Electronic Supplementary Material (ESI) for Analyst.

Real-time microfluidic recombinase polymerase amplification for the toxin B gene of *Clostridium difficile* on a SlipChip platform

Electronic Supplementary Information

Device design

The device consists of two separate plates with wells, ducts and holes patterned in each half. The top plate (55 mm x 60 mm) and bottom plate (64 mm x 45 mm) were fabricated from 1.0-mm-thick PMMA (figure S1).

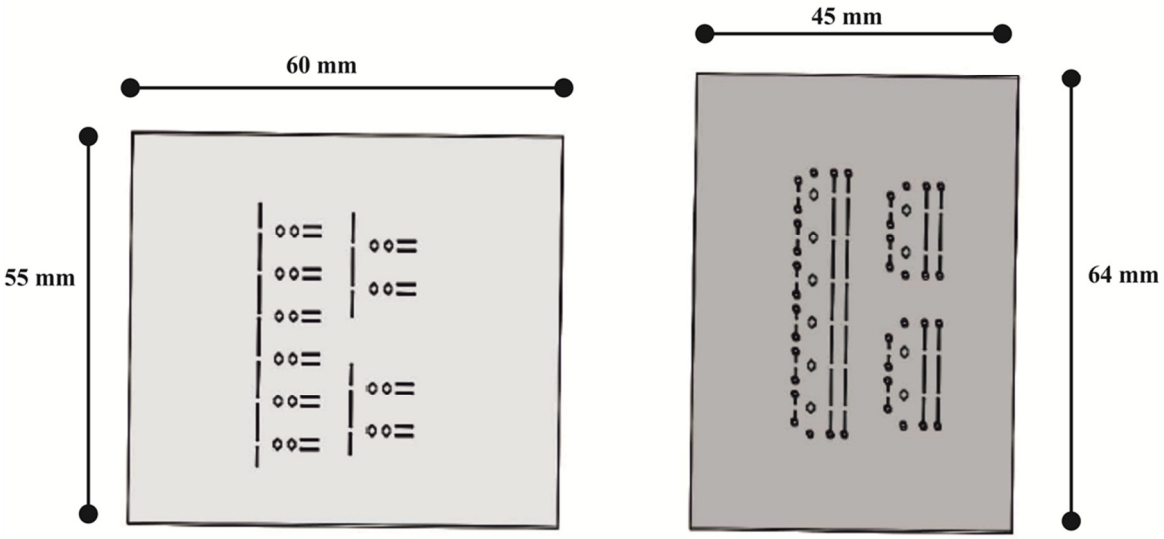

Fig. S1 Diagram of bottom plate (64 mm x 45 mm x 1 mm) and top plate (55 mm x 60 mm x 1 mm) of the SlipChip device.

Slipping protocol

The diagram in figure S3 summarises the top and bottom plate configuration during slipping. First, during the loading configuration the sample and reagents (magnesium acetate and RPA mastermix) are loaded on the device. Second, during the mixing configuration, the sample is incubation with the RPA master-mix. Third, during the incubation configuration, the chemical activation of the RPA reaction is undertaken using mixing with magnesium acetate. Reaction is then monitored for up to 1-hr under a fluorescence confocal microscope. Fourth and final, during the collection configuration, the sample is collected for further analysis.

