

R2010FSLUR001

Antiviral activity of VIRUPROTEC surface against human coronavirus HCoV-229E for the contact times of 2 and 24 hours.
According to ISO 21702 (2019) standard

| | |
|-------------------------------|---|
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Report includes 12 pages



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I. CONCLUSION

Antiviral activities of the VIRUPROTEC surface and inactive surface have been tested under conditions defined by the ISO 21702 (2019) protocol for the contact times of 2 and 24 hours against human coronavirus HCoV-229E.

The inactive surface is the control for this test.

- VIRUPROTEC surface, 2 hours of contact time

Under experimental conditions (25°C, 2 hours), the VIRUPROTEC surface shows an antiviral activity per cm² associated with a logarithmic reduction of 0.4log₁₀ (60.19%) efficiency under the ISO 21702 protocol.

| PRODUCT | Contact time (hours) | Antivirale activity R (log ₁₀ cm ²) | Antiviral activity (%) |
|------------|----------------------|--|------------------------|
| VIRUPROTEC | 2 hours | R= 0.4 | 60.19 |

- VIRUPROTEC surface, 24 hours of contact time

Under experimental conditions (25°C, 24 hours), the VIRUPROTEC surface shows an antiviral activity per cm² associated with a logarithmic reduction of 1.8 log₁₀ (98.42%) efficiency under the ISO 21702 protocol.

| PRODUCT | Contact time (hours) | Antivirale activity R (log ₁₀ cm ²) | Antiviral activity (%) |
|------------|----------------------|--|------------------------|
| VIRUPROTEC | 24 hours | R = 1.8 | 98.42 |



II. CONTRACTUAL DOCUMENTS

The present service is defined by the following contractual documents:

| | |
|--------------------|--------------------------------------|
| . Quotation | DEV0200 |
| . Order | Good for agreement date : 14/10/2020 |

III. TEST CONDITIONS AND SAMPLES DATA

III.1 Samples identification

| Surface | VIRUPROTEC | Inactive surface |
|----------------------------|--------------------------------|--------------------------------|
| Appearance | white | white |
| Size (cm/cm ²) | 5 cm x 5 cm/ 25cm ² | 5 cm x 5 cm/ 25cm ² |
| Thickness (mm) | 7 to 10 mm | 7 to 10 mm |
| Porous / non porous | non porous | non porous |

Manufacturer: LUREDERRA

Supplier: LUREDERRA

Storage conditions: room temperature

Evaluation period: 10/2020

III.2 Experimental conditions

Test surface: VIRUPROTEC

| Experimental Conditions | |
|-------------------------------|---|
| Date | - 16/10/2020 |
| Viral strain | Human coronavirus HCoV-229E |
| Inoculum volume | 400 μ L |
| Cover film (cm ²) | 4 cm x 4 cm = 16 cm ² |
| Temperature | 25°C |
| Humidity HR (%) | 90% |
| Contact time | 2 hours 24 hours |
| Neutralisation | 10mL of SCDLP medium |
| Quantification | endpoint titration on permissives cells |
| Number of wells per dilution | 8 |
| Incubation temperature | 34 \pm 1 °C |



IV. RESULTS

IV.1 Antiviral activity of the VIRUPROTEC surface against human coronavirus HCoV-229E for the contact times of 2 and 24 hours

a. Cell susceptibility

| Surface | Log ₁₀ TCID ₅₀ /mL |
|--|--|
| SCDLP medium | 6.3 |
| VIRUPROTEC | 6.0 |
| Inactive surface | 6.1 |
| Active Surface: Difference < 0.5 log ₁₀ <input checked="" type="checkbox"/> yes <input type="checkbox"/> no | |
| Inactive Surface: Difference < 0.5 log ₁₀ <input checked="" type="checkbox"/> yes <input type="checkbox"/> no | |

b. Cytotoxicity

The test surface cytotoxicity is determined by reading of cytopathic effect (CPE) on MRC5 permissive cells and quantified by TCID₅₀ technique.

For viral recuperation on surface, the surfaces are submerging in 10mL of SCDLP medium (recuperation buffer). The recuperation buffer cytotoxicity is determined by reading of cytopathic effect (CPE).

Under test conditions, the recuperations buffers from VIRUPROTEC and inactive reference surfaces did not show cytopathic effects on MRC5 cells for the contact times of 2 and 24 hours.

The test results are dependent on and take into account the cytotoxicity results.

c. Inactivation of antivirale activity

| Product | Log ₁₀ TCID ₅₀ /mL |
|--|---|
| S_n = SCDLP medium | 5.5 |
| S_t = active surface | 5.1 |
| S_u = inactive surface (reference) | 5.1 |
| $S_n - S_u \leq 0,5$ <input checked="" type="checkbox"/> yes <input type="checkbox"/> no | |
| $S_n - S_t \leq 0,5$ <input checked="" type="checkbox"/> yes <input type="checkbox"/> no | |

Explanations:

S_n : the average of the common logarithm of the infectivity titer of virus from three of the SCDLP broth for negative control.

