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EVALUATION OF THE VIRUCIDAL ACTIVITY OF PLASTIC PRODUCTS ACCORDING TO A METHOD BASED UPON JIS Z 2801: 2000 AND JIS Z 2801: 2006

Test report written by: Dr Marlène RICHARD

Marseilles: July 23rd 2009

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I : DESCRIPTION OF THE STUDY:

Title:	Evaluation of the virucidal activity of plastic products according to a method based upon JIS Z 2801: 2000 and JIS Z 2801: 2006.
Study n°:	546.LEG.07
Sponsor:	LEGRAND 128, av. du Maréchal de Lattre de Tassigny 87045 Limoges Cedex *Contact:* Mr. HASBROUCK
Test period:	From 24/04/2008 to 06/11/2008.
Study manager:	Dr Marlène RICHARD
Test laboratory:	Laboratoire FONDEREPHAR Laboratoire de bactériologie, virologie et Microbiologie Industrielle

II : AIM OF THE STUDY:

Determine, according to the experimental conditions described in the standards JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2], the ability of plastic products to reduce in 24 hours at 35±1°C of at least 10^2 times the number of infectious unit of *Enterovirus type I.*

III : MATERIEL:

a) Test pieces:

PC R903B test pieces
ABS test pieces
Polypropylene test pieces
SEBS test pieces

According to the experimental conditions described in the standards JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2], 3 antimicrobial test pieces and 6 untreated test pieces are necessary to evaluate the virucidal activity for each microorganism tested.

b) Virus strain :

Enterovirus type I: ----------AFSSAPS batch n° NIBSC 01/528

Maintaining and culture conditions of viral strains are those specified in the European standard NF EN 14476+ A1: 2007.

c) **Cells culture:**

VERO cells----------ATTC CLL-81, batch n° 3372621

The method of cell culture is consistent with the requirements of the European standard NF EN 14476 + A1: 2007.

d) **Diluent, culture medium and recovery solution:**

MEM containing 2% fetal calf serum (FCS).

IV : METHODE :

a) **Viral suspension:**

The titre of the virus suspension is adjusted between 5.6 and 6.0 log $TCID_{50}$ in MEM culture medium containing 2% FCS.

b) **Test pieces contamination:**

For each plastic product, the test pieces i.e 3 antimicrobial test pieces and 6 untreated test pieces are placed in a sterilized Petri dish with the test surface uppermost. 480 µl of the test suspension are deposited onto each test pieces and then cover by two cover glasses (50 x 50 mm) in order to spread the inoculum on a 2500 mm^2 surface.

c) **Incubation of the inoculated test pieces:**

After contamination, 3 antimicrobial test surfaces and 3 out of 6 untreated surfaces are incubated at 35±1°C for 24±1 hours. In parallel, the 3 other untreated test pieces are immediately washed out in order to determine the initial contamination level of the test pieces.

d) **Determination of the contamination level of the test pieces:**

Immediately after contamination or after incubation, 10 ml of the recovery solution (MEM + 2% FCS) were added over the bottom of the test surface. The surface of the test piece is then gentle scrapped with a cell scraper to resuspend the virus film.

Viruses are titrated according to the method described in the European standard NF EN 14476 + A1: 2007. Fourfold dilutions of the virus/recovery solution mixture are carried out in MEM + 2% FCS using glass test tubes in order to avoid virus adsorption on surfaces. Viruses are titrated on cells in suspension on microtiter plates.

Cytopathic effect is determined after 48 hours of incubation at 37°C and the estimated number of infectious units is determined by the Spaerman-Karber method by calculating the negative logarithm of the 50% titration endpoint for infectivity (lgTCID50) by the following formula:

$lgTCID_{50}$ = Negative logarithm of the highest virus concentration – [(sum of percentages affected at each dilution/100 – 0,5) x (log of the dilution)].

e) Test conditions :

According to JIS Z 2801: 2000[1], the tests are satisfactory if the three following test conditions are met:

i) The number of viable viruses on test pieces immediately after contamination from the untreated test pieces shall satisfy the following requirement:

$$(T_{max} - T_{min})/(T_{mean}) \leq 0,2$$

Where T_{max} : maximum logarithm of the number of viable viruses on untreated test pieces;

T_{min} : minimum logarithm of the number of viable viruses on untreated test pieces;

T_{mean} : average of the logarithm of the number of viable viruses on three untreated test pieces.

ii) Average of the number of viable viruses immediately after contamination of untreated test pieces shall be within the range of $1.0x10^5$ (5,0 log_{10}) and $4.0x10^5$ IU/test piece (5,6 log_{10})

iii) The number of viable viruses on an untreated test piece after 24 hours shall not be less than $1.0x10^4$ IU/test piece (4 log_{10})

f) Expression of virucidal activity:

When the test conditions are validated, the virucidal activity of the antimicrobial test pieces is calculated as follows:

$$R = [\log (B/A) - \log (C/A)] = [\log (B/C)]$$

With R: Value of virucidal activity;

A: Average number of viral infectious units 50% ($TCID_{50}$) immediately after contamination of untreated test pieces;

B: Average number of viral infectious units 50% ($TCID_{50}$) after 24 hours on untreated test pieces;

C: Average number of viral infectious units 50% ($TCID_{50}$) after 24 hours on the antimicrobial test pieces

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V : RESULTS:

a) Validation of test conditions :

Table I : Validation of the number of viable viruses immediately after contamination on untreated test pieces: $(T_{max} - T_{min})/T_{mean}) \leq 0,2$; T_{max}: maximum logarithm of the number of viable viruses on untreated test pieces; T_{min}: minimum logarithm of the number of viable viruses on untreated test pieces and T_{mean}: average of the logarithm of the number of viable viruses on three untreated test pieces

Plastic products	Assay 1 (T_{max})	Assay 2 (T_{min})	Assay 3	T_{mean}	$(T_{max} - T_{min})/(T_{mean})$
PC R903B	5.4	5.1	5.3	5.3	0.06
ABS	5.3	5.1	5.2	5.2	0.04
Polypropylene	5.1	5.1	5.1	5.1	0.00
SEBS	5.0	5.1	5.0	5.0	0.02

For each plastic product:

- ✓ $(T_{max} - T_{min})/(T_{meann})$ is lower than 0,2 (see table I) ;
- ✓ The average of the logarithm of the number of viable *Enterovirus type 1* viruses immediately after contamination of untreated test pieces are within the range of 5.0 log_{10} ($1.0x10^5$ IU/test piece) and 5.6 log_{10} ($4.0x10^5$ IU/test piece).

Test conditions are therefore validated for each plastic product tested.

b) Main tests :

Table II : Evaluation of the virucidal activity of plastic products against Enterovirus type I for a 24±1 hours contact time at 35±1°C. Log A : Log $TCID_{50}$ immediately after contamination on untreated test pieces ; log B : Log $TCID_{50}$ after 24 hours contact time on untreated test pieces ; log C : Log $TCID_{50}$ after 24 hours contact time on antimicrobial test pieces ; R : virucidal ativity.

Plastic products	Log A	Log B	Log C	R [Log B – Log C)]
PC R903B	5,3	4,6	3,6	1,0
ABS	5,2	4,2	4,0	0,2
Polypropylene	5,1	4,1	4,1	0,0
SEBS	5,0	4,1	3,9	0,2

According to the results presented in table II, a slight reduction of the number of unfectious units of *Enterovirus type 1* is observed ($0.0 \leq R \leq 1.0$)after a 24 hours contact time.

For the PC R903B plastic product, the viral titre reduction is not zero (1.0 log_{10}) but remains below the standards requirements (2 log_{10}).

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VI : CONCLUSIONS :

In the test conditions described in this test report, PC R903B, ABS, polypropylene and SEBS plastic products did not show a virucidal activity consistent with the JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2] requirements (i.e reduction of at least 10^2 times of the number of infectious unit deposited on test pieces for a 24 hours contact time at 35±1°C) when the virus strain is *Enterovirus type 1*.

Although virucidal activities observed are below the standard requirements, a slight virucidal activity is observed for the PC R903B plastic product with a reduction of 1.0 log_{10}.

VII : REFERENCES :

1. JIS Z 2801: 2000 – Antimicrobial products: Test for antimicrobial activity and efficacy.
2. JIS Z 2801 : 2006 – Antimicrobial products : Test for antimicrobial activity and efficacy, Amendment 1
3. NF EN 12353: 2006 – Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity.

