Target Malaria: Mosquito Classification Device

BIOEN 405: Bioengineering Team Design II

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Problem Statement and Description:

The goal of this project as stated earlier is to control and decrease the incidence of malaria, transmitted primarily via mosquitoes. It is estimated that in 2015, there were 438 thousand deaths resulting from malaria. 70% of these deaths were children under the age of 5 [4]. However, those that survive may have additional complications, decreasing their quality of life. These complications can include neurologic defects resulting in trouble speaking and moving, deafness, and blindness [5]. Additionally, *P. falciparum* can lead to severe anemia and premature delivery in pregnant women whereas *P. vivax* has been known to cause ruptured spleens. Both infections via *P. vivax* and *P. ovale* are known to cause relapses up to years after the initial infection due to dormant parasites.

In order to control the disease, Target Malaria has begun releasing transgenic mosquitoes, to decrease the fertility of female mosquitoes and bias the mosquito population towards males, into parts of Burkina Faso to investigate Gene Drive as a method for controlling mosquito populations. Gene drive is a tool that causes a target gene to spread throughout a population within a few generations. While this process is relatively straightforward on paper, there are challenges implementing it at scale and tracking how the gene drive project progresses. For example, researchers may release a large number of mosquitos at a designated location, but these transgenic mosquitoes will each migrate to different locations. Understanding how far the mosquitoes travel, how effective the transgene is spreading, and how many transgenic mosquitoes are required to affect the whole mosquito population in a given geographic area is essential for the success of the project.

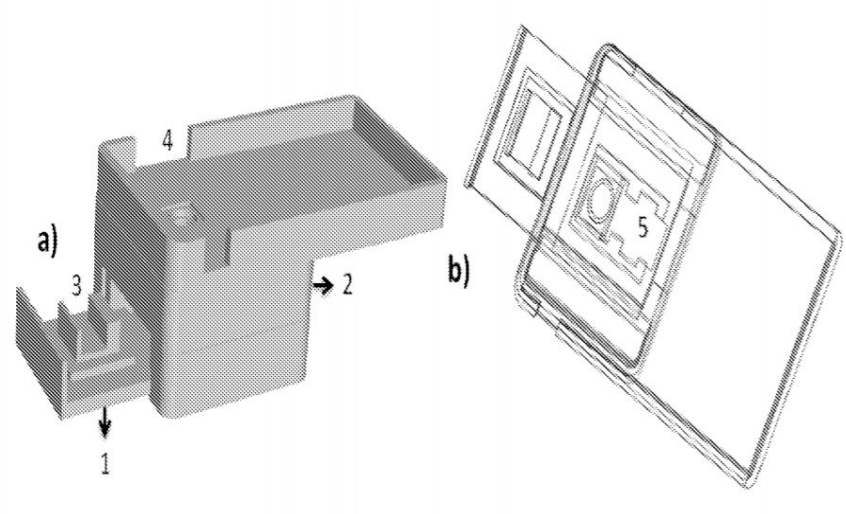
Currently mosquito samples from around Burkina Faso are collected from traps, separated into tubes, and then transported many miles to labs that are equipped to run PCR or LAMP assays. While this centralized method of running samples is well established and provides accurate and reliable results due to the training of the staff

and the reliability of the instruments used, it severely limits the number of samples that can be processed, requires significant person-hour investments and poses serious risks for chain-of-identity regarding the mosquito samples. To eliminate these risks and inefficiencies, Target Malaria needs a field deployable device that can perform a LAMP assay on mosquitoes in the field by their technicians without returning to the lab. Currently, there are prototypes made by researchers and hobbyists for running LAMP assay samples in the field, but none can run a significant number of samples nor has been tested in the field.

Prior Art:

There is a significant amount of prior art cited in patents for the LAMP assay itself as well as diagnostic devices integrated with smart phones. Patent application #W02017025984A1, titled Smartphone Integrated Real-time Molecular Diagnostic Device, describes a two module system that can be used to run an assay using its own battery power and analyze the results using a smartphone integrated imaging system. It outlines the modules used for maintaining samples at a constant temperature, as well as the series of filters, LEDs, and lenses used for reading the results of the assay. This system is only capable of analyzing one sample at a time meaning that this patent does not significantly encroach on our design space which consists of a design that must run 48 samples. In addition, this patent application clearly states that it is a smartphone integrated device which further separates our own which may be used in tandem with a smartphone, but will likely not be smartphone integrated.

The figure below shows the device in which a smartphone such as the iPhone 6 would rest. The smartphone sits camera is positioned to to be inline with the sample to



be imaged, and the heating module sits below the sample tray and imaging module.

A patent more focused on the LAMP assay itself is Patent Application #US20160076083A1, titled Methods and devices related to toehold-based strand displacement with loop-mediated isothermal amplification. This patent application claims a method of detecting nucleic acids. It specifically claims a two component system for amplifying a DNA sample through an isothermal amplification method, and subsequent detection through a strand displacement reporter. They further claim a method of detecting the target nucleotide using camera based imaging for real time analysis of a sample. The proposed detection device is claimed to either have separate imaging and amplification components or a fluid connection between amplification and imaging.

A Patent Application that more generally claims smartphone fluorescent imaging is #US20130157351A1, titled Compact wide-field fluorescent imaging on a mobile device. It claims a device that secures to a phone, allowing for the cross illumination of samples and detection of fluorescence resulting from fluorescence labeling of samples. The claimed device is specific to detection of cells that are labeled by antibody binding.

A combination of both diagnostic detection and a LAMP assay is implemented in a patent titled Portable Microfluidic chip LAMP visible detection and detection method thereof. This patent claims to have created this detection method that encompasses a microfluidic chip that is placed over a temperature controlled metal heating block that allows an isothermal LAMP assay to take place within the chip. This chip is visualized from above the casing by a visual detecting system including a microcam and ultraviolet fluorescent lamp. The patent also claims that the temperature controller contains a control unit, temperature display section, temperature measurement section, and siren. Included in the temperature controller is a time programmer. The patent also describes a dismountable cover plate over both the chip and heating block.

In addition to these patents, a research article that is particularly relevant to this project is Loop-mediated isothermal amplification shield for Arduino DNA detection. This article describes a prototype for a device that can run isothermal amplification for a LAMP assay. It includes an arduino uno with a shield attached that contains the heating circuit to maintain the temperature of a single PCR tube. Additionally, the code uploaded onto the Arduino is provided temperature control is given.

This article demonstrated the ability of Arduino enable circuits to control the heating of a sample for LAMP assays. Like the patents mentioned above, this device was only capable of running a LAMP assay for one sample meaning it is too small to be useful for Target Malaria without being adapted.

Design Specifications:

Need #	Design Constraint	Unit of Measure	Acceptable Value	Ideal Value
1	Lightweight	Pounds	3-5	3

Our goal is to design a device that can run a LAMP assay on 48 samples in addition to necessary control samples in a field setting with access to electricity from a car battery. The device must include a heating module for running the assay by maintaining the samples between 65°C and 70°C for at least an hour. This module must allow for heating to be delivered both to the bottom and top of the samples, to allow for consistent heat distribution. It must also include a module for image analysis of the sample for one, two or three analytes, each of which will have their own absorption and emission spectrum. Listed below are constraints and criteria to aid in the design of this device.

Constraints Table:

Constraint #	Constraint
1	Less expensive than current alternative methods, such as laboratory methods ELISA and PCR
2	Little training needed to use the device
3	Reusable
4	Enabled by 12V power supply from a car cigarette adapter
5	Machinery does not require calibration or maintenance over the

	course of a field trail
6	Displays temperature of the heating block
7	Wired connections and device can withstand vibrations from transportation
8	Compatible with sterilization methods
10	Contains both heating and imaging elements
11	Maintains block temperature between 60 - 70 C to facilitate LAMP activity
12	Hold and run assay on 48 thin-walled, 0.2mL tubes
13	Must have positive and negative controls for assay. (System for reading running control standards and/or space for control samples to be run alongside 48 samples to be analyzed.
14	Functions over ambient operating temperatures between 10-40 °C
15	Device can be manufactured at a scale of 100 units
16	Capable of detecting fluorescent emission of a specific wavelengths

Criterion Table:

Criterion #	Criterion	Quantitative Value
1	Control of temperature	Maintain 65°C +/- 5°C;

		enzyme stability from 50 - 70 °C
2	Time to heat to desired temperature	< 20 minutes
3	Dimensions	Each module < $6in^3$

Documentation of Solution Generation and Selection:

Our process for generating solution ideas began with meetings with our mentors as well as industry partners. These sessions allowed us to gain a clear vision of the constraints and criteria which may be necessary to build a successful device for Target Malaria. Additionally, we performed an exhaustive literature search to familiarize ourselves with similar devices in the DNA amplification space as well as received literature from our partners at the University of Texas regarding current work on the assay requirements and imaging. Finally, we sought out Dr. Neils to gain insight on circuitry, heating components and LED excitation.

