BME Design-Fall 2016 - Hunter Johnson
Complete Notebook

PDF Version generated by

Hunter Johnson

on

Oct 19, 2016 @02:09 PM CDT

Table of Contents

Team contact Information

▪ Joshua Liberko ▪ Sep 23, 2016 @01:43 PM CDT

Last Name	First Name	Role	E-mail	Phone	Office Room/Building
Puccinelli	John	Advisor	puccinelli@bme.wisc.edu	(608) 890-3573	2132 ECB
Kwa	Tim	Client	tkwa@ucdavis.edu		NA
Johnson	Hunter	Leader	hjjohnson3@wisc.edu	(608) 212-1461	NA
Feeney	Austin	Communicator	afeeney@wisc.edu		NA
Zachary	Hite	BSAC	zhite@wisc.edu	(651) 245-6642	NA
Jorgensen	James	BWIG	jejorgensen2@wisc.edu	(414) 940-4254	NA
Liberko	Josh	BPAG	jliberko@wisc.edu	(612) 600-4365	NA

Project description

▪ Hunter Johnson ▪ Oct 19, 2016 @10:33 AM CDT

Course Number:

BME 300/200

Project Name:

EWH: Micro-fluidics based point-of-care diagnostic devices for Ethiopia

Short Name:

ufluidic_poc

Project description/problem statement:

This fall Jimma University, Jimma, Ethiopia, are launching a thesis-based Master's program in Biomedical Engineering, and they are exploring the possibility and viability of microfluidics-based projects, for example, fabricating devices for point-of-care testing and comparing the results to the locally available tests. The UW BME design team will collaborate with Dr. Tim Kwa, Assistant Professor at Jimma University to design tools such as PDMS molds and others which will be useful for developing microfluidics devices in Ethiopia.

About the client:

Client:
The client is Dr. Timothy Kwa, a professor at Jimma University. Dr. Kwa received his PhD from UC-Davis in Biomedical Engineering and his research interests include improving healthcare through early diagnosis technology.

graduate studies : https://gradstudies.ucdavis.edu/news/timothy-kwa

Alternate Contacts:
Amit J Nimunkar
ajnimunkar@wisc.edu

Heidi Busse
hbusse@wisc.edu

2016_9_14 - First Client/Advisor Meeting

▪ Hunter Johnson ▪ Sep 14, 2016 @06:43 PM CDT

Title: First Client and Advisor Meeting

Date: 9/14/2016

Content by: Hunter

Present: Hunter, Josh, Zach, Austin, James

Goals:

- Meet client (via Skype)
- Explanation of project

Content:

- Introduction to client collaborator: Paddrick
  - BME undergrad and grad student at UW
  - Works as a faculty member at Jimma University in Ethiopia
  - Teach
    - Entrepreneurship, BME design, and thermodynamics
  - Starts 3rd academic school year
  - Involved with EWH and applying BME skills to BME global health projects
- Primary Client
  - Tim
  - Finished up PhD at UC Davis
  - Jimma university faculty member
  - PhD in point of care diagnostics
- Project Overview
  - Jimma University Ethiopia
    - Ethiopia in eastern Africa
    - Low income, wide variety of languages
    - Jimma, southwestern portion, in Aromia area of Ethiopia
    - Well known for Jimma University
      - Founded in the 50s
    - BME first program in country
      - Graduated 3 sets of classes (so 3 years)
    - 250,000 people, with the university, hospital
      - Has many patients, with only the most severe making it
      - Struggling for resources
    - Quickly growing city, just attained internet
  - Projects currently working on
    - Paddrick: working with hospital to make water distillation device.
      - Currently, the surgeons sterilize with autoclave
      - Use tap water, but should be using distilled water.
    - Undergrad senior design project: low cost kidney health analyzing device. Take small sample of urine and measures ALB concentration using spectrometer.
  - Tim's interest
    - Did PhD work in microfluidics
    - Interested in bringing POC diagnostics care in Jimma. Essentially setting up a lab at the university.
    - Wants to set up a facility or a research center withing the department for this use.
    - Resources available:
      - Major challenge, assess to internet (3G internet just arrived), so communications can be a challenge. Internet blackouts or electricity black outs.
  - Larger group on campus working with Ethiopia already on a emergency medicine program.
  - Our goals/questions
    - Understand diseases
    - Research point of care disease testing
    - Current tests available
    - Create a reproducible design that they could use/make in Ethiopia
    - What are the prototyping facilities available
      - Bare bones: screw drivers/hammers/wrenches etc, printers.
    - Beebe lab readings
      - General info
    - Possibly meet Scott, publications
  - BME 550
    - Attend labs or lectures
  - Bill and Melinda Gates Foundation
- Look up photolothography

Conclusions/action items:

- Email Tim
  - Let him know how we will learn this field
  - Let him know we need a tangible device
- Research
  - Beebe lab papers
  - Start formulating PDS, Problems in the field, formulating ideas
  - Meet every Friday 1:30 - 2:00, 3126.

2016_9_16 - Advisor Meeting 1

▪ James Jorgensen ▪ Sep 16, 2016 @02:16 PM CDT

Title: Advisor Meeting 1

Date: 9/16/16

Content by: James

Present: All

Goals: Meet with advisor to talk about our first progress report, future work on PDS, and direction of design

Content:

- No response from Tim yet in terms of the questions that we asked him
- Current work is preparing for when he responds and familiarizing ourselves within the field of microfluidics
- PDS can be worked on continuously, none of it is set in stone
    - Work can be started on it and then when we get specifics, it can be adjusted
- Progress report was a good start
    - It will be more substantial next week as more work is done
- Project status update was completed well
- Expectations:
    - Be together at weekly advisor meeting (Let him know in advance if we cannot make it so we can reschedule)
    - Notebooks are a heavy aspect of grades (Due to individual work)
    - Mid semester grades are more of a learning experience for the rest of the semesters work
    - Dividing and conquering is beneficial, but everyone in the group should have an understanding of research done (Do not just copy and paste)
    - MAKE SURE TO ADDRESS AND IMPROVE UPON THE COMMENTS MADE BY PUCCINELLI ON ALL WORKS
    - Only one person needs to take team meeting notes, but it can be helpful for everyone to have input so the information is as concrete as possible. Not everyone has the same perspectives on what was done so getting a wide range of input is positive.
- We will most likely get a list of diseases from Tim, and then we can choose the one we want to pursue creating a diagnostic device for
- Find syllabus for BME 550 class on microfluidics
- Check with Amit to see if Tim has another email address that we can use if we do not get a response
- Molds for microdevices can be made in very different ways that are accessible in Jimma

Conclusions/action items:

Continue to do research and add to labarchives so team mates can see what research has been done, and what still needs to be done. Establish a rough PDS outline and make sure that is finalized for the next adviser meeting. Follow up with Tim when/if he responds, and make sure to find a way to reach him if he does not get back to us shortly.

2016_9_23 - Advisor Meeting 2

▪ James Jorgensen ▪ Sep 23, 2016 @01:56 PM CDT

Title: Advisor Meeting 2

Date: 9/23/16

Content by: James

Present: All

Goals: Discuss current design goals as well as update Dr. Puccinelli with our information gained from Dr. Kwa

Content:

- Spent the introduction of the meeting explaining the information given by Dr. Kwa in our previous email
- One of the cheapest options available is a test strip, but has no
- The only fast acting test device approved for use in America is incredibly expensive, but very accurate. Can look more into this to gain information on how this design works.
- Patent research is very hard to analyze and focus is hard to determine
    - google.com/patents is a helpful tool to get easy and helpful results on existing patents
    - Can essentially do anything we want in terms of academia so patents are not the most important aspect to focus on, international patents can be tricky to find, however
- Ask Tim if there is somebody at JU that can give us more information on the types of POC devices used to detect malaria
- Main problem seems to be the detection method (technology wise) of the sample as opposed to being able to isolate blood that contains the disease
- Look into disposable/reusable aspects of our design that would be helpful for limited resources in Ethiopia
- Zach learning a lot in 550, spent lecture discussing silicon grinding and fabrication methods
- Notebooks look good thus far
    - approximately 2 research articles each week for each team member in order to keep pace with the project
- PDS
    - Shelf life - do not refer to specific methods. Look more into if it is multi-use or single-use. Saying to be able to store for a year is good though, keep in mind the different aspects of JU as opposed to here.
    - Convert temperature to SI units
    - Materials - Focus on what might be available in Jimma in terms of fabrication
    - Upload new PDS on site whenever we update it
- Testing would be more concept based as opposed to actually seeing if the device positively marks malaria
    - Testing the ability of the device to positively mark and identify a different substrate
- Begin thinking about design ideas for our preliminary presentation coming up in a few weeks

Conclusions/action items:

Continue to fine-tune the PDS as more research is done and we understand more about our specifications. Communicate with Tim in order to learn more and ask him the list of questions that we will produce. Come up with a few design ideas for next weeks advisor meeting. We can send our presentation to Dr. Puccinelli before the presentation in order to get extra feedback.

2016_9_29 - Advisor Meeting 3

▪ James Jorgensen ▪ Sep 29, 2016 @05:37 PM CDT

Title: Advisor Meeting 3

Date: 9/29/16

Content by: James

Present: James Hunter Josh

Goals: Discuss our potential design ideas with Dr. Puccinelli and get more research direction

Content:

- Began meeting by giving Dr. Puccinelli general overview of how malaria works and the developmental process of contracting the disease.
  - Discussion about the number of infected cells and infected cell properties were discussed
- Design 1:
  - PDMS can be cut small enough for this design to be feasible, but height of the channel would be a huge factor
  - A collection pool for each type of blood would be needed
- Design 2:
  - Main microfluidic POC device that just came to marked (lateral flow immunoassay)
  - 90-93% accuracy in current marketed US device
- Design 3:
  - Infected RBCs have a higher iron content than regular RBCs
  - Beebe lab magnetic treatment (Varifast)
- Design 4:
  - Life at low Reynold's numbers
  - Microfluidics and gravity do not go so well together
- Design 5:
  - Very new type of research using electrical properties of infected RBCs
- Look into the life cycle of malaria more in depth in order to see when the disease is detectable and still curable
- Gold nanoparticles conjugate different long chain molecules, self assembled monolayers (SAM)

Conclusions/action items:

Do more in depth research on the different life stages of malaria, and when it can be detected during those stages and how. Look into papers described above (Beebe lab and Reynold's numbers). Begin working on more defined prototypes for preliminary presentation in 2 weeks.

2016_10_7 - Advisor Meeting 4

▪ James Jorgensen ▪ Oct 07, 2016 @02:16 PM CDT

Title: Advisor Meeting 4

Date: 10/7/16

Content by: James

Present: All

Goals: Discuss the created design matrix as well as plans for preliminary presentation and paper

Content:

- Design matrix outline shown
- Will upload the design matrix after the meeting is complete
- Deformability Design:
  - Length and height of capillary important for separation of cells
  - Could test this using polystyrene beads as pseudo iRBCs
- Magnetic Design:
  - Look into patents about magnetic filtration being used currently
  - Talk to a physics professor about magnetic fields and equations found
- Electrical Design:
  - Hard to find research about this design
- Justifiable to use same weighting and categories
- Possibly adjust the weighting of cell deformation versatility because it has a higher capacity to test for other diseases
  - Could cell deformation be used to help stage the time periods of cellular diseases
- Likes the idea of gold nanoparticles (control pad in particular)
- Access to bioprinters in Williams Lab
  - For printing antibodies onto paper for detection
- Very solid design ideas thus far
- Try and get an edge on some of the existing technology that uses magnets
  - Applying a detection technique to this separation technique partially accomplishes this
- Remind Dr. Puccinelli about setting up a video camera on Friday for presentation
- Send slides to Dr. Puccinelli by Wednesday at the latest to review them before presentation

Conclusions/action items:

The designs are coming along nicely. Continue to narrow down focus of each design for presentation. Upload all deliverable before next Friday and send slides to Dr. Puccinelli by Wednesday. Look into current patent designs for magnetic filtration devices and determine how we can get a leg up on these design groups.

2016_9_14- Initial Client Questions

▪ James Jorgensen ▪ Sep 14, 2016 @03:44 PM CDT

Title: Initial Client Questions

Date: 9/14/16

Content by: James

Present: All

Goals: Create a list of questions related to our design project for our client (Tim Kwa) to give us direction in our research.

Content:

What are the locally available tests already in use (price, availability, etc)?

Where can we get the tests available there for comparison?

What illnesses are the most prominent for testing?

Specific disease in mind for device to identify?

What are the prototyping facilities available in Jimma for use in creating a prototype?

Conclusions/action items:

Email Tim this list of questions in order to get an understanding of what our design should be. When he responds, and even before, dive into the literature available online to familiarize ourselves with microfluidics, as well as the diseases in the area that need to be considered.

2015_9_28 - Preliminary Brainstorming

▪ Hunter Johnson ▪ Oct 19, 2016 @02:01 PM CDT

Title: Preliminary brain storming

Date: 9/28/16

Content by: James

Present: All

Goals: Brainstorm aspects of our design that we would like to incorporate into the prototype

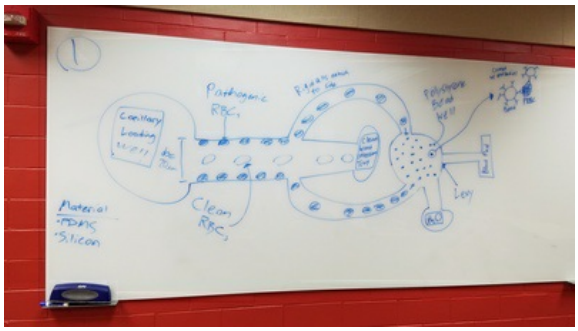
Content:

For preliminary design ideas see attached Images

Conclusions/action items:

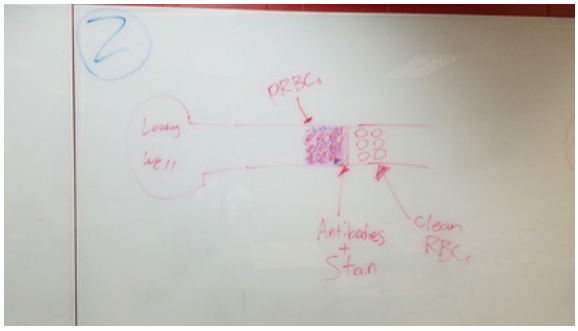
Discuss these possible brainstormed designs with our advisor, begin working on a design matrix.

▪ Hunter Johnson ▪ Oct 19, 2016 @02:01 PM CDT



20160928_184746.jpg(1.6 MB) - download Image 1 (Design 1): This design utilizes a loading well that the blood sample can be placed into. After loading into the microfluidic chamber, capillary action draws the sample through a channel. One aspect of blood infected with malaria is that the red blood cells become more rigid. This increased rigidity makes the infected red blood cells more likely to be drawn closer to the sides of the channel. On either end of the channel, secondary channels would collect the infected red blood cells and pool them in a chamber, while the clean red blood cells would continue forward into a separate chamber. The chamber containing the infected cells would also contain polystyrene beads coated with antibodies that would become activated when introduced to water. The red blood cells would form a conglomerate if infected and visual detection could be used to gauge if the disease is present or not. A release lever could also be used in order to help the cells be flushed from the chamber, as they would still be attached to the antibodies on the polystyrene beads. More research on the properties of infected red blood cells, antibodies to use, and polystyrene beads would need to be done in order to determine the feasibility of this design.

▪ Hunter Johnson ▪ Oct 19, 2016 @02:01 PM CDT

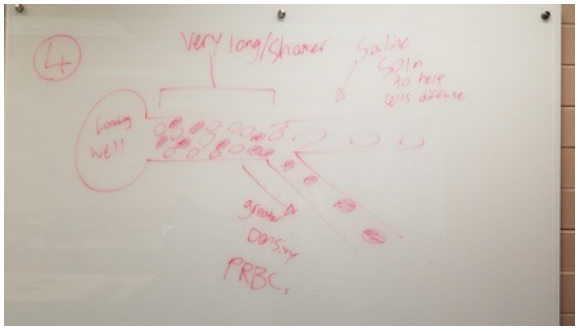


20160928_184758.jpg(1.4 MB) - download Image 2 (Design 2):this design utilizes a loading well for the blood sample to be placed into. After loading into the microfluidic chamber, capillary action draws the sample down a channel to a portion where antibodies have been adhered to the inside of the channel. These antibodies will bind to any infected red blood cells and stop them from continuing on down the channel. A stain could be utilized in this area to help indicate the presence of any infected red blood cells. More research on the type of antibody to use and whether or not blood will be able to continue to flow past this point if a conglomerate of infected red blood cells are blocking the path.

▪ Hunter Johnson ▪ Oct 19, 2016 @02:01 PM CDT

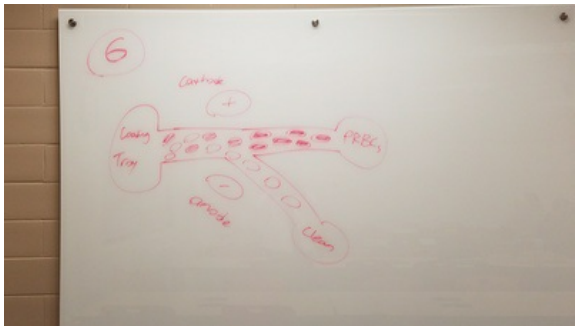
20160928_184807.jpg(1.4 MB) - download Image 3 (Design 3): This design utilizes a loading well for the blood sample to be placed into. After loading into the microfluidic chamber, capillary action would draw the sample through a channel that has a magnet placed along side of it. Infected red blood cells are known to have a greater iron content than regular red blood cells, and thus are more susceptible to be influenced by magnets. The infected red blood cells would hypothetically be repelled more strongly by the magnet and be pushed into a separate channel leading to a chamber. The regular red blood cells would continue to flow into a collecting chamber of its own kind. Testing using iron deficient animal blood and regular blood could be implemented to test the feasibility of this design. More research on the iron composition of infected red blood cells would need to be done to determine the feasibility of this design.

▪ Hunter Johnson ▪ Oct 19, 2016 @02:01 PM CDT



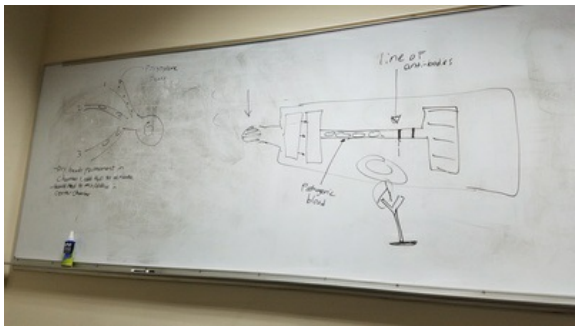
20160928_184816.jpg(1.5 MB) - download Image 4 (Design 4): This design utilizes a loading well for the blood sample to be placed into. After loading into the microfluidic chamber, capillary action would draw the sample through a rather long channel. This entire channel would be placed on some sort of shaker in order to expedite the process of more dense parasitic red blood cells to settle to the bottom layer, while the less dense regular blood cells would remain on the top layer inside the channel. A saline solution of some sort could also be added in order to help the cells diffuse more easily and settle. An indicator could be placed in the well parasitic red blood cells were expected to accumulate in to help identify if the sample has been infected. More research on the densities and length of channel would need to be done to move forward with this design.

▪ Hunter Johnson ▪ Oct 19, 2016 @02:01 PM CDT



20160928_184839.jpg(1.3 MB) - download Image 5 (Design 6): This design incorporates a loading well for the blood sample to be placed into. After the loading into the microfluidic chamber, capillary action would draw the sample through a channel with a anode and cathode on either side. Red blood cells infected with malaria are known to have a different electrical charge than standard red blood cells. The infected red blood cells hypothetically would be attracted to the positive charge while the regular red blood cells would be repelled into a separate channel. An indicator would be placed into the collecting well at the opposite end of the channel in order to determine if the blood does indeed contain the malaria parasite. More research into the feasibility of using electrical charges would need to be done in order to progress with this design.

▪ Hunter Johnson ▪ Oct 19, 2016 @02:01 PM CDT



20160928_175131_1_.jpg(1.8 MB) - download Image 6: This was our initial thought process for two of our designs drawn out (See images 1 and 2 above for more specific details)

2016_9_16 - Project Description and Research

▪ James Jorgensen ▪ Oct 19, 2016 @10:40 AM CDT

Title: Team Meeting 1

Date: 9/16/16

Content by: James

Present: All

Goals: Come up with research topics for the next week in order to help define our project, prepare for work on PDS.

Content:

- Each team mate searched the literature for information on microfluidic diagnostic devices and point of care (POC) devices already in use
- This information was recorded individually in each person's lab notebook for future use in prototype designing and meeting with our adviser and communicating with our client
- Work on the PDS will be done early next week
- Establish weekly meeting times on Monday nights and Friday afternoons before meeting with adviser

Conclusions/action items:

- Each team member will search literature for POC, and report their findings in their lab notebooks for our next team meeting.
- Begin work on PDS

2016_9_19 - Product Design Specification Work

▪ James Jorgensen ▪ Sep 19, 2016 @08:41 PM CDT

Title: Team Meeting 2 (PDS)

Date: 9/19/16

Content by: James

Present: All

Goals: Discuss research that was done over the weekend as well as begin work on the PDS

Content:

- The email we received from our client, Tim, over the weekend was very helpful in terms of giving us direction for our design, including:
  - Focusing mostly on Malaria
  - Many current lab tests take days to weeks to return results
  - No current fabrication facilities in Jimma, but a PDMS microfluidics foundry could be established
  - Accuracy of device should be >95%
  - Time to result should be less than 1 hour
  - Device ideally battery powered
  - Device should be rather small
  - Could hypothetically charge up to $5 per test
- Research done over the weekend can be seen in the individual folders of each team mate
- Work on the PDS can be seen in the attachment
- Josh was looking into the current patent designs and patents of microfluidic diagnostic devices

Conclusions/action items:

Turn in the PDS to the BME website.

Continue to do research on microfluidics in general, as well as already existing patents and devices, information on materials (such as PDMS), etc.

Set up a skype meeting with Dr. Kwa after more research has been done

2016_9_28 - Brainstorming

▪ Hunter Johnson ▪ Oct 19, 2016 @02:04 PM CDT

Title: Preliminary Design Brainstorming

Date: 9/28/2016

Content by: Hunter

Present: Team

Goals: Brainstorm and develop possible designs

Content:

- Reference brainstorming section to see results

Conclusions/action items:

- **Begin to research each preliminary idea**

2016_10_5 - Design Matrix Criteria

▪ James Jorgensen ▪ Oct 05, 2016 @07:44 PM CDT

Title: Team Meeting 4 (Design matrix and deliverables planning)

Date: 10/5/16

Content by: James

Present: All

Goals: Create categories and weighting for design matrix, begin planning steps in the next two weeks for completing deliverables (paper and presentation)

Content:

- Design Matrix Potential Categories and weighting
  - cost (4) - <$5 - 10%
  - sensitivity/specificity (1) - >95% - 25%
  - user friendly (3) - 20%
  - time to result (4) - <1 hour - 10%
  - Point of care feasibility (2) - 20%
  - ease of fabrication (4) - 10%
  - ~~testable before delivery~~
  - ~~fake positive rate~~
  - versatility (species of parasite as well as other diseases) (5) - 5%
- Separation Ideas
  - Cell Deformation Model
  - Magnetism Model
  - Electricity Model
- Detection
  - Antibody on Paper
  - Polystyrene beads coated with antibodies

- Gold Nanoparticles

- Category Descriptions
  - **Sensitivity/Specificity > 95% (25%):**

    Separation: The device should effectively separate 80% of infected RBCs into separate channels and should allow minimal passage of any uninfected RBCs into these channels.

    Detection: The detection method should be 95% effective in labeling the concentrated portals of infected RBCs. It would ideally detect all strains of malaria.

    Equipment Free / Portable / Ease of Detection (20%):

    Separation: The method of separation should be able to be transported long distances and be provided in a device smaller than a personal computer. Preference would be for the device to not need power.

    Detection: The detection method should be able to use the naked eye or a cheap and easy to use device in order to obtain an accurate reading of the state of the disease.

    User Friendly (20%):

    Separation: A small amount of sample and easy to handle reagents are necessary in the resource limited regions where this device would be implemented. Collection of sample should be safe for both the patient and the technician. Also should not require extensive training to be able to run many tests.

    Detection: The form of detection should be able to be monitored on site by a modestly trained technician, without the use of high tech laboratory equipment. A simple protocol for the type of detection would be provided.

    Ease of Fabrication (10%):

    Due to resource restrictions in Jimma at Jimma University, as well as the developing world in general, the microfluidic device protocol for fabrication should be relatively straightforward and not require high tech resources to produce. The design also needs to be able to be mass produced in order to be an effective POC diagnostic device.

    Cost (10%):

    The projected cost per device once the production is optimized should be less than $5.

    Time < 1 Hour (10%):

    Separation: The amount of time it will take to run the blood sample through the concentration device should be less than 20 minutes.

    Detection: The total time it would take for a patient to be diagnosed needs to be less than one hour.

    Versatility (5%):

    Separation: The ability for the concentration device to separate infected RBCs, specifically the malaria parasite, from healthy RBCs. It would be prefered for the device to be able to separate different strains of malaria or different diseases, but we will focus on the Plasmodium falciparum parasite which is specific to malaria.

    Detection: The ability to detect different strains of malaria parasites or different diseases with minor alterations to our final design would be useful. Patients presenting with mixed symptoms would be able to use the same device to test for multiple diseases.

Conclusions/action items:

Each team member needs to do more research on particular aspects of the possible designs that are shown above in the outline of the design matrix, this is to help further familiarize ourselves with the particular aspects of each design, and will help us rank each category for all of the designs. When research is done and the design matrix is complete, each team member will begin working on slides and portions of the paper that their research pertained to. Everyone should also begin to familiarize themselves with the research that others in the group have been doing.

2016_10_7 - Preliminary Design Development

▪ Hunter Johnson ▪ Oct 07, 2016 @01:42 PM CDT

Title: Preliminary Design Development and Design Matrix Weighting

Date: 10/7/16

Content by: Hunter

Present: Entire Team

Goals:

- Go over completed preliminary designs
- Complete Design Matrix

Content:

- Each team member completed the sketches and design evaluation for each of the designs, including:
  - Separation Preliminary Designs
    - Cell Deformation (Austin)
    - Magnetism (James)
    - Electricity (Josh)
  - Detection Preliminary Designs
    - BinaxNOW (Hunter)
    - Au LFIA (Hunter)
    - Polystyrene Beads (Zach)
- Evaluation of design criteria for each can be found in the design specification folder for each team member
- After collaboration, the design matrix was completed, and can be seen below

Design	*Separation*						*Detection*					
Criteria (weight)	Cell Deformation		Magnetic Separation		Electric Separation		BinaxNOW		PS Beads		GNPs	
Sensitivity (25)	3	15	5	25	4	20	5	25	4	20	5	25
Equipment Free/Usable in Field/Intuitive (20)	5	20	4	16	3	12	3	12	4	16	5	20
User-friendly (20)	5	20	5	20	3	12	4	16	4	16	5	20
Time (10)	2	4	4	8	4	8	4	8	5	10	4	8
Cost (10)	5	10	4	8	3	6	1	2	5	10	3	6
Ease of Fabrication (10)	4	8	4	8	3	6	5	10	3	6	2	4
Versatility (type of Malaria or other diseases) (5)	2	2	2	2	2	2	3	3	1	1	5	5
Total	79		87		66		76		79		88	

▪ Hunter Johnson ▪ Oct 07, 2016 @01:16 PM CDT

Conclusions/action items:

Divide and work on preliminary design report and presentation, as well as updating each notebook

2016_10_10 - Preliminary Presentation Work

▪ James Jorgensen ▪ Oct 10, 2016 @11:45 PM CDT

Title: Preliminary Presentation Work

Date: 10/10/16

Content by: Hunter, Austin, James, Zach

Present: Hunter, Austin, James, Zach

Goals: Begin work on preliminary presentation

Content:

- Hunter: Completed work on these slides: Malaria prevalence and pathology, BinaxNOW, and gold nanoparticles
- Zach: Completed work on these slides: Current diagnostic methods, polystyrene beads, and design matrix
- James: Completed work on these slides: Problem statement, Magnetic Detection method, and overview slides
- Austin: Completed work on these slides: Cell deformation detecction method, future work

Conclusions/action items:

Have Josh complete the following slides: PDS, electrical detection method. When the presentation is complete, send a sample into Dr. Puccinelli in order to review before preliminary presentation. Upload presentation to Labarchives as well as team website.

