Lab on a Chip

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PAPER

A miniaturized and integrated gel post platform for multiparameter PCR detection of herpes simplex viruses from raw genital swabs

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Herpes simplex virus (HSV) is one of the most prevalent viruses, with acute and recurrent infections in humans. The current gold standard for the diagnosis of HSV is viral culture which takes 2–14 days and has low sensitivity. In contrast, DNA amplification by polymerase chain reaction (PCR) can be performed within 1–2 h. We here describe a multiparameter PCR assay to simultaneously detect HSV-1 and HSV-2 DNA templates, together with integrated positive and negative controls, with product detection by melting curve analysis (MCA), in an array of semi-solid polyacrylamide gel posts. Each gel post is 0.67 μL in volume, and polymerized with all the components required for PCR. Both PCR and MCA can currently be performed in one hour and 20 min. Unprocessed genital swabs collected in universal transport medium were directly added to the reagents before or after polymerization, diffusing from atop the gel posts. The gel post platform detects HSV templates in as little as 2.5 nL of raw sample. In this study, 45 genital swab specimens were tested blindly as a preliminary validation of this platform. The concordance of PCR on gel posts with conventional PCR was 91%. The primer sequestration method introduced here (wherein different primers are placed in different sets of posts) enables the simultaneous detection of multiple pathogens for the same sample, together with positive and negative controls, on a single chip. This platform accepts unprocessed samples and is readily adaptable to detection of multiple different pathogens or biomarkers for point-of-care diagnostics.

Introduction

Herpes simplex virus (HSV) is a common human pathogen that exists as two genetically distinct viruses, HSV-1 and HSV-2. Infection with HSV-1 typically causes oral infections, whereas HSV-2 typically leads to genital infections. HSV diagnostic tests detect the presence of virus, viral antigens, or DNA in the swabs taken from the affected areas or lesions. The gold standard for diagnosing HSV is viral culture followed by immunofluorescent-antibody staining.[1,2] However, viral culture can give false negatives if the virus does not survive sample collection and storage until testing. If positive, it can accurately differentiate HSV-1 and HSV-2. Cullen *et al.* reported that the sensitivity of culture is 84.1% and the specificity is 100% as compared to a consensus result obtained from three different testing strategies.[3]

The antigen detection method has a sensitivity and specificity similar to viral culture.[4] These procedures take 2 to 14 days depending on the infectivity of the samples.

In contrast, genetic amplification of viral DNA by PCR is performed within a few hours. It is also a more sensitive detection method for HSV;[1,5,6] the rate of detection by PCR is 3.5 times higher than viral culture.[6] Compared with a multiplex PCR to detect both HSV-1 and HSV-2, the sensitivity of HSV culture for the same set of samples was only 71.8%.[2] Real-time PCR has been successfully used to detect HSV.[7–10] Adelson *et al.* developed a multiplex real-time PCR assay that identifies each infection as different fluorescently labelled products.[7] Pandori *et al.* tested un-purified genital swabs, which had real-time PCR crossing points about 1.5 cycles later than for purified DNA.[9] Even though PCR is a highly sensitive method for HSV detection, the lack of availability and the high cost of real-time PCR instruments restrict its use as a clinical diagnostic tool.[11]

In this paper, we used a low-cost prototype instrument (<$1000) for performing PCR and MCA with 45 unprocessed swab samples to simultaneously detect HSV-1 and HSV-2 on a novel multiparameter platform that integrates positive and

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negative controls. PCR and MCA are performed in an array of 0.67 μl polyacrylamide gel posts that incorporate all components of the PCR reaction including an intercalating dye for fluorescent detection. For a clinical diagnostic method to detect HSVs, samples containing templates to be amplified must be added to "ready to use" posts, immediately prior to running a test on a platform that also includes pre-loaded positive and negative controls. Here, the goal of simultaneous multiparameter PCR testing was achieved by a newly developed method for depositing different sets of primers in discrete sets of posts for HSV-1, HSV-2, positive and negative controls on the same gel post array.

This technology can be readily adapted for point-of-care diagnostics as a low-cost and faster alternative to conventional PCR or viral culture for simultaneous detection of HSV-1 and HSV-2 with no need for prior sample preparation.

Experimental

Real-time PCR and MCA were carried out in an acrylamide gel which has been photo-polymerized with 'azobis' (2,2′-azobis-(2-methyl-*N*-(2-hydroxyethyl) propionamide)) as the polymerization initiator, as previously described.[12] The experimental steps performed for detecting HSV-1 and HSV-2 are described below.

Mold and coverslip preparation

The mold with approximate size of 18 mm × 27 mm is made with a 1.1 mm thick glass slide (Schott Borofloat® 33) bonded to an another glass slide with 8 × 12 array of holes (Fig. 1(a)). Holes are conical in shape. Each hole is 1.1 mm deep and bottom and top diameters are 0.52 mm and 1.2 mm respectively. The schematic of a gel post is shown in Fig. 1(b). The surface of the mold is coated with safety coat (Cat# 4017-01, J.T.Baker, Phillisburg, NJ, USA) for 1 h and washed out with water so that the gel would not adhere to the mold. Coverslips (22 mm × 22 mm) were prepared as described previously.[12] After the mold is used once for detecting DNA, it is cleaned by submersion in Conflict detergent for ~1 h and then leaving it in 1M KOH in methanol for ~45 min and finally washing with water in order to remove any DNA that could contaminate the next array during PCR.

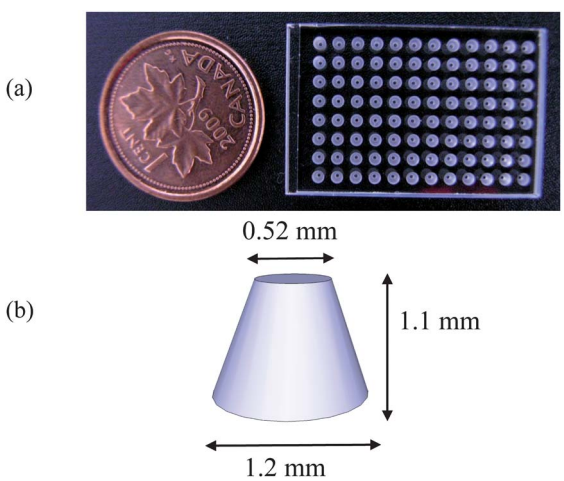

Fig. 1 (a) Photo of the 8 × 12 array glass mold with dimensions of 18 mm × 27 mm, (b) schematic of the gel post (volume ~0.67 μL) made with the mold in (a).

HSV samples

Clinical samples (HSV-1, HSV-2, and negative controls) were obtained from the Alberta Provincial Laboratory for Public Health. Genital swabs from patients were placed in universal transport media (UTM; Copan Diagnostics Inc., Murrieta, CA, USA) at the clinic and transported to the laboratory where they were frozen at −20 °C until use. For all experiments, unprocessed samples in UTM were used directly without any DNA extraction procedure. After establishing the conditions for PCR with known samples, a total of forty-five swab samples were assayed. The sample set was non-random and selected to include HSV-1+, HSV-2+ or HSV-negative samples based on testing using culture methods in the clinical laboratory; the sample set included 15 samples from each category. All 45 samples were tested in conventional PCR to confirm their status as HSV-1+, HSV-2+ or HSV-negative. They were then tested on gel posts, with the sample identity and PCR or culture status blinded to the operator.

In order to test the specificity of the HSV primers, purified DNA was extracted from cultures representing a panel of sexually transmitted infections, obtained from the Alberta Provincial Laboratory for Public Health.

Analysis of clinical specimens

Blinded analysis. PCR for each sample was performed by filling the four corners of the mold with 4 different reaction mixes as shown in Fig. 2.

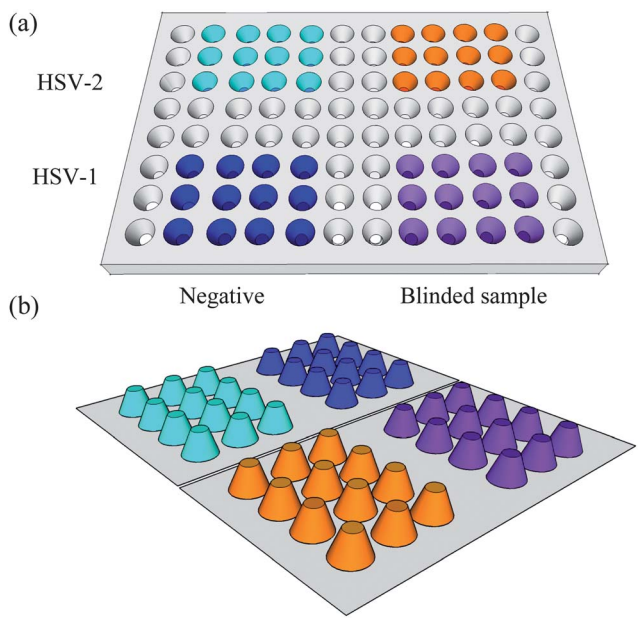

Fig. 2 Gel post configuration for sample polymerized within the gel posts (a) Schematic of sample and negative control areas of the post array (each post is 0.67 μL in volume) and (b) posts pulled out of the mold. In this approach, each gel post received ~26 nL of raw sample.

Separate reaction mixes were prepared with or without template for both HSV-1 and HSV-2, for all 45 genital swab specimens. Primers for HSV-1 targeting a 174 bp product from template encoded by the tegument protein (UL41) and HSV-2 targeting a 106 bp product of the DNA polymerase gene (UL30) are shown in Table 1. Each 100 μL reaction mix consisted of 20 μL of 5X PCR buffer (333 mM tris-sulfate, pH 8.6, 83 mM $(NH_4)_2SO_4$ (Sigma); and 40% sucrose (Sigma)), 4 μL of 50 mM $MgCl_2$ (Fluka), 2 μL of 10 mM [dNTP] (Sigma), 2 μL of 1%BSA (Sigma), 2 μL of 10 μM primer solution (Integrated DNA technologies, San Diego, CA) for each of the two primers, 4 μL HSV sample in UTM, 10 μL of 10X LC Green Plus (Idaho Technology Inc., Salt Lake City, Utah) and 3 μL of Taq polymerase (20 units/μL), 10 μL of a 40% acrylamide (Sigma, cat no. A9099) + 4% bis-acrylamide aqueous solution (*N*,*N*-methylene bisacrylamide, BioRad, Hercules, CA, cat no. BA05-1610201), 2 μL of 3% azobis (Wako, Richmond, cat no. VA-086), 1 μL of 10% TEMED (*N*,*N*,*N*′,*N*′ tetramethylethylenediamine, Sigma, cat no. T7024) and water. For the negative controls, 4 μL of water was added instead of an aliquot of sample in UTM. The mixes were vortexed, centrifuged, and loaded into the mold leaving two rows of holes between them in order to avoid cross-contamination (Fig. 2(a)).

