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Introduction

N-chlorotaurine (NCT), an endogenous chloramine compound produced by human phagocytes and its analogs, Aganocide compounds *N*-monochloro-2,2-dimethyltaurine (NVC-612) and *N,N*-dichloro-2,2-dimethyltaurine (NVC-422), were reported to have broad spectrum antiviral activity (1, 2). This study was aimed to evaluate the *in vitro* virucidal activity of NCT, NVC-612 and NVC-422 against several influenza H1N1 strains.

Materials & Methods

Influenza A strains H1N1 (Singapore / Hong Kong 2339, 2000; H. Katinger, Vienna), H1N1 (APR-8-38, MicroTest) and H1N1v (California, 2009; clinical isolate, Innsbruck) were grown on MDCK or Vero cells in RPMI 1640 (Fig. 1). Cell-free supernatants containing 10⁶ to 10⁹ plaque forming units (pfu/ml) were incubated at room temperature in solutions of test substances at different concentrations and times. Aqueous stock solutions of the test substances (~pH 7.4 in water or phosphate buffer without test substances for controls) were diluted ten-fold in the viral suspensions to the indicated end concentrations. After inactivation of the oxidants with sodium thiosulfate or FBS-containing medium, aliquots were transferred to monolayers of MDCK or Vero cells in serial ten-fold dilutions. Cells were incubated and monitored for 7-10 days for cytopathic effects.

Results

The three test substances inactivated all three influenza A strains, while taurine and dimethyltaurine did not reduce the viral titer. NCT and NVC-612 (0.1%) inactivated H1N1 Singapore to the detection limit after 5 min incubation time (reduction factor > 5 log₁₀) (Fig. 2A), while the H1N1v titer was reduced by approximately 2 log₁₀ after 5 min and 4 log₁₀ after 10 min (Fig. 2C).

Influenza strains H1N1 Singapore 2000 and California 2009 were inactivated to the detection limit by 55 mM NCT and NVC-612 within 1 min. A 10 min incubation with 10 and 30 mM NVC-422 reduced the titer of H1N1v by 1 and 2 log₁₀, respectively, the titer of strain Singapore by 2 log₁₀ (Fig. 2B and 2D). 1 hr treatment with 40 mM NVC-422 in saline pH 4 resulted in complete inactivation of the H1N1 strain Apr-8-38 (Fig 3).

The activity of all three test substances was significantly enhanced by the addition of ammonium chloride (Fig. 4), which can be explained by formation of the low molecular weight monochloramine.

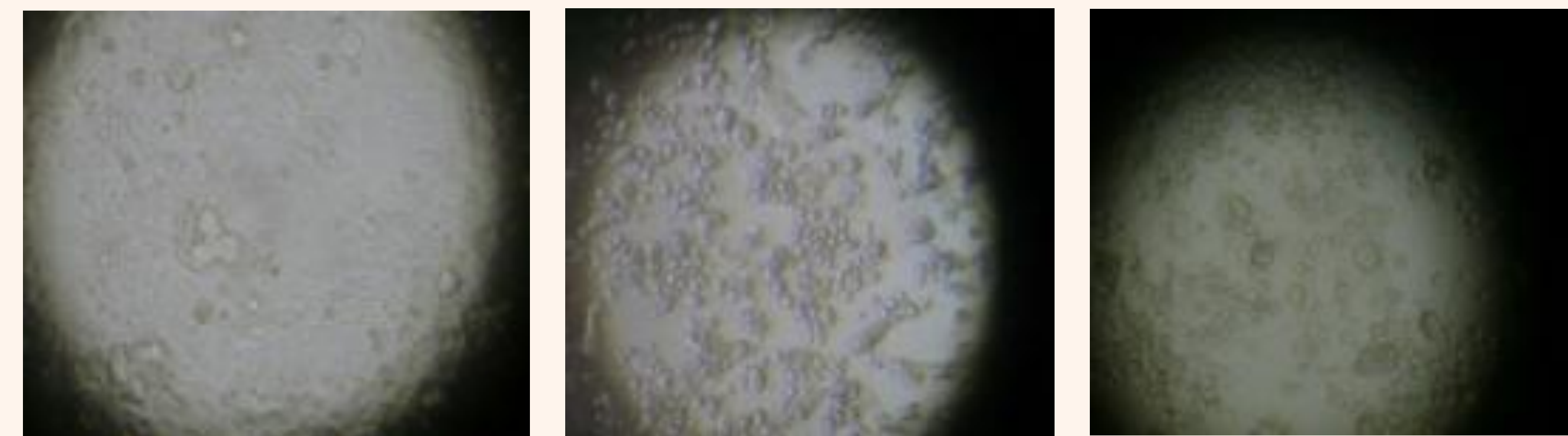
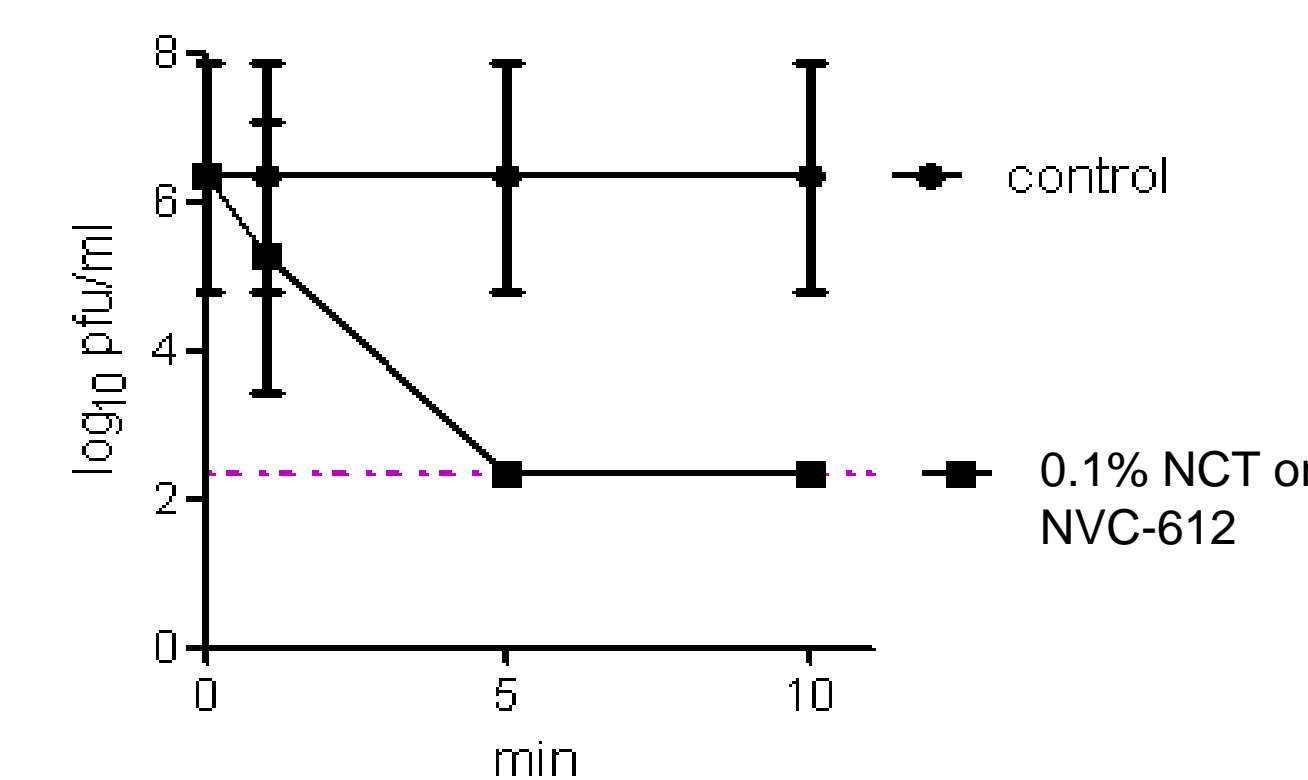


Fig.1: Cytopathic effect of Influenza H1N1 on MDCK cells. Left: uninfected control cells; center: infected with strain Singapore 2000; right: infected with strain California 2009.

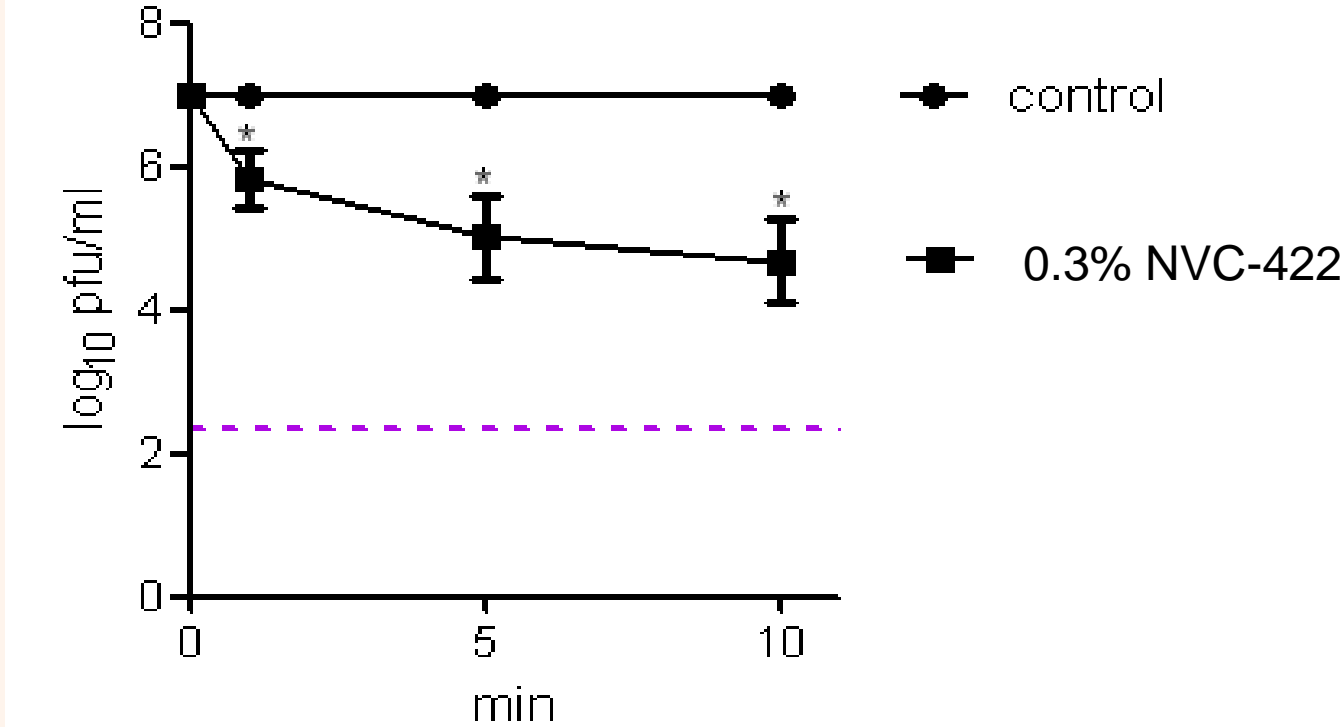
Singapore - 0.1% NCT or NVC-612



n = 3; mean values ± SD
* P < 0.01 after 5 and 10 min
..... detection limit 2.35 log₁₀

Fig. 2A. H1N1 Singapore is inactivated by 0.1% NCT or NVC-612 by 5 minutes.

