Supplementary information for:

A Fully-Integrated and Automated Testing Device for PCR-Free Viral Nucleic Acid Detection in Whole Blood

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Resistance calculations

Flow rate through a microfluidic channel under laminar flow is given by

$$Q = \frac{\Delta P}{R}$$

where Q is the volumetric flow rate, ΔP is the pressure difference and R is the channel resistance. The channel resistance for a rectangular channel can be approximated by the following equation¹

$$R = \frac{12\eta L}{wh^3 \left(1 - 0.63\frac{h}{w}\right)}$$

where η is the dynamic viscosity of the fluid flowing, and L, w, h are the channel dimensions. The resistance of a fluid flowing through a resistance channel with dimensions L=1E-2 m, w=2.5E-5 m, and h=2E-5 m is approximately 1.21E18* η Pa*s*m⁻³.

For the flow of liquid in timing and reagent storage channels to be laminar, a Reynolds number (Re) <2100 is needed. The Reynolds number is given by

$$Re = \frac{QD_H}{vA}$$

where Q is the volumetric flow rate, D_H is the hydraulic diameter, v is the kinematic viscosity of the fluid flowing, and A is the cross-sectional area of the channel.

$$D_H = \frac{4A}{P} ,$$

where, A is area and P is the perimeter wetted by the fluid.

The timing and reagent storage channels with w=1.6E-3 m and h=1.1E-3 m have a D_H of 1.30E-3 m. Using the flow rate when water is in the resistance channel, Re is calculated to be 5.89E-2, which means flow through the storage channels are laminar.

In the integrated PMMA device, the resistance of the timing liquid storage (L=6.56E-1 m, w=1.6E-3 and h=1.1E-3 m) is 6.52E12* η Pa*s*m⁻³. The resistance for the reagent storage (L=4.5E-1 m, w=1.6E-3 and h=1.1E-3 m) is 4.47E12* η Pa*s*m⁻³. The resistance for the heat/lysis region (L=2.5E-1 m, w=1.6E-3 and h=4E-4 m) is 3.48E13* η Pa*s*m⁻³. The resistance of the hybridization channel on top of the detection chip (L=2.5E-2 m, w=1.6E-3 and h=2E-4 m) is 2.54E13* η Pa*s*m⁻³. Compared with the resistance channel (L=6.4E-2 m, w=1.4E-4 and h=1.7E-5 m) which has a resistance of 1.21E18* η Pa*s*m⁻³, the sum of the resistances from the other device components is more than 10,000x lower. This means the resistance of the resistance channel will be the factor that dictates the flow rate of fluids moving through the system.



Fig. S1. Flow rate measurement of water flowing through resistance channel of varying geometries. Error bars represent standard error, n=5.

Membrane regulator behaviour

The use of different filter membranes as flow regulators is shown below (Figure S2). The hydrophobic PVDF is unable to form an air-tight seal when the sample was added. As a result, air enters the system through the filter and the preloaded liquid does not flow. The hydrophilic polycarbonate membrane, Isopore, is sufficiently hydrophilic to form the air-tight seal to allow the sample to be pulled into the channel. However, the membrane is very thin and the capillary force of the pores is insufficient to maintain the seal once the sample depletes from the membrane. This causes the flow of the preloaded liquid to stop. The asymmetrical polysulfone membrane, Vivid, and Whatman cellulose chromatography paper are both highly hydrophilic to allow the sample to be pulled into the channel and have sufficient capillary force to maintain the air seal to allow preloaded liquids to flow. For a membrane to be a successful regulator, it needs to be very hydrophilic and have sufficient capillary force to retain liquid in the pores after the sample depletes from the filter. Depending on the flow rate used and the capillary force of the filter, it may be necessary to have another small plug of liquid (2-3 μ L) loaded directly behind the filter, to wet the underside of the filter and initiate flow through it.



Fig. S2. Flow regulator using different filter membranes.



Fig. S3. IR measurement of the heat generated by the exothermic reaction as a function of time at the surface of the spacer layer. Black box represents heating region.



Fig. S4. Probe specificity at 25 °C using DNA oligomers. Concentration for complementary (COM), one mismatch (1 MM) and two mismatch (2 MM) oligomers are at 10 pM. Concentration for non-complementary (NC) oligomer is 10 nM. Error bars represent standard error, n=4.



Fig. S5. Time series of hybridization under flow using the resistance channel normalized to the current with no flow at 20 minutes. Error bars represent standard error, n=4.

Reference

1. Y.-H. Hsu, M. L. Moya, P. Abiri, C. C. W. Hughes, S. C. George and A. P. Lee, *Lab Chip*, 2013, **13**, 81–9.