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Ultrasensitive ELISA Detection of Inactivated Viruses

超高感度タンパク質測定法を用いた不活化ウイルスの測定

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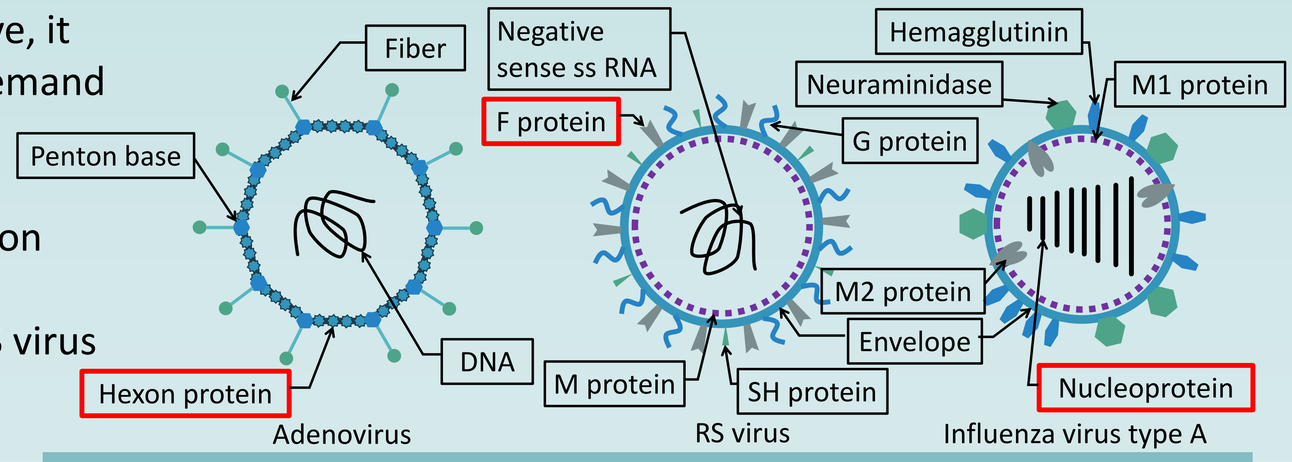
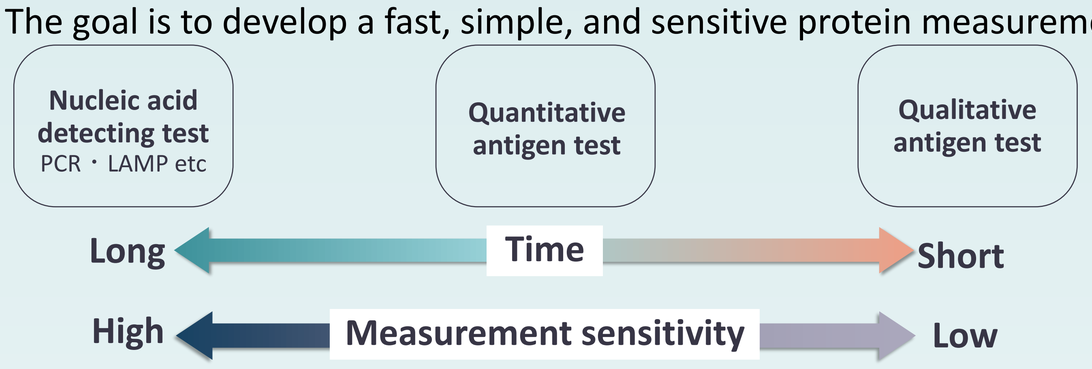
Abstract

- PCR is now often used for diagnosis; although PCR is highly sensitive, it also amplifies nucleic acids of dead viruses. Thus, there is a high demand for detecting proteins directly.
- For the measurement of viruses, we constructed ELISA to target nucleoprotein for influenza, hexon protein for adenovirus, and fusion protein for respiratory syncytial virus (RS virus).
- We have successfully measured influenza virus, adenovirus, and RS virus with high sensitivity, using thio-NAD cycling ELISA.



