FONdation pour le DEveloppement de la REcherche PHARmaceutique

Système de management de la qualité
Certifié ISO 9001

Reference 20-2657/20-2658

CERTIFICATE OF ANALYSIS

Society :
Address :

To the attention of : LOIC MARCHIN

Customer Reference:	
Fonderephar Sample Reference:	20-2658-2 / 20-2657 - 2
Date os sample receipt:	May 25th, 2020
Date of sample analysis:	May - June 2020
Date of certificate of analysis:	June 8th, 2020

Test
Evaluation of antimicrobial efficiency based on JIS Z2801 : 2010 for bacteria

Results: The results are given as log reduction R, corresponding to the value of antimicrobial activity

Escherichia coli CIP 53.126 R = 5,75 (after a contact time 24H)

Test
Evaluation of antivirucidal activity according to the methodology based on ISO 21702 : 2019 for virus

Results: The results are given as log reduction R, corresponding to the value of antivirucidal activity

Coronavirus Humain 229E R = 0,98 (after a contact time 1H)
R = 3,28 (after a contact time 24H)

Certified by Catherine FEUILLOLAY and Laila HADDIOUI
Test Managers

DEREPHAR - Faculté des Sciences Pharmaceutiques – 35, chemin des Maraîchers – 31062 TOULOUSE cedex 9 - Email : contact@fonderephar.com - www.fonderephar.com
Tél. +33 (0)5 62 25 68 60 - Fax. +33 (0)5 61 25 95 72 - Siret 328 649 538 000 27 - APE 7211Z - N° TVA : FR 80328649538 - Association Loi 1901 n° W313014485

FONdation pour le
DEveloppement de la
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PHARmaceutique

Système de management de la qualité
Certifié ISO 9001

Reference 20-2663

CERTIFICATE OF ANALYSIS

Society :
Address :

To the attention of : LOIC MARCHIN

Customer Reference:	
Fonderephar Samples Reference:	20-2663-2/20-2663-3/20-2663-4/20-2663-5
Date os sample receipt:	June 2nd, 2020
Date of sample analysis:	June 2020
Date of certificate of analysis:	June 17th, 2020

Test
Evaluation of antimicrobial efficiency based on JIS Z2801 : 2010 for bacteria

Results: The results are given as log reduction R, corresponding to the value of antimicrobial activity after a contact time 24H

Escherichia coli CIP 53.126

R = 5,86 without wash
R = 5,86 after 100 washes with isopropilic alcohol
R = 5,86 after 100 washes with detergent with bleach
R = 5,86 after 100 washes with Surfanios Premium

Certified by Catherine FEUILLOLAY
Test Manager

EPHAR - Faculté des Sciences Pharmaceutiques – 35, chemin des Maraîchers – 31062 TOULOUSE cedex 9 - Email : contact@fonderephar.com - www.fond
Tél. +33 (0)5 62 25 68 60 - Fax. +33 (0)5 61 25 95 72 - Siret 328 649 538 000 27 - APE 7211Z - N° TVA : FR 80328649538 - Association Loi 1901 n° W313014489

FONdation pour le DEveloppement de la REcherche PHARmaceutique

Système de management de la qualité
Certifié ISO 9001

Toulouse, June 4th 2020

STUDY 20-2657

EVALUATION OF THE VIRUCIDAL ACTIVITY OF NON-POROUS SURFACES AGAINST HUMAN CORONAVIRUS 229E ACCORDING TO THE METHODOLOGY OF STANDARD ISO 21702 MAY 2019

Client

Test laboratory FONDEREPHAR
Faculté des Sciences Pharmaceutiques
35 Chemin des Maraîchers
31062 TOULOUSE cedex 9
FRANCE

Dr Laïla HADDIOUI Assay Manager	Dr Jocelyne BACARIA Quality Manager

EPHAR - Faculté des Sciences Pharmaceutiques – 35, chemin des Maraîchers – 31062 TOULOUSE cedex 9 - Email : contact@fonderephar.com - www.fond
Tél. +33 (0)5 62 25 68 60 - Fax. +33 (0)5 61 25 95 72 - Siret 328 649 538 000 27 - APE 7211Z - N° TVA : FR 80328649538 - Association Loi 1901 n° W313014489

I - IDENTIFICATION OF SAMPLE

- Product name:	Film Gerg. Standard
- Reference:	Film N°3 STD A.CO
- Batch:	Film N°3 du 20.05.2020
- Date of receipt :	05-25-2020
- Internal code :	20-2657-1

- Product name :	Coversafe Film (Film Ger. ADD)
- Reference:	Film N°1 A.CO ADD2
- Batch:	Film N°3 du 20.05.2020
- Date of receipt :	05-25-2020
- Internal code :	20-2657-2

- Promotor :	PYLOTE
- Period of testing :	May- June 2020

II -VIRUS TEST:

II-1 Human Coronavirus

Name:	Human Coronavirus 229E
Origin:	ATCC
Reference:	VR-740
Supplier batch number:	58505270
Internal batch number:	SS-2-081216 (Passage N°2)

II-2- Recipient cells

Name:	Vero Cells
Origin:	ATCC
Reference:	CCl-81
Supplier batch number:	3372621
Internal batch number:	WCB-090708 (Passages N°24)

III - EXPERIMENTAL CONDITIONS

- Contact times: 20 minutes, 60 minutes et 24 hours

- Test temperature: 36°C ± 1°C

IV- IV- TEST METHOD

IV-1 Contact virus/Pieces

- Each sample (50mm/50mm) submitted to the test is placed in a sterile glass Petri dish.
- 400 µl of the previously adjusted viral film is added to the piece.
- The viral film is covered by a glass lamella

IV-2 Viral film recovery

After each incubation, the viral film is recovered by adding 2.6 ml of culture medium by gentle scraping with a cell scraper.
The titration of the residual viable viruses is then carried out immediately.

IV-3 Viral Load

The titration technique is the one indicated in standard NF EN 14476 + A2 (July 2019).
Dilutions of ratio 4 of the viral suspensions are carried out in the cell culture medium in neutral glass tubes in order to limit the phenomena of virus adsorption on the surfaces.
Titration is performed on 96-well microplates. Each dilution is performed 8 times.

IV-4 viral load calculation

The assay was performed by the microplate method of suspension cells. The cytopathic effect was determined at least 4 days of culture.
The number of infectious units is estimated by the SPEARMAN-KÄRBER method by calculating the negative logarithm of the 50% limit point ($lgDICT_{50}$) using the following formula:
$lgDICT_{50}$ = Negative logarithm of the highest concentration of virus used - [(Sum of % assigned to each dilution/100 - 0.5) X (lg of dilution)]

V- RESULTS

V-1 Contact time 20 min

V-1-1 Test validation

Control T0 :

- Control 1: lg $DICT_{50}$ = 3.98
- Control 2 : lg $DICT_{50}$ = 4.20
- Control 3 : lg $DICT_{50}$ = 4.13

Average lg $DICT_{50}$ Control T0 = 4.10

$$\frac{\text{Maximum viral load - Minimum viral load}}{\text{Average of the 3 viral loads.}} = 0.02$$

The loads (lg TCID50) of the 3 tests at T0 must be homogeneous.
Maximum viral load - Minimum viral load / Average of the 3 viral loads ≤ 0.2.

Control T20 min :

- Control 1 : lg $DICT_{50}$ = 4.43
- Control 2 : lg $DICT_{50}$ = 4.35
- Control 3 : lg $DICT_{50}$ = 4.43

Average lg $DICT_{50}$ Control 20 min = 4.40

$$\frac{\text{Maximum viral load - Minimum viral load}}{\text{Average of the 3 viral loads.}} = 0.05$$

The loads (lg TCID50) of the 3 tests at T20 min must be homogeneous.
Maximum viral load - Minimum viral load / Average of the 3 viral loads ≤ 0.2.

V-1-2 Test

- Test 1 : lg $DICT_{50}$ = 4.35
- Test 2 : lg $DICT_{50}$ = 4.13
- Test 3 : lg $DICT_{50}$ = 3.83

Average lg $DICT_{50}$ test = 4.10

R = Average lg $DICT_{50}$ test 20 min – Average lg $DICT_{50}$ control 20 min = 0.30 lg

V-2 Contact time 60 min

V-2-1 Test validation

Control T0 :

- Control 1 : lg $DICT_{50}$ = 3.98
- Control 2 : lg $DICT_{50}$ = 4.20
- Control 3 : lg $DICT_{50}$ = 4.13

Average lg $DICT_{50}$ T0 = 4.10

$$\frac{\text{Maximum viral load - Minimum viral load}}{\text{Average of the 3 viral loads.}} = 0.05$$

The loads (lg TCID50) of the 3 tests at T0 min must be homogeneous.
Maximum viral load - Minimum viral load / Average of the 3 viral loads ≤ 0.2.

Control T60 min :

- Control 1 : lg $DICT_{50}$ = 4.43
- Control 2 : lg $DICT_{50}$ = 4.50
- Control 3 : lg $DICT_{50}$ = 4.50

Average lg $DICT_{50}$ Control 60 min = 4.48

$$\frac{\text{Maximum viral load - Minimum viral load}}{\text{Average of the 3 viral loads.}} = 0.02$$

The loads (lg TCID50) of the 3 tests at T60 min must be homogeneous.
Maximum viral load - Minimum viral load / Average of the 3 viral loads ≤ 0.2.

V-2-2 Test

- Test 1 : lg $DICT_{50}$ = 3.45
- Test 2 : lg $DICT_{50}$ = 3.45
- Test 3 : lg $DICT_{50}$ = 3.60

Average lg $DICT_{50}$ Test = 3.50

R = Average lg $DICT_{50}$ test 60 min – Average lg $DICT_{50}$ control 60 min = 0.98 lg

V-3 Contact time 24 hours

V-3-1 test validation

Control T0 :

- Control 1 : lg $DICT_{50}$ = 3.98
- Control 2 : lg $DICT_{50}$ = 4.20
- Control 3 : lg $DICT_{50}$ = 4.13

Average lg $DICT_{50}$ T0 = 4.10

$$\frac{\text{Maximum viral load - Minimum viral load}}{\text{Average of the 3 viral loads.}} = 0.05$$

The loads (lg TCID50) of the 3 tests at T0 must be homogeneous.
Maximum viral load - Minimum viral load / Average of the 3 viral loads ≤ 0.2.

Control T24 hours :

- Control 1 : lg $DICT_{50}$ = 4.35
- Control 2 : lg $DICT_{50}$ = 4.13
- Control 3 : lg $DICT_{50}$ = 4.05

Average lg $DICT_{50}$ Control 24 hours = 4.18

$$\frac{\text{Maximum viral load - Minimum viral load}}{\text{Average of the 3 viral loads.}} = 0.07$$

The loads (lg TCID50) of the 3 tests at T24 hours must be homogeneous.
Maximum viral load - Minimum viral load / Average of the 3 viral loads ≤ 0.2.

V-2-2 Test

- Test 1 : lg $DICT_{50}$ = 0.90
- Test 2 : lg $DICT_{50}$ = 0.90
- Test 3 : lg $DICT_{50}$ = 0.90

Average lg $DICT_{50}$ test = 0.90

R = Average lg $DICT_{50}$ test 24 hours – Average lg $DICT_{50}$ control 24 hours = 3.28 log

VI-CONCLUSION

According to the methodology of the ISO 21702 standard (May 2019), the contact of the Coversafe Film (Film Ger. ADD) with the strain of human Coronavirus 229E Batch N°3 of 20/05/2020 induced:

- A reduction of the log viral load of 0.30 lg at 20 min contact time.

- A reduction of the viral load 0.98 lg at contact time 60 min.

- A viral load reduction of 3.28 lg at 24 hours contact time.

FONDEREPHAR
Faculté des Sciences Pharmaceutiques – 35 Chemin des Maraîchers – 31062 TOULOUSE Cedex 09
Tél. 05 62 25 68 60 Fax. 05 61 25 95 72 Email. contact@fonderephar.com

FONdation pour le DEveloppement de la REcherche PHARmaceutique

Système de management de la qualité
Certifié ISO 9001

Toulouse, June 16th 2020

STUDY 20 - 2658M

This report supersedes the precedent one (June 2nd 2020)

ANTIBACTERIAL PRODUCTS
TEST FOR ANTIBACTERIAL ACTIVITY AND EFFICACY
***Escherichia coli* CIP 53.126**
According to the methodology of standard JIS Z 2801: 2010

Client

Test laboratory **FONDEREPHAR**
Faculté des Sciences Pharmaceutiques
35 Chemin des Maraîchers
31062 TOULOUSE cedex 9
FRANCE

Dr Catherine FEUILLOLAY Assay Manager	**Dr Jocelyne BACARIA** Quality Manager

JIS Z 2801 : 2010. Antimicrobial products - Test for antimicrobial activity and efficacy.