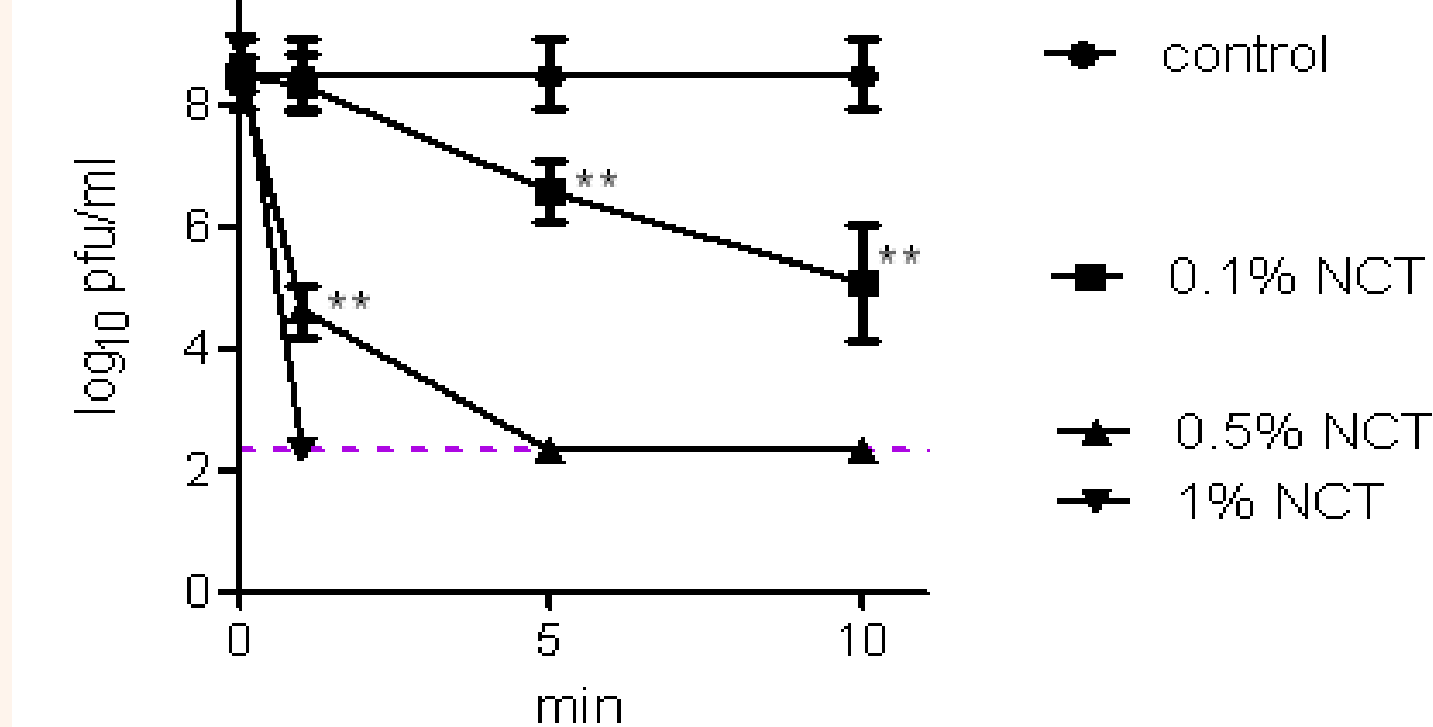
Singapore - 0.3% NVC-422



n = 3; mean values ± SD
* P < 0.05
..... detection limit 2.35 log₁₀

Fig. 2B. H1N1 Singapore was not inactivated by 0.3% NVC-422 within 10 minutes.

