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Ultrasonic welding for fast bonding of self-aligned structures in lab-on-a-chip systems *K. Kistrup C.E. Poulsen M.F. Hansen and A. Wolff* 



Fig. S1 Technical drawing of the tongue-and-groove joint used in Design A2. The overall design was based on the guides made available by Dukane's "Guide to Ultrasonic Plastics Assembly" (<u>http://www.dukane.com/us/DL\_DesignGuides.asp</u>, accessed March 03, 2015). The features themselves were generated by CNC micromachining in aluminium.



Fig. S2 Chip failure during pressure testing. To investigate the bonding strength of the welding seams pressurised air was applied to the chip inlet, while the other connectors were blocked. Design A1 failed after applying 3.5 bar (see photograph). The mode of failure was bursting of the 152  $\mu$ m thick foil. Note that the welding seam is intact, as is visible by the darker surface. Design A2 with the 500  $\mu$ m thick foil did not fail within the testing limits, which was up to 8.5 bar.

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Fig. S3 Various micrographs of the capillary microvalve of Design A1 and A2. First two rows are standard bright field images, with the contrast and colour adjusted for clarity. Third row is taken with fluorescence microscopy. Note that the foaming, especially present in Design A2, reflects the light emitted from the rhodamine B solution; however, this is distinctly different from where actual fluid is present around the edges of Design A1. Row four shows a 3D representation of confocal micrographs taken at the upper corner of the capillary microvalve as marked with the white squares in row three (Zeiss LSM 700 confocal microscope (20x/0.5, z-stack images, 1.590 µm sections). The bottom plane shows the outer foil surface and the upper plane the inner foil surface. Note that for Design A1 the corner of the main chip part is also visible due to the gap, whereas for Design A2, no such surface exists.

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## **Droplet PCR**

Droplets were produced off-chip using a Droplet Junction Chip (#3000301, Dolomite, UK) using 2 % (V/V) Pico-Surf<sup>TM</sup> 1 (Dolomite, UK) in 3M FC40 oil (Walbom A/S, Denmark) as the continuous phase. The dispersed phase consisted of PCR master mixture for detection of *Campylobacter* species using universal Campylobacter primers targeting a 300 bp of 16sRNA gene described previously<sup>1</sup>. 1 pm/µl TaqMan probe labelled with 5'6-FAM and 3' BBQ (DNA Technology, Denmark) was added to the PCR mixture to detect DNA amplification. 10 µM Sulphorhodamine 101 (Cat 80101, Biotium, VWR, Denmark) was also added for improved droplet detection and size statistics. Target DNA samples were 7.2 fM chromosomal DNA isolated from *Campylobacter jejuni*, strain NCTC-11284, using QIAamp DNA mini kit (Qiagen, Germany)<sup>1</sup>. Thermo-cycling was performed on a Bio-Rad DNA Engine PTC-200 flat-bed PCR thermo-cycler with the COC lid contacting the hot-plate, see Fig. S1. Temperature steps used were as described previously<sup>1</sup>. In brief, 5 minute hot start of 96 °C followed by 40 cycles consisting of a melting step at 94 °C for 15 seconds, an annealing step a 54 °C for 15 seconds.



Fig. S4 Mounting of chip B on flat-bed PCR thermo-cycler.

Using circular Hough transformation, 13438 droplets were analysed in a single chip to have a diameter of  $98.7 \pm 6.0 \,\mu\text{m}$  which fits well with the observed monolayer. After thermal cycling, 79% of the droplets were observed to fluoresce from the TaqMan probe (FAM/FITC channel), which corresponds to a concentration of 5.2 fM, estimated using the *Poisson* distribution. As discussed in other ddPCR studies, this underestimation of 28% may be ascribed to the sensitivity of the PCR reaction and sample loss in the upstream microfluidic system<sup>2–4</sup>.

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