EPHAR - Faculté des Sciences Pharmaceutiques – 35, chemin des Maraîchers – 31062 TOULOUSE cedex 9 - Email : contact@fonderephar.com - www.fond
Tél. +33 (0)5 62 25 68 60 - Fax. +33 (0)5 61 25 95 72 - Siret 328 649 538 000 27 - APE 7211Z - N° TVA : FR 80328649538 - Association Loi 1901 n° W313014489

1. Laboratory Test

FONDEREPHAR
Faculté des Sciences Pharmaceutiques
35 chemin des Maraîchers
31062 Toulouse cedex 9
France

2. Identification of samples

Product's name :	**Untreated test pieces – Film Gerg. Standard**
Reference :	FILM N°3 STD A.CO
Batch:	FILM N°3 - 20.05.2020
Date of receipt :	May/25/2020
Internal code :	**20-2658-1**

Product's name :	**Treated test pieces – Coversafe Film (Film Gerg. ADD)**
Reference :	FILM N°1 A.CO ADD
Batch:	FILM N°3 - 20.05.2020
Date of receipt :	May/25/2020
Internal code :	**20-2658-2**

Promotor :

Period of testing : May 2020

3. Experimental Conditions

*** Test Microorganism :**

Escherichia coli CIP 53.126

*** Preparation of test pieces :**

Test pieces (untreated and treated) were firstly treated with ethanol, rinsed with distilled sterile water, and then dried under microbiological safety cabinet before the test.
During the test, the inoculum was covered by a film (hydrophobic character of pieces)

0,4 mL of test inoculum have been put onto each test piece (= final concentration 10^5 CFU/piece).

*** Culture medium :**

The inoculum was prepared in 1/500 Nutrient Broth (Internal preparation - Batch 9409 Exp. June/02/2020).
The recovery solution used was SCDLP (Internal preparation - Batch 9364 Exp. June/04/2020).
The dilutions have been performed in PBS (SIGMA - Batch RNBJ0743 Exp. Dec/2021).

*** Agar Medium**

Tryptic-soy agar (Biomérieux - Batch 1007893660 Exp. Aug/15/2021).

*** Microorganism recovery**

- **Untreated and Treated pieces**: deposition of each piece in a sterile flask + 10mL SCDLP + sterile glass beads. Manual mix for 1 minute.

*** Conditions of the test**

- Temperature during the contact : 36 ± 1°C
- Relative humidity : > 90%
- Contact time : 24 hours

The test has been performed three times.

4. Results

- **Untreated pieces** (Area : 16 cm^2)

Inoculum/piece : $1,56.10^5$ CFU = $0,98.10^4$ CFU/cm^2

Untreated pieces	CFU	CFU/cm2	log CFU	log CFU/cm2
T0 - 1	$1,39.10^5$	$8,69.10^3$	5,14	3,94
T0 - 2	$1,52.10^5$	$9,50.10^3$	5,18	3,98
T0 - 3	$1,38.10^5$	$8,63.10^3$	5,14	3,94
Mean (U0=CFU/cm2)			5,15	3,95

Test validation :
(Lmax - Lmin)/(Lmean) ≤ 0,2
Number of viable bacteria shall be within the range $1,0 \times 10^5$ et $4,0 \times 10^5$ CFU / 16 cm^2

Untreated pieces	CFU	CFU/cm2	log CFU	log CFU/cm2
T24h - 1	$1,01.10^7$	$6,31.10^5$	7,00	5,80
T24h - 2	$7,80.10^6$	$4,88.10^5$	6,89	5,69
T24h - 3	$9,50.10^6$	$5,94.10^5$	6,98	5,77
Mean (Ut=CFU/cm^2)			6,96	5,75

Control / Petri dish	CFU	CFU/cm2	log CFU	log CFU/cm2
T24h	$8,90.10^6$	$5,56.10^5$	6,95	5,75

- **Treated pieces** (Area : 16 cm^2)

Treated pieces	CFU	CFU/cm2	log CFU	log CFU/cm2
E24h - 1	< 10	< 1	< 1,00	0
E24h - 2	< 10	< 1	< 1,00	0
E24h - 3	< 10	< 1	< 1,00	0
Mean (At=CFU/cm^2)			< 1,00	0