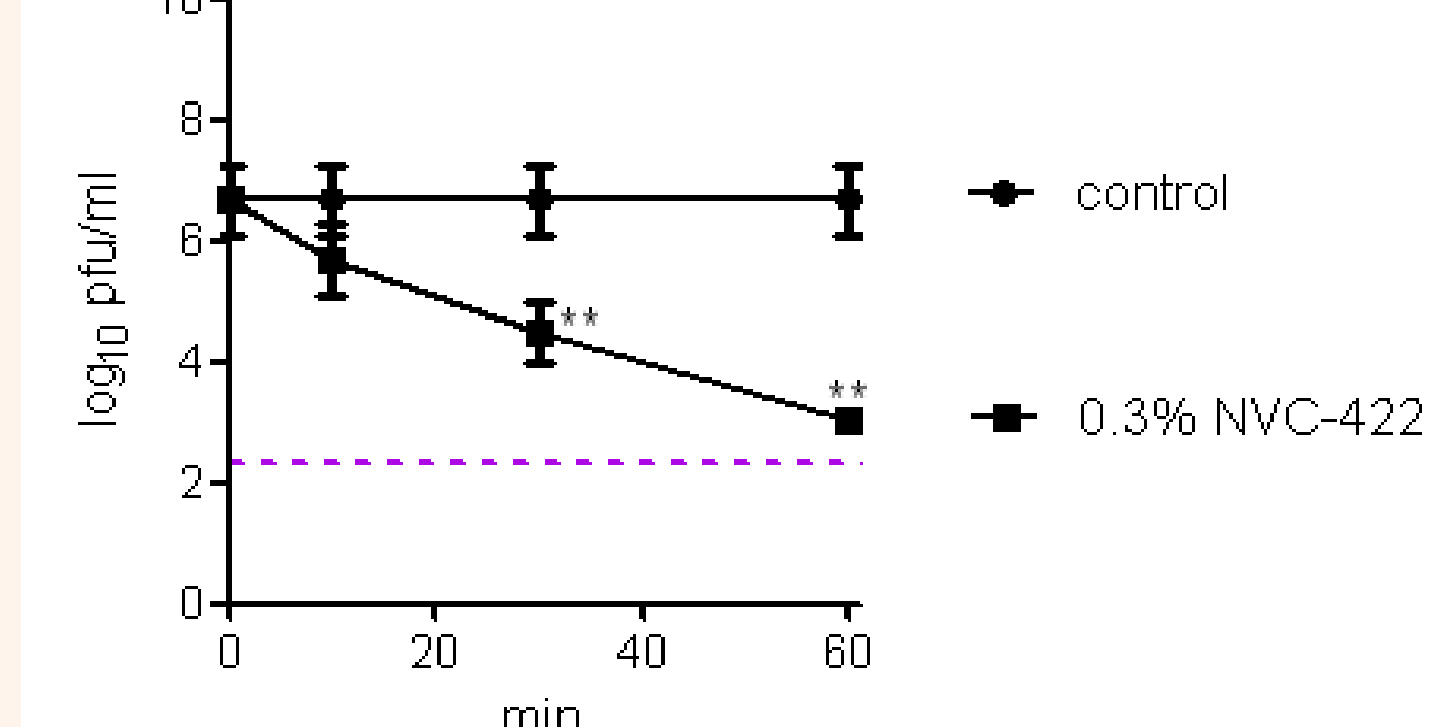
California - NCT



n = 4; mean values ± SD
** P < 0.01 versus control
..... detection limit 2.35 log₁₀

Fig. 2C. H1N1v California is inactivated by NCT within 5 minutes at 0.5% and 1%. 0.1% NCT failed to inactivate H1N1v.

California - NVC-422



n = 3; mean values ± SD
P < 0.01
..... detection limit 2.35 log₁₀

Fig. 2D. H1N1v California was not inactivated by 0.3% NVC-422 within 60 minutes.