The mold with the two halves of the coverslip atop of the gel mix was then exposed to a 360 nm UV lamp (~1 mW cm^{-2} on the posts) for 20 min in order to photopolymerize the acrylamide mixes to make 4% polyacrylamide gel posts. Once polymerized, both halves of the coverslip with gel posts attached to them were detached from the mold (Fig. 2(b)) and were immediately immersed in mineral oil (Sigma, cat no. M5904) in a shallow anodized aluminum 23 mm × 23 mm pan such that posts were facing up. The pan was then placed on a Peltier element for thermal cycling. After a pre-denaturation step of 2 min at 95 °C, 35 cycles of amplification was carried out at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, followed by final extension step of 120 s at 72 °C. MCA was performed from 60–99 °C in one degree intervals.

Conventional PCRs were performed in order to confirm the sample identity for the 45 samples. PCR reaction mix of 25 μL consisted of 5 μL of 5× PCR buffer, 1.5 μL of 50 mM $MgCl_2$, 0.5 μL of 10 mM [dNTP], 0.5 μL of 1% BSA, 0.5 μL of 10 μM each primer, 1 μL HSV sample in UTM, and 0.5 μL of Taq polymerase. Thermocycler conditions were similar to the gel amplification parameters. PCR for determining the primer specificity was also performed with similar reagent and thermal cycling conditions.

To reduce the cycling time for DNA amplification, we performed a 2-step PCR with a pre-denaturation step at 95 °C for 120 s, 35 cycles of amplification at 95 °C for 15 s and 64 °C for 30 s, followed by a final extension step at 72 °C for 120 s.

Sensitivity testing of the assay. In order to study the sensitivity of the HSV assay, purified HSV DNA preparations with a known viral copy number were obtained for use as a control (Vircell, Spain, HSV-1 cat. No. MBC023, HSV-2 cat. No. MBC024) and were tested using serial dilution in our prototype system. This allowed a calculation of the number of viral copies that could be detected per gel post. The PCR recipe and the thermal conditions were similar to the 3-step PCR recipe stated above. In order to test whether the UTM would reduce the PCR efficiency, we also performed PCR in gel posts with purified DNA controls to which a final concentration of 4% UTM was added (to mimic conditions for a sample in UTM) compared to purified DNA controls with no UTM.

Delivery of the sample to pre-polymerized posts. To test the efficiency of the PCR when the sample is delivered on top of preformed posts compared to tests where template-containing sample is added before polymerization occurs, a set of posts were made as shown in Fig. 2(b). Then 8 μL of diluted HSV-1 sample (1 μL of clinical sample + 10 μL of water) was added onto one of the half coverslips and allowed to diffuse in for 15 min before starting the PCR. Posts with no DNA were included as negative controls. If the entire sample added to the pre-polymerized posts is uniformly absorbed by the gel, each post should receive about 29 nL of undiluted sample.

Primer sequestering. In order to include positive and negative controls as well as detect an unknown sample, HSV-1 and HSV-2 primers were deposited into individual wells of the mold and dried in position before adding the gel/reaction mix. A primer sequestering mix of 100 μL for each HSV was made by adding 4 μL of 10 μM each reverse and forward primer to 30 μL of 1M trehalose (Cargill Inc, Canada) and water. Appropriate sections of the mold (Fig. 3(a) - sections for negative controls and sample), were filled with the trehalose/primer mix and were dried in the mold at room temperature for about 2 h before adding a mix of reaction components in gel reaction for polymerization without primers or DNA. Preliminary work showed that different dried primers are held within the dried spot during the addition of the gel/reaction mix into the wells with no detectable cross contamination: this was validated by the experiments reported here, where no cross contamination was observed between adjacent posts having different primer sets. Primers diffuse as the dried region gradually dissolves, and diffuse into the mix for each gel post during polymerization. The gel/reaction mix for the wells with sequestered primers is similar to the mix stated above but without the primers. The positive controls in the last two columns were polymerized with purified DNA and primers in the gel/reaction mix as shown in Fig. 3(a). Posts pulled out of the mold are shown in Fig. 3(b) and (c). The coverslip atop

Table 1 Primer sequences for HSV-1 and HSV-2 PCR amplifications

HSV-1 (174 bp)	F-5′ CGTCGCGGGTTGGCCACATA 3′ R-5′ CGCCGGCGGATACGAAGACG 3′
HSV-2 (106 bp)	F -5′ TACGACGGCCAGCAGATCCGCGTC 3′[13] R - 5′ CCTTGTCGAGGCCCCGAAACCG 3′[13]