Marlène RICHARD
Lab deputy manager
Signature _____ Date 23/07/2009

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Managing director
Signature _____ Date 23/07/2009

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Test report written by: Dr Marlène RICHARD

Marseilles: July 23rd 2009

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I : DESCRIPTION OF THE STUDY:

Title:	Evaluation of the antimicrobial activities of plastic products according to a method based upon JIS Z 2801: 2000 and JIS Z 2801: 2006.
Study n°:	546.LEG.07
Sponsor:	LEGRAND 128, av. du Maréchal de Lattre de Tassigny 87045 Limoges Cedex *Contact:* Mr. HASBROUCK
Test period:	From 11/02/2008 to 28/11/2008.
Study manager:	Dr Marlène RICHARD
Tests done by:	Isabelle SEVEROVIC
Test laboratory:	Laboratoire BIOTECH-GERMANDE Parc Scientifique de Luminy 163 Avenue de Luminy – Case 927 13288 Marseille Cedex 9

II : AIM OF THE STUDY:

Determine, according to the experimental conditions described in the standards JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2], the ability of plastic products to reduce within 24 hours at 35±1°C of at least 10^2 times the number of viable microorganisms deposited on test pieces.

III : MATERIAL:

a) Test pieces:

PC R903B test pieces
ABS test pieces
Polypropylene test pieces
SEBS test pieces

According to the experimental conditions described in the standards JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2], 3 antimicrobial test pieces and 6 untreated test pieces are necessary to evaluate the antimicrobial activity for each microorganism tested.

b) Microbial strains :

i) PC R903B and ABS surfaces:

The antimicrobial activity of PC R903B and ABS plastic products are evaluated against the 9 following microbial strains:

Pseudomonas aeruginosa CIP 103467
Staphylococcus aureus CIP 4.83
Escherichia coli CIP 54127
Candida albicans CIP 4872
Aspergillus niger CIP 1431.83 spores
Mycobacterium terrae CIP 104321
Mycobacterium avium CIP 105415
Bacillus cereus CIP 105151 spores
Bacillus subtilis CIP 52.62 spores

ii) Polypropylene and SEBS surfaces:

The antimicrobial activity of polypropylene and SEBS plastic products are evaluated against the 4 following microbial strains:

Pseudomonas aeruginosa CIP 103467
Staphylococcus aureus CIP 4.83
Escherichia coli CIP 54127
Mycobacterium terrae CIP 104321

The conditions of preservation of the microbial strains used for the determination of the bactericidal, mycobactericidal, sporicidal and fungicidal activities are those described in the European standard NF EN 12353: 2006[3].

c) Maintaining and counting medium:

For *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83, *Escherichia coli* CIP 54127, *Bacillus subtilis* CIP 52.62 spores and *Bacillus cereus* CIP 105151 spores:
Trypticase soja agar:
TS agar (BIOMERIEUX, 51044)----40 g
Distilled water: ----1000 ml
Steam sterilized at 121°C for 21 minutes.

For *Mycobacterium terrae* CIP 104321 and *Mycobacterium avium* CIP 105415:
7H10 agar
Mycobacteria 7H10 agar (Difco A5062717)----21 g
Glycerol (Sigma G5150)----5 ml
Middle Brook OADC Enrichment (Difco A5701886)----10% (v/v)
Distilled water: ----1000 ml
Steam sterilized at 121°C for 21 minutes.

For *Candida albicans* CIP 4872 and *Aspergillus niger* CIP 1431.83 spores:
Malt extract agar:
Soja peptone(SIGMA P-1265) :----3 g
Malt ectract (SIGMA M0383) : ----30g
Agar (SIGMA A5306) : ----15g
Distilled water: ----1000 ml
Steam sterilized at 121°C for 21 minutes.

d) Recovery solution:

Tween 80 (SIGMA P17-54) : ---------- 50 ml
Sodium thiosulfate (SIGMA S85-03) : ---------- 10g
Saponin (SIGMA S79-00) : ---------- 5g
Lecithin (SIGMA P53-94) : ---------- 10g
Trypticase soja broth: ---------- 500 ml
Steam sterilized at 121°C for 21 minutes.

e) Diluent :

Test suspensions are prepared in phosphate buffer 100 mM, pH 7.0 (III.e.ii).

i) Stock solution:

Phosphate buffer 10X (1000mM, pH 7.0):
Dibasic sodium phosphate, Na_2HPO_4 $7H_2O$: ---------- 144.5g
Monobasic potassium phosphate, KH_2PO_4: ---------- 71.2g
Distilled water: ---------- 1000 ml
Steam sterilized at 121°C for 21 minutes.

ii) Working solution:

Phosphate buffer 1X (100mM, pH 7.0):
Phosphate buffer 10X (1000mM, pH 7.0) : ---------- 100 ml
Distilled water: ---------- 1000 ml

IV : METHOD :

a) Test suspensions :

For each microorganism, the test suspension is prepared in the diluent (see III.e) and adjusted in order to contain between $2.5x10^5$ and $10x10^5$ cfu/ml, according to the JIS Z 2801: 2000[(1)] requirements. The population in the original inoculum (Tc) is counted for each assay using a validated and specific method for each microorganism.

b) Test pieces contamination:

For each microorganism, test pieces (i.e 3 antimicrobial test pieces and 6 untreated test pieces), are placed in a sterile Petri dish with the test surface uppermost. 400 µl of the test suspension are deposited onto each test pieces and then cover by two cover glasses (22 x 40 mm) in order to spread the test suspension on a 1760 mm^2 surface.

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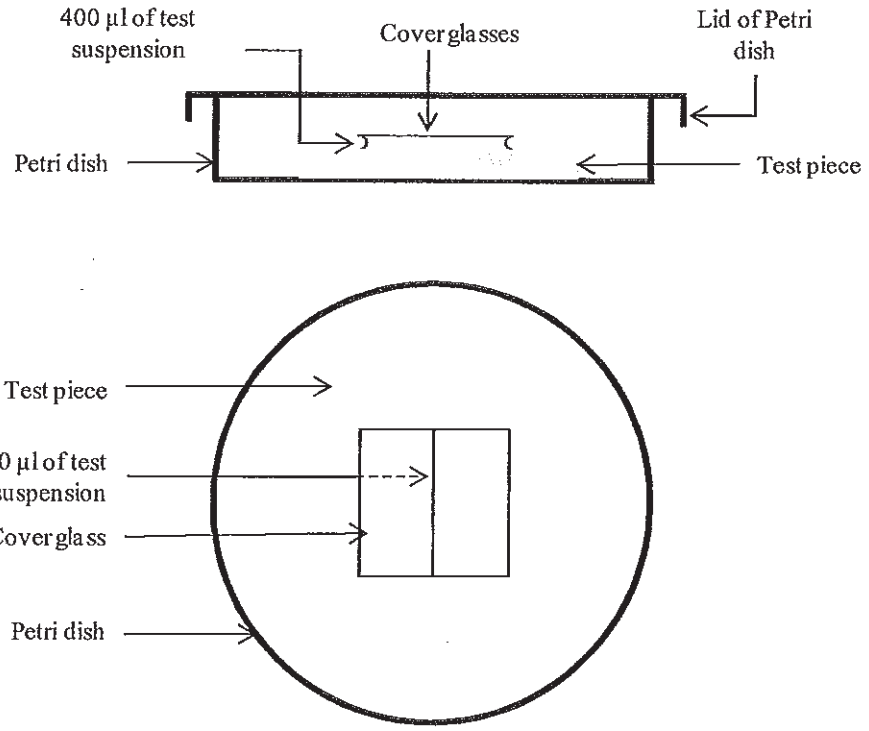

Figure 1 : Instillation of inoculums onto the test piece and coverglasses

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c) Incubation of the inoculated test pieces:

After contamination, 3 antimicrobial test surfaces and 3 out of 6 untreated surfaces are incubated at 35±1°C for 24±1 hours. In parallel, the 3 other untreated test pieces are immediately washed out in order to determine the initial contamination level of the test pieces (see IV.d).

d) Determination of the contamination level of the test pieces:

Immediately after contamination or after 24±1 hours of incubation of the test pieces, the residual number of viable microorganisms remaining on the test pieces are determined. For this, in a first step, the cover glasses are carefully removed from the surface and transferred into a test tube containing 10 ml of recovery solution and approximately 1 ml of glass beads from 0.25 to 0.50 mm in diameter. The test tubes containing the cover glasses are submitted to a manual agitation in order to remove the microorganisms from the surface. In a second step, the surface test piece is recovered with 5 ml of recovery solution and then scraped with a cell scraper and rinsed with additional 5 ml of recovery solution. The volume of the recovery solution containing the microorganisms collected in the Petri dish is transferred into the test tube containing the corresponding cover glasses. The number of viable microorganisms present per milliliter of reaction mixture is determined by successive tenfold dilution and inclusion of 1ml of each dilution in the counting medium specific of the test microorganism.
After incubation at the specific temperature and time of the test microorganism, the colonies are counted and the results are expressed as the number of cfu per test piece.

e) Test conditions :

According to JIS Z 2801: 2000[(1)], the tests are satisfactory if the three following test conditions are met:

i) Logarithmic value of the number of viable microorganisms immediately after contamination on untreated test pieces:

$$(L_{max} - L_{min})/(L_{mean}) \leq 0,2$$

With L_{max}: Maximum logarithm of the number of viable microorganisms on untreated test pieces;

L_{min}: Minimum logarithm of the number of viable microorganisms on untreated test pieces;

L_{mean}: Average of the logarithm of the number of viable microorganisms on three untreated test pieces.

ii) The average of the number of viable microorganisms immediately after contamination of untreated test pieces shall be within the range of $1.0x10^5$ (5,0 log_{10}) and $4.0x10^5$ cfu/test piece (5,6 log_{10}).

iii) The number of viable microorganisms on an untreated test piece after 24 hours shall not be less than $1.0x10^4$ cfu/test piece (4 log).

f) Expression of antimicrobial activity :

When the test conditions are validated, the antimicrobial activity of the antimicrobial test pieces is calculated as follows:

$$R = [\log (B/A) - \log (C/A)] = [\log (B/C)]$$

With R: Value of antimicrobial activity;

A: average of the number of viable microorganisms immediately after contamination on the untreated test pieces;

B: average of the number of viable microorganisms after 24 hours on the untreated test pieces;

C: average of the number of viable microorganisms after 24 hours on the antimicrobial test pieces.

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V : RESULTS :

a) PC R903B plastic products:

i) Test conditions validation:

Table I : Validation of the number of viable microorganisms immediately after contamination on untreated PC R903B test pieces: $(L_{max} - L_{min})/(L_{mean}) \leq 0,2$; L_{max}: maximum logarithm of the number of viable microorganisms on untreated test pieces; L_{min}: minimum logarithm of the number of viable microorganisms on untreated test pieces and L_{mean}: average of the logarithm of the number of viable microorganisms on three untreated test pieces.

	Assay 1 (L_{max})	Assay 2 (L_{min})	Assay 3	L_{mean}	$(L_{max} - L_{min})/(L_{mean})$
Pseudomonas aeruginosa CIP 103467	5.62	5.52	5.53	5.56	0.02
Staphylococcus aureus CIP 4.83	5.49	5.23	5.26	5.34	0.05
Escherichia coli CIP 54127	5.30	4.45	5.26	5.00	0.17
Mycobacterium terrae CIP 104321	5.67	5.23	5.40	5.47	0.08
Mycobacterium avium CIP 105415	5.83	5.57	5.65	5.70	0.05
Candida albicans CIP 4872	5.84	5.16	5.40	5.56	0.12
Aspergillus niger CIP 1431.83 spores	5.18	5.00	5.18	5.12	0.03
Bacillus cereus CIP 105151 spores	5.24	5.17	5.22	5.21	0.01
Bacillus subtilis CIP 52.62 spores	5.32	5.26	5.26	5.28	0.01

For each test microorganism:

- ✓ $(L_{max} - L_{min})/(L_{meann})$ is lower than 0,2 (table I);
- ✓ The average of the number of viable microorganisms immediately after contamination of untreated PC R903B test pieces is within the range of $1.0x10^5$ and $4.0x10^5$ cfu/test piece for each test microorganism with the exception of *Mycobacterium avium* CIP 105415. For this microorganism, the contamination level is slightly higher than the upper allowed limit ($5.0x10^5$ cfu/test piece instead of $4.0x10^5$ cfu/test piece). However, this value does not affect the validity of the test conditions.

The test conditions are therefore validated for each microorganism.

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ii) Main test:

Table II : Evaluation of the antimicrobial activity of PC R903B plastic products against each test microorganism for a 24±1 hours contact time at 35±1°C. A: average of the number of viable microorganisms immediately after contamination on the untreated test pieces; B: average of the number of viable microorganisms after 24 hours on the untreated test pieces; C: average of the number of viable microorganisms after 24 hours on the antimicrobial test pieces and R: Value of antimicrobial activity.

	A (Nb. cfu/test piece)	**B** (Nb. cfu/test piece)	**C** (Nb. cfu/test piece)	**R** [Log (B/C)]
Pseudomonas aeruginosa CIP 103467	$3.6x10^5$	$1.0x10^7$	$4.0x10^2$	**4.40**
Staphylococcus aureus CIP 4.83	$2.2x10^5$	$2.4x10^7$	$5.0x10^4$	**2.67**
Escherichia coli CIP 54127	$1.4x10^5$	$3.3x10^7$	$< 1.4x10^1$	**> 6.37**
Mycobacterium terrae CIP 104321	$3.0x10^5$	$9.9x10^5$	$6.0x10^4$	1.21
Mycobacterium avium CIP 105415	$5.0x10^5$	$3.6x10^5$	$3.2x10^5$	0.04
Candida albicans CIP 4872	$3.6x10^5$	$6.2x10^5$	$8.5x10^4$	0.86
Aspergillus niger CIP 1431.83 spores	$1.3x10^5$	$1.1x10^5$	$1.4x10^5$	0.00
Bacillus cereus CIP 105151 spores	$1.6x10^5$	$9.4x10^4$	$8.3x10^4$	0.05
Bacillus subtilis CIP 52.62 spores	$1.9x10^5$	$1.5x10^5$	$1.3x10^5$	0.09

For each microorganism, the average of the number of viable microorganisms after 24 hours on the untreated test pieces is higher than $1.0x10^4$ cfu/test piece (table II, B). The test conditions are therefore validated.

According to the results presented in table II, the antimicrobial activity of the PC R903B plastic product (cf. table II, C) leads to a reduction of at least 10^2 times the number of viable microorganisms when the test microorganisms are: *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83 and *Escherichia coli* CIP 54127. For those three strains, the logarithmic reductions are respectively 4.4 $\log_{10}$, 2.7 $\log_{10}$ and > 6.4 $\log_{10}$.

For *Mycobacterium terrae* CIP 104321 and *Candida albicans* CIP 4872, the reduction of the number of viable microorganisms is higher than 0.5 $\log_{10}$ (respectively 1.2 $\log_{10}$ and 0.86 $\log_{10}$) but remains below standards requirements (2 $\log_{10}$).

For the other test microorganisms (*Mycobacterium avium* CIP 105415, *Aspergillus niger* CIP 1431.83 spores, *Bacillus cereus* CIP 105151 spores and *Bacillus subtilis* CIP 52.62 spores), the reductions of the number of viable microorganisms related to the antimicrobial activity are lower than 0.1 $\log_{10}$.

b) ABS plastic products:

i) Test conditions validation:

Table III : Validation of the number of viable microorganisms immediately after contamination on untreated ABS test pieces: $(L_{max} - L_{min})/(L_{mean}) \leq 0,2$; L_{max}: maximum logarithm of the number of viable microorganisms on untreated test pieces; L_{min}: minimum logarithm of the number of viable microorganisms on untreated test pieces and L_{mean}: average of the logarithm of the number of viable microorganisms on three untreated test pieces.