H1N1 Apr-8-38

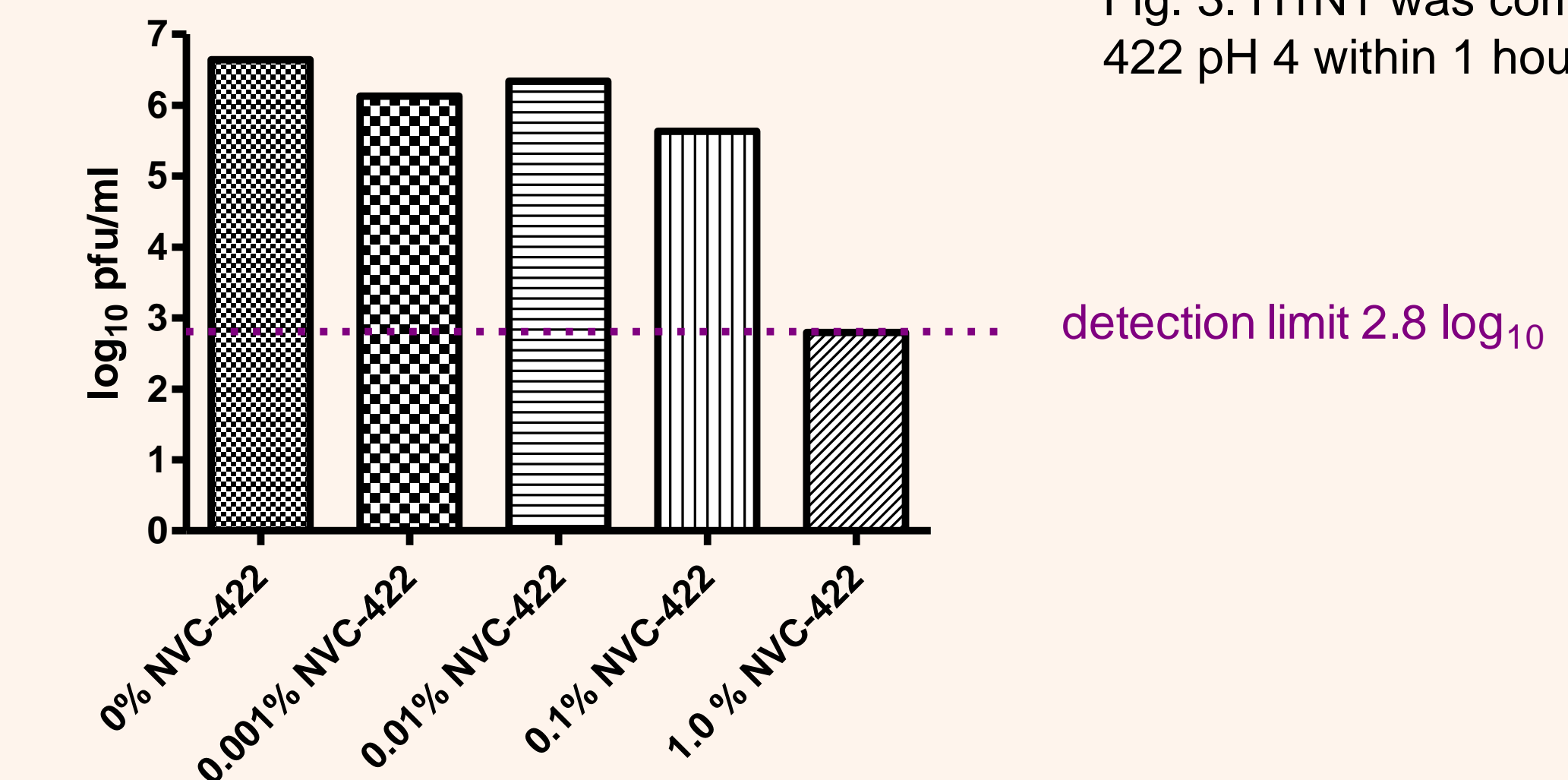


Fig. 3. H1N1 was completely inactivated by 1% NVC-422 pH 4 within 1 hour.