2016_10_17 - Preliminary Report Writing

▪ James Jorgensen ▪ Oct 18, 2016 @06:10 PM CDT

Title: Preliminary Report Writing

Date: 10/17/16

Content by: James

Present: All

Goals: Break up the preliminary report into sections and complete them individually before compiling into document

Content:

- Hunter - Completed work on the introduction and background sections
- Austin - Completed work on the preliminary design section
- James - Completed work on the preliminary methods/materials/testing sections, as well as compiling the final document
- Zach - Completed work on the preliminary design evaluation section
- Josh - Completed work on the discussion section
- See attached for work completed on this date

Conclusions/action items:

Finish revising the document, and add reference before submitting

2016_10_18 - Deliverables Work

▪ Hunter Johnson ▪ Oct 19, 2016 @10:37 AM CDT

Title: Preliminary Design Report and Notebooks

Date: 10/18/2016

Content by: Hunter Johnson

Present: All

Goals:

- Work to finish preliminary design intervals

Content:

- Met and worked on the preliminary design report as well as helped each other with Lab Archives updates

Conclusions/action items:

- Send preliminary deliverables to client and advisor

2016_9_21: Problem Research

▪ Hunter Johnson ▪ Sep 23, 2016 @01:01 AM CDT

Title: Design problem motivation and specification research

Date: 9/21/2016

Content by: Hunter

Present: Individual research

Goals:

- Find resources detailing disease diagnostic problems and motivations
- Find current shortcomings in the field and overarching goals
- Specify design criteria based on need and resources

Content:

Title: Requirements for high impact diagnostics in the developing world

Source: Urdea, M., et al. *Nature Diagnostics* 2006

Notes:

- Infection diseases continue to plague the developing world as there is a basic lack of diagnostic tests to identify these devastating diseases.
- Many harmful diseases are treatable, but they continue to spread due to lack of timely diagnosis
- A 90% effective malaria diagnostic test without the use of a laboratory could save approximately 2.2 million lives and prevent 447 million unnecessary treatments per year.
- For tests to be successful in developing countries they need to be accurately and easy to administer without the use of laboratory infrastructure.
- Most developing countries have a lack of clean water, dependable electricity, cold storage, and personal, so many of the current diagnostic tests do not work in these conditions.
- Clean water, through purification, is attainable through filtration devices, electricity can be subsidized though batteries or solar power, and the inability to keep solutions cold require immediate testing and reagents that are extremely thermo-stable.
- Our client is operating under the assumption of minimal laboratory infrastructure, as there is rural clinics around the area.
    - Thus reliable electricity and filter water may be an issue, as well as untrained personal, room temperature fluctuations, as well as timing.
        - Hopefully less than 1 hour
- With the current requirements, Malaria sample types include blood (finger prick), urine, or saliva. A possible screen could use parasite antigens for diagnosis (further research)

Conclusions/action items:

- Use this article to help define PDS
- Further research biology of Malaria, current diagnostic devices/procedures, and microfluidics as diagnostic tools

2016_9_22: Malaria Biology and Diagnosis

▪ Hunter Johnson ▪ Sep 27, 2016 @08:24 PM CDT

Title: Pathobiology of Malaria

Date: 9/24/16

Content by: Hunter Johnson

Present: Individual research

Goals:

- Understand the basic biology of Malaria, how it is transmitted, how it affects the host, current diagnostic methods and how it is treated.

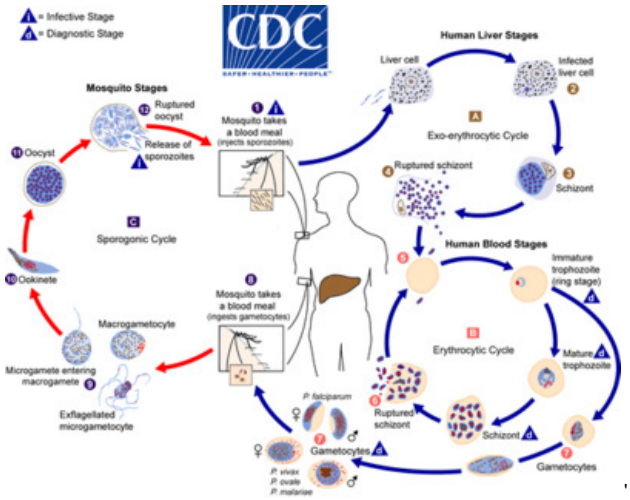
Content:

Source: https://www.cdc.gov/malaria/about/biology/index.html

Notes:

- Malaria is caused by a parasite that is transmitted to humans from a certain type of mosquito
- Symptoms include high fevers, shaking chills, and flu-like illness
- Malaria can be fatal, but death is usually preventable if diagnosed early
- Biology
  - The parasite usually infects *Anopheles* mosquitos first, which then transmit it to humans, or can be spread from human to human by blood contamination, usually thorough mosquitos also.
  - In humans, once infected the parasite attaches and multiplies within red blood cells, destroying them to release more newly synthesized parasites to continue the process
  - Blood stage parasites usually are what cause the symptoms of the disease.
- Malaria life cycle
  - The malaria parasite involves two stages in different hosts. In mosquitos it goes through the sporogonic cycle where it matures inside the salvary glands until the mosquito inoculates the sporozoites into the human host. From there the sporozoites infect the liver cell and mature into schizonts, which rupture and release merozoites into the blood stream. The parasite then undergoes asexual proliferation in the erythrocytes, as the merozoites continue to infect red blood cells, which then are responsible for the clinical manifestations of the disease.
  - The figure below summarizes this dual host lifecycle, adapted from the CDC.

▪ Hunter Johnson ▪ Sep 27, 2016 @08:25 PM CDT



malaria_lifecycle.gif(43.5 KB) - download Malaria life cycle. Explanation above

▪ Hunter Johnson ▪ Sep 27, 2016 @08:31 PM CDT

- Malaria is usually found around the tropical and subtropical areas where the specific mosquitos can survive and multiply, allowing the parasite to complete its growth cycle or incubation period.
- The highest transmitions areas are in the central parts of Africa, south of the Sahara desert.
- In the US Malaria is quickly diagnosed and treated.

Conclusions/action items:

- Research diagnostic approaches for malaria and current microfluidic based diagnostic devices

2016_9_24: General POC Devices

Title: General POC Devices Research

Date: 9/22/2016

Content by: Me

Present: Individual Research

Goals:

- Understand what Point of Care (POC) devices are.
- Understand limitations and advantages of POC devices
- Find current general methods used

Content:

Title: Point of care testing: The impact of nanotechnology

Source: Syedmoradi, L, et al. *Biosensors and Bioelectronics* 2016, 87 (2017) 373-387

Notes:

- The development of portable, reusable, and effective microplatforms for disease testing is what encompasses point of care systems.
- Provides near patient settings possibly without the use of clinics.
- Gives timely diagnostic information
- Affordable, sensitive, specific, user-friendly, rapid/robust, equipment free, and delivered to greatest need (ASSURED)
- We will focus on small hand held devices, instead of larger bench top ones.
- Types of POC devices
    - Strip-based POC assay: capillary flow tests
        - Dipsticks
            - paper based device, detect presence of simple compounds
        - Lateral flow tests
            - capillary flow platform. Liquid samples move along a solid paper-based membrane.
            - Add different components at different times and wait to see presence of analyte in specimen
            - Can use immunoassays in this test
- More on Lateral Flow tests:
    - Components can include samle pad, conjugate pad, reagent storage, loading, reaction, detection, and others
    - Label: ideally should contain amenable characteristics, useful in dynamic ranges, have low or no non-specific binding, and stable under routine storage.
    - Read out of test should be done by the naked eye
    - Paper materials more useful in our case, as they are inexpensive, requires no additional force beyond capillary control , and can be used for novel detection.
    - Use this paper for specifics in paper based POC device fabrication
        - Talks about specific molecules to use at each site.
- Printed Electrode based POC assay
    - Inexpensive way for rapid, low cost, on-site, real time analysis of a clinical biomarker.
    - Used for electrochemical reactions
    - Can modify surfaces and circuit construction, such as self assembled monolayers
        - conjugate various peptides and surface antigens for specific uses.
    - Usually used to integrate POC to technology as a biosensor
- Nanomaterial-based POC assay
    - Nano materials include carbon nanotubes, graphene, and metal nanoparicles.
    - Nanomaterials can be used as carriers to load signal markers, or ans signal reporters themselves.
    - Can alter size, shape, composition, construction, and other parameters
    - Can be used to improve chemical stability, electrical proerties, and surface to volume ratio
    - Very early stage in disease diagnosis
    - An example is the use of gold nano particles (GNP) to augment visable color intensity.
        - Can be used as labels in LFAs
        - Simple and inexpensive production and modification.
        - Coat with macromolecules for enzyme based signal enhancement.
        - Can also be sued in electrode printing an biosensor application with POC devics
    - Magnetic nanoparticles
        - Used in magnetic fluids, with biosensors
- Challenges with POC devices
    - Preventing non-specific adsorption
    - Integration of automation of technology
    - Standardization of practices and users

Conclusions/action items:

- **Further research how to diagnose malaria, and think about how to integrate diagnostic process to POC deviece method listed above.**

2016_10_7 - Current Diagnostic Techniques

▪ Hunter Johnson ▪ Oct 18, 2016 @06:03 PM CDT

Title: Current Malaria Diagnostic Methods

Date: 10/7/2016

Content by: Hunter Johnson

Present: Individual Research

Goals:

- Investigate current diagnostic methods used today
- Research existing products or patents regarding malaria diagnosis

Content:

Title: Advances in microfluidics in combating infectious diseases

Source: Biotechnology Advances 34 (2016) 404-421

Notes:

- Main method used for Malaria diagnosis is a blood smear using a Giemsa stain, which stains nuclei blue.
- Red blood cells do not have nuclei, so the stain only target the parasite nuclei and make small spots visible withing the blood cell, leading the technician to conclude that cell has the malaria parasite and the individual is then diagnosed with the disease.
- Though this is most robust and essentially the gold standard of diagnosis, the method requires a microscope and a trained technician, so it really is not feasible for the developing world or point of care testing.

Conclusions/action items:

Look into specific devices used to diagnose malaria

2016_10_7- BinaxNOW Malaria Detection

▪ Hunter Johnson ▪ Oct 07, 2016 @10:13 AM CDT

Title: BinaxNOW current Malaria detection device

Date: 10/7/16

Content by: Hunter Johnson

Present: Individual Research

Goals:

- Research and define current microfluidic devices used for POC malaria detection.

Content:

- Main device for POC microfluidic diagnosis of malaria is BinaxNOW Malaria made by alere
- It is an immunochromatographic assay for the qualitative detection of *Plasmodium* antigens in infected human red blood cels.
- It targest histidine-rich protein 2 (HRP2) andtigen specific to *Plasmodium falciparum* and a pan-malaria antigen found in all four malaria species.
- However, negative results must be confirmed by thin/thick smear, it is just meant for rapid detection of malaria.
- Benefits
    - Rapid
        - 15 minutes
    - Easy to use
        - only 1 reagent
        - small blood sample by finger prick
        - no need for skilled microscopist
    - Easy to interpret
        - qualitative results
        - easy to read
    - Sensitivity
        - Sensitivity for P. falciparum : 99.7%
        - Specificity for P. falciparum : 94.2%*
        - Sensitivity for P. vivax : 93.5%
        - Specificity for P. vivax : 99.8%
        - Needs parasite levels > 5,000 parasites/uL
- http://www.alere.com/en/home/product-details/binaxnow-malaria.html

▪ Hunter Johnson ▪ Oct 07, 2016 @10:13 AM CDT

Binax_NOW.jpg(29.9 KB) - download

▪ Hunter Johnson ▪ Oct 18, 2016 @06:35 PM CDT

Negatives

- 25 tests cost $809.92

Conclusions/action items:

- Though the device is extremely selective and a functional POC device, the main pitfall is the the high parasite/uL required volume as well as the limiting ability to only tell *P. faliciparium* specifically. Our design should focus on reducing its own cost, or possibly improving the BinaxNOW device, to increase demand, and lower the price of this already tested product
- For the future, look up exact mechanism used and current patent/patent laws

2016_10_8 - Rapid Diagnostic Tests

▪ Hunter Johnson ▪ Oct 18, 2016 @08:00 PM CDT

Title: Current Products: Rapid Diagnostic Tests (RDTs)

Date: 10/8/2016

Content by: Hunter Johnson, adapted from Zach Hite

Present: Individual Research

Goals:

- Research RDTs as a malaria detection device
- Research current patents that exist with RDTs

Content:

Source: A. Tay et al. / Biotechnology Advances 34 (2016) 404–421

Notes:

There are quite a few malaria diagnostic devices on the market today, for both rapid detection (RDTs) and definitive diagnosis, with varying levels of specificity, cost, training, and requirements.

Devices that currently exist:

- Amplino - portable PCR device
- NanoMal - finger prick device
- Sanitoets Home Testing Kit
- SD Bioline Malaria - dip stick
- ParaHIT ®
- First Response® Malaria
- CareStartTM Malaria HRP2/pLDH (Pf)

Many of these devices follow the same pattern: They either require advanced technician training and laboratory infrastructure or, for the field devices, can only detect malaria, not diagnose it. This means they are able to tell an individual that they may have malaria, but the individual must reaffirm these tests with a blood smear. All negative results seem to be unreliable, and the devices are designed just to detect malaria early on, which they seem to do pretty effectively. The other major obstacle is all of them are very expensive, deterring from their universal use.

Conclusions/action items:

There are current devices out there, but universal shortcomings still exist. To overcome this, our device be designed to be extremely efficient, ideally having the ability to diagnose malaria without a second referral. It should be able to detect all four strands of malaria, to actually diagnose the disease, rather than just telling the individual if they have it or not. Finally, the cost of the device needs to be low, <$5, to actually be used universally and make a difference.

2016_9_27: Microfluidics as Diagnostic Tools Research

▪ Hunter Johnson ▪ Sep 27, 2016 @07:22 PM CDT

Title: Microfluidic devices and combating infectious disease research

Date: 9/24/2016

Content by: Me

Present: Individual Research

Goals:

- Understand how microfluidic devices are being used today
- Learn general uses of microfluidic devices as they relate to infectious diseases
- Begin to look at Malaria and microfluidic devices specifically
- Start to generate possible design ideas, focusing on fabrication method and how it relates to our PDS

Content:

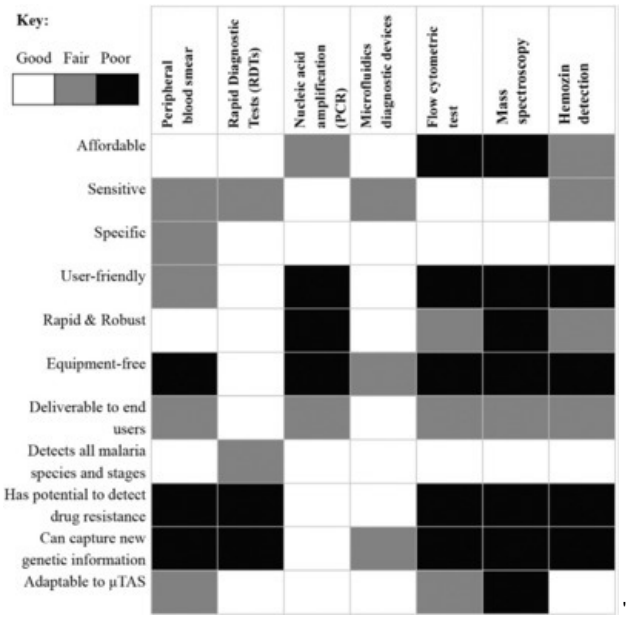
Title: Advances in microfluidics in combating infectious diseases

Source: Tay, A., et al. *Biotechnology Advances* 2016, 34: 404-421

Notes:

- Development of a low-cost, intuitive, and accurate technology is a main focus of science and engineering today.
- The microfluidics field has been growing in recent years and a prominent method for point of care diagnostic devices regarding infectious diseases.
- Microfluidics is the science or technology that deals with systems that manipulate small amounts of fluids, requiring low sample volume, high throughput design and increased accuracy.
    - It has been used in biosensors, analysis systems, sample mixing, and timely readout systems.
- The World Health Organization (WHO) recommends diagnostic devices for developing countries be
    - affordable, sensitive, specific, user-friendly, rapid, equipment-free, and deliverable to end-users (mentioned before: ASSURED).
- Malaria pathobiology:
    - The infection is caused by *Plasmodium* protozoa transmitted through an intermediate host such as the *Anopheles* mosquito.
    - This infection affects red blood cells and their ability to circulate throughout the body.
    - 600,000 people continue to die due to this disease, primarily due to late diagnosis.
    - The best method to diagnose malaria is through the microscope method, using a blood smear to visualize the protozoa.
        - Though the method itself is cheap, it requires a microscope and trained technician.
        - Also, bacteria and other impurities can be misinterpreted.
    - Rapid Diagnostic Tests are also an essential tool used today, which relies on antibody antigen reaction.
        - Requires 5-15 uL of blood with monoclonal antibodies specific to the target parasite antigen
            - Issues with this include chemical inactivation due to warm and humid conditions
            - Targets the HRP-2 receptor
        - There are also more than one type of malaria parasites that exist
    - If the parasite level is too low for other detection methods, PCR has been used but this requires many reagents, laboratory infrastructure and machinery, and trained personal.
    - Hemozin detection has also been used to diagnose malaria. This is essentially a functional test, as the protein is converted from the malaria protozoa, though to detect this degradation product of hemoglobin you need dark field microscopy, unrealistic for developing countries.
    - Symptom based malaria detection is not reliable as malaria share many clinical symptoms of many other common diseases in poverty stricken areas.
    - The figure below summarizes the advantages and disadvantages of each malaria diagnostic method aforementioned:

▪ Hunter Johnson ▪ Sep 27, 2016 @07:25 PM CDT



Malara_Diagnostic_Methods_Matrix.png(177.2 KB) - download Evaluation of the efficacy of different malaria diagnostic tools used today

- From the design matrix above, adapted from Gascoyne et al 2014, Rapid Diagnostic Tests and Microfluidic diagnostic devices seem to be the most realistic for developing areas, so we will focus on these two methods for future research.

Microfluidics diagnostic tools

- Microfluidic methods can be used to direct diagnosis, as there are processes that allow the separation of interfering blood cells and the ability to concentrate malaria parasites, to enhance diagnostic potential.
- Cell deformation has been shown as a preliminary step to concentrate malaria affected blood cells
    - Normally, red blood cells need to be deformable to travel through capillaries, which are often times smaller than the resting RBC diameter
    - iRBCs infected with the malaria parasite become less deformable as the disease matures
        - This is due to the parasites being non-deformable, which increases the internal viscosity of RBCs.
        - This is also due to the parasites causing oxidative stress on the host cell.
    - Hou et al. was able to make a microfluidic platform to somewhat separate and concentrate the non deformable infected blood cells from healthy cells.
    - This method, using channels, pushes the infected RBCs to the outside of the channels where they can be collected.
        - Refer to Hou, H.W., Bhagat, A.A.S., Chong, A.G.L., Mao, P., Tan, K.S.W., Han, J., et al., 2010. Deformability based cell margination—a simple microfluidic design for malaria infected erythrocyte separation. Lab Chip 10, 2605–2613. for specific fabrication/protocol
    - This is not always the most accurate method, as the most sever cases of Malaria may not exhibit this change in deformational ability.
- Electrical signature can also be use in microfluidic devices to concentrate the iRBCs.
    - electrical conductivity of iRBCs is significantly higher than healthy RBCs, so using gradients to force cell movement may be a way to isolate iRBCs, demonstrated by the Gascoyne et al 2004.
    - the issue with this method is it requires a stable form of electricity and it is very size dependent.
- Molecular analysis in microfluidic devices provides another outlet for malaria diagnosis.
    - microfluidic PCR is a new, more feasible form of PCR for diagnostic purposes.
    - There are many microPCR devices on the market, but none really have focused on malaria
    - The process of PCR is similar to microPCR, requiring cell lysates, primers and other reagents coupled with a heating and cooling cycle to magnify DNA in the sample. Specific Malaria diagnostic priers could be used.
    - A combination of methods should be used for this purpose
    - *Research more into microPCR products*
- Optics still provides the most tested and used method to diagnose malaria
    - Blood smears are still the most traditional way to diagnose malaria, though this requires use of a microscope, which as already been deemed to be unfeasible.
    - Other light detection methods may be useful though, including absorbance and chemiluminescence.
        - Malaria parasite enzyme activity has been used as a detection method, using the DNA cleavage-ligation event.
        - Hou, H.W., Bhagat, A.A.S., Chong, A.G.L., Mao, P., Tan, K.S.W., Han, J., et al., 2010. Deformability based cell margination—a simple microfluidic design for malariainfected erythrocyte separation. Lab Chip 10, 2605–2613.

Conclusions/action items:

- Research more into the actual biology of Malaria, and vital functions of the parasite.
- Find other detection methods, such as visual illumination, or other useful protocols for use in developing countries to possibly combine with these separation/purification methods.

2016_9_28: Lateral Flow POC Devices

Title: Microfluidic lateral flow POC diagnostic devices

Date: 9/28/16

Content by: Hunter Johnson

Present: Individual Research

Goals:

- Understand the general principles and uses of lateral flow POC devices
- Try to apply this method to malaria based devices

Content:

Title: Point-of-Care Diagnostic in Low Resource Settings: Present Status and Future Role of Microfluidics

Source: Sharma, S., et al. *Biosensors* 2015, 5: 577-601

Notes:

- Developing countries account for around 60% of the world population, 90% of the global disease burden, and only 12% of global health expenditure, creating a need for cheap, reliable POC testing devices for rural and low resource areas
- Lateral flow devices are one of the most common POC testing platforms used today
- Lateral flow immunoassays (LFIA) is among the most rapid and simplistic device platforms developed.
- Some attractive qualities for use in developing areas:
    - stability for more than one year
    - precise performance
    - interpretation by minimally trained users
    - versatility
    - no refrigeration required during shipping and storage
- Some current LFIA tools on the market for malaria
    - Binax NOW, made by Alere, uses the antigen *Plasmodium Ag*, requiring 15 uL of whole blood, taking 15 minutes, and is extremely sensitive and specific
- Principles of Lateral Flow Immunoassay
    - Uses nitrocellulose, polymer, paper, or other composite substrate membrane to separate the analytes of interest
    - Capilary to transport fluid using capillary action
    - Once loaded, the blood interacts with a pre-loaded antibody on the strip and migrates the strip, until the cell binds and immobilizes at that location. This location can be seen by the eye if enough cells are immobilized at that location. Control is present with non-specific antibody.
    - Thus the performance is contingent upon the biorecognition of the element on the cell. Antibodies are the best for this application.
    - Material choices
        - Sample application pad
            - Cellulose, glass fibre, rayon or cross-linked silica. Allows continually uniform migration of the sample to the conjugate pad.
        - Conjugate cap
            - travels from sample pad to this where it mixes with dried conjugate, telling shelf life of the test
        - Membrane
            - Usually a hydrophobic nitrocellulose as it has a high affinity to protein binding (requires blocking)
        - Capture line
            - specific antigen or antibodies immobilized to capture the target analyte and control line after
        - Absorbent Pad
            - high-density cellulose. Absorb the sample and doesn't allow bactrackage. Wicks up.
        - Backing for membrane
            - Everything mounted on to this material. Typically polystyrene or another type of plastics
    - Detection methods
        - Common detection methods are latex beads, colloidal carbon, colloidal gold, fluorescent tags, enzymes, streptavidin, or gold nanoparticles, conjugated with the antibody.
        - Latex beads combined with colored dyes are commercially available and can be conjugated. Different colors and couples can allow for multiple analysis.
        - Gold nanoparticles have strong optical properties, can enhance sensitivity. The intensity is higher than latex beads
    - Detection system
        - Visual detection is the most common method, especially for our purpose.
- Limitations of LFIA
    - The simplicity of this design limits it performance and in many cases is not as accurate. Regarding malaria, LFIA haven't really been promoted universally, as they are relatively new .
    - Restrictions in the limit of detection of these platforms also may be an issue.

Conclusions/action items:

Further research detection methods, the LFIA

2016_10_5: Gold Nanopartice LFIAs

▪ Hunter Johnson ▪ Oct 18, 2016 @05:41 PM CDT

Title: Gold Nanoparticles Background

Date: 10/5/16

Content by: Hunter

Present: Individual Research

Goals:

- Develop background regarding gold nanoparticles as a malaria detection method
- Explain the preliminary design from brainstorming session
- Evaluate feasibility of design, attain enough information to accurately determine design matrix

Content:

Background on gold nanoparticles

Title: Point of care testing: The impact of nanotechnology

Source: Biosensors and Bioelectronics 87 (2017) 373-387

Notes:

- Gold nanoparticles (GNPs) are by far the most preferred nanomaterial used in labeling, as it provides enhancement and various advantages over molecular reagents.
- They have excellent biocompatibility, good stability, and easy to functionalize.
- GNPs have optical properties (i.e. surface plasmon resonance and Raman scattering).
- Synthesized by colloidal or liquid chemical synthesis through the reduction of chloroauric acid solution in water, to generate Au0
- Spherical properties manifest as an intense visible color that changes upon dispersion/aggregation.
    - Used in colormetric assays
- Can be used to generate a strong visible signal in LFAs.
- High stability in dried form
    - Stability in liquid form is good, but can aggregate after several days
- Can be combined with silver ions to increase color as well.
- This method can improve detection sensitivity 1000x fold.
- In POC tests, used at a 2 step procedure, step 1 - 10 nm particle with primary antibody, step 2 - 40 nm particle with secondary antibody.

