Your notice of	Your reference	Date
31-05-2012	122cYR2012	06-08-2012

Analysis Report 12.02353.02

Required tests :

ISO 20743-2 (2007)	**antibacterial activity of antibacterial finished products – quantitative test**
ASTM G21 (2009)	**Standard Practice for determining resistance of Synthetic Polymeric Materials to Fungi**
BS 7175 (1989)	**Section 3 – Individual bedcovers - Ignition source no. 5**

Identification number	Information given by the client	Date of receipt
T1207428	PU-120	29-05-2012
T1207429	PU-150	29-05-2012
T1207430	PU-170	29-05-2012
T1207431	PU-210	29-05-2012
T1207432	PU-210 PA	29-05-2012
T1207433	PU-120 without antibacterial finish	29-05-2012

Yvette Rogister

Order responsible

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Reference: **T1207428 - PU-120**
T1207429 - PU-150
T1207430 - PU-170
T1207431 - PU-210
T1207432 - PU-210 PA
T1207433 - PU-120 without antibacterial finish

Antibacterial activity of antibacterial finished products – quantitative test

Date of ending the test	12-07-2012
Standard used	ISO 20743-2 (2007)

1. Method

ISO 20743 2007 : Textiles – Determination of antibacterial activity of antibacterial finished products – Part 2 : Transfer method.

Aim : This test is used to quantitatively measure the antimicrobial activity of antibacterial finished textile products including nonwovens.

Method and principle of the test :

- Treated samples and control samples are cut in pieces of 3.8 cm diameter
- The inoculation is achieved by transfer of bacteria from an agar plate onto samples : Each sample (as well as each control) is placed on the agar surface of an agar plate that has been previously inoculated (by first flooding the agar surface with 1 ml of a bacterial suspension adjusted to a concentration of 1 10^6 to 3 10^6 CFU /ml and then sucking up as much of the excess liquid as possible). A weight of 200g is applied on the sample for 60 seconds and the sample is afterwards placed in a Petri dish with the transferred surface face up.
- Six test samples in individual Petri dishes plus six separate Petri dishes with control samples constitute one test.
- Immediately after transfer ("0 contact time"), three of the six samples and three of the six controls are placed in a vial containing a neutralizing solution (1vial/sample) and are shaken out to extract the bacteria present on them. A counting on the extraction liquid is performed by Plate Count Method.
- The other samples and controls are incubated in their Petri dishes in a humidity chamber at 37 °C for 18 to 24 hours.
- After incubation, extractions and counting of the bacteria still present on the remaining samples (3 treated and 3 controls) are performed as for "0 contact time".
- The growth value and the activity values are then computed.

Strains mentioned in the protocol : *Klebsiella pneumoniae ATCC 4352*
Staphylococcus aureus ATCC 6538

The growth value is computed as followed :

$$F = C_t - C_0$$

Where , F : growth value on the control sample
C_t : average common logarithm for the number of bacteria obtained from three test samples of control fabric after 18 to 24 hours incubation
C_0 : average common logarithm for the number of bacteria obtained from three test samples of control fabric immediately after transfer to the control fabric

The test is judged to be effective, when the growth value is ≥ 1 and when the difference in extremes for the three controls immediately after transfer as well as after incubation is ≤ 1 $\log_{10}$

The **calculation of the activity values** is obtained according to the following formula :

$$A = (C_t - C_0) - (T_t - T_0) = F - G$$

Where , A : antibacterial activity value
F : growth value on the control fabric ($F = C_t - C_0$)
G : growth value on the antibacterial treated sample ($G = T_t - T_0$)
T_t : average common logarithm for the number of bacteria obtained from three antibacterial treated test samples after 18 to 24 hours incubation
T_0 : average common logarithm for the number of bacteria obtained from three antibacterial treated test samples immediately after transfer

2. Results

Technical data :

- Samples dimension : 3.8 cm diameter
- Volume of the inoculum used to flood the agar surface of the transfer agar plate : 1 ml
  - Composition of the inoculum solution : Peptone-Salt solution (Tryptone, pancreatic digest of casein 1 g/l, NaCl 8.5 g/l)
- Volume of the extraction solution (= neutralizing solution) : 20 ml
  - Composition of the neutralizing solution : Polysorbate 80 30 g/l, Lecithin 3 g/l, Histidine hydrochloride 1 g/l, Peptone 1 g/l, NaCl 4.3 g/l, Monopotassium phosphate 3.6 g/l, Disodium phosphate dihydrate 7.2 g/l
- Contact time used : 18 hours at 37°C
- Tested strains : *Staphylococcus aureus* ATCC 6538
  Methicillin Resistant Staphylococcus aureus – MRSA ATCC 33951
- Microbiological technique used for the determination of the viable cells : count of number of colonies of dilution series on Petri dishes (Plate Count Method)
- The samples are not sterilized before performing the test.