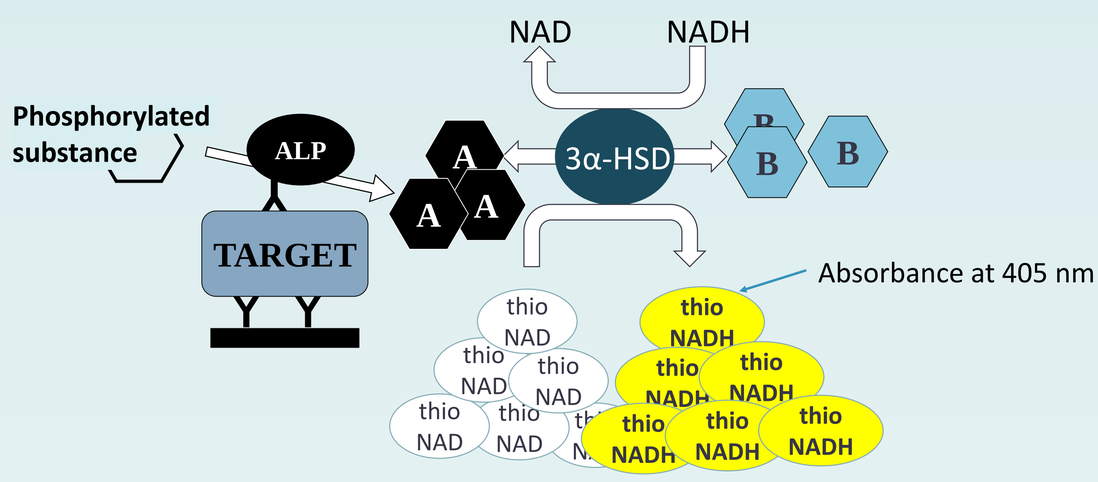
Introduction

- Nucleic acid detection tests and antigen tests are used for laboratory diagnosis, but they have a trade-off relationship in terms of time required and measurement sensitivity.
- Since nucleic acids are detected regardless of whether the virus is alive or dead, it is necessary to measure proteins to see actual biological phenomena.
- The goal is to develop a fast, simple, and sensitive protein measurement.

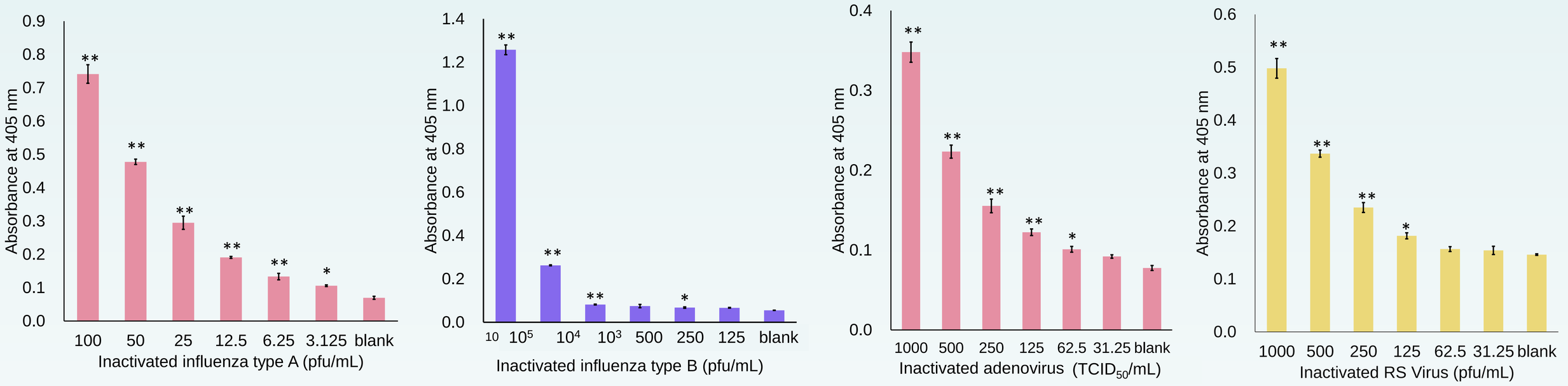


Methods

- By combining ELISA and thio-NAD cycling to develop thio-NAD cycling ELISA, the signal is amplified over the reaction time and that enabled ultra-sensitive measurements.



Results



Detection of surfactant-inactivated viruses.
P values were used to evaluate the significance of differences to blank at 60 min using one-way ANOVA with a post-hoc Holm test. *p<0.05, **p<0.01

- For the measurement of viruses, nucleoprotein for influenza virus, hexon protein for adenovirus, and fusion protein for RS virus were used as targets of ELISA, and virus cultures were surfactant-inactivated with extracts before measurement.
- Influenza virus type A was measured at a sensitivity of 3 pfu/mL, type B at 1000 pfu/mL, adenovirus at 62.5 $TCID_{50}$/mL, and RS virus at 125 pfu/mL.
- The sensitivity of influenza virus type A is up to 10^6 times more sensitive than available Kits, type B to 1000, adenovirus to 10^6, RS virus to 10^2.

Discussion

- Highly sensitive measurement of several inactivated viruses is possible in this study.
- We will try measurements with actual patient samples.