

Virucidal test 항바이러스, 살바이러스 효과 실험

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The schematic procedure of virucidal test

Cytopathic Effect (CPE)

- Some viruses kill the cells in which they replicate, and infected cells may eventually detach from the cell culture plate.
- As more cells are infected, the changes become visible and are called cytopathic effects.



Quantification of CPE



Cell Culture Infectious Dose 50 (CCID₅₀): a measure of virulence of virus

CCID₅₀ Procedure

- Count wells exhibiting CPE
- Calculate using the dilution factors to get infection rates of zero to 100 percent





Virucidal test

1) Virus:

Zika (MR766 Uganda); Influenza A/Duck/MN/1525/81 (H5N1)

2) Cells: MDCK cells for influenza and Vero 76 cells for Zika.

3) Cytopathic effect (CPE) : on day 3 for H5N1 and day 6 for Zika virus.

4) The Reed-Muench method : 50% cell culture infectious dose (CCID50) log reduction value (LRV)

Zika (MR766 Uganda)

Table 1. Virucidal efficacy of Puriton against Zika virus after 4 or 18 hrs liquid-liquid contact at $22 \pm 2^{\circ}$ C

| Contact time (hr) | 90% Puriton | 50% Puriton | 70% Ethanol | Water |
|----------------------|---------------------------|---|---|--|
| 4 | 0.7 ± 0.0*** | 0.7 ± 0.0*** | 0.8 ± 0.2*** | 5.0 ± 0.00 |
| 4 | >4.3 | >4.3 | 4.2 | n/a |
| 18 | ^b <0.7*** | 0.7 ± 0.0*** | ^b <0.7*** | 5.2 ± 0.3 |
| 18 | >4.5 | 4.5 | >4.5 | n/a |
| | time (hr) 4 4 18 | time (hr) Puriton 4 0.7 ± 0.0*** 4 >4.3 18 ^b <0.7*** | time (hr)PuritonPuriton4 $0.7 \pm 0.0^{***}$ $0.7 \pm 0.0^{***}$ 4>4.3>4.318 $^{b}<0.7^{***}$ $0.7 \pm 0.0^{***}$ | time (hr)PuritonPuritonEthanol4 $0.7 \pm 0.0^{***}$ $0.7 \pm 0.0^{***}$ $0.8 \pm 0.2^{***}$ 4>4.3>4.34.218 ${}^{b}<0.7^{***}$ $0.7 \pm 0.0^{***}$ ${}^{b}<0.7^{***}$ |

^b For statistical analysis "<" signs were ignored.

***P < 0.001 by one-way ANOVA and Dunnett post-test compared with untreated virus control (water)

Zika (MR766 Uganda)



Influenza A/Duck/MN/1525/81 (H5N1)

Table 2. Virucidal efficacy of Puriton against Influenza A(H5N1) virus after 4 or 18 hrs liquidliquid contact at $22 \pm 2^{\circ}$ C

| | Contact time (hr) | 90% Puriton | 50% Puriton | 70% Ethanol | Water |
|--|----------------------|----------------------|----------------|----------------------|-------------|
| ^a CCID ₅₀ per 100 μL | 4 | ^b <0.7*** | 1.9 ± 0.5*** | ^b <0.7*** | 4.5 ± 0.2 |
| Log reduction value | 4 | >3.8 | 2.6 | >3.8 | n/a |
| ^a CCID ₅₀ per 100 μL | 18 | ^b <0.7*** | <0.7*** | ^b <0.7*** | 4.1 ± 0.4 |
| Log reduction value | 18 | >3.4 | >3.4 | >3.4 | n/a |

^a Log_{10} CCID₅₀ of virus per 0.1 mL, average of 3 replicates ± standard deviation

^b For statistical analysis "<" signs were ignored.

***P < 0.001 by one-way ANOVA and Dunnett post-test compared with untreated virus control (water)

Influenza A/Duck/MN/1525/81 (H5N1)



Summary

1) Puriton 의 90% 와 50%에 지카 바이러스 (Zika virus)를 4시간, 18 시간 동안 Incubation 한 결과 모두에서 지카 바이러스를 죽이는 효과가 있음.

2) 조류 독감바이러스 (Influenza A (H5N1) 의 경우는 puriton 90% 에서 4시간, 18 시간 동안 Incubation 한 모두에서 바이러스를 죽이는 효과가 있음.

3) Puriton 50%에 조류 독감바이러스 (Influenza A (H5N1)를 18 시간 동안 둔 경우에도 바이러스를 죽이는 효과가 있음.

4) Puriton 50%에 조류 독감바이러스 를 4시간 둔 경우도 바이러스를 죽이는 효과가 있으나 18시간 동안 둔 경우보다 약간 작은 효과를 보임

5) Puriton은 지카 바이러스 (Zika virus)와 조류 독감바이러스(Influenza A (H5N1) 모두에 바이러스를 죽이는 효과가 있음.

End