Number of samples assessed:
- at "0" contact time, directly after transfer (inoculation)

- 3 control samples
- 3 treated samples

- after 18 hours of incubation

- 3 control samples
- 3 treated samples

Samples references	*Tested combination*
T1207428 **T1207429** **T1207430** **T1207431** **T1207432**	= treated samples
T1207433	= untreated sample
EMPA Cotton 100%	= Internal Centexbel Control sample

Remark : An internal Centexbel control has been assessed in order to check the growth and the behavior of the strain (internal Centexbel control : Fabric 100 % cotton 200 g/m2, EMPA Nr.221.)

The bacteria transfer has been done on the coated side of the fabric.

2.1 Results obtained with Staphylococcus aureus

Table 1 : Control of the growth value obtained on the untreated sample and on the internal Centexbel control sample on ***Staphylococcus aureus*** - Contact time : **18 hours**

Inoculum concentration : 1.6 10^6 CFU/ml

Sample identification	*Trial*	*0 contact time Number of viable cells **		*18 hours contact time Number of viable cells**	
		CFU	*Log CFU*	*CFU*	*Log CFU*
Untreated sample **T1207433**	1	4.0 10^4	4.60	5.4 10^4	4.73
	2	3.4 10^4	4.53	2.6 10^4	4.41
	3	2.2 10^4	4.34	5.8 10^4	4.76
Average (log)		4.5 ± 0.13		4.6 ± 0.19	
Growth value F		***0.1***			
Internal Centexbel control sample **EMPA Cotton 100 %**	1	5.4 10^4	4.73	4.4 10^7	7.64
	2	9.4 10^4	4.97	3.4 10^7	7.53
	3	5.8 10^4	4.76	2.6 10^7	7.41
Average (log)		4.8 ± 0.13		7.5 ± 0.12	
Growth value F		***2.7***			

**Colonies expressed per sample*

The growth value of the Internal Centexbel control sample is ≥ 1 and is comparable to the values generally obtained in the laboratory; it attests that the tests conditions were good during the test.

The untreated sample has not a normal behavior (growth value < 1), therefore, for the calculation of the antibacterial activities we have decided to compare to the Centexbel internal cotton control fabric.

Table 2 : Count of ***Staphylococcus aureus*** on the samples - Contact time : **18 hours**

Inoculum concentration : 1.6 10^6 CFU/ml

Sample identification	*Trial*	*0 contact time Number of viable cells **		*18 hours contact time Number of viable cells**	
		CFU	*Log CFU*	*CFU*	*Log CFU*
Internal Centexbel control sample **EMPA Cotton 100 %**	1	5.4 10^4	4.73	4.4 10^7	7.64
	2	9.4 10^4	4.97	3.4 10^7	7.53
	3	5.8 10^4	4.76	2.6 10^7	7.41
Average (log)		4.8 ± 0.13		7.5 ± 0.12	
Growth value F		***2.7***			
Treated T1207428	1	3.2 10^4	4.51	0**	0
	2	6.0 10^4	4.78	1.0 10^2	2.00
	3	3.2 10^4	4.51	1.4 10^2	2.15
Average (log)		4.6 ± 0.16		1.4 ± 1.2	
Growth value G		***-3.2***			
Treated T1207429	1	2.8 10^4	4.45	4.0 10^1	1.60
	2	6.2 10^4	4.79	0**	0
	3	2.6 10^4	4.41	2.0 10^1	1.30
Average (log)		4.6 ± 0.21		1.0 ± 0.85	
Growth value G		***-3.6***			
Treated T1207430	1	5.0 10^4	4.70	3.0 10^3	3.48
	2	2.8 10^4	4.45	1.5 10^3	3.18
	3	4.2 10^4	4.62	3.0 10^3	3.48
Average (log)		4.6 ± 0.13		3.4 ± 0.17	
Growth value G		***-1.2***			

