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- Nordic Wood 2

Project P 99095 «Wood in the Food»

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Part report no. 10

Wood, plastic and steel – a comparison of hygienic properties

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Foreword

This project is a separate co-ordinated part of the project: ”Wood in the Food Industry part 2, P99095”.

The Danish legislation regarding ”fresh meat” is based on EU directives, e.g. ”the fresh meat directive”.

These EU directives have been implemented in various laws, regulations, governmental circulars and guidance’s. The legislation in this area is very extensive. The most important are stipulated in the Danish Veterinary Department regulation number 351 from May 2nd 1996: Regulation regarding fresh meat, Regulation no. 673 from October 14th 1988 on the export of meat products and regulation number 1073 from October 12th 1996 on meat products etc.

Among other things the regulations stipulate that:
The use of wood (in tools, tabletops and shelves and other flat surfaces, that can come in contact with fresh meat) is forbidden except in rooms that only contain hygienically packaged fresh meat.
In connection to equipment, tools, tables, materials etcetera, the use of wood is forbidden.
Use of wood is permitted in rooms for smoking, curing, maturing and pickling, for storage of meat products and shipping if technical reasons exclude the use of other materials and if no risk of contamination is present.
In rooms such as these wooden pallets may only be used for transportation of packaged meat or meat products.

Additionally it should be noticed that the veterinary service is very strict towards the use of wood. This practise is based on a prejudice that the porous structure of wood is unhygienic.

It is clear that the wide spread use of wood in the food industry has been diminished and is almost 100% replaced by other materials.

Regardless of scientific findings that wood, in certain situations can compete with plastics with regards to hygienic properties, the use of wooden products has largely given way to plastic and steel.

Based on the above it can be concluded that the use of wood within the food industry is very restrictive. This can be based on both national and international legislation (both in the EU and the USA).

There is also a risk that the development within the industrial sector will result in further veterinary restrictions against the use of wood in industrial kitchens, restaurants, and

butchers etc. Similarly the use of wood in industries that handle vegetables could be threatened.

The present report is one in a series of reports presented in the Nordic Wood 2 project no. P 99095 ”Wood in the Food Industry part 2”.

The Nordic Industrial Fond, The National Forest and Nature Agency and the following Danish industries have sponsored the part 2 of the project:

Dansk Træemballage A/S represented by Peter Jensen

Junckers Industrier A/S represented by Anders Gottlieb

Aalborg Træindustri A/S represented by Søren Padkjær

Scanwood ApS represented by Jens Peter Møller

Bodum Træ A/S represented by Mads Bondesen

The Danish Veterinary and Food Administration – Regional Laboratory Northeast Zealand represented by Claus Jeppesen

The laboratory at the Danish Technological Institute, Centre for Biotechnology and The Danish Veterinary and Food Administration – Regional Laboratory Northeast Zealand has done the laboratory tests.

The steering committee of the project consisted of:

Dansk Træemballage A/S represented by Peter Jensen (DK)

Limtré h.f. represented by Bjarni Ingibergsson (I)

Åsljunga Pallen AB represented by Stefan Nilsson (S)

Otta Sag og Høvleri A/S represented by Dag Aasheim (N)

Knud Erik Kvist from the Danish Technological Institute has been the Danish project co-ordinator throughout most of the project. In the final stage this was taken over by Christian J. Kofod and Berit Lindegaard also from the Danish Technological Institute.

The participants would like to thank both the Nordic industrial Fond and The National Forest and Nature Agency for sponsoring the project.

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Contents

1 Summery

The aim of the project was to test the survival of selected bacteria, commonly found in the meat industry, on different wood species, plastic and stainless steel. The project included 4 parts.

In the first part, the survival of the Gram-positive *Bacillus subtilis* was tested on different wood species, plastic and steel. It can be concluded, that oak showed the best results in elimination of bacteria on the surface. A remarkably big difference between wooden samples and plastic and steel in the amount of bacteria survival on the surface of the samples was observed.

The second part involved the testing of the same wooden species, plastic and steel. The gram- negative bacterium, *Pseudomonas fluorescens*, was used to contaminate test samples. Oak was again showing the highest rate in bacterial decrease. There is not a big difference in bacterial survival between oil treated wooden samples and untreated wooden samples contaminated neither by *Bacillus subtilis*, nor by *Pseudomonas fluorescens*. The explanation could be that linseed oil did not really work as protection, but rather as nutrition. In the experiments with pine and spruce contaminated with both mentioned bacteria, pine is performing better than spruce both at low and high moisture content.

In the third part of the project the focus was to compare bacteria survival on new and artificially aged wood. New oil treated and untreated oak and plastic was used as reference material. Samples were contaminated with bacteria in 1% meat extract. The results showed only slight differences between new and artificially aged samples. But plastic was again showing best conditions for bacterial survival.

In the last part the efficiency of a cleaning programme, simulating the ordinary dish washing in the kitchen was tested. The cleaning did have a big influence on bacterial survival by using detergent even without antibacterial additives. The cleaning programme gave satisfactory results. We did not detect any amount of bacteria higher than 100 CFU/ml already after washing. Cleaning increased the speed of removal of bacteria.

In the final part of the project the Danish Veterinary and Food Administration – Regional Laboratory Northeast Zealand carried out a minor test programme for verification of results obtained at DTI. They repeated some of the tests using the same methods as DTI. The results showed a high correlation between the results obtained by the two laboratories.

2 Introduction

The aim of this project is to test the survival of selected bacteria, commonly found in the meat industry, on different wood species, plastic and stainless steel. The project includes testing of simulated used tabletops in comparison with new ones and the efficiency of a cleaning programme.

The background of this test programme is the obtained results from similar testing in Norway and Iceland. Different methods for testing the hygienic properties of wood in the food industry were developed and tested with special focus on the fish industry. The survival of selected bacteria is tested on a selection of wood species, plastic and stainless steel. From the former programme (Report 1/2000) two methods for analysis were selected, namely the swab method and the contact agar method.

The selection of wood species were discussed with the industrial partners and the decision was to examine planed beech, oak and ash, because these wood species are used as tabletops in general or in production line and as inventories, handles, cutting-boards etc. The wood species were tested with and without oil treatment. The industry has agreed to use linseed oil. For the packaging industry it was decided to test sawn, untreated sapwood of pine and spruce. Stainless steel and plastic were used as reference materials.

To simulate used tabletops, two "ageing machines" were constructed, one to give systematic scratches on the surface of wooden samples, and the other simulating the use of a meat hammer by producing dents in the surfaces. Aged samples were tested and compared with not aged ones.

To investigate the efficiency of ordinary kitchen dish washing, a common detergent without special antibacterial additives was used. The new and aged samples were washed a certain number of times with a sponge after contamination. Then the survival of bacteria on the surface of the cleaned wooden samples, were tested.

Some of the tests carried out at Danish Technological Institute were repeated at The Danish Veterinary and Food Administration – Regional Laboratory Northeast Zealand in order to verify the results and conclusions.

3 Laboratory analysis

3.1 Test samples

Both in former and in these experiments the size of the wooden samples was 50x50x20 mm. Stainless steel samples were 50x50 x1mm and plastic samples were 50x50x10mm.

In order to simulate moisture content of wood in practice but also make it possible to limit the number of test samples the following wood moisture contents were chosen:

Beech, oak and ash samples are simulating tabletops. They were prepared in 2 series of planed untreated and planed oil-treated samples. All these samples were stabilised at 7-11% w/w moisture content.

Spruce and pine are simulating the usage of pallets and therefore tested as dry and wet samples. Dry samples were stored in climatic chamber and stabilised at a wood moisture content of 7-11% w/w. Wet samples were prepared by spraying the wooden samples with demineralised water, pack the samples in autoclavable bags and sterilise the samples in autoclave at 12ºC for 15 minutes. Samples were stored in plastic bags to keep the wood moisture at 19-25% w/w during the whole experiment. The actual moisture was checked before and after experiment.

Samples of plastic (polyethylene) and stainless steel were wrapped in aluminium paper and packed in autoclavable bags. They were sterilised in autoclave at 121°C for 15 minutes.

Sterile plastic and steel are used as reference materials.

3.2 Bacteria cultures

Selected bacteria species were used in the tests as monocultures:

- *Bacillus subtilis* CCUG 10779 (G+ sporogenous rod-shaped bacteria)
- *Pseudomonas fluorescens* CCUG 1253 (G- rod-shaped bacteria)

These bacteria were chosen, because they represent different morphological characteristics, they can appear on meat products and they are traditional indicator bacteria. Experiences with gentle methods for conserving food show that G+ bacteria have a higher ability to survive than G- bacteria. G+ bacteria is known to survive longer on wood than G- bacteria.

3.2.1 Description of bacteria

Bacillus subtilis (G+, endo-spore forming rods)

Rod-shaped, straight-cells, vary widely in size (0.5 - 1 x 2 - 10 µm). Growth occurs at a water activivity of 0.901-0.931 and at a temperature range of 12- 55°C. The optimum temperature is 43-46°C. The pH range is 4.6-9.2. This bacteria forms endo-spores and they are widely distributed and occur in many heat-treated products. Spores are highly heat resistant.

Pseudomonas fluorescens (G-, psychrotrophic, aerobic spoilage bacteria)

It is found in soil and water. The organism is commonly associated with spoilage of foods (eggs, meat, fish and milk). Growth occurs at a water activity of 0.949-0.970 and at a temperature range of 4-40°C. The optimum temperature is 23-24°C.

3.3 Cultivation

3.3.1 Media and suspensions

The inoculum used to contaminate wooden samples with *Bacillus subtilis* and *Pseudomonas fluorescens* had been growing 24 hours in 100 ml of a media called Tryptic Soy Broth medium TSB). *Bacillus subtilis* was incubated at 37°C, *Pseudomonas fluorescens* at 22°C. The final cell concentration was about 10^7 CFU/ml (CFU=colony forming units pr ml).

The concentration of 100 ml of meat extract dry was 1% and 0.03%. They simulated a high and low degree of the contamination of tabletops.

Petri dishes with Plate Count Agar were used to test the amount of bacteria obtained by both, swab and contact method from contaminated samples.

3.3.2 Contamination

The 24 hours old inoculum was separated from TSB medium (100 ml) by centrifugation (4,000 rpm; 18°C; 10 minutes). The sediment was re-suspended in 100ml of 1% meat extract simulating a high degree of contamination, or in 0.03% meat extract simulating a low degree of contamination.

This kind of re-suspension of bacteria in meat extract was done, to simulate meat contamination on tabletops. Only ash, beech, oak, plastic and steel were tested that way. Just for comparison the contamination of mentioned samples was also carried out with TSB medium alone.

In the case of pine and spruce, 1 ml of the inoculum in TSB medium was used to contaminate wooden samples directly, what was supposed to symbolise contamination of wooden pallets.

A volume of 0.5ml (in the case of ash, beech and oak) was spread on the pith side of wooden samples with the side of the pipette. The same volume was spread on the Plastic and stainless steel samples. Because of a very fast suction in the case of sawn pine and spruce, the volume needed to spread was doubled to 1ml with the same concentration of bacteria.

3.3.3 Media and suspensions

To simulate the temperature on a production line, with wooden tabletops the incubation temperature for contaminated samples was 20°C. When pallets in industry contain packed meat/meals, the temperature is much lower, around 10°C and more variable. In this test programme we chose 20ºC for all test samples for comparison of results.

Sampling intervals are chosen to simulate cleaning intervals (2 hrs.), or cleaning after end of working day (5 hrs) and after 7 days. Intervals are chosen to examine sluggishness of bacteria in the wood and their ability to survive under the micro-aerophilic conditions, which can appear in the depth of wood. The interval after suction of bacterial suspension on the surface of wooden samples indicates the actual concentration of bacteria appearing on the samples after contamination. Each interval included series of 3 test samples.

After performing the swab and the contact methods, the Petri dishes were incubated at the optimum growing conditions for the tested microorganism. Plates contaminated with *Bacillus subtilis* were incubated in heating cabinet at 37°C. Plates contaminated with *Pseudomonas fluorescens* were incubated at 22°C.

3.3.4 Testing survival of the bacteria in wood

It was discussed if the bacteria could penetrate laterally from the surface into the wood. Therefore wooden samples were cut transversely and the new cut surfaces were analysed for bacteria using the contact method.

3.4 Analytical methods

The choice of methods to measure the bacterial survival was based on Report 1, 2000. The following methods have been used: Contact agar method and Swab method. The swab method is preferred. This method was chosen, because the obtained results at higher bacterial concentrations are more precise. The disadvantage is that the detection limits are **100-200 CFU/ml**. If lower concentrations were to be expected, e.g. after cleaning, the contact method by which under **100 bacteria/plate**, can be measured, was used.

The wood industry expressed the wish, also to examine bacterial sluggishness in the wood including their ability to survive in wood. That is why this programme includes surface as well as in depth examinations of inoculated wood samples. In depth examinations were only included in the first part of the project.

3.4.1 Swab method.

The swab-technique to prove the bacterial viability is described in Report 1/2000. After contamination, the surface of each test sample was swabbed by using a sterile cotton wool swab at the different time intervals. Before swabbing, the swab was dipped into a sterile peptone /salt water liquid. The swab was used to stroke over the contaminated surface according to a defined pattern. It is important to cover the total surface. During this process and finally, the swab was stirred in the sterile peptone/salt water liquid. From this solution, dilutions were made and spread on PCA medium. The number of microbes in the peptone/ salt liquid was determined by plate counting.

PCA plates were incubated 24-48 hours in heating cabinet at 22°C for *Pseudomonas fluorescens* and at 37°C for *Bacillus subtilis*.

The detection limits for swab method are **100-200 CFU/ml**.

3.4.2 Contact method

Contact samples were carried out on Petri dishes. One contact sample covers the area of 24cm^2. To prove the viability of the bacteria, plates with PCA medium were used. After contamination, the test samples were placed resting on the surface of a PCA agar plate for 1 minute. The samples were taken in the same time intervals as by the swab method. The same method was used for the in depth examinations. The samples were prepared for the test by cutting a transverse section through “the middle”. The inner side was placed resting on the surface of PCA agar plate for 1 minute. PCA plates were incubated 24-48 hours in a heating cabinet at 22°C for *Pseudomonas fluorescens* and at 37°C for *Bacillus subtilis.*

The contact method can be used, when the concentration of bacteria is under **100 bacteria/plate**.

3.5 Treatment of Samples

3.5.1 Oil treatment

The industry has agreed to use linseed oil. Oil treated ash, beech and oak should simulate tabletops used in food industry. The samples were prepared by the following procedure.

The weight and wood moisture were measured before treatment. Then the samples were placed fully soaked in linseed oil for 30 minutes. The treated samples were stored and left drying in climatic room for a couple of days until they obtained constant weight. Then the final constant weight and actual moisture was measured before sample contamination took place.

3.5.2 Cleaning programme

To simulate kitchen dish washing, warm water mixed with a detergent (1ml detergent/l water) was used. The detergent was ”Neutral” which is a common detergent without any additives with special antibacterial effects.

Each contaminated sample was separately washed after drying with a clean sponge a certain amount of times. Then they were left to dry again. After drying the contaminated and now cleaned samples were tested for bacterial survival at different intervals: after drying, after 2 hours, 5 hours and after 7 days from washing.

3.5.3 Artificial ageing

To prepare wooden and plastic samples simulating used tabletops, an apparatus (see annex 1), which could perform uniform scratches in samples in the same comparable way, was created. Each test-sample was aged 12 times at certain angles simulating cutting with a knife. Then the samples were hit in a different machine by a specified load, to simulate damage caused by a kitchen meat hammer. The machines were developed at the department for Wood Technology. Detailed description is given in annex 1.

3.6 Interpretation of results

Detailed results are given in the annex 2 and 3. In the main text the results are summarised. The number of + indicates the intensity of bacterial growth. Tests were carried out in series of 3 parallel samples.

++++++	More than 300 colonies/plate, which means maximum result in intensity of growth for all three samples.
+	Up to150 colonies/plate
<< (+)	Only one or few single colonies/plate
(-)	The amount of bacteria was not detectable by the swab method at the beginning of the experiment.

4 Tests at the Danish Technological Institute

Four test programmes were carried out:
1. Experiments with *Bacillus subtilis*
2. Experiments with *Pseudomonas fluorescens*
3. Bacterial testing with preceding artificial ageing
4. Experiments with cleaning after contamination

The test programme was only carried out once at the Danish Technological Institute. Where test results vary most or are unexpected parallel testing was executed at a reference laboratory.

4.1 Experiments with *Bacillus subtilis*

4.1.1 Materials and methods

The test samples were: ash, beech, oak, pine, spruce, plastic and stainless steel. Ash, beech and oak were tested as planed, untreated and treated with linseed oil. Pine and spruce were tested as sawn, untreated, dry and wet.

The bacterium was *Bacillus subtilis.*

Three different media were used to contaminate the wood samples:

- 0.03% meat extract should simulate low contamination degree in industry
- 1% meat extract should simulate high contamination degree
- TSB medium – the artificial nutrition – simulating laboratory conditions

The start concentration of *B. subtilis* was about 10^7 CFU / ml. Experiments were carried out in different dilutions to be able to count the single colonies. In the case of ash, beech and oak, the described intensity of growth is corresponding to dilution 10^{-2} and in case of plastic and steel this corresponds to dilution 10^{-3}, because of very intensive growth.

There is no suction on the surface of plastic and steel, and first interval for bacterial viability test is therefore 2 hours after sample contamination. For contamination, bacteria re-suspended in 1% of meat extract liquid (100 ml) are used.

4.1.2 Results

Detailed results are given in anex 2. Table 1 and 2 shows a summary of some results.

Table 1. Growth of *Bacillus subtilis* measured by the swab method. Comparison of untreated and oil treated samples. Contamination with 1% of meat extract. Number of + indicates the amount of bacteria.

Test samples	Dilution of bacterial suspension	Time after contamination (hours)			
		after suction	2hrs.	5hrs.	168hrs.
Untreated					
ash	10^{-2}	++++(+)	(+)	(+)	-
beech	10^{-2}	(+)	(+)	(+)	-
oak	10^{-2}	(-)	-	-	-
plastic	10^{-3}	no	++++	(+)	-
stainless steel	10^{-3}	no	++++++	+(+)	-
Oil treated					
ash	10^{-2}	++(+)	(+)	(+)	-
beech	10^{-2}	+	(+)	(+)	-
oak	10^{-2}	(+)	-	-	-

Table 2. Growth of *Bacillus subtilis* measured by the swab method. Comparison of dry and wet samples of pine and spruce. Contamination with the TSB medium. Number of + indicates the amount of bacteria.

Test Samples	Moisture content	Dilution of bacterial suspension	Time after contamination (hours)			
			after suction	2hrs.	5hrs.	168hrs.
spruce	7-10%	10^{-2}	++++++	(+)	-	-
pine	7-10%	10^{-2}	(+)	-	-	-
spruce	19-25%	10^{-2}	++			-
pine	19-25%	10^{-2}	+++	+	-	-

4.1.3 Discussion

Table 1 shows the results by using the swab method. Ash, beech and oak samples, untreated as well as oil treated are compared. The amount of *Bacillus subtilis* is highest at the beginning and is decreasing within time of testing. There is almost no difference in the rate of decrease between untreated and oil treated samples.

The results of using TSB medium and 0.03% meat extract to contaminate untreated ash, beech and oak are comparable (annex 2). Oak is giving the best results in bacterial viability test reducing the amount of bacteria very fast. Oak is followed by beech with slight better results than ash.

To test plastic and steel only the swab method was used because of intensive growth of *B. subtilis*. The results of contamination with 0.03% meat extract and TSB medium showed, that plastic and steel are almost equal in bacterial viability test and with a high amount of bacteria after 2 hours decreasing within the time, see annex 2. There is a big difference between plastic and steel and untreated/oil treated wood samples.

Comparison of wet and dry samples of pine and spruce, table 2, shows that there is a higher survival of bacteria on the wet surface. But in both cases the amount of bacteria is decreasing within the time.

4.1.4 Conclusion

It can be concluded, that in this experiment oak showed the best results in elimination of bacteria on the surface followed by beech and ash. Last came plastic followed by stainless steel. There was a remarkably big difference between wood samples and plastic and steel in the amount of bacteria surviving on the surface after contamination.

Because of almost no difference between untreated and oil treated planed samples, it can be concluded, that linseed oil did not work as a real protection, but rather as a nutrition.

It can be concluded that pine is better than spruce, in elimination of bacteria from the surface of sawn timber.

4.2 Experiments with Pseudomonas fluorescens

4.2.1 Materials and methods

The test samples were: ash, beech, oak, pine, spruce, plastic and stainless steel. The bacterium was *Pseudomonas fluorescens*. The procedure was the same as described in 3.1.

Ash, beech and oak were tested as planed, untreated and treated with linseed oil. Pine and spruce were tested as sawn, untreated, dry and wet. Three different media were used to contaminate ash, beech and oak:

- 0.03% meat extract should simulate low contamination degree
- 1% meat extract should simulate high contamination degree
- TSB medium presenting laboratory conditions, not the contamination in real life

The start concentration of *P. fluorescens* is about 10^7 CFU/ml. The experiment is carried out in the same dilutions as the first experiment. In the case of ash, beech and oak, the described intensity of growth is corresponding to dilution 10^{-2} and in case of plastic and steel is corresponding to dilution 10^{-3}, because of very intensive growth.

All samples were contaminated with bacteria *P. fluorescens* re-suspended in 1% meat extract liquid. Incubation of Petri dishes containing bacteria was carried out in heating cabinet at 22°C.

4.2.2 Results

Detailed results are given in annex 2. A summary of some results is given in table 3 and 4.

Table 3. Growth of *Pseudomonas fluorescens* measured by the swab- and contact method. Comparison of untreated and oil treated wood samples and plastic and steel. Contamination with bacteria re-suspended in 1% meat extract. Number of + indicates the amount of bacteria.

Test samples	Dilution of bacterial suspension	Time after contamination (hours)			
		after suction	2hrs.	5hrs.	168hrs.
Untreated		**Swab-method**			
ash	10^{-2}	++++++	(+)	(+)	-
beech	10^{-2}	++++	(+)	(+)	-
oak	10^{-2}	++++++	-	-	-
plastic	10^{-3}	no	++++++	++++++	-
steel	10^{-3}	no	++++++	++++++	++
Oil treated		**Swab-method**			
ash	10^{-2}	++++++	+++++	++	-
beech	10^{-2}	+++++(+)	+	(+)	-
oak	10^{-2}	++(+)	-	-	-
Untreated		**Contact-method**			
sh	10^{-2}	++++++	++++++	++++(+)	-
beech	10^{-2}	++++++	++++++	+(+)	-
oak	10^{-2}	++++++	++++	-	-
plastic	10^{-3}	no	++++++	++++++	(+)
steel	10^{-3}	no	++++++	++++++	+
Oil treated		**Contact-method**			
ash	10^{-2}	++++++	++++++	++++++	-
beech	10^{-2}	++++++	++++++	++++++	-
oak	10^{-2}	++++++	++++++	+	-

Table 4. Growth of *Pseudomonas fluorescens* according to the swab- and contact method. Comparison of dry and wet samples. Contamination with TSB medium. Number of + indicates the amount of bacteria.

Test samples	Moisture content	Dilution of bacterial suspension	Time after contamination (hours)			
			after suction	2hrs.	5hrs.	168hrs.
			Swab-method			
spruce	7-10%	10^{-2}	++++++	(+)	+	<(+)
pine	7-10%	10^{-2}	++++++	(+)	++(+)	-
spruce	19-25%	10^{-2}	++++++	+++	+	-
pine	19-25%	10^{-2}	++++++	+++	-	-
			Contact-method			
spruce	7-10%	10^{-2}	++++++	+++++	++++	-
pine	7-10%	10^{-2}	++++++	++++++	++(+)	-
spruce	19-25%	10^{-2}	++++++	++++++	(+)	-
pine	19-25%	10^{-2}	++++++	(+)	(+)	-

4.2.3 Discussion

Table 3 shows the results of the swab and the contact method used on untreated and oil treated wooden samples, plastic and steel. All the samples were inoculated with 0.5ml bacterial suspension from 1% meat extract. There is an evident difference between wooden samples and plastic and steel in the interval of 2 hours after contamination.

In the case of oil treated samples the bacterial amount is decreasing slower. There is not a big difference in bacterial survival between oil treated wooden samples in comparison with plastic and steel. But the difference can still be noticed. The amount of *P. fluorescens* on the surface of plastic and steel is 2-3 times higher than on the wooden samples. The bacteria survive longer on oil treated ash and beech than on untreated samples.

The contact method is a direct method, which is giving results in number of bacteria/plate. No dilutions are used. That is the reason why it seems, in table 3, as though there is a slower decrease, than in the case of using the swab method. But there is no evidence of bacteria after 7 days, except on plastic and steel surfaces. The best result in decrease of the amount of bacteria on the surface shows oak, followed by beech and ash, which slight differences almost are on the same level. Plastic and steel still have surviving bacteria after 168 hrs. There is a high correlation between the swab and the contact method. These two methods are giving basically the same results in evaluation of bacterial viability. Both

support the conclusion that on wood the amount of surviving bacteria is reduced faster than on plastic and steel.

From the annex it can be concluded that bacteria die faster by using 0.03 % meat extract as contaminant, than TSB medium. It can be explained by lower amount of enrichment in 0.03 % meat-extract medium. To show a worst case scenario the following tests were therefore carried out by using 1% of meat-extract liquid.

The tables 4 describe the results of swab and contact method used on pine and spruce contaminated with *P. fluorescens* in TSB medium. Samples were contaminated with 1ml bacterial suspension from TSB medium. The contamination of pallets in use was simulated by the use of samples at two different moisture contents – low and high. The samples were prepared the same way, as described in 3.1. In annex 2 results from a similar test but with 0.03% meat extract are shown. The obtained results show that pine is performing better than spruce both at low and high moisture content. For further details see annex 2.

4.2.4 Conclusion

The amount of *P. fluorescens* surviving on the surface of plastic and steel is 2-3 times higher than on the wooden samples and the bacteria survive much longer. Already after 2 hrs a big difference can be seen. Oiltreatment prolong the survival of bacteria especially on Ash and Beech.

The two test methods differ in the way that the contact method is a direct method, which is giving results in number of bacteria/plate whereas the swab method involve dilution. Therefore the results in table 3 and 4 cannot be compared directly. In both cases there are no evidence of bacteria after 168 hrs, except on plastic and steel surfaces.

The best result in decrease of the amount of bacteria on the surface shows oak, followed by beech and ash, which slight differences almost are on the same level.

Results of using the swab and contact method on pine and spruce contaminated with *P. fluorescens* in TSB medium show that pine is performing better than spruce both at low and high moisture content.

Oil treatment seem to further the survival of Pseudomonas fluorescens.

For further testing 1% meat extract as contaminant is preferred as a worst-case scenario.

4.3 Bacterial testing with preceding artificial ageing

4.3.1 Materials and methods

The survival of bacteria on new and artificially aged wood was compared by bacterial viability tests. Planed untreated and oil treated oak and plastic was chosen for this test since it showed the best results in the tests described in 3.1 and 3.2. The oak samples were contaminated with *Bacillus subtilis* and *Pseudomonas fluorescens* re-suspended in 1%

meat extract liquid to simulate a high degree of contamination. Oil treatment was carried out in the same way as described in 2.5.1. Bacterial viability was tested by swab and contact method.

4.3.2 Results

Detailed results are given in annex 2. A summary f some results is given in table 5 and 6.

Table 5. Growth of *Bacillus subtilis* measured by the swab- and contact-method. Comparison of untreated and oil treated, aged and new oak and plastic. Contamination with 1% of meat extract liquid. Number of + indicates the intensity of growth.

Test samples	Dilution of bacterial suspension	Time after contamination (hours)			
		after suction	2hrs.	5hrs.	168hrs.
Untreated		**Swab-method**			
aged oak	10^{-2}	+++	-	-	-
aged plastic	10^{-3}	no	++++++	(+)	-
new oak	10^{-2}	++(+)	-	-	-
new plastic	10^{-3}	no	++++++	(+)	-
Oil treated		**Swab-method**			
aged oak	10^{-2}	(+)	<(+)	-	-
aged plastic	10^{-3}	no	++++++	(+)	-
new oak	10^{-2}	++(+)	-	-	-
new plastic	10^{-3}	no	++++++	(+)	-
Untreated		**Contact method**			
aged oak	10^{-2}	++++++	++++(+)	++(+)	-
aged plastic	10^{-3}	no	++++++	++++++	<(+)
new oak	10^{-2}	++++++	+++++	+++(+)	<<(+)
new plastic	10^{-3}	no	++++++	++++++	-
Oil treated		**Contact method**			
aged oak	10^{-2}	++++++	+++++(+)	++++(+)	-
aged plastic	10^{-3}	no	++++++	++++++	<(+)

new oak	10^{-2}	++++++	++++++	+++(+)	-
new plastic	10^{-3}	no	++++++	++++++	-
aged oak	10^{-2}	++++++	++++(+)	++(+)	-

Table 6. Growth of *Pseudomonas fluorescens* measured by the swab- and contactmethod. Comparison of untreated and oil treated, new and aged oak and plastic. Contamination with bacteria re-suspended in 1% meat extract. Number of + indicates the intensity of bacteria growth.