5. Conclusion

The antibacterial activity (R) is based on logarithmic reduction/cm^2 of *E. coli* CIP 53.126 strain between standard and antimicrobial surfaces after 24H of contact according to the following matrix:

$$R = (Ut - U0) - (At - U0) = Ut - At$$

Antimicrobial activity	Result (log CFU/cm^2)	Specifications (log CFU/cm^2) JIS Z2801 :2010
Coversafe Film (FILM Gerg. ADD)	5,75	> 2

FONDEREPHAR
Faculté des Sciences Pharmaceutiques - 35 Chemin des Maraîchers - 31062 TOULOUSE Cedex 09
Tél. 05 62 25 68 60 Fax. 05 61 25 95 72 Email. contact@fonderephar.com

FONdation pour le
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PHARmaceutique

Système de management de la qualité
Certifié ISO 9001

Toulouse, June 16th 2020

STUDY 20 - 2663 - A

ANTIBACTERIAL PRODUCTS
TEST FOR ANTIBACTERIAL ACTIVITY AND EFFICACY
***Escherichia coli* CIP 53.126**
According to the methodology of standard JIS Z 2801: 2010

Client

Test laboratory

FONDEREPHAR
Faculté des Sciences Pharmaceutiques
35 Chemin des Maraîchers
31062 TOULOUSE cedex 9
FRANCE

Dr Catherine FEUILLOLAY Assay Manager	**Dr Jocelyne BACARIA** Quality Manager P. O

JIS Z 2801 : 2010. Antimicrobial products - Test for antimicrobial activity and efficacy.

EPHAR - Faculté des Sciences Pharmaceutiques – 35, chemin des Maraîchers – 31062 TOULOUSE cedex 9 - Email : contact@fonderephar.com - www.fon
Tél. +33 (0)5 62 25 68 60 - Fax. +33 (0)5 61 25 95 72 - Siret 328 649 538 000 27 - APE 7211Z - N° TVA : FR 80328649538 - Association Loi 1901 n° W313014489

1. Laboratory Test

FONDEREPHAR
Faculté des Sciences Pharmaceutiques
35 chemin des Maraîchers
31062 Toulouse cedex 9
France

2. Identification of samples

Product's name :	**Untreated test pieces - Film Gerg. Standard**
Reference :	FILM N°3 STD A.CO
Batch:	FILM N°3 - 20.05.2020
Date of receipt :	May/25/2020
Internal code :	**20-2663-1**

Product's name :	**Treated test pieces - Coversafe Film (Film Gerg. ADD) without wash**
Reference :	2000521/1
Batch:	Not indicated
Date of receipt :	June/02/2020
Internal code :	**20-2663-2**

Product's name :	**Treated test pieces** **Coversafe Film (Film Gerg. ADD) Isopropilic alcohol washes (100 times)**
Reference :	2000521/1 + 100 washes
Batch:	Not indicated
Date of receipt :	June/02/2020
Internal code :	**20-2663-3**

Product's name :	**Treated test pieces** **Coversafe Film (Film Gerg. ADD) detergent with bleach washes (100 times)**
Reference :	2000521/1 + 100 washes
Batch:	Not indicated
Date of receipt :	June/02/2020
Internal code :	**20-2663-4**

Product's name :	**Treated test pieces** **Coversafe Film (Film Gerg. ADD) Surfanios Premium washes (100 times)**
Reference :	2000521/1 + 100 washes
Batch:	Not indicated
Date of receipt :	June/02/2020
Internal code :	**20-2663-5**

Promotor : PYLOTE

Period of testing : June 2020

3. Experimental Conditions

*** Test Microorganism :**

Escherichia coli CIP 53.126

*** Preparation of test pieces :**

Test pieces (untreated and treated) were firstly treated with ethanol, rinsed with distilled sterile water, and then dried under microbiological safety cabinet before the test.
During the test, the inoculum was covered by a film (hydrophobic character of pieces)

0,4 mL of test inoculum have been put onto each test piece (= final concentration 10^5 CFU/piece).

*** Culture medium :**

The inoculum was prepared in 1/500 Nutrient Broth (Internal preparation - Batch 9436 Exp. June/10/2020).
The recovery solution used was SCDLP (Internal preparation - Batch 9434 Exp. July/03/2020).
The dilutions have been performed in PBS (SIGMA - Batch RNBJ0743 Exp. Dec/2021).