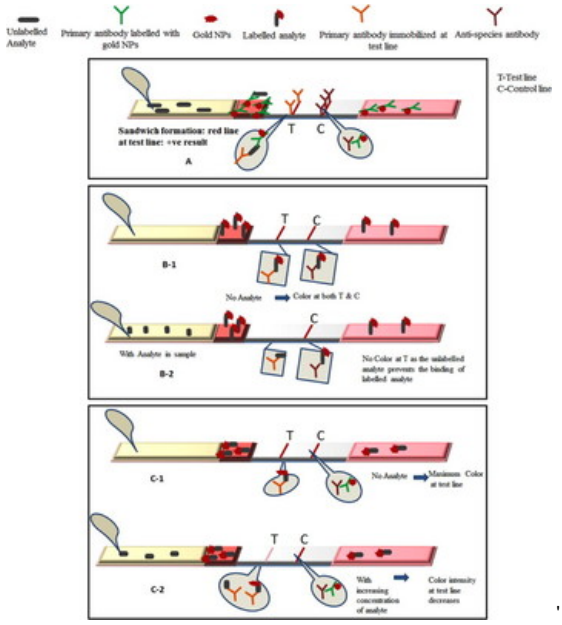
Title: Evaluation of gold nanoparticle based lateral flow assays for diagnosis of enterobacteriaceae members in food and water

Source: *Food Chemistry* (2015) 170: 470-483

Notes:

- Gold nanoparticles have been used in current LFIA assays in the Food chemistry industry to diagnose enterobacteriasceae members in food and water.
- Thus the concept is there it just needs to be adapted to uses in malaria detection.
- An example of AuNP based LFIA in the food industry is show below.

▪ Hunter Johnson ▪ Oct 07, 2016 @12:00 PM CDT



AuNPs.jpg(91.4 KB) - download

▪ Hunter Johnson ▪ Oct 07, 2016 @12:00 PM CDT

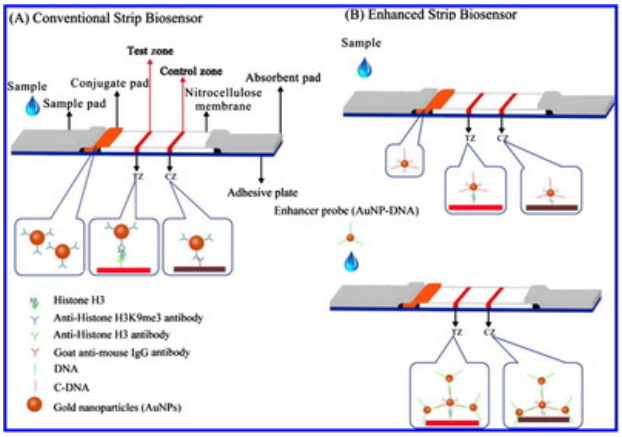
Title: Nanomaterial-enhanced paper-based biosensors

Source: *TrAc Trends in Analytical Chemistry*2014 58:31-39

Notes:

- Advantages of using colorimetric assay with AuNPs
  - Very fast and single signal reporter
  - The experimental result can be visible

▪ Hunter Johnson ▪ Oct 07, 2016 @12:00 PM CDT



GNP.jpg(87.8 KB) - download

▪ Hunter Johnson ▪ Oct 07, 2016 @12:05 PM CDT

Conclusions

Develop AuNP LFIA design

2016_10_12 - Cell Deformation Design

▪ Hunter Johnson ▪ Oct 18, 2016 @06:43 PM CDT

Title: Cell Deformation Design

Date: 10/12/2016

Content by: Hunter Johnson, adapted from Austin Feeney

Present: Individual

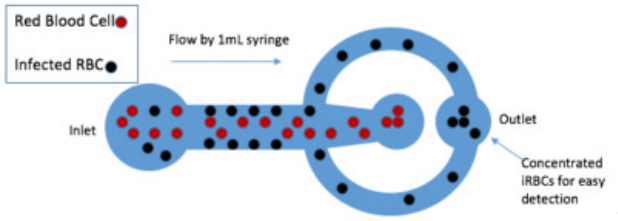
Goals:

- Develop and define cell deformation design

Content:

This separation design is based on changes in cell deformability caused to the host RBC upon infection with the malarial parasite. Parasitic proteins are secreted within the iRBC to make its membrane more adhesive as well as stiffer. The increased stiffness along with a reduction in the surface area to volume ratio contributes to a significant decrease in cell deformability especially in late stage iRBCs. Normal RBCs, however, remain highly deformable. It has been demonstrated that the stiffer iRBCs can separate towards the sidewalls of a long straight channel microfluidic device The diagram below illustrates the preliminary cell deformability design. It has an inlet, where a blood sample is loaded via a syringe. The blood cells then flow down the 3-centimeter-long channel channel (15um wide), which forces the iRBCs to the sidewalls. Finally, the iRBCs can be concentrated in separate outlet from the RBCs by the use of smaller channels on the outside walls. The innermost outlet would thus mostly contain RBCs, and the outer would contain mostly iRBCs.

▪ Hunter Johnson ▪ Oct 18, 2016 @06:44 PM CDT



Cell_deformation_design.tif(36.6 KB) - download

▪ Hunter Johnson ▪ Oct 18, 2016 @07:08 PM CDT

Pros of the design

- There are no additional equipment requirements when testing
- Easily testable on our end, to show proof of concept, using polystyrene beads, or something similar that is not deformable

Cons:

- Less effective in separating early stages of iRBCs
- Requires 40% blood hematocrit (reasonable, but can be high in some situations)

Conclusions/action items:

Evaluate this design according to the design matrix

2016_10_12 - Magnetic Separation Design

▪ Hunter Johnson ▪ Oct 18, 2016 @07:12 PM CDT

Title: Magnetic Separation Design

Date: 10/12/2016

Content by: Hunter Johnson, adapted from James Jorgensen

Present: Individual

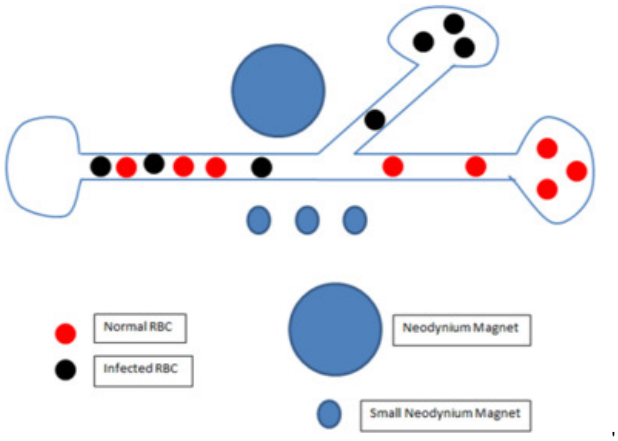
Goals:

- Explain Magnetic Separation Design

Content:

This separation design is based on changes in magnetic properties caused to the host RBC upon infection with the malarial parasite. When parasites infect and feed on RBCs they release iron, which is converted into hemazoin. Hemazoin is a weakly paramagnetic crystallite, which is produced during all life stages by all four strains of the disease. It interferes with the magnetic spins of hydrogen atoms, which align when exposed to a powerful magnetic field. However, hemazoin production in iRBCs causes iRBCs and RBCs to have distinguishable magnetic properties, which can be utilized to separate infected cells from uninfected cells in a highly specific manner in microfluidic device. Shown below is a preliminary design for magnetic separation of iRBCs involving neodynium magnets.

▪ Hunter Johnson ▪ Oct 18, 2016 @07:19 PM CDT



Magnetic_Design.png(50.6 KB) - download

▪ Hunter Johnson ▪ Oct 18, 2016 @07:22 PM CDT

Pros:

- Hemozoin produced at all life stages of malaria
- fast separation
- separation possible with minimal amounts of iRBCs

Cons:

- Complicated magnetic field calculations to determine correct placement of magnets
- Requires extra fabrication and equipment during testing

Conclusions/Action Items

- Evaluate Design according to design matrix

2016_10_12 - Electrical Separation Design

▪ Hunter Johnson ▪ Oct 18, 2016 @07:24 PM CDT

Title: Electrical Separation Design

Date: 10/12/2016

Content by: Hunter Johnson, adapted from Josh Liberko

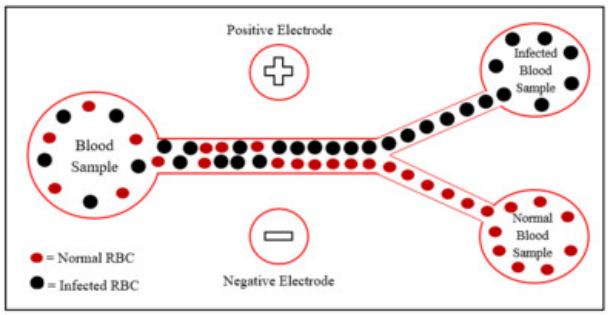
Present: Individual

Goals: Explain Electrical Separation Design

Content:

This separation design is based on changes in electrical properties caused to the host RBC upon infection with the malarial parasite. The electrical conductivity of iRBCs is significantly higher than normal RBCs, which allows for electrical separation of these cells. Dielectrophoretic (DEP) forces are non-uniform electric fields that can be created by cells when subject to direct or alternating current. Different DEP properties of iRBCs and RBCs contributes to their unique conductivities. This can be utilized to separate iRBCs and RBCs in a microfluidic device. The diagram below illustrates the concept of electrical stimulation in a microfluidic device for the function of separating RBCs.

▪ Hunter Johnson ▪ Oct 18, 2016 @07:24 PM CDT



Electrical_Design.png(40.3 KB) - download

▪ Hunter Johnson ▪ Oct 18, 2016 @07:26 PM CDT

Pros:

- High specificity for iRBCs
- iRBCs very sensitive to positive charge

Cons:

- Electrical difficulties for POC
- High cost due to batteries

Conclusions/Action Items

- **Evaluate Design according to design matrix evaluation**

2016_10_5 - Gold Nanoparticle Design

▪ Hunter Johnson ▪ Oct 07, 2016 @12:03 PM CDT

Title: Gold Nanoparticle Preliminary Design

Date: 10/5/16

Content by: Hunter

Present: Individual Research

Goals:

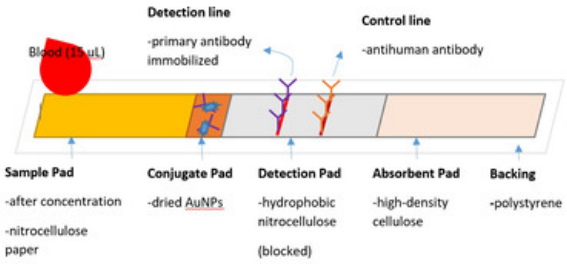
- Develop the gold nanoparticles (AuNP) lateral flow immunoassay (LFIA) design as a malaria detection method
- Explain the preliminary design from brainstorming session
- Evaluate feasibility of design, attain enough information to accurately determine design matrix

Content:

Our Design:

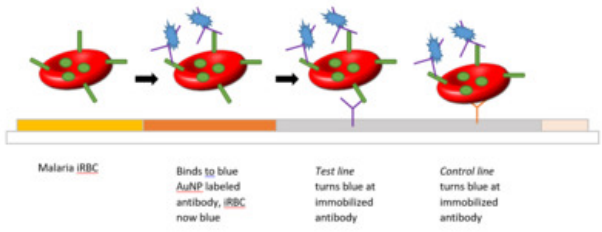
- This LFIA AuNP design would adapt the current design of lateral flow immunoassays to fit the malaria diagnosis model, utilizing gold nanoparticles.
- The primary antibody would either be histidine-rich protein 2 (HRP2), found in the *P. falciparum* malaria parasite, coupled with pan-malaria antigen, which targets all four species.
    - These antibodies are used in the current BinaxNOW malaria test with:
    - § Sensitivity for P. falciparum : 99.7%
    - § Specificity for P. falciparum : 94.2%*
    - § Sensitivity for P. vivax : 93.5%
    - § Specificity for P. vivax : 99.8%
    - This test currently needs levels of >5,000 parasites/uL
- Another antibody that could be used is *P. falciparum* Heat Showck Protein 70 (*Pf*Hsp70) antigen or *Plasmodium Ag* antigen.
- Based on current LFIA AuNP devices in the food and water chemistry field, as well as BinaxNOW, this device could theoretically
    - Only need 15 uL whole blood
    - Take 15 minutes to deliver results
    - Be extremely sentitive, as well as specific
    - Cost as low as $1 a test, when mass produced
- Diagram of design shown below

▪ James Jorgensen ▪ Oct 19, 2016 @10:43 AM CDT



AuNP_LFIA_Design_Diagram.PNG(160.5 KB) - download

▪ Hunter Johnson ▪ Oct 07, 2016 @11:41 AM CDT



Mechanism_of_AuNP_LFIA_Design.PNG(82.2 KB) - download

▪ Hunter Johnson ▪ Oct 07, 2016 @11:59 AM CDT

Design Matrix Criteria Evaluation AuNP LFIA Design

- Sensitivity
  - Depending on the antibody, this design has the potential to be extremely sensitive
- Equipment Free
  - Once crafted, there is no equipment necessary
- Userfriendly
  - Extremely user friendly, as there is only a need for a finger prick
- Time
  - This could take longer, depending on how long the concentration method takes, as there should be 5000 parasites per microliter
- Cost
  - Unknown at this point, current AuNP LFIAs are being used in the food and water chemistry industry, with very low costs
- Ease of fabrication
  - Low, as we would have to start from scratch, essentially
- Verstility
  - Very high, as this has the potential to determine every type of malaria.

Conclusions

Inform team of this design, and weight it against the other detection designs in a design matrix. Try to find more information on cost and time on the device.

2016_10_10 - BinaxNOW Improvement Design

▪ Hunter Johnson ▪ Oct 18, 2016 @07:52 PM CDT

Title: BinaxNOW improvement design

Date: 10/10/16

Content by: Hunter Johnson

Present: Individual

Goals: Explain BinaxNOW improvement design

Content:

BinaxNOW is a currently available diagnostic tool for malaria, reference the "BinaxNOW" section in competing designs. Its major pitfall is the necessity of 5,000 parasites/uL in blood samples as well as an extremely high and unfeasible cost. The separation techniques described before would act in concentrating the parasites for easier and more successful detection. This assay takes 15 minutes to run. When combining BinaxNOW with one of the separation techniques, this tool could become more successful in diagnosing malaria.

Thus the design idea is to pair the selected concentration technique with BinaxNOW, allowing the test to diagnose malaria earlier and more robustly. This would hopefully increase the relevance and demand for the product and subsequently decrease the cost of the assay.

Pros:

- The assay is already tested, rapid (15 min), requires a small blood volume, easy to interpret, and extremely senstitive

Cons:

- Price is still the main concern

Conclusions/action items:

- Evaluate design according to the design matrix

2016_10_12 - Polystyrene Bead Design

▪ Hunter Johnson ▪ Oct 18, 2016 @07:56 PM CDT

Title: Polystyrene Bead Design

Date: 10/12/2016

Content by: Hunter Johnson (adapted from Zach Hite)

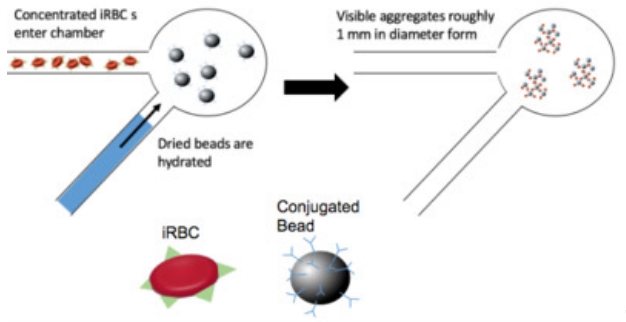
Present: Individual

Goals: Explain Polystyrene Bead Design

Content:

This method of malaria detection is based on an antigen-antibody interaction whereby polystyrene beads are conjugated with an antibody and interact with specific iRBC antigens. The beads and iRBCs would then form a visible aggregate in the well in a process known as immunoagglutination. The dried beads could be could be coated in the well and rehydrated upon use. The aggregation would occur within 2 minutes and would be visible without a microscope, visualized by the figure below

▪ Hunter Johnson ▪ Oct 18, 2016 @07:57 PM CDT



Polystyrene_beads.tif(82.5 KB) - download

▪ Hunter Johnson ▪ Oct 18, 2016 @08:02 PM CDT

Pros:

- Two minute rapid diagnosis
- Requires only 2 uL of whole blood

Cons:

- Testing for multiple strains would be cumbersome, requiring separate beads and wells for each
- only about 80% specific

Conclusions/action items:

- Evaluate design according to design matrix criteria

2016_10_12 - Design Matrix Criteria

▪ Hunter Johnson ▪ Oct 18, 2016 @08:20 PM CDT

Title: Design Matrix Criteria

Date: 10/12/2016

Content by: Hunter Johnson

Present: Entire Team

Goals: Establish and explain criteria used in the design matrix criteria

Content:

Design matrix criteria decided upon by team with explanations:

- **Sensitivity/Specificity > 95% (25%):**
  - *Separation:* The device should effectively separate 80% of infected RBCs into separate channels and should allow minimal passage of any uninfected RBCs into these channels.
  - *Detection:* The detection method should be 95% effective in labeling the concentrated portals of infected RBCs. It would ideally detect all strains of malaria.

- **Equipment Free / Portable / Ease of Detection (20%):**
  - *Separation:* The method of separation should be able to be transported long distances and be provided in a device smaller than a personal computer. Preference would be for the device to not need power.
  - *Detection:* The detection method should be able to use the naked eye or a cheap and easy to use device in order to obtain an accurate reading of the state of the disease.

- **User Friendly (20%):**
  - *Separation:* A small amount of sample and easy to handle reagents are necessary in the resource limited regions where this device would be implemented. Collection of sample should be safe for both the patient and the technician. Also should not require extensive training to be able to run many tests.
  - *Detection:* The form of detection should be able to be monitored on site by a modestly trained technician, without the use of high tech laboratory equipment. A simple protocol for the type of detection would be provided.

- **Ease of Fabrication (10%):**
  - Due to resource restrictions in Jimma at Jimma University, as well as the developing world in general, the microfluidic device protocol for fabrication should be relatively straightforward and not require high tech resources to produce. The design also needs to be able to be mass produced in order to be an effective POC diagnostic device.

- **Cost (10%):**
  - The projected cost per device once the production is optimized should be less than $5.

- **Time < 1 Hour (10%):**
  - *Separation:* The amount of time it will take to run the blood sample through the concentration device should be less than 20 minutes.
  - *Detection:* The total time it would take for a patient to be diagnosed needs to be less than one hour.

- **Versatility (5%):**
  - *Separation:* The ability for the concentration device to separate infected RBCs, specifically the malaria parasite, from healthy RBCs. It would be prefered for the device to be able to separate different strains of malaria or different diseases, but we will focus on the Plasmodium falciparum parasite which is specific to malaria.
  - *Detection:* The ability to detect different strains of malaria parasites or different diseases with minor alterations to our final design would be useful. Patients presenting with mixed symptoms would be able to use the same device to test for multiple diseases.

Conclusions/action items:

Evaluate each design according to these criteria

2016_10_12 - Design Matrix and Evaluation

▪ Hunter Johnson ▪ Oct 18, 2016 @08:22 PM CDT

Title: Design Matrix Evaluation

Date: 10/12/2016

Content by: Hunter

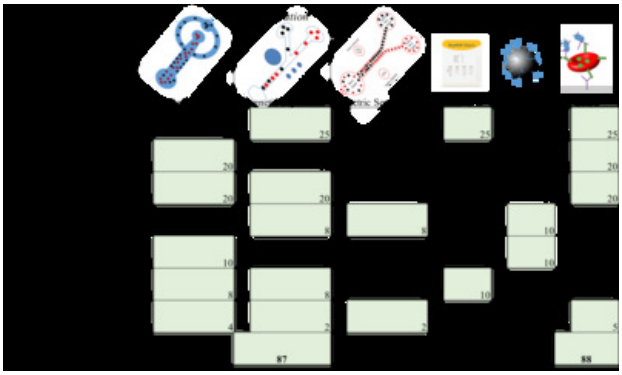
Present: Completed by all

Goals: Evaluate each design according to the design matrix and choose a final separation and detection design.

Content:

Design matrix evaluation below

▪ Hunter Johnson ▪ Oct 18, 2016 @08:23 PM CDT



Design_Matrix.png(213.2 KB) - download

▪ Hunter Johnson ▪ Oct 18, 2016 @08:41 PM CDT

Design matrix explanations

For the separation techniques the magnetic separation method scored highest because the magnetic properties of cells are specific to the diseased state. Research shows that the cell deformation method would separate any cells with different surface properties than uninfected red blood cells, where diseases such as diabetes would interfere with results.The cell deformation design scored highest in ease of use and fabrication as it would consist solely of PDMS that could be manufactured in bulk off site and transported in, where the electric separation method scored lowest because they would require batteries and a base with electrodes to be transported to the clinic. To be user friendly the method must be able to concentrate cells without training, the electric separation method doesn't pass this test because tuning the electrodes for optimal separation would take constant supervision. Cell deformation scored the lowest for time because the small channels require a low flow rate of liquid that is not as fast as capillary action in the other designs. Lastly for ease of fabrication the device must be able to be fabricated and assembled on site, the magnetic separation only had one downfall which was figuring out the placement of the magnets for optimal cell separation, where the cell deformation design needed a very thin channel to be fabricated out of PDMS which can pose problems. The best separation method was deemed to be the magnetic design, it did the best in four out of six of the criteria and second best in the other two.

The detection methods were evaluated with the same criteria as the separation techniques. Regarding sensitivity, BinaxNOW is the top performer, with over 99% accuracy. Gold nanoparticles have the possibility to be just as sensitive, if they use the same antibodies, so they received the same rating. They also won in ease of use and user-friendliness as it can be read in 15 min by looking for colored bands. The polystyrene bead design is the top performer in time and cost because the test takes two minutes to finish and is made from the cheapest and easiest to assemble materials (except for the preassembled BinaxNOW). The major drawback of adapting BinaxNOW to the microfluidic device is the cost of about $40 per test. The gold nanoparticles were the chosen detection method, as they have the highest versatility to detect for all four strains of malaria on one strip test. One challenge of this design is the complexity of fabrication, this will be simplified if a printer can be converted to lay antibodies on nitrocellulose.

Conclusions/Action Items

Develop final design

2016_10_16 - Final Design Idea

▪ Hunter Johnson ▪ Oct 18, 2016 @09:17 PM CDT

Title: Final Design chosen

Date: 10/16/2016

Content by: Hunter Johnson

Present: Individual

Goals: State final design, as determined by the design matrix

Content:

The proposed final design would consist of two connected designs: the magnetic separation portion, and the gold nanoparticle detection portion. The separation portion of the design utilizes a polydimethylsiloxane (PDMS) channel and loading/separation wells cast from a master mold shaped with the proper dimensions for the microfluidic channel (master mold could be any generic material), a large neodymium magnet capable of producing strong magnetic fields and multiple other smaller neodymium magnets or ferromagnetic wires capable of orienting the magnetic field in the proper manner for separation of iRBCs from RBCs. Immediately attached to the iRBCs collection well, there would be the detection portion of the design. The detection portion of the design utilizes lateral flow immunoassay (LFIA) and gold nanoparticles (AuNPs). Separated blood containing iRBCs would be placed on a nitrocellulose paper, which would be attached to a pad containing dried AuNPs conjugated with malaria antigen detecting primary antibodies (Multiple strains). Multiple lines of primary antibodies that are antigen specific to differing malarial antigens would be printed into separate lines following the conjugate pad. An absorbent pad and backing made of high density cellulose will follow for excess runoff. Many more details regarding the specifics of materials to purchase need to be defined by the design team in order to move forward with these plans.

Conclusions/action items:

Begin fabrication research and prototyping

2016 Malaria and POC Paper

▪ Zachary Hite ▪ Oct 19, 2016 @03:23 AM CDT

Title:

Malaria and POC Device Recent Advancements

Date:

10/18/2016

Content by:

Zach Hite

Goals:

Record the current ideas out there for POC devices that can detect malaria

Content:

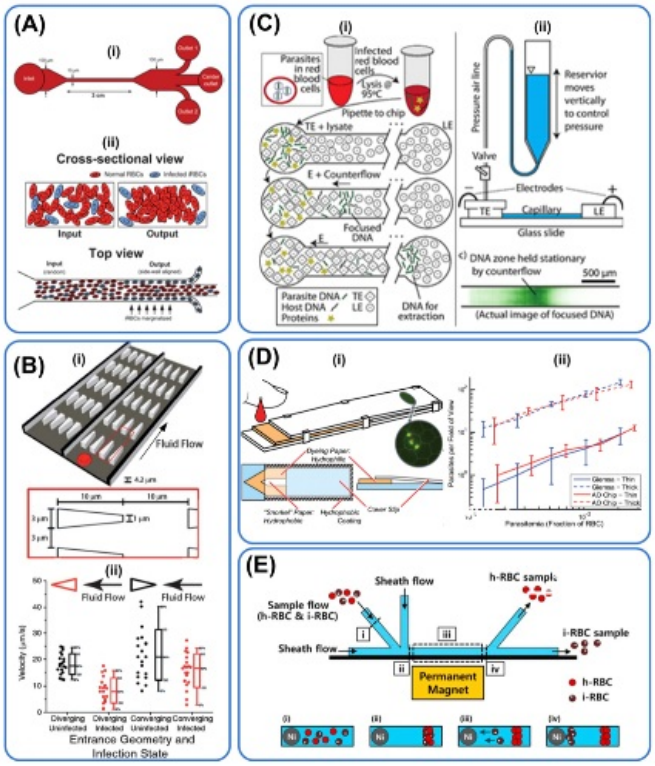
Journal: www.elsevier.com/biotechadv

Paper Title: Advances in Microfluidics in Combating Infectious Diseases

- In order to replace the gold standard blood smear test a device needs to be quick, accurate, cheap, easy to run, and deliverable in its final form
- These requirements lead to many detection methods that require lab equipment but one way to circumvent to common shortcomings is to make a microfluidic device
    - These devices take very little sample to diagnose
    - The reagents can be fully contained within the device
    - Inside of device can be sterilized and brought out into the field without contamination
    - Easy disposal
    - Faster kinetics lead to quick readings
- Currently used are rapid diagnostic tests (RDTs) which are great except for the very low accuracy
    - This leads to misdiagnosis, treating a healthy person can lead to disease strengthening
    - Multiple strains of the parasite that require different treatments to be fully effective cannot be distinguished with accuracy
    - Tests are not thermal-stable or resistant to humidity which leads to degradation
- Symptom based diagnosis is also common in rural communities
    - This is a poor method due to the similarities between malarial infection and other common infectious diseases in the area
    - Anything that causes a fever (influenza, dengue fever) can be misdiagnosed
    - at certain hospitals, 40% of patients leave with a malaria diagnosis

- There are three separation techniques mentioned in this paper that are especially exciting for our project
    - Separation allows for more of the infected red blood cells (iRBCs) to be concentrated for a more specific detection
    - Concentration will lead to tests that can detect parasites at much lower than the current 5,000 parasites/uL
- Cell Deformation (Figures A and B)
    - The iRBCs are less deformable than their healthy counterparts due to oxidation of cell membrane components and a lower surface area to volume ratio
    - The cells are filled with many new proteins and structures necessary for the proliferation of the parasite
    - Figure A depicts a way of taking advantage of the changes in the cell membrane composition
        - The cells that are less deformable are pushed towards the edge and are eventually collected in the top and bottom chambers as the uninfected cells will be directed down the middle of the channel.
    - Figure B depicts a method of forcing cells through small openings to concentrate the iRBCs to the edge of the device again.
        - This is not optimal because PDMS is not easily made into rigid precise structures like this and silicon is not a material used in Ethiopia due to the high cost and the need for a clean room.
- Magnetic Separation
    - Infected cells are consumed for energy and one of the most abundant structures in red blood cells is the hemoglobin complex
    - This complex coordinates an iron 2+ atom in the center to help binding of oxygen, and once degraded the iron is exposed to the cytoplasm where it oxidizes to iron 3+. This form is more paramagnetic than the 2+ atom.
    - In Figure E it is shown that depending on the orientation of a set of magnets you could attract/repel iRBCs into a special chamber
        - This would work well as a mold could be placed on top of the magnets and the test would run by capillary action doing to completion by itself
- Electric Separation
    - This method does not have a diagram but is similar to the concept for magnetic separation, the iRBCs have a higher conductivity due to a few factors including membrane permeability and shape.
    - These properties would allow for selective separation of iRBCs, however the drawbacks are that many diseased states have altered RBCs that may interfere with the purification.
    - This is not as specific as the paramagnetic properties of the cells.
    - This would also require more equipment and power to run, the optimal current and frequency would need to be derived with samples and tuned at the test site.

