Poster No

373

Development of multiplex loop-mediated isothermal amplification (LAMP) based on a microfluidic chip for detection of Human herpesvirus type 1 (HSV-1) and -2 and Varicella zoster virus (VZV).

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Abstract

Aim of this study was to develop LAMP method which detect HSV-1, HSV-2, and VZV DNAs in skin swab sample simultaneously using microfluidic chips. Detection limits of HSV-1 and 2 and VZV DNA by a microfluidic chip were 500-1000 copies per reaction. In addition, a microfluidic chip amplified each viral DNA specifically. The results of microfluidic LAMPs using clinical specimens were consistent with those of conventional LAMPs. If real-time PCR was used as a standard method to detect viral DNA in swab samples, sensitivity of HSV-1, HSV-2, and VZV LAMPs based on microfluidic chip were 90%, 100%, and 100%, and the specificity of three methods were 96%, 100% and 100%, respectively.

Advantages of LAMP method based on microfluidic chip

1.Advantages for microfluidic chip technology

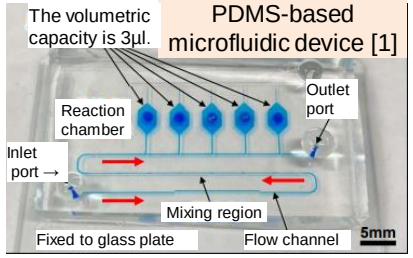
Microfluidic chips have the distinct advantage of integrating multiple chambers for different targets into a single chip, significantly reducing reagent, sample usage, and cost. Therefore, this assay can be performed with high throughput testing by multiplex assay.

2. Advantages for LAMP method

The LAMP can amplify DNA with high specificity and efficiency under isothermal conditions. Therefore, the LAMP can be performed in a simple hot-water bath instead of expensive instrumentation for amplifying target gene. Moreover, it is possible to detect LAMP products by the naked eye as an increase in turbidity in a reaction tube.

This approach utilizes the advantages of the LAMP method and microfluidic devices to amplify and detect multiple targets simultaneously.

Microfluidic chip

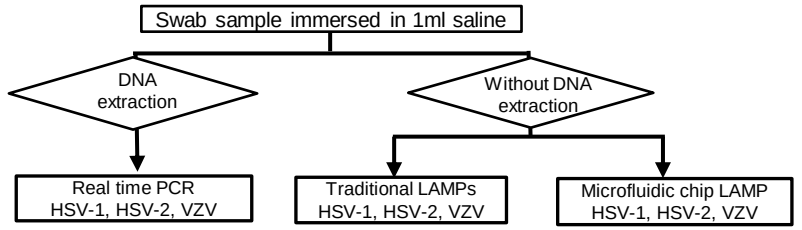


Microfluidic chip was made from polydimethylsiloxane (PDMS). The size of device was approximately 25 mm×50 mm. The width and height of the microchannel were approximately 200 μm and 50 μm, respectively. Only 25 microliters of volume was required to fill five microchambers. This volume was same as one conventional LAMP reaction.

Patients and samples

Thirty-five swab samples were collected from 13 male and 22 female patients who were suspected of HSV infection.

Virological examination



Method

1.Real-time PCRs

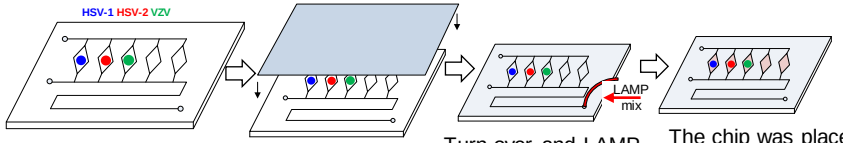
Primers and probes of real-time PCR were described (HSV-1and HSV-2 [2], VZV [3]).

2. Conventional LAMPs

Primers and LAMP condition were described (HSV-1 and HSV-2[4],VZV[5]).

3. Microfluidic chip LAMP

Primers and LAMP reaction condition were same as conventional LAMP. .



Each primers were dried on chip(HSV-1,HSV-2,VZV).

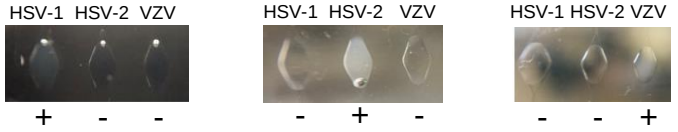
Chip was covered with glass.

Turn over and LAMP mixture was injected into the chip.

The chip was placed into the hot-water bath, and incubated at 65° C for 60 min.

Results

1. Visual detection of microfluidic chip LAMP products in reaction chambers



The positive result was defined increase turbidity of reaction chamber using naked eye.

2. Initial validation analysis of microfluidic chip LAMP (sensitivity)

The plasmids containing target sequences were used to determine the detection limit of microfluidic LAMP (five times measurement).

	HSV-1	HSV-2	VZV
1000 copies*/reaction	4 / 5	5 / 5	5 / 5
500 copies/reaction	3 / 5	4 / 5	5 / 5

*Because primers and LAMP conditions were same to conventional LAMPs, the plasmid of sensitivity levels for conventional LAMPs was used.

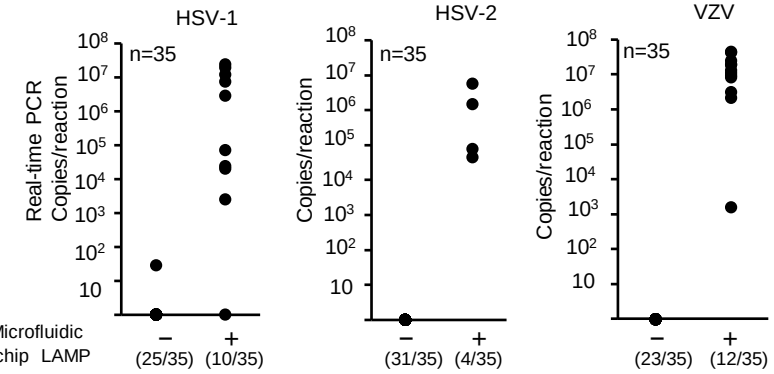
2. Reliability of microfluidic chip LAMP for analyzing clinical specimens.

- The positive rate was compared between conventional and microfluidic LAMP

	HSV-1	HSV-2	VZV
Conventional LAMPs	9* / 35	4 / 35	12 / 35
Microfluidic chip LAMP	10 / 35	4 / 35	12 / 35

*1 sample positive microfluidic chip LAMP and not with conventional LAMP

- Copy numbers of each viral DNA in microfluidic chip LAMP positive and negative samples were measured by real-time PCR.



- The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of microfluidic chip LAMP (Real-time PCR was used as a standard.).

	HSV-1	HSV-2	VZV		HSV-1	HSV-2	VZV
Sensitivity	90.0	100	100	PPV	90.0	100	100
Specificity	96.0	100	100	NPV	96.0	100	100

Conclusions

1.Detection limits of HSV-1, HSV-2, and VZV microfluidic chip LAMP were approximately 500-1000 copies per reaction.

2.The results of microfluidic LAMPs were consistent with that of conventional LAMPs and real-time PCRs on analysis of clinical swab samples.

Multiplex LAMP based on the microfluidic chip may be a useful rapid diagnostic method that can save cost and sample volume compared with conventional LAMPs and real-time PCRs. In the future, it is necessary to consider automating of microfluidic chip LAMP method.

Reference

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