Dissolvable bridges for manipulating fluid volumes in paper networks

*Jared Houghtaling, Tinny Liang, Gregory Thiessen, and Elain Fu**

Box 355061, Department of Bioengineering, University of Washington, Seattle, WA, USA;

*Corresponding author:
Elain Fu
School of Chemical, Biological, and Environmental Engineering
Oregon State University
elain.fu@oregonstate.edu

Supporting Information for Publication

Malaria protein *Pf*HRP2 assay

Assays were fabricated from nitrocellulose strips (Millipore, Billerica, MA), glass fiber source/storage pads (Ahlstrom, Helsinki, Finland), and a cellulose wicking pad (Millipore, Billerica, MA). All components were cut to appropriate shapes on a CO_2 laser cutting system (Universal Laser Systems, Scottsdale, AZ). A high-resolution scanner (Epson, Nagano, Japan) was used to acquire images and intensity data was extracted using a custom analysis program (MATLAB, Natick, MA). The assay used a standard sandwich format. A murine antibody to *Pf*HRP2 (National Bioproducts Institute, Pinetown, South Africa) was patterned with a piezoelectric inkjet printer (Scienion, Berlin, Germany) at a concentration of 1 mg/mL onto the detection region of a paper strip at the test line. An antimouse antibody was patterned at a concentration of 0.5 mg/mL at the control line. After patterning, the substrates were dried at 37 °C for 1 hour. Patterned membranes were treated to minimize nonspecific adsorption by soaking in a solution of 0.25% BSA, 0.25% PVP, 5% sucrose, and 15 mM NaN_3 in phosphate buffered saline for 30 min and then dried at 37 °C overnight. A second murine antibody to *Pf*HRP2 (National Bioproducts Institute, Pinetown, South Africa) conjugated to a gold nanoparticle (BBInternational, Cardiff, UK) served as a label. Tris-buffered saline with Tween 20 (TBST) was used as the rinse buffer (50 μL).

Effect of sugar concentration on assay signal generation

The effect of high concentrations of trehalose on signal generation in an assay was investigated. Mock samples were created by spiking 20 ng/mL of recombinant *Pf*HRP2 (CTK

Biotech, San Diego, CA) into fetal bovine serum (Invitrogen, Carlsbad, CA) containing 0%, 10%, 20%, and 30% trehalose by weight. The results of the assay are shown in Figure S1. The plot shows the normalized intensity vs. time in the test line for a *Pf*HRP2 concentration of 20 ng/mL in 0%, 10%, 20%, and 30% trehalose by weight. At 25 minutes, the signal was lower for higher concentrations of trehalose. However, by 45 minutes, the signal became comparable for all trehalose concentrations including 0%. These results indicate that the main effect of the trehalose is to slow down the timescale of signal generation at higher trehalose concentrations, so that a longer time to result will be required to achieve a signal comparable to the control with 0% trehalose. For assays with additional downstream sample processing, it will be critical to test for compatibility between the higher sugar concentrations and the downtream reagents.

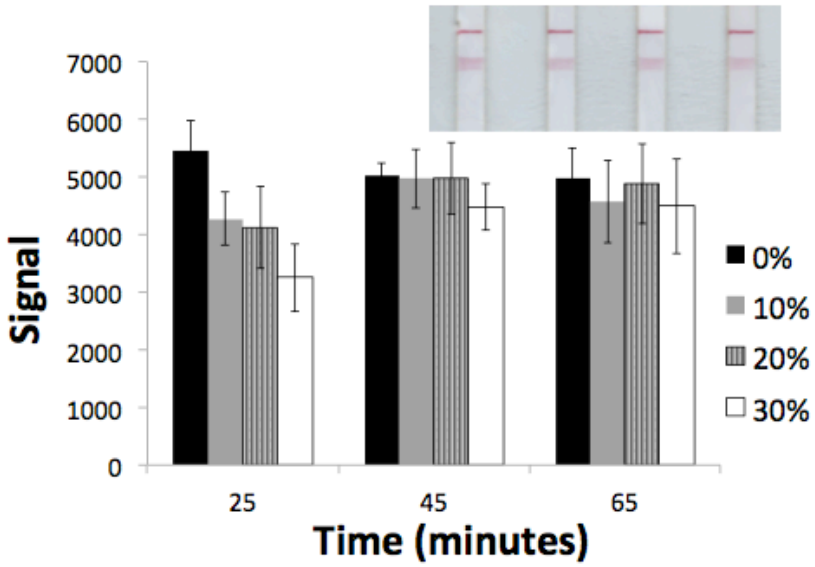

Figure S1. The plot shows the background substracted signal vs. time in the test line for a *Pf*HRP2 concentration of 20 ng/mL in 0%, 10%, 20%, and 30% trehalose by weight. The error bars represent the standard deviation of N=4 replicate measurements. The signal was lower for higher concentrations of trehalose at 25 minutes, but became comparable for all trehalose

concentrations by 45 minutes. The inset image shows the test (lower) and control (upper) lines at 65 minutes for 0%, 10%, 20%, and 30% trehalose by weight, from left to right, respectively.

