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Virucidal activity of the Agivir surface on Human coronavirus HCoV-229E
for a contact time of 15 and 60min.

Adapted protocol from ISO 21702 (201) standard

CLIENT

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SUMMARY

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I. CONCLUSION

Virucidal activity of the Agivir surface and non-active surface have been tested under conditions defined by the ISO 21702 (2019) adapted protocol for contact times of 15 and 60 minutes on the human coronavirus HCoV-229E with the chosen interfering substance (mucus/saliva mixture or BSA 0,3g/L)

The non-active surface is the control for this test

- Agivir, 15 minutes of contact time

Under experimental conditions, (20°C, 15 minutes, Mucus/saliva interfering substance), the Agivir surface shows a virucidal activity associated with a logarithmic reduction of 1.30 log₁₀ which is equivalent to a 94.99% efficiency under the ISO 21702 adapted protocol.

- Agivir, 60 minutes of contact time

Under experimental conditions, (20°C, 60 minutes, Mucus/saliva interfering substance), the Agivir surface shows a virucidal activity associated with a logarithmic reduction of 2.27 log₁₀ which is equivalent to a 99.46% efficiency under the ISO 21702 adapted protocol.

| PRODUCT | Contact time (min) | Interfering substance | Logarithmic reduction (Log10) | Virucidal efficiency (%) |
|---------|--------------------|-----------------------|-------------------------------|--------------------------|
| Agivir | 15 | Mucus/ saliva | 1.30 | 94.99% |
| | 60 | | 2.27 | 99.46% |

- Agivir, 15 minutes of contact time

Under experimental conditions, (20°C, 15 minutes, BSA 0,3g/L interfering substance), the Agivir surface shows a virucidal activity associated with a logarithmic reduction of 2.0 log₁₀ which is equivalent to a 99% efficiency under the ISO 21702 adapted protocol.

- Agivir, 60 minutes of contact time

Under experimental conditions, (20°C, 60 minutes, BSA 0,3g/L interfering substance), the Agivir surface shows a virucidal activity associated with a logarithmic reduction of 2.66 log₁₀ which is equivalent to a 99.78% efficiency under the ISO 21702 adapted protocol.

| PRODUCT | Contact time (min) | Interfering substance | Logarithmic reduction (Log ₁₀) | Virucidal efficiency (%) |
|---------|--------------------|-----------------------|--|--------------------------|
| Agivir | 15 | BSA 0,3g/L | 1.30 | 99% |
| | 60 | | 2.27 | 99.78% |



II. CONTRACTUAL DOCUMENTS

The present service is defined by the following contractual documents:

- . **Quotation:** 2002FER002
- . **Order:** Good for agreement date: 27/03/20

III. TEST CONDITIONS AND SAMPLES DATA

III.1 Samples identification

Test surface : Agivir

Control surface : non-active surface

Product appearance: white, smooth and non-porous

Manufacturer : SERGE FERRARI

Supplier : SERGE FERRARI

Storage conditions : room temperature

Evaluation period : 04/2020

II.1 Experimental conditions

Test surface : surface top coat 51166

| Conditions expérimentales | |
|----------------------------------|--|
| Date | - 04/14/2020 |
| Viral strain | - Human coronavirus HCoV-229E |
| Sample size (cm ²) | - 1.5 cm x 1.5 cm = 2.25 cm ² |
| Inoculum size (cm ²) | - 1 cm x 1 cm = 1cm ² |
| Inoculum volume | - 50uL |
| Temperature | 20°C |
| Interfering substance | Mucus/saliva or BSA 0,3g/L |
| Contact time | 15 et 60 minutes |
| Neutralisation | - 2 mL of infection medium without FCS |
| Quantification | -endpoint titration on permissives cells |
| Number of wells per dilution | 4 |
| Incubation temperature | 34 ± 1 °C |

II. RESULTS

Virucidal activity of the Agivir surface on human coronavirus HCoV-229E for a contact time of 15 and 60 minutes

a. Cell susceptibility

| Surface | LOG TCID50/mL |
|--|---------------|
| Agivir | 5.7 |
| Non-active surface | 6.0 |
| Différence < 1 log <input checked="" type="checkbox"/> oui <input type="checkbox"/> no | |

Comparative titre of human coronavirus HCoV-229E on MRC5 cells inoculated with Agivir surface and non-active surface recuperation buffer show a difference less than 1log.

