Electronic Supplementary Material (ESI) for Analyst.

A Flexible Paper-Based Platform for Point-of-Care Detection of Viral RNA

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Supplementary Information

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1. RT and RT-PCR product with RNA template extract from Raji B cells

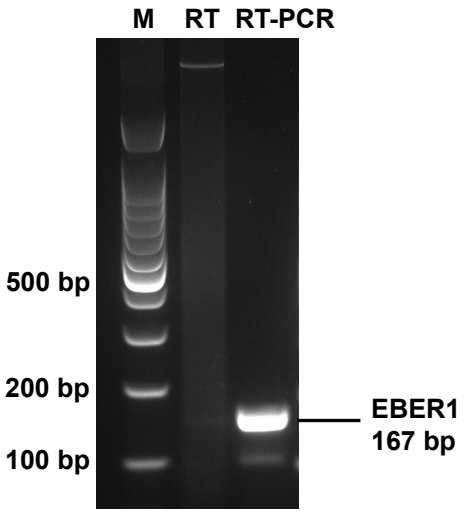

Figure S1. RT and RT-PCR product using total RNA extract from Raji B cells as template. As shown in lane 2, the variable length of RT products is due to non-specific primer-binding. This will not affect the assay since the capture and reporter probes are selective for the correct RT product. In lane 3, the desired 167 bp PCR product is visible.

2. Result analysis from LI-COR image

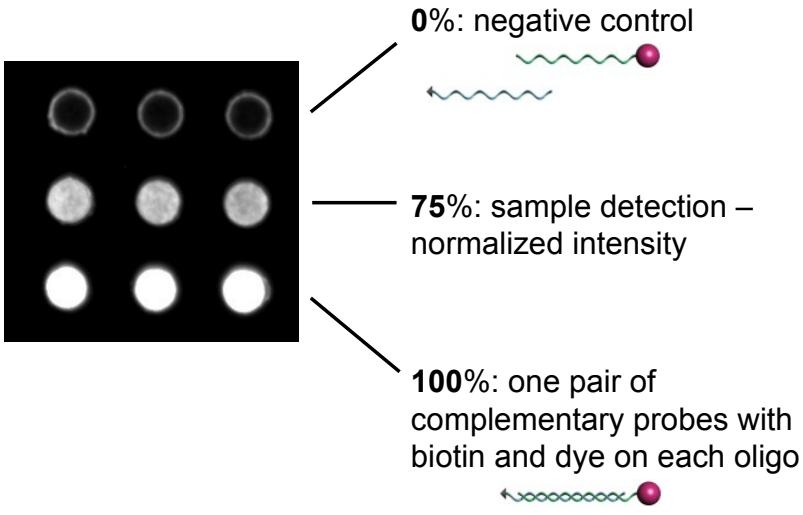

Figure S2. Results analysis with ImageJ software. The 0% and 100% intensity are defined as the intensity of negative control (I_0) and a pair of complementary probes with Alexa Fluor 647 (which introduced a fixed amount of dye in each experiment, I_{100}). The normalized intensity of sample was calculated with the following equation:

Normalized intensity = $\frac{I - I_0}{I_{100}} \times 100\%$

3. Pre-loaded sample detection with paper microzone

A 4 μL aliquot of 4.2 μM (0.25 mg/mL) NA was allowed to adsorb for 30 min in each fabricated reaction zone. After washing once with PBS buffer (10 mM sodium phosphate, 150 mM sodium chloride pH 7.2), a 4 μL aliquot of 4 μM b-Tar was incubated in the well for 15 min. Then, the wells were washed once with PBS and blocked for 40 min, followed by drying for 20 min under ambient conditions. A 128 μL aliquot of Tar or Ran-Tar (7 nM to 1 mM, 5'-TTATACAGATGATATTAGATGATCTCA-ATAGGAGCATCATTACTATATTAA-3') was loaded and dried under ambient conditions. Then, a 4 μL aliquot of PBS was added to each well and incubated for 30 min, followed by a 30-min hybridization to the Alexa Fluor 647-conjugated oligo. The result was imaged using a LI-COR Odyssey Fc imaging system, and target wells were normalized against the positive control well. As shown in Figure 5, Ran-Tar could be easily distinguished even in high concentrations. The signal from 1 μM (1.28 pmol) of pre-dried target was 91.8% of the expected value. However, the signal for the same amount of target was only 82.3% as calculated by the linear calibration curve showed in Figure 2. This increased signal may due to the longer total hybridization time (time for sample drying process plus 30-min hybridization). This result supports the utility of this fabricated paper microzone platform for sample storage prior to detection.

Specificity study.

A 1 μM (128 μL) aliquot of Tar (GACGTGTGTGGCTGTAGCCACCCGTCCCGGGTACAA-GTCCCGGGTGGTGAG) or Ran-Tar (5'-TTATACAGATGATATTAGATGATCTCAATAGGAGC-ATCATTACTATATTAA-3') was loaded on the NA and a 4 μL aliquot of 4 μM b-Tar-treated paper microzone. Then, a 30-min hybridization to the Alexa Fluor 647-conjugated oligo was performed. The result was imaged using a LI-COR Odyssey Fc imaging system (Figure S3).

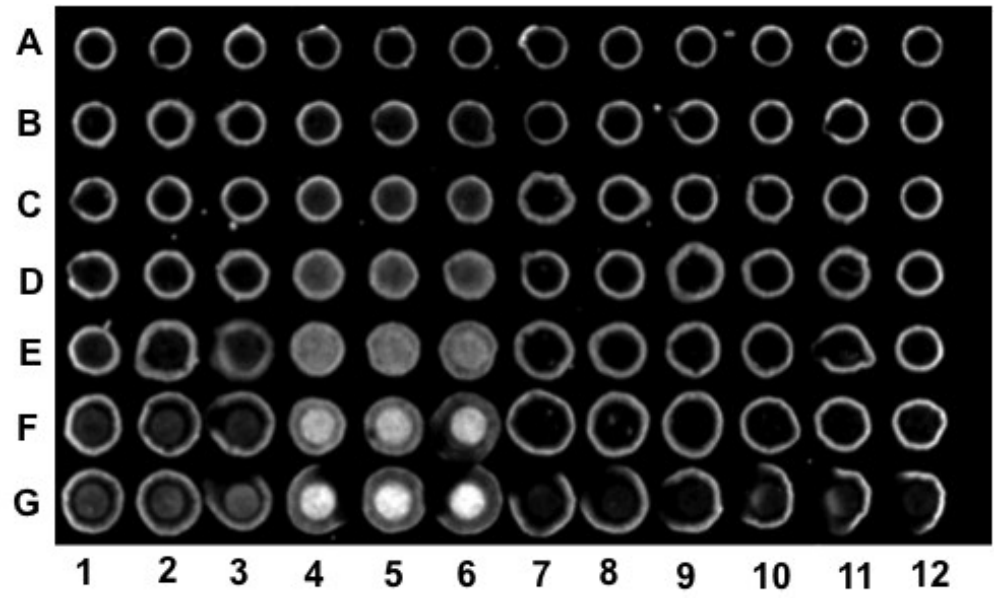

Figure S3. Wells A1-G6 demonstrate response when target is used at different concentrations (A1-G3, A4-G6). Lanes A7-H11 represent the response when Ran-Tar is used at different concentrations (A7-G9, A 10-G12).