Conclusions/action items:



Malaria Biology

▪ Zachary Hite ▪ Oct 19, 2016 @04:50 AM CDT

Title:

Biology and Physiology Research of Malaria (General)

Date:

10/18/16

Content by:

Zach Hite

Goals:

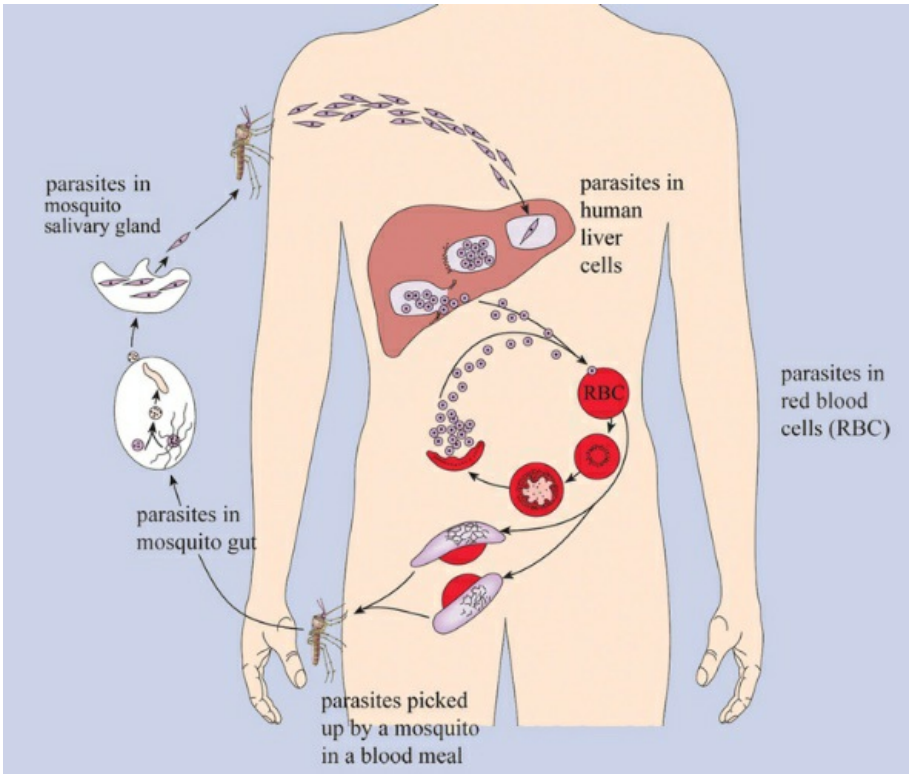
Learn about how malaria effects the body for possible uses in detection, and how the malarial parasite life cycle involves humans

Content:

General Malarial Biology

- The cycle starts when an infectious mosquito injects Plasmodium parasites from its salivary gland into the blood stream.
- These immature parasites migrate to the liver where they multiply rapidly and grow.
    - They also have the option of staying in the liver tissue dormant for up to years at a time, this makes treatment hard as the disease usually comes in waves.
- Once ready the parasitized blood cells will rupture releasing many parasites from each cell.
- These parasites are now ready to infect red blood cells
- This is the mature stage of the life cycle and is ready to be:
    - Infecting red blood cells and digesting the components especially hemoglobin, producing heme (toxic byproduct)
    - Or taken back up by mosquitos
- If taken back up by mosquitos the parasite will reproduce and move back to the salivary gland where it can infect another human.

- The most dangerous part of this cycle is when the parasite is digesting the red blood cells
- At times the waves of rupture are synced up and every 24-48 hours there will be a rush of cell failure.
    - These events lead to a lot of vascular issues
        - The cell membranes are sort of "sticky" and will grab on to the cells lining capillaries causing blockage.
        - This can get really bad in the liver and pancreas.
        - Anemia is a common side effect
    - If this goes on long enough a person may not be treatable.
        - This is a reason why our diagnostic device should be able to detect malaria before people show up to the hospital because of consequences from the disease



Conclusions/action items:

Keep reading up on the physiology in humans of malarial infection to see if there are any other useful biomarkers of the disease!

Title:

Rapid POC Isothermal Amplification Assay for Detection of Malaria

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4721682/

Date:

9/19/2016

Content by:

Zach Hite

Present:

N/A

Goals:

Learn about a new method published about a method to amplify the Malarial nucleic acids without the steps required for PCR.

Content:

This method doesn't need the equipment or time

- Strands of DNA are able to self separate
- Gene replicated are for the cytochrome oxidase subunit 1, this is unique to*Plasmodium falciparum* (hard to distinguish from *P. vivax*)
- 5 min @ 90 degrees C to lyse cells and denature
- 30 min @ 65 degrees C to complete reaction
- 1000x dilution in PBS was the best to minimize fluorescent quenching by blood proteins
    - They were able to use pure blood without purifying for nucleic acids before analysis because of the dilution
- Detection level was 1.5 parasites/uL

Saliva Test also included

Conclusions/action items:

This method seems useful but the temperature and cost are worries. Not sure of the price for the primers and the storage of the primers.

Very Specific. Time is on the right scale. Very low detection level.

Market Research

Title:

Market Research on POC device specifications and pricing

Date:

9/19/2016

Content by:

Zach Hite

Present:

N/A

Goals:

Learn more about the current market to inform our later brainstorming and problem design statement

Content:

Amplino - portable PCR device

- Pros
  - Cheaper than normal PCR
  - Detects very low levels of parasite DNA
- Cons
  - Time it takes to run test is very long
  - Lab equipment

NanoMal

- Pros
  - Diagnosis 10-15 min
  - Can identify specific strain to provide treatment options
  - Touchscreen + battery make it easy to operate
  - Finger prick test
- Cons
  - up to $20 per test
  - Initial device will cost > $500

Sanitoets Home Test Kit

- Pros
  - Can be ran by anyone at home
  - Easy to read results
  - Thermostable
- Cons
  - $126 for two tests
  - Accuracy is unknown

Other Cheap and Popular Devices and Manufacturers

- SD Bioline Malaria
- ParaHIT ®
- First Response® Malaria
- CareStartTM Malaria HRP2/pLDH (Pf)

From A. Tay et al. / Biotechnology Advances 34 (2016) 404–421

- These devices usually run around $0.55 to $1.50 per test
- Quality control does not exist because there are not standards followed by the manufacturers

Conclusions/action items:

This list will give us something to talk about because there is a lot of room in the market if we can in fact make a device that is as cheap and accurate as our design coordinator thinks is possible. Research in this area is being pursued from many directions but one that seems to be exciting is microfluidics. The companies all seemed to have a base device to be able to read the result of the test with a disposable chip that the reaction takes place in.

Currently available RDT's

▪ Zachary Hite ▪ Oct 19, 2016 @03:58 AM CDT

Title:

Research on currently available/used POC devices.

Date:

10/7/16

Content by:
Zach Hite

Present:

N/A

Goals:

Learn about our number one competitor in implementing our device. Also to see what flaws we can specifically focus on improving to make our design better than what is used.

Content:

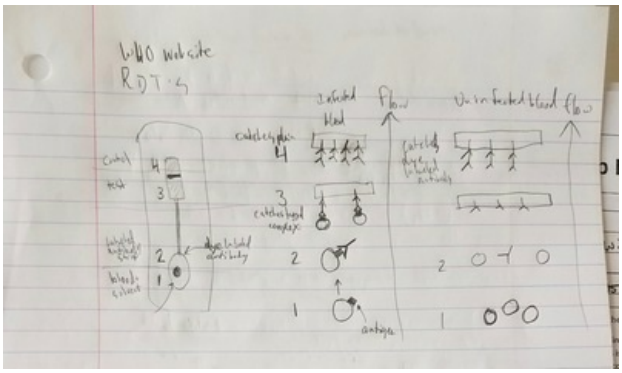
Source WHO : http://www.who.int/malaria/areas/diagnosis/rapid-diagnostic-tests/about-rdt/en/

A picture of the process is attached below.

- Materials
  - Bottom layer is nitrocellulose or other cellulose polymer that works through capillary action
  - Labeled part two is a layer of free antibodies that attach to specific antigens on the surface of the infected red blood cells
    - These antibodies are dyed so detection can be made with the naked eye
  - Line three is an immobilized line of antibodies that binds to the same antigen as the antibody in part two to detect disease
  - Line four is a strip of antibodies that binds the free dye labeled antibodies as a control line

- Logistics
  - First the blood is loaded into a spot on the test strip, this spot is then soaked with a buffer that helps the capillary action of the paper do the work in moving blood up the strip.
  - The blood comes in contact with the line of free antibodies at point two.
  - If the blood is infected then the colored antibodies bind to it, if not the antibodies get washed up the strip with the rest of the buffer and blood
  - Next the blood comes into contact with the test line at three. If the blood is infected, once again the antigen binds, the blood with the labeled antigen is now bound to the strip and this line is visible.
  - Next the left over antibodies and cells come into contact with the control line, any antibodies left will bind to the immobilized line and show up as another visible line.
- A test with two lines is positive and one with one line on the control is a negative result

▪ Zachary Hite ▪ Oct 10, 2016 @02:09 PM CDT



10_1_.jpg(846.3 KB) - download

▪ Zachary Hite ▪ Oct 18, 2016 @10:10 PM CDT

Conclusions/action items:

First Day of Attendance

Title:

BME 550 Lecture 9/20/2016 Notes

Date:

9/20/2016

Content by:

Zach Hite

Present:

550 Lecture Class

Goals:

Learn a little about production of micro devices and talk to Prof. Justin Williams about course specifics and how they apply to our project.

Content:

Prof. Justin Williams will be granting me access to their course webpage which has many resources including journal articles, and the syllabus for the class indicating when relevant information will be covered. I will be reviewing the course page to obtain the relevant information from the first days of class I missed.

Silicon as MEMS Materisl

- Pure
- Multiple crystalline forms
  - Amorphous
  - Polycrystalline
  - Crystal
    - entire solid is one ordered array
- Strong
- Light
- Semiconductor
  - Control of density
    - 100 (etches very quickly / low density)
    - 110
    - 111 (etches 30x slower than 100)
- Photoresistant will not bind water or etch, also will not allow for boron to diffuse through

Etching of silicon

- Etching with a flat bottom will result from no agitation (sedimentation will occur)
- Etching equally (forming semicircles) will result from

- isotropic (using a single density of silicon
- anisotropic (make new structure can look like channels)

Doping silicon

- Boron is used or phosphorus (
  - gas phase,
- Electrical Properties
  - Can become a semiconductor
- Mechanical properties
  - Resistant to etching by acid
  - Etch until you want it to stop.
- Two ways to perform this
  - Diffusion
  - High Velocity
    - bury a profile of boron
    - this allows for etching to a certain point

Conclusions/action items:

9/29/16 PDMS Lecture

Title:

First Lecture on soft lithography in BME 550

Date:

9/29/16

Content by:

Zach Hite

Present:

Lecture of 550

Goals:

Learn about the properties of PDMS, and how we could use these to pattern our device.

Molding with photoresist.

Also gave ideas for how we could detect and diagnose malaria.

Content:

Advantages to soft over silicon in bioMEMS devices

- Cultures
- Etching and materials needed for silicon are harsh and expensive
- Building is easier
- Transparency (TESTING)
- no clean room requirements
- Three Dimensional structures

What properties can we use

- Patterns can be curved
- membranes, valves (deformable surfaces)
- Optically transparent

Soft lithography examples

- Starting point is photolithography
- Microstamping
    - place any material that you want onto the surface of the device
- Microfluidic patterning
    - make a mold, fill with material that will solidify and create pattern

Patterning PDMS (silicone!)

- Photoresist that is patterned
- this will make the master mold. can be used multiple times
- silicone will bind to itself
- you can make masters from your final device of silicone (silane treatment sublimates in vacuum desiccator)
- don't need to do photolithography to make new masters!
- Watch out for aspect ratios

PDMS

- inexpensive
- elastic/soft
- transparent to 300nm
- hydrophobic / but can be made hydrophilic
- can be stuck to itself and other
- can be oxidized or etched
- swells when in contact with solvents (alcohol or acetone)
- insulator
- Gas is able to diffuse through

Conclusions/action items:

Magnetic Separation

▪ Zachary Hite ▪ Oct 19, 2016 @01:32 PM CDT

Title:

Magnetic Separation Design

Date:

10/18/16

Content by:

Zach Hite adapted figure and information from James Jorgensen

Present:

Me and James

Goals:

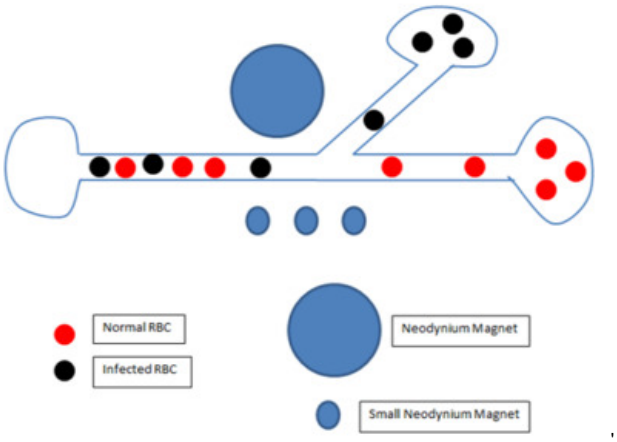
To explain how the magnetic separation method works to see if this design can be used in our final product

Content:

- Magnetic separation works on the premise that when the malaria parasite enters red blood cells they begin digesting hemoglobin which alters the oxidation state of iron bound in heme
- This oxidation makes the iRBCs more paramagnetic so they can be deflected by magnetic fields.
- In theory we could use this to concentrate the infected cells into a separate well

- Pros
  - This is a specific process with no diseases found that effect hemoglobin in this way
  - Once the base magnet array is set up it would be very easy to build the channels of the microfluidic device around it.
  - Reusable base and disposable fluidic devices
  - No power or training required to operate
  - easy to test (paramagnetic microparticles mixed with blood
- Cons
  - Layout of magnets will be challenging, requires extensive knowledge of magnetic field equations
  - Testing required to optimize the process

▪ Zachary Hite ▪ Oct 19, 2016 @01:22 PM CDT



Magnetic_Design.png(50.6 KB) - download

▪ Zachary Hite ▪ Oct 19, 2016 @01:19 PM CDT

Electric Separation

▪ Zachary Hite ▪ Oct 19, 2016 @02:00 PM CDT

Title:

Electric separation design

Date:

10/18/16

Content by:

Zach Hite figures and information adapted from Josh Liberko

Present:

Me and Josh

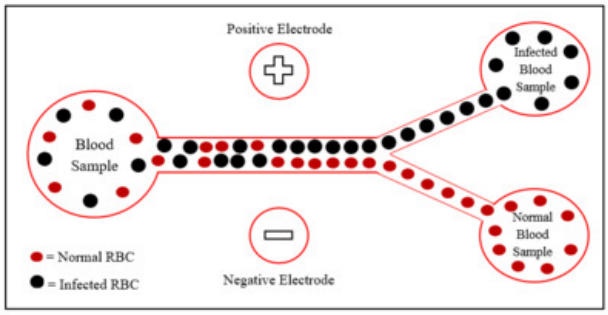
Goals:

Explain how the electric separation method proposed would work and then evaluate this idea

Content:

- The premise behind this design is that infected red blood cells have different membrane properties that change their conductivity. This change in conductivity can be exploited to sort the cells by the forces exerted on them by the current.
- The altered surface is not unique to a Plasmodium infection which could cause error.
- The design would require batteries to run the cathode and anode that would subject the infected cells to force.
- Pros
  - Easy setup, no field equations. Optimization could be found quick.
  - Fast test, capillary action is enough to allow the cells to separate
- Cons
  - Equipment required (batteries, resistors, possibly circuits to tune the current)
  - Not specific to malaria infection
  - Membrane shape can alter results (sickle cell)

▪ Zachary Hite ▪ Oct 19, 2016 @01:46 PM CDT



Electrical_Design.png(40.3 KB) - download

*Cell Deformation

▪ Zachary Hite ▪ Oct 19, 2016 @02:08 PM CDT

Title:

Cell deformation design

Date:

10/18/16

Content by:

Zach Hite figures and information adapted from Austin Feeney

Present:

Me and Austin

Goals:

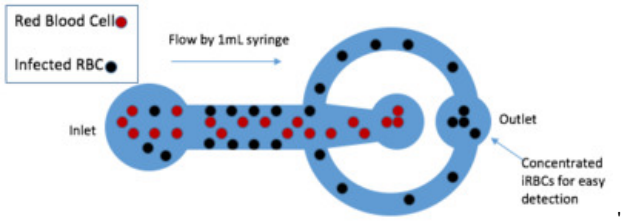
Show the pros and cons of separating infected cells by the principle of deformability

Content:

- This method works on the principle that when red blood cells are infected their surface properties change in a predictable manner.
  - The surface area to volume ratio decreases
  - The membrane hardens due to oxidation of membrane components
- These changes lead to an overall more "sticky" membrane that is less deformable as a whole
- When ran through capillary tubes the infected cells are likely to be pushed to the sides as the more deformable uninfected cells move down the middle of the channel
- If the capillary is long enough eventually you should be able to collect the concentrated infected cells

- Pros
  - Easy to fabricate once the dimensions are figured out
  - Easy and quick to run, very cheap device made solely out of PDMS
  -

▪ Zachary Hite ▪ Oct 19, 2016 @02:02 PM CDT



Cell_deformation_design.tif(36.6 KB) - download

10/7/16 Polystyrene Bead Aggregation

▪ Zachary Hite ▪ Oct 10, 2016 @02:19 PM CDT

Title:

Polystyrene bead design matrix research

Date:

10/7/16

Content by:

Zach Hite

Present:

N/A

Goals:

Find missing information on how possible this detection method is.

Content:

This is the design matrix

1. sensitivity/specificity (1) - >95% - 25%
2. user friendly (3) - 20%
3. Point of care feasibility (2) - 20%
4. time to result (4) - <1 hour - 10%
5. cost (4) - <$5 - 10%
6. ease of fabrication (4) - 10%
7. versatility (species of parasite as well as other diseases) (5) - 5%

This method currently has 1 paper describing the mechanism and methods for testing malarial infection with agglutination readings

1. Detection of Malaria Infection via Latex Agglutination Assay - Polpanich 2007

- Assay takes 2 min to read
- Needs only 2 uL of plasma diluted by 1000x
- Measurement can be qualitative (with the naked eye) or read with a cheap optical microscope
    - This could be integrated with an app for smartphone and optimized
    - Aggregates are roughly 1 mm in diameter
- Specific Measurement of P. falciparum possible
    - Not made to measure for different strains, in theory with multiple chambers one could try
- 350 nm polystyrene beads used optimal
- Non-specific binding of proteins is a problem so the beads need to be hydrophilic to enhance specificity

- Results
- 16 of the 19 cases of Malaria were detected (blood smear to find value of 19)
    - 84% accuracy
- 2 of the 10 patients negative for infection were incorrectly diagnosed
    - 80% specificity
- This would not be good enough for identification (much too many false positives)

Other sources were explored to find missing info for design matrix!

1. From: [Lateral Flow Tests//Bangs Laboratories Inc. pdf]

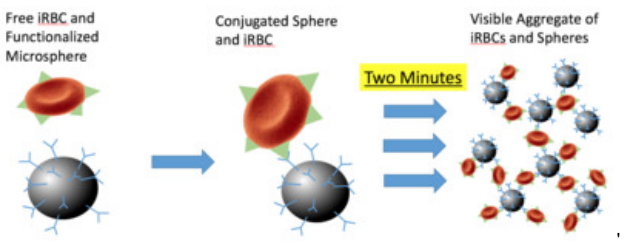
- Dried conjugated polystyrene beads have a shelf life of 1 year!
- Price of test would be < $1 and probably close to $0.20 once optimized

2. From: Polysciences Inc. Microspheres and Particle Handling Guide

http://www.polysciences.com/skin/frontend/default/polysciences/pdf/Microparticles%20Guide.pdf

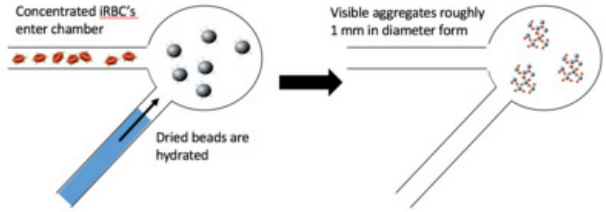
- Need very pure DI water for good shelf life
- Bead density = 1.05 g/ml which is the same as cells and plasma - good mixture

▪ Zachary Hite ▪ Oct 10, 2016 @02:39 PM CDT



designAnimation1.png(521.1 KB) - download

▪ Zachary Hite ▪ Oct 10, 2016 @02:39 PM CDT



designAnimation2.png(134.9 KB) - download

▪ Zachary Hite ▪ Oct 10, 2016 @02:19 PM CDT

Conclusions/action items:

Have all the data necessary for the matrix and because this method has not been strongly researched it may be a hard starting point for us to optimize.

We are also missing data for how detection of P. falciparum would differ from P. vivax, one of the parts of the design matrix.

The fact this can be read with the naked eye in 2 min using only 2 uL of blood is a huge upside!

Gold Nanoparticles

▪ Zachary Hite ▪ Oct 19, 2016 @01:43 PM CDT

Title:

Gold nanoparticle design information

Date:

10/18/16

Content by:

Zach Hite adapted information and figures from Hunter Johnson

Present:

Me and Hunter

Goals:

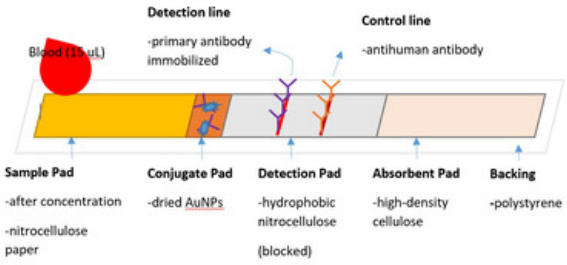
Explain our findings for diagnosis with gold nanoparticle paper test

Content:

- This design is very similar to the current pregnancy test
- The blood flows up a nitrocellulose strip from capillary action into a conjugate pad where gold nanoparticles (AuNP) conjugated with antibodies lie
    - The gold particles can have antibodies for specific proteins on all 4 different strains of malaria
    - The particles can also be different colors by controlling their dimensions
    - This means that we can test for all 4 strains separately on a single platform
- If the antibodies bind to the blood cells this means they are infected, they will then be trapped on a line of immobilized printed antibodies. If a colored line shows up then the corresponding parasite is infecting the patients blood.

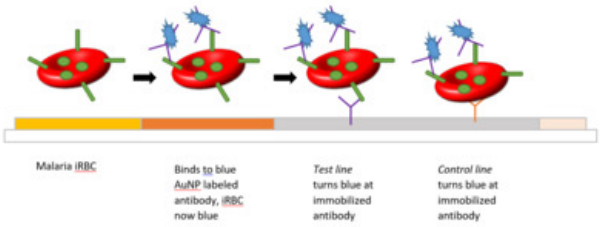
- Pros
    - Small volume of blood
    - Very specific
    - Colors make the test easy to read
    - Versatility of testing for all diseases on the same strip is a huge improvement
- Cons
    - Fast but not amazing (~15 min)
    - The production of this device on a small scale can be challenging due to fabrication restraints
        - printing antibody lines
        - conjugating and placing AuNPs on the strip

▪ Zachary Hite ▪ Oct 19, 2016 @01:34 PM CDT



AuNP_LFIA_Design_Diagram.PNG(160.5 KB) - download

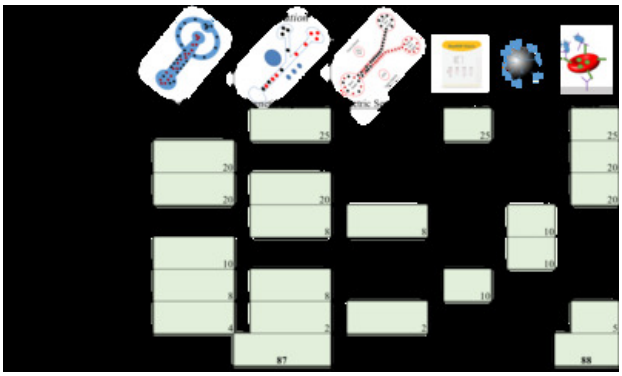
▪ Zachary Hite ▪ Oct 19, 2016 @01:35 PM CDT



Mechanism_of_AuNP_LFIA_Design.PNG(82.2 KB) - download

Design Matrix

▪ Zachary Hite ▪ Oct 19, 2016 @02:01 PM CDT



designMatrix.png(203.4 KB) - download

Meeting 1 : 9/16/16

▪ Zachary Hite ▪ Sep 16, 2016 @12:51 PM CDT

Title:

BSAC Meeting 1

Date:

9/16/16

Content by:

Zach Hite

Present:

BSAC People

Goals:

Share and collect info

Content:

Selection Day

- Took too long to
- Notification Earlier of the details of the draft for 200 students

New Admittance Program

- Freshman (high school) apply to degree program
- They are admitted first if they meet the 3.5
- other engineers get next priority
- then outside the school transfers

Mentoring

- Matched up with a freshman
- Set up date (force their hand)

- Initial meeting with Freshmen Ideas
  - BBQ
  - Film with WUD and food
  - Nerf War

New Website

- Handbook will be interactive (selection and looking at descriptions of all courses)

Electives

- Talk to Freshmen about CS 310 (MatLab) vs 302 (java), bioinstrumentation is the only one that needs 302

Conclusions/action items:

Group should talk to mentees, so that they have good contact at school for any questions and feel open to talking.

9.12.16 Overview Paper on Microfluidic Design Requirements

▪ Austin Feeney ▪ Oct 18, 2016 @06:34 PM CDT

Title: Overview Paper on Microfluidic Design Requirements

Date: 9.12.16

Content by: Austin Feeney

Paper Location: http://www.nature.com/nature/journal/v444/n1s/full/nature05448.html

Present: Austin

Goals: To explore the requirements for high impact diagnostics in the developing world and further understand the scope of our design project.

Content:

High Impact diagnostics:

- There is a necessity for high impact diagnostic tests at low infrastructure sites.
- High infancy death from pneumonia and malaria at these sites.
- HIV and infection and syphilis also contribute to deaths at these sites.
- Additionally, malaria drug resistance has been acquired by the parasite, thus early diagnosis is important.