Sample identification	*Trial*	*0 contact time Number of viable cells **		*18 hours contact time Number of viable cells**	
		CFU	*Log CFU*	*CFU*	*Log CFU*
Treated T1207431	1	$3.6\ 10^4$	4.56	$2.2\ 10^3$	3.34
	2	$3.0\ 10^4$	4.48	$1.4\ 10^3$	3.16
	3	$5.6\ 10^4$	4.75	$5.0\ 10^2$	2.70
Average (log)		4.6 ± 0.14		3.1 ± 0.33	
Growth value G		***-1.5***			
Treated T1207432	1	$4.2\ 10^4$	4.62	0**	0
	2	$5.2\ 10^4$	4.72	0**	0
	3	$4.2\ 10^4$	4.62	0**	0
Average (log)		4.7 ± 0.06		0	
Growth value G		***-4.7***			

**Colonies expressed per sample*
***0 signifies that no colony has been counted (should normally be written < 20 because the count is performed on 1 ml coming from the wash solution (20 ml)) When we obtain 0 colony, log(CFU) has been arbitrarily fixed to 0.*

Table 3 : Antibacterial activity value with ***Staphylococcus aureus : A = F - G***

Inoculum concentration : 1.6 10^6 CFU/ml

Sample identification	***Antibacterial activity A after 18 hours*** *(compared to the EMPA cotton 100 % - Internal control sample – 18 hours)* ***Mean*** *[Min ; Max]*
T1207428	***5.9*** *[5.2 ; 7.3]*
T1207429	***6.3*** *[5.7 ; 7.3]*
T1207430	***3.9*** *[3.8 ; 4.1]*
T1207431	***4.2*** *[4.0 ; 4.6]*
T1207432	***7.4*** *[7.4 ; 7.4]*
T1207433 - untreated sample	***2.6*** *[2.44 ; 2.79]*

Conclusion :

According to the ISO 20743 – Transfer methods all the tested samples show an antibacterial activity against *Staphylococcus aureus*.
The highest antibacterial activity value corresponds to the strongest activity.

The treated samples T1207432, T1207429 and T1207428 show a very strong activity (nearly all the bacteria are killed) whereas the samples T 1207430 and T1207431 show a less high

activity (especially if the results are compared to the untreated sample T1207433 which gives a slight antibacterial activity compared to the Internal cotton sample due to the bad growth obtained after 18 hours).

2.2 Results obtained with Methicillin Resistant Staphylococcus aureus - MRSA

Table 4 : Control of the growth value obtained on the untreated sample and on the internal Centexbel control sample on ***MRSA*** - Contact time : 18 hours

Inoculum concentration : 2.2 10^6 CFU/ml

Sample identification	*Trial*	*0 contact time Number of viable cells **		*18 hours contact time Number of viable cells**	
		CFU	*Log CFU*	*CFU*	*Log CFU*
Untreated sample **T1207433**	1	1.3 10^4	4.11	1.1 10^6	6.03
	2	2.6 10^4	4.41	7.2 10^5	5.86
	3	1.6 10^5	5.21	5.2 10^4	4.72
Average (log)		4.6 ± 0.57		5.5 ± 0.71	
Growth value F		*0.9*			
Internal Centexbel control sample **EMPA Cotton 100 %**	1	6.6 10^4	4.82	5.4 10^7	7.73
	2	7.0 10^4	4.85	4.6 10^7	7.66
	3	6.4 10^4	4.81	/	/
Average (log)		4.8 ± 0.02		7.70 ± 0.05	
Growth value F		*2.9*			

**Colonies expressed per sample*

The growth value of the Internal Centexbel control sample is ≥ 1 and is comparable to the values generally obtained in the laboratory; it attests that the tests conditions were good during the test.

The untreated sample gives very variable results and the average growth value < 1, therefore, for the calculation of the antibacterial activities we have decided to compare to the Centexbel internal cotton control fabric.