S_u : the average of the common logarithm of the infectivity titer of virus recovered from three of the untreated test specimens;

S_t : the average of the common logarithm of the infectivity titer of virus recovered from three of the test specimens.

d. Test

Raw data for antiviral activity of VIRUPROTEC and inactive reference surfaces against human coronavirus HCoV-229E under test conditions (25°C, 2 and 24 hours) are presented in appendices.

Results have been determined by visual reading of cytopathic effects (CPE) and quantified by TCID₅₀ technique on MRC5 cells.

| Surface | Cytotoxicity (log ₁₀ TCID ₅₀) | Specimen | U ₀ (log ₁₀ TCID ₅₀ /cm ²) | U _{t2} (log ₁₀ TCID ₅₀ /cm ²) | U _{t24} (log ₁₀ TCID ₅₀ /cm ²) |
|-------------------------|--|----------------|---|--|---|
| Inactive surface | 0.5 | L1 | 5,3 | 4,9 | 4,5 |
| | | L2 | 5,3 | 4,9 | 4,1 |
| | | L3 | 5,0 | 4,9 | 4,4 |
| | | <i>Average</i> | 5,2 | 4,9 | 4,3 |

| Surface | Cytotoxicity (log ₁₀ TCID ₅₀) | Specimen | A ₀ (log ₁₀ TCID ₅₀ /cm ²) | A _{t2} (log ₁₀ TCID ₅₀ /cm ²) | A _{t24} (log ₁₀ TCID ₅₀ /cm ²) |
|-------------------|--|---|---|--|---|
| VIRUPROTEC | 0.5 | L1 | 5,4 | 4,0 | 2,5 |
| | | L2 | 4,9 | 4,8 | 2,5 |
| | | L3 | 5,1 | 4,9 | 2,5 |
| | | <i>Average</i> | 5,1 | 4,5 | 2,5 |
| | | R (log ₁₀ TCID ₅₀ /cm ²)* | / | 0,4 | 1,8 |

R is the antiviral activity

U₀ is the average of the common logarithm of the number of TCID₅₀ recovered from three untreated test specimens immediately after inoculation

U_t is the average of the common logarithm of the number of TCID₅₀ recovered from three untreated test specimens

A₀ is the average of the common logarithm of the number of TCID₅₀ recovered from three treated test specimens immediately after inoculation

A_t is the average of the common logarithm of the number of TCID₅₀ recovered from three treated test specimens.

The logarithmic value of the number of TCID₅₀ recovered immediately after inoculation from test specimens satisfies the requirement below: $(L_{max} - L_{min}) / (L_{mean}) \leq 0.2$.

The number of plaques recovered from each untreated test specimen after contacting for 24 h is not less than $6,2 \times 10^2$ TCID₅₀/cm².



V. CONCLUSION

VIRUPROTEC surface shows antiviral activity of $0.4 \log_{10}$ TCID₅₀/cm² (60.19%) and of $1.8 \log_{10}$ TCID₅₀/cm² (98.42%) against human coronavirus HCoV-229E after the contact times of 2 and 24 hours respectively at 25°C and 90% RH.

VI. ANNEXES

VI.1 Materials and reagents

- Cell line

Name : MRC5 ATCC® CCL-171™

Number of passages: 23

Culture medium: EMEM (Lonza, lot n°0000757679, 11/2020) avec 10% of SVF (Dutscher, lot n° S16529S1810, 09/2022), 1% of antibiotics (Gibco, lot n° 2145466, 12/2020) et 1% of L-glutamine (Gibco, lot n° 2091579, 22/2021)

- Viral strain

Name: human coronavirus 229E ATCC® VR-740™

Viral test suspension: 4.22×10^7 (batch number: 102020229-2)

Quantification technique :

- Successive tenfold dilution in infection medium: EMEM (Lonza, lot n°0000757679, 11/2020) avec 2% of SVF (Dutscher, lot n° S16529S1810, 09/2022), 1% of antibiotics (Gibco, lot n° 2145466, 12/2020) et 1% of L-glutamine (Gibco, lot n° 2091579, 22/2021)
- Add 100µL of every dilution on 8 wells on a 96 plate.
- Incubate 7 days at 34°C, 5% CO₂

- Cover film :
 - PE film, 40 mm square, 0,15 mm thick (FILM-PE-1, Toutembal)

VI.2 RAW DATA: TCID₅₀ quantification of human coronavirus HCoV-229E after 2 and 24 hours, visual reading of cytopathic effects (8 wells per dilutions)

