CUSTOMER REPORT

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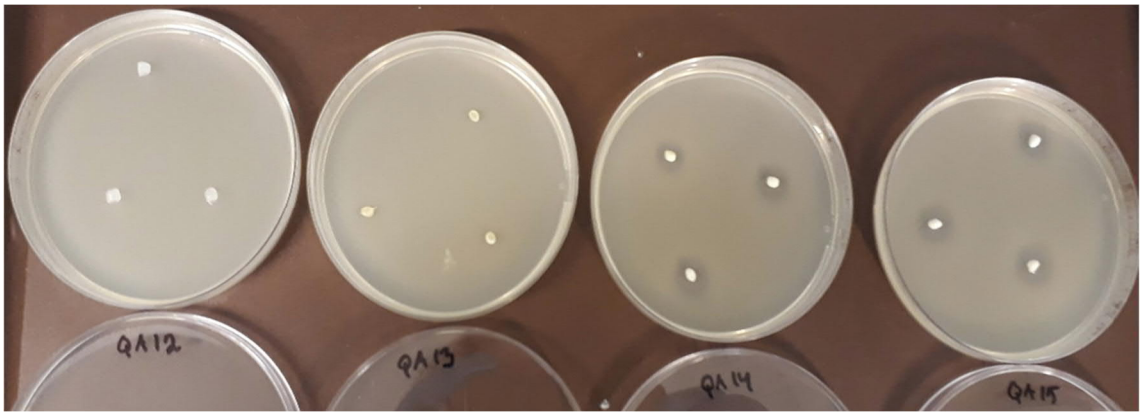

Antibacterial efficacy of plastic samples

Authors: Satu Salo, Hanna-Leena Alakomi

Confidentiality: Confidential

Report's title

Antibacterial efficacy of plastic samples

Customer, contact person, address	**Order reference**
Premix Henrik Yli-Seppälä Muovitie 4 05201 Rajamäki	
Project name	**Project number/Short name**
AntiMRSA	VTT-V-125943-20

Summary

The aim was to define antibacterial efficacy of plastic granulates and films with Methicillin-resistant *Staphylococcus aureus* (MRSA) VTT E-183582 strain. Test methods were modified from standards; for granulates EN 1104:2005 "Paper and board intended to come into contact with foodstuffs. Determination of the transfer of antimicrobial constituents." and for plastic films ISO 22196:2011(E) "Measurement of antibacterial activity on plastics and other non-porous surfaces".

Results of this research study demonstrated clearly that both granulates and plastic films had antibacterial efficacy against Methicillin-resistant *Staphylococcus aureus* (MRSA) VTT E-183582 strain.

Espoo 27.5.2020

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Distribution (customer and VTT)

Customer (Henrik Yli-Seppälä) and VTT (archive, Satu Salo)

1. Description and objectives

The aim of this project was to define antibacterial efficacy of plastic granulates and films provided by the customer. Tests were performed only with Methicillin-resistant *Staphylococcus aureus* and test methods were modified from standards; for granulates EN 1104:2005 ”Paper and board intended to come into contact with foodstuffs. Determination of the transfer of antimicrobial constituents.” and for plastic films ISO 22196:2011(E) “Measurement of antibacterial activity on plastics and other non-porous surfaces”. Test method used for defining antibacterial efficacy of plastic granulates revealed the transfer of antimicrobial constituents in nutrient media and test method used for defining antibacterial efficacy of plastic films revealed the antimicrobial efficacy against microbes in contact point. These two methods are both used generally for studying microbicidal efficacy but the results from these two methods are not supposed to be compared with each other. Several other standardized methods for studying antibacterial efficacy are available and these standards were selected togher with customer based on earlier studies.