Results showed that recuperation buffers of the test surfaces didn't affect the MRC5 susceptibility to human coronavirus HCoV-229E under test conditions.

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 2mL of infection medium without FCS (recuperation buffer). The recuperation buffer cytotoxicity is determined by reading of cytopathic effect (CPE)

Under test conditions, with artificial mucus / saliva mix interference or BSA 0,3g/L, the recuperations buffers from Agivir and non-active surfaces didn't show cytopathic effects on MRC5 cells for a contact time of 15 and 60 minutes.

The test results are dependant and take into account the cytotoxicity results.

c. test

Raw data for virucidal activity of Agivir and non-active surfaces on human coronavirus HCoV-229E under test conditions (20°C, 5, 15 and 60 minutes, interference Mucus/Saliva or BSA 0,3g/L) are presented in appendices

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

| Surface | Interfering substance | Cytotoxicity (log ₁₀ TCID50) | Support | T0 (log ₁₀ TCID50) | T15 (log ₁₀ TCID50) | T60 (log ₁₀ TCID50) |
|--------------------|-----------------------|---|----------------------------|-------------------------------|--------------------------------|--------------------------------|
| Non-active surface | 0,3 g/L BSA | 0.5 | S1 | 5.5 | 5.5 | 5.3 |
| | | | S2 | 5.7 | 5.7 | 5.5 |
| | | | S3 | 5.7 | 5.5 | 5.5 |
| | | | Average N1 | 5.63 | 5.57 | 5.43 |
| | | | <i>SD</i> | 0.12 | 0.12 | 0.12 |
| Non-active surface | Mucus/saliva | 0.5 | S1 | 5.7 | 5.5 | 5.7 |
| | | | S2 | 6 | 6 | 5.7 |
| | | | S3 | 6 | 5.7 | 5.7 |
| | | | Average N1' | 5.90 | 5.73 | 5.70 |
| | | | <i>SD</i> | 0.17 | 0.25 | 0.00 |
| Surface Agivir | 0,3 g/L BSA | 0.5 | S1 | 6 | 3.5 | 3.3 |
| | | | S2 | 6 | 3.5 | 2.5 |
| | | | S3 | 5.7 | 3.7 | 2.5 |
| | | | Average N2 | 5.90 | 3.57 | 2.77 |
| | | | <i>SD</i> | 0.17 | 0.12 | 0.46 |
| | | | Reduction D1 (log DICT50)* | / | 2 | 2.66 |
| Surface Agivir | Mucus/saliva | 0.5 | S1 | 6 | 4.5 | 3.3 |
| | | | S2 | 6 | 4.5 | 3.5 |
| | | | S3 | 6 | 4.3 | 3.5 |
| | | | Average N2' | 6 | 4.43 | 3.43 |
| | | | <i>SD</i> | 0 | 0.12 | 0.12 |
| | | | Reduction D2 (log DICT50)* | / | 1.3 | 2.27 |

N1 = viral quantity in log₁₀ (average of triplicate) non-active surface (BSA 0,3g/L)

N1' = viral quantity in log₁₀ (average of triplicate) non-active surface (mucus/saliva)

N2 = viral quantity in log₁₀ (average of triplicate) Agivir (BSA 0,3g/L)

N2' = viral quantity in log₁₀ (average of triplicate) Agivir (Mucus/ saliva)

* *D1* : virucidal activity for every contact time (logarithmic reduction in log₁₀)

D2 : virucidal activity for every contact time (logarithmic reduction in log₁₀)

$D1 = N1 - N2$ $D2 = N1' - N2'$



V. CONCLUSION

Agivir surface shows virucidal efficiency of 99.00% (2.00 Log₁₀ TCID₅₀) and 99.78% (2.66 log₁₀ TCID₅₀) on human coronavirus HCoV-229E respectively after a contact time of 15 and 60 minutes with BSA 0,3g/L interference

