IN-GEL PCR AMPLIFICATION OF HERPES SIMPLEX VIRUS

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ABSTRACT

We present an in-gel DNA amplification method for detecting HSV-1 and HSV-2 in unprocessed genital swabs using a low cost prototype instrument that employs a CCD camera, Peltier element and diode laser. Polymerase chain reaction (PCR) and melting curve analysis (MCA) are performed in an array of polyacrylamide gel posts less than 1 μL in volume that incorporate all components of the PCR reaction. HSV DNA templates directly from genital swabs were used for this assay and we successfully detected HSV-1, HSV-2, and negative samples. This HSV detection assay can readily be developed to use in point-of-care devices for clinical use.

Keywords: genetic amplification (PCR), miniaturization, pathogen detection, point-of-care.

INTRODUCTION

Herpes, a widespread sexually transmitted disease, is caused by a herpes simplex viruses type 1 (HSV-1) or type 2 (HSV-2). Current routinely used detection methods include detection of viral antigens or viral culture [1]. Depending on the severity of the infection, the viral culture can take up to 10 days. Polymerase chain reaction (PCR), on the other hand, can be performed in few hours or less for detecting HSV virions [1,2]. We have developed a miniaturized in-gel DNA amplification assay for performing HSV-1 and HSV-2 PCR and MCA simultaneously in an array of polyacrylamide semi-solid gel posts on an inexpensive prototype instrument. HSV samples were added to the mixture prior to polymerization or added atop the gel posts and allowed to diffuse into the gel. We believe that this platform will lead to faster, more affordable pathogen detection for clinical diagnostics.

EXPERIMENTAL PROCEDURE FOR HSV DETECTION

Acrylamide gel posts were cast in a glass mold (Fig. 1(a)), 18 mm x 27 mm, covered with a treated coverslip that adheres to the gel during the photopolymerization. Each post is 0.64 μL in volume. Prior to the preparation of the gel, primers for HSV-1 or HSV-2 in an "isolator" solution containing sugars were placed in designated wells of the mold and dried in position. The reaction mixtures with swab templates were then added into the mold. Purified HSV-1 or HSV-2 DNA was added to the last two columns as positive controls. LC Green Plus, an intercalating fluorescent dye, was added to the reaction mixture of all the posts [3]. After photopolymerization for 20 min., the cover slip with the posts adhered was removed (Fig. 1b). Before starting the PCR, an unknown sample was added after polymerization to the middle three columns of posts. The cover slip with the post array was then placed in a pan and immersed in oil for PCR/MCA. The prototype instrument configuration is shown in Fig. 2. It consists of a diode laser for fluorescence excitation, a Peltier element for thermal cycling, and a CCD camera for fluorescence detection during the PCR and MCA, all of which are run by a microcontroller.

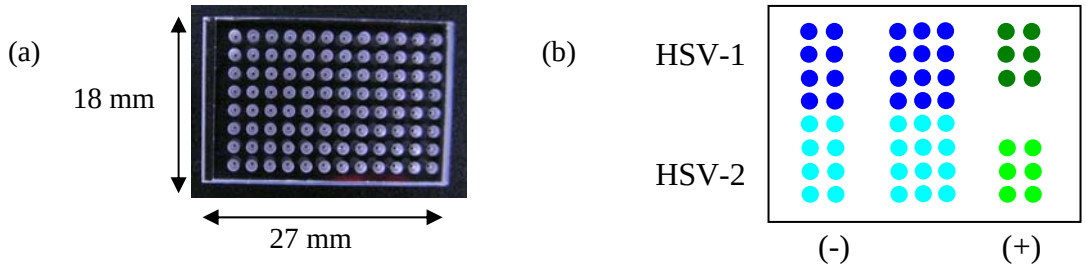

Figure 1: (a) Photo of the glass mold, (b) HSV assay arrangement on a 22 mm x 2mm cover slip. The first two and last two columns are negative and positive controls. Sample with DNA template was added to the middle three columns.

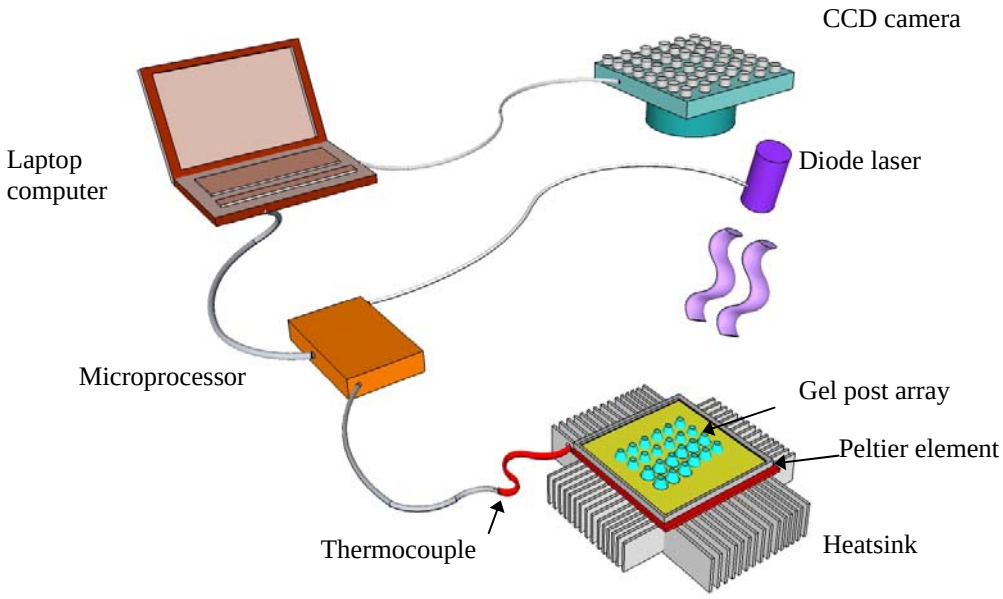

Figure 2: Schematic diagram of the instrument used for performing PCR and MCA.