Test samples	**Dilution of bacterial suspension**	**Time after contamination (hours)**			
		after suction	**2hrs.**	**5hrs.**	**168hrs.**
Untreated		**Swab-method**			
Aged oak	10^{-2}	++(+)	-	-	-
Aged plastic	10^{-3}	no	+	(+)	-
new oak	10^{-2}	++(+)	-	-	-
new plastic	10^{-3}	no	+++	+++(+)	-
Oil treated		**Swab-method**			
aged oak	10^{-2}	(-)	-	-	-
aged plastic	10^{-3}	no	+	(+)	-
new oak	10^{-2}	++(+)	-	-	-
new plastic	10^{-3}	no	+++	+++(+)	-
Untreated		**Contact-method**			
aged oak	10^{-2}	+++(+)	(+)	<(+)	-
aged plastic	10^{-3}	no	++++++	++++++	-
new oak	10^{-2}	++++(+)	(+)	<(+)	-
new plastic	10^{-3}	no	++++++	++++(+)	-
Oil treated		**Contact-method**			
aged oak	10^{-2}	++++++	++++(+)	++(+)	-
aged plastic	10^{-3}	no	++++++	++++++	-

new oak	10^{-2}	++++++	+++++	+++(+)	-
new plastic	10^{-3}	no	++++++	++++(+)	-

4.3.3 Discussion

Table 5 shows the results of contamination with *Bacillus subtilis.* In the series of untreated oak tested with the swab-method, there are almost no differences between aged and new oak. New oil treated oak samples perform slightly better than aged samples. Using the contact-method the decrease is slightly faster for aged untreated oak than for new oak but opposite trend is seen for oil treated samples.

On plastic, there is a higher survival of bacteria, both on aged and new samples using both testmethods. There were no big differences between new and aged plastic. We have to be aware, that in the case of plastic we used the dilution 10^{-3}, because of very intensive bacteria growth. In the case of oak samples we worked with dilution 10^{-2}.

Table 6 show the results of contamination of new and aged wood and plastic samples by *Pseudomonas fluorescens*. By swab method we can see a fast decrease of bacteria within one hour, except for plastic where there is no suction. New plastic is having worse results than aged ones. It can possibly be explained by the fact that a certain amount of bacteria hiding in scratches could not be detected. The wet cotton-wool could not catch all bacteria, only those on the surface.

The contact method detects that the bacterial decrease is happening faster on the surface of untreated oak samples. And again, plastic is showing to have the best conditions for bacteria survival. After 7 days, there are no bacteria found on the surface.

As in test 1 and 2, the treatment with linseed oil did not show remarkable antibacterial protection to wooden samples.

4.3.4 Conclusion

The survival of both bacteria is slightly less on oil treated aged oak compared to new samples shown by the contact method. A slightly better survival of Bacillus on untreated new oak samples is shown by the contact method.

Oil treatment cannot be recommended as an antibacterial treatment. There is a tendency to see better survival of bacteria on oil treated samples than on untreated wood.

For plastic aged samples show better results using the contact-method and worse results using the swab-method. The explanation could be that bacteria hiding in the scratches are easier to catch with the swab. The same tendency is seen on oak samples.

4.4 Efficiency of cleaning programme

4.4.1 Materials and methods

To investigate the efficiency of ordinary kitchen dish washing, warm water mixed with a common detergent without special antibacterial additives was used. New and aged samples were washed after contamination a certain number of times with a kitchen sponge, which had showed to be the most effective. Then the survival of bacteria on the surface on wooden samples was tested by the swab method and the contact method.

Untreated and oil treated oak, beech and plastic was tested. Series of aged and new samples of each kind were tested. The samples were contaminated with *Bacillus subtilis* re-suspended in 1 % of meat extract liquid. The procedure for cultivation described in 2.3 is followed.

After contamination of samples the bacterial suspension was allowed to suck into the samples from the surface. Then each sample was washed individually in 1 litre warm water, containing 1 ml of Neutral detergent. A sponge was used to wash the surface of a sample (single forward and backward movement = 1 time). After washing the samples were left to dry and then the test started immediately after drying, then after 2 hours, 5 hours and 7 days from washing. Before washing a sample was taken from the surface to prove the efficiency of the cleaning. Only the surfaces were tested using the swab and the contact method. Petri dishes were incubated at 37°C 24-48 hours.

A pilot project aiming at finding the most effective number of times of washing the surface by a sponge was performed.

A sponge was dipped into the detergent water solution after every single cleaning to avoid contamination. Washing the samples by different number of times: 2 times, 4 times and 6 times, was tested. Results are given in figure 1, which shows, that washing the contaminated samples 4 times is the most effective way. The whole cleaning programme was then carried out by “4 times washing”.

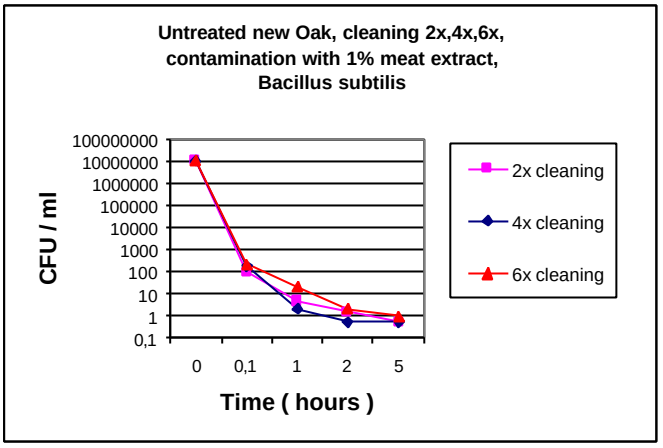


Figure 1. Results of preliminary testing of 3 different cleaning procedures.

4.4.2 Results

Detailed results are gien in annex 2. A summary of some results is given in table 712.

Table 7. Growth of *Bacillus subtilis* measured by the swab method. Comparison of untreated/oil treated wood and plastic, aged and new before and after cleaning. Contamination with *Bacillus subtilis* re-suspended in 1% of meat extract liquid. Number of + indicates the amount of bacteria.

Test samples	Dilution of bacterial suspension	Time after contamination (hours)				
		after suction before cleaning	after suction after cleaning	2hrs.	5hrs.	168hrs.
Untreated						
Aged oak	10^{-2}	< (+)	-	-	-	-
Aged beech	10^{-2}	(+)	-	-	-	-
Aged plastic	10^{-3}	++++	-	-	-	-
New oak	10^{-2}	(+)	-	-	-	-
New beech	10^{-2}	<(+)	-	-	-	-
New plastic	10^{-3}	++(+)	-	-	-	-
Oil treated						
Aged oak	10^{-2}	++(+)	-	-	-	-
Aged beech	10^{-2}	+(+)	-	-	-	-
Aged plastic	10^{-3}	++++	-	-	-	-

New oak	10^{-2}	< (+)	-	-	-	-
New beech	10^{-2}	<(+)	-	-	-	-
New plastic	10^{-3}	++(+)	-	-	-	-

Table 8. Growth of *Bacillus subtilis* measured by the contact method. Comparison of aged and new untreated/oiltreated wood and plastic before and after cleaning. Contamination with *Bacillus subtilis* re- suspended in 1% of meat extract liquid. Number of + indicates the amount of bacteria.

Test samples	**Dilution of bacterial suspension**	**Time after contamination (hours)**				
		after suction before cleaning	**after suction after cleaning**	**2hrs.**	**5hrs.**	**168hrs.**
Untreated						
Aged oak	10^{-2}	++++	-	-	-	-
Aged beech	10^{-2}	++++	-	-	-	-
Aged plastic	10^{-3}	++++++	<(+)	<<(+)	-	-
New oak	10^{-2}	++++	-	-	-	-
New beech	10^{-2}	++++	<<(+)	<<(+)	-	-
New plastic	10^{-3}	++++++	-	<<(+)	-	<<(+)
Oil treated						
Aged oak	10^{-2}	++++	-	-	-	-
Aged beech	10^{-2}	++++	-	<<(+)	-	-
Aged plastic	10^{-3}	++++++	<(+)	<<(+)	-	-
New oak	10^{-2}	++++	-	-	-	-
New beech	10^{-2}	++++	-	<<(+)	-	-
New plastic	10^{-3}	++++++	-	<<(+)	-	<<(+)

4.4.3 Discussion

The cleaning has had a big influence on bacterial survival. It seems to be enough to wash every sample 4 times with a sponge. A concentration of 1 ml *Neutral* detergent/liter warm water was sufficient, to give satisfactory results. Table 7 shows, that already after washing, any amount of bacteria higher than 100 CFU/ml could not be identified. Only by the

contact method (table 8), results showed the presence of few single colonies on the surface of especially plastic samples after cleaning. Bacteria are dying on the surface of samples because of drying and insufficient water activity. Cleaning is increasing the efficiency of bacterial removal.

4.4.4 Conclusion

Cleaning after contamination of plastic and wood with normal detergant without antibacterial additives is effective. Woodsamples perform slightly better than plastic and oak slightly better than beech.

5 Verification of laboratory results

5.1 Tests at the Danish Veterinary and Food Administration – Regional Laboratory Northeast Zealand.

To check some of the results obtained by Biotechnology, Danish Technological Institute during the project, the Danish Veterinary and Food Administration – Regional Laboratory Northeast Zealand, DVF, was asked to carry out experiments, where the already obtained results were more variable than expected.

5.1.1 Materials and methods

Four experiments were repeated using the same methods, substrates and conditions as in DTI-tests. The following experiments had been repeated:

1. Oil treated oak, aged and new, contaminated with *Bacillus subtilis* re-suspended in 1% of meat extract

2. Oil treated oak, aged and new, contaminated with *Pseudomonas fluorescens* re-suspended in 1% of meat extract

3. Plastic, aged and new, contaminated with *Pseudomonas fluorescens* re-suspended in 1% of meat extract

4. Spruce and pine, ordinary moisture (7-10%) contaminated with *Pseudomonas fluorescens* in TSB medium.

5.1.2 Results

Detailed results are given in anex 2. A summary of some results are given in table 9-12.

Table 9. The growth of *Bacillus subtilis* measured by the swab and contact methods on oil treated, aged and new oak, carried out by DVF and DTI. Contamination with 1% of meat extract liquid. Number of + indicates the amount of bacteria.

Lab./ Method	Test samples	Dilution of bacterial suspension	Time after contamination (hours)			
			after suction	2hrs.	5hrs.	168hrs.
DVF/	aged oak	10^{-2}	<<(+)	<<(+)	-	-
Swab	new oak	10^{-2}	<(+)	<(+)	<<(+)	-
DTI/	aged oak	10^{-2}	(+)	<(+)	-	-
Swab	new oak	10^{-2}	++(+)	-	-	-
DVF/	aged oak	10^{-2}	++++	++++	-	-
Contact	new oak	10^{-2}	++++++	++++(+)	++++++	-
DTI/	aged oak	10^{-2}	++++++	+++++(+)	++++(+)	-
Contact	new oak	10^{-2}	++++++	++++++	+++(+)	-

Table 10. The growth of *Pseudomonas fluorescens* measured by the swab and contact methods on oil treated, aged and new oak, carried out by DVF and DTI. Contamination with1% of meat extract liquid. Number of + indicates the amount of bacteria.

Lab./ method	Testsamples	Dilution of bacterial suspension	Time after contamination (hours)			
			after suction	2hrs.	5hrs.	168hrs.
DVF/	aged oak	10^{-2}	(-)	-	-	-
Swab	new oak	10^{-2}	+(+)	<<(+)	-	<<(+)
DTI/ swab	aged oak	10^{-2}	(-)	-	-	-
	new oak	10^{-2}	++(+)	-	-	-
DVF/	aged oak	10^{-2}	+++(+)	+++++	+(+)	-
Contact	new oak	10^{-2}	+++(+)	+++(+)	++(+)	-
DTI/	aged oak	10^{-2}	++++++	++++(+)	++(+)	-
contact	new oak	10^{-2}	++++++	++++	+++(+)	-

Table 11. The growth of *Pseudomonas fluorescens* measured by the swab and the contact methods on aged and new plastic, carried out by DVF and DTI. Contamination with1% of meat extract liquid. Number of + indicates the amount of bacteria.

Lab./ method	Test samples	Dilution of bacterial suspension	Time after contamination (hours)			
			after suction	2hrs.	5hrs.	168hrs.
DVF/ swab	aged plastic	10^{-3}	no	+(+)	(+)	-
	new plastic	10^{-3}	no	++++(+)	(+)	-
DTI/ swab	aged plastic	10^{-3}	no	+	(+)	-
	new plastic	10^{-3}	no	+++	+++(+)	-
DVF/ contact	aged plastic	10^{-3}	no	++++++	++++++	-
	new plastic	10^{-3}	no	++++++	++++++	+++
DTI/ contact	aged plastic	10^{-3}	no	++++++	++++++	-
	new plastic	10^{-3}	no	++++++	++++(+)	-

Table 12. The growth of *Pseudomonas fluorescens* measured by the swab and the contact methods on dry pine and spruce with 7-10% moisture, carried out by DVF and DTI. Contamination with1% of meat extract liquid. Number of + indicates the amount of bacteria.

Lab./ method	Test samples	Dilution of bacterial suspension	Time after contamination (hours)			
			after suction	2hrs.	5hrs.	168hrs.
DVF/ swab	spruce	10^{-2}	++++++	<(+)	<(+)	-
	pine	10^{-2}	++++++	<(+)	-	-
DTI/ swab	spruce	10^{-2}	++++++	(+)	+	<(+)
	pine	10^{-2}	++++++	(+)	++(+)	-
DVF/ contact	spruce	10^{-2}	++++++	++++++	(+)	-
	pine	10^{-2}	++++++	++++++	(+)	-
DTI/ contact	spruce	10^{-2}	++++++	+++++	++++	-
	pine	10^{-2}	++++++	++++++	++(+)	-

5.1.3 Discussion

Table 9 – 12 shows the comparison of results obtained by DTI and by DVF.

In table 9 the start concentration of *B. subtilis* was in both cases about 10^7 CFU/ml. The described intensity of growth detected by the swab method corresponds to dilution 10^{-2}. There is a high correlation of results of aged oak and less correlation of results of new oak. In the DTI experiment, the amount of bacteria on the surface of new oak was decreasing rapidly and could not be detected already after suction, while in in DVF experiment few single colonies were still appearing on the surface of samples after 2 hours and 5 hours from contamination.

Results from using the contact method are similar, with slight differences in the interval of 5 hours after contamination. No bacteria were detected after seven days from sample contamination.

The correlation of results detected by the swab and by the contact method on samples contaminated with *Pseudomonas fluorescens* was very high, table 10. DVF detected a few single colonies on the surface of new oak using the swab method. Results from using the contact method didn't show any remarkable difference between the 2 laboratories.

The results from using the swab method to collect *Pseudomonas fluorescens* on plastic, table 11, show, that aged plastic is actually better than new. Whereas an aged surface is giving the bacteria opportunity to "hide" and not to be detected by the swab method, the smooth surface doesn't give any advantages. All bacteria can be removed from the surface by the swab method and also detected by the contact method. Sometimes it was difficult to spread a whole drop of bacterial suspension evenly on the surface of plastic resulting in one spot with high concentration of bacteria. *P. fluorescens* is a bacterium of slimy consistence. The slime can work as a protection and the bacteria can survive for a long period. This could be the reason that bacteria on the smooth surface are detected even after 7 days in the DVF test. There is a good correlation in results from the two laboratories.