Table 5 : Count of *MRSA* on the samples - Contact time : **18 hours**

Inoculum concentration : 2.2 10^6 CFU/ml

Sample identification	*Trial*	*0 contact time Number of viable cells **		*18 hours contact time Number of viable cells**	
		CFU	*Log CFU*	*CFU*	*Log CFU*
Internal Centexbel control sample **EMPA Cotton 100 %**	1	6.6 10^4	4.82	5.4 10^7	7.73
	2	7.0 10^4	4.85	4.6 10^7	7.66
	3	6.4 10^4	4.81	/	/
Average (log)		4.8 ± 0.02		7.7 ± 0.05	
Growth value F		***2.9***			
Treated T1207428	1	1.3 10^5	5.11	0**	0
	2	3.2 10^4	4.51	0**	0
	3	2.6 10^4	4.41	1.0 10^2	2.00
Average (log)		4.7 ± 0.38		0.7 ± 1.15	
Growth value G		***-4***			
Treated T1207429	1	7.2 10^4	4.86	0**	0
	2	8.8 10^4	4.94	2.0 10^1	1.30
	3	2.0 10^4	4.30	0**	0
Average (log)		4.7 ± 0.35		0.4 ± 0.75	
Growth value G		***-4.3***			
Treated T1207430	1	2.8 10^4	4.45	5.0 10^2	2.70
	2	2.6 10^4	4.41	1.0 10^3	3.01
	3	3.0 10^4	4.48	1.6 10^3	3.20
Average (log)		4.4 ± 0.04		3.0 ± 0.25	
Growth value G		***-1.4***			
Treated T1207431	1	6.8 10^4	4.83	8.0 10^1	1.90
	2	2.0 10^4	4.29	2.8 10^2	2.45
	3	4.0 10^4	4.60	4.0 10^1	1.60
Average (log)		4.6 ± 0.27		2.0 ± 0.43	
Growth value G		***-2.6***			
Treated T1207432	1	3.8 10^4	4.58	0**	0
	2	2.2 10^4	4.34	6.0 10^1	1.78
	3	4.8 10^4	4.68	2.0 10^1	1.30
Average (log)		4.5 ± 0.17		1.0 ± 0.92	
Growth value G		***-3.5***			

**Colonies expressed per sample*

***0 signifies that no colony has been counted (should normally be written < 20 because the count is performed on 1 ml coming from the wash solution (20 ml)) When we obtain 0 colony, log(CFU) has been arbitrarily fixed to 0.*

Table 6 : Antibacterial activity value with ***MRSA: A = F - G***

Inoculum concentration : 2.2 10^6 CFU/ml

Sample identification	***Antibacterial activity A after 18 hours*** *(compared to the EMPA cotton 100 % - Internal control sample – 18 hours)* ***Mean*** *[Min ; Max]*
T1207428	***6.9*** *[5.6 ; 7.6]*
T1207429	***7.2*** *[6.3 ; 7.6]*
T1207430	***4.3*** *[4.1 ; 4.6]*
T1207431	***5.5*** *[5.1 ; 5.9]*
T1207432	***6.4*** *[5.6 ; 7.4]*
T1207433 - untreated sample	***2.0*** *[1.5 ; 2.8]*

Conclusion :

According to the ISO 20743 – Transfer methods all the tested samples show an antibacterial activity against *Methicillin Resistant Staphylococcus aureus – MRSA* .
The highest antibacterial activity value corresponds to the strongest activity.

The strongest activities are obtained by the samples T1207428, T1207429 and T1207432.
The samples T1207430 and T1207431 seem to have a less strong activity but that activity is still important.

Performed in the microbiological lab under the responsibility of Yvette Rogister

Reference: **T1207428 - PU-120**
T1207429 - PU-150
T1207430 - PU-170
T1207431 - PU-210
T1207432 - PU-210 PA

Standard Practice for determining resistance of Synthetic Polymeric Materials to Fungi

Date of ending the test 19-07-2012
Standard used ASTM G21 (2009)

1. Method

Standard followed: ASTM G 21 : 2009 – Standard Practice for determining resistance of Synthetic Polymeric Materials to Fungi

Description of the method :

The test specimens are exposed on an incomplete nutritive medium (agar medium without a carbon source) to a standardized mixed spore suspension of the test fungi. The test fungi can only grow at the expense of the material. After incubation at 28 to 30°C and not less than 85% relative humidity for 28 days, the fungi growth on the specimen is evaluated by growth rating (visual and microscopic examination).

