polymer surfaces.

Conclusions: Biocontamination and the adhesion of microorganisms to chemically inert solid depend not only on the roughness of the surface but also on the surface properties of the material concerned. To reduce and optimise cleaning and disinfecting procedures in the food industry, a more thorough knowledge of adhesion processes is required. This implies more knowledge of the surface, its cleanability and disinfectability, the appropriate design of equipment, a better understanding of the cleaning fluids and disinfectants, and effective control of cleaning and disinfecting processes. Early studies showed that it was possible to modify the energy characteristics of a solid surface by preferential surface adsorption of organic molecules from foods, cleaning agents or disinfectants. This can be used to promote the adhesion of specific microorganisms at the expense of others whilst it can be assumed that surfaces conditioned with a multimolecule film of disinfectant will be less favourable to the proliferation of bacteria than surfaces conditioned with molecules of a cleaning agent.

From a more scientific standpoint, when conducting comparative studies of adhesion or biofilm development on stainless steel, it is important to specify the surface finishes concerned and the method (or methods) used to count the adhering microorganisms. When choosing a material, it is best to examine several of its properties besides microbiological cleanability, *e.g.* the presence or absence of metal elements released by the solid when in contact with biological substances or foods Certain elements released can be toxic or, on the contrary, promote bacterial growth. It is also useful to examine the mechanical resistance of the material and its resistance to corrosion over a period of time. Lastly, recyclability is becoming a decisive argument in choosing a material.

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List of Standards for Testing of Food Contact Materials

CEN/TS 13130-9 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 9: Determination of acetic acid, vinyl ester in food simulants

CEN/TS 13130-10 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 10: Determination of acrylamide in food simulants

CEN/TS 13130-11 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 11: Determination of 11-aminoundecanoic acid in food simulants

CEN/TS 13130-12 Material and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 12: Determination of 1,3-benzenedimethanamine in food simulants

CEN/TS 13130-13 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 13: Determination of 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A) in food simulants

CEN/TS 13130-14 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 14: Determination of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indoline in food simulants

CEN/TS 13130-15 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 15: Determination of 1,3-butadiene in food simulants

CEN/TS 13130-16 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 16: Determination of caprolactam and caprolactam salt in food simulants

CEN/TS 13130-17 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 17: Determination of carbonyl chloride in plastics

CEN/TS 13130-18 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 18: Determination of 1,2-dihydroxybenzene, 1,3-dihydroxybenzene, 1,4-dihydroxybenzene, 4,4"-dihydroxybenzophenone and 4,4"dihydroxybiphenyl in food simulants

CEN/TS 13130-19 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 19: Determination of dimethylaminoethanol in food simulants

CEN/TS 13130-20 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 20: Determination of epichlorohydrin in plastics

CEN/TS 13130-21 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 21: Determination of ethylenediamine and hexamethylenediamine in food simulants

CEN/TS 13130-22 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 22: Determination of ethylene oxide and propylene oxide in plastics

CEN/TS 13130-23 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 23: Determination of formaldehyde and hexamethylenetetramine in food simulants

CEN/TS 13130-24 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 24: Determination of maleic acid and maleic anhydride in food simulants

CEN/TS 13130-25 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 25: Determination of 4-methyl-1-pentene in food simulants

CEN/TS 13130-26 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 26: Determination of 1-octene and tetrahydrofuran in food simulants

CEN/TS 13130-27 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 27: Determination of 2,4,6-triamino-1,3,5-triazine in food simulants

CEN/TS 13130-28 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 28: Determination of 1,1,1-trimethylolpropane in food simulants.

CEN/TS 14234 Materials and articles in contact with foodstuffs. Polymeric coatings on paper and board- Guide to the selection of conditions and test methods for overall migration

CEN/TS 14235 Materials and articles in contact with foodstuffs. Polymeric coatings on metal substrates. Guide to the selection of conditions and test methods for overall migration

CEN/TS 14577 Materials and articles in contact with foodstuffs. Plastics. Polymeric additives. Test method for the determination of the mass fraction of a polymeric additive that lies below 1000 Daltons

EN 631-1 Materials and articles in contact with foodstuffs. Catering containers. Part 1: Dimensions of containers

EN 1183 Materials and articles in contact with foodstuffs. Test methods for thermal shock and thermal shock endurance

EN 1184 Materials and articles in contact with foodstuffs. Test methods for translucency of ceramic articles

EN 1388-1 Materials and articles in contact with foodstuffs. Silicate surfaces. Part 1: Determination of the release of lead and cadmium from ceramic ware

EN 1388-2 Materials and articles in contact with foodstuffs. Silicate surfaces. Part 2: Determination of the release of lead and cadmium from silicate surfaces other than ceramic ware

EN 1900 Materials and articles in contact with foodstuffs. Non-metallic tableware. Terminology

EN 12546-1 Materials and articles in contact with foodstuffs. Insulated containers for domestic use. Part 1: Specification for vacuum ware, insulated flasks and jugs

EN 12546-2 Materials and articles in contact with foodstuffs. Insulated containers for domestic use. Part 2: Specification for insulated bags and boxes

EN 12546-3 Materials and articles in contact with foodstuffs. Insulated containers for domestic use. Part 3: Specification for thermal packs

EN 12980 Materials and articles in contact with foodstuffs. Non-metallic articles for catering and industrial use. Method of test for the determination of impact resistance

EN ISO 8442-5 Materials and articles in contact with foodstuffs. Cutlery and table holloware. Part 5: Specification for sharpness and edge retention test of cutlery (ISO 8442-5:2004)

EN ISO 8442-6 Materials and articles in contact with foodstuffs. Cutlery and table holloware. Part 6: Lightly silver plated table holloware protected by lacquer (ISO 8442-6:2000)

EN ISO 8442-7 Materials and articles in contact with foodstuffs. Cutlery and table holloware. Part 7: Specification for table cutlery made of silver, other precious metals and their alloys (ISO 8442-7:2000)

EN ISO 8442-8 Materials and articles in contact with foodstuffs. Cutlery and table holloware. Part 8: Specification for silver table and decorative holloware (ISO 8442-8:2000)

EN 1186-1 Materials and articles in contact with foodstuffs. Plastics. Part 1: Guide to the selection of conditions and test methods for overall migration

EN 1186-2 Materials and articles in contact with foodstuffs. Plastics. Part 2: Test methods for overall migration into olive oil by total immersion

EN 1186-3 Materials and articles in contact with foodstuffs. Plastics. Part 3: Test methods for overall migration into aqueous food simulants by total immersion

EN 1186-4 Materials and articles in contact with foodstuffs. Plastics. Part 4: Test methods for overall migration into olive oil by cell

EN 1186-5 Materials and articles in contact with foodstuffs. Plastics. Part 5: Test methods for overall migration into aqueous food simulants by cell

EN 1186-6 Materials and articles in contact with foodstuffs. Plastics. Part 6: Test methods for overall migration into olive oil using a pouch

EN 1186-7 Materials and articles in contact with foodstuffs. Plastics. Part 7: Test methods for overall migration into aqueous food simulants using a pouch

EN 1186-8 Materials and articles in contact with foodstuffs. Plastics. Part 8: Test methods for overall migration into olive oil by article filling

EN 1186-9 Materials and articles in contact with foodstuffs. Plastics. Part 9: Test methods for overall migration into aqueous food simulants by article filling

EN 1186-11 Materials and articles in contact with foodstuffs. Plastics. Part 11: Test methods for overall migration into mixtures of C-labelled synthetic triglycerides

EN 1186-12 Materials and articles in contact with foodstuffs. Plastics. Part 12: Test methods for overall migration at low temperatures

EN 1186-13 Materials and articles in contact with foodstuffs. Plastics. Part 13: Test methods for overall migration at high temperatures

EN 1186-14 Materials and articles in contact with foodstuffs. Plastics. Part 14: Test methods for "substitute tests" for overall migration from plastics intended to come into contact with fatty foodstuffs using test media iso-octane and 95 % ethanol

EN 1186-15 Materials and articles in contact with foodstuffs. Plastics. Part 15: Alternative test methods to migration into fatty food simulants by rapid extraction into iso-octane and/or 95 % ethanol

EN 13130-1 Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 1: Guide to test methods for the specific migration of substances from plastics to foods and food simulants and the determination of substances in plastics and the selection of conditions of exposure to food simulants