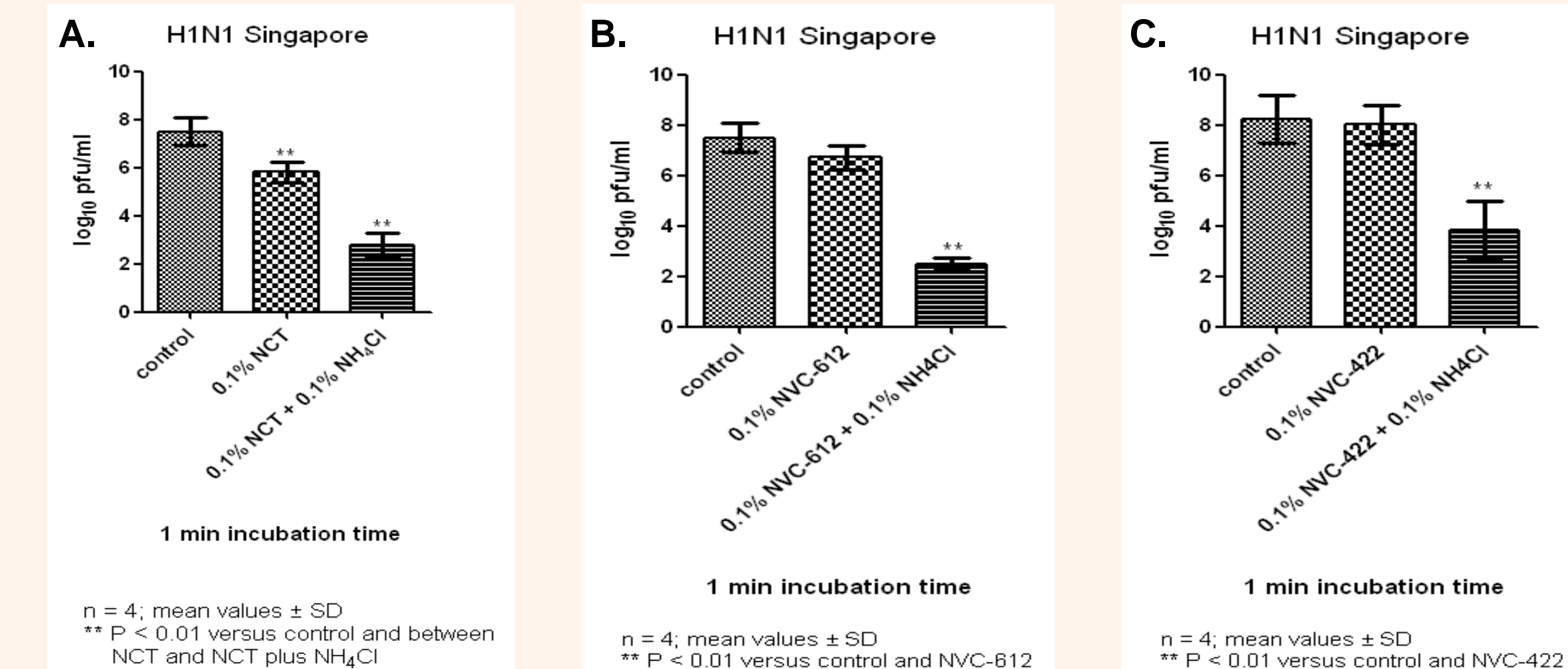


Fig. 4. The addition of NH₄Cl enhances H1N1 inactivation by 0.1% NCT, NVC-612 and NVC-422. A) 0.1% NCT pH 7; B) 0.1% NVC-612 pH 7; C) 0.1% NVC-422 pH 7.4

Conclusion

- NCT, NVC-612, and NVC-422 all demonstrated virucidal activity against influenza A.
- 1% NVC-422 achieved complete inactivation of H1N1 virus (Fig. 3).
- Interestingly, addition of ammonium chloride showed an accelerated time-kill for all three compounds, which is presumably due to the formation of the low molecular weight monochloramine, warranting further investigation.
- The *in vitro* data suggest that H1N1 (Singapore 2000) is slightly more susceptible than H1N1v (California 2009).
- Further investigation into potential consumer products and therapeutic use of these agents are warranted.

References

1. C. Celeri, D. Debabov, M. Flagella, S. Rani, N. Alvarez, M. Zuck, and B. Belisle. Biological Characterization of *N,N*-dichloro-2,2-dimethyltaurine (NVC-422). ICAAC, Washington, DC October 25-28, 2008.
2. K.K. Najafi, S.S. Wilmarth, R. Najafi, B. Khosrovi, M. Anderson, C. Celeri, D. Debabov, M. Chowhan, D.W. Stroman. *In vitro* Microbicidal Activity in Tears of *N,N*-dichloro-2,2-dimethyltaurine (NVC-422, AL-46383A), a Novel Topical Ophthalmic Agent. ARVO, Fort Lauderdale, May 2-6 2010.