2. Methods

Customer provided following samples (four granulates and three plastic films) 14th January 2020 (Figures 1-7):

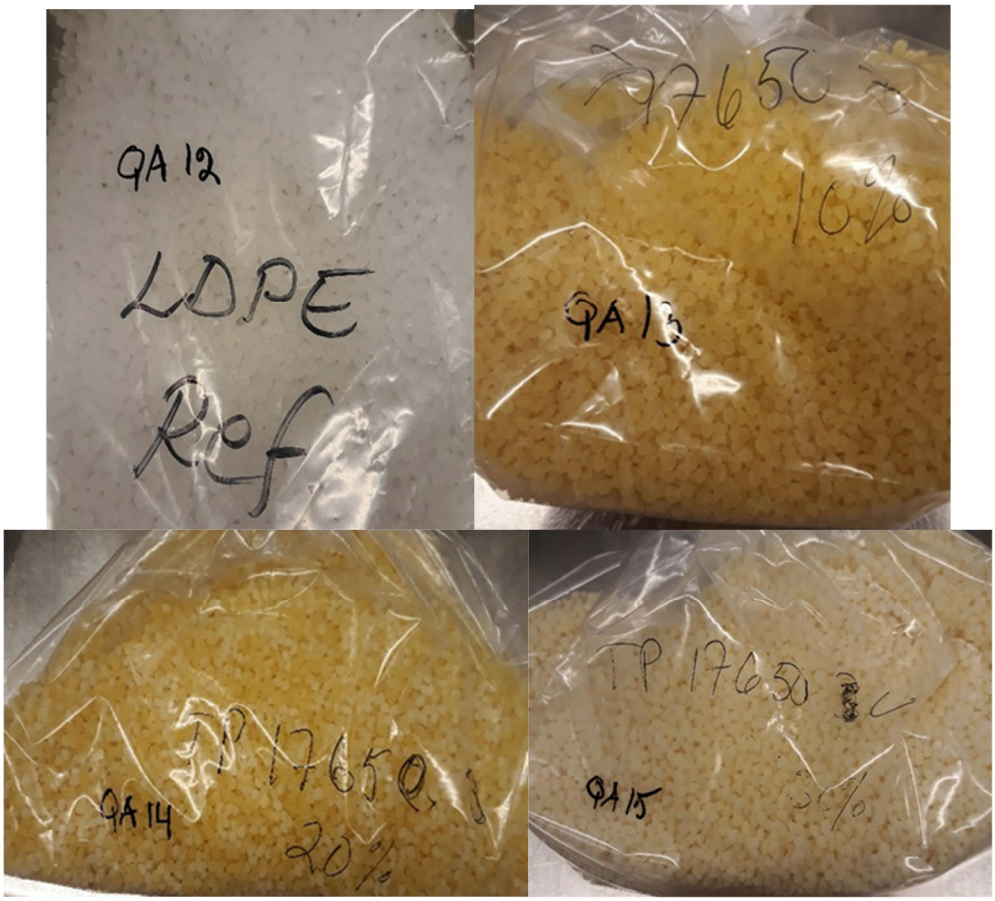
Plastic granulates

- LDPE ref; QA 12, Figure 1
- TP 17650 10%; QA 13, Figure 2
- TP 17650 20%; QA 14, Figure 3

