Microfluidic PDMS on Paper (POP) Devices
Supporting information

Reagents for liver function analysis

The test reagents and principles proposed by S. J. Villa et al have been adopted.
All the reagents were loaded to the testing points by shooting star process.

Total levels of proteins (Protein): To measure the total levels of proteins, reagents in the following order were loaded onto the testing points: i) 250-mM citrate buffer solution (pH 1.8) containing Triton X-100 (two times); ii) 6.0 mM tetrabromophenol blue (TBPB) solution in 4% ethanol in water, iii) 250-mM citrate buffer solution (pH 1.8) containing Triton X- 100 (three times). Ten minutes of nitrogen-drying at 25 °C after each step of reagent loading was taken.

Aspartate aminotransferase (AST): Reagents in the following order were loaded onto the testing points: i) solution of 10% trehalose in water; ii) substrate solution containing cysteine sulfinic acid (CSA) (306 mg), α-ketoglutarate (34 mg), ethylene diaminetetraacetic acid (EDTA) (1.6 mg) in TRIS buffer (400 mM) (1 mL); iii) reagent solution containing 1% polyvinyl alcohol (250 mg), 0.4 % methyl green (100 mg), 0.2% rhodamine B (50 mg), zinc chloride (2.75 mg), triton X-100 (1 drop) in water (25.0 mL); iv) reagent solution containing 1% polyvinyl alcohol (250 mg), 0.1 % methyl green (25 mg), 0.1% rhodamine B (25 mg), zinc chloride (2.75 mg), triton X-100 (1 drop) in water (25.0 mL). Ten minutes of nitrogen-drying at 25 °C after each step of reagent loading was taken.

Alkaline phosphatase (ALP): In the experiment we used BCIP/NBT Alkaline Phosphatase Color Development from Keygen Biotech Corporation for ALP analysis. A BCIP/NBT staining solution was prepared by mixed reagents in the following order: i) 750 μL of alkaline phosphatase developing buffer; ii) 2.5 μL of a BCIP solution (300X); iii) 5 μL of a NBT solution (150X). The resulting BCIP/NBT staining solution was loaded onto the testing points. Ten minutes of nitrogen-drying at 25 °C after reagent loading was taken.
Figure S1. PDMS curing under different temperatures. A. Curing time analysis under different temperatures for PDMS droplets; B. Penetration depth and the diameters for the droplets cured under different temperatures; C. Curing time analysis under different temperatures for PDMS thin layer.

Figure S2. shooting star mode liquid flowing process. A1-4. Red ink shooting through the channel for sample loading; B1-4. Transparent distilled water shooting through colorful testing pads array.

Figure S2A is the real experiment time serial images for the shooting-star liquid loading process. After the column of liquid was pressed throughout the channel, it was selectively trapped in the PES paper region, while no liquid was trapped on PDMS. This is mainly because PES paper is hydrophilic and PDMS is hydrophobic. After liquid flowing through the region, discrete testing points were generated automatically.

Experiment shown in figure S2B measured the possible contamination caused by dissolution of preloaded reagents from PES paper to the sample liquid. As it depicted, the exposed PES testing points were preloaded with differently colored inks, a transparent liquid sample was pushed to quickly shoot throughout the channel. The results show no
observable color change occurred to the preloaded inks on PES testing points, and the color of the sample liquid changed very little. The results imply in a fast shooting star sample loading process, the contamination caused from the testing reagents to the sample can be neglected for colorimetric analysis.
### Technical summary:

#### Table 1. Summary of existing techniques for PDMS patterning on paper:

<table>
<thead>
<tr>
<th>Patterning technique</th>
<th>Composition</th>
<th>Curing method</th>
<th>Curing time</th>
<th>Minimum feature (μm)</th>
<th>Penetration depth control</th>
<th>Surface smoothness</th>
<th>Coupling with reagent &amp; channels</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desk plotter with special pen</td>
<td>PDMS mixture: hexane (3:1), On filter paper</td>
<td>70 °C</td>
<td>1 hr</td>
<td>~1000</td>
<td>No</td>
<td>Rough</td>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>Flexographic printing</td>
<td>PDMS mixture On filter paper, printing paper and PET</td>
<td>Transfer to Oven 150 °C or infrared drier 180 °C</td>
<td>15 s 10 s</td>
<td>&gt;700</td>
<td><strong>Yes</strong></td>
<td>Rough on filter paper and printing paper</td>
<td>No</td>
<td>3</td>
</tr>
<tr>
<td>Inkjet printing</td>
<td>Dilute with xylene On filter paper, printing paper and PET</td>
<td>Transfer to Oven 150 °C or infrared drier 180 °C</td>
<td>15 s 10 s</td>
<td>700</td>
<td>No</td>
<td>Rough on filter paper and printing paper</td>
<td>No</td>
<td>3</td>
</tr>
<tr>
<td>Screen printing</td>
<td>PDMS mixture On filter paper</td>
<td>Transfer to Oven 120 °C</td>
<td>30 min</td>
<td>~200 for surface patterning ~600 for penetrated patterning</td>
<td>No</td>
<td>Rough</td>
<td>No</td>
<td>4</td>
</tr>
<tr>
<td>Screen printing</td>
<td>PDMS mixture On PES paper, printing paper and filter paper</td>
<td><strong>Immediate transfer (~5 s) to Hot plate 150 °C</strong></td>
<td>9 s</td>
<td>~60 for surface patterning ~150 for penetrated patterning</td>
<td><strong>Yes</strong></td>
<td>Rough on filter paper and printing paper,</td>
<td>No</td>
<td>In this paper</td>
</tr>
</tbody>
</table>

*Smooth on PES paper Yes, POP devices*
References:


