



Toulouse, February 4th 2021

STUDY 20-2818/3

REPORT 21-1634

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

Client RuKaInnovation B.V.
Zuidendijk 393,
3317 NS, Dordrecht,
Netherlands

NEOTENY / (주)네오테니
8th Floor, Seoul Finance Center,
136, Sejong-daero, Jung-gu,
04520 Seoul, Republic of Korea

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

Dr Laïla HADDIOUI
Study Manager

Dr Jocelyne BACARIA
Quality Manager

I- TEST LABORATORY IDENTIFICATION

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

II -SAMPLE IDENTIFICATION

- Name of support: PROMAXSILVER-ACLIVSilver PET
- Batch number: 200606-04
- Date of receipt: 26/11/2020
- Internal code: 20-2818-3

- Name of support: TEMOIN PET
- Batch number: not communicated
- Date of receipt: 26/11/2020
- Internal code: 20-2818-6

- Supplier: RuKaInnovation b.v. - NEOTENY

- Period of testing: January 2021

III - TEST METHOD

III-1 VIRUS

Name: Human Coronavirus 229E
Origin: ATCC
Reference: VR-740
Supplier batch number: 58505270
Internal batch number: SS-2-210920 (Passage N°2)

III-2- Recipient cells

Name: Vero Cells
Origin: ATCC
Reference: CCI-81
Supplier batch number: 3372621
Internal batch number: WCB-090708 (Passage N°20 et 27)

IV -TEST CONDITIONS

- Contact times: 30 minutes, 1 hour and 24 hours

- Test temperature: 25°C ± 1°C

V- TEST METHOD

V-1 Control of cytotoxicity

2.5 ml of neutralizing medium are added to 3 untreated and 3 treated samples. The samples are washed 4 times with the neutralizing medium.

A ten-fold serial dilution is made to check the absence of cellular cytotoxicity.

V-2 Control of the sensitivity of the cells to the virus and stopping the antiviral activity

2.5 ml of neutralizing medium are added to 3 untreated and 3 treated samples.

The samples are washed 4 times with the neutralizing medium. Then 1.98 ml of recovery medium are mixed with 20 µl of the virus suspension prepared at a concentration of 4 to 6 .10⁶ TCID₅₀/ml. After 30 min of 25°C incubation, tubes with virus solution are maintained in ice before titration.

V-3 Contact virus/surface

Each sample with a surface area of 5 cm x 5 cm (control and test samples) is placed in a sterile glass Petri dish.

- 400 µl of the viral suspension are deposited on each surface and spread over 16 cm² using a 4 x 4 cm film to reduce desiccation of the inoculum.

V-4 Recovery of the viral film

After incubation, 3.6 ml of a neutralizing solution (frozen culture medium) are added to the samples in order to recover viable viruses.

The titration of the remaining viable viruses is then carried out immediately.

V-5 Viral titer

The titration technique is indicated in the standard NF EN 14476 + A2 (July 2019).

A ten-fold serial dilution of the viral suspensions is made 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 transfer in 8 wells.

V-6 Viral titer calculation

The assay is performed by the microplate method of suspension cells. The cytopathic effect is determined at least 4 days of culture.

The number of infectious units is estimated with the SPEARMAN-KÄRBER method by calculating the negative logarithm of the 50% limit point (lgTCID₅₀) using the following formula:

$\text{lgTCID}_{50} = \text{Negative logarithm of the highest concentration of virus used} - [(\text{Sum of \% assigned to each dilution}/100 - 0.5) \times (\text{lg of dilution})]$

The following tests are carried out 3 times.

VI- RESULTS

VI-1 Validations 30 min and 1 hour

VI-1-1 Control of cytotoxicity

No cytotoxicity was observed on the cells after contact of the culture medium with treated and untreated samples.

VI-1-2 Control of the sensitivity of cells to viruses and cessation of virucidal activity

The difference between the average titers (lg TCID₅₀) of the neutralizing solution controls of and the sensitivity titers average of the treated and untreated surfaces must be less than or equal to 0.5 lg.