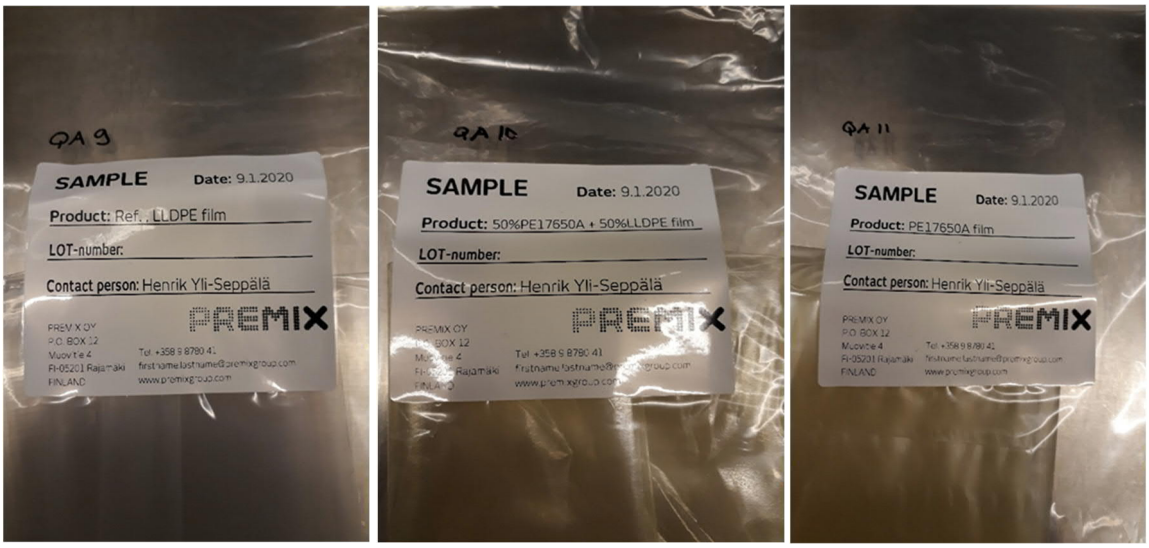
TP 17650 30%; QA 15, Figure 4

Plastic films

- Ref.LLDPE film; QA 9, Figure 5
- 50%PE17650A + 50%LLDPE film; QA 10, Figure 6
- PE17650A film; QA 11, Figure 7



Figures 1-4. Plastic granulates



Figures 5-7. Plastic films

Tests were performed with Methicillin-resistant *Staphylococcus aureus* (MRSA) strain VTT E-183582. This strain is purchased from DSMZ-German Collection of Microorganisms and Cell Cultures and named: *Staphylococcus aureus* Rosenbach 1884. DSM collection strain number is 11822 (other collection number: ICB 25701) and strain is isolated from clinical material.

Tests were initiated 23.1.2020.

Inhibition zone test for plastic granulates

Inhibition zone test was based on standard EN 1104:2005 ”Paper and board intended to come into contact with foodstuffs. Determination of the transfer of antimicrobial constituents.” Antibacterial efficacy of granulates was studied by preparing Plate Count Agar (PCA) and inoculating it with MRSA suspension containing 90 000 000 colony forming units (cfu) ml^{-1}. Inoculated agar was plated on Petri dishes and granulates were added to semisolid agar and plates were incubated at 37°C for 2 d. Inhibition zones were measured after 2 day incubation period. Test was performed with 9 parallel granulates.

Measurement of antibacterial activity of plastic films

Measurement of antimicrobial activity of plastic films was based on standard ISO 22196:2011(E) “*Measurement of antibacterial activity on plastics and other non-porous surfaces*”. Antibacterial efficacy of plastic films was studied using pieces size 5 cm x 5 cm (two layers). Test surfaces were cut from plastic films and 0.4 ml of MRSA suspension containing 900 000 cfu ml^{-1} was pipetted on each test surface and covered with piece sterile plastic (4 cm x 4 cm). Figure 8 shows inoculation of test specimen and placement of cover film. Number of viable MRSA cells were determined from surfaces after 2 h, 6 h, 12 h and 24 h incubation. During incubation samples were stored at 37°C in closed Minigrip bag with moistured paper in order to maintain moisture level. Culturing was done by adding 20 ml sterile peptone saline on Petri dish containing surface samples and orbital shaking 5 min before culturing on PCA plates. Culturing was performed by spreading 0.1 ml of sample on PCA and additionally for 2 h, 6 h and 12 h samples 1 ml of sample was used to pour plate technique to achieve detection limit 20 cfu/sample. Plates were incubated at 37°C for 2 d. Test series were performed using 3 replicates.

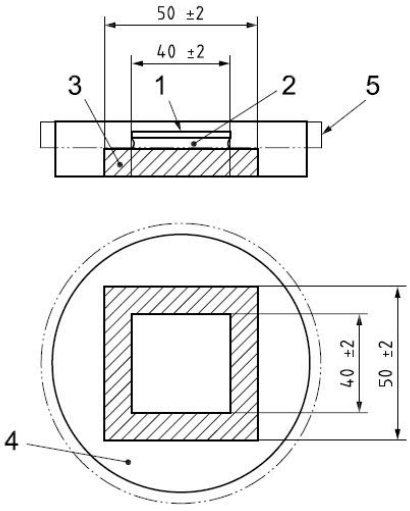


Figure 8. Inoculation of test specimen and placement of cover film from standard ISO 22196. Numbers: 1 = cover film, 2 = test inoculum (0.4 ml), 3 = test specimen, 4 = Petri dish and 5 = lid of Petri dish. Dimensions are in millimetres.