Characteristics of Diagnostics for the Developing World:

- The design requirements of these tests include:
    - affordable
    - sensitive
    - specific
    - user-friendly
    - rapid
    - equipment free
    - delivered

Conclusions/action items:

A simple test that we could design would ideally require minimal lab equipment, could possibly involve a finger prick for blood chemistry, and could possibly test for multiple diseases in one assay. Additionally, this device would not require electricity or infringe on any patents. Finally, it would be made of recyclable materials and be biodegradable. This paper was useful in laying out design requirements for devices for many different diseases. It will be especially useful to refer to when we find out which disease to design our microfluidic device for. It has laid out our basic product design specifications for many different diseases.

9.23.16 Biology of Malaria

▪ Austin Feeney ▪ Oct 19, 2016 @12:52 AM CDT

Title: Biology of Malaria

Date: 9.23.16

Content by: Austin

Paper Location: https://www.cdc.gov/malaria/about/biology/index.html

Present: Austin

Goals: To understand the biology of malaria and current forms of diagnosis as our client has decided that our device should focus on the diagnosis of malaria.

Content:

- Malaria involves parasites that infect two type of hosts.
    - *Anopheles* mosquitoes and humans
- The mosquito carries the disease from human to human and thus acts acts as a vector. The mosquito does not feel the effects of the parasite. It merely transmits the disease.
- Additionally, uninfected mosquitoes can contract the parasites from humans are infected to increase the spread of the disease.
- In humans, the parasites grow and multiply in the liver and then the red blood cells (RBCs).
- These parasites then grow and destroy the blood cells and then release of merozoites (daughter parasites).
- The released merozoites then infect other RBCs and worsen the disease.
- This infection of RBCs causes the symptoms of malaria.
- The life cycle of malaria involves different life stages of parasites which cause further infection and transmission from human to mosquito and vice versa.
- Refer to this paper for any specifics in these stages.

Conclusions/action items:

This article from the CDC is useful in understanding how malaria affects its human host. It provides the basis for possible detection of the disease by looking at infected host red blood cells.

9.23.16 Point of Care Testing

Title: POC testing: Impact of Nanotechnology

Date: 9.23

Content by: Austin

Paper Location: http://www.sciencedirect.com/science/article/pii/S0956566316308417

Present: Austin

Goals: Research POC testing.

Content:

- POC = rapid and accurate results; low cost, ease of use
- Goal: chip based
- Examples: paper based and printed electrode technologies
- Want portable, reusable, and effective POC system
- ASSURED - affordable, sensitivem specific, user-friendly, rapid, equipment free, delivered
- TWO KINDS:
    - Handheld: state of art microfabrication techniques
    - Benchtop: Reduced versions of large central lab equipments
    - Global POC diagnostics is expected to reach 27.5 billion by 2018
- Dipsticks
    - paper based indicators
    - simple
- Lateral flow tests
    - capillary flow platform, (LFA or test strip)
    - POC or at home use
    - This design seems favorable because minimal technique for user
    - 5-15 minutes for analysis
    - LF immunoassays are among most commercially successful microfluidic POC devices
- Targeting with antibody
    - Antigen/antibody reaction is something to be explored for analyte in malaria
    - Multi analyte testing is a possibility for better success
    - Example biomarkers: pH changes in sweat or saliva; vitamin D levels and detection of alk phos activity
    - Should target: drug and toxin, tumor markers, protein, DNA, virus, pathogen, and hormone
- Paper is attractive because its cheap
    - Microchannels on paper
    - **Look up these fab techniques**
    - **Photolithography, etching, writing, dipping, printing, stamping, and spraying**
    - **also look at Alkyl ketene dimer ink jet printing and wax printing**
- Detection methods
    - colorimetric, electrochemical, fluorescence, chemiluminescence, electrochemiluminescence
    - fluorescence and electrochemistry based designs are more sensitive and specific
    - colorimetric is good because can used with camera phone
- Printed POC based assays are somewhat possible refer back if necessaru
- Nanomaterial Based POC Assays are most likely out of our cost range

Conclusions/action items:

This article was very useful in giving background in different microfluidic assays. After reading this I think that our ideal assay would be a paper-based colorimetric assay. This would be the simplest design. Nanoparticle designs also sound interesting but out of our range in terms of cost. Refer back if necessary.

9.23.16 Physics and Applications of Microfluidics in Bio (Beebe)

▪ Austin Feeney ▪ Oct 18, 2016 @07:34 PM CDT

Title: Physics and Applications of Microfluidics

Date: 9.23.16

Content by: Austin

Paper Location: http://www.annualreviews.org/doi/full/10.1146/annurev.bioeng.4.112601.125916

Present: Austin

Goals: To further understand microfluidics as a field and how it works

Content:

- Microfluidics provides ability to work with smaller reagent volumes, shorter reaction time, entire lab onto a chip
- Chip based capillary electrophoresis devices
- Laminar Flow: two microchannels only mix by diffusion
- Good schematics in this paper with relevant physics
- Want large surface area to volume ratio for lots of capillary based tests
- Surface tension is important at the microscale

Fabrication methods

- Micromachining - too costly and over-precise
    - silicon not ideal for microfluidic material because of cost
    - glass based devices are well suited to chemistry applications that require strong solvents
- **Soft Lithography - PDMS**
    - soft material from a lithographic master
    - pattern surfaces via stamping
    - microchannels using molding and embossing
    - molding a two part polymer (elastomer and curing agent)
    - PDMS using photoresist masters (can also be done using phototihography)
    - can even have multi-d layers
    - **soft lithography is fast and less expensive and suitable for biology**
    - masters are expensive though
- In situ (looks a little difficult)
- Micromolding (looks more complicated than necesary)
- **Components of devices**
    - channel network first and foremost (sometimes a T shape is enough)
    - **Actuators**
        - **valves**
        - manipulate flow
        - passive valves = no energy
        - limit flow to one direction
        - **mixers**
        - passive mixers increase diffusion between two substances by channel geometry - ie a curve
        - **pumps**
        - magnetic or electrical to move fluids
        - passive pump - large drop vs small drop surface tensio
        - other passive pumps to look up -**bubble pump, osmotic based pump, evaporation**
    - Sensors
        - measure device or system output
        - fluid output
        - fluorescent tags
- Macromolecular Analysis
    - microfluidic devices that mix DNA and a restriction enzyme and separates fragments
    - **Enzyme Assays**
        - determine reaction kinetics of enzyme with B-gal
        - fluorescent immunoassays
    - Cell Culture with microfluidics (NA)

Conclusions/action items:

There is a lot of good information here about fabrication and microscale physics. It is important to look up biomarkers of malaria from here and fabrication techniques for microfluidics devices.

Look up the Whitesides Group!

9.26.16 Microfluidic design and fabrication research

▪ Austin Feeney ▪ Sep 26, 2016 @07:56 PM CDT

Title: Microfluidic design and fabrication research

Date: 9.26.16

Content by: Austin

Paper Location: http://www.nature.com/protocolexchange/system/uploads/3745/original/Microfluidic_device_design_fabrication_and_testing_protocols_protex2015_069.pdf?1436957242

Present: Austin

Goals: To better understand microfluidic fabrication for the purpose of developing potential designs for Friday 9.30

Content:

Protocols for making master molds and a PDMS device and how to test it

- Trap, deform, and sort cells
- Figure 1
  - For master
    - spin on photoresist
    - align and expose mask to UV light
    - develop pattern
  - For device
    - relief mold master
    - bond on substrate and punch access holes
- AutoCAD design techniques **(do we have this software somewhere?)**
  - lines vs polylines
  - slightly confused on this topic
- SU-8 polymer can become crosslinked by UV exposure to be raised and create microfluidic channels using PDMS
- **Soft Lithography**
- stay within these aspect ratios of height width (1:10) and (4:1)
- flow is controlled in many devices by **syringe pumps**
- Bonded (PDMS) to glass coverslips
- Leave 2mm border on all sides of each device (make this where razor guidelines are)
- Design a single device or multiple to fit on a cover slip (24mmx60mm)
  - leave 2mm on all sides
- SECTION2 Master Fabrication Protocols
  - Review specifics when developing protocols and designs
  - A lot info in here may not be relevant (could be simplified)
- SECTION3 Microfluidic Device Fabrication
  - silanize master before applying PDMS to make easier to pull off
  - Review specifics when developing designs
  - in section 4 can bond device to glass by corona discharge

Conclusions/action items:

This paper seems highly technical and I am still slightly confused on fabricating a microfluidic design which makes it hard to develop relevant designs for our project. I am going to look more into device fabrication and relevant biomarkers to assess for malaria.

10.5.16 Deformability Separation Research: Margination

Title: Deformability Separation Research

Date: 10.5.16

Content by: Austin

Paper Location: https://www.ncbi.nlm.nih.gov/pubmed/20689864

Present: Austin

Goals: To further understand the deformability design of infected cell separation for evaluating the possible design in our design matrix.

Content:

Abstract

- less than 300um: RBC's migrate to center while leukocytes go the edges
- Known as margination
- malaria infected RBC's behave similar to leukocytes
- contributes to their adherence to cell walls

Intro

- 3.3 billion at risk; 1 to 2 million deaths annually
- 4 species of malaria
- Plasmodium falciparum is most deadly
- Infected blood cells change in 48 hours
- Gold standard: **Giemsa-stained blood smear**
    - **requires technician**
- ICS lateral flow-strip test (promising alternative)
- INTERESTING possible combination with separation by size: microfluidic colorimetric immunoassay (Stevens et al)
- Gascoyne et al: dieelectrophoresis (separation by charge differences)
- Zimmerman et al: magnetic deposition microscopy
- Could test with pig blood and possibly look under microscope to see the different sizes of molecules that are separated
- POSSIBLY apply to sickle cell and leukemia, with maybe different size channels
- Where we come in is to design a new detection technique for this separation technique
- reduced surface area to volume=less deformable
- less deformable also because of parasitic proteins

Design

- 3cm long
- (15 by 10um) width by height
- 100um width down to 15um
- infected cells are close to 3um
- PDMS (soft lithographic techniques)
- Pretty good information on fabrication here
- silicon etching was used
- 2 hours in trimethylchlorosilane to release mold
- cured at 70 for 2.5 hours
- maybe want a top (channels inside)? if possible
- mold (negative image of mold)
- information on bonding to glass slides here

Conclusions/action items:

This is a great paper and offers many other separation techniques other than deformability. I will continue to look up information on more ways to fabricate a similar design. It would be ideal to collect the infected RBCs and test them directly in the device.

10.5.16 Deformability Separation Research: Non-inertial Hydrodynamic Lift

▪ Austin Feeney ▪ Oct 18, 2016 @07:39 PM CDT

Title: Deformabilty Separation Research #2 (2014 article)

Date: 10.5.16

Content by: Austin

Paper Location: https://malariajournal.biomedcentral.com/articles/10.1186/1475-2875-13-375

Present: Austin

Goals: To further understand more recent approaches to deformability separation of infected red blood cells.

Content:

- Used PDMS and soft lithographic techniques
- It has two inlets and two outlets
- Each inlet driven by a syringe pump connected by PTFE tubes
- syringes, tubes, and microchannel can be autoclaved
- microchannel width 91um
- height 102um
- 20mm long separation channel
- outlet 2 at angle of 49 deg
- both outlets at 250um
- they demonstrate out how cell separation based purely on margination is slightly less effective

Conclusions/action items:

This again shows it would be great if we could combine this technique with something else to create an all in one device for separation and detection. It also demonstrates that separation by non-inertial lift is a little more specific than purely on margination. However, this design seems to be a bit more technical than we might able to fabricate in one semester.

10.18.16 Isothermal Amplification for Detection of Malaria

▪ Austin Feeney ▪ Oct 18, 2016 @08:19 PM CDT

Title: Isothermal Amplification for Detection of Malaria

Date: 10.18.16

Content by: Adapted from Zach Hite

Paper Location: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4721682/

Present: Austin

Goals:

To learn about a method for amplifying Malarial nucleic acids without the use of a PCR machine.

Content:

- In this method DNA strands are able to separate without the use of a lot of time or a PCR machine
- It allows for the gene replication of a specific marker unique to Plasmodium falciparum (one of the malarial parasites).
- The method took 35 minutes in total which required lysing and denaturing cells in order to complete a 35 minute reaction.
- The test is able to use pure blood and can detect parasites as low as 1.5 parasites/uL

Conclusions/action items:

I did not perform research on this topic but rather had this information explained to me by Zach Hite at a team meeting. It seems as though this method does not meet our design criteria as the assay requires lab space and heating solutions up to 90 degrees Celsius. It is a very sensitive assay in that it can test for small levels of infection; however, it is specific to only one strain of the disease. Additionally, it does not seem to be an easy detection method to carry out.

10.18.16 General Malaria Market for Rapid Diagnostics

▪ Austin Feeney ▪ Oct 18, 2016 @08:35 PM CDT

Title: General Malaria Market for Rapid Diagnostics

Date: 10.18.16

Content by: Adapted from Zach Hite

Paper Location: http://www.sciencedirect.com/science/article/pii/S073497501630009X

Present: Austin

Goals: To learn more about the market on current malaria diagnostic tools and evaluate pitfalls of these designs.

Content:

Here are the current Rapid Diagnostic Tools and other diagnostic methods on the market:

- Giemsa Nuclear stain
- Amplino - Portable PCR
- NanoMal
- Sanitoets Home Test Kit
- SD Bioline Malaria
- ParaHIT ®
- First Response® Malaria
- CareStartTM Malaria HRP2/pLDH (Pf)

These are the many tools that exist on the market today. However, many still face shortcomings that we look to overcome involving the need for trained technicians, lack of strain specificity, and high cost. Many of these techniques must be confirmed with the the current gold standard, which is a Giemsa blood stain. Giemsa Stain stains parasitic nuclei. However, human technician error and the need for a skilled technician causes this method to have limits as a global diagnostic method.

Conclusions/action items:

Our device should not require skilled users and should cost less than 5 dollars. Additionally, it should not require affirmation from a Giemsa stain and should diagnose all 4 strains of the disease.

10.7.16 Separation: Design Idea One – Cell Deformability

▪ Austin Feeney ▪ Oct 18, 2016 @08:39 PM CDT

▪ Austin Feeney ▪ Oct 18, 2016 @09:01 PM CDT

Title: Separation: Design Idea One – Cell Deformability

Date: 10.7.16

Content by: Austin

Design by: Austin

Present: Austin

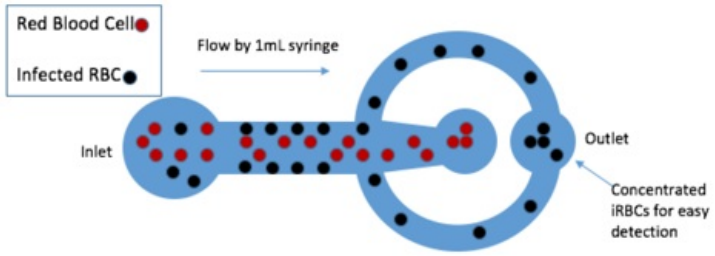
Goals: To illustrate a design I created for a separation technique based on cell deformability research I performed.

Content:

This separation design is based on changes in cell deformability caused to the host RBC upon infection with the malarial parasite. Parasitic proteins are secreted within the iRBC to make its membrane more adhesive as well as stiffer. The increased stiffness along with a reduction in the surface area to volume ratio contributes to a significant decrease in cell deformability especially in late stage iRBCs. Normal RBCs, however, remain highly deformable. It has been demonstrated that the stiffer iRBCs can separate towards the sidewalls of a long straight channel microfluidic device The diagram below illustrates the preliminary cell deformability design. It has an inlet, where a blood sample is loaded via a syringe. The blood cells then flow down the 3-centimeter-long channel channel (15um wide), which forces the iRBCs to the sidewalls. Finally, the iRBCs can be concentrated in separate outlet from the RBCs by the use of smaller channels on the outside walls. The innermost outlet would thus mostly contain RBCs, and the outer would contain mostly iRBCs.

Conclusions/action items:

This content was written by me as well as the design. The design is pictured below.



10.14.16 Separation: Design Idea Two – Magnetism

▪ Austin Feeney ▪ Oct 18, 2016 @09:04 PM CDT

Title: Separation: Design Idea Two – Magnetism

Date: 10.14.16

Content by: Austin

Design by: James Jorgensen

Present: Austin

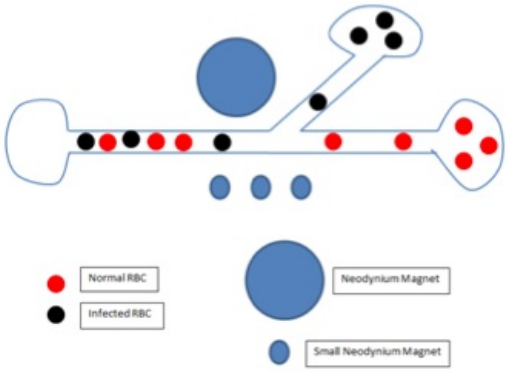
Goals: To illustrate a design James created for a separation technique based on magnetism research he performed.

Content:

This separation design is based on changes in magnetic properties caused to the host RBC upon infection with the malarial parasite. When parasites infect and feed on RBCs they release iron, which is converted into hemazoin. Hemazoin is a weakly paramagnetic crystallite, which is produced during all life stages by all four strains of the disease. It interferes with the magnetic spins of hydrogen atoms, which align when exposed to a powerful magnetic field. However, hemazoin production in iRBCs causes iRBCs and RBCs to have distinguishable magnetic properties, which can be utilized to separate infected cells from uninfected cells in a highly specific manner in microfluidic device. Shown below is a preliminary design for magnetic separation of iRBCs involving neodynium magnets.

Conclusions/action items:

This content was written by me and the design was created by James. The design is pictured below.



10.14.16 Separation: Design Idea Three – Electrical

▪ Austin Feeney ▪ Oct 18, 2016 @09:06 PM CDT

Title: Separation: Design Idea Three – Electrical

Date: 10.14.16

Content by: Austin

Design by: Joshua Liberko

Present: Austin

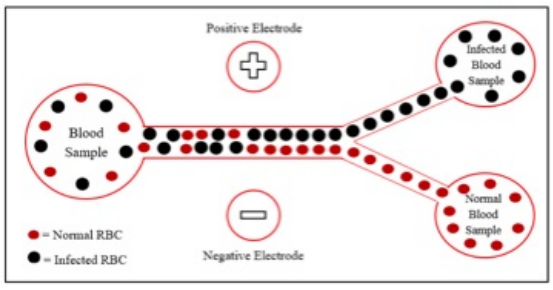
Goals: To illustrate a design Josh created for a separation technique based on electrical conductivity research he performed.

Content:

This separation design is based on changes in electrical properties caused to the host RBC upon infection with the malarial parasite. The electrical conductivity of iRBCs is significantly higher than normal RBCs, which allows for electrical separation of these cells. Dielectrophoretic (DEP) forces are non-uniform electric fields that can be created by cells when subject to direct or alternating current. Different DEP properties of iRBCs and RBCs contributes to their unique conductivities [Tay Malaria and POC]. This can be utilized to separate iRBCs and RBCs in a microfluidic device. The diagram below illustrates the concept of electrical stimulation in a microfluidic device for the function of separating RBCs.

Conclusions/action items:

This content was written by me and the design was created by Josh. The design is pictured below.



10.14.16 Detection: Design Idea One – BinaxNOW

▪ Austin Feeney ▪ Oct 18, 2016 @09:10 PM CDT

Title: Detection: Design Idea One – BinaxNOW

Date: 10.14.16

Content by: Austin

Design by: Hunter/BinaxNOW

Present: Austin

Goals: To illustrate a design Hunter created/proposed for a detection technique based on BinaxNOW.

Content:

BinaxNOW is a currently available diagnostic tool for malaria as described by Hunter. Its major pitfall is the necessity of 5,000 parasites/uL in blood samples. The separation techniques described would act in concentrating the parasites for easier and more successful detection. This assay takes 15 minutes to run. When combining BinaxNOW with one of the separation techniques, this tool could become more successful in diagnosing malaria.

Conclusions/action items:

This content was written by me and the design was created/proposed by Hunter.

10.14.16 Detection: Design Idea Two - Polystyrene Beads

▪ Austin Feeney ▪ Oct 18, 2016 @09:09 PM CDT

Title: Detection: Design Idea Two - Polystyrene Beads

Date: 10.14.16

Content by: Austin

Design by: Zach Hite

Present: Austin

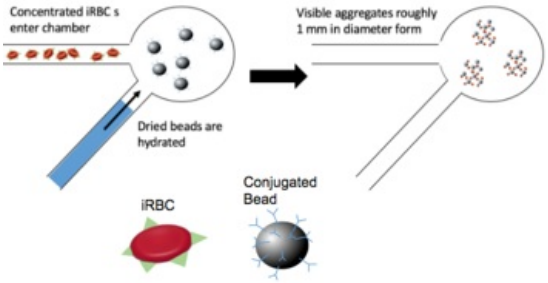
Goals: To illustrate a design Zach created for a detection technique based on immunoagglutination research he performed.

Content:

This method of malaria detection is based on an antigen-antibody interaction whereby polystyrene beads are conjugated with an antibody and interact with specific iRBC antigens. The beads and iRBCs would then form a visible aggregate in the well in a process known as immunoagglutination. The dried beads could be could be coated in the well and rehydrated upon use. The aggregation would occur within 2 minutes and would be visible without a microscope.

Conclusions/action items:

This content was written by me and the design was created by Zach. The design is pictured below.



10.14.16 Detection: Design Idea Three - Gold Nanoparticles

▪ Austin Feeney ▪ Oct 18, 2016 @09:14 PM CDT

Title: Detection: Design Idea Three - Gold Nanoparticles

Date: 10.14.16

Content by: Austin

Design by: Hunter Johnson

Present: Austin

Goals: To illustrate a design Hunter created for a detection technique based on gold nanoparticle research he performed.

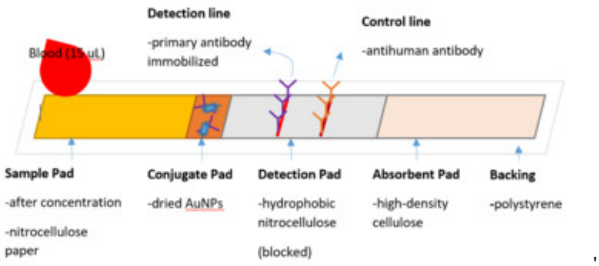
Content:

This method of malaria detection is a lateral flow immunoassay whereby iRBCs are detected using unique antigen-antibody interactions. The concentrated blood sample would be placed on the sample pad and run down the nitrocellulose paper to pass through the conjugated gold nanoparticle pad. Gold nanoparticles, would be conjugated with an antibody specific to iRBC antigens and would change the color of iRBCs. The antibodies present on the gold nanoparticles would also be immobilized on the detection. Thus, colored iRBCs would cause a colored line at this junction to signify detection of the disease. A conjugated gold nanoparticle for each strain of malaria could be placed in the conjugate pad. Thus, the actual design would contain four separate detection lines for each strain of the disease in addition to the antihuman control line. The antihuman antibody control line would always be detected and is present to demonstrate antibody integrity.

Conclusions/action items:

This content was written by me and the design was created by Hunter. The design is pictured below.

▪ Austin Feeney ▪ Oct 18, 2016 @09:16 PM CDT



Detection-GNP.png(95.1 KB) - download

10.14.16 Design Matrix Evaluation and Final Design

▪ Austin Feeney ▪ Oct 18, 2016 @09:27 PM CDT

Title: Design Matrix Evaluation and Final Design

Date: 10.14.16

Content by: Austin

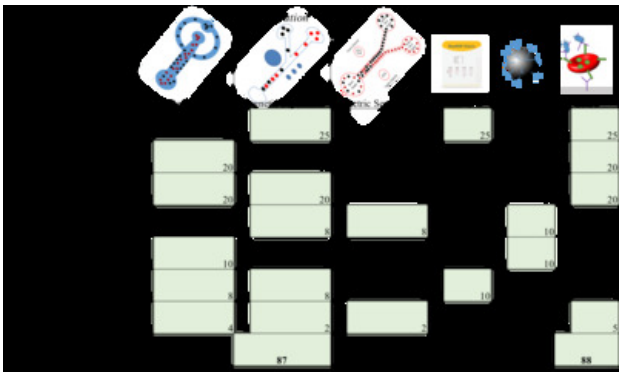
Present: All

Goals: To evaluate each design in accordance with the design matrix criteria as seen in the Design Matrix Criteria entry. To choose a final design for each of the separation and detection designs.

Content:

The design matrix and its evaluation are shown below. Additionally it must be noted that the proposed final design will consist of an integrated design for the magnetic separation and gold nanoparticle detection methods.

▪ Austin Feeney ▪ Oct 18, 2016 @09:19 PM CDT



Design_Matrix.png(213.2 KB) - download

▪ Austin Feeney ▪ Oct 18, 2016 @09:53 PM CDT

Design Matrix Evaluation: (all designs were evaluated using the same criteria from the Design Matrix Criteria entry)

The magnetic separation technique scored the highest because the hemazoin is secreted in all stages of the diseased state for all four strains of the disease and would thus have the capacity to be very successful in iRBC separation. The cell deformation design scored the highest in fabrication and ease of use because it would not require magnets or any sort of current as part of the design. It could be fabricated as a stand alone PDMS device, which would be easiest to use because no magnets or batteries would be necessary in setup of the assay. The electric separation method thus scored lowest in the user-friendly category because of battery replacement. Ultimately, the cell deformation design did not win because of its low score in sensitivity, or the ability to effectively separate iRBCs from RBCs. This design could easily have RBCs travel to the wrong outlets. The electric separation design ultimately did not win because of the electricity requirement, which poses problems for use in rural areas and replacement of batteries. The magnetic design was deemed as the winner and won five out of the seven categories in the design matrix.

Currently, BinaxNOW is the most sensitive design on the market with 99% accuracy and could become more effective when coupled with on our separation techniques. However, BinaxNOW would be difficult to recreate regarding patent issues and the purchasing the actual product would be expensive. Gold nanoparticles were deemed as the winning design because they could be just as accurate as BinaxNOW and have the possibility to be much cheaper when mass produced. The gold nanoparticle design was also deemed the best in the user friendly category because of the easy colored bands for detection. The polystyrene bead design ranked highest in cost because the materials are cheaper than BinaxNOW and gold nanoparticles. It additionally ranked highest in time because the assay takes merely 2 minutes to run. The gold nanoparticle design was deemed as the winner and won four out of the seven categories in the design matrix.

Final Design:

The final design will consist of an integrated design containing the magnetic separation and gold nanoparticle detection methods.

Conclusions/Action Items

Develop the integrated final design.

10.14.16 Design Matrix Criteria

▪ Austin Feeney ▪ Oct 18, 2016 @09:56 PM CDT

Title: Design Matrix Criteria

Date: 10.14.16

Content by: All

Present: All

Goals: To develop and demonstrate the design matrix criteria.

Content:

Sensitivity/Specificity > 95% (25%):

Separation: The device should effectively separate 80% of infected RBCs into separate channels and should allow minimal passage of any uninfected RBCs into these channels.

Detection: The detection method should be 95% effective in labeling the concentrated portals of infected RBCs. It would ideally detect all strains of malaria.

Equipment Free / Portable / Ease of Detection (20%):

Separation: The method of separation should be able to be transported long distances and be provided in a device smaller than a personal computer. Preference would be for the device to not need power.

Detection: The detection method should be able to use the naked eye or a cheap and easy to use device in order to obtain an accurate reading of the state of the disease.

User Friendly (20%):

Separation: A small amount of sample and easy to handle reagents are necessary in the resource limited regions where this device would be implemented. Collection of sample should be safe for both the patient and the technician. Also should not require extensive training to be able to run many tests.

Detection: The form of detection should be able to be monitored on site by a modestly trained technician, without the use of high tech laboratory equipment. A simple protocol for the type of detection would be provided.

Ease of Fabrication (10%):

Due to resource restrictions in Jimma at Jimma University, as well as the developing world in general, the microfluidic device protocol for fabrication should be relatively straightforward and not require high tech resources to produce. The design also needs to be able to be mass produced in order to be an effective POC diagnostic device.

Cost (10%):

The projected cost per device once the production is optimized should be less than $5.

Time < 1 Hour (10%):

Separation: The amount of time it will take to run the blood sample through the concentration device should be less than 20 minutes.

Detection: The total time it would take for a patient to be diagnosed needs to be less than one hour.

Versatility (5%):

Separation: The ability for the concentration device to separate infected RBCs, specifically the malaria parasite, from healthy RBCs. It would be prefered for the device to be able to separate different strains of malaria or different diseases, but we will focus on the Plasmodium falciparum parasite which is specific to malaria.

Detection: The ability to detect different strains of malaria parasites or different diseases with minor alterations to our final design would be useful. Patients presenting with mixed symptoms would be able to use the same device to test for multiple diseases.

Conclusions/action items:

Evaluate designs based on these criteria.

Advising Day 9.23.16

▪ Austin Feeney ▪ Oct 18, 2016 @07:43 PM CDT

Title: Advising Day

Date: 9.23.16

Content by: Austin

Present: 300/200

Goals: Learn about future opportunities

Content:

- Get - CO-ops
- Prepare for MCAT or GRE - summer before senior year
- Do your homework on post degree options
- Writing your story
    - Personal statement
    - write current story
    - tie parts of story together
    - your passion and your proposed goals
- 24 credit masters
    - 1 year: great option
    - keep your research advisor
    - stepping stone for Medical School, PhD school and industry
    - PhD - independent researcher, grants, academia
    - MS makes you more desirable
    - 10,000 dollars more
    - Can co-op during MS as well
    - time to find the dream job
    - SAVE ADVANCED BME COURSE for graduate
    - can do 6 credits of BME 699 research
- How to apply for masters
    - www.grad.wisc.edu
    - 3 letters of rec
    - GRE or MCAT scores
    - application fee
    - Application deadline is February 1st (guaranteed admission for 3.0 or higher)
    - Can start this in spring summer or fall! Deadline for January admission is October 1st
- MS funding
    - Teaching Assistant - Stipend of $31,000
        - if more than 1/3 time then get tuition paid for
    - Research Assistant (NIH, NSF grant) probably not (normally PhD)
    - Project Assistant (revamping courses: grant)
- Can do masters in mechanical engineering
- Can start in masters program and continue in the PhD program here
- PhD
    - BME
    - utilize your lab PI here
    - Build your resume
    - Honors in research program (look at this)
    - Look at REUs and SURE programs
    - External funding NSF - GFRP
        - www.nsfgrfp.org/
        - UW hosts workshops - watch for my emails
    - Apply Early
    - Connect with someone at the institution through REU experience
- Medical School
    - {requirements for most Medical School)
    - Prehealth.wisc.edu
    - UW Madison - must now take I/A and CommB
    - MCAT - psychology and sociology and biochemistry (required for MCAT) soc134
    - **Work at a nursing home**
- Beyond Coursework
    - volunteer
    - shadow physicians
    - build relationships
    - use design experiences
    - research is great experience

Conclusions/action items:

Join REU, Join honors in research program, Stem Cell Certificate, UW Masters program

Don't spread yourself too thin

Classwork is most important!

Background on Malaria

▪ Joshua Liberko ▪ Oct 19, 2016 @01:05 PM CDT

Title: Background on Malaria

Date: 10/19/16

Content by: Josh Liberko

Present: Me

Goals: Generate a basic understanding of what malaria is, its symptoms, how it forms, and the different strains of it.

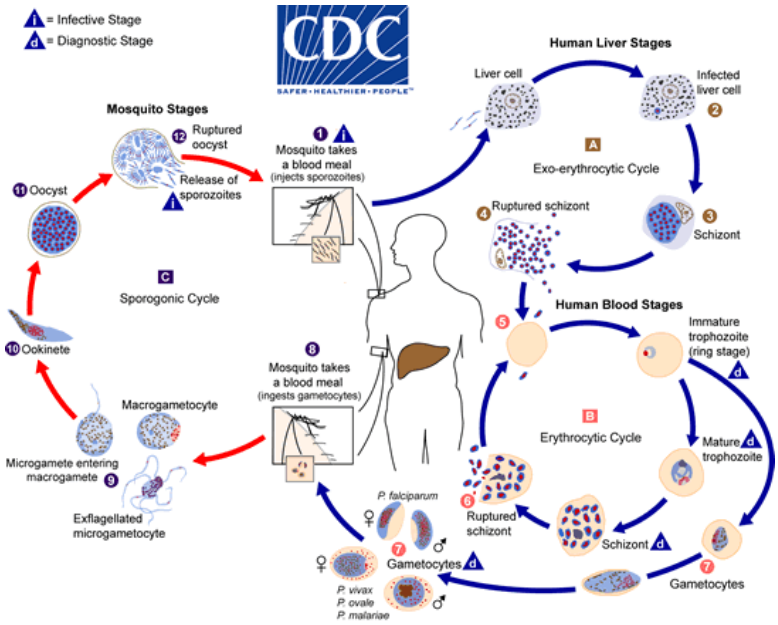
Content:

Malaria- is a serious and potentially fatal disease that is cause by a parasite that commonly infects female mosquitoes which feed on humans. This mosquito is known as the Anopheles mosquito. Approximately 3.2 billion people live in areas at risk of malaria transmission. This includes 106 countries and territories. It is estimated by the World Heath Organization that malaria caused 214 million clinical episodes and 438,000 deaths in 2015 alone (http://www.cdc.gov/malaria/about/facts.html).

Symptoms- a classical attack typically last 6-10 hours and consists of...

- Fever
- Sweats
- Nausea/Vomiting
- Chills
- Headaches
- Bodyaches
- General malaise

How it forms- in humans the parasite grows and multiplies in the live and then ruptures into the blood stream and infecting red blood cells. When a female mosquito bites an infected human the cycle basically starts over again, however, mosquitoes do not feel the negative affects of the parasite like humans too. A good depiction of the parasite life cycle is depicted below...



Species/Strains

- Plasmodium falciparum: is the most dangerous and deadly form of malaria
- Plasmodium vivax: is the most frequent and widely distributed cause of recurring malaria
- Plasmodium ovale: is a more rare species of malaria and is less dangerous compared to falciparum and vivax
- Plasmodium malariae: similar to ovale, malariae is more rare and less dangerous

Conclusions/action items:

Malaria is a very serious disease that effects millions of people and therefore needs to be more easily detectable and treatable. The design project we have inherited will hopefully lead to a better POC detection method.

Malaria Diagnosis

▪ Joshua Liberko ▪ Sep 19, 2016 @05:34 PM CDT

Title: Malaria Diagnosis

Date: 9/19/2016

Content by: Josh Liberko

Present: Myself

Goals: Learn about simple ways to detect malaria

Content:

Malaria parasites can be detected in a patient's blood under a microscope by using a dye (Giemsa stain) to give the cells a distinctive blue/purple appearance.

Various test kits are available to detect antigens of malaria parasites. These immunochromatographic tests usually involve a dipstick and provide feedback in 2-15mins. However, to become more universal the tests need to become more accurate, cheaper, and easier to use in less developed places.

Serology: detects antibodies against malaria parasites by indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). However, serology only detects past exposer not current.

Conclusions/action items:

The current tests are not very accurate and need to be supplied cheaper. We need to detect some enzyme or protein in a malaria parasite that when mixed with a chemical will react to prove that the patient has the disease.

Charge of Malaria

▪ Joshua Liberko ▪ Oct 05, 2016 @04:39 PM CDT

Title: Detection of malaria parasites by magnetic charge

Date: 9/28/16

Content by: Josh Liberko

Present: Me

Goals: Determine an easy way to detect malaria with the use of magnet charge

Content:

Malaria detection has not changed much over the past decade as the most accurate testing method is still examining a smear of blood under a microscope by a trained physician. However, this factors in a great deal of human error in the detection method.

A research team at the Singapore-MIT Alliance for Research and Technology (SMART) has come up with a method that uses magnetic resonance relaxometry (MRR), which is very similar to an magnetic resonance imaging (MRI), that is able to detect the waste product found in the blood of infected patients. This method offers a reliable way to detect malaria and also provides a field deployable system because there is no need for labels or dyes. The SMART system detects the parasitic waste product known as hemozin. When the malaria parasite infects red blue cells, it feed on the hemoglobin carried by health red blood cells. When hemoglobin breaks down it released iron which the parasite converts into hemozin, a weakly paramagnetic crystallite.

If hydrogen atoms are exposed to a powerful magnetic field, the spins all align, however, the hemozin interferes with the magnetic spins of the hydrogen atoms. When introduced to a smaller magnetic field the hemozin disrupts the atoms spin change, whereas normal atoms should show a synchronized change. This disruption is called relation. The researchers in this study used a 0.5 tesla magnet, but more research is being done to reduce the size and portability of the system. The procedure takes about 10 minutes, cost about 10 cents, and only requires about 10 microliters of blood, a fingerprint's worth.

*hemozin crystals are produced in all four stages of malaria infection

*hemozin is generated by all known species of Plasmodium parasite

*the amount of hemozin present can reveal the severity of the infection and if it responding to treatment

*Plasmodium falciparum is the most dangerous form of the parasite

URL: https://www.sciencedaily.com/releases/2014/08/140831150339.htm

Conclusions/action items:

The tracking of hemozin crystals by magnetic fields could be a viable option for our group to do more research on and possibly base our experiment off of. However, the magnet the research team used in this experiment was not cheap and could influence our design. I think we should do more research into this design idea however we should also look for other methods and are easier to perform and control.

Patent for Microfluidics and Malaria Detection

▪ Joshua Liberko ▪ Sep 26, 2016 @05:38 PM CDT

Title: Patent for Microfluidics and Malaria Detection

Date: 9/26/16

Content by: Josh Liberko

Present: Me

Goals: Discover if our project will be interfered by any existing patents, and to learn from the methods that these researchers have already conducted.

Content:

Patent US8628972 B2: Microfluidic devices and methods for malaria detection

The detection method includes a contact surface that is hydrophilic and/or roughened and when a clean blood sample passes through the device nothing sticks to the contact surface. However, if a malaria infected blood sample is used the malaria infected red blood cells (miRBCs) interact with the contact surface, temporarily immobilizing them.

Claim 1 States, "A method of identifying infection by a malaria parasite comprising: obtaining a sample of whole blood comprising RBCs from a subject; flowing the sample through a microfluidics device having a channel comprising a contact surface at a flow rate sufficient to produce a shear rate between about 2.1 sec^{-1} and 3.2 sec^{-1}, the contact surface being at least one of a hydrophilic contact surface and a roughened contact surface; and identifying the presence of infected RBCs, wherein RBC's infected with the malaria parasite are at least temporarily trapped on the contact surface and indicate infection and wherein RBC's not infected with the malaria parasite flow past the contact surface and are not trapped on the same."

Background from patent source-

The most accurate approaches to malaria detection are based on a polymerase chain reaction (PCR) and Giemsa-stained blood smears. PCR provide the highest sensitivity of 0.004 to 5 parasites per µl of blood, however, are not portable and therefore not accessible in rural areas. Giemsa-stain tests are the next highest in sensitivity of 5 to 20 µl of blood, however, require a carefully prepared slide and must be examined by a specialist under 1000x magnification. Other travelers kits available include: ICT Malaria Pf/Pv®, Parasight®-F, and OptiMAL®. These kits are portable and provide quick analysis, however, lack in sensitivity and specificity making them inadequate for early stage malaria detection.

URL for patent: https://www.google.com/patents/US8628972?dq=microfluidic+malaria+detection&hl=en&sa=X&ved=0ahUKEwjcvu3v_63PAhWk3YMKHXFiAH8Q6AEIHDAA

Conclusions/action items:

I do not believe that our project will interfere with this patent as it requires a pump to push the blood sample into the device and, from the specification pictures, appears to difficult for us to construct. However, I do feel that there are very useful stats and methods that may be able to be applied to our project. We could try to incorporate some area on the microfluidics device to capture the infected malaria parasites. Also, I believe the next step is to look into the other travelers kits that were mentioned in this patent and see how they operate and detect the malaria parasites.

List of Articles to read and document in the future

▪ Joshua Liberko ▪ Oct 19, 2016 @04:16 AM CDT

Title: List of Articles to Reference

Date: 10/07/06

Content by: Josh Liberko

Present: Me

Goals: Generate a list cache of potentially helpful articles

Content:

https://www.sciencedaily.com/releases/2012/05/120510095616.htm

https://www.sciencedaily.com/releases/2014/06/140625101556.htm

https://www.sciencedaily.com/releases/2013/04/130415094458.htm

https://www.sciencedaily.com/releases/2008/07/080728192813.htm

http://www.sciencedirect.com/science/article/pii/S0092867413005047

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3415751/

https://www.ncbi.nlm.nih.gov/pubmed/1231059

*** https://www.researchgate.net/publication/244479356_Magnetic_Separation_of_Malaria-Infected_Red_Blood_Cells_in_Various_Developmental_Stages

Conclusions/action items:

Read and document all of the articles listed above

Cell Deformation Separation Design

▪ Joshua Liberko ▪ Oct 19, 2016 @03:28 AM CDT

Title: Cell Deformation Separation Design

Date: 10/18/16

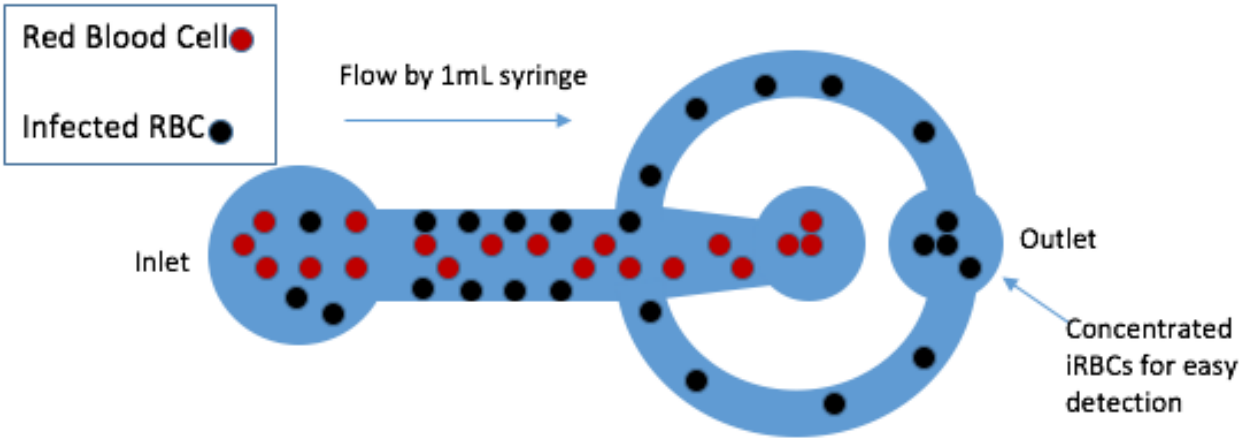
Content by: Josh Liberko (adapted from Austin Feeney)

Present: Me

Goals: To determine the pros and cons of the cell deformation separation design

Content:

Red blood cells are known to have a high degree of deformability/malleability, however, when infected by a parasite like malaria, RBCs lose some of their power to deform. The proteins secreted into the RBC by a parasite increase the stiffness of the cell membrane. The reduced surface area to volume ratio of iRBCs also adds to the decrease in the deformability of the cell. Relating to microfluidics channel, it has been proven that stiffer walled cells will be forced to the edged of the channels and the more deformable cells will reside in the center. With these properties of the cell in mind, we were able to construct a design that would collect the iRBCs of the by two channels connected to the sides of the main channel. As shown in the picture below, the blood sample is loaded into the inlet and then allowed to travel down the channel by capillary action. The 3cm x 15 um should provide enough room and time for the iRBCs to congregate to the outer walls of the channel and then exit through the top and bottom channel which connect at the outlet. The healthy RBCs are crammed in the middle this whole time so they end up congregating in a separate outlet in the middle of the device.



Pros:

- This design does not have any additional requirements like the magnetic and electric method do.
- Testing can be easily accomplished with polystyrene beads

Cons:

- iRBCs have less deformability the later the stage they are found in and because of this it may be hard to detect early stage malaria
- Requires 40% blood hematocrit

Conclusions/action items:

The cell deformation separation design would be only of the easier proposed designs to construct, however, it may have trouble detecting early stage malaria. More research needs to be conducted on the fabrication of this type of device and how good of a job it can do on concentrating early stage iRBCs.

Magnetic Separation Method

▪ Joshua Liberko ▪ Oct 19, 2016 @02:50 AM CDT

Title: Magnetic Separation Method

Date: 10/18/16

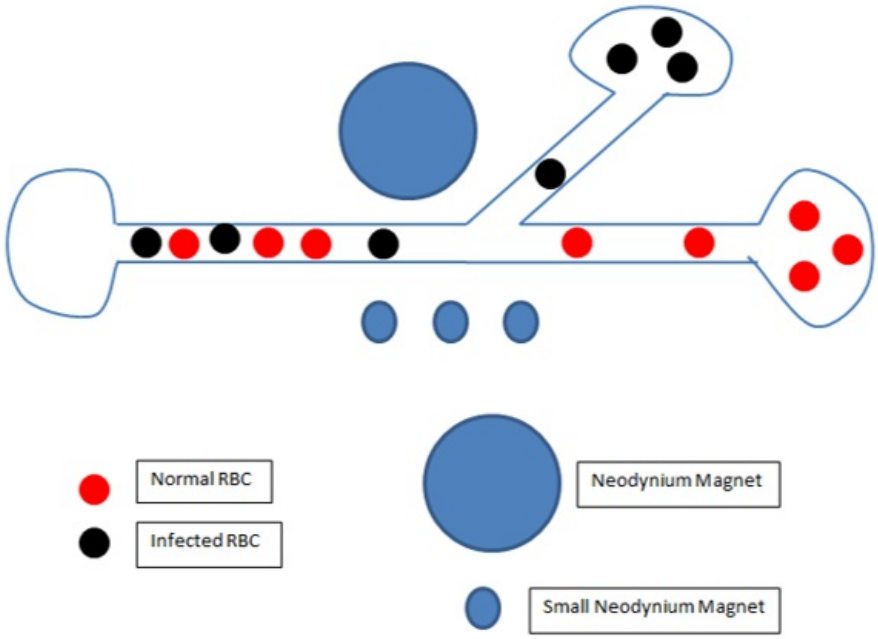
Content by: Josh Liberko (adapted from James Jorgensen)

Present: Me

Goals: To determine the pros and cons of this separation method

Content:

When a parasite infects a RBC, it converts the hemoglobin into a paramagnetic crystalline material known as hemozoin. The magnetic properties of the iRBCs can be exploited when exposed to a strong magnetic/electric field. Under a strong magnetic field, the Hydrogen atom spins in the iRBC align allowing the cell to be attracted to a certain pole (+) of the magnet. This technique can be used to separated iRBCs from healthy RBCs as shown in the picture below.



*Neodymium Magnet is the correct spelling

Pros:

- Hemozoin is produced at all stages of life of a parasite infected RBC
- Fast detection
- Separation is possible with even with a small concentration of iRBCs

Cons:

- The magnetic field calculations are very extensive and complicated
- Requires extra fabrication

Conclusions/action items:

The magnetic separation device offers many fabrications components that would be cheap and easy to construct. However, placement of the magnets will be crucial to the effectiveness of this device, so a lot more research and advising will need to occur before fabrication of this model could begin.

Electric Separation Method

▪ Joshua Liberko ▪ Oct 18, 2016 @11:02 PM CDT

Title: Electric Separation Method

Date: 10/18/16

Content by: Josh Liberko

Present: Me

Goals: To determine the pros and cons of this separation method

Content:

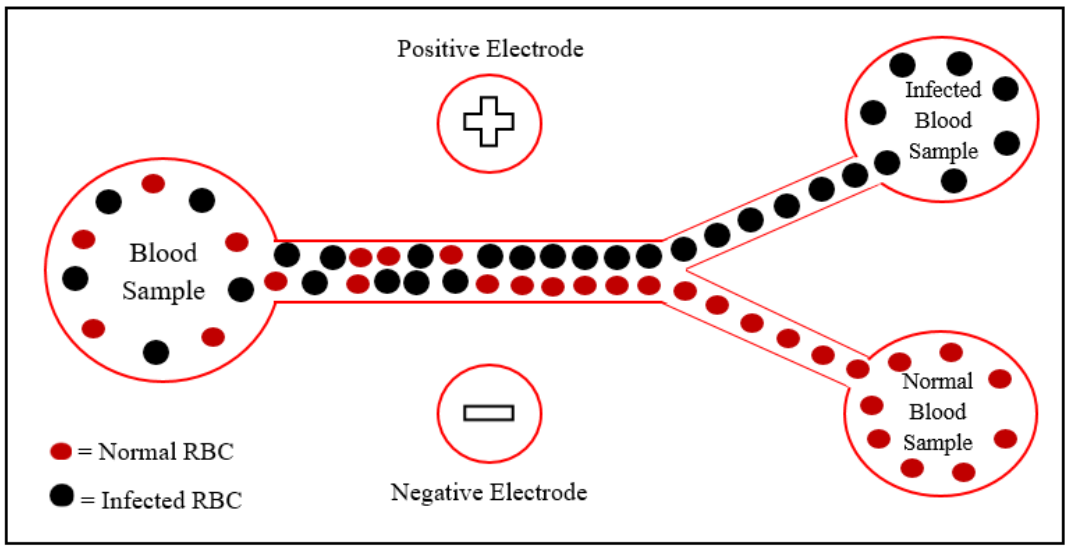
The idea of the electric separation method comes from infected red bloods cells having a paramagnetic characteristic. When the malaria parasite infects a blood cell it converts the hemoglobin into hemozin, which is weakly paramagnetic. Similar to the magnetic separation method, it is believed that electrodes could be used to separate the infected blood cells from the normal RBCs. Hemozin has a negative characteristic to it, so in theory it should be attracted to the positive cathode and the normal RBCs would not be attracted so they would get pushed to the other side of the channel by the iRBCs which would effectively separate the cells.

Pros:

- High Specificity
- iRBCs are sensitive to + charges (conductivity)

Cons:

- Electrical difficulties as this is supposed to be a POC device
- Would have to include batteries which would drive up the cost of the device.



Above is a simple diagram of what this electric separation method would look like.

Conclusions/action items:

This method of separation poses feasible construction, however, the fact that it uses electricity and therefore must include batteries almost single handedly rules this design out. It would be fun to build and test this design, but our time may be better spent on other designs.

BinaxNOW Detection Design

▪ Joshua Liberko ▪ Oct 19, 2016 @04:06 AM CDT

Title: BinaxNOW Detection Design

Date: 10/18/16

Content by: Josh Liberko

Present: Me

Goals: Describe how the BinaxNOW device works and how we can extrapolate off of it

Content:

BinaxNOW is currently the only RDT device that is FDA approved and sold in the United States. A package of 12 tests costs ~$400, so it is a very expensive RDT that is unfeasible to the citizens of Ethiopia. Although it does have an extremely high cost to use, it is also a very sensitive and accurate test with a 99% specificity for Plasmodium falciparum. The downfall of the test is that it requires a concentration of 5,000 parasites/uL in blood samples. This is where the idea for a separation (concentration) and diagnostic device comes in handy. If one of the proposed separation methods works effectively, there should be a high enough concentration of parasites/uL. The total for running this device should be under 15 mins.

Pros:

- Researchers have already conducted and been working on this product, making it extremely sensitive and easy to interpret.

Cons:

- The high price of this design is the main concert

Conclusions/action items:

More research can be conducted on how exactly the antibodies bond on this design, and steps need to be taken to determine what could reduce the price of a devices that is very similar to this BinaxNOW device.

Polystyrene Bead Detection Design

▪ Joshua Liberko ▪ Oct 19, 2016 @09:32 AM CDT

Title: Polystyrene Bead Detection Design

Date: 10/18/16

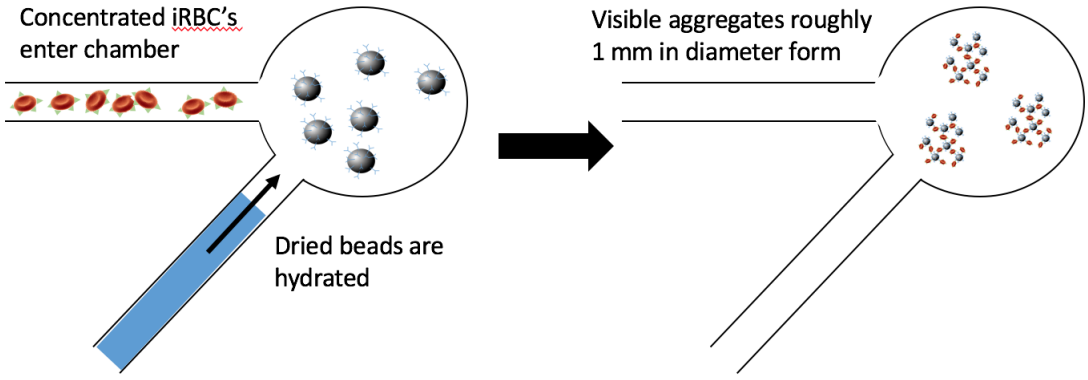
Content by: Josh Liberko (adapted from Zach Hite)

Present: Me

Goals: To examine the pros and cons of the PS bead detection method

Content:

Polystyrene beads can be easily conjugated with antibodies which can then interact with specific antigens, in this case iRBC antigens. After the blood passes through the channel and enters the chamber where the polystyrene beads are, the antigens covering the iRBCs would bind with specific antibodies of the polystyrene beads (PS) forming a visible coagulation in the chamber. Originally the beads would remain dry in the chamber, but they would be hydrated with water just before the blood sample reaches them. Aggregation would occur quickly, within ~2 mins, and would be visible to the naked eye, removing the need for a microscope. This would also add to the credibility of this method being a POC design.



Pros:

- Very rapid diagnosis, ~2 minutes
- A small blood sample would be needed, ~2uL which is a tiny fingerprint

Cons:

- This design is only able to test for one specific strain at a time, so to be able to test for all four strains would require four chambers and four differently coated PS beads... cumbersome!
- Only about 80% specific so it does not meet the design specification required by Dr. Kwa

Conclusions/action items:

Evaluate this design according to the design matrix and perform more research on this type of detection method.

Gold Nanoparticle Detection Design

Title: Gold Nanoparticle Detection Design

Date: 10/18/16

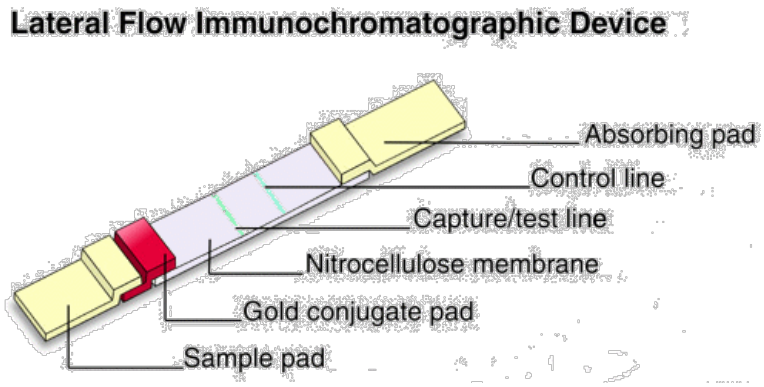
Content by: Josh Liberko (adapted from Hunter Johnson)

Present: Me

Goals: To determine the pros and cons of the gold nanoparticle design

Content:

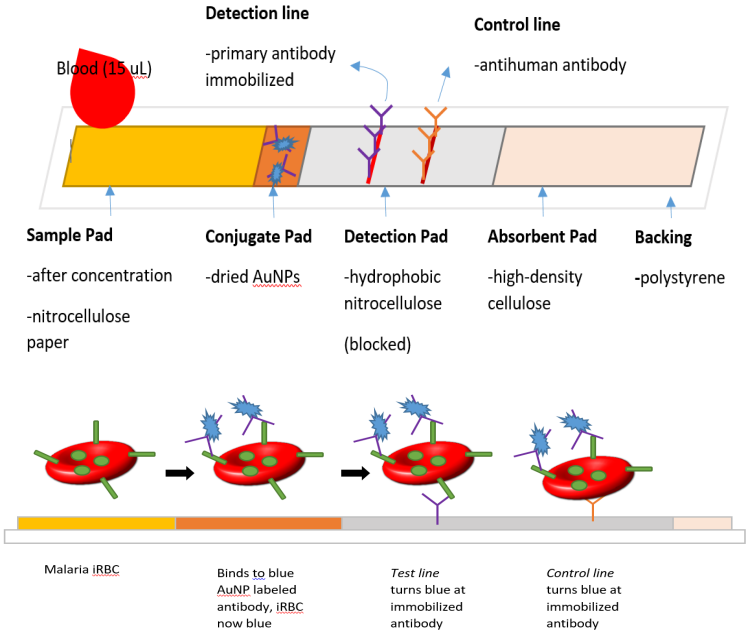
This design would incorporate lateral flow immunoassays which are depicted below...



In short the blood sample would be placed at sample pad end and flow laterally to the right, as depicted, through the structures shown in the picture above.

The gold nanoparticles contain primary antibodies that can be created to bind with a specific malaria antigen, so when the blood sample flows through the gold conjugate pad, only the iRBCs will be attached to these gold nanoparticles. Traveling down the nitrocellulose membrane the bound AuNPs and iRBCs will be captured on specific test lines whereas the normal RBCs will pass smoothly through the entire device and collected in the absorbing pad. The gold conjugate pad will contain dried gold nanoparticles, but these particles will by hydrated when they make contact with the blood sample. If a line appears at the capture line specific to that species of malaria, the blood sample contains that strain of malaria. If no lines show up, the blood is clean.

Another diagram of how this reaction works is shown below...



Pros:

- This is a demonstrated method in other areas of detection so it definitely works, however, it will be unique to detecting malaria parasites
- Low detection time
- High degree of accuracy
- Every capture line can be specific to a different species of malaria

Cons:

- Expensive design without mass production
- There may be fabrication difficulties when trying to create this design

Conclusions/action items:

The gold nanoparticle design may be difficult to construct, however, the fact that it would be able to detect all four strains of malaria at once makes it a very considerable option.

More research needs to be done on what antigens and antibodies to use and how to fabricate this design. Finally, this design needs to be examined by the design matrix.

Design Matrix Criteria

▪ Joshua Liberko ▪ Oct 19, 2016 @04:13 AM CDT

Title: Design Matrix Criteria

Date: 10/18/2016

Content by: Josh Liberko

Present: Entire Team

Goals: Generate a list of criteria and rank it for use in the design matrix

Content:

Design matrix criteria:

- **Sensitivity/Specificity > 95% (25%):**
  - *Separation:* The device should effectively separate 80% of infected RBCs into separate channels and should allow minimal passage of any uninfected RBCs into these channels.
  - *Detection:* The detection method should be 95% effective in labeling the concentrated portals of infected RBCs. It would ideally detect all strains of malaria.
- **Equipment Free / Portable / Ease of Detection (20%):**
  - *Separation:* The method of separation should be able to be transported long distances and be provided in a device smaller than a personal computer. Preference would be for the device to not need power.
  - *Detection:* The detection method should be able to use the naked eye or a cheap and easy to use device in order to obtain an accurate reading of the state of the disease.
- **User Friendly (20%):**
  - *Separation:* A small amount of sample and easy to handle reagents are necessary in the resource limited regions where this device would be implemented. Collection of sample should be safe for both the patient and the technician. Also should not require extensive training to be able to run many tests.
  - *Detection:* The form of detection should be able to be monitored on site by a modestly trained technician, without the use of high tech laboratory equipment. A simple protocol for the type of detection would be provided.
- **Ease of Fabrication (10%):**
  - Due to resource restrictions in Jimma at Jimma University, as well as the developing world in general, the microfluidic device protocol for fabrication should be relatively straightforward and not require high tech resources to produce. The design also needs to be able to be mass produced in order to be an effective POC diagnostic device.
- **Cost (10%):**
  - The projected cost per device once the production is optimized should be less than $5.
- **Time < 1 Hour (10%):**
  - *Separation:* The amount of time it will take to run the blood sample through the concentration device should be less than 20 minutes.
  - *Detection:* The total time it would take for a patient to be diagnosed needs to be less than one hour.
- **Versatility (5%):**
  - *Separation:* The ability for the concentration device to separate infected RBCs, specifically the malaria parasite, from healthy RBCs. It would be prefered for the device to be able to separate different strains of malaria or different diseases, but we will focus on the Plasmodium falciparum parasite which is specific to malaria.
  - *Detection:* The ability to detect different strains of malaria parasites or different diseases with minor alterations to our final design would be useful. Patients presenting with mixed symptoms would be able to use the same device to test for multiple diseases.

Conclusions/action items:

We need to now evaluate each design according to this criteria to help us decide which design idea to pursue.

Design Matrix Evaluation

▪ Joshua Liberko ▪ Oct 19, 2016 @09:15 AM CDT

Title: Design Matrix Evaluation

Date: 10/18/16

Content by: Josh Liberko

Present: Agreed on by all teammates

Goals: To determine the best separation device and the best detection method.

Content:

Here is the final design matrix...

Design	*Separation*						*Detection*					
Criteria (weight)	**Cell Deformation**		**Magnetic Searation**		**Electric Separation**		**BinaxNOW**		**PS Beads**		**GNPs**	
Sensitivity (25)	3	15	5	25	4	20	5	25	4	20	5	25
Equipment Free/Usable in Field/Intuitive (20)	5	20	4	16	3	12	3	12	4	16	5	20
Userfriendly (20)	5	20	5	20	3	12	4	16	4	16	5	20
Time (10)	2	4	4	8	4	8	4	8	5	10	4	8
Cost (10)	5	10	4	8	3	6	1	2	5	10	3	6
Ease of Fabrication (10)	4	8	4	8	3	6	5	10	3	6	2	4
Versitility (type of Malaria or other diseases) (5)	2	2	2	2	2	2	3	3	1	1	5	5
Total	79		87		66		76		79		88	

Upon evaluation of the separation methods, the magnetic separation technique proved to score the highest. In the category with the most weight, Sensitivity (25%), the magnetic separation method scored highest by a substantial amount compared to cell deformation and electric separation. The magnetic separation also scored or tied for the most points in user-friendliness, time, ease of fabrication, and versatility. The cell deformation column accumulated the most points in being equipment free, user-friendliness, cost, ease of fabrication, and versatility. Finally, the electric separation method only tied for the most points in the time and versatility categories. Both the magnetic and cell deformation scored the highest in five categories, however, the magnetic separation won by a substantial amount in the sensitivity category. Since the magnetic separation method scored the highest of the three it will be the idea that will be advance to the fabrication process.

With adherence to the detection methods, diagnosing with gold nanoparticle appears to be the winning idea. The AuNPs scored higher than the BinaxNOW and the polystyrene beads in equipment free, user-friendly, and versatility categories. The BinaxNOW proved to be the easiest to fabricate because the product already exists so a similar construction would just have to be copied. The polystyrene beads acquired the most points compared to the other designs in the time and cost categories, as polystyrene beads are relatively cheap and when bonded with the malaria antigens would form large conjugates in the blood sample that can be scene by the naked eye, therefore reducing the amount of time to diagnose. The gold nanoparticles design scored the highest in the design matrix, so it will be the detection method that first be tried in the fabrication process.

Conclusions/action items:

To form a final design based on the magnetic separation and gold nanoparticle detection and begin fabrication and prototyping based on that design.

Final Design Idea

▪ Joshua Liberko ▪ Oct 19, 2016 @10:06 AM CDT

Title: Final Design Idea

Date: 10/18/16

Content by: Josh Liberko

Present: Me

Goals: To describe, based off the design matrix which designs will be the most applicable to our design specifications.

Content:

The final design will be a combination of the magnetic separation design and the gold nanoparticles design. These two methods combined will be able to produce accurate results in detecting the multiple different strains of malaria. The separation method will include a PDMS channel and epoxy (or some similar type of material) mold of the loading well and separation wells. A strong neodymium magnet will be placed on one side of the channel with smaller magnets or ferromagnetic wires. This will orient the magnetic field to properly separate the iRBCs from the normal RBCs. The gold nanoparticle detection portion will then be attached the well holding the iRBCs. Using lateral flow immunoassay (LFIA), the nitrocellulose paper that the iRBCs in the separated well are in would basically wick the cells down the detection pad. This pad would contain dried gold nanoparticles that are manufactured with malaria detecting antibodies. These primary antibodies can be created to be specific to a specific malaria antigen and would be printed on separate lines following the conjugate pad. So, in short, the iRBCs will be separated using magnets, the collected blood sample containing the iRBCs will then travel down a conjugate pad moistening the dried gold nanoparticles, the gold nanoparticles would then attached to a specific malaria antigen stopping and conjugating them at specific malaria antigen lines, and the excess runoff would collected by an absorbent pad.

Conclusions/action items:

The design team will need to begin prototyping and fabrication asap, and will also need to look into the specific materials, like the magnets, that will need to be ordered.

Individual Notes Initial Client Meeting

▪ Joshua Liberko ▪ Oct 06, 2016 @11:49 AM CDT

Title: Individual Notes on Initial Client Meeting

Date: 9/14/16

Content by: Josh Liberko

Present: All group members

Goals: To determine what our project is about, who it is for, and what our initial steps will be to move forward.

Content:

Dr. Padraic Casserly: a UW-Madison Alumni who majored in BME-tissue engineering. Also did some work with Engineers Without Boarders which is how he became interested in helping people in third world countries, like Ethiopia.

Dr. Amit Nimunkar (collaborator): introduced us to Dr. Casserly and kept our meeting running smoothly. Will be a good contact for questions about the project.

Dr. Tim Kwa (Primary Client): acquired his PhD at UC-Davis in BME, focusing on point of care diagnostics (microfluidics).

Background on Ethiopia and Jimma University: Jimma has approximately 250,000 people in the SW, Jimma University was founded in the 50s and holds the first BME program in the country. The hospital associated with the University is struggling in a resource perspective. It has very poor conditions, is out of date, and is poorly maintained. 3G internet is recently added to the town, but the city is showing rapid progress in all fronts.

Jimma Projects:

- Water Distillation Device- surgeons sterilize devices using and auto-clave and are supposed to use DI water but they lack DI water so they have been using tap water instead. However, tap water leaves minerals behind which is ruining their devices.
- Kidney Health Analysis Device- low cost

Dr. Tim Kwa's Project- wants to set up a lab or facility with low cost tests and diagnostics.

Resources available:

- A big challenge is access to internet as it is recently available and therefore has frequent internet and electricity blackouts, especially in the months of May-September.
- DI water is insufficient
- Low amount of tools
- Small prototyping lab

Funding: Bill Gates Foundation in Ethiopia. There are also many other funding companies that we can look into.

Questions:

- What diseases should we be focusing our research on and testing for?
    - Malaria and others like HIV is possible
- With electricity as a problem how should we power our equipment?
    - Battery powered operation or a battery backup system would be a good solution
- What equipment do they have at their prototyping facilities?
    - Multi-meters, hammers, nails, and 2-D printers...

We want to make our design easy to translate the knowledge so the people in Ethiopia can actually use it.

Conclusions/action items:

A major difficulty of this project is going to be communicating with Dr. Kwa and they frequently lose access to the internet.

Steps we need to take:

- Figure out testable diseases
- Generate a second, more specific, list of questions for Dr. Kwa
- Read the BB lab webpage ==> lab general info ==> physics/micro-fluids info.
- Read publications from Dr. Kwa
- Read about other projects on point of care technology

BPAG Notes 10/7/16

▪ Joshua Liberko ▪ Oct 07, 2016 @12:19 PM CDT

Title: BPAG Meeting Notes 10/7/16

Date: 10/7/16

Content by: Josh Liberko

Present: Me

Goals: To figure out the BPAG role and the work it requires

Content:

Best thing to do would be to get the client to make a purchase for you. Otherwise you buy it and get reimbursed.

All Clients:

- Directly by personal check, credic car, or cash

UW Based Clients:

- UW credit card, (aka ProCard, PCard...
- Shop@UW (aka MDS) or DoIT accounts.
- Provide CoE Student Shop with their**funding information**

Vendors Available to UW Clients

- Shop@UW Complete list of vendors- http://www.bussvc.wisc.edu/shopuw/vendors.html

Reimbursement:

- BPAG Person is the only one that can get reimbursed for the team.
- E-Reimbursement from UW-Clients
    - BPAG must provide original, hard copy receipts of their purchase.

General Notes:

- No purchases from McMaster Carr
- Save all original receipts. Seek reimbursement monthly
- Talk to Susan Sauer (BME Accountant) with questions about purchases.

Conclusions/action items:

I don't think we will need to purchase anything.

2016_9_23 - Malaria Diagnoses

▪ James Jorgensen ▪ Sep 23, 2016 @11:36 AM CDT

Title: Malaria Diagnoses

Date: 9/23/16

Content by: James

Present: NA

Goals: Research biological markers of malaria, how they can be indicated, and their properties

Content:

- https://www.cdc.gov/malaria/diagnosis_treatment/diagnosis.html
  - Malaria parasites can be seen underneath a microscope using a stain known as Giemsa stain. This is widely claimed as the gold standard of lab confirmation of malaria. Relies heavily on quality of stain and technician ability.
  - Antigen detection is also very prominent, and dipstick tests can give results in as little as 2-15 minutes. Some problems remaining are accuracy, cost and quality performance under adverse testing environments.
  - Serology is the diagnosis of blood serum, and is used to detect malaria via the antigen plasmodium. The homologous antibody is introduced and will form a complex with the antigen if they are present. A fluorescent label is then added and will attach to the complex and can be examined with a fluorescent microscope. Serology does not detect current infection, only previous exposures.
- http://ijs.sagepub.com/content/15/3/292.long
  - Giemsa Stain
  - Requires microscope and advanced technician abilities
  - Marks the parasite in malarial blood
- Look into how long it takes malarial antibodies to build up in the blood.

Conclusions/action items:

IFA (Immunoflouresense) and ELISA can only determine if the malarial antibody is in the blood, so more research needs to be done on how long it takes to build up the antibodies in the blood before this method can be delved into more deeply. Giemsa stain could contribute benefits if we can incorporate some sort of fluorescence detection capabilities within the design.

2016_10_3 - Blood Content of Malaria

▪ James Jorgensen ▪ Oct 12, 2016 @11:21 PM CDT

Title: Blood Content of Malaria and magnetic properties Research

Date: 10/3/16

Content by: James

Present: NA

Goals: Learn more about how red blood cells of people infected with malaria differ from regular red blood cells, and interesting characteristics of blood with the parasite

Content:

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3699179/ - Malaria and Human Red Blood Cells, Mohandas and An 2012
  - RBCs of people with malaria are typically more rigid, adhesive to endothelial substrates, and increases permeability to ions and other species.
  - Infected RBCs are adhesive to both other infected RBCs and **non infected RBCs**
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4039013/ - Iron, anemia and hepcidin in Malaria, Spottiswoode et al. 2014
  - Plasmodium proliferation in the liver developmental stage and the disease-associated phase requires iron, and iron chelators have been shown to inhibit pathogen growth in animal models.
  - It is yet unknown how plasmodium parasites get the iron that they need to proliferate
- http://www.sciencedirect.com/science/article/pii/S0925443908002238 - Magnetic susceptibility of iron in malaria-infected red blood cells, Hackett et al. 2009
  - Malaria parasites catabolize up to 80% of cellular haemoglobin
  - High grade magnetic gradients have been used to extract deoxygenated erythrocytes from whole blood as well as cells containing adult stage parasites.
  - Moore et al. demonstrated that cells containing sufficient methaemoglobin concentrations[8] or containing mature parasites [9] will migrate in the direction of increasing field strength, indicating that these cells contain paramagnetic iron species.
  - Malaria parasites penetrate erythrocytes and digest haemoglobin as a main source of amino acids. Heme is released and oxidized to hemetin
  - Study used magnetic fractionation to differentiate the normal RBCs from those containing the parasite
  - Were able to extract parasites after fractionation by a mean value of 22 fold vs unfractioned blood
  - Magnetic susceptibility of fractioned cells is 6 times higher than unfractioned cells
  - the magnetic susceptibility of mature cells increases most likely because of digestion of haemoglobin rather than the detoxification of haem
- http://news.mit.edu/2014/new-method-diagnose-malaria-0831
  - When parasites infect RBCs, they feed on hemoglobin, break it down into hemozin (Detectable with magnets)
  - Can do this test in under 1 minute, for less than 10 cents
  - Uses 0.5 tesla magnet
  - Spins down the blood first in order to concentrate red blood cells
  - http://scitation.aip.org/content/aip/journal/rsi/83/9/10.1063/1.4754296
    - Paper describing the setup of their magnetic filtration device.
- http://malariajournal.biomedcentral.com/articles/10.1186/1475-2875-9-17
  - Uses neodynium earth magnets to explore magnetic fields and the relationship between recovery of infected RBCs
  - This design worked better than other commercially available devices

Conclusions/action items:

Based off of this research done, the feasibility of using magnets to separate infected RBCs from normal RBCs is high. In order to learn more about the types of magnets to use and how they work, research on magnetic field strength as well as how strongly hemozin will be effected by said magnets. More research will need to be done on magnetic fractionation.

2016_10_19 - Hemozoin Research

▪ James Jorgensen ▪ Oct 19, 2016 @11:55 AM CDT

Title: Hemozoin Detection Research

Date: 10/19/16

Content by: James

Present: NA

Goals: Learn about the specifics of how hemozoin can be used to help diagnose malaria

Content:

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4049529/ - Malarial Hemozoin: From Target to Tool (Coronado et al. 2014)
  - Hemoglobin is the principal component of red blood cells, composing approximately 95% of the proteins of the cytosol
  - During the intra-erythrocytic stages of malaria infection, up to 80% of the cytoplasm of the host is consumed
  - IR spectroscopy methods have outlined the composition and structure of hemozoin
    - Structure - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4049529/figure/F3/
  - Paramagnetism in hemozoin arises from the unpaired electrons of the *Fe3+* species of iron
  - The magnetic moment value for hemozoin in 5.1 Bohr magneton units
  - When exposed to a magnetic filed of 1 T, it took only 100 us for the paramagnetic molecules to line up
  - In order to line up, the field strength must be strong enough to overcome thermal diffusion
  - Due to the rod shape of hemozoin, it is twice as magnetically susceptible than spherical paramagnetic molecules

Conclusions/action items:

The magnetic separation methods of iRBCs using hemozoin as a marker are broadly outlined, and soon a magnetic field equation can be created for specific use in our design.

2016_9_18 - POC Research

Title: POC Device Research Notes

Date: 9/18/16

Content by: James Jorgensen

Present: NA

Goals: Familiarize myself and learn more about current microfluidic point of care devices that are being produced

Content:

- Microfluidics-based diagnostics of infectious diseases in the developing world (Chin et al, 2011)
  - Many current microfluidics based diagnostic techniques require large, clinical based tests that are ill suited for POC testing in underdeveloped areas of the world
  - Trying to decrease the price of creating high-throughput manufacturing of microfluidic cassettes. Injection molding is more scalable and cheaper than other techniques such as glass, silicon, and PDMS.
  - Automated delivery of multiple reagents for multistep reactions, Introduced via ELISA to add 14 different reagents at different times without any electricity or external instrumentation. Use bubble-based method of delivery that requires workers to manually introduce the reagents.
  - Signal amplification and detection using minimal instrumentation. ELISA used with silver and gold nanoparticles lead to cheap and easy signal detection.
  - Testing of this new device in Rwanda had a clinical completion time of 15 minutes for HIV detection, whereas current methods in Rwanda had turn around times of up to weeks.
- Point of care testing: The impact of nanotechnology (Syedmoradi et al, 2016)
  - Goal is to create a small and portable device that allows for assay of many different analytes.
  - Dipsticks and paper based testing have increased in relevance over the last few years. They can be used to test for a wide range of analytes due to simple design and manufacturability.
  - Lateral flow tests utilize capillary flow. Usually these types of test require simply a very small amount of liquid and time for the detection and analysis of the analyte.
- Advances in microfluidics in combating infectious diseases (Tay et al, 2016)
  - Malaria leads to infected RBCs that disrupt microcirculation resulting in anemia and organ failure. Current gold standard test for malaria is using a microscopic blood smear (too heavily relies on microscope quality and skill of technician)
  - Can also be detected through hemozin detection. Hemozin is converted by malaria parasite from degraded product of hemoglobin (heme). Can be problematic due to detection levels in small quantities and early on during stages of malaria.
  - Look into Rapid diagnostic tests
  - PCR can be used with microfluidics in two ways: Stationary and continuous flow. (Main difference is in when and where the fluid is heated and cooled)
  - Look into magnetic testing (Not yet has much been done)

Conclusions/action items:

Try and find more information on different ways that malaria could be detected. Incorporate ideas from current designs into our design while brainstorming. Try and find papers that have looked into magnetic testing in terms of identifying malaria. Share all of this information and research articles with the rest of the team.

2016_10_5 - Magnetic Fractionation Research

▪ James Jorgensen ▪ Oct 05, 2016 @12:24 PM CDT

Title: General Magnetic Fractionation Research

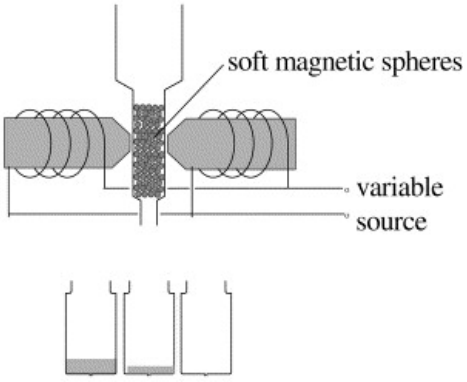
Date: 10/5/16

Content by: James

Present: NA

Goals: Learn more about magnetic fractionation and how it works

Content:

- http://www.sciencedirect.com/science/article/pii/S030488530000439X, Magnetic Fractionation of magnetic fluids Rheinlander et al. 2000
  - Properties of magnetic nanoparticles show a wider range of properties and a broad distribution, and therefore only a small amount of particles contribute to the desired field
  - Magnetic methods thus far have only been used to separate aggregate (magnetic filtration)
  - magnetic fractionation separates magenetic nanoparticles based on their magnetic moments (proportional to core volume)
  - 

    - Experimental setup used in this study for magnetic fractionation. Iron spheres were used. Magnetic fluid was poured into the column at highest magnetic flux density, was then washed with aqueous solution until collected feed-through was almost colorless (IE no magnetic particles coming through). This procedure was repeated until the magnetic flux density was taken down to 0, and no fluid was remaining unfractioned.

Conclusions/action items:

Look more into magnetic filtration techniques, as this seems to be the process by which we would like our magnetic separation prototype to follow. More research should be done on on the fractionation of blood, and techniques used to do it.

2016_10_5 - Magnetic Filtration of Malaria Infected Blood

▪ James Jorgensen ▪ Oct 07, 2016 @12:51 PM CDT

Title: Magnetic Filtration of Malaria Infected Blood

Date: 10/5/16

Content by: James

Present: NA

Goals: Learn more about current methods of separating iRBCs from whole blood using magnets

Content:

- http://www.cmu.edu/news/stories/archives/2016/june/malaria-magnetic-treatment.html
  - Prototype that uses a dialysis like function to separate iRBCs out of whole blood, and remove them from the individual, before placing the blood back into the body.
  - Design implements many small magnets instead of one large magnet to increase magnetic field.
  - Able to separate 20% of infected cells on the first pass alone.
  - This design is currently under further prototyping and development.
- http://www.europeanpharmaceuticalreview.com/34040/news/industry-news/magnetic-blood-filter-to-tackle-drug-resistant-malaria/
  - Initial testing shows that MediSieves magnetic filtration device could potentially remove 90% of infected cells from a person infected with the parasite.
  - Similar to dialysis, passes through an external loop
  - Regular treatments with this device could help keep malaria at bay indefinitely if treatments are continued
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3278042/
  - Many factors contribute to how effective magnetic filtration devices are at removing infected RBCs (Magnetic pole pitch array, chamber height, flow rate, etc.)
  - This paper models iRBCs as paramagnetic particles suspended in Newtonian fluid
    - Intensely complicated mathematical models used (First order stiff differential equations)
  - Severe malaria = >5% infected RBCs
  - Magnetic field applied with a stationary 0.3 Tesla magnet adjacent to an array of micro ferromagnetic structures
    - 1 T = V*s/m^2 (V = Volt, s = seconds, m =meters)

Conclusions/action items:

The feasibility of this type of design is highly feasible. More information on types of magnets to use, as well as learning about the math behind particles in fluid need to be done.