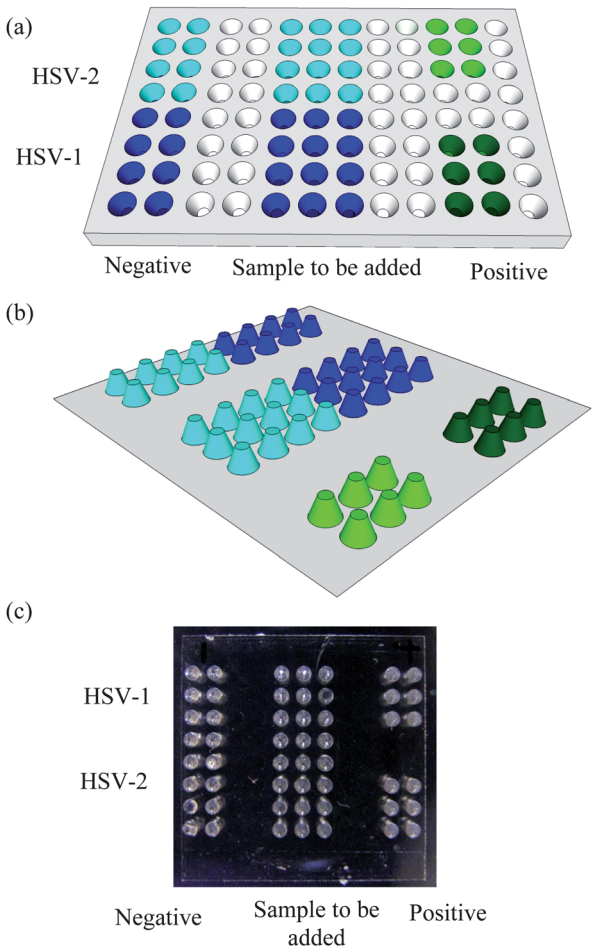

Fig. 3 Gel post configuration with positive and negative controls, for post-polymerization addition of sample to test posts. (a) Schematic of sample and assay control areas in the post array (each post is 0.67 μL in volume). No primers were sequestered in wells colored light gray. (b) Schematic and (c) photo of the gel posts (4% polyacrylamide) pulled out of the mold onto a coverslip (22 mm × 22 mm). The sample to be tested was added atop the gel posts after polymerization, to the middle three columns of posts and allowed to diffuse into posts before starting the PCR.

of the mold was slipped from the negative side to the positive side to avoid contaminating the negative controls.

Specimen testing. Ten μL of water and 1 μL of sample were added atop of the polymerized posts containing HSV-1 and HSV-2 primers in the middle three columns (Fig. 3(c)) and allowed to diffuse into the gel for 15 min. The coverslip was then immersed in an oil-filled pan for performing PCR. The hydrophobic nature of the treated glass coverslip prevents sample from flowing into the other regions of the coverslip.

Detection criteria. For a sample to be scored as positive for either pathogen, both HSV-1 and HSV-2 negative control posts should show no melt peaks and only one of the HSVs should show melt peaks at their correct temperature as shown in Fig. 5. For a sample to be negative, the posts in all four sections in Fig. 2 or the negative sections and the sections labelled as "sample to be added" in Fig. 3 should show no melt peaks at the correct temperatures. If a sample showed random peaks at different temperatures for HSV-1 and for HSV-2 reactions, out of range of the correct temperatures, the sample was deemed negative. Blinded samples were scored as positive or negative in the absence of any information on the identity of the sample or its HSV status as determined by conventional PCR. The sample code was broken only after final conclusions had been reached on their HSV status as determined on the gel post platform.

The specificity of the assay was calculated by dividing the number of true negatives by the sum of true negatives and false positives. Sensitivity of the assay was calculated by dividing the number of true positives by the sum of true positives and false negatives.

PCR instrument. The prototype instrument used for both PCR and MCA was described in detail previously.[12] A simplified diagram of the experimental set up is shown in Fig. 4. Briefly, it consists of a Peltier element for thermal cycling, a diode laser (405 nm) as an excitation source and a CCD camera for acquiring fluorescence images all of which are controlled by a microprocessor. The instrument costs ~$1000. An inexpensive laptop computer (~$300 netbook) was employed for the collection of images and data processing. The data processing algorithm was described previously.[12]

Results and discussion

MCA of HSV-1 and HSV-2

Meting curve analysis is routinely used to verify amplicon identity in commercial real-time quantitative PCR instruments. LC Green, a dye that binds to double stranded DNA, fluoresces when bound to DNA. When DNA denatures, dye binding and hence fluorescence is lost. MCA measures the denaturation of the amplicon with increasing temperature *via* the loss of fluorescence as the intercalating dye is released from the melting DNA. The melt temperature is a function of amplicon size and GC content.

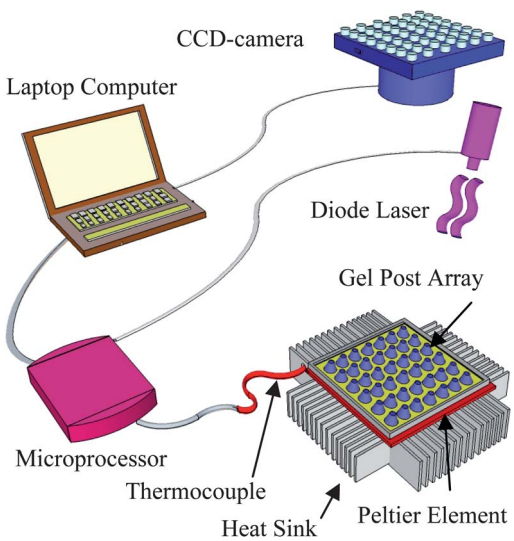

Fig. 4 A schematic of the prototype instrument used for PCR and MCA.

