Designing Microfluidic PCR Chip Device Using CFD Software for the Detection of Malaria

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SUPPLEMENTARY MATERIAL

S1: Appendix A

**Optimized Real-Time PCR Procedure for Malaria Detection in Low Level Parasite**

Blood samples of 1 mL were drawn into sterile tubes containing EDTA. An aliquot of 500 μL were stored and used for DNA extraction. The DNA was extracted using500 μLTE buffer.

Low concentration of primer sequence of M60 and M61 and real time probe M62 were used as PCR mixture. The PCR mixture contain 500 nM of each primer M60 (5’ACA TGG CTA TGA CGG GTA ACG3’) and M61 (5’TGC CTT CCT TAG ATG TGG TAG CTA3’) and 300 nM of M62 (5’FAMTM-TCA GGC TCC CTC TCC GGA ATC GA-TAMRATM3’). The thermal cycler was set at 95oC for 10s (denaturation), 58oC for 15s (annealing) and 72oC (extension) for 20s. The PCR mixture of 25 μL was added to 5 μL DNA sample.

S2: Appendix B

**Viscosity Calculation**

Falkenhagen Theory was used to evaluate the viscosity of PCR mixture and sample [1].

**μsolution**(P) is the viscosity of the solution.

**μsolvent**(P) is the viscosity of the solvent.

**Csolute**(M) is the molar concentration of the solution.

**A** is a constant, dependent on the electrostatic forces of attraction.

 A = 0.1365

*For PCR mixture:*

Results show that the viscosity of the PCR mixture is the same as the viscosity of the solvent water.

For sample concentration calculation:

For Sample:

To calculate for the viscosity of the solution (*vsolution*) containing the sample and PCR mixture REFUTAS equation was used.

Where:

 *Wsample* is the mass fraction of the sample in the solution.

*WPCRmix* is the mass fraction of PCR mixture in the solution.

*VBI* is the viscosity blending index calculated using the formula.

Where:

 *v* is the kinematic viscosity which is the given by the equation below

Kinematic viscosity:

**Viscosity Binding Index**

The viscosity of water at 60 oC is 0.467 P and the calculated viscosity of solution is 0.463 P. Since the viscosity difference is minimal, water properties were used in the simulation.

S3: Appendix C

**Design Calculation**

To calculate for the time consumed in each cycle:

Where:

t is the time consumed in second.

tAis the time consumed for annealing in second.

tEis the time consumed for annealing in second.

tDis the time consumed for annealing in second.

V is the velocity in micrometer per second.

VFR is the volumetric flowrate in cubic micrometer per second.

LD is the length of the denaturation copper plate in micrometer.

LE is the length of the extension copper plate in micrometer.

LA is the length of the annealing copper plate in micrometer.

LEG is the length of the edge gap of the fluid path to the edge of annealing copper plate in micrometer.

LS is the length of the space in micrometer.

rp is the radius of the path design in micrometer.

rc is the radius of the fluid path cross section in micrometer.

Lc and Wc are the length and width of the fluid path cross section in micrometer.

ACS is the cross-sectional area of the fluid path in square micrometer.

CNo is the number of cycles in the design.

Reference

[1] X.D. Yang, R.V.N. Melnik, Effect of internal viscosity on Brownian dynamics of DNA molecules in shear flow, Comput Biol Chem. 31 (2007) 110–114. https://doi.org/10.1016/J.COMPBIOLCHEM.2007.02.010.