RESULTS AND DISCUSSION

Fig. 3 shows an example of both HSV-1 (left) and HSV-2 (right) MCA curves where known DNA samples were added to the reaction mix before the polymerization. These MCA curves serve as known standards for evaluating results from unknown samples.

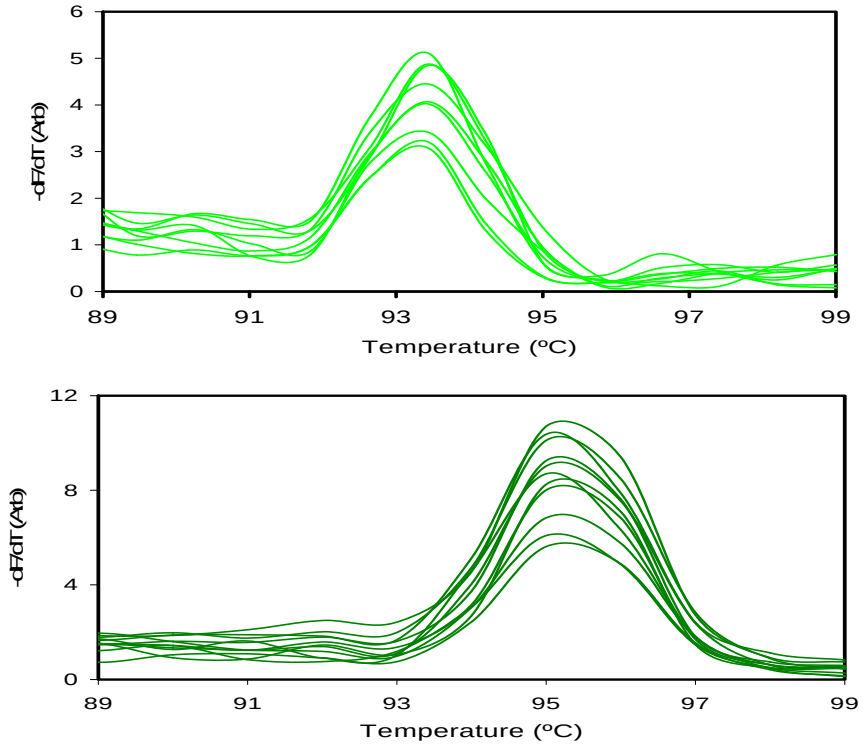

Figure 3: Melting curves of HSV-2 (a) and HSV-1 (b).

Fig. 4 shows the MCA curves for the gel posts shown in Fig. 1(b). Melt peaks were obtained with HSV-2 primers but not for HSV-1 primers, indicating that the sample tested was positive for HSV-2 and negative for HSV-1. The sugars used to sequester the primers shifted the melt peak of the unknown sample compared to the positive control.

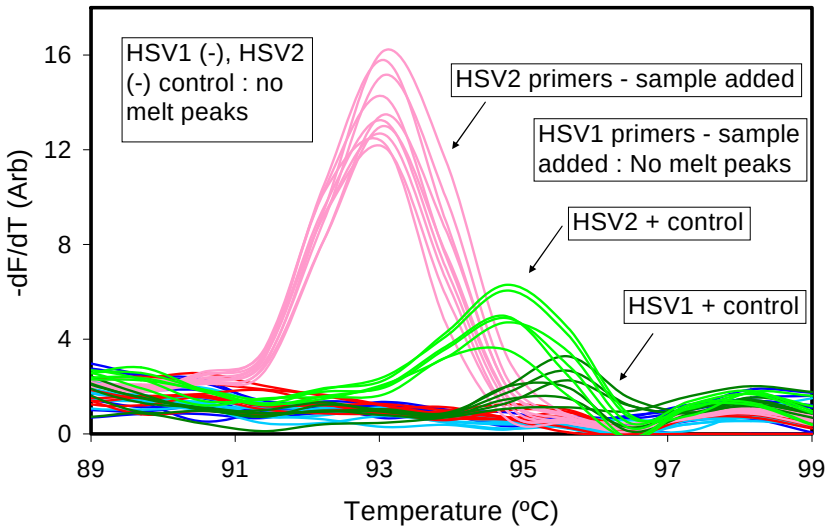

Figure 4: MCA results from a gel post array shown in Fig. 1(b).

CONCLUSIONS

With in-gel PCR where each reaction occurs in a gel post less than 1 μL in volume, we have successfully detected HSV with unprocessed genital swab samples taken in the clinic. Both HSV-1 and HSV-2 are detected simultaneously in different sets of gel posts on a single cover slip where positive and negative controls were also incorporated in separate sets of gel posts. The prototype instrument used for the assay is inexpensive and suitable for development as a portable device. Our platform can be further extended to simultaneously detect a wider range of pathogens. This technology can be used to miniaturize molecular detection of pathogens (nucleic acid testing) for low cost, automated diagnostics.

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