Electronic Supplementary Material (ESI) for Lab on a Chip

Novel method for sample delivery and testing of whole blood: gel strip PCR for point of care (POC) molecular diagnostics

Recipe for the PCR/gel reaction mix

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, St. Louis, MO.); and 40% sucrose (Sigma)), 30 µL of 40% trehalose (Cargill Inc. Canada), 4 µL of 50mM $MgCl_2$ (Fluka, Buchs), 2 µL of 10 mM [dNTP] (Sigma), 2 µL of 1% BSA (Sigma), 4 µL of 10 µM primer solution (Integrated DNA technologies, San Diego, CA) for each of the two primers, 4 µL of 1000X SYBR Green I (Life Technologies, Grand Island, NY) and 5 µL of Taq polymerase (20 units/µL), 10 µL of a 40% acrylamide (Sigma) + 4% bis-acrylamide aqueous solution (N,N-methylene bisacrylamide, BioRad, Hercules, CA), 2 µL of 3% azobis (Wako, Richmond), 1 µL of 10% TEMED (N,N,N9,N9-tetramethylethylenediamine, Sigma) and water.

Samples with BKV titers were verified using conventional qPCR (off chip), or TaqMan PCR.

Inhibitory components in whole blood or plasma are known to inhibit Taq polymerase. Use of whole blood in gel strip PCR requires a polymerase that can suppress the inhibitors in blood or plasma. We have previously published a method to perform whole blood PCR in liquid media[7] using a commercially available polymerase and detection of amplicons by on-chip capillary electrophoresis. Recently, a study was conducted to detect malaria in unprocessed blood by conventional PCR with a proprietary buffer and polymerase sold commercially for use with high concentrations of SYBR Green I intercalator[8]. Here we used a custom-constructed DNA polymerase that is resistant to inhibition by whole blood and can be used with inexpensive buffers and a variety of intercalator dyes (in preparation).

Preparation of the gel cassette

750 µL of molten wax (70°C) was placed on the aluminum pan and a PDMS seal was pressed into the pan with wax to make the imprint of the channels. The pan and the seal was allowed to cool to room temperature and the PDMS seal was peeled away from the pan. 5mm wide coverslips were then placed on the channels and a soldering iron was used to apply the heat to the coverslip on top of the trenches in order to melt the wax underneath the coverslip. The method ensures that the coverslip is attached to the wax to create trenches.

During the PCR and MCA, the gel but not the coverslip is totally immersed in melted wax. The amount of wax that is placed in the pan is sufficient to cover the gel strips but not enough to overflow onto the coverslip. Melted wax also tends to stay around the coverslip rather than flowing onto the surface of the coverslip. The gel does not deform after the PCR; it remains fully hydrated when surrounded by the molten wax, hence maintaining its rectangular shape.