	Assay 1 (L_{max})	Assay 2 (L_{min})	Assay 3	L_{mean}	$(L_{max} - L_{min})/(L_{mean})$
Pseudomonas aeruginosa CIP 103467	5.24	5.22	5.24	5.23	0.01
Staphylococcus aureus CIP 4.83	5.64	5.44	5.61	5.56	0.03
Escherichia coli CIP 54127	5.52	5.21	5.30	5.34	0.06
Mycobacterium terrae CIP 104321	5.53	5.37	5.44	5.45	0.03
Mycobacterium avium CIP 105415	5.99	5.55	5.86	5.80	0.10
Candida albicans CIP 4872	4.86	4.84	4.84	4.85	0.01
Aspergillus niger CIP 1431.83 spores	4.83	4.73	4.81	4.79	0.02
Bacillus cereus CIP 105151 spores	4.95	4.86	4.91	4.90	0.02
Bacillus subtilis CIP 52.62 spores	4.81	4.72	4.74	4.76	0.02

For each test microorganism:

- ✓ $(L_{max} - L_{min})/(L_{meann})$ is lower than 0,2 (table III);
- ✓ The average of the number of viable microorganisms immediately after contamination of untreated ABS test pieces is within the range of $1.0x10^5$ and $4.0x10^5$ cfu/test piece for *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83, *Escherichia coli* CIP 54127 and *Mycobacterium terrae* CIP 104321. For *Mycobacterium avium* CIP 105415, the contamination level is slightly higher than the upper allowed limit ($6.9x10^5$ cfu/test piece instead of $4.0x10^5$ cfu/test piece). For *Candida albicans* CIP 4872, *Aspergillus niger* CIP 1431.83spores, *Bacillus cereus* CIP 105151 spores and *Bacillus subtilis* CIP 52.62 spores, the average number of viable microorganisms immediately after contamination of untreated test pieces is slightly lower than the minimal recommended value ($5.7x10^4$ cfu/test piece for *Bacillus subtilis* CIP 52.62 instead of $4.0x10^5$ cfu/test piece). These slight deviations from JIS Z 2801: 2000 requirements do not affect the validity of the test conditions since for each test microorganism, the average number of viable microorganisms found on the untreated test pieces after 24 hours incubation is greater than $1.0x10^4$ cfu/test piece (table IV, B).

The test conditions are therefore validated for each microorganism.

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ii) Main test :

Table IV : Evaluation of the antimicrobial activity of ABS plastic products against each test microorganism for a 24±1 hours contact time at 35±1°C. A: average of the number of viable microorganisms immediately after contamination on the untreated test pieces; B: average of the number of viable microorganisms after 24 hours on the untreated test pieces; C: average of the number of viable microorganisms after 24 hours on the antimicrobial test pieces and R: Value of antimicrobial activity.

	A (Nb. cfu/test piece)	**B** (Nb. cfu/test piece)	**C** (Nb. cfu/test piece)	**R** [Log (B/C)]
Pseudomonas aeruginosa CIP 103467	$1.7x10^5$	$1.1x10^7$	< 14	**> 5.88**
Staphylococcus aureus CIP 4.83	$3.7x10^5$	$1.7x10^5$	$1.6x10^1$	**4.02**
Escherichia coli CIP 54127	$2.5x10^5$	$8.5x10^5$	< 14	**> 4.80**
Mycobacterium terrae CIP 104321	$2.8x10^5$	$2.1x10^5$	$8.7x10^3$	1.45
Mycobacterium avium CIP 105415	$6.9x10^5$	$1.0x10^5$	$2.3x10^3$	1.61
Candida albicans CIP 4872	$7.0x10^4$	$1.1x10^5$	$8.2x10^3$	1.11
Aspergillus niger CIP 1431.83 spores	$6.2x10^4$	$9.0x10^4$	$8.2x10^4$	0.04
Bacillus cereus CIP 105151 spores	$8.1x10^4$	$5.1x10^4$	$1.9x10^4$	0.44
Bacillus subtilis CIP 52.62 spores	$5.7x10^4$	$4.7x10^4$	$3.9x10^4$	0.13

According to the results presented in table IV, the antimicrobial activity of the ABS plastic product (cf. table IV, C) leads to a reduction of at least 10^2 times the number of viable microorganisms when the test microorganisms are: *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83 and *Escherichia coli* CIP 54127. For those three strains, the logarithmic reductions are respectively >5.88 log_{10}, 4.02 log_{10} and > 4.80 log_{10}.

For *Mycobacterium terrae* CIP 104321, *Mycobacterium avium* CIP 105415 and *Candida albicans* CIP 4872, the reduction of the number of viable microorganisms is higher than 1.0 log_{10} (respectively 1.45 log_{10}, 1.61 log_{10} et 1.11 log_{10}) but remains below standards requirements (2 log_{10}).

For the other test microorganisms (*Aspergillus niger* CIP 1431.83spores, *Bacillus cereus* CIP 105151 spores and *Bacillus subtilis* CIP 52.62 spores) the reductions of the number of viable microorganisms related to the antimicrobial activity are lower than 0.5 log_{10}.

c) Polypropylene plastic products:

i) Test conditions validation:

Table V : Validation of the number of viable microorganisms immediately after contamination on untreated polypropylene test pieces: $(L_{max} - L_{min})/(L_{mean}) \leq 0,2$; L_{max}: maximum logarithm of the number of viable microorganisms on untreated test pieces; L_{min}: minimum logarithm of the number of viable microorganisms on untreated test pieces and L_{mean}: average of the logarithm of the number of viable microorganisms on three untreated test pieces.

	Assay 1 (L_{max})	Assay 2 (L_{min})	Assay 3	L_{mean}	$(L_{max} - L_{min})/(L_{mean})$
Pseudomonas aeruginosa CIP 103467	5.20	5.16	5.19	5.19	0.01
Staphylococcus aureus CIP 4.83	5.41	5.30	5.39	5.37	0.02
Escherichia coli CIP 54127	5.43	5.36	5.37	5.39	0.01
Mycobacterium terrae CIP 104321	5.07	4.69	4.99	4.92	0.08

For each test microorganism:

- ✓ $(L_{max} - L_{min})/(L_{meann})$ is lower than 0,2 (table V) ;
- ✓ The average of the number of viable microorganisms immediately after contamination of untreated polypropylene test pieces is within the range of $1.0x10^5$ and $4.0x10^5$ cfu/test piece for *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83 and *Escherichia coli* CIP 54127. For *Mycobacterium terrae* CIP 104321, the average number of viable microorganisms immediately after contamination of untreated test pieces is slightly lower than the minimal recommended value ($8.8x10^4$ cfu/test piece instead of $1.0x10^5$ cfu/test piece). These slight deviations from JIS Z 2801: 2000 requirements do not affect the validity of the test conditions since for each test microorganism, the average number of viable microorganisms found on the untreated test pieces after 24 hours incubation is greater than $1.0x10^4$ cfu/test piece (table VI, B).

The test conditions are therefore validated for each microorganism.

ii) Main test :

Table VI : Evaluation of the antimicrobial activity of polypropylene plastic products against each test microorganism for a 24±1 hours contact time at 35±1°C. A: average of the number of viable microorganisms immediately after contamination on the untreated test pieces; B: average of the number of viable microorganisms after 24 hours on the untreated test pieces; C: average of the number of viable microorganisms after 24 hours on the antimicrobial test pieces and R: Value of antimicrobial activity.

	A (Nb. cfu/test piece)	B (Nb. cfu/test piece)	C (Nb. cfu/test piece)	R [Log (B/C)]
Pseudomonas aeruginosa CIP 103467	$1.5x10^5$	$2.1x10^7$	$4.4x10^5$	1.7
Staphylococcus aureus CIP 4.83	$2.3x10^5$	$1.8x10^5$	$2.1x10^3$	**2.1**
Escherichia coli CIP 54127	$2.5x10^5$	$7.9x10^5$	$< 1.4x10^2$	**> 3.7**
Mycobacterium terrae CIP 104321	$8.8x10^4$	$2.3x10^4$	$3.7x10^2$	1.8

According to the results presented in table VI, the antimicrobial activity of the polypropylene plastic product (cf. table VI, C) leads to a reduction of at least 10^2 times the number of viable microorganisms when the test microorganisms are: *Staphylococcus aureus* CIP 4.83 et *Escherichia coli* CIP 54127. For those two strains, the logarithmic reductions are respectively 2.1 $\log_{10}$ and > 3.7 $\log_{10}$.