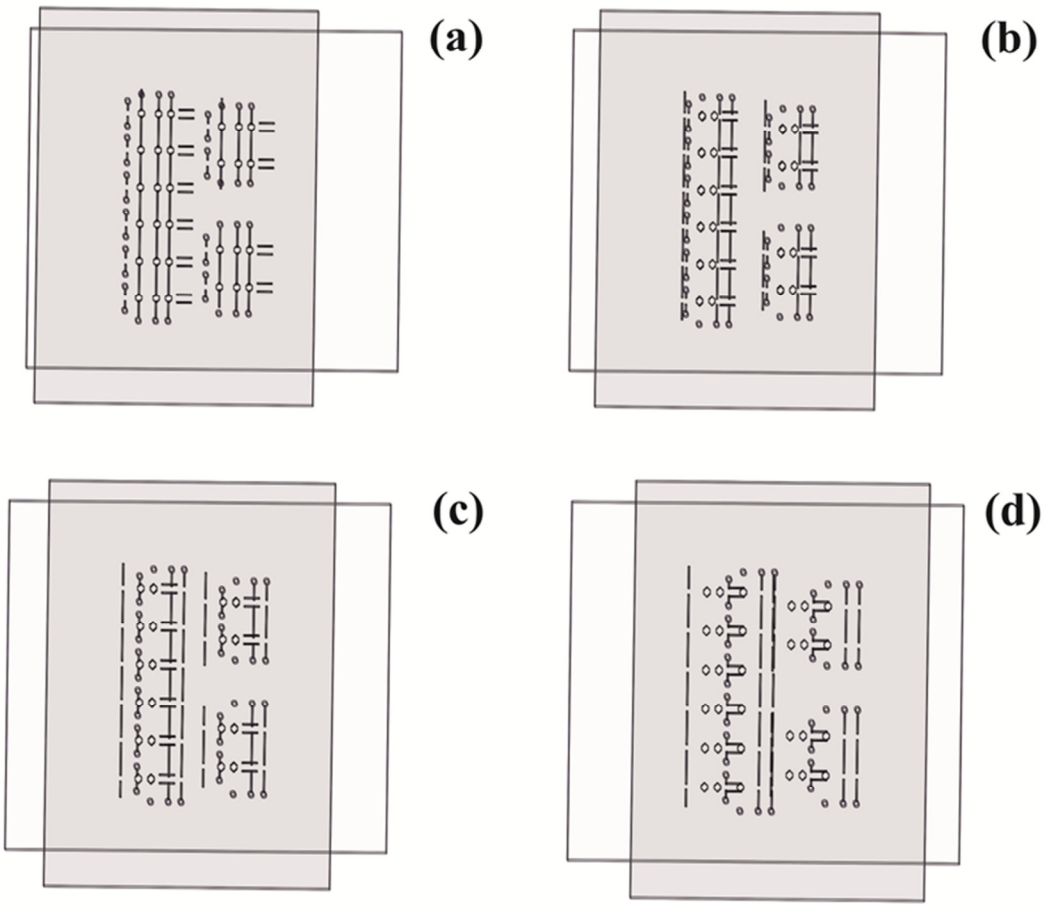

Fig. S2 Top and bottom plates of the SlipChip during the RPA reaction. (a) Loading configuration. (b) Mixing configuration. (c) Incubation configuration. (d) Collection configuration.

Thermal control

The RPA reaction was carried out at 39°C using a top plate heated via a 64.0 mm x 45.0 mm x 2.0 mm aluminium plate (figure S3a). A silicon self-adhesive heating mat (50 mm, 2 W, 12 V DC, RS Components, UK) covered the aluminium block (figure S3b) and thermoregulation to within 0.1°C was achieved using an analogue proportional-integrative-derivative (PID) control system (230 VAC, Emko, RS Components, UK). Temperature was monitored with a rubber patch thermocouple attached at the top of the heating mat for feedback temperature control. Before use, each device was tested for temperature control reliability inside the reaction chambers using an integrated 0.075 mm probe thermocouple.

