Abstract: In this research, we proposed a microfluidic chip to pretreat the samples for genetic analysis of infectious viruses. The microfluidic chip has the following three functions; (1) Virus purification by hydroxyapatite-packed microcolumn, (2) Viral RNA extraction by silica-packed microcolumn, and (3) Capture of the targeted virus genome by PNA-immobilized glass substrate. Each function has been demonstrated separately using microfluidic chips.

1. Background

Genetic Analysis of Infectious Viruses

DNA Sequencer
- High throughput
- Diagnosis of multiple diseases

Pretreatment of clinical sample
- Virus Purification and Enrichment
- Viral DNA/RNA Extraction

Cumbersome processes by human hand

2. Concept

On-chip Sample Pretreatment

1. Virus Purification by Hydroxyapatite-packed Microcolumn

Sample

Elution Buffer

Hydroxyapatite

Micropillar

100 μm

2. Viral RNA Extraction by Silica-packed Microcolumn

Lysis Buffer

Elution Buffer

Silica

Micropillar

3. Virus Detection (Targeted Genome Capture)

Virus genome

PNA

Horseradish peroxidase (HRP)

Luminol substrate

3-aminophthalic acid dianion

(Ex: 430, Em: 460 nm)

3. Results

1. Virus Purification

1. Introduce a mixture of NDV (Newcastle Disease Virus) and FBS proteins
2. Introduce 500 mM KCl to elute the FBS proteins
3. Introduce 1 M Phosphate buffer to elute the NDVs
4. Hemagglutination Reaction

RNA Collection Rate: 70.8 %

2. RNA Extraction

1. Lyse a 10⁴ pfu/mL NDV suspension
2. Introduce the lysate
3. Introduce the wash buffer
4. Introduce the elution buffer

Experimental Result

Fluorescence Signal

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Cycle Number

0 10 20 30 40 50

Virus Titer [pfu/mL]

0 10⁴ 5x10³ 1x10³


3. Virus Detection

PNA selectively captured influenza A/H1N1 virus genome.

The fluorescence intensity became stronger as the virus titer increased.

4. Conclusion

- Three different functions of the microfluidic chip have been demonstrated separately.
- In our future work, we will integrate all the functions in one chip.

References: