Microfluidic Device to Diagnose Malaria in Rural Locations

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Abstract

Malaria is a severe parasitic disease that is often deadly due to delayed diagnosis in rural areas, lack of laboratory infrastructure, equipment and training. This design is a point of care (POC) device that combines a separation technique based on the magnetic properties of infected red blood cells and a detection portion that uses a lateral flow immunoassay method paired with gold nanoparticles to diagnose malaria in rural locations. Device tested with diluted porcine blood showed the 50, 80 and 100 um constriction points are feasible for use.

Introduction

Problem Statement

To create a device to diagnosis malaria for point of care (POC) testing in developing countries.

Project Motivation[1]

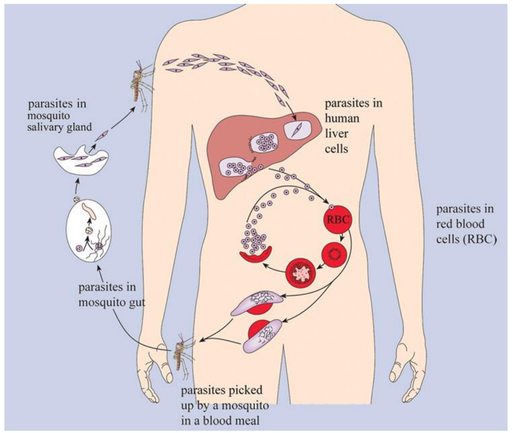
- Poor diagnosis allows treatable diseases to become deadly
- Nearly 3.2 billion people are at risk of contracting malaria
- Approximately 438,000 malaria related deaths annually
- An accurate, cheap, and easily administered test could save thousands of lives

Design Criteria

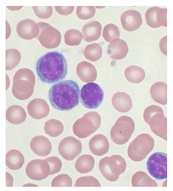
- > 95% accuracy rating
- Results within an hour
- Small in size, usable in rural locations without lab technician
- Under $5 per test
- Able to detect and distinguish the main strains of malaria
- Fabrication with limited laboratory equipment

Malaria Background

From female mosquitos – infects red blood cells, 4 strains[3]



Competing Designs/Current Methods



Histology[3] Blood Smear

BinaxNow

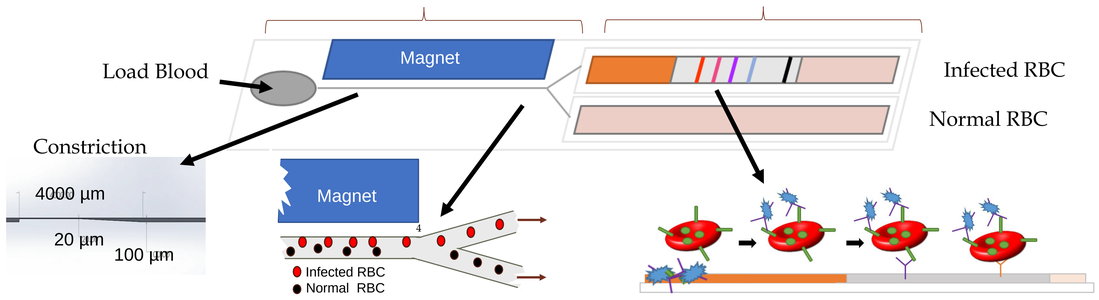
Pitfalls

Histology
- Need Lab technician/ equipment

BinaxNow
- Not all strains detected
- Very expensive

Final Design – Malaria POC Diagnostic Device

Separation	Detection
• Concentration of Malaria infected red blood cells (iRBCs) • Parasite converts hemoglobin to hemozoin (paramagnetic) • Device pulls iRBCs toward magnet to detection portion	• Lateral Flow Immunoassay (LFIA) detection • Immobilized antibody paper assay, uses conjugated gold-nanoparticles (Au-NPs) –Ab detection. Visible by eye



POC Device Operation

1) Load 50 µL whole blood sample
2) Constriction controls flow rate
3) Magnet separates iRBCs
4) Channel divides and separates iRBCs
5) Blood wicks down detection strip
6) Blood rehydrates Au-NPs-Antibody
7) Au-NPs tagged iRBCs bind to immobilized antibody line(s)
8) Control line appears and if iRBCs are present the specific infection line(s) appear

Antibody Color Code

Malaria species	Antibody	Au-NP d	Color
P. falciparum	HRP2	5 nm	
P. vivax	P. vivax ARP	30 nm	
P. ovale	P. ovale MAbs	50 nm	
P. malariae	Pan Malaria**	80 nm	

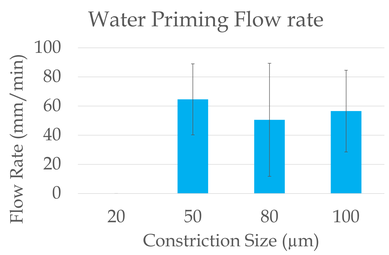
Cost Per Device

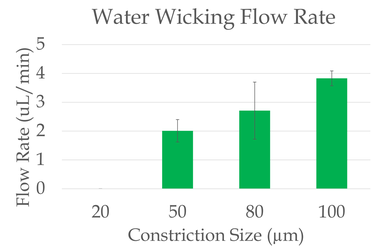
Material	Cost
Fisherbrand Glass Slide	$0.87
PDMS (one channel)	$0.25
LFIA Pads	$0.033
Disposable Finger Prick	$0.08
Antibodies	$1.00
Gold Nano-particles	$0.40
Total	$2.63

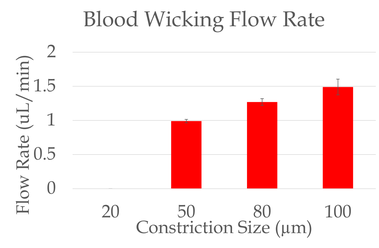
Testing and Results

Flow rates were measured for both priming and wicking through four different channel constriction sizes: 20, 50, 80, and 100 µm.