In a plot of first derivative of fluorescence *vs.* temperature, a single peak at the approximate expected melt temperature (which varies with salt concentration and other conditions) indicates a clean PCR reaction with a single product. Examples of MCA curves obtained after the amplification of template from known HSV-1 and HSV-2 genital swabs polymerized in the gel are shown in Fig. 5(a) and (b). These curves served as a guide to identify the melt peaks in samples blinded to the operator. Fig. 5(a) shows the drop in fluorescence of HSV products with increasing temperature. The first derivative of the fluorescence data in Fig. 5(a) is shown in Fig. 5(b) where the melting points of the HSV products are obtained. The melting point of the product is defined as the temperature where 50% of the PCR products are melted. Across different experiments, the melt temperature range of the HSV-1 product is 94.2 ± 0.8 °C and of the HSV-2 product is 92.3 ± 0.9 °C.

Vertical acrylamide gel electrophoresis was performed with PCR amplified products in gel posts to confirm the size (Fig. 5(c)). They were also sequenced to confirm their identity.

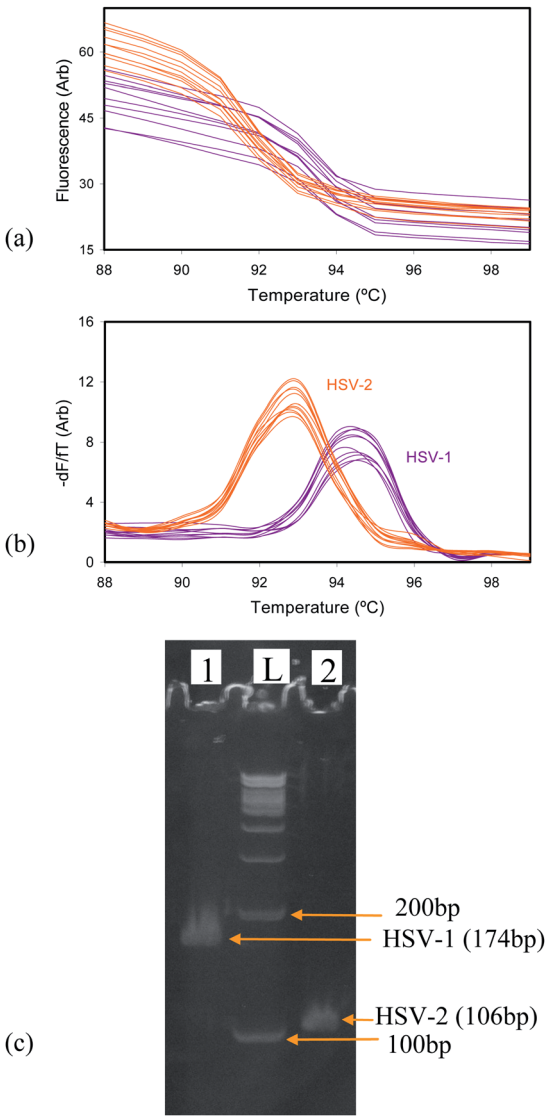

Fig. 5 Melting curve analysis: (a) drop in fluorescence with the increasing temperature and (b) negative derivative of (a) for HSV-1 and HSV-2. Each line represents the melt curve for one gel post. Purple lines represent HSV-1 reactions and orange lines represent HSV-2 reactions. (c) Confirmation of the product size by gel electrophoresis. Lane 1 and 2 show HSV-1 (174 bp) and HSV-2 (106 bp) products from gel posts respectively. L – 100bp DNA ladder (exACTGeneTM, Low Range Plus DNA ladder, Fisher).

Assay sensitivity

MCA data for HSV-1 and HSV-2 samples is shown in Fig. 6 where the sample was serially diluted to determine the sensitivity of the assay. The volume of clinical sample used for the blinded panel was 4% of the PCR/gel reaction mix. The results in Fig. 6 show that we can detect as little as 0.4% of the raw sample volume, equivalent to ~2.5 nL of raw sample per post.

Detection limits for HSV amplification in gel posts using purified DNA with a known viral copy number

To accurately determine the number of viral DNA copies that were detectable on gel posts, purified DNA preparations with a known number of viral DNA copies were purchased and tested in serial dilution on gel posts, thereby providing a known number of templates applied per post at each dilution. MCA data for the PCR performed with serial dilutions of control DNA with and without UTM in the reaction mix is shown in Fig. 7. Dilutions range in concentration from 513 to 32 copies per post. We reliably detected 64 copies per post with confidence, with or without UTM added to the reaction mixture. This may improve with further optimization of the platform. The MCA signal remained approximately the same with or without UTM, at 64 copies per post. We cannot