Neutralizing solution control

- Control 1 : lg TCID₅₀ = 3.63
- Control 2 : lg TCID₅₀ = 3.88
- Control 3 : lg TCID₅₀ = 3.75

lg TCID₅₀ neutralizing solution control average = 3.75

Control Sensitivity of untreated surfaces

- Control 1 : lg TCID₅₀ = 3.88
- Control 2 : lg TCID₅₀ = 3.75
- Control 3 : lg TCID₅₀ = 3.75

lg TCID₅₀ Sensitivity of untreated surfaces average = 3.79

Titer neutralizing solution average - Sensitivity of untreated surfaces average = 0.04

Difference ≤ 0.5 lg (verification valid)

Test Sensitivity of treated surfaces PROMAXSILVER-ACLIVSilver PET

- Control 1 : lg TCID₅₀ = 3.75
- Control 2 : lg TCID₅₀ = 3.75
- Control 3 : lg TCID₅₀ = 3.63

lg TCID₅₀ Control Sensitivity average = 3.71

Titer neutralizing solution average - Sensitivity of treated surfaces average = 0.04

Difference \leq 0.5 lg (verification valid)

VI-1-3 T0 control

- Control 1 : lg TCID₅₀ = 5.88
- Control 2 : lg TCID₅₀ = 5.63
- Control 3 : lg TCID₅₀ = 5.50

lg TCID₅₀ T0 average = 5.67

Maximum viral title - Minimum viral title = 0.07

Average of the 3 viral titles

The titer (lg DICT₅₀) of the 3 tests at T0 must be homogeneous

Maximum viral titer - Minimum viral titer / Average of the 3 viral title \leq 0,2.

TCID₅₀ average /ml = 4.68 10⁶

Average TCID₅₀ /ml = 10^{average log₁₀ DICT₅₀} × 10

Infectivity titer (TCID₅₀/cm²) =

$$\frac{\text{TCID}_{50}/\text{ml} * \text{Volume de récupération (4ml)}}{\text{Surface (16 cm}^2\text{)}} = 1.17 \cdot 10^6$$

Infectivity titer at T0 (TCID₅₀/cm²) must be between 8.94 10⁵ and 4.46 10⁶

VI-2 Validations 24 hours

VI-2-1 Control of cytotoxicity

No cytotoxicity was observed on the cells after contact of the culture medium with treated and untreated samples.

VI-2-2 Control of the sensitivity of cells to viruses and cessation of virucidal activity

The difference between the average titers (lg TCID₅₀) of the neutralizing solution controls of and the sensitivity titers average of the treated and untreated surfaces must be less than or equal to 0.5 lg.

Neutralizing solution control

- Control 1 : lg TCID₅₀ = 3.88
- Control 2 : lg TCID₅₀ = 4.00
- Control 3 : lg TCID₅₀ = 3.75

lg TCID₅₀ neutralizing solution control average = 3.92

Control Sensitivity of untreated surfaces

- Control 1 : lg TCID₅₀ = 3.88
- Control 2 : lg TCID₅₀ = 3.75
- Control 3 : lg TCID₅₀ = 3.75

lg TCID₅₀ Sensitivity of untreated surfaces average = 3.79

Titer neutralizing solution average - Sensitivity of untreated surfaces average = 0.13

Difference ≤ 0.5 lg (verification valid)

Test Sensitivity of treated surfaces PROMAXSILVER - ACLIVSilver PET

- Control 1 : lg TCID₅₀ = 3.75
- Control 2 : lg TCID₅₀ = 3.63
- Control 3 : lg TCID₅₀ = 3.88

lg TCID₅₀ Control Sensitivity average = 3.75

Titer neutralizing solution average - Sensitivity of treated surfaces average = 0.13

Difference ≤ 0.5 lg (verification valid)

VI-2-3 TO control

- Control 1 : lg TCID₅₀ = 5.88
- Control 2 : lg TCID₅₀ = 6.00
- Control 3 : lg TCID₅₀ = 5.75

lg TCID₅₀ TO average = 5.88

Maximum viral title - Minimum viral title = 0.04
Average of the 3 viral titles

The titer (lg DICT₅₀) of the 3 tests at TO must be homogeneous
Maximum viral titer - Minimum viral titer / Average of the 3 viral title ≤ 0,2.