Effect of bridge drying time on bridge function reproducibility

The effect of one hour vs. overnight bridge drying time on the average volume delivered by the bridge and the reproducibility of the volume delivered by the bridge was investigated. Fetal bovine serum was used as the fluid. For N=3 or 4 replicate measurements, the average volume of fluid delivered was smaller in the case of the one hour dried bridges, 28 µl, vs. the overnight dried bridges, 32 µl. The reproducibility of the volume delivered in the two cases was comparable with coefficients of variation of 5% and 7%, for one hour and overnight bridge drying time, respectively.

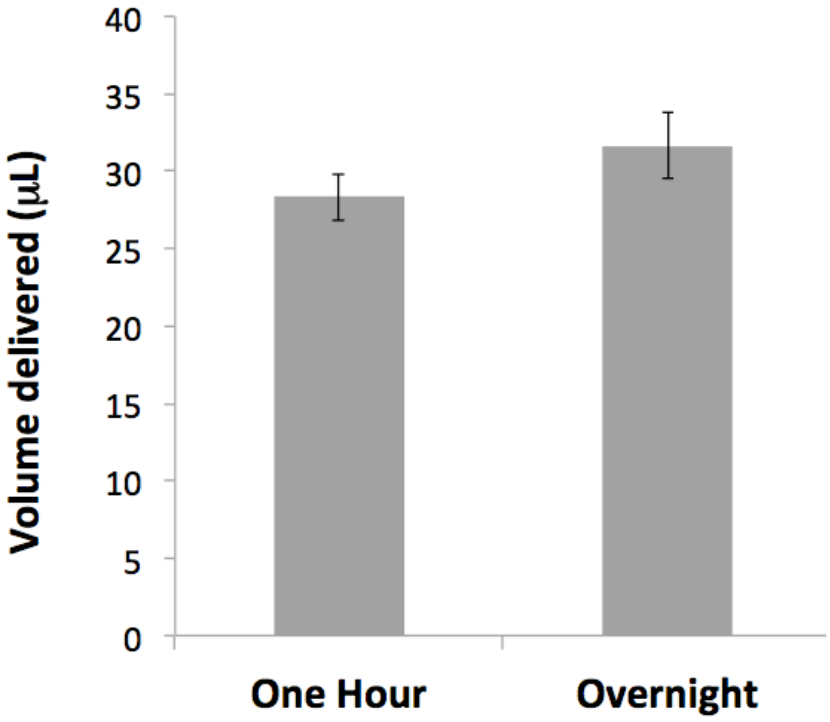

Figure S2. The plot shows the volume delivered in bridge circuits with a feeder material of treated polyester and a delivery material of nitrocellulose. The bridges were dried for either one

hour or overnight and then evaluated using fetal bovine serum as the fluid. The error bars represent the standard deviation of N=4 measurements for the one hour bridge drying time and N=3 measurements for the overnight drying time (one of the bridge cards failed to operate correctly).

Reproducibility of assay signal downstream of a bridge

Mock samples were created by spiking 80 ng/mL of recombinant *Pf*HRP2 (CTK Biotech, San Diego, CA) into fetal bovine serum (Invitrogen, Carlsbad, CA). An excess volume of ~720 μL was added to a single glass fiber source pad that led to four parallel bridge circuits in a folding card (similar to that shown in Fig. 4). The card was closed and the sample fluid was allowed to run through the bridge circuit to the detection region of the assay and the bridges were allowed to break over a time of 15 minutes. The card was then opened, and a glass fiber source pad placed immediately upstream of each nitrocellulose strip to accept a 50 μL volume of TBST rinse fluid. The detection region was scanned 25 minutes after folding the card closed. The average background subtracted signal was 7500±720 (N=4). This corresponds to a coefficient of variation of 10%.

Table S1. Material properties as supplied by the manufacturers.

	Thickness (μm)	**Porosity**	**Weight (g/m^2)**	**Water Absorption Capacity ($\mu l/cm^2$)**	**Composition**
Alhstrom GR 8975 Thin Glass Fiber	310	Not available	50	19	Borosilicate glass fiber with PVA binder
Millipore HF13502XSS Nitrocellulose	135	0.83	35	11	Nitrocellulose
Ahlstrom GR 6613 Polyester	420	Not available	100	Not available	Polyester (subsequently treated with

					Tris-based solution)