Compiled Chip for All Pretreatment Processes of Virus Gene Analysis

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For Detection of Virus Subtype in One Chip!!

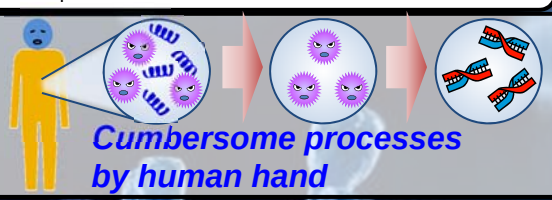
Abstract: In this research, we proposed a microfluidic chip to pretreat the samples for genetic analysis of infectious viruses. The microfluidic chip has the following three functions; (1) Virus purification by hydroxyapatite-packed microcolumn, (2) Viral RNA extraction by silica-packed microcolumn, 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

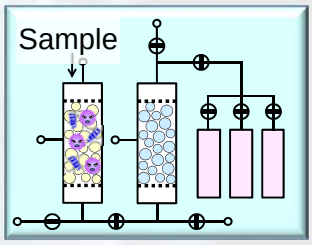


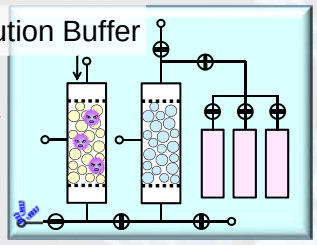
Cumbersome processes by human hand

2. Concept

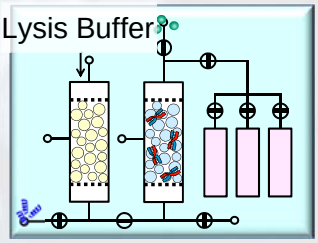
On-chip Sample Pretreatment

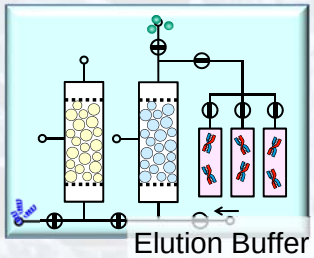
1. Virus Purification by Hydroxyapatite-packed Microcolumn

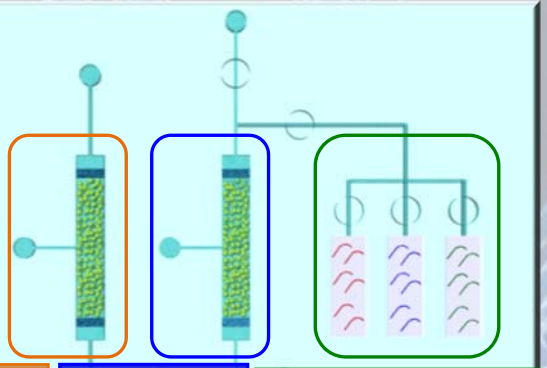




2. Viral RNA Extraction by Silica-packed Microcolumn

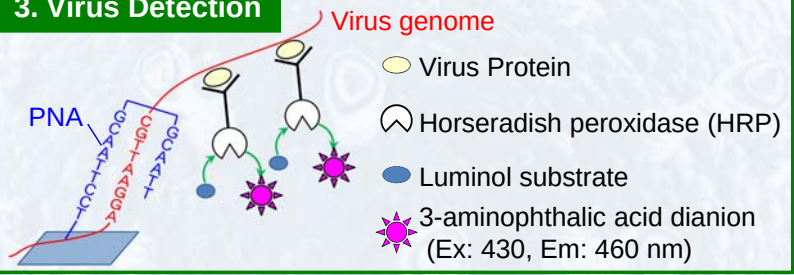






1. Virus Purification | 2. Viral RNA Extraction | 3. Virus Detection (Targeted Genome Capture)

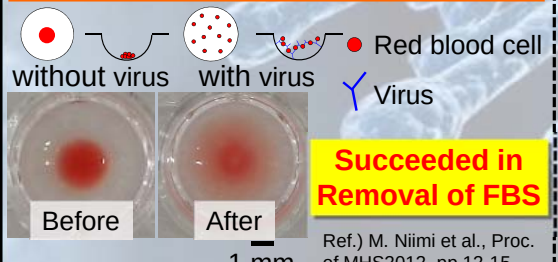
3. Virus Detection



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



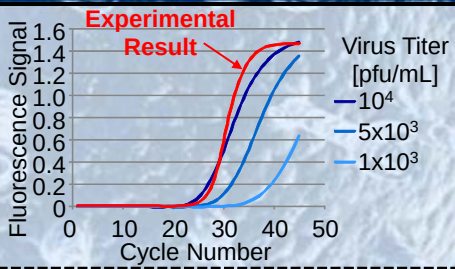
Succeeded in Removal of FBS

Ref.) M. Niimi et al., Proc. of MHS2012, pp.12-15

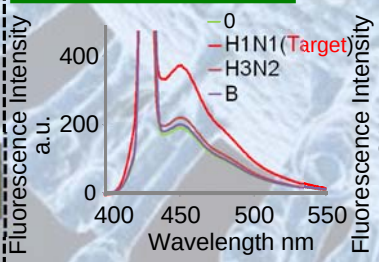
2. RNA Extraction

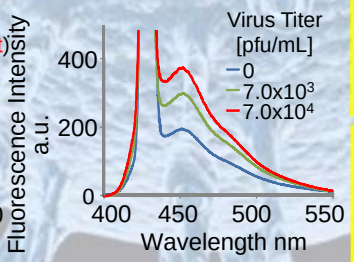
1. Lyse a 10^4 pfu/mL NDV suspension
2. Introduce the lysate.
3. Introduce the wash buffer.
4. Introduce the elution buffer.

RNA Collection Rate: 70.8 %



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.

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References :
1. M. Niimi, et. al., 17th International Conference on Miniaturized Systems for Chemistry and Life Sciences (Micro TAS 2013), pp. 482-484, 2013
2. M. Niimi, et. al., 24th Micro-NanoMechatronics and Human Science (MHS 2013), pp. 248-249, 2013