TCID₅₀ average /ml = 7.59 10⁶

Average TCID₅₀ /ml = 10^{average log₁₀ DCICT₅₀} × 10

Infectivity titer (TCID₅₀/cm²) =

$$\frac{\text{TCID}_{50}/\text{ml} * \text{Volume de récupération (4ml)}}{\text{Surface (16 cm}^2\text{)}} = 1.89 \cdot 10^6$$

Infectivity titer at T0 (TCID₅₀/cm²) must be between 8.94 10⁵ and 4.46 10⁶

VI-3 Tests

VI-3-1 Test 30 minutes

Control 30 minutes:

- Control 1 : lg TCID₅₀ = 5.50
- Control 2 : lg TCID₅₀ = 5.63
- Control 3 : lg TCID₅₀ = 5.38

lg TCID₅₀ T30 min average = 5.50

Moyenne TCID₅₀ /ml = 3.17 10⁶

Moyenne TCID₅₀ /ml = 10^{Moyenne log₁₀ DCICT₅₀} × 10

Infectivity titer (TCID₅₀/cm²) =

$$\frac{\text{TCID}_{50}/\text{ml} * \text{Recovery volume (4ml)}}{\text{Surface (16 cm}^2\text{)}} = 7.91 \cdot 10^5$$

Infectivity titer at contact time 30 min (TCID₅₀/cm²) must be greater than 2.21 10³

Test 30 minutes

- Assay 1 : lg TCID₅₀ = 3.25
- Assay 2 : lg TCID₅₀ = 3.50
- Assay 3 : lg TCID₅₀ = 3.38

lg TCID₅₀ Assay average = 3.38

**R = lg TCID₅₀ control 30 minutes average - lg TCID₅₀ Test 30 minutes average = 2.12
log**

VI-3-2 Test 1 hour**Control 1 hour:**

- Control 1 : lg TCID₅₀ = 5.50
- Control 2 : lg TCID₅₀ = 5.63
- Control 3 : lg TCID₅₀ = 5.38

lg TCID₅₀ T0 average = 5.50

Moyenne TCID₅₀ /ml = $3.17 \cdot 10^6$
 Moyenne TCID₅₀ /ml = $10^{\text{Moyenne log}_{10} \text{DCICT}_{50}} \times 10$

Infectivity titer (TCID₅₀/cm²) =

$$\frac{\text{TCID}_{50}/\text{ml} * \text{Recovery volume (4ml)}}{\text{Surface (16 cm}^2)} = 7.91 \cdot 10^5$$

Infectivity titer at contact time 1 hour (TCID₅₀/cm²) must be greater than $2.21 \cdot 10^3$

Test 1 hour

- Assay 1 : lg TCID₅₀ = 2.75
- Assay 2 : lg TCID₅₀ = 3.00
- Assay 3 : lg TCID₅₀ = 2.88

lg TCID₅₀ Assay average = 2.88

R = lg TCID₅₀ control 1 hour average - lg TCID₅₀ Test 1hour average = 2.62 log

VI-3-3 Test 24 hours**Control 24 hours:**

- Control 1 : lg TCID₅₀ = 5.50
- Control 2 : lg TCID₅₀ = 5.63
- Control 3 : lg TCID₅₀ = 5.63

lg TCID₅₀ T24h average = 5.59

Moyenne TCID₅₀ /ml = $3.89 \cdot 10^6$
 Moyenne TCID₅₀ /ml = $10^{\text{Moyenne log}_{10} \text{DCICT}_{50}} \times 10$

Infectivity titer (TCID₅₀/cm²) =

$$\frac{\text{TCID}_{50}/\text{ml} * \text{Recovery volume (4ml)}}{\text{Surface (16 cm}^2)} = 9.73 \cdot 10^5$$

Infectivity titer at contact time 24 hours (TCID₅₀/cm²) must be greater than $2.21 \cdot 10^3$

Test 24 hours

- Assay 1 : lg TCID₅₀ = 2.13
- Assay 2 : lg TCID₅₀ = 2.00
- Assay 3 : lg TCID₅₀ = 2.25

lg TCID₅₀ Assay average = 2.13

R = lg TCID₅₀ control 24 hours average - lg TCID₅₀ Test 24 hours average = 3.46 log

VII-CONCLUSION

According to the methodology of the ISO 21702 standard (May 2019), contact of treated support PROMAXSILVER-ACLIVSilver PET with the strain of Human Coronavirus 229E induces a reduction of the viral titer of 2.12 lg at contact time 30 minutes, 2.62 lg at contact time 1 hour, and 3.46 lg at the contact time 24 hours

The treatment of supports PROMAXSILVER-ACLIVSilver PET induces a reduction of the viral load of 99.24% at contact time 30 minutes, 99.76% at contact time 1 hour, and 99.97% at contact time 24 hours.