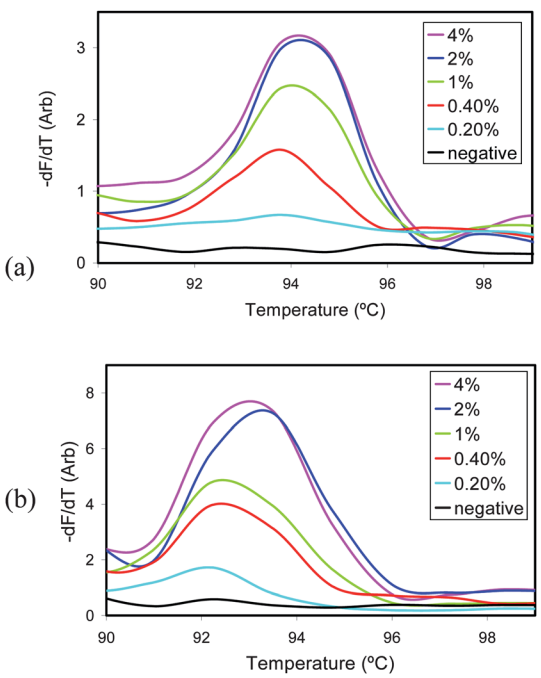

Fig. 6 MCA for (a) HSV-1 and (b) HSV-2 samples with different sample volumes in the reaction mix.

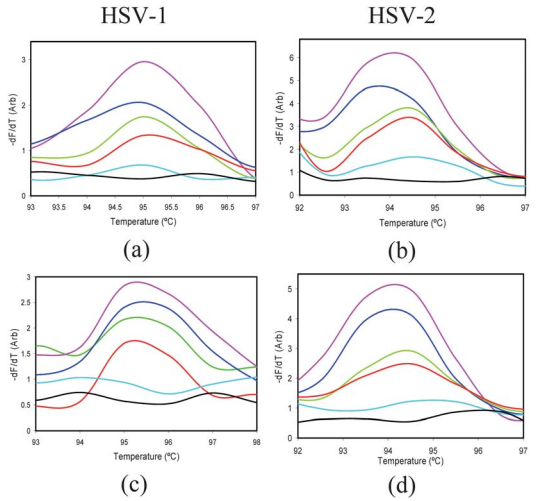

Fig. 7 MCA for HSV-1 (a and c) and HSV-2 (b and d) for the serial dilutions performed with (c and d) and without (a and b) the presence of UTM. Each gel post contained 513 (magenta), 256 (blue), 128 (green), 64 (red), 32 (light blue) or 0 (black) copies.

ascertain the extent to which purified DNA in UTM reflects the conditions for unpurified swabs collected in UTM. It is not clear the extent to which viral DNA in a swab exists as free DNA or in a viral capsid, and hence how much of the DNA in a swab is available for PCR amplification. Nevertheless, we are able to detect a melt peak for HSV amplicons in the range of clinical samples tested here suggesting that the amount of DNA available from swabs in UTM is sufficient for detecting HSV.

Specificity of the primers

In order to confirm the specificity of the primers for HSV-1 and HSV-2, conventional PCRs were performed with each primer set against all the others with direct clinical samples dissolved in UTM. No cross-reactivity was detected, which shows the absence of any detectable cross-contamination between primer sets. DNA extracted from clinical samples with various sexually transmitted infections (Table 2) were also performed to confirm the specificity of the primers.

Analysis of blinded clinical samples

Example of melt peaks acquired with unknown clinical samples and later identified as HSV-1 and HSV-2 are shown in Fig. 8. Real-time PCR data, which does not discriminate between misprimed double stranded fragments (*e.g.* primer dimers) and the correct double stranded amplicon, was not used as a sample validation tool. Misprimed peaks with lower melting temperatures can be formed for some samples (Fig. 8). The amplification of these misprimed peaks to form double stranded products can lead to increased fluorescence during the real time PCR. Therefore, a genital swab sample was identified as HSV-1 or HSV-2 positive based solely on the melting curve analysis.

To determine the HSV status of 45 genital swab samples, aliquots of sample were added to the reaction mixture prior to polymerization, followed by PCR and MCA. Most samples

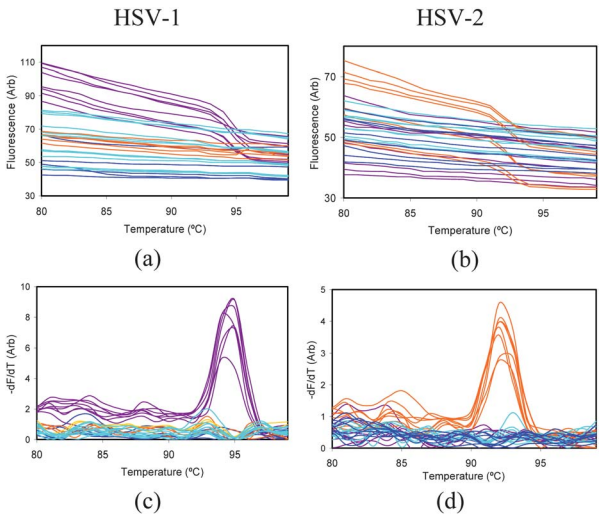

Fig. 8 Melt curve analysis of two unknown samples that were identified as HSV-1 and HSV-2. (a) and (b): measurement of fluorescence as a function of increasing temperature, (c) and (d): melt curves for HSV-1 and HSV-2. Colors here match the colors in Fig. 2. Dark blue: HSV-1 (-), Light blue: HSV-2 (-), Purple: HSV-1 primers with the sample, and Orange: HSV-2 primers with the sample. Only one set of primers amplified the DNA and showed the correct melt peaks. No melt peaks were observed in the sections with the other HSV primer or the negative controls. The small peaks at lower temperatures (<90 °C) are due to misprimed products.

