OSTTHÜRINGISCHE
MATERIALPRÜFGESELLSCHAFT
für Textil und Kunststoffe mbH
Breitscheidstraße 97
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Your letter of	Your ref	Our ref	phone extension	date
09.10.2018, 03.01.2019		**4.5/B61/2018-A-EN**	03672 379-450	08.03.2019

TEST REPORT

1. General

Test report – No.:	4.5/B61/2018-A-EN This copy completely replaces the test report 4.5 / B61 / 2018 dated 06.12.2018.
Commissioned by:	E. Epple & Co. GmbH, Ms. Dr. Claudia Eisermann
Objects tested:	**Internal lab number**
Sample 1	epple 46-neu / V1 / grau — 3702
Sampling:	by client
Test:	„Plastics - Evaluation of the action of microorganisms"; according to DIN EN ISO 846:1997, Method A: Resistance to fungi (growth test) Method C: Resistance to bacteria
Date received:	16.10.2018
Test period:	29.10. – 05.12.2018
Processed by:	Ms. C. Reichmann
Subcontractors:	none
Test procedure:	1) DIN EN ISO 846:1997
Remarks:	Reason for the re-issue of the test report: The sample designation was subsequently changed at the request of the client (e-mails from 12.12.2018 and 03.01.2019).
Report copies:	1 copy for client 1 copy for OMPG

The tests were carried out between the date of receipt and the report date. The results of the measurements and analysis refer exclusively to the test samples. This test report is only valid with the signature of the laboratory director or his representative legal. It may only be fully reproduced. Copying of excerpts require a written approval of the laboratory.

2. *Test method*

Materials, which can be metabolized by microorganisms are tested by incubation together with fungi (method A) or bacteria (method C) on Carbon-deficient nutrient media. Visual inspection is carried out to evaluate microbial growth next to and on the samples.

Method A: Resistance to fungi (growth test)

Materials and test conditions:

Samples:	NC	glass object slide, Marienfeld
	PC	cellulose filter soaked with 10 % glycerine, 0,5 % malt extract
	3702	epple 46-neu / V1 / grau
Test organism:	*Aspergillus niger* DSM 1957 (ATCC 6275)	
Sample preparation:	disinfection with 70 % ethanol	
Dimension of sample:	approx. 30 mm x 30 mm (irregular cut by customer)	
Nutrient media & cultivation:		
pre-incubation	on oatmeal malt extract agar at 29 °C	
spore preparation	mineral salt solution with mit wetting agent	
medium for inoculation	mineral salt solution	
incubation media	mineral salt agar	
control media	mineral salt agar with glucose	
incubation temperature	29 °C	
incubation time	4 weeks	
Inoculation spore number:	$1 \cdot 10^6$ cfu/ml (set: $1 \cdot 10^6$ cfu/ml)	

Method description:

Preparation of the samples:

- the samples were disinfected by dipping into 70 % ethanol for 1 min and dried in a petri dish (∅ 90 mm) for at least 4 h at 45 °C

Preparation of inoculum:

- cultivation of *Aspergillus niger* DSM 1957 on oatmeal malt extract agar until spore formation at 29 °C
- extraction of the spores by washing the spores with mineral salt solution with wetting agent and separation of remaining mycelium by centrifuging
- adjusting the spore count to $1 \cdot 10^6$ cfu / ml in mineral saline solution

Inoculation and incubation of the samples:

- adding and plating out 100 µl of the spore suspension onto mineral salt agar
- placing of specimens on the cooled agar
- approach of 5 parallel samples
- incubation for 4 weeks at 29 ± 1 ° C and a relative humidity of 90 %

Controls:

1. Growth control:

- 100 µl of the inoculate were plated on mineral salt agar with glucose and incubated for 3-4 days at 29 ° C

2. Sterile control:

- placing of samples on mineral salt agar without fungal spores
- approach of 5 parallel samples
- incubation for 4 weeks at 29 ± 1 ° C and a relative humidity of 90 %

3. Storage under standard climatic conditions

- 5 test samples were stored without nutrient medium under standard climatic conditions (23 ° C and 50 % rH)

Method C: Resistance to bacteria

Materials and test conditions:

Samples	NC	glass object slide, Marienfeld
	PC	cellulose filter soaked with 10 % glycerine, 0,5 % malt extract
	3702	epple 46-neu / V1 / grau
Test organism:	*Pseudomonas aeruginosa* DSM 1253	
Sample preparation:	disinfektion with 70 % ethanol	
Dimension of sample:	approx. 30 mm x 30 mm (irregular cut by customer)	
Nutrient media & cultivation: pre-incubation medium for inoculation incubation media incubation temperature incubation time	 in tryptone-soy-bouillon (TSB, Carl Roth) at 37 °C and 110 rpm, for 24 h buffer solution mineral salt-agar 29 °C 4 weeks	
Inoculation cell number:	$9{,}5 \cdot 10^5$ KBE/ml (set: $4 \cdot 10^5 - 2 \cdot 10^6$ KBE/ml)	