3. Results

Inhibition zone test for granulates

Inhibition zones were measured after 2 day incubation and results are shown in Table 1. Image of inhibition zones (Figure 9) shows clearly the inhibition zones around granulate samples TP 17650 20%; QA 14 and TP 17650 30%; QA 15, but only minor reduction of microbial growth

next to granulate TP 17650 10%; QA 13 was observed. Inhibition zone for the positive control, Fennosan IT 21, diluted 1:100, was 20.9 mm. Granulates were not totally emerged into the agar (Figure 9).

Table 1. Inhibition zone test for granulates observed after 2 day incubation.

sample	Inhibition zone (mm)
Ref. LDPE; QA 12	no zone
TP 17650 10%; QA 13	reduction in growth next to granulate
TP 17650 20%; QA 14	9.3 ± 1.3 mm
TP 17650 30%; QA 15	11.2 ± 0.7 mm

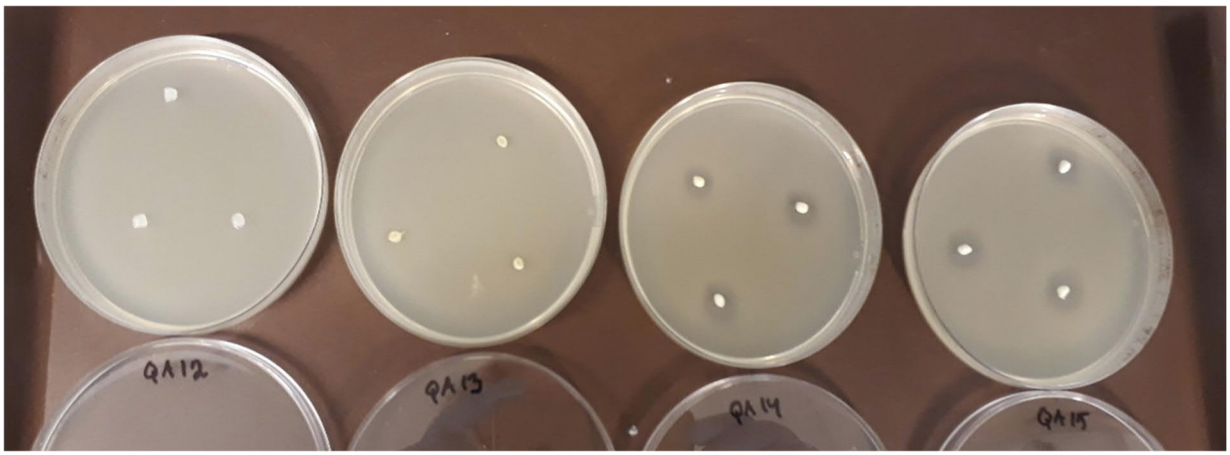


Figure 9. Inhibition zones of tested granulates; granulates from left to right: Ref. LDPE; QA 12, TP 17650 10%; QA 13, TP 17650 20%; QA 14 and TP 17650 30%; QA 15

Measurement of antibacterial activity of plastic films

Number of viable MRSA bacteria were determined from plastic surfaces after 2 h, 6 h, 12 h and 24 h contact time. Results are shown in Figure 10 and Table 2. Additionally for 2 h and 12 h samples the viability of cells was determined by pouring agar directly on the surface of the sample to achieve detection limit 1 cfu/sample. According to this method growth was detected at timepoint 2 h from control sample and 50%PE17650A + 50%LLDPE sample but not from PE17650A and at timepoint 12 h growth was detected from control sample but not from 50%PE17650A + 50%LLDPE sample and PE17650A sample.