The DVF results obtained by the swab method confirm, that pine is better than spruce, even though in DTI-tests the bacterial decrease was a little bit slower. There are some differences in the case of contact method. In one experiment pine is better than spruce, in the other experiment, no differences were detected. In such a case we have to decide, which method is more precise. The amount of bacteria detected by the contact method was over 300 bacteria/ plate in first 2 intervals, then the number started to decrease. In the case of swab method the amount of bacteria started to decrease already after suction. According to detection limits, the swab method is a more precise method in this case.

5.1.4 Conclusion

Selected tests have been repeated at DVF in order to verify the results obtained at DTI. It can be concluded that there is a very good correlation between the results from the two laboratories and the conclusions drawn from the testing is therefore the same.

6 Summarised discussion and conclusion

From the laboratory tests that have been carried out at the Danish Technological Institute centre for Biotechnology and at the Danish Veterinary and Food Administration – regional laboratory Northeast Zealand it can be concluded that there is a big difference in survival of the test bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* on different wood species and plastic and steel.

- Bacterial survival is lower on wood than on plastic and steel.
- Bacterial survival is lower on planed oak compared to beech and ash.
- For pallets sawn pine is performing better than spruce both at high and low moisture content and with both test bacteria.
- Oil treatment with linseed oil does not improve the wooden product with regards to hygienic properties maybe rather opposite. But oil treatment might improve the appearance of the finish.
- Artificial ageing does not change the overall conclusions on the hygienic properties of wood, plastic and steel.
- Normal dish washing exemplified by the use of a common detergent wihout antibacterial additives and 4 times washing with a sponge proved to be an adequate cleaning procedure for wood items as well as for plastic under the given testconditions. Wood was slightly easier to clean than plastic.
- There is a good correlation between the work carried out at the two different laboratories DVF and DTI.

7 Co-ordination with other R&D projects

7.1 Braunschweig

June 13th 2001 the project group participated in a workshop in Braunschweig. Apart from members from the Nordic project the workshop also included participants from Germany, who presented their projects and results. The agenda for the workshop is shown in annex 4.

The goal of the workshop was to present the various projects and to discuss results and needs for future investigations. (kek skriv et par kloge ord).

7.2 C.E.I.-Bois

During the project members of the project group have participated in meetings of the C.E.I.-Bois (European Confederation of Woodworking Industries), thereby ensuring that the results from the project were communicated within the european union. At the same time information regarding other european R&D inititiatives were collected.

7.3 Microwave technology

DICAM A/S is a Danish company that among other things has specialised in producing microwave ovens for sterilisation of used egg trays. During the project this company was invited to meetings and a group from the project visited the company.

The use of heat treatment for disenfection is an old known widely used technique. The use of microwave technique is also known from e.g. wooden building structures, where dry rot can be fought with portable microwave equipment. Where normal warm air heats from the outside, microwaves heat the material from the inside (Koch, A. P., 2001).

DICAM A/S was contacted, because the project group were interested in how the technology and equipment performed and seeing if the technology could be used to ”clean” wood.

At DICAM A/S 11 test specimens of wood were treated in the microwave oven in order to test the potential of the technology and equipment.

6 of these test specimens were directly connected to this project. The 5 remaining test specimens were from a wooden construction, which had been infected with fungus and blue rot. These extra test specimens were chosen as the results could be used to draw conclusion regarding wooden pallets.

The test specimens were treated in an older pilot oven. It was not possible to control temperature or time precisely, why some of the test specimens were burnt on the surface.

The test results are shown in the following tables.

Table 13. Microwave treated wood infected in a building by mouldfungi.

Test specimen no.	Fungus before treatment		Fungus after treatment	
1	3	Alternaria sp.	1	Arthrinium phaeospermum
	1	Aspergillus niger	1	Drechslera sp.
	>100	Penicillium sp.	37	Penicillium sp.
			3	Triocosporon pullullans
2	2	Alternaria sp.	1	Penicillium sp.
	>50	Yeast		
	29	Penicillium sp.		
3	5	Acremonium sp.	2	Chaetomium sp.
	1	Aspergillus niger	2	Sordaria sp.
	1	Aspergillus ustus	18	Penicillium sp.
	9	Penicillium sp.		
	3	Trichoderma viride/harzianum		
	3	Ulocladium sp.		
4	>50	Acremonium sp.	5	Aureobasidium pullulans
	5	Chaetomium sp.	1	Epicocum sp.
	18	Yeast	10	Yeast
	>30	Penicillium sp.	25	Penicillium sp.
			6	Triocosporon pullullans
5	2	Alternaria sp.	10	Acremonium sp.
	1	Aspergillus versicolor	>50	Penicillium sp.
	1	Aspergillus niger		
	5	Paecilomyces farinosus		
	>100	Penicillium sp.		

Table 14. Microwave treated wood, infected with a monoculture of *Bacillus cereus.*

Test specimen no.	CFU before treatment	CFU after treatment
1 Beech	29	3*
2 Beech	8	31*
3 Beech	5	6
1 pine	>300	3*
2 pine	>300	0
3 pine	>300	3*

* infected with coccus

Based on the experiments it is hardly possible to draw any specific conclusions. Following statements should be noted:

- The company has a modern oven that rotates the objects in order to treat all the surfaces
- As with any other kind of heat treatment, microwave technique can kill organisms if time and temperature are optimised.
- These makeshift experiments have shown a considerable reduction in the overall germ count
- The results confirm that a verifiable experiment in a modern oven should be carried out.
- The technique is assessed as having a solid potential for the use of reducing the germ count of pallets whereby increasing the usability and life span of wooden pallets n the food industry.
- The technique is also assessed as having a future potential for the use in the food industry for disinfection of other wooden products.

8 Communication of test results

An internal success factor throughout the project has been the ability to attract public awareness. Therefore a great deal of resources have been allocated to.

- Writing articles
- Giving interviews
- Supplying material for external information sources
- The final conference

In this chapter a brief overview of the results of this work is resented.

8.1 Articles and interviews

The project has delivered information to the following articles:

Mad og Sundhed (Internet information source)

Jyllands-Posten (News paper), 2001-11-28

Levnedsmiddel Blader (Technical periodical) 11, 2001

Ingeniøren (Technical periodical), 2001-11-29

Regional Avisen Lørdag (Regional newspaper), 2002-01-05

Århus Stifttidende (Local news paper), 2002-01-12

Fyns Amts Avis (Regional newspaper), 2002-01-02

Nordjyske Stift, Søndag (Regional newspaper), 2002-01-13

Politiken (National newspaper), 2001-11-29

Træ og Industri (Technical periodical)

Scandinavian Food and Drink (Technical periodical) 2002-01-25

Plus Process (Technical periodical) 3 2002

Kalundborg Folkeblad (Local news paper) 2002-02-23

Kolding Ugeavis (Local news paper) 2002-04-07

Midtsjællands Folkeblad (Regional newspaper) 2002-04-04

Midtjyllands Avis (Regional newspaper) 2002-04-09

Træ & Industri (Technical periodical) 2002-03-05

Århus Stiftstidende (Regional newspaper) 09-04-02

Berlingske Tidende (National newspaper) 08-04-2002 (Ritzau)

Politiken (National newspaper) 08-04-2002

Træ er miljø (www.trae.net) Internet information soruce), 2000-01-30, 2000-01-12, 2001-03-11, 2001-11-20, 2001-11-24, 2002-02-14

Furthermore Træ er Miljø has initiated a campaign to increase the use of wood in the food industry.

Apart from these Danish references the project has also been described in various journals in the other participating Nordic countries. These references have not been monitored from Denmark and are therefore not brought here.

9 Conclusions and discussion regarding further actions

The aim of the project was to test the survival of selected bacteria, commonly found in the meat industry, on different wood species, plastic and stainless steel. The project included 4 parts.

In the first part, the survival of the Gram-positive *Bacillus subtilis* was tested on different wood species, plastic and steel. It can be concluded, that oak showed the best results in elimination of bacteria on the surface. A remarkably big difference between wooden samples and plastic and steel in the amount of bacteria survival on the surface of the samples was observed.

The second part involved the testing of the same wooden species, plastic and steel. The gram- negative bacterium, *Pseudomonas fluorescens*, was used to contaminate test samples. Oak was again showing the highest rate in bacterial decrease. There is not a big difference in bacterial survival between oil treated wooden samples and untreated wooden samples contaminated neither by *Bacillus subtilis*, nor by *Pseudomonas fluorescens*. The explanation could be that linseed oil did not really work as protection, but rather as nutrition. In the experiments with pine and spruce contaminated with both mentioned bacteria, pine is performing better than spruce both at low and high moisture content.

In the third part of the project the focus was to compare bacteria survival on new and artificially aged wood. New oil treated and untreated oak and plastic was used as reference material. Samples were contaminated with bacteria in 1% meat extract. The results showed

only slight differences between new and artificially aged samples. But plastic was again showing best conditions for bacterial survival.

In the last part the efficiency of a cleaning programme, simulating the ordinary dish washing in the kitchen was tested. The cleaning did have a big influence on bacterial survival by using detergent even without antibacterial additives. The cleaning programme gave satisfactory results. We did not detect any amount of bacteria higher than 100 CFU/ ml already after washing. Cleaning increased the speed of removal of bacteria.

In the final part of the project the Danish Veterinary and Food Administration – Regional Laboratory Northeast Zealand carried out a minor test programme for verification of results obtained at DTI. They repeated some of the tests using the same methods as DTI. The results showed a high correlation between the results obtained by the two laboratories.

10 References

Koch, A. P., 2001	Koch, A. P.: ”Mikrobehandling af Træ”, Notat, Teknologisk Institut, Bioteknik, 2001

Annex 1

Danish Technological Institute, Wood Technology 2002.01.04 ebl

Apparatus for manual cutting/scratching of wood, plastic and metal samples

Description of apparatus

Knife blade: Scalpel blade Swann-Morton Product no. 3031 BS2982 ISO 7740 Small Fitting

Actual load on knife:1650 g

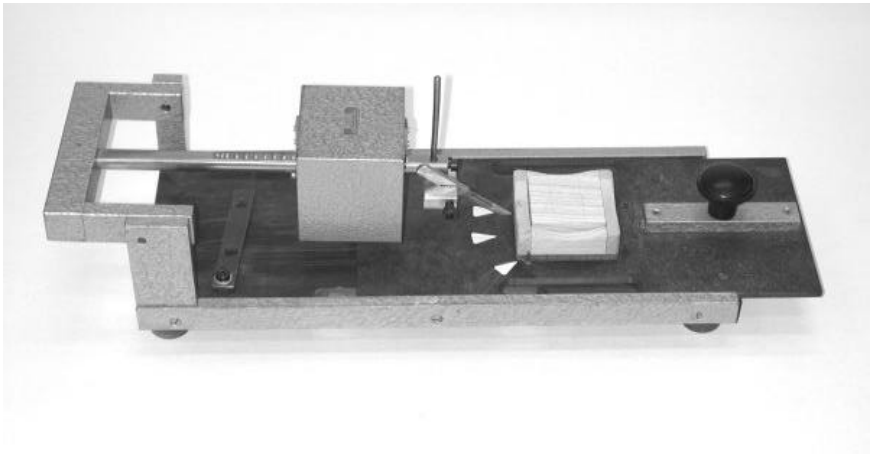
Length: 82 mm.

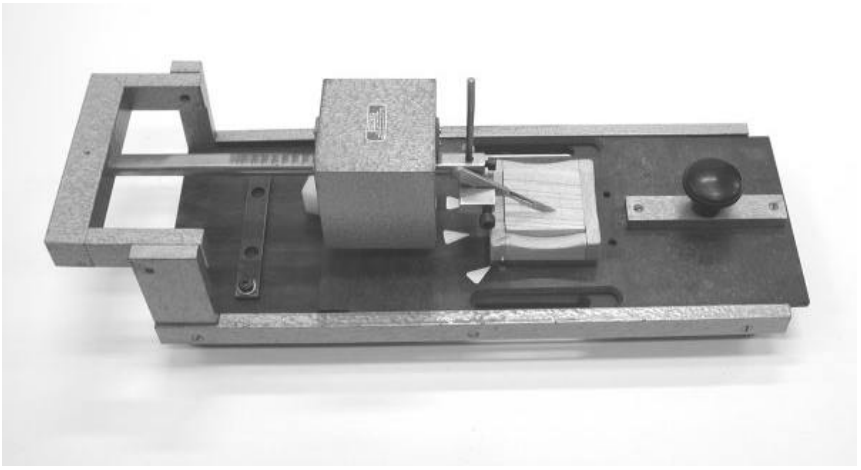
Angel: ca. 30°

Number of samples: 50 x 50 mm

Number of cut per sample: 12 stk.

Number of scratch per knife blade in wood and plastic: 600 = 50 samples





Apparatus for manual impact loading of wood, plastic and metal samples

Description of apparatus

Load, dimension: Ø 60 mm, height 136 mm.

Load, weight: 3000g

Actual load height: 45 mm.