*** Agar Medium**

Tryptic-soy agar (Biomérieux - Batch 1007893660 Exp. Aug/15/2021).

*** Microorganism recovery**

- **Untreated and Treated pieces**: deposition of each piece in a sterile flask + 10mL SCDLP + sterile glass beads. Manual mix for 1 minute.

*** Conditions of the test**

- Temperature during the contact : 36 ± 1°C
- Relative humidity : > 90%
- Contact time : 24 hours

The test has been performed three times.

4. Results

- **Untreated pieces** (Area : 16 cm^2)

Inoculum/piece : $2,63.10^5$ CFU = $1,64.10^5$ CFU/cm^2

Untreated pieces	CFU	CFU/cm2	log CFU	log CFU/cm2
T0 - 1	$1,89.10^5$	$1,18.10^4$	5,28	4,07
T0 - 2	$1,79.10^5$	$1,12.10^4$	5,25	4,05
T0 - 3	$1,79.10^5$	$1,12.10^4$	5,25	4,05
Mean (U0=CFU/cm2)			5,26	4,06

Test validation :
(Lmax - Lmin)/(Lmean) ≤ 0,2
Number of viable bacteria shall be within the range $1,0 \times 10^5$ et $4,0 \times 10^5$ CFU / 16 cm^2

Untreated pieces	CFU	CFU/cm2	log CFU	log CFU/cm2
T24h - 1	$1,00.10^7$	$6,25.10^5$	7,00	5,80
T24h - 2	$1,42.10^7$	$8,88.10^5$	7,15	5,95
T24h - 3	$1,12.10^7$	$7,00.10^5$	7,05	5,85
Mean (Ut=CFU/cm^2)			7,07	5,86

- **Treated pieces - Coversafe Film (Film Gerg. ADD) without wash** (Area : 16 cm^2)

Treated pieces	CFU	CFU/cm2	log CFU	log CFU/cm2
E24h - 1	< 10	< 1	< 1,00	0
E24h - 2	10	< 1	1,00	0
E24h - 3	< 10	< 1	< 1,00	0
Mean (At=CFU/cm^2)			< 1,00	0

- **Treated pieces - Coversafe Film (Film Gerg. ADD) Isopropilic alcohol washes** (Area : 16 cm^2)

Treated pieces	CFU	CFU/cm2	log CFU	log CFU/cm2
E24h - 1	< 10	< 1	< 1,00	0
E24h - 2	< 10	< 1	< 1,00	0
E24h - 3	< 10	< 1	< 1,00	0
Mean (At=CFU/cm^2)			< 1,00	0

- **Treated pieces - Coversafe Film (Film Gerg. ADD) detergent with bleach washes** (Area : 16 cm^2)

Treated pieces	CFU	CFU/cm2	log CFU	log CFU/cm2
E24h - 1	< 10	< 1	< 1,00	0
E24h - 2	< 10	< 1	< 1,00	0
E24h - 3	< 10	< 1	< 1,00	0
Mean (At=CFU/cm^2)			< 1,00	0

- **Treated pieces - Coversafe Film (Film Gerg. ADD) Surfanios Premium washes** (Area : 16 cm^2)

Treated pieces	CFU	CFU/cm2	log CFU	log CFU/cm2
E24h - 1	< 10	< 1	< 1,00	0
E24h - 2	< 10	< 1	< 1,00	0
E24h - 3	< 10	< 1	< 1,00	0
Mean (At=CFU/cm^2)			< 1,00	0

5. Conclusion

The antibacterial activity (R) is based on logarithmic reduction/cm^2 of *E. coli* CIP 53.126 strain between standard and antimicrobial surfaces after 24H of contact according to the following matrix:

$$R = (Ut - U0) - (At - U0) = Ut - At$$

Antimicrobial activity	Result (log CFU/cm^2)	Specifications (log CFU/cm^2) JIS Z2801 :2010
Coversafe Film (Film Gerg. ADD) without wash	5,86	> 2
Coversafe Film (Film Gerg. ADD) Isopropilic alcohol washes	5,86	> 2
Coversafe Film (Film Gerg. ADD) detergent with bleach washes	5,86	> 2
Coversafe Film (Film Gerg. ADD) Surfanios Premium washes	5,86	> 2