For *Pseudomonas aeruginosa* CIP 103467 and *Mycobacterium terrae* CIP 104321, the reductions of the number of viable microorganisms related to the antimicrobial activity are higher than 1.5 $\log_{10}$ (respectively 1.7 $\log_{10}$ and 1.8 $\log_{10}$) but remains below standards requirements (2 $\log_{10}$).

d) SEBS plastic products:

i) Test conditions validation:

Table VII : Validation of the number of viable microorganisms immediately after contamination on untreated SEBS test pieces: $(L_{max} - L_{min})/(L_{mean}) \leq 0,2$; L_{max}: maximum logarithm of the number of viable microorganisms on untreated test pieces; L_{min}: minimum logarithm of the number of viable microorganisms on untreated test pieces and L_{mean}: average of the logarithm of the number of viable microorganisms on three untreated test pieces

	Assay 1 (L_{max})	Assay 2 (L_{min})	Assay 3	L_{mean}	$(L_{max} - L_{min})/(L_{mean})$
Pseudomonas aeruginosa CIP 103467	5.94	5.83	5.87	5.88	0.02
Staphylococcus aureus CIP 4.83	5.47	5.42	5.43	5.44	0.01
Escherichia coli CIP 54127	5.96	5.80	5.92	5.89	0.03
Mycobacterium terrae CIP 104321	5.39	5.29	5.35	5.34	0.02

For each test microorganism:

- ✓ $(L_{max} - L_{min})/(L_{meann})$ is lower than 0,2 (table VII) ;
- ✓ The average of the number of viable microorganisms immediately after contamination of untreated SEBS test pieces is within the range of $1.0x10^5$ and $4.0x10^5$ cfu/test piece for *Staphylococcus aureus* CIP 4.83 and *Mycobacterium terrae* CIP 104321. For *Pseudomonas aeruginosa* CIP 103467 and *Escherichia coli* CIP 54127, the contamination level is slightly higher than the upper allowed limit ($7.7x10^5$ ufc/test piece for *Pseudomonas aeruginosa* CIP 103467 and $7.9x10^5$ cfu/test piece for *Escherichia coli* CIP 54127 instead of $4.0x10^5$ cfu/test piece). These slight deviations from JIS Z 2801: 2000 requirements do not affect the validity of the test conditions. The number of viable microorganisms found on the untreated test pieces after 24 hours incubation is greater than $1.0x10^4$ cfu/test piece (table VIII, B) for *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83 and *Escherichia coli* CIP 54127. The test conditions are validated for those three stains. On the other hand, the number of viable microorganisms found on the untreated test pieces after 24 hours incubation is lower than $1.0x10^4$ cfu/test piece for *Mycobacterium terrae* CIP 104321 ($7.2x10^2$ cfu/test piece). The test conditions cannot be validated for this test microorganism.

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ii) Main test :

Table VIII : Evaluation of the antimicrobial activity of SEBS plastic products against each test microorganism for a 24±1 hours contact time at 35±1°C. A: average of the number of viable microorganisms immediately after contamination on the untreated test pieces; B: average of the number of viable microorganisms after 24 hours on the untreated test pieces; C: average of the number of viable microorganisms after 24 hours on the antimicrobial test pieces and R: Value of antimicrobial activity.

	A (Nb. cfu/test piece)	**B** (Nb. cfu/test piece)	**C** (Nb. cfu/test piece)	**R** [Log (B/C)]
Pseudomonas aeruginosa CIP 103467	7.7×10^5	1.1×10^7	1.1×10^7	0.0
Staphylococcus aureus CIP 4.83	2.8×10^5	2.1×10^5	8.6×10^4	0.4
Escherichia coli CIP 54127	7.9×10^5	7.6×10^5	$< 1.4 \times 10^1$	**> 4.6**
Mycobacterium terrae CIP 104321	2.2×10^5	7.2×10^2	3.1×10^2	0.9

According to the results presented in table VIII, the antimicrobial activity of the SEBS plastic product (cf. table VIII, C) leads to a reduction of at least 10^2 times the number of viable microorganisms when the test microorganisms is *Escherichia coli* CIP 54127. For this strain, the logarithmic reduction is > 4.6 $\log_{10}$.
For *Pseudomonas aeruginosa* CIP 103467 and *Staphylococcus aureus* CIP 4.83, the reductions of the number of viable microorganisms related to the antimicrobial activity are lower than 0.5 $\log_{10}$.
Although test conditions are not validated for *Mycobacterium terrae* CIP 104321, reduction of the number of viable microorganisms (0.9 $\log_{10}$) remains below the standard requirements (2 $\log_{10}$) and this effect observed cannot be attributed to the antimicrobial agent.

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VI : CONCLUSIONS :

In the test conditions described in this test report, an antimicrobial activity consistent with the JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2] requirements (i.e reduction of at least 10^2 times of the number of viable microorganisms deposited on test pieces for 24 hours contact time at 35±1°C) is observed for:
- PC R903B and ABS plastic products when microorganisms are *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83 and *Escherichia coli* CIP 54127;
- Polypropylene plastic products when the tests microorganisms are *Staphylococcus aureus* CIP 4.83 and *Escherichia coli* CIP 54127;
- SEBS plastic products when the test microorganism is *Escherichia coli* CIP 54127.

Even if they are lower than the standard requirements, a significant antimicrobial activity is also observed for:
- PC R903B plastic products against *Mycobacterium terrae* CIP 104321 (reduction of 1.21 $\log_{10}$) and *Candida albicans* CIP 4872 (reduction of 0.86 $\log_{10}$);
- ABS plastic products against *Mycobacterium terrae* CIP 104321 (reduction of 1.45 $\log_{10}$), *Mycobacterium avium* CIP 105415 (reduction of 1.61 $\log_{10}$) and *Candida albicans* CIP 4872 (reduction of 1.11 $\log_{10}$);
- Polypropylene plastic products against *Pseudomonas aeruginosa* CIP 103467 (reduction of 1.7 $\log_{10}$) and *Mycobacterium terrae* CIP 104321 (reduction of 1.8 $\log_{10}$).

VII : REFERENCES :

1. JIS Z 2801: 2000 – Antimicrobial products: Test for antimicrobial activity and efficacy.
2. JIS Z 2801 : 2006 – Antimicrobial products : Test for antimicrobial activity and efficacy, Amendment 1
3. NF EN 12353: 2006 – Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity.

VIII : STATEMENT OF GOOD LABORATORY PRACTICE:

The study was conducted according to NF EN ISO/CEN 17025 (2005) General requirements for the competence of testing and calibration laboratories.
The Quality Assurance Unit (QAU) has reviewed this report and determined it accurately describes the procedures used and that the results and conclusions herein accurately reflect the raw data from the study. Applicable Standard Operating Procedures and Good Laboratory Practice were followed in this study.
The original records of this report, the notebooks, protocol, and final study report are stored in the archives of Biotech-Germande "546.LEG.07".

Marlène RICHARD Lab deputy manager	Signature		Date 23/07/2009
Christine AH-DIP Quality Assurance Unit manager	Signature		Date 23/07/2009
Lionel PINEAU Managing director	Signature		Date 23/07/2009

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EVALUATION OF THE ANTIMICROBIAL ACTIVITIES OF PAD PRINTING-LABELED ABS SURFACES ACCORDING TO A METHOD BASED UPON JIS Z 2801 : 2000 AND JIS Z 2801 : 2006

This test report is a translation of the test report 815.LEG.09.1 edited on November 30th 2009

Test report written by: Dr Marlène RICHARD

Marseilles: March 25th 2010

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CONTENT

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I : DESCRIPTION OF THE STUDY:

Title:	Evaluation of the antimicrobial activities of pad printing-labeled ABS surfaces according to a method based upon JIS Z 2801: 2000 and JIS Z 2801: 2006.
Study n°:	815.LEG.09
Sponsor:	LEGRAND 128, av. du Maréchal de Lattre de Tassigny 87045 Limoges Cedex *Contact:* Mme ARBOGAST
Test period:	From 02/11/2009 to 26/11/2009
Study manager:	Dr Marlène RICHARD
Tests done by:	Isabelle SEVEROVIC
Test laboratory:	Laboratoire BIOTECH-GERMANDE Parc Scientifique de Luminy 163 Avenue de Luminy – Case 927 13288 Marseille Cedex 9

II : AIM OF THE STUDY:

Determine, according to the experimental conditions described in the standards JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2], the ability of pad printing-labeled ABS surfaces to reduce within 24 hours at 35±1°C of at least 10^2 times the number of viable microorganisms spread on test pieces.