Size of ball: Ø 4,67 mm

Number of balls: 5 stk. (fra bundleje i kontorstol)

Distance between balls: 9,0 mm

Balls inside circle: Ø 16,0 mm

Inside diameter of guide tube: Ø 60 mm


FREM

Annex 2

1. Experiment with Bacillus subtilis

Untreated Ash, Beech, Oak, Pine and Spruce; ordinary moisture (7-10%), (inoculum from TSB)

ASH

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus subtilis	*8,70%* (10,8,7,11, 8,8)	*44,4min* (45,52,40, 40,45)	10(-5)= 151	10(-2)	0, 0, 0	0, 0, 0	0, 17, 17	0, 1, 0	0, 5, 5	0, 0, 0	0, 0, 16	0, 0, 0	3, 1, 0	0, 0, 0	0, 0, 0
			10(-6)= 30												
			10(-7)=0	10(-3)	0, 0, 0		0, 4, 3		0, 1, 1		0, 0, 2		2, 0, 0		

BEECH

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus subtilis	*9,30%* (10,9,10,8, 9,10)	*41,1min* (40,52,30, 40,45)	10(-5)= 151	10(-2)	0, 0, 0	0, 0, 0	tntc, 67, 269	1, 0, 0	0, 0, 0	1, 0, 0	1, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-6)= 30												
			10(-7)=0	10(-3)	0, 0, 0		289, 6, 14		0, 0, 0		0, 0, 0		0, 0, 0		

OAK

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus subtilis	*11,20%* (12,11,11,11, 10,12)	*56min* (55,70,45, 55,55)	10(-5)= 151	10(-2)	0, 0, 0	0, 0, 0	1, 2, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-6)= 30												
			10(-7)=0	10(-3)	0, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		

SPRUCE

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples			After 0 hours			After 2 hours			After 5 hours			After 7 days		
					Swab test	Contact metho	Contact metho	Swab test	Contact method		Swab test	Contact method		Swab test	Contact	Contact method	Swab test	Contact method	
					surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	urface	depth
Bacillus subtilis	*12,30%* (13,13,13,13, 11,11,12)	*39,4min* (60,50,40,40, 48,13,30)		10(-2)	0, 0, 0	- - -	0, 0, 0	tntc, 495,tntc	tntc,91, 300-400	0, 136, 13	2, 0, 0	130, 300-400, 42	0, 1, 1	0, 0, 0	165, 53, 22	0, 0, 0	0, 0, 0	1, 1, 1	0, 0, 0
			10(-6)=23																
			10(-7)=4	10(-3)	0, 0, 0			490, 53, tntc			0, 0, 0			0, 0, 0			0, 0, 0		

PINE

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples			After 0 hours			After 2 hours			urs	After 7 days				
					Swab test	Contact metho	Contact metho	Swab test	Contact method		Swab test	Contact method		Swab test	Contact	Contact method	Swab test	Contact method	
					surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	urface	depth
Bacillus subtilis	*12,30%* (12,12,12,12, 12,13,13,12,5)	*20,6min* (16,27,27,22 15,12,25)		10(-2)	0, 0, 0	- - -	0, 0, 0	10, 39, 1	6, 16, 59	0, 0, 0	0, 0, 0	11, 16, 1	1, 0, 0	0, 0, 0	1, 0, 0	0, 0, 0	0, 0, 0	1, 1, 0	0, 1, 0
			10(-6)=23																
			10(-7)=4	10(-3)	0, 0, 0			2, 2, 0			0, 0, 0			0, 0, 0			0, 0, 0		

Annex 2

Untreated ASH, BEECH and OAK, contaminated with 1 % meat extract, Bacillus subtilis

CFU 1 TSB medium 10(-6)= 36 colonies
10(-7)= 0 col.

CFU 2 (1% meat extract) 10(-6)= 27 colonies
10(-7)= 1 col.

ASH

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus	10,10%	61,3min	10)-6)=27	10(-2)	0, 0, 0	0, 0, 0	79,tntc,tntc	1, 0, 0	0, 9, 9	0, 0, 0	0, 2, 0	0, 0, 1	0, 0, 0	0, 0, 0	0, 0, 0
	(10,5;11,11,	(65,65,55,60)													
subtilis	10,8)		10(-7)= 1	10(-3)	0, 0, 0		6,147, 712		0, 4, 0		0, 0, 0		0, 0, 0		

BEECH

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus	11,75%	46min	10)-6)=27	10(-2)	0, 0, 0	0, 0, 0	1, 6, 98	0, --, 0	1, 45, 3	0, 2, 1	2, 11, 0	0, 0, 0	0, 0, 0	1, 0, 1	0, 0, 0
	(11,12,12,	(45,40,45,55,45)													
subtilis	13,11,11,5)		10(-7)= 1	10(-3)	0, 0, 0		1, 0, 10		0, 2, 0		0, 2, 0		1, 0, 0		

OAK

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus	12%	51min	10)-6)=27	10(-2)	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	(11,12,12	(45,50,60,50,50)													
subtilis	13,12)		10(-7)= 1	10(-3)	0, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		

Untreated ASH, BEECH and OAK, contaminated with 0,03 % meat extract, Bacillus subtilis

CFU 1 (TSB) 10(-6) = 19 col.
10(-7) = 3 col.

CFU 2 (0,03% KE) 10(-6) = 49 col.
10(-7) = 2 col.

ASH

					Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
Number of samples					1, 2, 3		4, 5, 6		7, 8, 9		10, 11, 12		13, 14, 15,	16, 17, 18	
	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus			10)-6)=49	10(-2)	0, 0, 0	0,0, 0	tntc,12,258	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0,0,0
	9,50%	48 min													
	(9, 10)	(40,46,51,													
subtilis		48,55)	10(-7)= 2	10(-3)	0, 0, 0		149, 0, 25		0, 0, 0		0, 0, 0		0, 0, 0		

BEECH

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus			10)-6)=49	10(-2)	0, 0, 0	0, 0	31, 16, 0	0, 1, xx	1, 0, 0	0, 0, 0	0, 0, 0	xx,0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	9%	30,6 min													
		(40,31,26,													
subtilis		23,33)	10(-7)= 2	10(-3)	0, 0, 0		4, 1, 0		0, 0, 0		0, 0, 0		0, 0, 0		

xx-too hard to cut

OAK

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus			10)-6)=49	10(-2)	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0 , 0, 0	0, 0, 0
	11,50%	59,4 min													
		(55,61,61,													
subtilis		57,63)	10(-7)= 2	10(-3)	0, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		

Annex 2

Untreated PINE and SPRUCE, HIGH MOISTURE (19-25%), TSB medium, Bacillus subtilis

SPRUCE

	Træfugtihed (%)	Suction periode (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days	
					Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method
					surface	surface depth	surface	surface depth	surface	surface depth	surface	surface depth	surface	surface depth
Bacillus	**20,43%**	**34,3min**	**10(-6)=32**	**10(-2)**	0, 0, 0	0, 0, 0	1, 17,tntc	tntc 70, 50, 3*	tntc,259,30	tntc 40*, 3, 5	10,29,56	ca180,41,68 0,0,0	0, 0, 0	0,0,0 0,0,0
subtilis	(19,20,19, 20,22,23,20)	(30,47,48,32 11,40,28,38)	**10(-7)=3**	**10(-3)**	0, 0, 0		0, 3, tntc		415,35, 1		2, 2, 10		0, 0, 0	

tntc= 300

Moisture after 7 days
20%,25%, 26%

PINE

	Træfugtihed (%)	Suction periode (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days	
					Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method
					surface	surface depth	surface	surface depth	surface	surface depth	surface	surface depth	surface	surface depth
Bacillus	**20,33%**	**32,1min**	**10(-6)=32col.**	**10(-2)**	0, 0, 0	1, 0, 0 0, 0, 0	64,tntc,81	tntc 25 ,42,30	12,60, 1	32,17, 3 0,0,0	0, 0, 0	0, 0, 0 0, 0, 0	0, 0, 0	0,0,0 0,0,0
subtilis	(18,19,20 20,24,21)	(15,40,52,47 11,40,26,26)	**10(-7)=3col.**	**10(-3)**	0, 0, 0		4, 249, 8		1,15 ,1		0, 0*, 0		0, 0, 1	

Moisture after 7 days
20%, 40%, 20%

Oil treateted Ash, Beech and Oak, ordinary moisture (7-10%) , TSB medium, Bacillus subtilis

(18.9.- 25.9.2001)

ASH

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus		**45,8 min**	**10(-6)- 8**	**10(-2)**	0, 0, 0	0, 0, 0	158,tntc,tntc	2, 1, 30	26,53,26	0 , 5, 0	39,31,21	2, 0, 0	0, 0, 0	0 , 0 , 0	0,0,0
subtilis		(30,50,60,42,47)	**10(-7)- 0**	**10(-3)**	0, 0, 0		14,592,253		1, 4, 0		1, 1, 0		0, 0, 0		

BEECH

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus		**41,2 min**	**10(-6)-8 col.**	**10(-2)**	0, 0, 0	0, 0, 0	tntc,365,tntc	4, 0, 31	12,4, 1	0, 0, 0	2,13,70	0, 0, 0	0, 0, 0	0 , 0 , 0	0,0,0
subtilis		(32,3842,44,50)	**10(-7)- 0col.**	**10(-3)**	0, 0, 0		204,18,180		0 , 0, 0		1,4,7		0, 0, 0		

OAK

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus		**63 min**	**10(-6)-8col.**	**10(-2)**	0, 0, 0	0, 0, 0	12,tntc,tntc	2, 0, 0	1, 2, 0	0 , 0, 1	1, 0, 0	0, 0, 0	0, 0, 0	0 , 0 , 0	0,0,0
subtilis		(60,58,>70)	**10(-7)- 0col.**	**10(-3)**	0, 0, 0		0,4 72, 99		0, 0, 0		0, 0, 0		0, 0, 0		

Annex 2

Oil treated ASH, BEECH and OAK, contaminated with 1 % meat extract, Bacillus subtilis

CFU 1 **10(-6) = 24 colonies**
10(-7) = 2 colonies

CFU 2 **10(-6) = 20 colonies**
10(-7) = 3 colonies

ASH

Number of samples	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples 1, 2, 3		After 0 hours 4, 5, 6		After 2 hours 7, 8, 9		After 5 hours 10, 11, 12		After 7 days 13, 14, 15, 16, 17, 18		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus	***10,90%*** (10,11,105;	**52,3 min** (60,55,47,	**10)-6)=20**	**10(-2)**	0,0, 0	0, 0, 0	80,tntc,16	79, 0, 0	6, 24, 1	17, 86, 0	30, 3, 23	0, 0, 1	0, 0, 0	1 , 0, 0	0, 0, 1
subtilis	11,12,11,105)	47)	**10(-7)= 3**	**10(-3)**	0, 0, 0		36, 49, 2		1, 5, 0		2, 1, 2		0, 0, 0		

BEECH

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus	***11,14%*** (12,11,12,11	**47,3 min** (40,55,47	**10)-6)=20**	**10(-2)**	0, 0, 0	0, 0, 0	13, 72, 62	0, 1, 0	3, 14, 87	0, 0, 0	15, 18, 5	0, 2, 0	0, 0, 0	0, 0, 0	0, 0, 0
subtilis	11,5,10,5,10)	47)	**10(-7)= 3**	**10(-3)**	0, 0, 0		1, 18, 4		0, 1, 11		0, 1, 0		0, 0, 0		

OAK

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus	***12,14%*** (12,5;11,5;11	**69,8 min** (60,55,67	**10)-6)=20**	**10(-2)**	0, 0, 0	0, 0, 0	31, 2, 1	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	1, 0, 0	0, 0, 0
subtilis	3x12,5)	97)	**10(-7)= 3**	**10(-3)**	0, 0, 0		4, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		

Oil treated ASH, BEECH and OAK, contaminated with 0,03 % meat extract, Bacillus subtilis

CFU 1 (TSB) **10(-6) = 37 colonies**
10(-7) = 2 col.

CFU 2 (0,03%) **10(-6) = 35 colonies**
10(-7) = 2 col.

ASH

Number of samples	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					1,2,3		4,5,6		7,8,9		10,11,12		13,14,15	16,17,18	
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus	***11,40%*** (11,12,11,12	**47 min** (67,28,53,46	**10)-6)=35**	**10(-2)**	0, 0, 0	0, 0, 0	51, 1, 12	0, 0 , 0	1, 0, 0	1, 1, 2	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
subtilis	10,5,12)	41)	**10(-7)= 2**	**10(-3)**	0, 0, 0		1, 0, 2		0, 1, 0		0, 0, 0		0, 0, 0		

BEECH

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus	***10,30%*** (11,8,11,8,11	**43 min** (27,43,53,46,	**10)-6)=35**	**10(-2)**	0, 0, 0	0, 0, 0	3, ca15, 15	0, 0, 0	1, 0, 2	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 2, 0	0, 0, 0
subtilis	12,11)	46)	**10(-7)= 2**	**10(-3)**	0, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		

OAK

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours		After 2 hours		After 5 hours		After 7 days		
					Swab test	Contact metho	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	Swab test	Contact method	
					surface	depth	surface	depth	surface	depth	surface	depth	surface	surface	depth
Bacillus	***11,90%*** (12x4, 11,11,	**62,3 min** (62,48,78,61)	**10)-6)=35**	**10(-2)**	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
subtilis	13)		**10(-7)= 2**	**10(-3)**	0, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		0, 0, 0		

Annex 2

Plastic and stainless steel tested with Bacillus subtilis

inoculum- 24t, TSB medium

	After 2 hours		After 5 hours			CFU
Bacillus subtilis	**Swab test surface**		**Contact metho surface**	**Swab test surface**		**Contact method surface**	10(-6) = **17 col.**
	10 (-2)	**10 (-3)**	**10 (-2)**	**10 (-2)**	**10 (-3)**	**10 (-2)**	10 (-7) = **0 col.**
Plastik	47, 62, tntc	4, 8, 528	tntc,tntc,tntc	tntc, 2, 0	179, 0, 0	tntc, tntc, >100	
Rust fri stål	tntc,tntc,tntc	tntc,548, 710	tntc,tntc,tntc	0, 0, 3	0, 1, 0	48, 37, tntc	

Prøv Dilution 10 (-4) and 10 (-5)

Plastic and Steel, contaminated with TSB medium, Bacillus subtilis

	Control samples			After 2 hours			After 5 hours			After 7 days			
Bacillus subtilis	**Swab test surface**		**Contact m. surface**	**Swab test surface**			**Swab test surface**			**Swab test surface**			
	10 (-2)	**10 (-3)**		**10 (-3)**	**10 (-4)**	**10 (-5)**	**10 (-3)**	**10 (-4)**	**10 (-5)**	**10 (-2)**	**10 (-3)**	**10 (-4)**	**10 (-5)**
Plastic	0, 1, 0	0, 0, 0	0, 1, 0	tntc,28 ,tntc	27, 4, 75	2, 1, 12	0, 2, ca 5-10	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	1, 0, 0
Stainless steel	0, 0, 0	0, 0, 0	0, 4, 0	tntc,tntc,tntc	93, 133, 102	15, 10, 6	2, 0, ca. 10	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0

CFU **10(-6) = 6 colonies**
10(-7) = 2 colonies

Plastic and Steel, contaminated with 1% meat extract, Bacillus subtilis

	Control samples				After 2 hours			After 5 hours			After 7 days			
Bacillus subtilis	**Swab test surface**			**Contact m. surface**	**Swab test surface**			**Swab test surface**			**Swab test surface**			
	10 (-3)	**10 (-4)**	**10 (-5)**		**10 (-3)**	**10 (-4)**	**10 (-5)**	**10 (-3)**	**10 (-4)**	**10 (-5)**	**10 (-2)**	**10 (-3)**	**10 (-4)**	**10 (-5)**
Plastic	0, 0, 0	0, 0, 0	0, 0, 0	2, 3, 0	tntc ,tntc, 19	281, 146, 2	19, 18, 0	2, 14, 8	0, 2, 2	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
Stainless steel	1, 0, 0	0, 0, 0	0, 0, 0	1, 0, 0	3xtntc	193, 261, 103	14, 20, 7	259,ca.10,0	50, 4, 2	8,ca.10, 0	0, 2, 0	0, 0, 0	0, 0, 0	0, 0, 0

CFU 1 10(-5) = 87 colonies
10(-6) = 9 col.
10(-7) = 1 col.

CFU 2 10(-5) = 60 col.
10(-6) = 5 col.
10(-7) = 1 col.