Table 2 Primers are specific for HSV and do not amplify DNA from other sexually transmitted infections

Pathogen	Agent	HSV-1 primers	HSV-2 primers
Coxsackie virus A16	Virus	—	—
Enterovirus 71	Virus	—	—
Human papilloma virus 18	Virus	—	—
N. gonorrhea	Bacteria	—	—
C. trachomatis	Bacteria	—	—
T. pallidum	Bacteria	—	—
U. urealyticum	Bacteria	—	—
Group A Strep.	Bacteria	—	—
Staphylococcus epidermidis	Bacteria	—	—
Staphylococcus aureus	Bacteria	—	—
HSV-1	Virus	+	—
HSV-2	Virus	—	+

were tested several times using prototype instruments, all performed "blind". The MCA plot for each sample was examined manually to determine the presence of HSV-1 or HSV-2. Once a final conclusion was reached regarding the HSV status of each sample, the code was broken and test results using the gel post platform were compared to those from the conventional PCR analysis of each sample as the gold standard. Our results for all 45 samples are shown in Table 3. We found that the concordance of the gel post assay with the same samples tested by conventional PCR is 91%. Specificity of HSV-1 and HSV-2 are 93% and 96% respectively. Sensitivity is 93% for both HSV-1 and HSV-2. This set of experiments further confirmed the absence of cross-contamination between posts with different primer sets. We have begun exploring methods of automated data analysis (*e.g.* Decision Support System or DSS) to help reduce or eliminate human bias in the evaluation of MCA plots to determine HSV status.

Addition of raw sample to preformed gel posts

Detection sensitivity of PCR products with MCA. Clinical testing is feasible only if the sample can be added to preformed gel posts. We determined the extent to which PCR with sample incorporated into the gel posts was comparable to PCR with sample added atop preformed gel posts. For an HSV-1+ clinical sample, Fig. 9 shows a comparison of the MCA data where the sample was polymerized in the gel along with the other reagents (Fig. 9(a)) *vs.* the sample added atop of the pre-polymerized gel posts and allowed to diffuse into the gel (Fig. 9(b)).

The amount of sample when added into the polymerization mixture (pre-polymerization) is about 26 nL per post. If the entire sample added atop a previously polymerized post were uniformly absorbed into each post, it would be about 29 nL of sample per post. Results in Fig. 9 show that the PCR efficiencies of template delivery methods are comparable; both produced melt peaks with similar intensities. This confirms that delivery of sample to gel posts is clinically feasible. Previously, we have also shown that the sample added atop of the posts and allowed to diffuse into the gel posts shows the same amplification as that observed for an aliquot of the same sample polymerized into the gel posts.[12]

A clinically feasible test strategy that integrates positive and negative controls

For a clinically useful test, an HSV sample should be added to a gel post array having integrated positive and negative

Table 3 Blinded HSV analysis of raw samples on gel posts. Fourteen samples out of 15 were correctly identified for each HSV while 13 out of 15 were correctly identified for the negative samples

	Gel post PCR (n)		
Conventional PCR (n)	HSV-1	HSV-2	Negative
HSV-1 (15)	14	1	0
HSV-2 (15)	0	14	1
Negative (15)	1	1	13

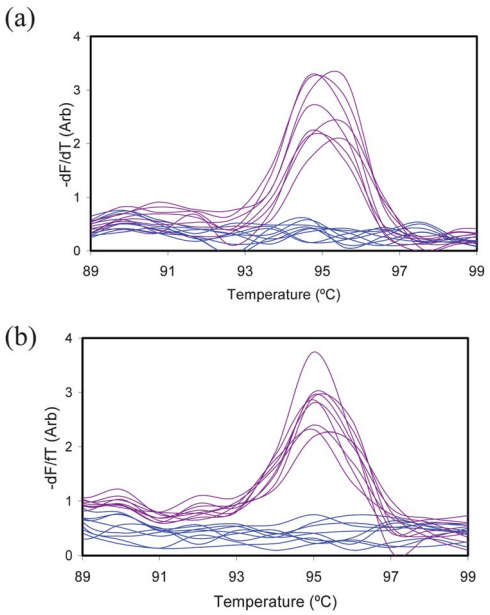

Fig. 9 MCA for a HSV-1 sample where (a) the sample was polymerized with the reagents and (b) the sample was added atop of preformed posts and was allowed to diffuse into gel posts. Blue lines show the HSV-1 negative controls.