III : MATERIAL:

a) Test pieces:

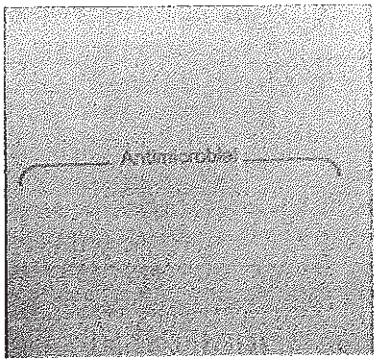

Figure 1 : Pad printing-labeled ABS surfaces

According to the experimental conditions described in the standards JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2], 3 antimicrobial pad printing-labeled ABS surfaces and 6 untreated pad printing-labeled ABS surfaces are necessary to evaluate the antimicrobial activity for each microorganism tested.

b) Microbial strains :

Pseudomonas aeruginosa CIP 103467
Staphylococcus aureus CIP 4.83
Candida albicans CIP 4872
Escherichia coli CIP 54127
Mycobacterium terrae CIP 104321

The conditions of preservation of the microbial strains used for the determination of the bactericidal, mycobactericidal, sporicidal and fungicidal activities are those described in the European standard NF EN 12353: 2006[3].

c) Maintaining and counting medium:

For *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83 and *Escherichia coli* CIP 54127:
Trypticase soja agar:
TS agar (BIOMERIEUX, 51044)----40 g
Distilled water: ----1000 ml
Steam sterilized at 121°C for 21 minutes.

For *Mycobacterium terrae* CIP 104321:
7H10 agar
Mycobacteria 7H10 agar (Difco A5062717)----21 g
Glycerol (Sigma G5150)----5 ml
Middle Brook OADC Enrichment (Difco A5701886)----10% (v/v)
Distilled water: ----1000 ml
Steam sterilized at 121°C for 21 minutes.

For *Candida albicans* CIP 4872:
Malt extract agar:
Soja peptone(SIGMA P-1265) :----3 g
Malt ectract (SIGMA M0383) : ----30 g
Agar (SIGMA A5306) : ----15 g
Distilled water: ----1000 ml
Steam sterilized at 121°C for 21 minutes.

d) Recovery solution:

Tween 80 (SIGMA P17-54) :----50 ml
Sodium thiosulfate (SIGMA S85-03) : ----10 g
Saponin (SIGMA S79-00) : ----5 g
Lecithin (SIGMA P53-94) : ----10 g
Trypticase soja broth: ----500 ml
Steam sterilized at 121°C for 21 minutes.

e) **Diluent :**

Test suspensions are prepared in phosphate buffer 100 mM, pH 7.0.

i) Stock solution:

Phosphate buffer 10X (1000mM, pH 7.0):
Dibasic sodium phosphate, Na_2HPO_4 $7H_2O$:----------144.5g
Monobasic potassium phosphate, KH_2PO_4:----------71.2g
Distilled water: ----------1000 ml
Steam sterilized at 121°C for 21 minutes.

ii) Working solution:

Phosphate buffer 1X (100mM, pH 7.0):
Phosphate buffer 10X (1000mM, pH 7.0) :----------100 ml
Distilled water: ----------1000 ml

IV : METHOD :

a) **Test suspensions :**

For each microorganism, the test suspension is prepared in the diluent (see III.e) and adjusted in order to contain between $2.5x10^5$ and $10x10^5$ cfu/ml, according to the JIS Z 2801: 2000[1] requirements. The number of viable microorganisms in the test suspension (Tc) is verified for each assay using a validated and specific counting method for each microorganism.

b) **Test pieces contamination:**

For each microorganism, test pieces (i.e 3 antimicrobial pad printing-labeled ABS surfaces and 6 untreated test pieces), are placed in a sterile Petri dish with the treated surface facing up. 400 µl of the test suspension are deposited onto each test pieces and then cover by two cover glasses (22 x 40 mm) in order to spread the test suspension on a 1760 mm² surface.

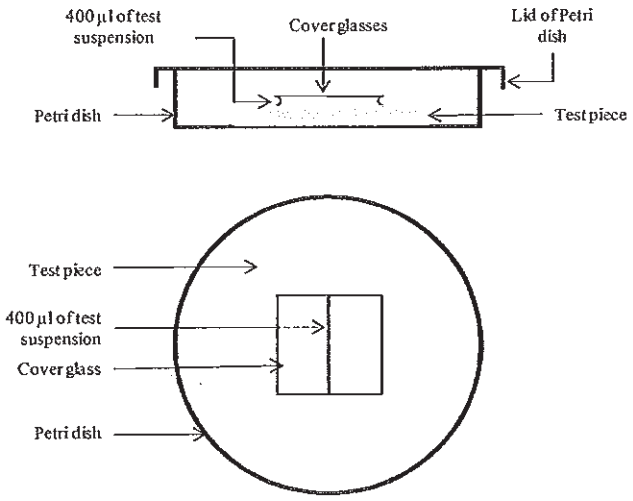

Figure 2 : Instillation of inoculums between the test piece and cover glasses

c) Incubation of the inoculated test pieces:

After contamination, 3 antimicrobial pad printing-labeled ABS surfaces and 3 out of 6 untreated surfaces are incubated at 35±1°C for 24±1 hours. In parallel, the 3 other untreated test pieces are immediately washed out in order to determine the initial contamination level of the test pieces (see IV.d).

d) Determination of the contamination level of the test pieces:

Immediately after contamination or after 24±1 hours of incubation of the test pieces, the residual number of viable microorganisms remaining on the test pieces are determined. Firstly, the cover glasses are carefully removed from the surface and transferred into a test tube containing 10 ml of recovery solution and approximately 1 ml of glass beads from 0.25 to 0.50 mm in diameter. The test tubes containing the cover glasses are submitted to a manual agitation in order to remove the microorganisms from the surface. In a second step, the surface test piece is recovered with 5 ml of recovery solution and then scraped with a cell scraper and rinsed with additional 5 ml of recovery solution. The volume of the recovery solution containing the microorganisms collected in the Petri dish is transferred into the test tube containing the corresponding cover glasses. The number of viable microorganisms present per milliliter of test mixture is determined by successive tenfold dilution and inclusion of 1ml of each dilution in the counting medium specific of the test microorganism.
After incubation at the specific temperature and time of the test microorganism, colonies are counted and the results are expressed as the number of cfu per test piece.

e) Test conditions :

According to JIS Z 2801: 2000[1], the tests are satisfactory if the three following test conditions are met:

i) Logarithmic value of the number of viable microorganisms immediately after contamination on untreated test pieces:

$$(L_{max} - L_{min})/(L_{mean}) \leq 0,2$$

Where L_{max}: Maximum logarithm of the number of viable microorganisms on untreated test pieces;

L_{min}: Minimum logarithm of the number of viable microorganisms on untreated test pieces;

L_{mean}: Average of the logarithm of the number of viable microorganisms on three untreated test pieces.

ii) The average of the number of viable microorganisms immediately after contamination of untreated test pieces shall be within the range of $1.0x10^5$ (5,0 $\log_{10}$) and $4.0x10^5$ cfu/test piece (5,6 $\log_{10}$).

iii) The number of viable microorganisms on an untreated test piece after 24 hours shall not be less than $1.0x10^4$ cfu/test piece (4 log).

f) Expression of antimicrobial activity :

When test conditions are validated, the antimicrobial activity of the antimicrobial test pieces is calculated as follows:

$$R = [\log (B/A) - \log (C/A)] = [\log (B/C)]$$

With R: Value of antimicrobial activity;

A: average of the number of viable microorganisms immediately after contamination on the untreated test pieces;

B: average of the number of viable microorganisms after 24 hours on the untreated test pieces;

C: average of the number of viable microorganisms after 24 hours on the antimicrobial test pieces.

V : RESULTS :

a) Test conditions validation:

Table I : Validation of the number of viable microorganisms on untreated pad printing-labeled ABS surfaces immediately after contamination: $(L_{max} - L_{min})/(L_{mean}) \leq 0,2$; L_{max}: maximum logarithm of the number of viable microorganisms on untreated test pieces; L_{min}: minimum logarithm of the number of viable microorganisms on untreated test pieces and L_{mean}: average of the logarithm of the number of viable microorganisms on three untreated test pieces.