Plastic and Steel, contaminated with 0,03% meat extract, Bacillus subtilis

	Control samples			After 2 hours			After 5 hours			After 7 days			
Bacillus subtilis	**Swab test surface**		**Contact metho surface**	**Swab test surface**			**Swab test surface**			**Swab test surface**			
	10 (-2)	**10 (-3)**	**10 (-2)**	**10 (-3)**	**10 (-4)**	**10 (-5)**	**10 (-3)**	**10 (-4)**	**10 (-5)**	**10 (-2)**	**10 (-3)**	**10 (-4)**	**10 (-5)**
Plastic	0, 0, 0	0, 0, 0	0, 0, 0	TNTC	175, 144, 129	7, 10, ca.40	18, tntc, tntc	0,312,tntc	0,41,ca100	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
Stainless steel	0, 2, 0	0, 0, 0	0, 0, 0	TNTC	193, 116, 139	25, ca.24, 12	2, 0, 0	0, ca.13, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0

CFU 10(-5) = 215 col.
10(-6) = 24(?) col.
10(-7) = 0 col.

CFU 2 10(-5) = ca.300 col.
10(-6) = 18 col.
10(-7) = 2 col.

Annex 2

2. Experiment with Pseudomonas fluorescens

Untreated Ash, Beech, Oak , ordinary moisture (inoculum from TSB), Pseudomonas fluorescens

CFU 10(-5) = tntc
10(-6) = 188 colonies
10(-7) = 11 col.

Ash

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**10,20%** (10,10,11, 10,5,9,5)	**36,3min**	**10(-5)=tntc**	**10(-2)**			121,tntc,tntc	TNTC	0, 0, 0	4, 1, 24	TNTC	0, 0, 0	191, 2, 1	tntc,123 52	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-6)=188															
			10(-7)=11	**10(-3)**			8,tntc,145			0, 0, 1			7, 0, 1			0, 0, 0		

tntc - too nummerous to count
Contact method - direct, without dilution

Beech

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	***9,90%*** (9,12,8, 10,10,5)	**32,2min**	**10(-5)=tntc**	**10(-2)**			119,35,tntc	TNTC*	0, 0, x	4, 0, 2	tntc, 257	0, 0, 0	1, 0, 0	tntc,20,128	0, 0, 0	0, **, 0	0, 0, 0	0, 0, 0
			10(-6)=188															
			10(-7)=11	**10(-3)**			18 ,2, 42			0, 1, 0			0, 0, 0			0, 0, 0		

Oak

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	***11,50%*** (12,11,12, 11,11,5)	***57,7min***	**10(-5)=tntc**	**10(-2)**			0, 0, 0	tntc,tntc,45	0, 0, 0	0, 0, 0	52,136, 0	0, 0, 0	0, 0, 0	33, 0 ,9	0, 0, 0	0, 0, **	0, 0 , 0	0, 0 , 0
			10(-6)=188															
			10(-7)=11	**10(-3)**			0, 0, 0			0, 0, 0			0, 0, 0			0, 0, **		

Untreated Ash, Beech, Oak , ordinary moisture, 1% meat extract contamination, Pseudomonas fluorescens

CFU 1 10(-6)= 252 col.
10(-7)= 24 col.

CFU 2 10(-6)= 380 col.
10(-7)= 39 col.

Ash

c0 = 195 * 10 E6

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**9,60%** (10, 10,9 9, 10)%	**39,3min** (34,35,42,39 51,40,33 40)	**10(-6)=380**	**10(-2)**			TNTC	TNTC	0, 0, 4-5	18, 2, 20	TNTC	0, 0, 0	0, 6, 33	tntc,296, 126	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-7)=39	**10(-3)**			210, 115, tntc			3, 0, 2			0, 0, 2			0, 0, 0		

Beech

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**9,80%** (9, 4x10)	**38,5min** (39,31,39, 41,51,35,44 28)	**10(-6)=380**	**10(-2)**			92, tntc, 233	TNTC	0 , 0 , 0	0, 5, 8	TNTC	0, 0, 0	0, 0, 3	48,135,41	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-7)=39	**10(-3)**			13, tntc, 18			0, 0, 0			0, 0, 0			0, 0, 0		

Oak

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**10,90%** (4x11, 10,5)	**45,6min** 40,42,55,42 51,46,46,43)	**10(-6)=380**	**10(-2)**			TNTC	TNTC	21, 0, 0	0, 0, 0	2,tntc,tntc	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0 , 0
			10(-7)=39	**10(-3)**			81, tntc, 47			0, 0, 0			0, 0, 0			0, 0, 0		

TNTC = 3 samples with > 300 colonies

Annex 2

Untreated Ash, Beech, Oak, contaminated with 0,03% meat extract, Pseudomonas fluorescens

Ash

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours			After 2 hours			After 5 hours			After 6 days: After 7 days		
					Swab	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact
					surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth
Pseudomonas fluorescens	**10,40%** (3X10, 2X11)	**41,3min**	**10(-6)=tntc**	**10(-2)**			tntc, 18, ca.250	TNTC	0, 0, 0	0, 0, 1	71, 135, ca.204	0, 0, 0	1, 1, 0	1, 2, 31	0, 0, 0	0, 0, 0	0, 0 , 0	0, 0, 0
			10(-7)=47	**10(-3)**			tntc, 0, 33			0, 0, 0			0, 0, 0			0, 0, 0		

Beech

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours			After 2 hours			After 5 hours			After 7 days		
					Swab	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact
					surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth
Pseudomonas fluorescens	**9,83%** (10,8,11,9 11,10)	**30,5min**	**10(-6)=tntc**	**10(-2)**			ca.207, 29, , 41	TNTC	0, 0, 0	0, 3, 0	TNTC	0, 0, 0	0, 0, 0	169, 65, 54	0, 0, 0	0, 0, 0	0, 0 , 0	0, 0, 0
			10(-7)=47	**10(-3)**			110, 1, 0			0, 0, 0			0, 0, 0			0, 0, 0		

Oak

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours			After 2 hours			After 5 hours			After 7 days		
					Swab	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact
					surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth
Pseudomonas fluorescens	**10,50%** (5x10,11,12)	**56,4min**	**10(-6)=tntc**	**10(-2)**			0, 146, 35	tntc, tntc, , 194	0, 0, 0	0, 0, 0	2, 0, 7	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0 , 0	0, 0, 0
			10(-7)=47	**10(-3)**			0, 16, 3			0, 0, 0			0, 0, 0			0, 0, 0		

Oil treated ASH, BEECH, OAK, Ordinary moisture (7 - 10 %), TSB medium, Pseudomonas fluorescens

Ash

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours			After 2 hours			After 5 hours			After 7 days		
					Swab	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact
					surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth
Pseudomonas fluorescens	**v scheme**	**56,3min**	**10(-6)=227**	**10(-2)**			TNTC	TNTC	0, 0, 0	TNTC	TNTC	0, 0, 2	256, tntc, tntc	TNTC	0, 0, 6	0, 0, 0	0, 0, 0	0, 0, 0
			10(-7)=21	**10(-3)**			289, 2x tntc			147,225,52			12,26,20			- , - , -		

Beech

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours			After 2 hours			After 5 hours			After 7 days		
					Swab	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact
					surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth
Pseudomonas fluorescens		**25,4min**	**10(-6)=227**	**10(-2)**			TNTC	TNTC	2, 0, 1	109, 169, tntc	TNTC	13, 0, 8	7, 14, 14	1, 3, 1	TNTC	0, 0, 0	0 , 0, 0	0, 0, 0
			10(-7)=21	**10(-3)**			tntc, tntc, , 126			10, 11, 46			1, 1, 1			-, -, -		

Oak

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours			After 2 hours			After 5 hours			After 7 days		
					Swab	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact
					surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth
Pseudomonas fluorescens		**62,3min**	**10(-6)=227**	**10(-2)**			tntc, 261, 101	TNTC	0, 0, 0	18, 55, 78	TNTC	0, 0, 0	31, 5, 1	TNTC	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-7)=21col.	**10(-3)**			tntc, 21, 11			0, 8, 9			3, 0, 0			-, -, -		

Annex 2

Oil treated Ash, Beech, Oak, contaminated with 1% meat extract, Pseudomonas fluorescens

Ash — c0 = 15,5 * 10E7

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**9,83%** (11,9,11,9, 10,9)%	**47,8min**	**10(-6)=353**	**10(-2)**			TNTC	TNTC	23, 0, 0	tntc, 202, 297	TNTC	66, 0, 25	79, 46, 146	TNTC	7, 53, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-7)=31	**10(-3)**			tntc, 187, 96			52, 13, 30			5, 4, 9			0, 0, 0		

KIM 1 241col. / 19 colonies
KIM 2 353col. / 31col.

TNTC a > TNTC Beech

Beech

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**10,33%** (11,9,11,11, 10,10)	**26,5min**	**10(-6)=353**	**10(-2)**			tntc,275,283	TNTC	0,1, 1	24, 78, 68	TNTC	1, 4 , 0	4, 5, 0	TNTC	11 , 1, 6	0, 0, 0	0, 0, 0	0, 0, 0
			10(-7)=31	**10(-3)**			tntc, 25, 12			2, 2, 6			0, 1, 0			0, 0, 0		

Oak

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**11,00%** (10,11,11,10, 12,11,12)	**65min**	**10(-6)=353**	**10(-2)**			tntc, 12, 39	TNTC	0, 0, 1	0, 0, 0	tntc, tntc, ca.300	0, 0, 0	0, 0, 0	0, 156, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-7)=31	**10(-3)**			206, 2, 3			0, 0, 0			0, 0, 0			0, 0, 0		

Oil treated ASH, Beech and Oak,contaminated with 0,03% meat extract , P. fluorescens

KIM1 10(-6)= 244 col. 10(-7)= 22 col. KIM2 10(-6)= 464 col. 10(-7)= 46 col.

Ash — c0 = 23 * 10E7

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**9,67%** (4x10,9,9)	**47,3min**	**10(-6)=464**	**10(-2)**			TNTC	TNTC	0, 0, 0	0, 34, 5	tntc, 274 , , tntc	0, 0, 0	0, 0, 0	tntc,161,75	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-7)= 46	**10(-3)**			tntc*, 14, 11			0, 0, 0			0, 0, 0			0, 0, 0		

*- ca.300col.

Beech

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**10,33%** (3x11,10,10, 9)	**33,2min**	**10(-6)=464**	**10(-2)**			8, 100-110, 9	TNTC	0, 2, 0	36, 4, 0	tntc, 174 , tntc	0, 0, 0	0, 0, 0	38 ,107, 59	0, 0, 0	0, 0, 0	0, 0, 0	0 ,0 , 0
			10(-7)= 46	**10(-3)**			0, 1, 0			0, 0, 0			0, 0, 0			0, 0, 0		

Oak

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact depth	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**11,50%** (3x11, 3x12)	**76,5min**	**10(-6)=464**	**10(-2)**			0, 188, 305	tntc,tntc,64	0, 0, 0	0, 0, 0	1, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-7)= 46	**10(-3)**			0, 49, 29			0, 0, 0			0, 0, 0			0, 0, 0		

Annex 2

Pine and Spruce, ordinary moisture 7-10%, contaminated with TSB medium, Pseudomonas fluorescens

SPRUCE — c0 = 13 * 10E7

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples		After 0 hours			After 2 hours			After 5 hours			After 7 days		
							Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	Contact	Swab test	Contact	
							surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth
Pseudomonas fluorescens	**11,50%** (12,5,11,12,5 11,11,11)	**8,71min** (10,20,5,4,9, 5,8)	**10(-5)-tntc**	**10(-2)**			TNTC	TNTC	30, 20, 27	8, 11, 76	tntc,tntc,221	ca70, ca50, 6	0, 4, 3	188,178,257	0, 2, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-6)189															
			10(-7)-13	**10(-3)**			88,tntc,tntc			0, 1, 4			0, 0, 0			0, 0, 0		

PINE

	Træmoisture (%)	Nedsugnings-periode (min)	CFU	Fortyd-ning	Control samples		After 0 hours			After 2 hours			After 5 hours			After 7 days		
							Swab test	Contact test		Swab test	Contact test		Swab test	Contact test		Swab test	Contact test	
							surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth
Pseudomonas fluorescens	**12,70%** (13,13,12,12,5 12,5,13)	**8,57min** (11,8,12,4,17, 5,3)	**10(-5)-tntc**	**10(-2)**			TNTC	TNTC	30, 11, 27	4, 38, 15	TNTC	2, 2, 1	32,tntc,20,	3, 160, ca150	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-6)189															
			10(-7)-13	**10(-3)**			TNTC			1, 2, 3			0, 50, 1			0, 0, 0		

PINE AND SPRUCE, 19-25% high moisture, contaminated with TSB medium, Pseudomonas fluorescens

SPRUCE — c0 = 25 * 10E7

	Tree moisture (%)	Suction period(min)	CFU	Dilution	After 0 hours			After 2 hours			After 5 hours			After 7 days			
					Swab test	Contact test		Swab test	Contact test		Swab test	Co	Contact test		Swab test	Contact test		moisture
					surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth		33, 49, 52%
Pseudomonas fluorescens	**27,30%** (23,27,21, 19,5;35,26,32 35)	**21min** (15,16,30,32, 23,10)	**10(-5)=tntc**	**10(-2)**	TNTC	TNTC	ca.115, 0, 93	211,tntc, tntc	TNTC	22,14,1	tntc,tntc, ca.130		TNTC	2,ca70, 0	0, tntc (1), tntc	tntc,0 , tntc	tntc, 0, ca.30	
			10(-6)=313-350col. 10(-7)=25	**10(-3)**	TNTC			23,tntc,tntc			tntc,tntc, ca.50				0, tntc(2), tntc			

(1)- > 500col., (2) - ca 460 col.