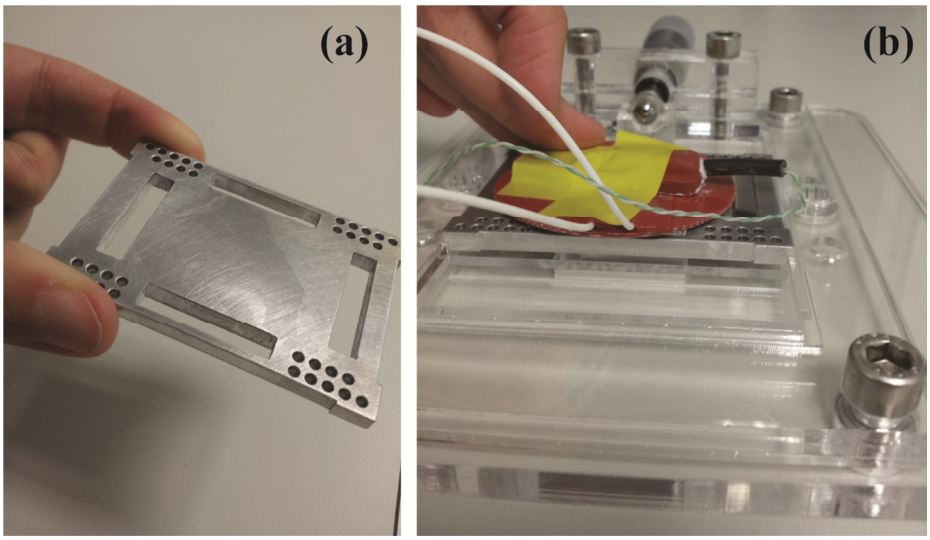

Fig. S3 (a) Photograph of the aluminium plate used for thermoregulation. The plate fits at the top of device and has inserts for the magnets. (b) Assembled heated top-plate with silicon heating mat and rubber thermocouple.

Optical and Mechanical System

Optical and x/y-axes controls were PC-interfaced with a NI BNC-2120 connector block (National Instruments, Texas, USA) connected via a SHC68-68-EPM 68-pin cable (National Instruments, Texas, USA) to a PCI-6259 data acquisition card (National Instruments, Texas, USA). A LabVIEW™-based program was developed to enable time series measurements of multiple positions. Excitation light at 635 nm from a 5 mW laser diode (LDM635, Thorlabs, UK) was immediately filtered through a 624 nm band-pass filter (40 nm band-pass, optical density > 6 in out-of-band region, 25 mm diameter, 67-035, Edmund Optics, USA). The light was then passed through a plano-concave lens (f = 200 mm, 25 mm diameter, LA1253-A, Thorlabs, UK). A motorized beam shutter (SH05, Thorlabs, UK) and controller (SC10, Thorlabs, UK) were used to control the laser beam exposure time at 15 ms to reduce photo bleaching of the fluorescent probes. The input laser light was then passed through a 10x beam expander (BE10X, Thorlabs, UK), reflected from the first broadband dielectric mirror (BB1-E01, Thorlabs, UK) and dual-band dichroic filter (Z488/633RDC, Chroma Technology Corporation, USA), before being reflected vertically from a second mirror (BB1-E01, Thorlabs, UK) and then focused into the SlipChip by a 10x Nikon Plan Fluor objective (N10X-PF, 0.3 NA, Thorlabs, UK). The base holder housing the SlipChip device was mounted on two motorized translation stages (MTS50/M-Z8E, Thorlabs, UK) and a manual z-stage. Adjustment of focus of the laser beam in the reaction chamber was performed using a separate imaging system above the sample, consisting of a zoom lens (MVL7000, Thorlabs, UK), camera (EC1280, Prosilica, Stemmer Imaging, UK) and collimated red light-emitting diode (M625L2, Thorlabs, UK) with an aspheric condenser lens (ACL2520-DG6-A, f = 20 mm, Thorlabs, UK) controlled by a constant current driver (LEDD1B, Thorlabs, UK). Excitation light was excluded from fluorescence detection using one notch filter (NF633-25) and one fluorescence filter centred at 692 nm (67-038, Edmund Optics, UK). The fluorescence signal was detected with a compact H7710-03 photomultiplier tube (Hamamatsu, Japan) with a C7319 amplifier (Hamamatsu, Japan).