	Assay 1 (L_{max})	Assay 2 (L_{min})	Assay 3	L_{mean}	$(L_{max} - L_{min})/(L_{mean})$
Pseudomonas aeruginosa CIP 103467	5.03	4.91	5.00	4.98	0.025
Staphylococcus aureus CIP 4.83	4.93	4.88	4.92	4.91	0.001
Escherichia coli CIP 54127	6.07	6.01	6.04	6.04	0.009
Mycobacterium terrae CIP 104321	4.45	4.11	4.24	4.27	0.081
Candida albicans CIP 4872	6.04	5.81	5.93	5.93	0.040

For each test microorganism:

- ✓ $(L_{max} - L_{min})/(L_{meann})$ is lower than 0,2 (table I);
- ✓ The average of the number of viable microorganisms immediately after contamination of untreated pas printing-labeled ABS test pieces is within the range of 5.0 $\log_{10}$ $1.0x10^5$ and $4.0x10^5$ cfu/test piece for *Pseudomonas aeruginosa* CIP 103467.
  For *Escherichia coli* CIP 54127 and *Candida albicans* CIP 4872, the contamination level is higher than the upper allowed limit (respectively $1.1x10^6$ cfu/test and piece $8.7x10^5$ cfu/test instead of $4.0x10^5$ cfu/test piece). For *Staphylococcus aureus* CIP 4.83 and *Mycobacterium terrae* CIP 104321, the average number of viable microorganisms immediately after contamination of untreated test pieces is slightly lower than

the minimal recommended value (respectively $8.1x10^4$ cfu/test piece and $2.0x10^4$ cfu/test piece instead of $4.0x10^5$ cfu/test piece). These slight deviations from JIS Z 2801: 2000 requirements do not affect the validity of the test conditions since for each test microorganism, the average number of viable microorganisms found on the untreated test pieces after 24 hours incubation is greater than $1.0x10^4$ cfu/test piece (table II, B).

The test conditions are therefore validated for each microorganism.

b) Main test :

Table II : Evaluation of the antimicrobial activity of pad printing-labeled ABS surfaces against each test microorganism for a 24±1 hours contact time at 35±1°C. A: average of the number of viable microorganisms immediately after contamination on the untreated test pieces; B: average of the number of viable microorganisms after 24 hours on the untreated test pieces; C: average of the number of viable microorganisms after 24 hours on the antimicrobial test pieces and R: Value of antimicrobial activity.

	A (Nb. cfu/test piece)	B (Nb. cfu/test piece)	C (Nb. cfu/test piece)	R [Log (B/C)]
Pseudomonas aeruginosa CIP 103467	$9.6x10^4$	$4.7x10^6$	< 14	> 5.5
Staphylococcus aureus CIP 4.83	$8.1x10^4$	$1.8x10^4$	$2.1x10^1$	3.0
Escherichia coli CIP 54127	$1.1x10^6$	$7.2x10^5$	$3.0x10^2$	3.4
Mycobacterium terrae CIP 104321	$2.0x10^4$	$2.1x10^4$	< 14	> 3.2
Candida albicans CIP 4872	$8.7x10^5$	$6.8x10^5$	$1.9x10^5$	0.6

According to the results presented in table II, the antimicrobial activity of the pad printing-labeled ABS surfaces (cf. table II, C) leads to a reduction of at least 10^2 times the number of viable microorganisms when the test microorganisms are: *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83, *Escherichia coli* CIP 54127 and *Mycobacterium terrae* CIP 104321. For those four strains, the logarithmic reductions are respectively >5.5 $\log_{10}$, 3.0 $\log_{10}$, 3.4 $\log_{10}$ and > 3.2 $\log_{10}$.

For *Candida albicans* CIP 4872, the reduction of the number of viable microorganisms (0.6 $\log_{10}$) remains below the standard requirements (2 $\log_{10}$).

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VI : CONCLUSIONS :

In the test conditions described in this test report, pad printing-labeled ABS surfaces show an antimicrobial activity consistent with the JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2] requirements (i.e reduction of at least 10^2 times of the number of infectious unit deposited on test pieces for a 24 hours contact time at 35±1°C) when the strains are *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83, *Escherichia coli* CIP 54127 and *Mycobacterium terrae* CIP 104321

Even if it is lower than the standard requirements, a slight antimicrobial activity is also observed for *Candida albicans* CIP 4872 (reduction of 0.6 $\log_{10}$).

VII : REFERENCES :

1. JIS Z 2801: 2000 – Antimicrobial products: Test for antimicrobial activity and efficacy.
2. JIS Z 2801 : 2006 – Antimicrobial products : Test for antimicrobial activity and efficacy, Amendment 1
3. NF EN 12353: 2006 – Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity.

VIII : STATEMENT OF GOOD LABORATORY PRACTICE:

The study was conducted according to NF EN ISO/CEI 17025 (2005) General requirements for the competence of testing and calibration laboratories.
The Quality Assurance Unit (QAU) has reviewed this report and determined it accurately describes the procedures used and that the results and conclusions herein accurately reflect the raw data from the study. Applicable Standard Operating Procedures and Good Laboratory Practice were followed in this study.
The original records of this report, the notebooks, protocol, and final study report are stored in the archives of Biotech-Germande "815.LEG.09".

Marlène RICHARD
Lab deputy manager — Signature — Date 25/03/2010

Christine AH-DIP
Quality Assurance Unit manager — Signature — Date 25/03/2010

Lionel PINEAU
Managing director — Signature — Date 25/03/2010

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HYGIENE-FORMATION-EVALUATION
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EVALUATION OF THE VIRUCIDAL ACTIVITY OF PAD PRINTING-LABELED ABS SURFACES ACCORDING TO A METHOD BASED UPON JIS Z 2801: 2000 AND JIS Z 2801: 2006

This test report is a translation of the test report 815.LEG.09.2 edited on November 30th 2009

Test report written by: Dr Marlène RICHARD

Marseilles: March 25th 2010

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I : DESCRIPTION OF THE STUDY:

Title:	Evaluation of the virucidal activity of pad printing-labeled ABS surfaces according to a method based upon JIS Z 2801: 2000 and JIS Z 2801: 2006.
Study n°:	815.LEG.09
Sponsor:	LEGRAND 128, av. du Maréchal de Lattre de Tassigny 87045 Limoges Cedex *Contact:* Mme ARBOGAST
Test period:	From 06/11/2009 to 23/11/2009.
Study manager:	Dr Marlène RICHARD
Test laboratory:	Laboratoire FONDEREPHAR Laboratoire de bactériologie, virologie et Microbiologie Industrielle

II : AIM OF THE STUDY:

Determine, according to the experimental conditions described in the standards JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2], the ability of pad printing-labeled ABS surfaces to reduce in 24 hours at 35±1°C of at least 10^2 times the number of infectious unit of *Enterovirus type 1* and *Influenza virus A* (H1N1)

III : MATERIEL:

a) **Test pieces:**

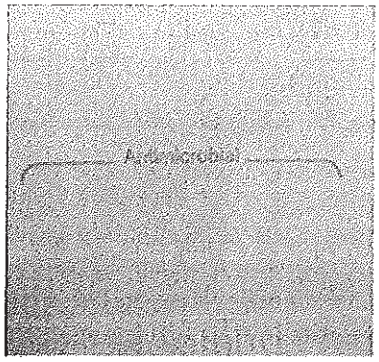

Figure 1 : Pad printing-labeled ABS surfaces

According to the experimental conditions described in the standards JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2], 3 antimicrobial pad printing-labeled ABS surfaces and 6 untreated pad printing-labeled ABS surfaces are necessary to evaluate the virucidal activity for each microorganism tested.

b) Virus strain :

Enterovirus type I: ----------AFSSAPS batch n° NIBSC 01/528
Influenza virus A (H1N1): ----------ATCC (VR-1520) batch n°7534335

Maintaining and culture conditions of viral strains are those specified in the European standard NF EN 14476+ A1: 2007.

c) Cells culture:

VERO cells----------ATTC CLL-81, batch n° 3372621
MDCK cells----------ATTC CLL-34, batch n° 58078519

The method of cell culture is consistent with the requirements of the European standard NF EN 14476 + A1: 2007.

d) Diluent, culture medium and recovery solution:

MEM containing 2% fetal calf serum (FCS).

IV : METHOD:

a) Viral suspension:

The titre of the virus suspension is adjusted between 5.6 and 6.0 log $TCID_{50}$ in MEM culture medium containing 2% FCS.

b) Test pieces contamination:

The test pieces i.e 3 antimicrobial pad printing-labeled ABS surfaces and 6 untreated test pieces are placed in a sterilized Petri dish with the treated surface facing up. 480 µl of the test suspension are deposited onto each test pieces and then cover by two cover glasses (50 x 50 mm) in order to spread the inoculum on a 2500 mm^2 surface.

c) Incubation of the inoculated test pieces:

After contamination, 3 antimicrobial test surfaces and 3 out of 6 untreated surfaces are incubated at 35±1°C for 24±1 hours. In parallel, the 3 other untreated test pieces are immediately washed out in order to determine the initial contamination level of the test pieces.

d) Determination of the contamination level of the test pieces:

Immediately after contamination or after incubation, 10 ml of the recovery solution (MEM + 2% FCS) were added over the bottom of the test surface. The surface of the test piece is then gentle scrapped with a cell scraper to resuspend the virus film.