PINE

	Tree moisture (%)	Suction period(min)	CFU	Dilution	After 0 hours			After 2 hours			After 5 hours		After 7 days					
					Swab test	Contact test		Swab test	Contact test		Swab test	Co	Contact test		Swab test	Contact test		moisture
					surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth		18,27,21%
Pseudomonas fluorescens	**21,00%** (21,20,21,22, 21,21,21)	**12,5min** (15,11,8,6,11, 17,23,9)	**10(-5)=tntc**	**10(-2)**	TNTC	TNTC	0,10, 0	tntc,165,0	0, 101, 0	0, 0, 0	0, 0, 0		0, ca 68, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	
			10(-6)=313-350col.															
			10(-7)=25	**10(-3)**	TNTC (>300)			tntc,9, 0			0, 0, 0				0, 0, 0			

Repeated high moisture

SPRUCE — c0 = 19 * 10E7

	Tree moisture (%)	Suction period(min)	CFU	Dilution	After 0 hours			After 2 hours			After 5 hours			After 7 days			
					Swab test	Contact test		Swab test	Contact test		Swab test	Contact test		Swab test	Contact test		moisture
					surface	surface	depth	surface	surface	depth	surface	surface	depth	surface	surface	depth	
Pseudomonas fluorescens	**22,60%** (26,23,28,29, 28,29,19,21, 18,19,17,20)	**17,2min** (11,29,28,8, 10,17,)	**10(-6)=189**	**10(-2)**	TNTC	TNTC	42, tntc, 26	tntc,89, 102	TNTC	2, 0,0	0, 0, 151	30 , 3, >40-80	8 , 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	22,23,22, 27,22,23,22%
			10(-7)=19	**10(-3)**	TNTC			68, 9, 11			0, 0, 20			0, 0, 0			

Annex 2

Plastic and Steel, TSB medium, Pseudomonas fluorescens

CFU **10(-6)= 117 col.**
10(-7)= 10 col.

c0 = 5 * 10 E7

	Control samples			After 2 hours				After 5 hours				After 7 days		
	Swab test surface		Contact surface	Swab test surface			Contact test surface	Swab test surface			Contact test surface	Swab test surface		Contact surface
Pseudomonas fluorescens	**10 (-2)**	**10 (-3)**	**direkt**	**10 (-3)**	**10 (-4)**	**10 (-5)**		**10 (-3)**	**10 (-4)**	**10 (-5)**		**10 (-2)**	**10 (-3)**	
Plastic				tntc, tntc,	72, tntc,	5, 25, 0	TNTC	111, 41, 251	11, 2, 18	1, 0, 0	TNTC	0, 0, 0	0, 0, 0	35, 13, 51
				21	4									
									ca.11, ca.27,					
				85,tntc,	18, tntc*,									
Stainless steel				tntc	tntc	4, 55, 148	TNTC	27, tntc, tntc	222	0, 23, 8	TNTC	0, 0, 0	0, 0, 0	75, 23, 60

*- ca 400col.

TNTCplastic > TNTCsteel

Plastic and Steel, 1% meat extract, Pseudomonas fluorescens

CFU 10(-6) = 294 col. **CFU 2** **10(-6) = TNTC (ca 300 col.)**
10(-7) = ca. 100 col. **10(-7) = 52 col.**

c0 = 26 * 10E7

	Control samples			After 2 hours				After 5 hours				After 7 days		
	Swab test surface		Contact test surface	Swab test surface			Contact test surface	Swab test surface			Contact test surface	Swab test surface		Contact surface
Pseudomonas fluorescens	**10 (-2)**	**10 (-3)**	**direkt**	**10 (-3)**	**10 (-4)**	**10 (-5)**		**10 (-3)**	**10 (-4)**	**10 (-5)**		**10 (-2)**	**10 (-3)**	
Plastic				TNTC	TNTC	TNTC	TNTC	TNTC	259,258,276	42, 20, 15	TNTC	0, 0, 0	0, 0, 0	18, 1, 16
											*			
Stainless steel				TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	100,41,145	TNTC	147, 167, 3,	17, 13, 0	33, 44, 95

*- TNTC plastic > TNTC steel

Plastic and Steel, 0,03% meat extract, Pseudomonas fluorescens

c0 = 8 * 10E7

	Control samples			After 2 hours				After 5 hours				After 7 days		
	Swab test surface		Contact surface	Swab test surface			Contact surface	Swab test surface			Contact surface	Swab test surface		Contact surface
Pseudomonas fluorescens	**10 (-2)**	**10 (-3)**	**direkt**	**10 (-3)**	**10 (-4)**	**10 (-5)**		**10 (-3)**	**10 (-4)**	**10 (-5)**		**10 (-2)**	**10 (-3)**	
Plastic				TNTC	TNTC	129, 285	TNTC	tntc, 33,	94, 3, 19	5, 0, 0	TNTC	0, 0, 0	0, 0, 0	0, 0, 0
						159		162						
Stainless steel				TNTC	TNTC	TNTC	TNTC	96, 13, 1	2, 2, 0	1, 1, 0	tntc,tntc, 0	0, 0, 0	0, 0, 0	0, 0, 0

KIM 1 **10(-6)= 303 col.** **KIM 2** **10(-6)= 158 col.** TNTCp = TNTC s
10(-7)= 18 col. **10(-7)= 16 col.**

Annex 2

3. Experiment with new and scratched samples contaminated with 1 % meat extract liquid.

Untreated oak, with and without scars on the surface, contamination with 1% meat extract, Bacillus subtilis

R = With scratched surface

Oak KIM 2

R	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact surface	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Bacillus subtilis	***10,43%*** (4x10,3x11)	***ca. 60min***	**10(-5)=138** **10(-6)=13** **10(-7)= 1kol.**	**10(-2)** **10(-3)**	0, 0 0, 0	0 , 0	28, 306, 127 5, 34, 7	TNTC	0, 0, 0	0, 0, 0 0, 0, 0	271, tntc, 139	0, 0, 0	0, 0, 0 0, 0, 0	163, 35, 139	0, 0, 0	0, 0, 0 0, 0, 0	0 , 0, 0	0, 0, 0

Note ! TNTC - to numerous to count

10(-5)=148	**KIM 1**
10(-6)=18	
10(-7)= 1kol.	

N = without scratches

OAK

N	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact surface	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Bacillus subtilis	***11,43%*** (4x11, 3x12)	**ca 67min**	**10(-5)=138** **10(-6)=13** **10(-7)=1 kol**	**10(-2)** **10(-3)**	0, 0 0, 0	0 , 0	124, 241, 1 14, 56, 0	TNTC	0, 0, 0	0, 0, 0 0, 0, 0	154,tntc,tntc	0, 0, 0	0, 0, 0 0, 0, 0	245, 257, 62	0, 0, 0	0, 0, 0 0, 0, 0	1, 0, 0	0, 0, 0

Untreated oak, with and without scars on the surface, contamination with 1% meat extract Pseudomonas fluorescens

Temperarure = 22 dOAKrees

Oak

R	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact surface	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**10,50%** (4x11,10,9)	**ca.33min**	**10(-5)=tntc** **10(-6)=343** **10(-7)=38kol**	**10(-2)** **10(-3)**	0, 0 0, 0	0, 0	tntc, 0, 15 95, 0, 0	tntc, 110,125	0, 0, 0	0, 0, 0 0, 0, 0	12, 15, 6	0, 0, 0	0, 0, 0 0, 0, 0	1, 1, 0	0, 0, 0	0, 0, 0 0, 0, 0	0, 0, 0	0, 0, 0

KIM 1	**10(-5)=tntc**
	10(-6)= 105
	10(-7)= 16 kol.

OAK

N	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact surface	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**9,40%** (3x9, 2x10)	**ca.40min**	**10(-5)=tntc** **10(-6)=343** **10(-7)=38kol**	**10(-2)** **10(-3)**			tntc,11, 1 207, 1, 0	tntc, 62, tntc,	0, 0, 0	0, 0, 0 0, 0, 0	0, 1, 26	0, 0, 0	0, 0, 0 0, 0, 0	5, 0, 0	0, 0, 0	0, 0, 0 0, 0, 0	0, 0, 0	0, 0, 0

Annex 2

Plastic witch and without scratches on the surface,contamination with 1% meat extract, Bacillus subtilis

	Control samples			After 2 hours				After 5 hours				After 7 days		
	Swab test		Contact	Swab test			Contact	Swab test			Contact	Swab test		Contact
Bacillus subtilis	surface		surface	surface			surface	surface			surface	surface		surface
	10 (-2)	10 (-3)	direkt	10 (-3)	10 (-4)	10 (-5)		10 (-3)	10 (-4)	10 (-5)		10 (-2)	10 (-3)	
Scratched plastic- R	0, 0	0, 0	0, 0	TNTC	281, 289,	37, 30 33	TNTC	4, 6, 3	0, 1, 0	0, 0, 0	TNTC	**	**	0, 1, 0
					286							**	**	
												**	**	
					tntc, tnc,							**	**	
New plastic - N	0, 0	0, 0	1, 0	TNTC	254	35, 46, 33	TNTC*	8, 9, 5	1, 0, 0	0, 0, 1	TNTC	**	**	0, 0, 0

TNTCn > TNTC r

TNTC r > TNTC n

Start concentration

KIM 1 10(-5)= 209 kol.
10(-6)= 22 kol.
10(-7)= 3 kol.

KIM2 10(-5)= 219 kol.
10(-6)= 5 kol.
10(-7)= 2 kol.

Plastic witch and without scratches on the surface, contamination with 1% meat extract, Pseudomonas fluorescens

	Control samples			After 2 hours				After 5 hours				After 7 days		
Pseudomonas fluorescens	Swab test		Contact	Swab test			Contact	Swab test			Contact	Swab test		Contact
	surface		surface	surface			surface	surface			surface	surface		surface
	10 (-2)	10 (-3)	direkt	10 (-3)	10 (-4)	10 (-5)		10 (-3)	10 (-4)	10 (-5)		10 (-2)	10 (-3)	
Scratched plastic - R			0, 0, 0	75, 54	13, 4	1, 1	TNTC	13, 3, 2	0, 0, 0	0, 0, 0	TNTC	**	**	0, 0, 0
												**	**	
												**	**	
												**	**	
New plastic - N			0, 0, 0	tntc, 164	tntc, 14	90, 2	TNTC	190, 11,tntc	8, 5, 50	1, 0, 1	94, 2 x tntc	**	**	0, 0, 0

Start concentration

KIM 1 10(-5)= tntc kol.
10(-6)= 136 kol.
10(-7)= 6 kol.

KIM2 10(-5)= tntc kol.
10(-6)= 231 kol.
10(-7)= 7 kol.

Oil treated OAK, with and without scars on the surface, contaminated with 1% meat extract, Bacillus subtilis

Oak

	Tree	Suction			Control samples		After 0 hours			After 2 hours			After 5 hours			After 7 days		
R	moisture (%)	interval (min)	CFU	Dilution	Swab surface	Contact surface	Swab test surface	Contact surface	Contact depth	Swab test surface	Contact surface	Contact depth	Swab test surface	Contact surface	Contact depth	Swab test surface	Contact surface	Contact depth
Bacillus subtilis	**10,60%** (9,10,11,11 12)	**ca.45min**	**10(-5)= 79**	**10(-2)**			0, 44, 0	TNTC	0, 0, 0	2, 0, 0	tntc, 269 ,tntc	0, 0, 0	0, 0, 0	53, tntc,tntc	0, 0, 0	0, 0, 0	0, ?, 0	0, 0, 0
			10(-6)= 6															
			10(-7)= 1	**10(-3)**			0, 5, 0			0, 0, 0			0, 0, 0			0, 0, 0		

KIM 1 (-5)= 116
10(-6)= 14 kol.
10(-7)= 3 kol.

TNTCr < TNTC n

OAK

mr. 76-99

	Tree	Suction			Control samples		After 0 hours			After 2 hours			After 5 hours			After 7 days		
N	moisture (%)	interval (min)	CFU	Dilution	Swab surface	Contact surface	Swab test surface	Contact surface	Contact depth	Swab test surface	Contact surface	Contact depth	Swab test surface	Contact surface	Contact depth	Swab test surface	Contact surface	Contact depth
Bacillus subtilis	**10,50%** (3x10, 3x11)	**>45min**	**10(-5)= 79**	**10(-2)**			4, 126, tntc	TNTC	0, 0, 0	0, 0, 0	tntc,301,tntc	0, 0, 0	0, 0, 0	tntc,265, 20	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-6)= 6															
			10(-7)= 1	**10(-3)**			1, 11, 25			0, 0, 0			0, 0, 0			0, 0, 0		

Annex 2

OIL TREATED OAK, with and without scars on the surface, contaminated with 1% meat extract, Pseudomonas fluorescens

Oak

R	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact surface	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**11,00%**	**ca.60min**	**10(-5)= tntc**	**10(-2)**	0, 0,		0, 0, 0	32, 20, 20	0, 0, 0	0, 0, 0	7, 0, 0	0, 0, 0	0, 0, 0	1, 1, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-6)= 264															
			10(-7)=26 kol	**10(-3)**	0, 0		0, 0, 0			0, 0, 0			0, 0, 0			0, 0, 0		

KIM 1 10(-5)= 195
10(-6)= 44
10(-7)= 2 kol.

OAK

N	Tree moisture (%)	Suction interval (min)	CFU	Dilution	Control samples Swab surface	Control samples Contact surface	After 0 hours Swab test surface	After 0 hours Contact surface	After 0 hours Contact depth	After 2 hours Swab test surface	After 2 hours Contact surface	After 2 hours Contact depth	After 5 hours Swab test surface	After 5 hours Contact surface	After 5 hours Contact depth	After 7 days Swab test surface	After 7 days Contact surface	After 7 days Contact depth
Pseudomonas fluorescens	**10,60%** (3x11, 2x10)	**ca.60min**	**10(-5)= tntc**	**10(-2)**	0, 0		tntc, 55, 1	92, 5, 2	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
			10(-6)= 264															
			10(-7)=26 kol	**10(-3)**	0, 0		84, 1, 0			0, 0, 0			0, 0, 0			0, 0, 0		

4. Efficiency of cleaning program

Pilot test

1.TEST

ONLY UNTREATED BEECH TESTED, BACILLUS SUBTILIS, CLEANING 2X, 4X, 6X. - TIMES

Only contact method after cleaning

CLEANED 2X

BEECH — KIM 2 — before cleaning — After cleaning — After cleaning — after cleaning — after cleaning

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours Swab test surface	After 0 hours Contact surface	After 1 hour Swab test surface	After 1 hour Contact surface	After 2 hours Swab test surface	After 2 hours Contact surface	After 5 hours Swab test surface	After 5 hours Contact surface	After 7 days, Swab test surface	After 7 days, Contact surface
Bacillus subtilis	**ca.11%**		**10(-5)=64**	**10(-2)**	15, 110	tntc, tntc		132, 71		1 , 8		1, 2		1, 0		0, 0
			10(-6)=11													
			10(-7)= 0kol.	**10(-3)**	2, 6											

CLEANED 4X

BEECH — After cleaning — After cleaning — After cleaning — after cleaning — after cleaning

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours Swab test surface	After 0 hours Contact surface	After 1 hour Swab test surface	After 1 hour Contact surface	After 2 hours Swab test surface	After 2 hours Contact surface	After 5 hours Swab test surface	After 5 hours Contact surface	After 7 days, Swab test surface	After 7 days, Contact surface
Bacillus subtilis			**10(-5)=64**	**10(-2)**	15, 110	tntc, tntc		tntc, 67		3, 1		1, 0		1, 0		0, 0
			10(-6)=11													
			10(-7)= 0kol.	**10(-3)**	2, 6											

CLEANED 6X

BEECH — After cleaning — After cleaning — After cleaning — after cleaning — after cleaning

	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours Swab test surface	After 0 hours Contact surface	After 1 hour Swab test surface	After 1 hour Contact surface	After 2 hours Swab test surface	After 2 hours Contact surface	After 5 hours Swab test surface	After 5 hours Contact surface	After 7 days, Swab test surface	After 7 days, Contact surface
Bacillus subtilis			**10(-5)=64**	**10(-2)**	15, 110	tntc, tntc		tntc, 135		4, 38		0, 4		1, 1		0, 0
			10(-6)=11													
			10(-7)= 0kol.	**10(-3)**	2, 6											

KIM 1 — 10(-5)= 76
10(-6)= 19
10(-7)= 0 kol.