2016_10_11-Magnetic Filtration General Information

▪ James Jorgensen ▪ Oct 19, 2016 @11:15 AM CDT

Title: Magnetic Filtration General Information

Date: 10/11/16

Content by: James

Present: NA

Goals: Learn more about how to diagram magnetic filtration and how to orient magnetic fields

Content:

- http://www.sciencedirect.com/science/article/pii/0304885384900507 - Magnetic filtration of particles in laminar flow through a bed of spheres
  - Particles less than 200 um in diameter, inertial and gravitational forces are negligible
  - Viscous drag force is modeled via stokes' law
    - $f_d = -6\pi\eta r_p(v - v_f),$
  - For a small particle immersed in a fluid, the force experienced due to a magnetic field H is:
    - $f_m = (2\pi/3) r_p^3 \mu_0 (\chi_p - \chi_f) \nabla H^2.$
    - $X_p > X_f$ =paramagnetic (IE Hemozin in iRBCs)
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4049529/ - Malarial Hemozoin: From Target to Tool (Coronado *et al.* 2014)
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3205221/ - Rare Cell Separation and Analysis by Magnetic Sorting (Zborowski *et al.* 2011)

Conclusions/action items:

Research the two articles listed above for further, more useful information

2016_10_12-Existing Magnetic Filtration Device Description

▪ James Jorgensen ▪ Oct 19, 2016 @11:14 AM CDT

Title: Existing Magnetic Filtration Device Description

Date: 10-12-16

Content by: James

Present: NA

Goals: Describe a magnetic filtration device that has been designed by a team at MIT

Content:

- http://scitation.aip.org/content/aip/journal/rsi/83/9/10.1063/1.4754296 - Development of a miniature, portable magnetic resonance relaxometry sytem for point-of-care diagnostic systems (Peng *et al.* 2012)
  - (Abstract): A novel, compact-sized (19 cm × 16 cm) and portable (500 g)magnetic resonance relaxometry system is designed and developed. We overcame several key engineering barriers so that magnetic resonance technology can be potentially used for disease diagnosis-monitoring in point-of-care settings, directly on biological cells and tissues. The whole system consists of a coin-sized permanent magnet (0.76 T), miniaturized radio-frequency microcoil probe, compact lumped-circuit duplexer, and single board 1-W power amplifier, in which a field programmable gate array -based spectrometer is used for pulse excitation, signal acquisition, and data processing. We show that by measuring the proton transverse relaxation rates from a large pool of natural abundance proton-nuclei presence in less than 1 μL of red blood cells, one can indirectly deduce the relative magnetic susceptibility of the bulk cells within a few minutes of signal acquisition time. Such rapid and sensitive blood screening system can be used to monitor the fluctuation of the bulk magnetic susceptibility of the biological cells (e.g., human blood cells), where unusual state of the bulkmagnetic susceptibility is related to a number of diseases.
  - This design is rather similar to ours, so it would be beneficial to research the equations and methods that they used to orient the magnets that they implemented.
  - This design appears to be use an electronic diagnosis method, which would distinguish our design from it, which will use gold nanoparticles to diagnose the disease in a more cheap and POC idealized manner.

Conclusions/action items:

A free version of the article should be found for further research.

2016_10_7 - Separation: Magnetic Design

▪ James Jorgensen ▪ Oct 19, 2016 @02:44 AM CDT

Title: Magnetic Separation Device Initial Drawup

Date: 10/7/16

Content by: James Jorgensen

Present: NA

Goals: Draw a rough, initial sketch of the magnetic separation design idea for the design matrix and preliminary presentation

Content:

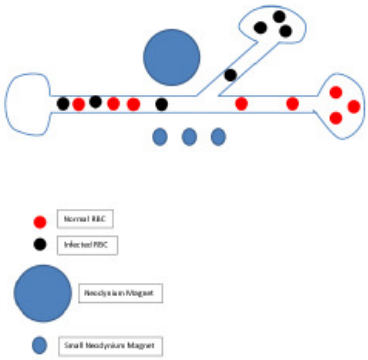
This design works using the unique physical properties of infected red blood cells. When a cell becomes infected with the malarial parasite hemoglobin is metabolized into heme, and then finally hemozoin. Hemozoin is a paramagnetic crystallite, while red blood cells are diamagnetic. This means that when they are exposed to an electric field they will align facing opposite directions. If a magnetic field is applied to a mixture of iRBCs and normal RBCs, they can be separated based on magnetism.

See Attachment

Conclusions/action items:

Fine tune this design by determining the size constraints of the chambers and capillaries, as well as the size and strength of magnets.

▪ James Jorgensen ▪ Oct 07, 2016 @01:15 PM CDT



Magnetic_Diagram.pdf(97.7 KB) - download

2016_10_19 - Separation: Cell Deformation Design

▪ James Jorgensen ▪ Oct 19, 2016 @02:48 AM CDT

Title: Cell deformation separation design

Date: 10/19/16

Content by: James, adapted from Austin Feeney

Present: NA

Goals: Document design of cell deformation separation

Content:

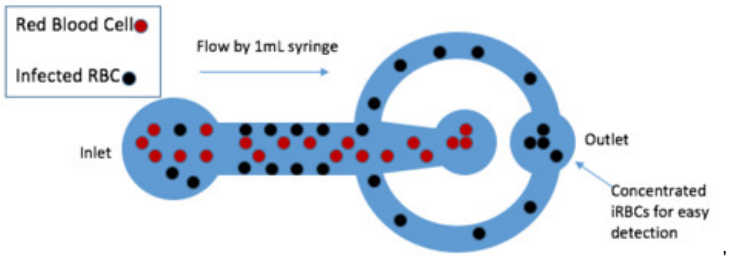
When a red blood cell becomes infected with the malarial parasite, they exhibit different physical properties, such as becoming less deformable, more stiff, and more adhesive to surfaces. If a blood mixture of iRBCs and normal RBCs are ran through a long and narrow channel, the iRBCs will attach to the sides of the channel and be able to separate the infected cells from the normal cells via this attachment. The infected cells could then be collected in a separate well stemming from two branched arms from the main channel for easier detection.

See attached image

Conclusions/action items:

Fine tune the dimensions of this image as well as determine a higher degree of feasibility for the design.

▪ James Jorgensen ▪ Oct 19, 2016 @02:49 AM CDT



1476841517642.png(37.4 KB) - download

2016_10_19 - Separation: Electrical Design

▪ James Jorgensen ▪ Oct 19, 2016 @03:05 AM CDT

Title: Electrical Separation Design

Date: 10/19/16

Content by: James, adapted from Josh Liberko

Present: NA

Goals: Present the design idea for electrical separation

Content:

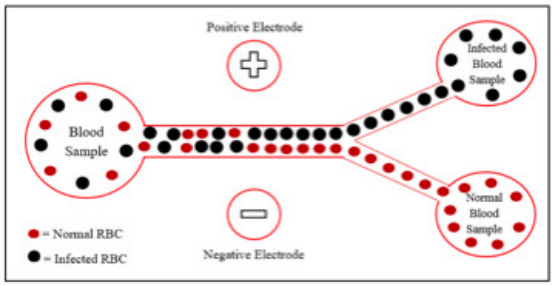
The electrical separation design is based off of the increased conductivity of iRBCs as compared to normal RBCs. Due to this increased conductivity, when an electrical current is run near the channel containing the blood sample the iRBCs will be drawn towards the + current, while the normal RBCs would be driven towards the negative current. This would allow for the two different groups of RBCs to be collected in separate chambers.

See attached image

Conclusions/action items:

More research needs to be done on the exact amount of electricity that would be required in order to proceed with the feasibility of this design, as access to electricity in rural areas such as Ethiopia is limited.

▪ James Jorgensen ▪ Oct 19, 2016 @02:49 AM CDT



147684226673.png(34.7 KB) - download

2016_10_19 - Detection: BinaxNow

▪ James Jorgensen ▪ Oct 19, 2016 @10:25 AM CDT

Title: BinaxNow Improvement Detection Design

Date: 10/19/16

Content by: James, adapted form Hunter Johnson

Present: NA

Goals: Explain the BinaxNow design

Content:

The BinaxNow is a currently marketed malaria detection device that is used in POC applications. The largest downfalls of this design are that it requires a rather high blood concentration of parasites (5000/uL blood) in order to detect malaria and positively diagnose it. It is also very expensive, especially considering that it is only a one time use device. If paired with a successful concentration device oulined in this notebook, the likelihood of detection could increase.

Conclusions/action items:

Research more on if prices could be dropped, otherwise this design is far too expensive.

2016_10_19 - Detection: Polystyrene Beads

▪ James Jorgensen ▪ Oct 19, 2016 @10:52 AM CDT

Title: Polystyrene Bead Detection Design

Date: 10/19/16

Content by: James, adapted from Zach Hite

Present: NA

Goals: Outline the polystyrene bead detection design

Content:

The polystyrene bead detection method utilizes a antibody-antigen interaction where small beads are conjugated with antibodies that bind to the *Plasmodium* antigens. Once forming a complex the beads will continuously bind to other bead-antigen complexes and form a large, visible aggregate (Immunoaggulation). Dried beads would be coated with the antibody and activated via hydration prior to blood interaction. This is a rapid diagnosis method as it would only take approximately 2 mins to diagnose a positive result.

See attached image

Conclusions/action items:

Research the conjugation method for polystyrene beads

2016_10_19 - Detection: Gold Nanoparticles

▪ James Jorgensen ▪ Oct 19, 2016 @10:43 AM CDT

Title: Gold Nanoparticle Detection Design

Date: 10/19/16

Content by: James, Adapted from Hunter Johnson

Present: NA

Goals: Outline the Gold Nanoparticle Design

Content:

The gold nanoparticles detection design utilizes lateral-flow immunassay (LFIA) in order to detect the antigens present on the iRBCs. The medium is a cellulose based paper conjugated with antibodies specific to different *Plasmodium* antigens. The nanoparticles change the color of the antigen-antibody complex when they bind which allows for easier detection by the user.

The primary antibody would either be histidine-rich protein 2 (HRP2), found in the*P. falciparum* malaria parasite, coupled with pan-malaria antigen, which targets all four species.

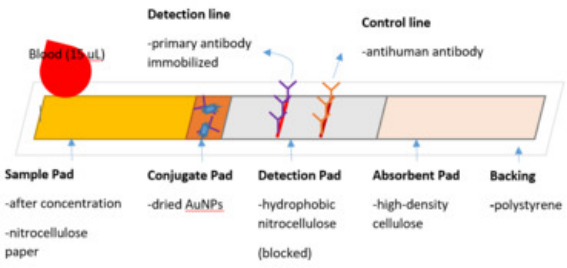
- These antibodies are used in the current BinaxNOW malaria test with:
- § Sensitivity for P. falciparum : 99.7%
  § Specificity for P. falciparum : 94.2%*
  § Sensitivity for P. vivax : 93.5%
  § Specificity for P. vivax : 99.8%
- This test currently needs levels of >5,000 parasites/uL
- Once mass produced this design could cost as little as $1 to produce

See attached image

Conclusions/action items:

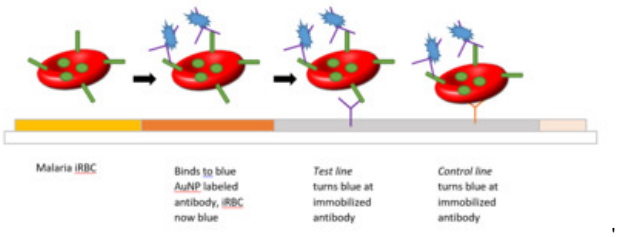
Research conjugation methods as well as different antibodies that could be used.

▪ James Jorgensen ▪ Oct 19, 2016 @10:44 AM CDT



AuNP_LFIA_Design_Diagram.PNG(45.2 KB) - download

▪ James Jorgensen ▪ Oct 19, 2016 @10:44 AM CDT



Mechanism_of_AuNP_LFIA_Design.PNG(82.2 KB) - download

2016_10_19 - Design Matrix Criteria

▪ James Jorgensen ▪ Oct 19, 2016 @03:11 AM CDT

Title: Design Matrix Criteria

Date: 10/19/16

Content by: James, adapted from group work

Present: NA

Goals: Outline the criteria categories for the design matrix

Content:

Sensitivity/Specificity > 95% (25%):

Separation: The device should effectively separate 80% of infected RBCs into separate channels and should allow minimal passage of any uninfected RBCs into these channels.

Detection: The detection method should be 95% effective in labeling the concentrated portals of infected RBCs. It would ideally detect all strains of malaria.

Equipment Free / Portable / Ease of Detection (20%):

Separation: The method of separation should be able to be transported long distances and be provided in a device smaller than a personal computer. Preference would be for the device to not need power.

Detection: The detection method should be able to use the naked eye or a cheap and easy to use device in order to obtain an accurate reading of the state of the disease.

User Friendly (20%):

Separation: A small amount of sample and easy to handle reagents are necessary in the resource limited regions where this device would be implemented. Collection of sample should be safe for both the patient and the technician. Also should not require extensive training to be able to run many tests.

Detection: The form of detection should be able to be monitored on site by a modestly trained technician, without the use of high tech laboratory equipment. A simple protocol for the type of detection would be provided.

Ease of Fabrication (10%):

Due to resource restrictions in Jimma at Jimma University, as well as the developing world in general, the microfluidic device protocol for fabrication should be relatively straightforward and not require high tech resources to produce. The design also needs to be able to be mass produced in order to be an effective POC diagnostic device.

Cost (10%):

The projected cost per device once the production is optimized should be less than $5.

Time < 1 Hour (10%):

Separation: The amount of time it will take to run the blood sample through the concentration device should be less than 20 minutes.

Detection: The total time it would take for a patient to be diagnosed needs to be less than one hour.

Versatility (5%):

Separation: The ability for the concentration device to separate infected RBCs, specifically the malaria parasite, from healthy RBCs. It would be prefered for the device to be able to separate different strains of malaria or different diseases, but we will focus on the Plasmodium falciparum parasite which is specific to malaria.

Detection: The ability to detect different strains of malaria parasites or different diseases with minor alterations to our final design would be useful. Patients presenting with mixed symptoms would be able to use the same device to test for multiple diseases.

Conclusions/action items:

These criteria have been assigned and referenced to each design and the designs have been weighted based upon the judgement of the team members.

2016_10_19 - Design Matrix Evaluation

Title: Design Matrix Evaluation

Date: 10/19/16

Content by: James, adapted form team collaboration

Present: NA

Goals: Assign ranks and values of every criteria for each design in order to quantitatively determine which design best suits the product design specifications

Content:

Design	*Separation*						*Detection*					
Criteria (weight)	Cell Deformation		Magnetic Separation		Electric Separation		BinaxNOW		PS Beads		GNPs	
Sensitivity (25)	3	15	5	25	4	20	5	25	4	20	5	25
Equipment Free/Usable in Field/Intuitive (20)	5	20	4	16	3	12	3	12	4	16	5	20
Userfriendly (20)	5	20	5	20	3	12	4	16	4	16	5	20
Time (10)	2	4	4	8	4	8	4	8	5	10	4	8
Cost (10)	5	10	4	8	3	6	1	2	5	10	3	6
Ease of Fabrication (10)	4	8	4	8	3	6	5	10	3	6	2	4
Versatility (type of Malaria or other diseases) (5)	4	4	2	2	2	2	3	3	1	1	5	5
Total	81		**87**		66		76		79		**88**	

For the separation techniques the magnetic separation method scored highest because the magnetic properties of cells are specific to the diseased state in all four stages. Research shows that the cell deformation method would separate any cells with different surface properties than uninfected red blood cells, where diseases such as diabetes would interfere with results.The cell deformation design scored highest in ease of use and fabrication as it would consist solely of PDMS that could be manufactured in bulk off site and transported in. The electric separation scored the lowest in this category due to the requirement of electricity and batteries, which would complicate the process as a POC device. To be user friendly the method must be able to concentrate cells without training, the electric separation method doesn't pass this test because tuning the electrodes for optimal separation would take constant supervision. Even though the magnetic separation design requires a complex layout of magnets, a base can be designed for simple placement and instruction for placement of said magnets. Cell deformation scored the lowest for time because the small channels require a low flow rate of liquid that is not as fast as capillary action in the other designs. Lastly for ease of fabrication the device must be able to be fabricated and assembled on site, the magnetic separation only had one downfall which was figuring out the placement of the magnets for optimal cell separation, where the cell deformation design needed a very thin channel to be fabricated out of PDMS which could pose problems in the fabrication process. The best separation method was deemed to be the magnetic design, it did the best in four out of six of the criteria and second best in the other two, thus this design will be pursued further.

The detection methods were evaluated with the same criteria as the separation techniques. Regarding sensitivity, BinaxNOW is the top performer, with over 99% accuracy. Gold nanoparticles have the possibility to be just as sensitive if properly conjugated and printed onto an ideal cellulose medium, if they use the same antibodies, so they received the same rating. Gold nanoparticles also scored highly in ease of use and user-friendliness as it can be read in 15 min by looking for colored bands without the requirement of any lab equipment. The polystyrene bead design is the top performer in time and cost because the test takes two minutes to finish and is made from the cheapest and easiest material to fabricate, excluding the fact that the BinaxNOW comes pre-assembled. The major drawback of adapting BinaxNOW to the microfluidic device is the cost of about $40 per test, which is unfeasible for a POC device. The gold nanoparticles were the chosen detection method, as they have the highest versatility to detect for all four strains of malaria on one strip test, in a rather inexpensive way. One challenge of this design is the complexity of fabrication, this will be simplified though using an inkjet printer that can immobilize antibodies onto a cellulose medium.

Conclusions/action items:

Create a design that successfully implements both the separation and detection designs outlined above into one entity.

2016_10_18 - PDMS Fabrication Research

▪ James Jorgensen ▪ Oct 19, 2016 @02:32 AM CDT

Title: PDMS Fabrication Research

Date: 10/18/16

Content by: James

Present: NA

Goals: Learn about how PDMS molds for microfluidic designs are made

Content:

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2917889/ - Fabrication of Microfluidic Devices using polydimethylsiloxane (Friend *et al. 2010)*
  - Polydimethylsiloxane (PDMS) has become the gold standard for microfluidic devices due to its physical properties, ease of fabrication, and economy.
  - PDMS is created by mixing together a crosslinker/curing agent and siloxane. After mixing, the PDMS must be degassed in a vacuum chamber (Must be used 2 hours after mixing before it sets)
  - A mold can be created using photolithography, but typically a negative casting mold is used by which the PDMS is poured over a master mold. This method limits the potential configuration of the mold in specific ways.
  - The casting mold can be made of many different materials, but this paper chose to use SU-8 (MicroChem, Newton MA, USA). This particular casting mold limits the thickness to around 250 um.
    - Outline of Process:
    - SU-8 poured onto water substrate, spin at varius rpm rates for varying amounts of time
    - Remove any bubbles
    - Prebake in order to evaporate solvent
    - Expose to UV radiation (350-400 nm)
    - Post exposure baking to increase crosslinking
    - Immerse in SU-8 developer
  - Casting process is straightforward, simply pour PDMS onto casting mold and place in oven at 65 C for 24 hours
  - In order to bond the mold to a surface, expose the side of the mold to be bonded to oxygen plasma for 10 mins, then immediately place onto surface to bind to (Si, glass, etc.)

Conclusions/action items:

In order to take this process of PDMS fabrication to the next level, more in depth research on the particular size of our microfluidic channel must be done. The resources available to us at UW-Madison must also be investigated. The resources available for reproduction in Jimma must also be investigated.

2016_10_18 - Gold Nanoparticle Fabrication Research

▪ James Jorgensen ▪ Oct 18, 2016 @10:27 PM CDT

Title: Gold Nanoparticles Fabrication Research

Date: 10/18/16

Content by: James

Present: NA

Goals: Perform research about how gold nanoparticles are conjugated with primary antibodies

Content:

- http://www.sciencedirect.com/science/article/pii/S0167577X09004194 - Preparation of antibody-conjugated gold nanoparticles (Di Pasqua *et al.* 2009)
  - The gold nanoparticles in this experiment were approximately 10 nm in diameter, and conjugated with an antibody for *E. coli.*
  - The gold nanoparticles first need to be functionalized with 11-mercapto-1-undecanol and 16-mercaptohexadecanoic acid using methods reported by Laaksonen et. al 2006 (**Stability and electrostatics of mercaptoundecanoic acid-capped gold nanoparticles with varying counterion size)**
  - The gold nanoparticles were then added to this solution along with ethanol. $NaBH_4$ was added dropwise while stirring. After stirring this for 3 hours the solution was washed with ethanol, centrifuged and decanted off excess solution.
  - In order to conjugate the *E. coli* antibodies onto the functionalized gold nanoparticles, a method was adapted from methods used by Zhao *et. al 2004 (***A rapid bioassay for single bacterial cell quantitation using bioconjugated nanoparticles)**
    - The particles were added to a solution of EDC and PBS before being added to a solution containing the goat *E. coli* antibody. The particles were then washed with PBS and ethanol before being centrifuged down and collected.

Conclusions/action items:

More research needs to be done on the two research papers listed above to further outline the methods of which this process is completed. The concentrations, volumes, and masses of reagents also need to be categorized for the specifics of our design scope. The type of antibody and antigen that would be ideal for controlled lab testing needs to be researched more thoroughly.

2016_10_18 - LFIA Fabrication Research

▪ James Jorgensen ▪ Oct 18, 2016 @09:39 PM CDT

Title: LFIA Fabrication Research

Date: 10/18/16

Content by: James

Present: NA

Goals: Research how LFIAs are produced

Content:

- http://pubs.rsc.org/EN/content/articlepdf/2015/ra/c5ra03442f - Photo-assisted inkjet printing of antibodies onto cellulose for the eco-friendly preparation of immunoassay membranes (Credou *et al.* 2015)
    - Cellulose was originally chosen as the medium for LFIAs due to its near inexhaustible quantities.
    - Cellulose also has biocompatible properties, and can come highly differentiated in terms of pore size and and sheet thicknesses.
    - Due to the complexity of older forms of antibody immobilization, inkjet printing was created in order to decrease the cost and complexity of the more antiquated forms.
    - This method is also considered the most environmentally friendly and cost efficient form of immobilization
    - Direct immobilization onto cellulose can result in up to 40% loss of antibodies, therefore cellulose activation/functionalization is required
    - For this experiment, antibodies were printed onto nitrocellulose paper using a laboratory piezoelectric drop-on-demand inkjet printer Dimantix Materials Printer DMP-2831
    - Antibodies were printed onto non-treated and pre-treated (with glucose) nitrocellulose sheets. The drop volume was 10pL and the band size was 600 um wide.
    - In order to immobilize the antibodies they were first dried at 37 C for 15 mins, then irradiated at 365 nm for 2 hours and 40 mins, and then thoroughly rinsed with a phosphate buffer solution.

Conclusions/action items:

The methods that this experiment outlines could most likely be reproduced by the design team with different variations of antibodies as well as antigens in order to test the feasibility of this aspect of the detection design. More research on the resources available to the university and the types of antibodies/antigens that can be used need to be done in order to proceed with this testing.

2016_10_19 - Neodymium Magnets

▪ James Jorgensen ▪ Oct 19, 2016 @11:27 AM CDT

Title: Neodymium Magnets Link

Date: 10/19/16

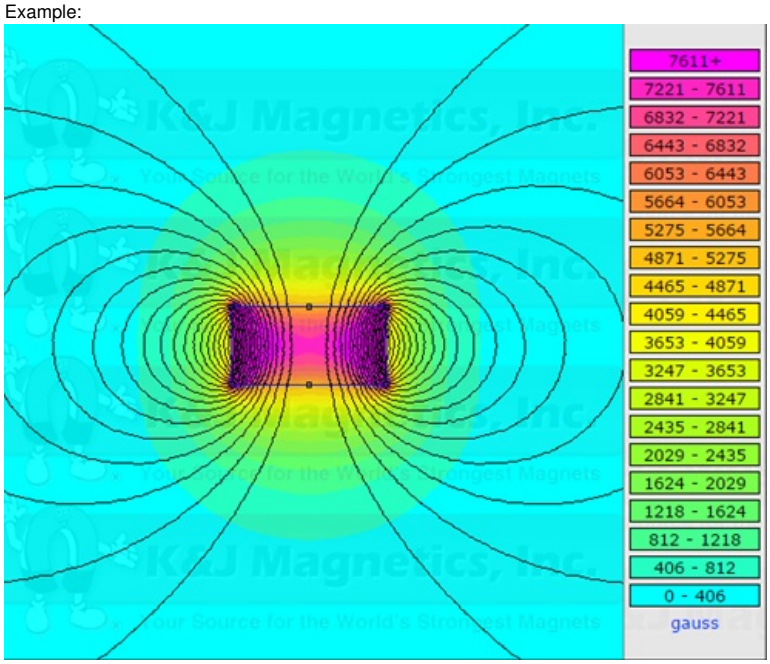
Content by: James

Present: NA

Goals: Outline a website that neodymium magnets can be purchased from for the teams separation design method

Content:

https://www.kjmagnetics.com/products.asp?cat=1

- This site has hundreds a different deodymium magnets ranging from approximately 0.5 T - 1 T (Most magnetic separation designs utilize magnets within this magnetic field strength range)
- The sizes of the magnets also very greatly so that a proper size can easily be found for the small size of our microfluidic channel
- Each magnet also has an calculated image showing the approximate range of strength of the magnetic field that the magnet produces
    - Example:
    - 

Conclusions/action items:

This site will be useful when a magnet needs to be purchased for the design. More research on the specificity of what magnet needs to be used will be done.

2016_9_14-Initial Client Meeting

▪ James Jorgensen ▪ Sep 14, 2016 @06:45 PM CDT

Title: Initial Client Meeting

Date: 9/14/16

Content by: James

Present: All

Goals: Get to know one of the people we will be working with (Patrick) and learn about our role in this design project. Receive information about direction to begin project in.

Content:

Talking to Patrick (Past BME student at UW)

Client – Tim (Jimma University) Newer faculty member

-Microfluidics: Point of care diagnostic abilities

-Wants new research lab/facility

-Point of Care diagnostics

Jimma: Most known for the university (flagship of country) – first BME department in country

-Has a regional hospital (most severe illnesses), struggling from a resource perspective, equipment is under-maintained.

-Design courses: Water distillation device (surgeons sterilize with autoclave, needs di water), results in mineral deposit from use of tap water. Kidney health analyzing device via measuring of albumin and kidney health and processing capabilities.

Resources in Jimma: Huge challenge, access to internet (3G), electricity can be knocked out, lack of prototyping tools

There is a larger network of people behind this project to help the development of BME in Jimma

Tim is meeting with Doctors in the regional hospital to find out what we need to do

Questions for client:

Locally available tests already (price, availability, etc)?

Where can we get the tests available there for comparison?

What illnesses are the most prominent for testing?

Specific disease in mind for device to identify?

PDMS molds (what kind, re-usable, etc.)?

-Removable parts that can be easily replaced so that it can be reused?

Responses:

-Ideally battery powered, or some sort of backup power supply

-Tests in Jimma: Information forthcoming from Tim after he speaks with the local doctors

-University Partnership: Teaching them about what we make and being able to translate the knowledge to be able to solve problems there.

-Creating a protocol that can be used to produce these prototypes over in Jimma (limited resources naturally)

-Bill and Melinda Gates?

Conclusions/action items:

Actions:

-Email Tim with a list of questions that can help us get a footing

-Visit the Beebe lab and see what is available to us for modeling (Webpage with info on microfluidics)

-Small lab space so limited availability for our usage

-Teaching lab in ECB has most of what we could need to use

-Prof. Justin Williams BME 550 (Microfluidics) sit in on class or see lab operations

-Weekly meetings at 1:30 with Pucc room 3126 ME

-Photolithography

2016/09/05-Entry guidelines

▪ John Puccinelli ▪ Sep 05, 2016 @01:18 PM CDT

Use this as a guide for every entry

- Every text entry of your notebook should have the **bold titles** below.
- Every page/entry should be **named starting with the date** of the entry's first creation/activity, subsequent material from future dates can be added later.

You can create a copy of the blank template by first opening the desired folder, clicking on "New", selecting "Copy Existing Page...", and then select "2014/11/03-Template")

Title: Descriptive title (i.e. Client Meeting)

Date: 9/5/2016

Content by: The one person who wrote the content

Present: Names of those present if more than just you (not necessary for individual work)

Goals: Establish clear goals for all text entries (meetings, individual work, etc.).

Content:

Contains clear and organized notes (also includes any references used)

Conclusions/action items:

Recap only the most significant findings and/or action items resulting from the entry.

2014/11/03-Template

▪ Austin Feeney ▪ Oct 18, 2016 @08:54 PM CDT

Title:

Date:

Content by:

Present:

Goals:

Content:

Conclusions/action items: