Creating an improved workflow for paper-based malaria diagnostics by integrating total lysis of whole blood

Supplementary Information

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Figure S1. Device absorption capacity

The absorption capacity was studied for the wax-printed devices (design 3 with saponin).

(a) Calibration curves of hemoglobin recovered on different types of saponin-treated paper from increasing blood volumes. For lysis devices produced on Whatman CF12 (blue) and Munktell TFN (red) paper, the absorption capacity of the outlet was determined by obtaining disks 5 mm in diameter using a punch from each material. These disks were then treated with 7 µL of 50% (w/v) saponin solution and were dried at 56 ºC for 5 minutes. Volumes from 4–13 µL of whole blood were spotted in these paper disks. The blood was then eluted by incubating the disks for 30 min at 300 rpm in 1 mL of Drabkin's reagent and the concentration of hemoglobin recovered was quantitated. In both types of paper, the concentration of hemoglobin measured increased with the volume of blood spotted up to 11 µL (CF12) and 12 µL (TFN). Error bars, which denote standard deviation for each measured hemoglobin concentration, reveal that hemoglobin recovery was more reproducible in TFN than in Whatman CF12.

(b) Extent of sample recovery percentage for different sample volumes. For the absorption capacity of the whole lysis device, different volumes of whole blood were pipetted onto the inlet and lysed blood was then eluted from the outlet. Lysis efficiency was then estimated by comparing the amount of hemoglobin detected in the blood before and after lysis. Complete lysis, and thus recovery of 100% of the hemoglobin, was only achieved for sample volumes above 50 µL (equivalent to approximately 1–2 drops of blood). Error bars denote standard deviation for each measured hemoglobin concentration.

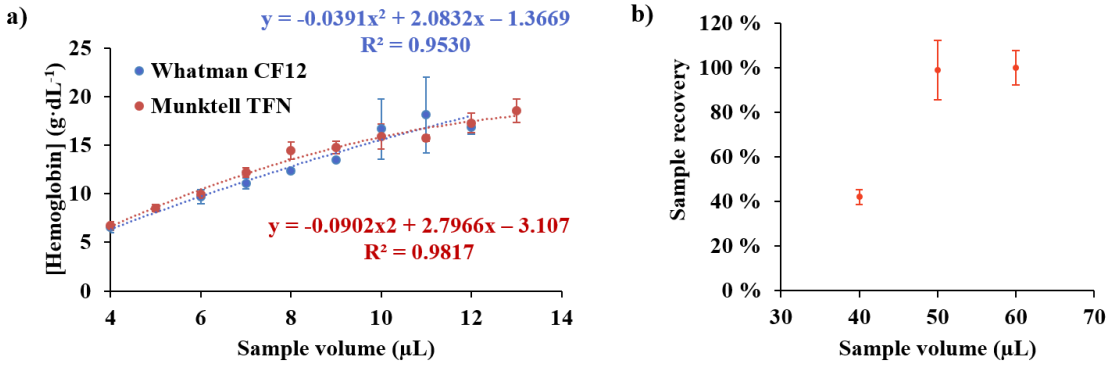
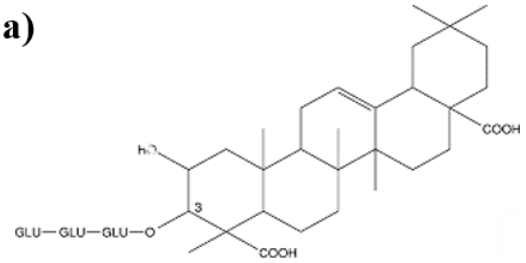


Figure S2. Effect of different hemolytic reagents in whole blood lysis.

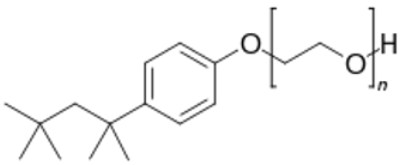
a) Chemical structure of saponin and Triton X-100.

b) Table summarizing the different lysis reagents tested and the extent of RBC lysis achieved. Briefly, blood samples were mixed gently with the corresponding hemolytic reagent. These lysed samples were then supplemented with Ficoll, a dense polymer, and were centrifuged inside microhematocrit capillary tubes at 800 × g for 60 minutes at 4 °C to separate the plasma from the cells. The extent of hemolysis was calculated by measuring the length occupied in the capillary by the packed RBCs in hemolyzed samples compared to that in the control samples of blood that experienced no hemolysis. The results disclosed that a minimum of either 5% saponin (w/v), 0.5% Triton X-100 (v/v), or mixed 0.25% Triton X-100 and 2.5% saponin were necessary to achieve 100 % of RBC lysis, while lower concentrations only produced partial lysis.

a)



Saponin



Triton X-100
(n=100)

b)

Lysis reagent	Concentration (v/v)	RBC % lysis (Ficoll)
Saponin	5 %	100 %
Triton X-100	0.1 %	5 %
	0.5 %	100 %
Triton X-100 + Saponin	0.25 % (T) & 2.5 % (S)	100 %
Nothing (control)	0 %	0 %

Figure S3. Performance of the wax-printed lysis device with different hemolyzing reagents

Design 3 ensured total device saturation for all hemoglobin hematocrits when saponin was used as a lysis reagent, which was evaluated by punching the outlet, extracting the lysed blood and measuring the hemoglobin concentration collected there in comparison to the initial sample's hemoglobin concentration.

In contrast, the use of Triton X-100 allowed the permeation of blood through the wax barrier (white arrows), which resulted in decreased flow of lysed blood towards the outlet and final lysed blood recovery.

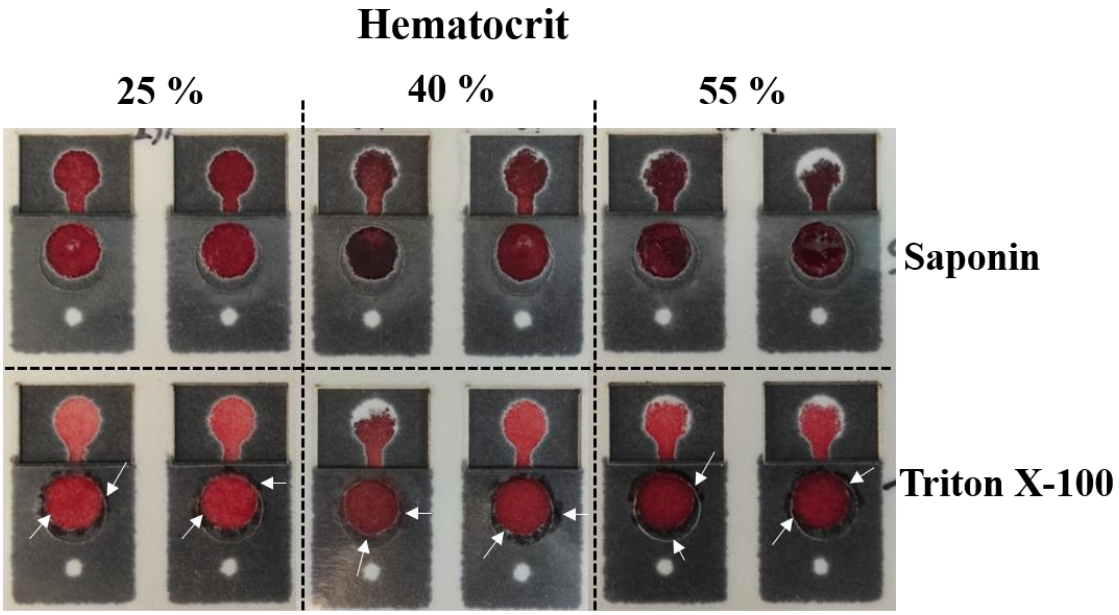


Figure S4. Anticoagulant dependency

Wax-printed lysis devices were used to lyse in parallel venous whole blood collected in EDTA tubes, venous whole blood collected in heparin tubes, and capillary blood collected by fingerstick with no anticoagulant. The three samples were applied to the lysis devices, and the extent of lysis was evaluated (n=4 for anticoagulated blood, and n=2 for the capillary blood). In all cases, after 10 minutes of flow the outlet was filled completely and could be punched for lysate recovery. When hemoglobin concentration was measured, comparable values were obtained for the three types of samples, except of EDTA blood that generated slightly lower and more variable values. These results showed that incorporating an anticoagulant to the lysis layer of the devices was not necessary.

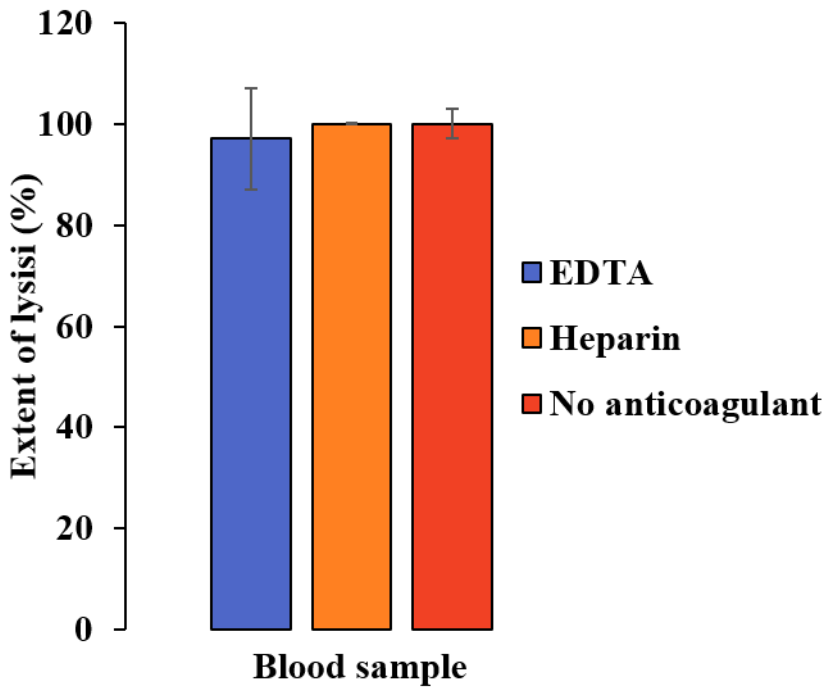


Figure S5. Stability of dried recombinant Pf-LDH and *Plasmodium* parasites at different temperatures for 1 month.

Recombinant Pf-LDH protein spiked in whole blood and native Pf-LDH in *Plasmodium* grown in human red blood cells were lysed in the lysis devices and dried overnight at 4 ºC. Once dried, they were vacuum-packed in sealed bags with silica gel and stored at 4 ºC or 25 ºC. Lysed samples were eluted and evaluated after 24 h, 48 h, 1 week and 1 month of storage.

Recombinant Pf-LDH protein was more sensitive to temperature than the native protein in the parasites. Dried recombinant Pf-LDH was stable for a week at 4 ºC, while 50% of the protein was lost after storage at 25 ºC. For native Pf-LDH in the parasites, stability was guaranteed at 25 ºC for up to one week, whereas detection at 1 month was observed only at high parasitemias.