Electronic Supplementary Material (ESI) for Analyst.

Magnetically-Enabled Biomarker Extraction and Delivery System: Towards Integrated ASSURED Diagnostic Tools

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Supplemental Information:

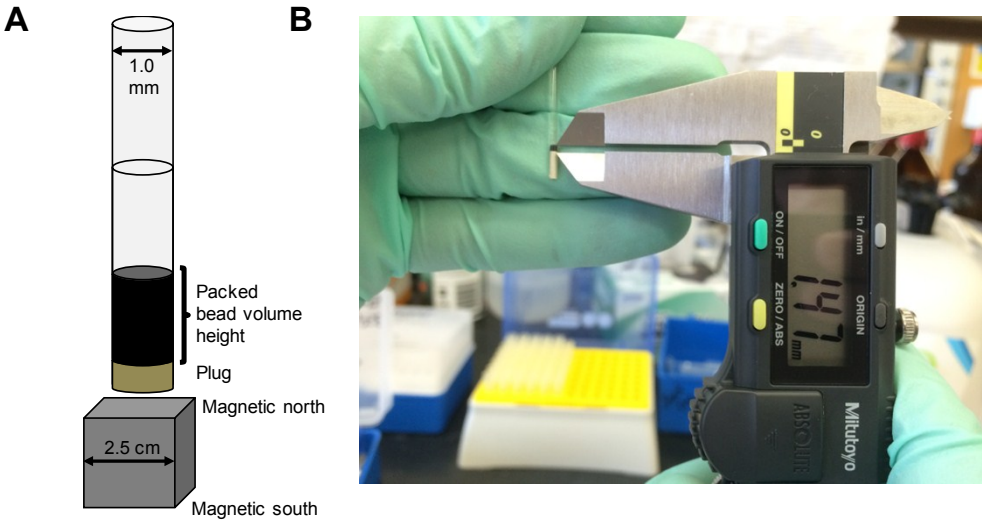


Figure S1. The method used to standardize the packed volume of beads from each manufacturer for performance comparisons where **A)** 10 µL of homogenized bead solution from the stock bead solution were added to a capillary tube, plugged with cha seal, and magnetically packed using a 1 inch^3 Neodymium magnet. **B)** The height of the magnetically packed beads is measured using a caliper.

Table S1. Bead characterization data gathered from manufacturer datasheets and generated by flow cytometry.

Manufacturer	Name of bead chemistry	Magnetic bead size range	Bead composition	Stock bead concentration	Divalent metal loading capacity
Qiagen	Ni-NTA magnetic agarose beads	20 – 70 µm	Agarose	8 beads/µL	N/A
CubeBiotech	Ni-NTA PureCube MagBeads	25-30 µm	Agarose	203 beads/µL	12 µeqv/mL resin
Clonetech	Ni-NTA His60 Ni Magnetic Beads	20-75 µm	Agarose	2 beads/µL	>20 µmol/g of beads
Bioneer	Ni-NTA AccuNanoBeads Magnetic	400 nm	Agarose	504 beads/µL	N/A

Table S2. Data from the magnetically packed bead experiment to determine the magnetically packed volume of beads from separate bead manufactures. The height was then used to determine the standardized volume of stock beads to achieve 5.5 mm^3 magnetically packed volume of beads.

Manufacturer	Volume of stock beads added to capillary tube (µL)	Average packing volume height (mm)	Packed bead volume (mm^3)	Normalized packed volume (mm^3)	Standardized volume of stock bead solution used per assay
Qiagen	10	7.0 ± 0.1	5.5	1	10
Cube Biotech	10	22.6 ± 0.1	26.7	0.21	2.06
Clonetech	10	14.8 ± 0.0	5.8	0.95	9.48
Bioneer	10	24.5 ± 0.1	9.6	0.57	5.71

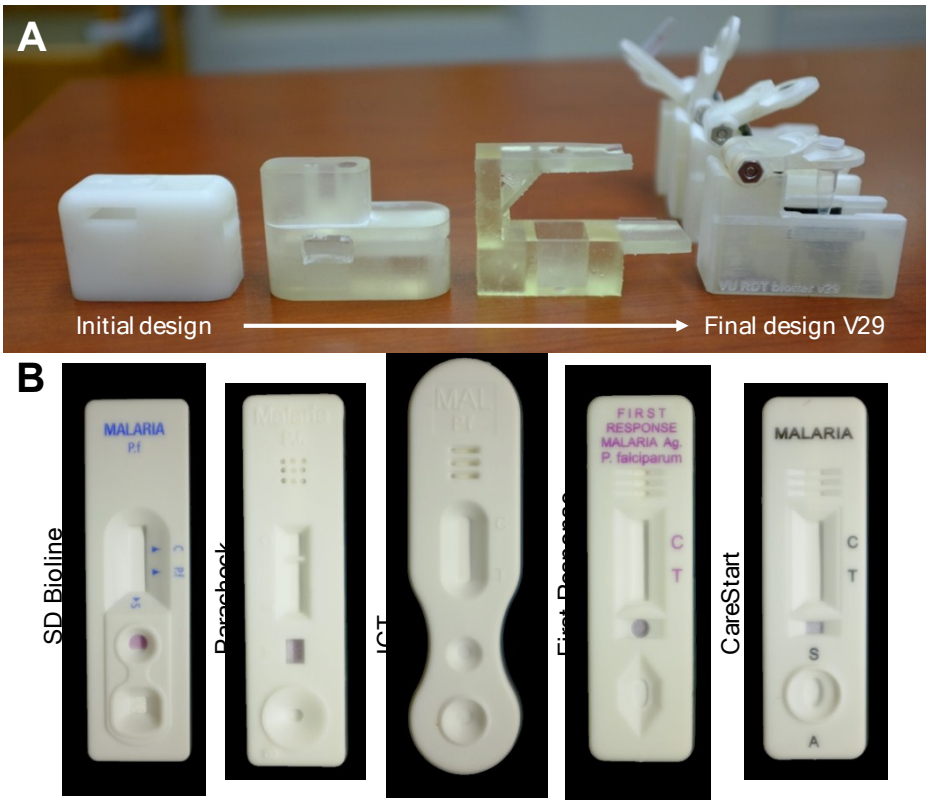


Figure S2. The mBEADS device and compatible RDTs. A) A photograph showing multiple iterations of the universal deposition device and B) photographs of the different compatible RDTs depicted in Figure 1.

Table S3. Percent residual HRP2 in the sample supernatant and eluted from the beads remaining on beads following the bead deposition step for the Zn-NTA bead type.

	Percent Residual HRP2				
Mixing Time (min)	0.5	1.0	2.0	5.0	10.0
Zn-NTA Supernatant	3.0 ± 1.0	1.0 ± 1.0	3.0 ± 2.0	1.0 ± 1.0	1.0 ± 1.0
Zn-NTA Bead Elution	22 ± 8	40 ± 20	9 ± 2	33 ± 10	20 ± 5

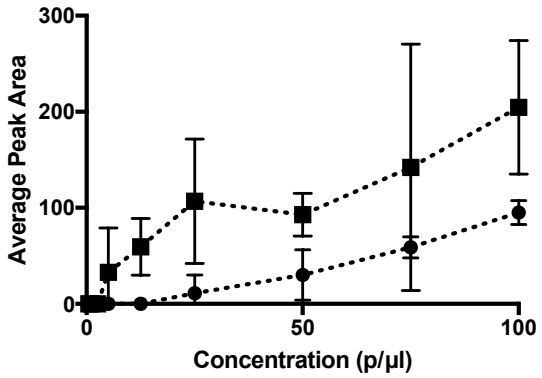


Figure S3. ICT-Pf dose response curve. test line signals (Average Peak Area) after employing mBEADS to process 50 µL whole blood samples spiked to parasitemias ranging from 1 - 200 parasites μL^{-1} (squares). Control samples (triangles) were processed in parallel per manufacturer suggested protocol

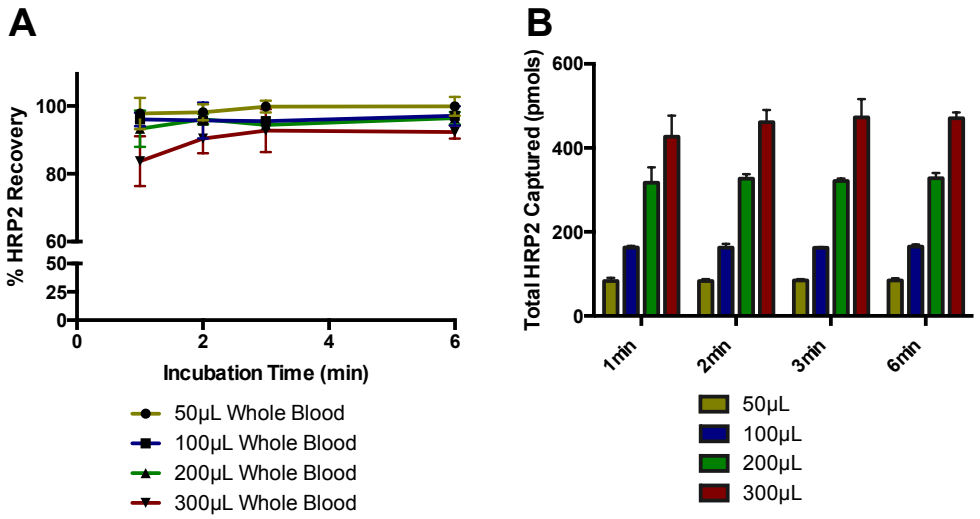


Figure S4. A) The percent HRP2 capture for increased volumes of mock clinical samples spiked to a parasitemia of 50 parasites μL^{-1} as a function of time employing single-use Zn-NTA tubes**. B)** The correlated total HRP2 captured.

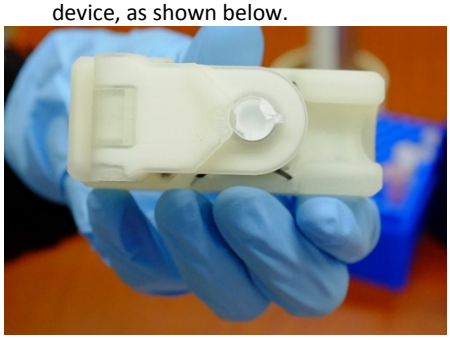
Video S1. Training video for mBEADS system:
https://vanderbilt.box.com/s/cjdkz3h5ytykk5xz5rnbdplgoa62ehu3

Supplemental Methods:

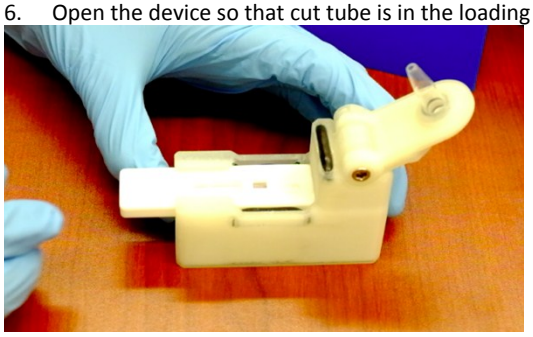
Detailed mBEADS Protocol. A 14-step protocol for correct mBEADS use with visuals for assistance.

1. Add 50 µL lysis buffer to sample tube (containing lyophilized beads).
2. Vortex for 5 seconds.
3. Add 50 µL of prepared blood into the sample tube (1:1 ratio with buffer).
4. Put sample tube onto VU Vortexer for 1 min. Push sample tube halfway into the VU Vortexer head so that it is firmly in place, but not pushed all the way down. Proper tube vortexing tube position shown below.

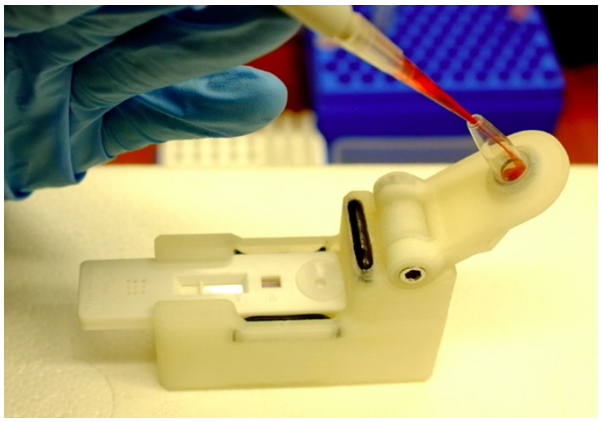
5. Insert cut tube into the top of device. The nose of the cut tube should be rotated under the catch in the top of the device, as shown below.



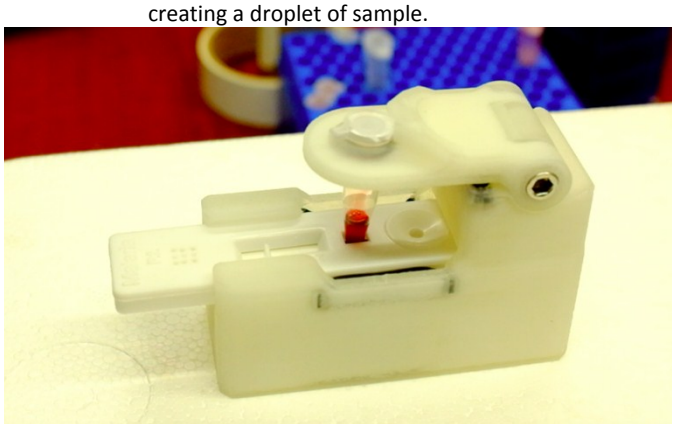
6. Open the device so that cut tube is in the loading position. Load RDT into device, running buffer port first.



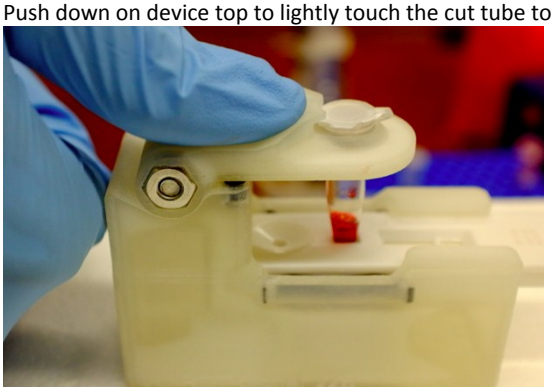
7. Manually re-suspend magnetic beads in solution via pipetting up and down 3 times. Collect full sample into pipette tip and aiming at the topside of the cut tube rapidly pipette the sample into the opening of the cut tube
    a. Note: fast sample addition prevents bubble formation at the opening of tube.



8. Flip the device top into position, allowing the cut tube to hover above the sample pad of the RDT. Ensure meniscus formation at bottom of cut tube.
9. Suspend sample over the magnet for 1 min, without touching the RDT, to collect magnetic beads.
    a. Note: If there is no meniscus at the tube opening, touch the side of the cut tube to warm the interior, creating a droplet of sample.



10. Push down on device top to lightly touch the cut tube to the RDT sample pad.



11. Open the device. Remove RDT from mBEADS device and add modified developing buffer.

12. Allow test to develop for 30 min. If using Dynabeads for SD Bioline RDT, place test on developing tray to prevent bead migration.
13. Open cassette and remove wicking and conjugate pads from RDT strip.
14. Insert into LFA reader for measurements.