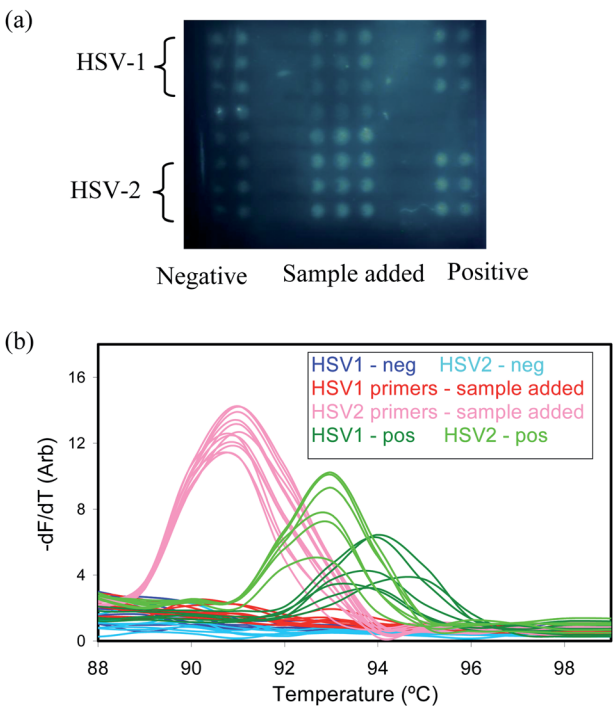

Fig. 10 Analysis of HSV 1 and 2 with integrated controls: (a) Image of the posts shown in Fig. 3(c) taken during the MCA. Fluorescence represents all dye bound DNA, not specific amplicons. (b) Melting curves of the in-gel PCR from posts shown in (a). The shift in melting temperature observed for the sample, as compared to the positive controls is due to the sugars (trehalose) used to sequester the primers in the test posts. For the positive control, primers were incorporated into the gel posts.

controls. A CCD image of the gel post array made with the configuration of Fig. 3 is shown in Fig. 10(a). In order to simultaneously test for both HSV-1 and HSV-2, primers were sequestered in the mold before making the gel posts. An aliquot of genital swab was added atop the gel posts, followed by PCR and MCA.

MCA data for the gel post array shown in Fig. 10(a) is shown in Fig. 10(b). When the code was broken, this "blinded" sample scored positive for HSV-2 and negative for HSV-1 (consistent with the status detected by conventional PCR). This experiment also shows the absence of any cross contamination between posts having HSV-1 primers and those having HSV-2 primers. The absence of a peak in the HSV-1 primed reaction (red lines) shows that HSV-2 primers did not contaminate posts with HSV-1 primers. This confirms that sequestered primers for one HSV type did not cross to the adjacent posts holding the alternate HSV primers. Positive control posts scored positive indicating that PCR on this platform was successful when the appropriate template was present. HSV-1 and HSV-2 negative controls showed no melt peaks, further confirming the absence of cross contamination and the absence of amplification when no template was present.

Two step PCR amplification

For faster PCR, we implemented a 2-step PCR that requires less time per cycle. MCA for the products of 2-step PCR are shown in Fig. 11. The total time for the PCR was 1 h; the subsequent MCA took 20 min. Two-step PCR and MCA are being further optimized to improve the speed of testing.

Conclusions

Using an array of gel posts, with each post serving as an individual reaction chamber, we amplified HSV-1 and HSV-2 directly from unprocessed genital swabs. Gel post arrays include sets of semi-solid gel posts with a volume of 0.67 μL. On the same array, as little as 2.5 nL of raw sample containing HSV-1 or HSV-2 can be simultaneously detected in different sets of gel posts, with separate areas of the gel post array reserved for positive and negative controls. Swab samples can be polymerized into the gel along with other reaction components, or alternatively they can be added atop preformed gel posts, a strategy compatible with the clinical use of this platform. Comparable results were obtained with either method. This platform measures PCR product through binding and dissociation of the dye LC Green to double stranded amplicons, followed by melting curve analysis to verify amplicons. *In situ* product detection is seamless with no need for channels, pumps or valves. Forty-five clinical swabs, including HSV-1+, HSV-2+ and negative samples as determined by conventional PCR, were tested on gel posts with their identity blinded to the operator. Each was subjected to multiparameter testing for HSV-1 and HSV-2 in tandem with negative controls. On unblinding, concordance with conventional PCR results was 91%. Sensitivity and specificity were $\geqslant$93%. Our primer sequestering technology used to facilitate multi-parameter testing allows us to detect more than one pathogen at the same time, with each test performed in a separate gel post, thereby avoiding the complexity of primer design inherent in multiplex testing where 2 or more primer sets are used in the same reaction vessel. Although applied only for HSV-1 and HSV-2, the multi-parameter technology can be extended to sequester primers for multiple pathogens or targets, enabling simultaneous screening for a panel of related pathogens or disease biomarkers on the same gel post array. Overall, our technology for detecting HSV has good concordance with conventional PCR, at ~1 h is considerably faster than culture methods that take several days to weeks, is amenable to further improvements in accuracy and speed, and can easily be adapted for medical diagnostics performed at the clinic.

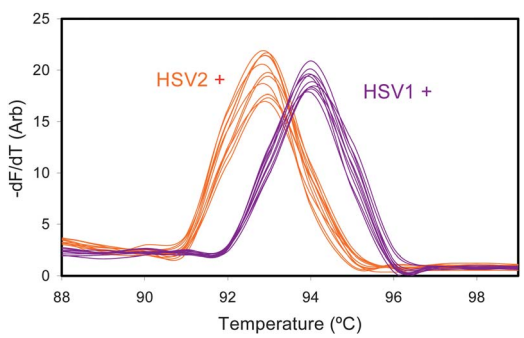

Fig. 11 MCA for a HSV-1 and HSV-2 samples from a 2-step PCR (1 h).

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