Viruses are titrated according to the method described in the European standard NF EN 14476 + A1: 2007. Fourfold dilutions of the virus/recovery solution mixture are carried out in MEM + 2% FCS using glass test tubes in order to avoid virus adsorption on surfaces. Viruses are titrated on cells in suspension on microtiter plates.

Cytopathic effect is determined after 48 hours of incubation at 37°C and the estimated number of infectious units is determined by the Spaerman-Karber method by calculating the negative logarithm of the 50% titration endpoint for infectivity (lgTCID50) by the following formula:

$lgTCID_{50}$ = Negative logarithm of the highest virus concentration – [(sum of percentages affected at each dilution/100 – 0,5) x (log of the dilution)].

e) Test conditions :

According to JIS Z 2801: 2000[1], the tests are satisfactory if the three following test conditions are met:

i) The number of viable viruses on test pieces immediately after contamination from the untreated test pieces shall satisfy the following requirement:

$$(T_{max} - T_{min})/(T_{mean}) \leq 0,2$$

Where T_{max} : maximum logarithm of the number of viable viruses on untreated test pieces;

T_{min} : minimum logarithm of the number of viable viruses on untreated test pieces;

T_{mean} : average of the logarithm of the number of viable viruses on three untreated test pieces.

ii) Average of the number of viable viruses immediately after contamination of untreated test pieces shall be within the range of $1.0x10^5$ (5,0 log_{10}) and $4.0x10^5$ IU/test piece (5,6 log_{10})

iii) The number of viable viruses on an untreated test piece after 24 hours shall not be less than $1.0x10^4$ IU/test piece (4 log_{10})

f) Expression of virucidal activity:

When the test conditions are validated, the virucidal activity of the antimicrobial test pieces is calculated as follows:

$$R = [\log (B/A) - \log (C/A)] = [\log (B/C)]$$

With R: Value of virucidal activity;

A: Average number of viral infectious units 50% ($TCID_{50}$) immediately after contamination of untreated test pieces;

B: Average number of viral infectious units 50% ($TCID_{50}$) after 24 hours on untreated test pieces;

C: Average number of viral infectious units 50% ($TCID_{50}$) after 24 hours on the antimicrobial test pieces

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V : RESULTS:

a) Validation of test conditions:

Table I : Validation of the number of viable viruses on untreated pad printing-labeled ABS surfaces immediately after contamination: $(T_{max} - T_{min})/T_{mean}) \leq 0,2$; T_{max}: maximum logarithm of the number of viable viruses on untreated test pieces; T_{min}: minimum logarithm of the number of viable viruses on untreated test pieces and T_{mean}: average of the logarithm of the number of viable viruses on three untreated test pieces

Viruses	Assay 1 (T_{max})	Assay 2 (T_{min})	Assay 3	T_{mean}	$(T_{max} - T_{min})/(T_{mean})$
Enterovirus type I	5.5	5.3	5.5	5.4	0.04
Influenza virus A (H1N1)	5.2	5.1	5.1	5.1	0.02

For each plastic virus:

- ✓ $(T_{max} - T_{min})/(T_{meann})$ is lower than 0,2 (see table I) ;
- ✓ The average of the logarithm of the number of viable *Enterovirus type I* and *Influenza virus A* (H1N1) viruses immediately after contamination of untreated test pieces are within the range of 5.0 log_{10} ($1.0x10^5$ IU/test piece) and 5.6 log_{10} ($4.0x10^5$ IU/test piece).

Test conditions are therefore validated for pad printing-labeled ABS surfaces.

b) Main tests :

Table II : Evaluation of the virucidal activity of pad printing-labeled ABS surfaces against *Enterovirus type I* and *Influenza virus A* (H1N1) for a 24±1 hours contact time at 35±1°C. Log A : Log $TCID_{50}$ immediately after contamination on untreated test pieces ; log B : Log $TCID_{50}$ after 24 hours contact time on untreated test pieces ; log C : Log $TCID_{50}$ after 24 hours contact time on antimicrobial test pieces ; R : virucidal ativity.

Viruses	Log A	Log B	Log C	R [Log B – Log C)]
Enterovirus type I	5.4	5.3	5.1	0.2
Influenza virus A (H1N1)	5.1	4.3	2.2	**2.1**

According to the results presented in table II, pad printing-labeled ABS surfaces are able to reduce, after 24 hours contact time at 35±1°C, of at least 10^2 times the number of infectious unit of *Influenza virus A* (H1N1).

For *Enterovirus type I*, the viral titre reduction is almost zero (0.2 log_{10}) and is below the standards requirements (2 log_{10}).

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VI : CONCLUSIONS :

In the test conditions described in this test report, pad printing-labeled ABS surfaces show a virucidal activity consistent with the JIS Z 2801: 2000[1] and JIS Z 2801: 2006[2] requirements (i.e reduction of at least 10^2 times of the number of infectious unit deposited on test pieces for a 24 hours contact time at 35±1°C) when the virus strain is *Influenza virus A* (H1N1).

However, an almost negligible activity is observed against *Enterovirus type 1* with a reduction of only 0.2 log_{10}.

VII : REFERENCES :

1. JIS Z 2801: 2000 – Antimicrobial products: Test for antimicrobial activity and efficacy.
2. JIS Z 2801 : 2006 – Antimicrobial products : Test for antimicrobial activity and efficacy, Amendment 1
3. NF EN 12353: 2006 – Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity.

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Date 25/03/2010

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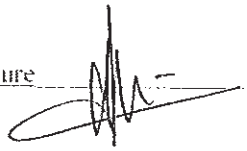
Signature 

Date 25/03/2010

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List of the references using the following antimicrobial materials

Référence	ABS	PC	PP	SEBS
067740	X			
067741	X			
067742	X			
067743	X			
067744		X		
067745	X			
067746	X			
067747	X			
067748	X			
067749	X			
067750	X			
067760		X		
069001	X	X		
069002	X	X		
069003	X	X		
070711	X		X	
070712	X		X	
070721	X		X	
070722	X		X	
070724			X	
070725			X	
070726	X		X	
070727	X		X	
070730	X		X	
070732	X	X	X	
070733	X	X	X	
070741	X		X	X
070742	X		X	X
070743	X		X	X
070749	X		X	X
070761	X		X	X
070771	X		X	X
070772	X		X	X
070773	X		X	X
070782	X	X	X	X
070783	X	X	X	X
070788	X		X	X
070792	X		X	X
070793	X		X	X
070794	X		X	X
076581		X		
076582		X		
076583		X		
076584		X		
076666	X	X		
076677	X		X	
076685	X	X		
077027	X			
077043	X	X		
077080	X			
077112		X		
077115		X		
077116		X		
077117		X		
077118		X		
077126		X		
077131	X	X		

List of the references using the following antimicrobial materials

Référence	ABS	PC	PP	SEBS
077132	X	X		
077133		X		
077141		X		
077146		X		
077147		X		
077150		X		
077198	X	X		
077216		X		
077217		X		
077219		X		
077220		X		
077231	X	X		
078204	X	X		
078240	X			X
078242	X			X
078244	X			X
078246	X	X		
078247	X	X		
078248	X	X	X	
078249	X	X	X	X
078305		X		
078329		X		
078330		X		
078331	X	X		
078332	X	X		
078334	X	X		
078335	X	X		
078336	X	X		
078375	X			
078376	X			
078387	X	X	X	
078700	X			
078701		X		
078702		X		
078703	X	X		
078704		X		
078705	X			
078706		X		
078707		X		
078709		X		
078710	X			
078711	X			
078712	X	X		
078713		X		
078714	X			
078715	X			
078716	X	X		
078720	X			
078721	X			
078722	X	X		
078723	X	X		
078724	X	X		
078725	X	X		
078726	X	X		
078880	X		X	X
099629	X	X		
099639	X			

List of the references using the following antimicrobial materials

Référence	ABS	PC	PP	SEBS
674420		X		
678023		X		
678024	X		X	X
678025		X		
678026		X		
678117	X			
678129		X		
678130		X		
678145	X			
678148		X		
678201	X			
678203	X			
678206	X			
678221	X			
678222	X			
678224	X			