Agivir surface shows virucidal efficiency of 94.99% (1.30 Log₁₀ TCID₅₀) and 99.46% (2.27 log₁₀ TCID₅₀) on human coronavirus HCoV-229E respectively after a contact time of 15 and 60 minutes with Mucus/ saliva mixture interference

VI. ANNEXES

V.1 Matériels et réactifs

- Cell line

Name: MRC5 ATCC® CCL-171™

Number of passages: 12

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

- Viral strain

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

Viral test suspension: 7.50×10^7 (batch number: 032020229-1)

Quantification technique:

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

V.2 Reagent preparation

- 0,3g/L of BSA: Dissolve 0,3g/L of BSA (SIGMA ALDRICH: batch n° SLB26632) in 100mL of distilled water. Sterilized by membrane filtration 0.2 µm

- mix 1mL of artificial saliva (ASTM2721) with 1mL of nasal epithelium mucus (EPI 118, Epithelix)

V.3 RAW DATA : TCID50 quantification of human coronavirus 229^E after 5, 15 and 60 minutes after Visual reading of cytophatic effects (4 wells per dilutions)

- Table 1 : stopping activity control

| | Product | interfering substance | contact time (min) | dilutions (-log) | | | | | | | | |
|--------------------|--------------------|-----------------------|--------------------|------------------|---|---|---|---|---|---|---|---|
| | | | | p | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| TO | Agivir | bsa | 0 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 3 | 0 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 3 | 2 | 0 | 0 | |
| | surface non-active | | 0 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 3 | 1 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | |
| | Agivir | mucus | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 3 | 2 | 0 | 0 | |
| surface non-active | 0 | | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | | |
| | 0 | | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | | |
| | 0 | | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | | |
| | 0 | | 4 | 4 | 4 | 4 | 4 | 2 | 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 : residual activity control

| | Product | interfering substance | contact time (min) | dilutions (-log) | | | | | | | | |
|--------------------|--------------------|-----------------------|--------------------|------------------|---|---|---|---|---|---|---|---|
| | | | | p | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| residual activity | Agivir | BSA | 0 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 1 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | |
| | surface non-active | | 0 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 3 | 1 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 3 | 1 | 0 | 0 | |
| | Agivir | mucus | 0 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 1 | 0 | 0 | |
| | | | 0 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | |
| surface non-active | 0 | | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | |
| | 0 | | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | | |
| | 0 | | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | | |
| | 0 | | 4 | 4 | 4 | 4 | 3 | 2 | 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 : test

| | Product | interfering substance | contact time (min) | dilutions (-log) | | | | | | | | |
|--------|---------|-----------------------|--------------------|------------------|---|---|---|---|---|---|---|---|
| | | | | p | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Essais | Agivir | bsa | 15 | 4 | 4 | 3 | 1 | 0 | 0 | 0 | 0 | 0 |
| | | | 15 | 4 | 4 | 4 | 1 | 0 | 0 | 0 | 0 | 0 |
| | | | 15 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 60 | | 4 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 60 | | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 60 | | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 15 | | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | |
| | 15 | | 4 | 4 | 4 | 4 | 3 | 2 | 0 | 0 | 0 | |
| | 15 | | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | |
| | 60 | | 4 | 4 | 4 | 4 | 3 | 1 | 0 | 0 | 0 | |
| | 60 | | 4 | 4 | 4 | 4 | 4 | 1 | 0 | 0 | 0 | |
| | 60 | | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | 0 | |
| | Agivir | mucus | 15 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 |
| | | | 15 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 |
| | | | 15 | 4 | 4 | 4 | 3 | 0 | 0 | 0 | 0 | 0 |
| | | | 60 | 4 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | 60 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | 60 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | surface non-active | 15 | 4 | 4 | 4 | 4 | 3 | 0 | 0 | 0 | 0 |
| | | | 15 | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 |
| | | | 15 | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 |
| | | | 60 | 4 | 4 | 4 | 4 | 3 | 2 | 0 | 0 | 0 |
| | | | 60 | 4 | 4 | 4 | 4 | 4 | 1 | 0 | 0 | 0 |
| | | | 60 | 4 | 4 | 4 | 4 | 4 | 1 | 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