Annex 2

UNTREATED OAK AND BEECH, CLEANING TEST (4x), CONTAMINATION WITH 1% MEAT EXTRACT. Bacillus subtilis

10(-7)= 2 kol.

R — KIM 2 — before cleaning — after cleaning — after cleaning — after cleaning — after cleaning

OAK	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours Swab test surface	After 0 hours Contact surface	After 2 hours,after cleaning Swab test surface	After 2 hours,after cleaning Contact surface	After 5 hours, after cleaning Swab test surface	After 5 hours, after cleaning Contact surface	After 7 days, after cleaning Swab test surface	After 7 days, after cleaning Contact surface
Bacillus subtilis	ca 11%		10(-5)= 37 10(-6)= 5 10(-7)= 0 kol.	10(-2) 10(-3)	0, 2 0, 0	tntc, tntc	0, 0, 0 0, 0, 0	0, 0	0, 0, 0 0, 0, 0	0, 0, 0	0, 0, 0 0, 0, 0	0, 0, 0	0, 0 0, 0	0, 0*

TNTC new>> TNTC scratched

NEW

OAK	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours, after cleaning Swab test surface	After 0 hours, after cleaning Contact surface	After 2 hours,after cleaning Swab test surface	After 2 hours,after cleaning Contact surface	After 5 hours, after cleaning Swab test surface	After 5 hours, after cleaning Contact surface	After 7 days, after cleaning Swab test surface	After 7 days, after cleaning Contact surface
Bacillus subtilis	ca 11%		10(-5)= 37 10(-6)= 5 10(-7)= 0 kol.	10(-2) 10(-3)	73, 6 1, 0	tntc, tntc	0, 0, 0 0, 0, 0	0, 0, 0	0, 0, 0 1, 0, 0	0, 0, 0	0, 0, 0 0, 0, 0	0, 0, 0	0, 0, 0, 0,	0, 0

R

BEECH	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours, after cleaning Swab test surface	After 0 hours, after cleaning Contact surface	After 2 hours,after cleaning Swab test surface	After 2 hours,after cleaning Contact surface	After 5 hours, after cleaning Swab test surface	After 5 hours, after cleaning Contact surface	After 7 days, after cleaning Swab test surface	After 7 days, after cleaning Contact surface
Bacillus subtilis	ca 11%		10(-5)= 37 10(-6)= 5 10(-7)= 0 kol.	10(-2) 10(-3)	9, 64 0, 9	tntc, tntc	0, 0, 0 0, 0, 0	1, 11	0, 0, 0 0, 0, 0	1, 3, 12	0, 0, 0 0, 0, 0	0, 0, 0	0, 0 0, 0	0, 0

NEW

BEECH	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours, after cleaning Swab test surface	After 0 hours, after cleaning Contact surface	After 2 hours,after cleaning Swab test surface	After 2 hours,after cleaning Contact surface	After 5 hours, after cleaning Swab test surface	After 5 hours, after cleaning Contact surface	After 7 days, after cleaning Swab test surface	After 7 days, after cleaning Contact surface
Bacillus subtilis	ca 11%		10(-5)= 37 10(-6)= 5 10(-7)= 0 kol.	10(-2) 10(-3)	0, 3 1, 0	tntc, tntc	0, 0, 0 0, 0, 0	0, 1, 3	0, 0, 0 0, 0, 0	0, 0, 2	0, 0, 0	0, 0, 0	0, 0 0, 0	0, 0

OIL TREATED OAK AND BEECH, CLEANING TEST (4x), CONTAMINATION WITH 1% MEAT EXTRACT

R

OAK	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours, after cleaning Swab test surface	After 0 hours, after cleaning Contact surface	After 2 hours,after cleaning Swab test surface	After 2 hours,after cleaning Contact surface	After 5 hours, after cleaning Swab test surface	After 5 hours, after cleaning Contact surface	After 7 days, after cleaning Swab test surface	After 7 days, after cleaning Contact surface
Bacillus subtilis	ca 11%		10(-5)=25 10(-6)= 3 10(-7)= 1 kol.	10(-2) 10(-3)	11, 283, 139 0, 17, 11	tntc, tntc	0, 0 0, 0	0, 0	0, 0, 0 0, 0, 0	0, 0, 0	0, 0, 0 0, 0, 0	0, 0, 0	0, 0 0, 0	0, 0

NEW

OAK	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours, after cleaning Swab test surface	After 0 hours, after cleaning Contact surface	After 2 hours,after cleaning Swab test surface	After 2 hours,after cleaning Contact surface	After 5 hours, after cleaning Swab test surface	After 5 hours, after cleaning Contact surface	After 7 days, after cleaning Swab test surface	After 7 days, after cleaning Contact surface
Bacillus subtilis	ca. 11%		10(-5)=25 10(-6)= 3 10(-7)= 1 kol.	10(-2) 10(-3)	7,, 0 0, 0	tntc, tntc	0, 0, 0 0, 0, 0	0, 0	0, 0, 0 0, ,0 , 0	0, 0, 0	0, 0, 0 0, 0, 0	0, 0, 0	0, 0 0, 0	0, 0

R

BEECH	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours, after cleaning Swab test surface	After 0 hours, after cleaning Contact surface	After 2 hours,after cleaning Swab test surface	After 2 hours,after cleaning Contact surface	After 5 hours, after cleaning Swab test surface	After 5 hours, after cleaning Contact surface	After 7 days, after cleaning Swab test surface	After 7 days, after cleaning Contact surface
Bacillus subtilis	ca. 11%		10(-5)=25 10(-6)= 3 10(-7)= 1 kol.	10(-2) 10(-3)	184, 30 19, 4	tntc, tntc	0, 0, 0 0, 0, 0	0, 0	0, 0, 0 0, 0, 0	1, 0, 1	0, 0, 0 0, 0 , 0	0, 0, 0	0, 0 0, 0	0, 0

NEW

BEECH	Tree moisture (%)	Suction interval (min)	CFU	Dilution	After 0 hours, before cleaning Swab surface	After 0 hours, before cleaning Contact surface	After 0 hours, after cleaning Swab test surface	After 0 hours, after cleaning Contact surface	After 2 hours,after cleaning Swab test surface	After 2 hours,after cleaning Contact surface	After 5 hours, after cleaning Swab test surface	After 5 hours, after cleaning Contact surface	After 7 days, after cleaning Swab test surface	After 7 days, after cleaning Contact surface
Bacillus subtilis	ca. 11%		10(-5)=25 10(-6)= 3 10(-7)= 1 kol.	10(-2) 10(-3)	7, 15 0, 1	tntc, tntc	0, 0, 0 0, 0, 0	0, 0	0, 0, 0 0, 0, 0	1, 2, 0	0, 0, 0 0, 0, 0	0, 0, 0	0, 0 0, 0	0, 0

Annex 2

Plastic with and without scratches on the surface,contamination with 1% meat extract, Bacillus subtilis cleaning 4- times

	Before cleaning, after drying				After cleaning, after 0 hour				After cleaning, after 2 hours			
	Swab test			Contact	Swab test			Contact	Swab test			Contact
Bacillus **subtilis**	surface			surface	surface			surface	surface			surface
	10 (-3)	10 (-4)	10 (-5)		10(-2)	10 (-3)	10 (-4)		10(-2)	10 (-3)	10 (-4)	
Scrached plastic- R	tntc, tntc	239, 171	24, 19	TNTC	0, 0, 0	0, 0, 0	0, 0, 0	4, 11	0, 0, 0	0, 0, 0	0, 0, 0	0, 1, 0
New plastic - N	tntc, 31	227, 4	28, 0	TNTC	0, 0, 0	0, 0, 0	0, 0, 0	0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 1, 0

KIM 1
10(-5)= 91
10(-6)= 5
10(-7)= 1 kol.

KIM 2
10(-5)= 170
10(-6)= 14
10(-7)= 2 kol.

After cleaning, after 5 hours				After cleaning, after 7 days		
Swab test			Contact	Swab test		Contact
surface			surface	surface		surface
10(-2)	10 (-3)	10 (-4)		10 (-2)	10 (-3)	
0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0	2, 0	0, 0
0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0	0, 0	0, 1

Annex 3

5. Experiments carried out by DVF, Rødovre. Correlation of results.

1.) Oil treated, scratched and new Oak,contaminated with 1% meat extract, Bacillus subtilis

OAK KIM2

R- scratched	CFU	Dilution	After 0 hours		After 2 hours		After 5 hours		After 7 days	
			Swab test	Contact	Swab test	Contact	Swab test	Contact	Swab test	Contact
			surface	surface	surface	surface	surface	surface	surface	surface
Bacillus subtilis	**10(-5)= 236**	**10(-2)**	0, 1	40, 280	3, 0	tntc,tntc	0, 0	0, 0	0	0, 0
	10(-6)= 36									
	10(-7)= 3	**10(-3)**	0, 0		0, 0		0, 0			

KIM 1 (TSB + bacteria) 196/ 34/ 2 kol.
KIM 2 (1% KE + bacteria) = Start concentration 236/ 36/ 3

OAK KIM 2

N- new	CFU	Dilution	After 0 hours		After 2 hours		After 5 hours		After 7 days	
			Swab test	Contact	Swab test	Contact	Swab test	Contact	Swab test	Contact
			surface	surface	surface	surface	surface	surface	surface	surface
Bacillus subtilis	**10(-5)= 236**	**10(-2)**	24, 5, 5	TNTC	4, 8, 4	31, 300, 300	1, 0, 0	TNTC	0, 0, 0	0, 0, 0
	10(-6)= 36									
	10(-7)= 3	**10(-3)**	3, 0, 0		0, 2, 0		0, 0, 0			

Annex 3

2.) Oil treated, scratched and new Oak, contaminated with 1% meat extract, Pseudomonas fluorescens.

OAK KIM2

R- scratched	CFU	Dilution	After 0 hours		After 2 hours		After 5 hours		After 7 days	
			Swab test	Contact	Swab test	Contact	Swab test	Contact	Swab test	Contact
			surface	surface	surface	surface	surface	surface	surface	surface
Pseudomonas fluorescens	**10(-5)= >300**	**10(-2)**	0, 0, 0	223, >300, 37	0, 0, 0	199, >300, 260	0, 0, 0	0, 49, 146	0, 0, 0	0, 0, 0
	10(-6)= 132									
	10(-7)=13	**10(-3)**	0, 0, 0		0, 0, 0		0, 0, 0			

OAK KIM 2

N - new	CFU	Dilution	After 0 hours		After 2 hours		After 5 hours		After 7 days	
			Swab test	Contact	Swab test	Contact	Swab test	Contact	Swab test	Contact
			surface	surface	surface	surface	surface	surface	surface	surface
Pseudomonas fluorescens	**10(-5)= >300**	**10(-2)**	6, 23, 211	>300, 106,153	1, 1, 0	142, >300, 36	0, 0, 0	0, 230, 164	6, 0, 3	0, 0, 0
	10(-6)= 132									
	10(-7)=13	**10(-3)**	2, 1, 25		1, 0, 0		0, 0, 0		1, 0, 0	

Annex 3

3, Plastic, scratched and new, contaminated with 1% meat extract, Pseudomonas fluorescens

Pseudomonas fluorescens	**After 2 hours**				**After 5 hours**				**After 7 days**		
	Swab test			**Contact**	**Swab test**			**Contact**	**Swab test**		**Contact**
	surface			surface	surface			surface	surface		surface
	10 (-2)	**10 (-3)**	**10 (-4)**		**10 (-2)**	**10 (-3)**	**10 (-4)**		**10 (-2)**	**10 (-3)**	
Scratched plastic	TNTC	98,60,28	7, 5, 4	TNTC	122,68,53	24,11, 4	0, 1, 0	TNTC	2, 1, 0	0, 0, 0	0, 0, 0
New plastic - N	TNTC	101,300,300	13,46, 49	TNTC	32,285,13	35, 34, 60	0, 1, 1	TNTC	2, 0, 0	0, 0, 0	5,210,ca200

KIM 1	*tntc*	*KIM 2*	10(-5)= tntc
	tntc		10(-6)= tntc
	50		10(-7)= 53 kol.

Annex 3

4, Pine and Spruce,ordinary moisture 7-10%, contaminated with TSB medium, Pseudomonas fluorescens

SPRUCE	KIM									
			After 0 hours		**After 2 hours**		**After 5 hours**		**After 7 days**	
	KIM TAL	**Dilution**	**Swab test**	**Contact tes**	**Swab test**	**Contact tes**	**Swab test**	**Contact test**	**Swab test**	**Contact**
			surface	surface	surface	surface	surface	surface	surface	surface
Pseudomonas fluorescens	**10(-5)=tntc**	**10(-2)**	TNTC	TNTC	1, 1, 3	TNTC	1, 5, 0	39, 49, 25	0, 0	0, 0, 0,
	10(-6)= tntc									
	10(-7)= 51,	**10(-3)**	tntc, ,279,146,		0, 0, 0		0, 0, 1		0, 0	

PINE										
			After 0 hours		**After 2 hours**		**After 5 hours**		**After 7 days**	
	KIM TAL	**Dilution**	**Swab test**	**Contact**	**Swab test**	**Contact**	**Swab test**	**Contact test**	**Swab test**	**Contact**
			surface	surface	surface	surface	surface	surface	surface	surface
Pseudomonas fluorescens	**10(-5)=tntc**	**10(-2)**	TNTC	TNTC	2, 4, 0	TNTC	0, 0, 0	20, 68, 47	0, 0, 0	0, 0, 0
	10(-6)= tntc									
	10(-7)= 51,	**10(-3)**	300 , , 108,191,		0, 1, 0		0, 0, 0		0, 0, 0	

Annex 4

Federal Biological Research Center for Agriculture and Forestry,

Messeweg 11/12, D 38104 Braunschweig

Agenda for the workshop on phytosanitary and hygienic aspects of wood and products

13th June 2001, Begin 9:00, End 16:00 (approximately)

Welcome and opening	Wolf
Introduction to the project ”wood in food”	Beyer
Test methods and results done in the nordic project	Gundjörnsdottir
Status report for the testing program in Denmark	Kvist
Proposed cleanwood project	Dickinson
Hygienic characteristics of wood and wood products – Laboratory data	Schönwälder
Hygienic properties of plastic and wooden pallets – Test in practice	Steinkamp
Possible conseqences of new results for administrative Regulations	v. Lauvenberg
Futher presentations and discussion.	