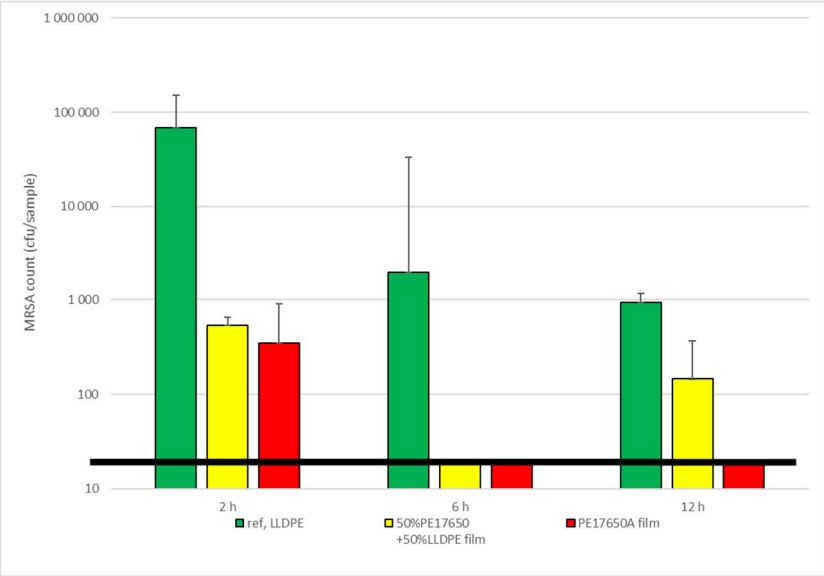


Figure 10. Viability of MRSA on samples after two day incubation at 37 °C. Thin black lines show deviations. Thick black line = detection limit 20 cfu/sample.

Table 2. Viability of MRSA on samples after 2 h, 6 h, 12 h and 24 h contact time with plastic films; cfu = colony forming units

survived cells/sample	2 h		6 h		12 h		24 h	
	average cfu/sample (cfu/cm²)	st deviation	average cfu/sample (cfu/cm²)	st deviation	average cfu/sample (cfu/cm²)	st deviation	average cfu/sample (cfu/cm²)	st deviation
ref, LLDPE	68 667 (4 292)	82 567	2 013 (126)	31 103	933 (58)	231	< 200 (< 13)	0
50%PE17650+ 50%LLDPE film	533 (33)	115	< 20 (< 1)	0	< 146 (< 9)	220	< 200 (< 13)	0
PE17650A film	< 346 (< 22)	566	< 20 (< 1)	0	< 20 (< 1)	0	< 200 (< 13)	0

Antibacterial activity is counted in table 3. Logarithmic reduction of MRSA bacteria was counted by dividing growth in reference sample with growth in antibacterial sample and taking that to logarithmic value:

Antibacterial activity = log (cfu reference sample/ cfu test sample)

Table 3. Microbicidal efficacy of plastic films containing PE17650. Results are given as reduction of survived MRSA amounts in logarithmic units; log (cfu on control sample/cfu on antimicrobial sample)

Antibacterial activity	2 h	6 h	12 h	24 h
50%PE17650 +50%LLDPE film	2.1	> 2.0	> 0.8	Indeterminate; control sample below detection limit
PE17650A film	> 2.3	> 2.0	> 1.7	

4. Conclusions

The results of this research study showed clearly that both granulates and plastic films had antibacterial efficacy against Methicilin Resistant *Staphylococcus aureus* (MRSA) strain VTT E-183582.

Inhibition zone test for granulates clearly demonstrated that granulates with higher amount of the effective incredient had larger inhibition zones. Variation between parallel samples was small.

In the plastic film test viability of the MRSA strain decreased during the incubation period also in the control samples. Hence, analysis of the viability at different time points is vital. Results from contact times 2 h, 6 h and 12 h showed clearly that both studied plastic films containing PE17650A had antibacterial efficacy against MRSA. PE17650A samples were slightly more effective than 50%PE17650+50%LLDPE film sample.

Survival of MRSA on surfaces was low and after 24 hours number of viable cells also on the control film had decreased below detection limit. In future studies it is recommended to study antimicrobial activity and adherence of the cells in early phase, e.g. 20 min, 1 h and 6 h. It is also possible to examine effect of dirt on the survival of the cells. In addition, additional target microbes incl. *Salmonella, Listeria, S. aureus* and *Pseudomonas aeruginosa* can be utilized and are available in VTT Culture Collection. Selection of test microbes and other test parameters such as contact time, temperature of environment, additional dirt load and level of microbe contamination should be linked to possible application. List of possible applications and their characteristics could help in selection of parameters for further studies.

References

Standards:

ISO 22196:2011(E) “Measurement of antibacterial activity on plastics and other non-porous surfaces”

SFS-EN 1104:2005 ”Paper and board intended to come into contact with foodstuffs. Determination of the transfer of antimicrobial constituents.”

SFS-EN 1104:2018 “Paper and board intended to come into contact with foodstuffs. Determination of the transfer of antimicrobial constituents.”