Fungal growth on test specimens shall be assessed according to the evaluation scheme shown in table 1.

Table 1 : Visual assessment

Observed growth on specimens (sporulating or non-sporulating, or both)	
Evaluation	***Growth rating***
None	0
Traces of growth (less than 10%)	1
Light growth (10 to 30%)	2
Medium growth (30 to 60%)	3
Heavy growth (60% to complete coverage)	4

2. Results

Dimensions and number of specimens :
6 specimens of 5 cm x 5 cm.

Tested side :
Coated side

Mixed spores suspension of the test fungi :

- *Aspergillus niger* ATCC 9642
- *Penicillium pinophilum* ATCC 11797
- *Aureobasidium pullulans* ATCC 18556
- *Gliocladium virens* ATCC 9645
- *Chaetomium globosum* ATCC 6205

Each suspension is adjusted at ± 1 10^6 CFU/ml and mix to obtain the mixed spores suspension. During the mixing an equal volume of each fungus is used.

Inoculation :

- 0.15 to 0.2 ml of the mixed spores suspension of the test fungi are evenly distributed (by spraying) on the sample and the agar in a plastic Petri dish (diameter of the Petri dish : ± 9 cm)
- 1 sample per Petri dish

Incubation time : 28 days

Incubation conditions : 28 ± 2° C - > 85% RH

Evaluation :

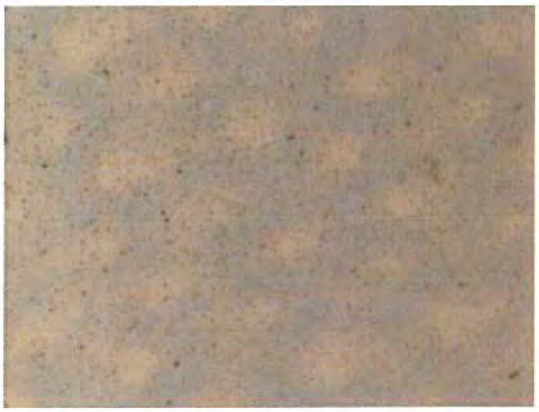
Fungal growth on test specimens has been assessed according to the evaluation scheme shown in table 1. Results are mentioned in the table 2.

Table 2 : Visible effects - Results obtained with the samples - Assessment after an incubation of **28 days**.

Sample identification	*Specimen*	*Growth rating**
Cellulose paper filter (control)	1	4
	2	4
T1207428	1	1
	2	1
	3	1
	4	1
	5	1
	6	1
T1207429	1	1
	2	1
	3	1
	4	1
	5	1
	6	1
T1207430	1	1
	2	1
	3	1
	4	1
	5	1
	6	1
T1207431	1	1
	2	1
	3	1
	4	1
	5	1
	6	1
T1207432	1	1
	2	2
	3	2
	4	2
	5	2
	6	2

-For the cellulose paper filter a copious growth was already observed after 14 days incubation (grade 3) → Validation of the test

Illustration of the results

	
T1207428 Magnification : 30 X - Growth rating 1	**T1207429** Magnification : 30 X - Growth rating 1
	
T1207430 Magnification : 30 X - Growth rating 1	**T1207431** Magnification : 30 X - Growth rating 1

Performed in the microbiological lab under the responsibility of Yvette Rogister.

	
T1207432 Magnification : 30 X - Growth rating 1	**T1207432** Magnification : 30 X - Growth rating 2

Conclusion :

According to the ASTM G21 the fungal growth is very limited on all the tested samples.

Traces of growth are observed under the binocular at magnification 30 for the samples T1207428 to T1207431 (growth rating 1). Some areas of the sample T1207432 show a growth slightly superior (growth rating = 2).

Performed in the microbiological lab under the responsibility of Yvette Rogister.

Reference: **T1207428 - PU-120**

Section 3 – Individual bedcovers - Ignition source no. 5

Date of ending the test 15-06-2012
Standard used BS 7175 (1989)

Deviation from the standard -

Conditioning 20°C, relative humidity 65%

The following test results relate only to the ignitability of the combination of materials under the particular conditions of test ; they are not intended as a means of assessing the full potential fire hazard of the materials in use.