• Table 1: Inactivation of antiviral activity

| | Product | contact time (hours) | dilutions (-log) | | | | | | | |
|------------------------------------|------------|----------------------|------------------|----------|----------|----------|---|---|---|---|
| | | | p | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Inactivation of antiviral activity | SCDLP | / | 44444444 | 44444444 | 44444444 | 22324121 | 0 | 0 | 0 | 0 |
| | | / | 44444444 | 44444444 | 44444444 | 11212111 | 0 | 0 | 0 | 0 |
| | | / | 44444444 | 44444444 | 44444444 | 11221210 | 0 | 0 | 0 | 0 |
| | untreated | / | 44444444 | 44444444 | 44444444 | 10010011 | 0 | 0 | 0 | 0 |
| | | / | 44444444 | 44444444 | 44444444 | 11121000 | 0 | 0 | 0 | 0 |
| | | / | 44444444 | 44444444 | 44444444 | 22101210 | 0 | 0 | 0 | 0 |
| | VIRUPROTEC | / | 44444444 | 44444444 | 44444444 | 01010011 | 0 | 0 | 0 | 0 |
| | | / | 44444444 | 44444444 | 44444444 | 11110010 | 0 | 0 | 0 | 0 |
| | | / | 44444444 | 44444444 | 44444444 | 11000111 | 0 | 0 | 0 | 0 |

Explanations:

- 1-4: degrees of CPE in 8 cell culture unit (microtiter plate)
- 0: no virus present
- n.a: not applicable
- n.d: not done

• Table 2 : Va/Va'

| | Product | contact time (hours) | dilutions (-log) | | | | | | | |
|--------|------------|----------------------|------------------|----------|----------|----------|---|---|---|---|
| | | | p | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Va/Va' | untreated | 0 | 44444444 | 44444444 | 44444444 | 11200111 | 0 | 0 | 0 | 0 |
| | | 0 | 44444444 | 44444444 | 44444444 | 11213001 | 0 | 0 | 0 | 0 |
| | | 0 | 44444444 | 44444444 | 44444444 | 00212100 | 0 | 0 | 0 | 0 |
| | VIRUPROTEC | 0 | 44444444 | 44444444 | 44444444 | 22101121 | 0 | 0 | 0 | 0 |
| | | 0 | 44444444 | 44444444 | 44444444 | 00000111 | 0 | 0 | 0 | 0 |
| | | 0 | 44444444 | 44444444 | 44444444 | 11203200 | 0 | 0 | 0 | 0 |

Explanations:

- 1-4: degrees of CPE in 8 cell culture unit (microtiter plate)
- 0: no virus present
- n.a: not applicable
- n.d: not done

• Table 3 : cytotoxicity

| | Product | contact time (hours) | dilutions (-log) | | | | | | | |
|--------------|------------|----------------------|------------------|---|---|---|---|---|---|---|
| | | | p | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| cytotoxicity | untreated | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | VIRUPROTEC | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | untreated | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | VIRUPROTEC | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Explanations:

- 1-4: degrees of CPE in 8 cell culture unit (microtiter plate)
- 0: no virus present
- n.a: not applicable
- n.d: not done

Table 4 : cell susceptibility

| | Product | contact time (hours) | dilutions (-log) | | | | | | | |
|---------------------|------------|----------------------|------------------|----------|----------|----------|----------|---|---|---|
| | | | p | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| cell susceptibility | SCDLP | / | 44444444 | 44444444 | 44444444 | 44444444 | 12320220 | 0 | 0 | 0 |
| | untreated | / | 44444444 | 44444444 | 44444444 | 44444444 | 10110011 | 0 | 0 | 0 |
| | VIRUPROTEC | / | 44444444 | 44444444 | 44444444 | 44444444 | 00111100 | 0 | 0 | 0 |

Explanations:

- 1-4: degrees of CPE in 8 cell culture unit (microtiter plate)
- 0: no virus present
- n.a: not applicable
- n.d: not done

• Table 5 : test

| | Product | contact time (hours) | dilutions (-log) | | | | | | | |
|------|------------|----------------------|------------------|----------|----------|----------|---|---|---|---|
| | | | p | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| test | untreated | 2 | 44444444 | 44444444 | 44444444 | 00001011 | 0 | 0 | 0 | 0 |
| | | 2 | 44444444 | 44444444 | 44444444 | 01000011 | 0 | 0 | 0 | 0 |
| | | 2 | 44444444 | 44444444 | 44444444 | 00011010 | 0 | 0 | 0 | 0 |
| | VIRUPROTEC | 2 | 44444444 | 44444444 | 10011100 | 0 | 0 | 0 | 0 | 0 |
| | | 2 | 44444444 | 44444444 | 11111111 | 11000000 | 0 | 0 | 0 | 0 |
| | | 2 | 44444444 | 44444444 | 11121412 | 0110010 | 0 | 0 | 0 | 0 |
| | untreated | 24 | 44444444 | 44444444 | 22121222 | 0 | 0 | 0 | 0 | 0 |
| | | 24 | 44444444 | 44444444 | 22130010 | 0 | 0 | 0 | 0 | 0 |
| | | 24 | 44444444 | 44444444 | 11214440 | 0 | 0 | 0 | 0 | 0 |
| | VIRUPROTEC | 24 | 44444444 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 24 | 44444444 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 24 | 44444444 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Explanations:

- 1-4: degrees of CPE in 8 cell culture unit (microtiter plate)
- 0: no virus present
- n.a: not applicable
- n.d: not done