Supplementary information for LC-ART-12-2012-041419

Primer name	Sequence $(5' - 3')$
HSV-1	F-GGGCCATTTTACGAGGAGGA
(147bp)	R-GGAACGCACCACACAAAAGA
HSV-2	F-GTTTGGCGTGTGTCTCTGAA
(150bp)	R-CTTTTATCCCCGGCACACAG
UU	F-GGAATGACACACGATAAACCCT
(219bp)	R-TGACAATCGCGCTTCTGTATAA
MH	F-AACGTAGGTTGTACTCCGTAGA
(129bp)	R-AAGTCGGTTTGCTAACCTCG

Table 1: Primers used for amplification of HSV-1, HSV-2, UU and MH targets.

Appendix 1

Different capillary arrangements are possible in the pan; for example, 4 trenches are shown in Fig. 2b(i) and 2b(ii). Pans with 6 trenches have also been successfully tested. Fig. 2b(i) can have four 6mm capillaries with 4 different primers in one trench. It is also possible to arrange 6 different capillaries in one trench using 4 mm capillaries (accepting 4 µl of sample) (not shown). Capillaries in the first three trenches accept delivery of samples and one trench is used for the negative control capillaries. Capillaries in trench 4 have DNA template polymerized in the mixture for use as positive controls. A cassette with the arrangement in Fig. 2b(i) but with 6 trenches is shown in Fig. 1(b). In Fig. 2b(i), the first two capillaries in each trench can have a primer set to detect HSV-1 or HSV-2, respectively, while the 3rd and 4th capillaries in the trench can have primers for detection of UU or MH. The first two capillaries can be spaced such that the sample can be loaded from either side of the cassette to allow delivery of two different sample types (see below).

As an example of an alternate geometry, the cassette in Fig. 2b(ii) accepts only two 7 mm capillaries in each trench. The layout for another alternative is a cassette with 10 trenches and two 6 mm capillaries in each trench (Fig. 2b(iii)). Each trench has one capillary to detect HSV-1 and another for HSV-2. The first 9 trenches have capillaries with no DNA while the last trench has capillaries with DNA polymerized in the capillaries as positive controls. This particular cassette can test 8 patients at a time where 8 samples are introduced to the first 8 trenches. The 9th and 10th trenches receive water to hydrate the gels; trench 9 contains the negative control capillaries and trench 10 contains the positive control capillaries.

Appendix 2

In order to perform PCR and MCA in a pan with gel capillaries, the prototype instrument shown in Fig. 1(f) was used. This instrument incorporates off the self components to measure the fluorescence from gel capillaries for real time PCR and MCA, collecting images with a CCD camera (Point Grey Research) and a 445 nm laser (Ultralasers Inc) for excitation of the LCGreen dye. A ring illuminator (Dolen Jenner) diffuses the laser light onto the pan for even illumination. It uses a Peltier element for heating and cooling during the thermocycling. The Peltier element, CCD camera and the laser are controlled by a microprocessor. A 12.5 mm lens made by Fujinon is used to focus the fluorescence light onto the CCD camera. A 30nm wide band-pass interference filter centred at 530 nm (Chroma Technology) is placed in front of the camera. A laptop computer running a customized Java-based program is used to control the instrument. The user enters the PCR and MCA parameters as well as the camera parameters. During the PCR, at the extension phase of each cycle, the laser switches on and an image of illuminated capillaries is captured, to collect the information for real time PCR analysis. During MCA, the laser is continuously switched on and images are acquired at 0.2 °C intervals with the temperature increment set at 0.1 °C/s. The system is calibrated with a K-type thermocouple (Omega Engineering Inc.) placed inside a gel-filled capillary.

Appendix 3



Comparison of oil and wax as a vapour barrier for gel capillaries. Capillary arrangement in the pan (a) in oil and (b) in wax, CCD images at 35^{th} cycle of PCR (c) in oil and (d) in wax, real-time PCR data of all 4 capillaries (e) in oil and (f) in wax, MCA data of all 4 capillaries (g) in oil and (h) in wax. The melting temperatures of HSV-1 and HSV-2 are 82 ± 0.7 °C and 85 ± 0.6 °C, respectively.

Appendix 4