Specimen Mattress cover
Testing substrate Mineral wool fibre pad

crib 5 positioned above the test specimen

	1	2
Unsafe escalating combustion	no	no
Test assembly consumed	no	no
Smouldering	no	no
Flames to extremities	no	no
Max destroyed distance (mm)	115	120
Flame time >10 min	no	no
Flame time	2 min 50 s	2 min 42 s
	non-ignition	non-ignition

Conclusion **Non-ignition**

Performed in the fire lab under the responsibility of Pros Van Hoeyland

Reference: **T1207429 - PU-150**

Section 3 – Individual bedcovers - Ignition source no. 5

Date of ending the test 15-06-2012
Standard used BS 7175 (1989)

Deviation from the standard -

Conditioning 20°C, relative humidity 65%

The following test results relate only to the ignitability of the combination of materials under the particular conditions of test ; they are not intended as a means of assessing the full potential fire hazard of the materials in use.

Specimen Mattress cover
Testing substrate Mineral wool fibre pad

crib 5 positioned above the test specimen

	1	2
Unsafe escalating combustion	no	no
Test assembly consumed	no	no
Smouldering	no	no
Flames to extremities	no	no
Max destroyed distance (mm)	120	110
Flame time >10 min	no	no
Flame time	2 min 46 s	3 min 01 s
	non-ignition	non-ignition

Conclusion **Non-ignition**

Performed in the fire lab under the responsibility of Pros Van Hoeyland

Reference: **T1207430 - PU-170**

Section 3 – Individual bedcovers - Ignition source no. 5

Date of ending the test 15-06-2012
Standard used BS 7175 (1989)

Deviation from the standard -

Conditioning 20°C, relative humidity 65%

The following test results relate only to the ignitability of the combination of materials under the particular conditions of test ; they are not intended as a means of assessing the full potential fire hazard of the materials in use.

Specimen Mattress cover
Testing substrate Mineral wool fibre pad

crib 5 positioned above the test specimen

	1	2
Unsafe escalating combustion	no	no
Test assembly consumed	no	no
Smouldering	no	no
Flames to extremities	no	no
Max destroyed distance (mm)	110	120
Flame time >10 min	no	no
Flame time	3 min 55 s	3 min 54 s
	non-ignition	non-ignition

Conclusion **Non-ignition**

Performed in the fire lab under the responsibility of Pros Van Hoeyland

Reference: **T1207431 - PU-210**

Section 3 – Individual bedcovers - Ignition source no. 5

Date of ending the test 15-06-2012
Standard used BS 7175 (1989)

Deviation from the standard -

Conditioning 20°C, relative humidity 65%

The following test results relate only to the ignitability of the combination of materials under the particular conditions of test ; they are not intended as a means of assessing the full potential fire hazard of the materials in use.

Specimen Mattress cover
Testing substrate Mineral wool fibre pad

crib 5 positioned above the test specimen

	1	2
Unsafe escalating combustion	no	no
Test assembly consumed	no	no
Smouldering	no	no
Flames to extremities	no	no
Max destroyed distance (mm)	110	140
Flame time >10 min	no	no
Flame time	3 min 48 s	4 min 57 s
	non-ignition	non-ignition

Conclusion **Non-ignition**

Performed in the fire lab under the responsibility of Pros Van Hoeyland

Reference: **T1207432 - PU-210 PA**

Section 3 – Individual bedcovers - Ignition source no. 5

Date of ending the test 15-06-2012
Standard used BS 7175 (1989)

Deviation from the standard -

Conditioning 20°C, relative humidity 65%

The following test results relate only to the ignitability of the combination of materials under the particular conditions of test ; they are not intended as a means of assessing the full potential fire hazard of the materials in use.

Specimen Mattress cover
Testing substrate Mineral wool fibre pad

crib 5 positioned above the test specimen

	1	2
Unsafe escalating combustion	no	no
Test assembly consumed	no	no
Smouldering	no	no
Flames to extremities	no	no
Max destroyed distance (mm)	145	175
Flame time >10 min	no	no
Flame time	3 min 41 s	5 min 05 s
	non-ignition	non-ignition

Conclusion **Non-ignition**

Performed in the fire lab under the responsibility of Pros Van Hoeyland