Method description:

Preparation of the samples:

- the samples were disinfected by dipping into 70 % ethanol for 1 min and dried in a petri dish (∅ 90 mm) for at least 4 h at 45 °C

Preparation of inoculum:

- cultivation of *Pseudomonas aeruginosa* DSM 1253 in TSB for 24 h at 37 °C and 110 rpm
- adjusting the cell count in sterile buffer solution

Inoculation and incubation of the samples:

- inoculation of the mineral salt agar, which was liquefied and cooled to 45 ° C, with the inoculum
- transfer of inoculated agar into sterile Petri dishes (Ø 90 mm)
- placing of the specimens on the cooled agar
- approach of 5 parallel samples
- pouring over the test pieces with inoculated agar until the samples are completely covered
- incubation for 4 weeks at 29 ± 1 °C and a relative humidity of 90 %

Controls:

1. Growth control:

- 100 µl of inoculum were plated on PCA and incubated for 24-48 h at 29 °C

2. Sterile control:

- placing of samples on mineral salt agar without bacterium into sterile Petri dishes (Ø 90 mm)
- placing of the specimens on the cooled agar
- approach of 5 parallel samples
- pouring over the test pieces with uninoculated agar until the samples are completely covered
- incubation for 4 weeks at 29 ± 1 °C and a relative humidity of 90 %

3. Storage under standard climatic conditions

- 5 test samples were stored without nutrient medium under standard climatic conditions (23 ° C and 50 % rH)

3. Assessment criteria:

Growth intensity	Assessment
0	no observable growth neither by naked eye nor microscopy
1	visible growth only with microscopy
2	visible growth up to 25 % sample surface
3	visible growth up to 50 % sample surface
4	extensive growth on more than 50 % sample surface
5	strong growth on whole sample surface

4. *Test results*

Test results are shown in table 1 and 2.

Table 1: Visual assessment of bacterial growth of *A. niger*, method A

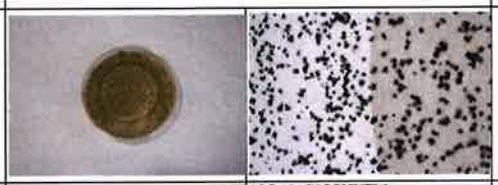
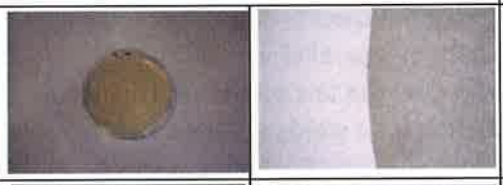
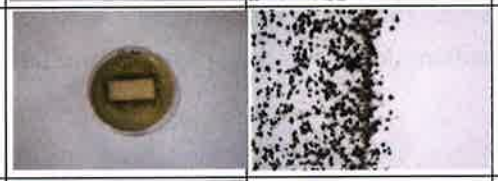
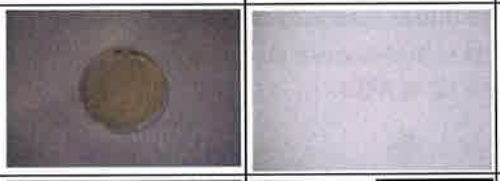
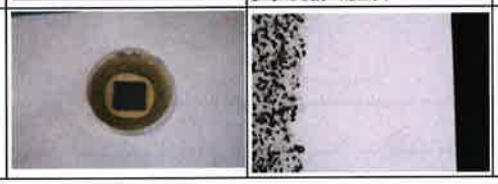
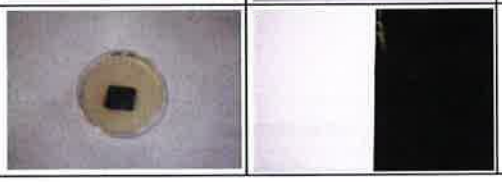
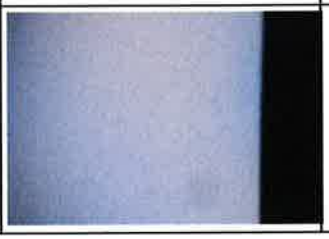
Sample	Inoculated sample	sterile control	growth intensity
PC			5
NC			0
3702			0

Table 2: Visual assessment of bacterial growth of *P.aeruginosa*, method C

Sample	Inoculated sample		sterile control	growth intensity
PC		homogeneously distrubuted, large cfu		sterile
NC		homogeneously distrubuted, small cfu		sterile
3702		zone of inhibition on the edge		sterile

3. Assessment

The sample „epple 46-neu / V1 / grau" (3702) shows no enhanced growth of *A. niger* DSM 1957 (ISO 846-A) and *P. aeruginosa* DSM 1253 (ISO 846-C), but even showed an inhibitory effect on both germs in the border area.

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