- 20 µm constriction shown not to allow for passage of water or blood through channel.
- Blood diluted to ¼ concentration with water, required positive pressure for priming.
- Blood and water wicking follow flow rates follow same trend (blood ~1/2 rate of water).



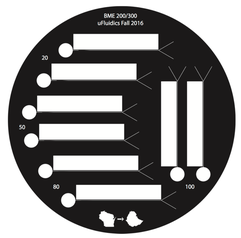




The error bars +/- 1 SEM

Materials and Methods

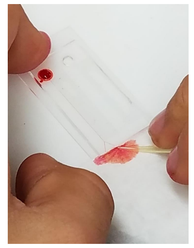
Photoresist Mask



Fabrication
- Patterned silicon wafer with SU-8 photoresist
  Exposed wafer to UV through the photomask (left)
- PDMS was polymerized on top of the wafer mold
- Channels were cut out and plasma treated to bond to a glass slide
- Outlets were cut and placed on wicking pad

Testing
- Priming velocity - the well was filled and the time the fluid took to run to the channel divide was measured
- Wicking flow rate - a drop (~8 uL) was placed in the loading well, the time it took for the drop to run through was measured (water and 25%porcine blood)

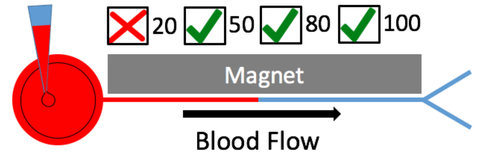
Wicking Flow Rate Test



Discussion

Channel Fluid Flow

Water = Passive
Blood = Positive Pressure



Priming velocity inconsistent
- Possibly due to inconsistent fabrication methods
  - Wafer placement or time after plasma treatment
    - Samples tested too long after plasma treatment

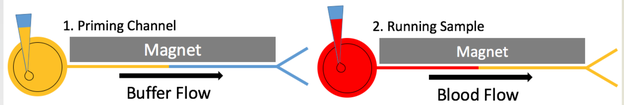
Testing Conclusions

Constriction Size (um)	50	80	100
Time to run assay (min)	50.6	39.3	33.6

100 um fastest but all sizes can be used for a <1 hour test.

- Size choice cannot rely on flow rate alone; magnetic separation efficiency needs to be tested with malaria iRBCs

Potential Priming Solution



Prime channel with solution first, followed by sample.

Method Limitations

This design only focuses on flow rate and wicking rate. Magnetic separation and disease detecting/diagnosis cannot be determined with these methods.

Design Specifications Met

< 1 hour	< $5 per test	all 4 strains	>95% Accuracy	Easily used POC
Yes*	Yes	Yes*	N/A	No

Future Work

1) Test blood flow rate immediately after plasma treatment.
2) Redesign blood sample addition component to allow flow without priming and to hold entire blood volume.
3) Begin work on detection portion
   1) Conjugating and drying Au-NPs
   2) Antibody immobilization
   3) Long term storage
4) Test design with malaria infected blood
   1) Magnetic separation ability
   2) Antibody specification
   3) Accuracy and time of malaria diagnosis
5) Refining fabrication for use in Ethiopia

Acknowledgements & References

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References

[1] WHO, "Malaria," in *World Health Organization*, World Health Organization, 2016. [Online]. Available: http://www.who.int/malaria/en/. Accessed: Oct. 11, 2016.
[2] D. Polpanich et al. "Detection of malaria infection via latex agglutination assay," *Analytical chemistry.*, vol. 79, no. 12, pp. 4690–5, May 2007. [Online]. Available: https://www.ncbi.nlm.nih.gov/pubmed/17511424. Accessed: Oct. 11, 2016.
[3] H. Hou *et al.*, "Deformability based cell margination--a simple microfluidic design for malaria-infected erythrocyte separation," *Lab on a chip.*, vol. 10, no. 19, pp. 2605–13, Aug. 2010. [Online]. Available: https://www.ncbi.nlm.nih.gov/pubmed/20689864. Accessed: Oct. 11, 2016.
[4] A. Trafton and M. N. Office, "A new way to diagnose malaria," MIT News, 2014. [Online]. Available: http://news.mit.edu/2014/new-method-diagnose-malaria-0831. Accessed: Oct. 11, 2016.
[5] W. K. Peng, L. Chen, and J. Han, "Development of miniaturized, portable magnetic resonance relaxometry system for point-of-care medical diagnosis," *Review of Scientific Instruments*, vol. 83, no. 9, p. 95115, Sep. 2012. [Online]. Available: http://scitation.aip.org/content/aip/journal/rsi/83/9/10.1063/1.4754296. Accessed: Oct. 14, 2016.
[6] A. Tay, A. Pavesi, S. R. Yazdi, C. T. Lim, and M. E. Warkiani, "Advances in microfluidics in combating infectious diseases," *Biotechnology Advances*, vol. 34, no. 4, pp. 404–421, Jul. 2016.