With this information in mind, we met as a group to flush out a draft of our constraints and criteria for the device as whole as well as the two separate components (imaging and heating). Moving forward, we spent time independently generating possible solutions, based upon agreed upon specifications. This allowed for us to develop unique solutions, to later be discussed, evaluated, and combined as a group. **The figures below are three of the initial solution designs for both the heating and imaging components.**

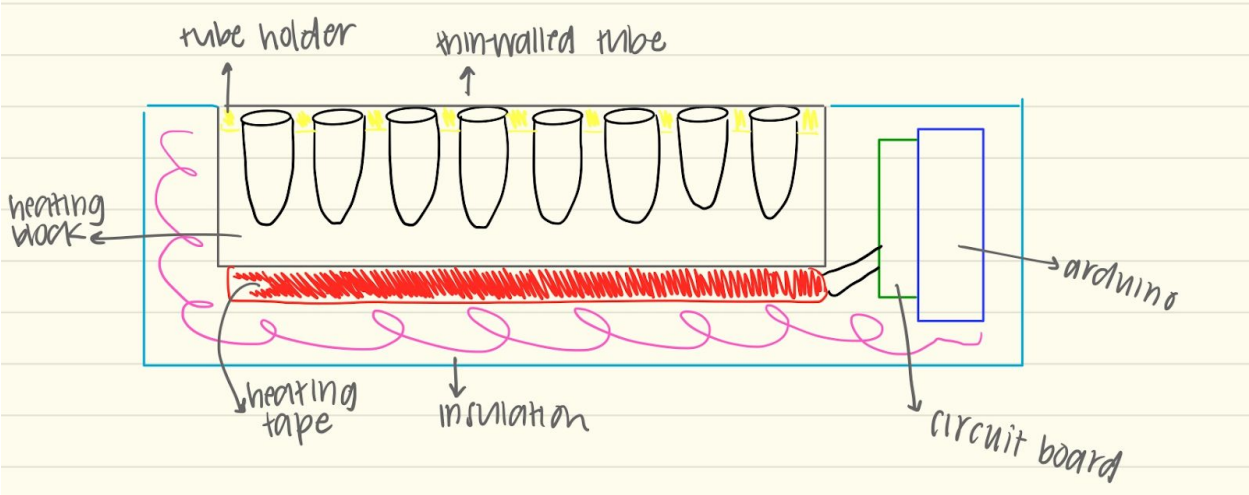


Figure 1: Anna's First Heating Module Design Iteration

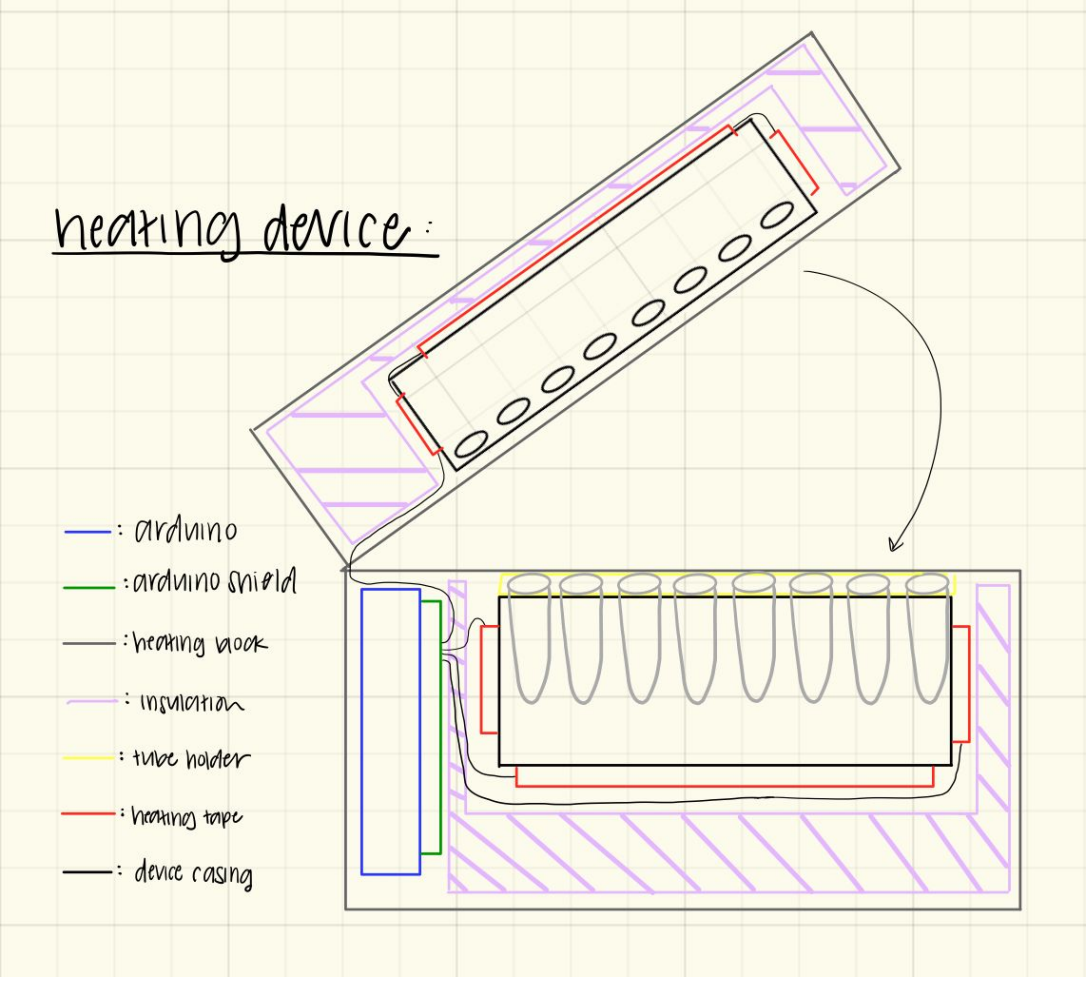


Figure 2: Anna's Second Heating Module Design Iteration

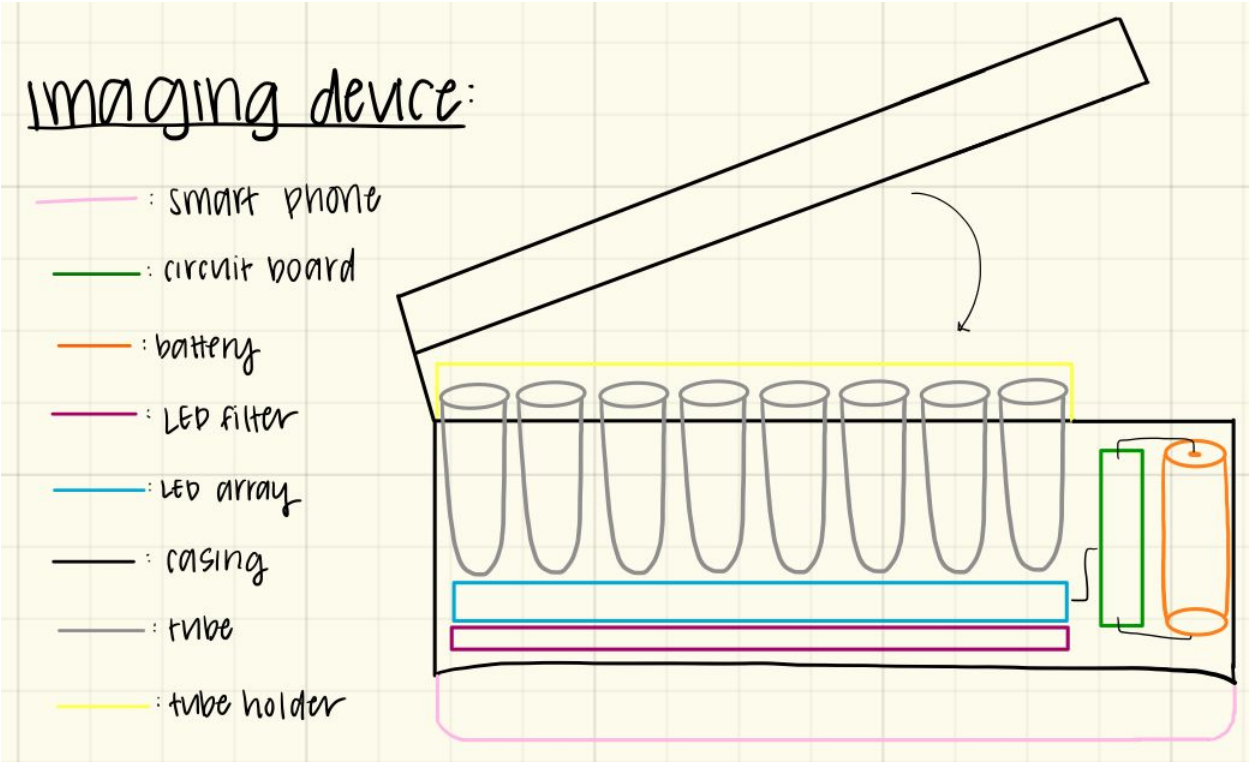


Figure 3: Anna's First Imaging Device Design Iteration

Due to the constraints as a result of COVID-19, we were only able to make significant progress on the in silico prototyping of the heating module. The design comparison matrix for this device is as follows.

Heating Component Pugh Chart

Criteria	Weight (1-5)	Anna D.1	Anna D.2
48 samples	5	+	+
Displays temperature	4	-	-
Contains space for positive and negative controls	5	+	+
Time to temperature	2	n/a	n/a

Maintains indicated temperature with minimal fluctuations	5	n/a	n/a
Reusable	4	+	+
Compatible with Arduino	2	+	+
Total			

Solution evaluation:

Moving forward we were able to build our proof of concept heat circuit design prior to the stay at home order as well as implement our proposed heating device design in SOLIDWORKS.

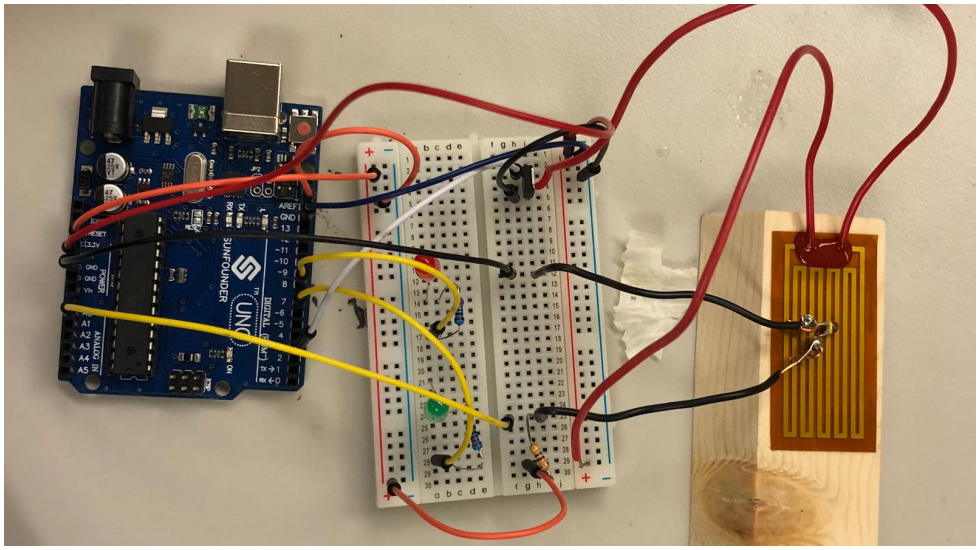


Figure 4: Proof of concept circuit

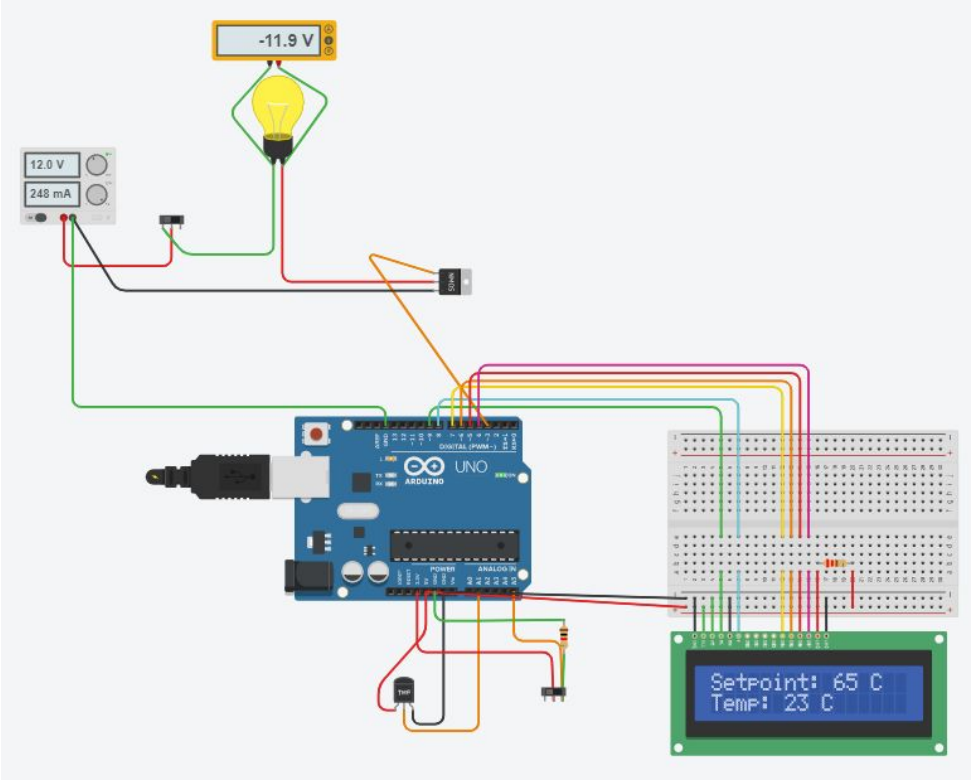


Figure 5: Iterated Circuit Design

We also put a great deal of work into improving the design of our heating device, casing and block, to ensure that the design was effective and easy to use. In the beginning stages of in silico modeling, our industry partners informed us that the heating device would have to accommodate for both 0.5 and 1.5 mL tubes. This caused us to create great changes to our device casing from the original sketches and SOLIDWORKS drawings. In doing so, we added a holder for the heat block, made from the same material as the block, to which the heating tape is attached. This eliminates the risk that the user places the heating tape incorrectly on block, compromising the heat distribution, and extends the shelf life of the heating tape. These factors were deemed more important than the additional weight as well as increase in device size that is incurred due to the holder. The original SOLIDWORKS designs for the casing can be found in **Figures 6-8.** In addition to adding the block holder, we modified the original

heating block to become two separate blocks, one to accommodate each tube size. This can be seen in **Figure 6.**

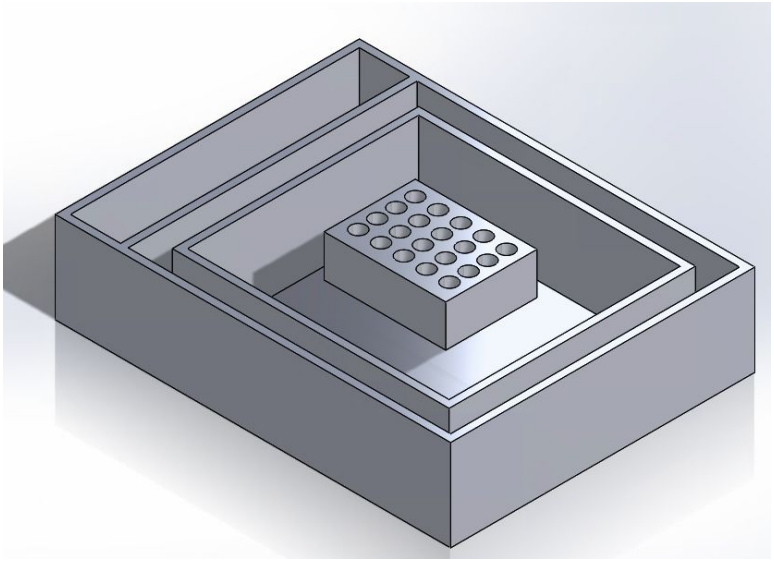


Figure 6: Bottom Casing and Heat Block (not to scale) for Heating Device

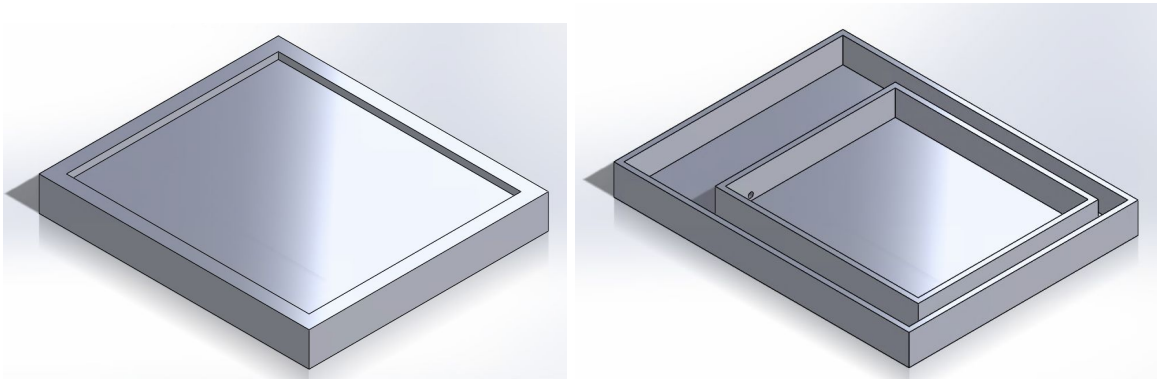


Figure 7: Top Casing

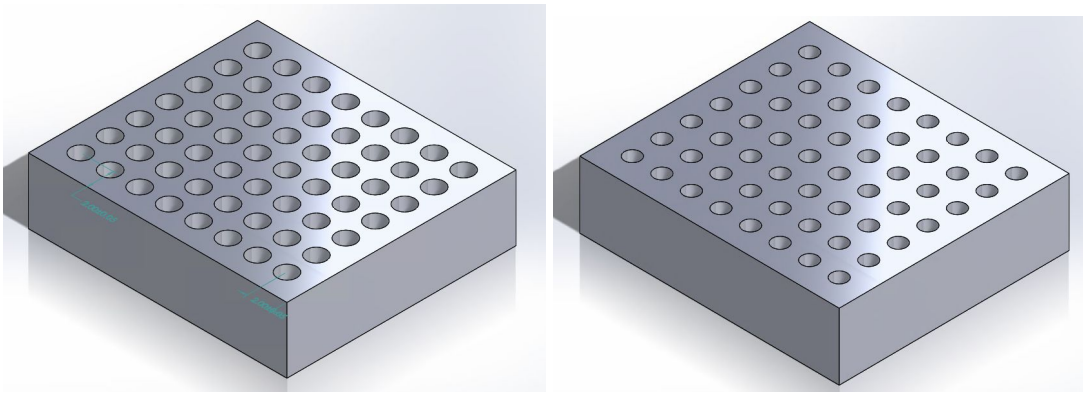


Figure 8: Iteration of Heating Blocks

The final changes made to our design for the heating component were made to increase the use for the end users. Some of these changes include adding a sample handling tray, attaching the lid via a hinge joint, adding placeholders for the user interface, space for thermocouple and temperature.

Results - Design Description:

Heating Module

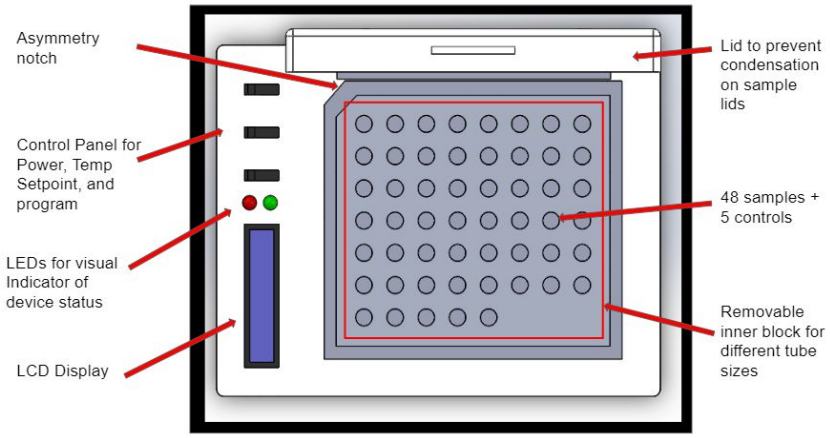


Figure 9: Top Down view of Design

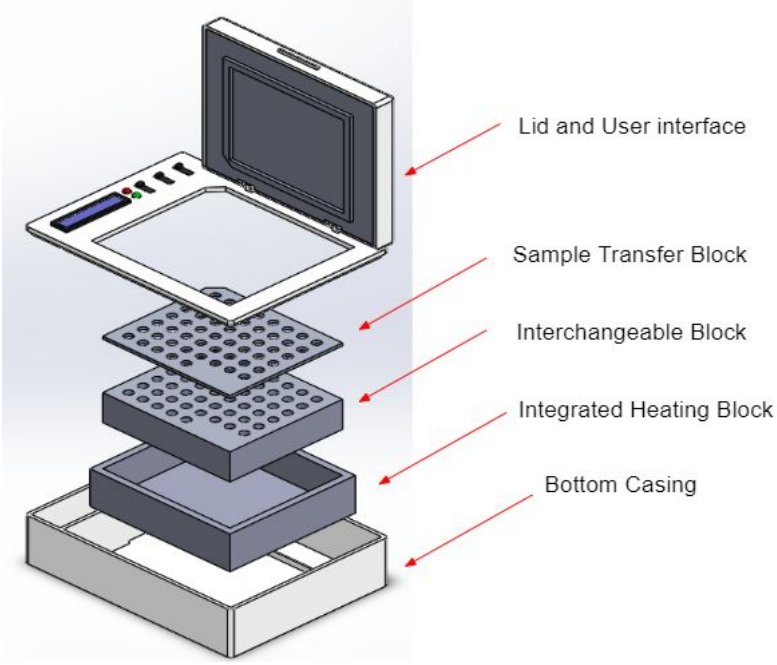


Figure 10: Exploded Isometric View of Heating Module

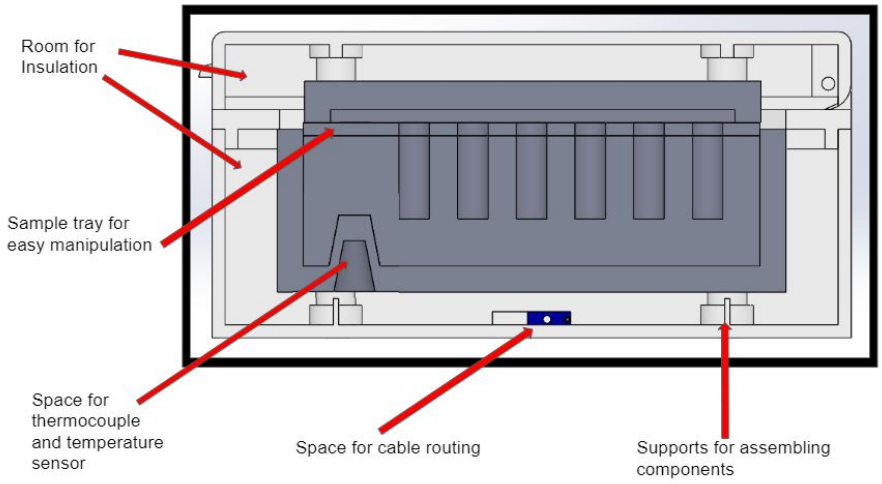


Figure 11: Cross Sectional View of Heating Module

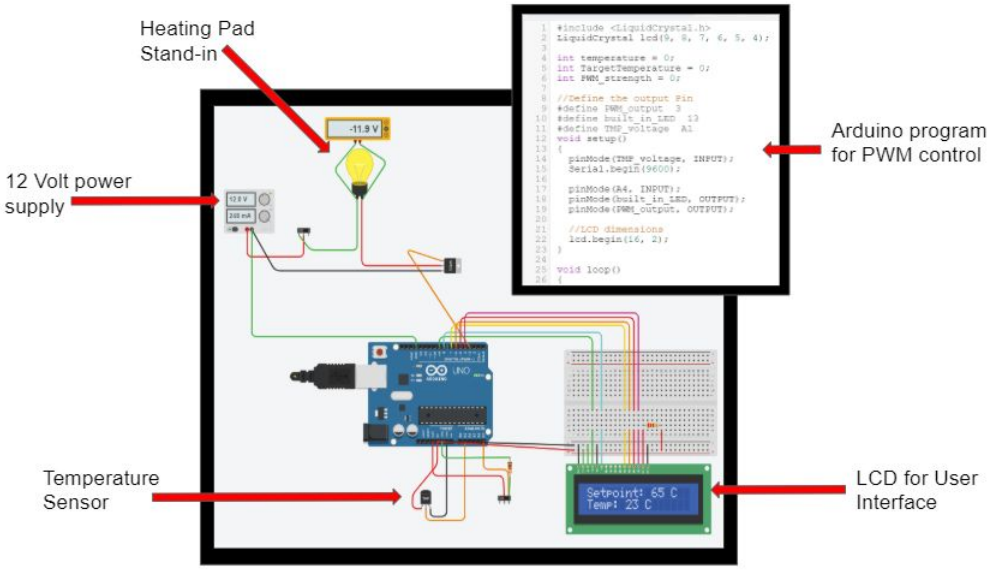


Figure 12: In-Silico Circuit Design

The Lid and User Interface consist of a control system with an LCD display, LEDs, and 3- toggle switches for controlling power and cycle settings like temperature set point and timing. The lid itself has an aluminum block shown in gray that acts as a

heat conductive top for the heating block, preventing condensation in the samples. The circuit will not be attached to this part of the block but could be if necessary.

The Sample Transfer Block is used with the device until samples need to be transferred from the Heating Module to the Imaging Module. This part helps the user quickly and easily move samples between systems.

The Interchangeable Block can be swapped to accommodate the 1.5 mL tubes required for the first step in the assay workflow and the other block that is used for running the LAMP assay in the 0.5 mL tubes. This component can run 48 samples and 5 controls simultaneously. Keeping the controls out of the 48 was to allow for more samples to be assayed at one time but isn't necessary. In the extra space where the three sample holes are missing as seen in **figure x** there is a conical cut fitting with an extrusion of the integrated heating block to allow for space to create a good thermocouple between the block and the circuit. This feature could be modified. Currently the block only contains sample ports as cylinders although to maximize heat transfer a draft angle should be added to the bottom to fit the tubes used. This will need to be optimized as it may impact the manufacturing methods of the blocks since milling machines may find it challenging to match the desired shape.

The Integrated Heating Block is screwed through the bottom of the casing to be fully integrated into the device. On the outside it will be attached to the heating pads that will control the block temperature. Maximal coverage of the block with heating pads is desired.

The Bottom Casing allows for 1.5 cm of room for insulation to surround the sides and bottom of the device to protect the user from burning themselves. Special care will be required to bury the screw heads to ensure they do not conduct heat to the user.

Aluminum or thermal conductive resin will be used for the block manufacturing of each heating component which includes the Heat Conductive lid, sample transfer block, interchangeable heating block and integrated heating block.

Thermally resistant materials will need to be used for the casing material in the final device since the assay will get up to 95 C. For prototyping, depending on the

quality of insulation ABS or PLA could be used since they are stable up to 80 C and we hope to maintain the device below 65 C following engineering standards.

The heating circuit as seen in **Figure X** shows our final circuit design based off of the Arduino Uno system. It contains a simple LCD display and a TMP36 temperature sensor which is accurate between 0 and 120 C. The voltage through the TMP36 scales linearly with the temperature making it easy to use. An alternative would be to use a thermistor and using its thermal properties to implement a function in the Arduino code to convert voltage to temperature. This approach was taken in Velders et al. The code displays the measured temperature and the target setpoint. An LED on the board is used to indicate whether the heating pin is outputting power. A MOSFET transistor is used to control the output. In the model we used a lightbulb to indicate the heating pads. When prototyping this part the lightbulb and the heating pads in parallel would be interchanged.

There are multiple options for ultimately powering our device. We will run this device off of cars meaning that it needs to be compatible with 12 volts. The Arduino device runs off of 5 volts however. This means there needs to either be a voltage regulator added to the device in order to convert the 12v to 5 for a sub-circuit powering the arduino or the Arduino needs to be run off of a battery pack. The heating circuit could be run off of 5v but many 5v car adaptors cannot accommodate enough current to get enough power to heat our device.

Imaging Module

The imaging module will be a device that allows the sample transfer block to be placed into it. An LED array will be placed on the bottom of the device shining up through the samples. On the sample lid will be a port for viewing that samples inside. This port will be covered with theater filters from LEE's filters, or a wheel of bandpass filters. This will allow for the detection of our target fluorophores by a phone camera placed on top of the filter. LED, filter, and fluorophore pairs need to be chosen. For

prototyping 495 nm LEDs, Fluorescein, and Lees Orange 105 filter or 770 Burnt Yellow filter are sets that are promising for future experimentation.

Assay Workflow Overview:

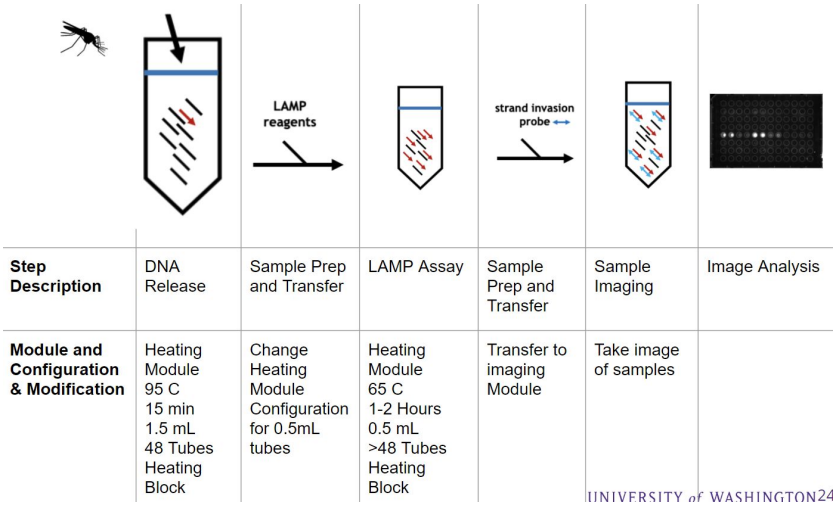


Figure 13

Figure X outlines the basic assay procedure. Mosquitos will be loaded into 1.5mL eppendorf tubes with water and heated to 95C in the heating module to release the DNA. Then these tubes will be used to prepare LAMP assay samples which will be run after changing the block in the heating module. A strand invasion probe will be added to the samples and they will be transferred to the imaging module for imaging.

Results: Test Data/Engineering Analysis:

The specifications that we prioritized for testing were the time to temperature, control of temperature, and the detection of fluorescent signals. These specifications were chosen, because if these are not met the product won't meet the engineering need. The time to temperature is important because if the initialization of the device takes too long the throughput of sample processing decreases, which can greatly hinder the application of the device. Temperature control is important because extreme deviations from the control temperature can either cause the reaction to not run or the enzymes in

the reaction can denature, leading to false negatives from the assay. This would be a serious failure from the device, as the Target Malaria researchers need to have confidence that the results that they are getting are from the state of the samples, not from the function of the device. Similarly, the ability to detect fluorescence from the assay is critical.

In silico we were able to test aspects of the heating block in COMSOL. The tests that we selected helped to inform design decisions about orientation and size of heating elements, block material, and other factors. Further, we evaluated our criterion for time to temperature through COMSOL models with reasonable parameters and materials. For temperature control we were able to test the circuit behavior such as maintaining a setpoint temperature in silico in TinkerCAD.

Due to circumstances there were tests that we were unable to perform but instead have proposed as future work. These tests mostly revolve around the imaging module, but some are also characterization of the heating module. For the imaging module we plan on testing design aspects such as distance between the samples and the camera, different designs for illuminating the samples, and testing filter sets to drive design decisions. Once settled on a promising design from these initial tests we propose further testing with a dilution series to test the sensitivity at which a signal can be detected in our system. These tests would help to test our detection of fluorescent signal specification. For the heating module we propose testing the control of the system through measuring temperature to verify the behavior that we theoretically tested in silico. These tests will inform on whether the temperature control specification is met.

Since the classification performed by our device is on mosquitos and its intent is in a research context, it may be exempt from the ISO regulatory pathways for in vitro diagnostic devices. However, through testing we would like to demonstrate the validity and verify the device behavior as outlined in ISO 13485 standard for engineering medical devices, and 18113-1 standard for in vitro diagnostic medical devices. To

satisfy these standards we need to prove that assays run by the device consistently classify the mosquito samples correctly.

Specific Tests Executed and Planned

Heating

Testing Time to Temperature

This test is to answer the question: How long does it take for the block to get to the indicated temperature with even distribution? Characterization of this property is important as it will inform the workflow for Target Malaria's teams.

Computational implementation: equipment includes COMSOL. Through the COMSOL modeling software we tested the time to temperature under a variety of power levels that are physically reasonable. This allowed us to fine tune our design such that it was within our specifications, without having to spend the time and resources of building and testing each configuration. Through our studies, we observed that heating equivalent to 60W per side was required to heat the tubes in our model to the set point of 95°C. However, the model used was unable to capture effects such as heat transfer through the air and that the temperature of the block would not exceed 95°C. If considering only the block, the power required to reach 95°C is roughly half, around 30W per side. Considering these factors we assume that the actual power required is between these values. This investigation drove design decisions on how much power we needed to stay within our specification that we propose would be validated in a physical prototype.

Physical implementation (Proposed Future Work) : equipment includes Arduino, heating circuit (thermal tape, resistors, transistors, thermistor), computer, timer, infrared thermometer, heating block. This test will be executed by placing the heating tape on the block, turning on the circuit controlled via the Arduino code and timing via the timer the time in which it takes for the block to get to both set points 65°C and 95°C. Once the circuit indicates the block is at temperature, the infrared thermometer will be used to measure the temperature at three other locations on the block to ensure even heat

distribution. Controls for this test include keeping the three other locations constant for each trial and allowing components to cool to the same starting temperature between trials. This test will be conducted at least 5 times so that an average time can be measured. This will produce a value that can act as a rough benchmark for time to temperature. While it would be favorable to run more tests to increase confidence in results, due to the nature of this test taking a long time per trial, likely more than 20 minutes per run, 5 tests should be sufficient to validate the behaviors observed in silico.

The metric measured from this test is the time until the block reaches the set point. This metric will further inform design decisions on power requirements and insulation. Further, this metric will inform decisions about workflow for the Target Malaria teams.

Accuracy at Control Temperature

This test is to answer the question: Is the heating block able to accurately remain at the set temperature over time?

Physical implementation (Proposed Future Work): Arduino, heating circuit, heating block, computer, timer, infrared thermometer. The set up for this test will be similar to the previous tests, where the heating circuit is connected to the prototype block. Using the readings from the thermometer we would begin timing once the block reaches temperature, and monitor the temperature every minute in trials that lasted for 30 minutes at 95°C and 2 hours at 65°C. These time windows represent 2 times the operating time for normal LAMP experiments at each of these temperatures. These measurements should be taken in triplicate to ensure that the measurements taken accurately represent the block temperature. These locations will be held as constants in this test. Due to the length of this experiment we will run three trials for each test group, with the two test groups being at 95°C and 65°C.

The metric from this test will be the average distance from the setpoint the block temperature is at. This can be represented as the root mean squared error of the data

from a constant line at the setpoint. This metric is important for showing consistency of performance in the device and will help to validate our design.

Heating Pad Coverage

This test is to answer the question: Does the configuration of heating elements, specifically the area covered make a big difference to heating efficiency?

Computational implementation: The equipment needed for this implementation is COMSOL software. In COMSOL we tested different areas of coverage for the heating elements ranging from 100% coverage to 60% coverage of the sides of the block. In these simulations we controlled for all aspects of the model other than the area that was covered by the simulated heating element.

The metric for this test was the temperature that was observed after 20 minutes in the model. This metric is important in driving design decisions and it gives us insight into some non-trivial behaviors. Specifically, this test highlighted a linear relationship between the area covered by the heating elements and the heating rate. With the same amount of power applied to each side, and the same amount of power between trials, when the coverage of the heat source was reduced from 100% to 60% we observe 40% reduction in the temperature of the block at 20 minutes of simulation. This is seen in figures 14 and 15 that follow. An assumption that we had going into this test that was so long as the overall power applied to the device was the same that the heating dynamics would be constant. One interesting implication of this finding is that power alone is not the only factor driving heating efficiency. This drove our design to use heating elements that are evenly applied, so that we can maximize the efficiency of the power that is put into the system.

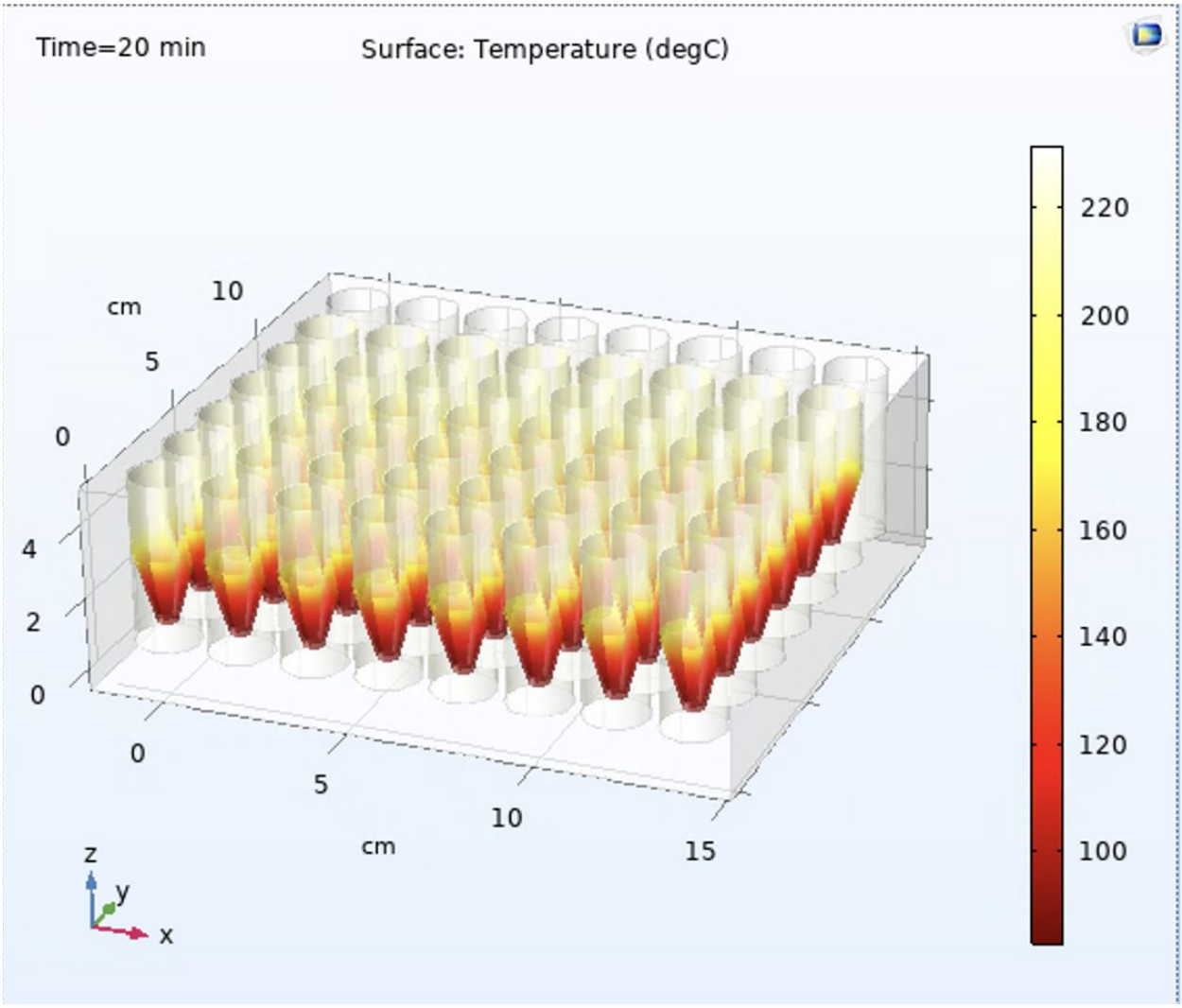


Figure 14. Heating pad orientation with heating flux of 7500 W/m^2 applied over 100% of the sides (approx 60W per side). Due to limitations of the model heat distribution to the tubes is likely inaccurate as described in previous sections. Maximum heat in the block is 225°C

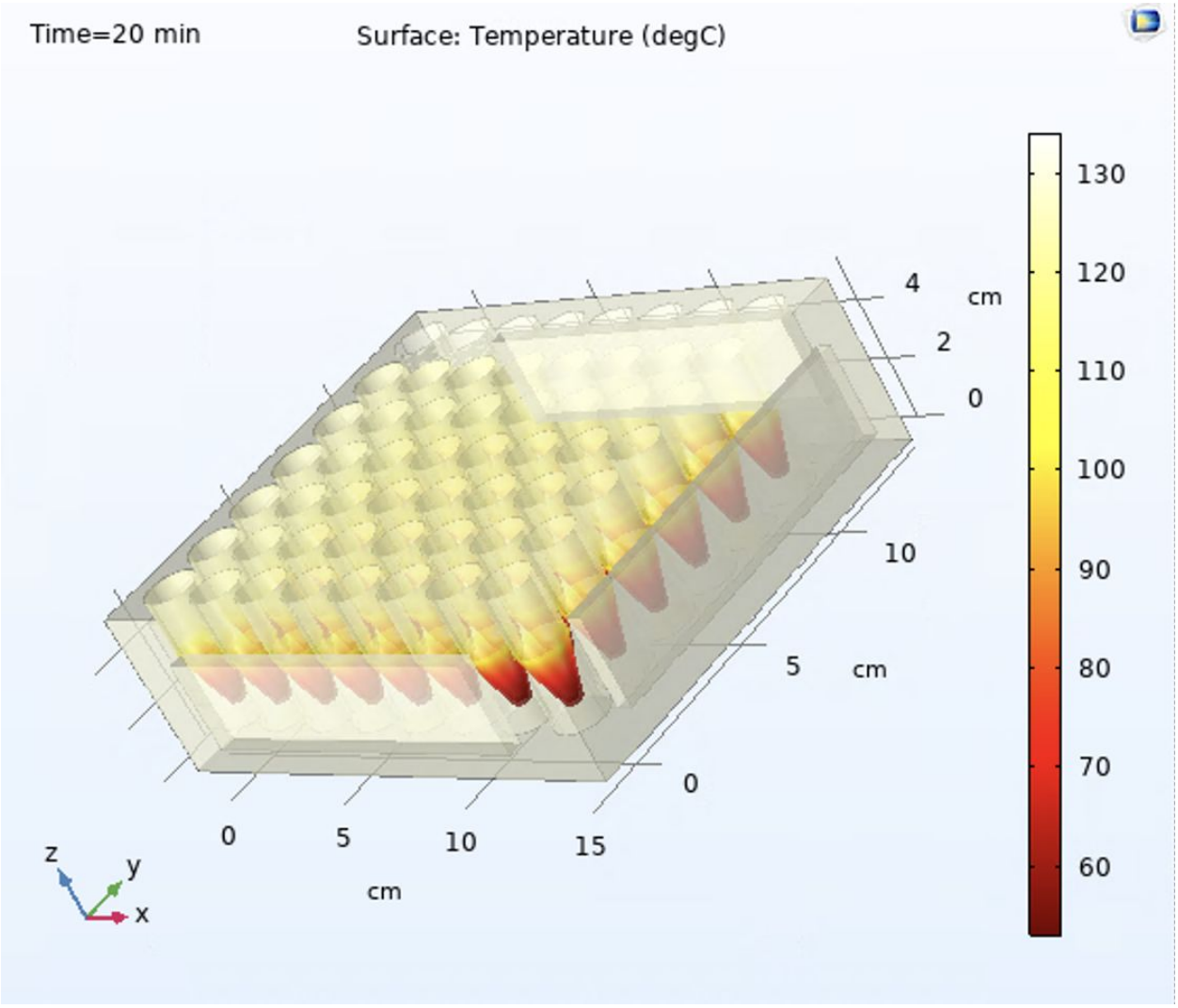


Figure 15. Heating pad orientation with heating flux of 13750 W/m^2 applied over 60% of the surface of the sides (approx 60W per side). Due to limitations of the model heat distribution to the tubes is likely inaccurate as described in previous sections. Maximum heat observed in the block is 135°C

Imaging (Proposed Future Work)

Configuration of LEDs

This test is to answer the question: How does the arrangement of LEDs relative to the sample affect the output of the system?

Physical implementation: This experiment would include fluorescein, blue LEDs, resistors and power source to power LEDs and a prototype of the imaging box that holds the samples, LEDs, has a holder for a smartphone, and filters for image capture. Multiple orientations would be tested, with LEDs illuminating below the samples, orthogonal to the samples, and in different ratios of LED to sample. These experiments would be run with 48 samples per trial, two trials per orientation. This would provide us with a statistically significant number of samples to compare between orientations. For this experiment the controls would be the smartphone and imaging box, the type of tube, concentration of fluorescent dye per tube, volume per tube, and the LEDs used in each configuration.

The metric produced by this experiment would be the fluorescent intensity of each sample. By calculating statistics such as mean and standard deviation of the samples we can compare different configurations based on their mean intensity and use this information to drive design decisions about LED configuration in the imaging module.

Detection Sensitivity

This test is to answer the question: What is our lower bound for detection sensitivity?

Physical implementation: equipment needed includes stock fluorescein, an orange filter from Lee's Filters, and a 495 nm LED for excitation, the imaging module, and a cellphone camera for taking images of the samples. The fluorescein would be diluted from a 100uM stock fluorescein by 5x, 10x, 50x, 100x, 500x, and 1000x. The diluted samples would be placed over an array of 495 nm LEDS and a filter would be placed between the samples and the camera. This experiment would be done with 8 of each concentration for 48 total samples. The controls for this experiment would be all of the equipment, LED configuration, and sample volume.

The metric from this test would be the cutoff concentration for detection, which is the sensitivity of the assay to fluorescence. After the initial dilution series another series can be narrowed around the cutoff concentration to better characterize the system. This

metric will allow us to compare our method to the sensitivity of already existing methods.

Placement of smartphone (distance from tubes/orientation)

This test is to answer the question: What placement of the smartphone allows for a single image to capture all samples at once, if any?

Physical implementation: equipment required includes fluorescein, LEDs, imaging circuit, power source, smart phone, tube holder, and the casing. This test will include imaging the samples both from above and below the tubes at three distances for each direction for a total of six conditions. The controls for this trial are the experimental set up, smart phone, and fluorescence concentration between samples. Three trials will be conducted, each with 48 samples.

The metric for this test is the number of tubes seen per image. The ideal configuration for this device is such that all 48 samples are visible. This is important, as the chain of identity between samples becomes a concern when multiple images are required to cover the entire block.

Whole Assay (Proposed Future Work)

Testing the whole assay attempts to address the question: Does the device output the expected LAMP assay results?

Physical implementation: equipment includes both modules, mosquito samples with and without transgene (or a sufficient assay analog such as DNA samples with and without the transgene), LAMP reagents, spectrophotometer. Based on a power analysis, assuming that the prevalence of the transgene in mosquitos is low, we would want to perform at least 100 trails to demonstrate that our assay device works and is both sensitive and selective. The experiment would consist of running the assay from start to finish in the device and comparing the output classification with the true identity of each sample. The controls for this experiment would be the device, cell phone camera used, and the parameters of the assay.

The metric from this test would be the percentage of mosquitos correctly classified. This metric is important for comparison to existing methods, and for determining confidence in the function of the device and the results it outputs.

Ethical Considerations:

Ethical considerations are especially relevant to Target Malaria due to the use of a gene drive. Implementing such a tool, in which new genes are spread throughout a population, causes a great deal of controversy due to the serious implications if not effectively managed to minimize the impact on the ecosystem and limit the spread outside the region [6]. Additionally, there is some concern regarding the potential for a future global extinction of mosquitoes. However, due to monetary cost and direct loss of life associated with malaria, this technology continues to be explored under strict regulations outlined by the World Health Organizations publication in 2014, *The Guidance Framework for testing genetically modified mosquitoes*, as well as a gene drive report released by the National Academics of Science, Engineering and Medicine [7]. These publications outline a multiphase methodology to evaluate safety and efficacy of the gene drive product. Our project assists Target Malaria in their compliance with these regulations as it provides a device in which captured mosquitos are classified as to whether or not they are positive for the transgene. This data is essential in tracking and reporting the spread of the transgene throughout the population.

There is also the potential for Target Malaria to have societal complications with stakeholders in the African communities. In order for mosquito samples to be collected, members of Target Malaria must enter communities in the participating areas to do so [4]. The collection of samples from homes or compounds requires Target Malaria to obtain individual consent as well as village level consent to collect larval sites sampling. However, to combat this Target Malaria has a very active stakeholder engagement team to engage with communities to inform community members of the research and goals in depth, prior to asking for a group decision to allow research in the area.

Further, the team stays in contact with members of the community via a grievance mechanism, to allow residents to address their concerns. It is through this team's efforts for clear communication and transparency that Target Malaria has achieved a strong positive relationship with their stakeholders, which has greatly diminished concerns in device implementation.

Global and Societal Impact:

The control of malaria via gene drive stands to have a substantial impact throughout the globe as it is estimated that individuals up to 3.4 billion within 92 countries are at risk of infection (https://www.who.int/gho/malaria/en/). While malaria is a global issue, it disproportionately impacts the African region. It is in this region as well as India that 80% of the deaths due to malaria occurred in 2017. Currently it is estimated that 25% of child mortality for children under 5 is due to malaria in Ghana (https://www.ncbi.nlm.nih.gov/books/NBK1712/). By decreasing the incidence of malaria in Africa, there will be a significant decrease in child mortality as well as increase in quality of life, as less suffer the long term effects that can follow infection.

Additionally, there is a substantial economic cost associated with the prevention, treatment, and loss of life due to malaria. Currently $5.1 billion per year is required to sustain efforts to reduce the incidence of malaria, including mosquito nets, insecticides, prevention drugs [4]. Unfortunately, the funding for these resources is not readily available nor are these prevention methodologies effective, for a multitude of reasons. While mosquito nets are typically effective, they are only used throughout the night, mosquitoes develop resistance to insecticides, and preventative drugs aren't recommended for long-term use. In addition, all methods are expensive to maintain. Cost of treatment, transportation to and from treatment, days absent from work or school, and expenses related to burials are all compounding factors in overall cost of malaria to individuals [5]. It is estimated by the CDC that the direct cost due to malaria annually is $12 billion, not including losses in economic growth. However, with the

control of malaria, there is the potential for strong economic growth in Africa. Through a multitude of studies the World Health Organization has concluded that the elimination of malaria by 1965 would have resulted in a 32% increase in sub-Saharan Africa's gross domestic product by 2000 (https://www.ncbi.nlm.nih.gov/books/NBK1712/). To this effect, the current GDP at the time would be increased by $100 billion, showing the significant positive impact of eradicating malaria on the African economy.

Description of Relevant Engineering Standards:

Standards for engineering medical devices set by the International Organization for Standardization indicate the quality management systems – requirements for regulatory purposes via ISO 13485. This standard is especially relevant to the testing and prototyping process for our device. In particular the ISO states that there must be clear design and development planning documents maintained including necessary validation tests done as well as the resources and competence needed throughout the process. The validation and verification tests used must have appropriate methods, acceptance criteria and statistical techniques to rationalize a sample size. These processes are extremely important to our device currently as well as moving forward. As we are completing *in silico* prototyping of the device which will later be implemented by BWB or others at Target Malaria to physically prototype the device, it is vital that we accurately document our progress so that it can be iterated upon in the future. Additionally, it is necessary that the intermediate as well as end goal tests are accurately verified to ensure that the imaging component accurately identifies whether or not a given sample has the transgene.

Future Work:

There is substantial interest by Target Malaria in bringing this device concept to a stage that is field enabled. The most obvious extension of the work that we have completed here is the development of the imaging module. While we have done research into feasible components and brainstormed designs amongst our team this

project would essentially be de novo design of an imaging module. When considering this module we envisioned a box that was fitted for the sample handling tray from the heating module with an array of LEDs to illuminate the samples from below and a holder on the lid that would image the samples through theater lenses to isolate the emission wavelengths of the samples. Because band pass filters in general are expensive, we envisioned a filter wheel with high pass filters that were compatible with common fluorophores and illumination of one fluorophore at a time. A particularly interesting feature would be an integrated circuit that turns on the proper excitation LED when the filter wheel is set to the corresponding emission spectra, eliminating chances for user error in filter selection.

Other future work on this project would be testing and optimization of the heating block design. Most of the tests that we anticipate being useful for both modules are outlined in the results section above. Some of the design feedback that we received but have yet to incorporate is that our design should consider moving away from components such as plastic switches on the control panel because these elements are prone to break in the field. Our Target Malaria partners also thought that the design should be reevaluated and tested in conditions that are dusty or wet, to ensure that the design would stand up to work in the field.

A large space for optimization in this design is cost. When doing initial price assessments for the heating module it appears that manufacturing (materials and machining) of the heating block would come in around $800. While this marks an improvement over the cost of equipment that is currently used in centralized laboratories, such as thermocyclers. While we could drive down the cost by taking advantage of economies of scale, we also believe that there is room for improvement within our design. For example, the design currently requires aluminum blocks that have a depth much greater than the standard. Creative design changes involving stacking block components or reducing the depth required for the block are some potential solutions to this problem. Further, there are aspects to our design that can be optimized

for CNC, as well as a wealth of online guides that outline methods of driving down costs such as this article from 3D HUBS.

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Appendix (2 pages)

Week	Due	Description	Tasks	Completed?
Winter Quarter: Week 1	01/08		Begin lit and patent search Initial search for arduino and device specifics	X
Week 2	01/15	Literature and Patent Search due	Needs finding Draft constraints and criteria Establish pipeline/contacts for troubleshooting with circuitry	X
Week 3	01/22	Progress presentation and slides	Update Ray and Jamie on progress in preparation for Target Malaria meeting Have proposal outline done (tentative)	X
Week 4			Brainstorm Solutions	X
Week 5	02/05	Proposal outline	Begin drawing device design and deciding imaging method	X
Week 6			Assess multiple design options with mentors.	X
Week 7			Begin prototyping (Tentative) Finalize draft proposal	X

Week 8	02/28	Proposal draft 2 due	Finalize presentation	X
Week 9	03/06	Presentation Due	Finish proposal	X
Week 10	03/13	Final proposal due		X
Spring *Quarter:* Week 1		Re-scope project	Meet with group as well as mentors to develop a new in silico plan for the project	X
Week 2		Re-scope meetings with Alyssa	Finalize re-scope plans Familiarize team with remote desktop	X
Week 3	4/14	Imaging component work Explore COMSOL testing Team Contract due Project Update (re-scope) due	Shift CAD drawings to SOLIDWORKS from Inventor and iterate Determine necessary tests for in silico tests Rebuild prototype circuit in Tinkercad	X

Week 4		COMSOL work Imaging component work	Build COMSOL model Adjust SOLIDWORKS files for recorded measurement constraints Add additional desirable features to Tinkercad circuit	X
Week 5	4/28	Imaging component work Heating Component work Reflection after Richard Ladner's Talks due Universal Design Group Assignment due	Iterate SOLIDWORKS files to fit heating circuit requirements Build top/lid of heating component Make rough imaging component experiments for one sample processing using leds, filter, etc. Draft requirements for full scale imaging component Meet as a group to discuss universal design	X
Week 6		Imaging Component work	Continue drafting potential testing methods for imaging component	X

Week 7		Evaluation	Testing in COMSOL Make adjustments if necessary	X
Week 8	5/19	Evaluation cont. Reflection I due	Final evaluations of design Draft presentation slides	X
Week 9	5/26	Engineering Analysis and Testing due Check-in Summary of Engineering Standards due Reflection II due	Present practice presentation in class Make presentations according to feedback Begin FInal Report	X
Week 10	06/01	Capstone Symposium/Final Presentation	Present presentation Finalize Final Report and complete peer evaluation form	X
Finals week	06/08	Final Report due Peer Evaluation due		X

Material Selection

- Heating tape
- Aluminum block or Heat Conductive Resin block
    - Wax block for prototyping
- PLA for printing housing and prototypes
- Circuit elements (thermistors, mosfets, resistors)
- Insulation - porous PLA structure
- Arduino
- LEDs for excitation of samples (465 nm) that can be made into an array (https://www.adafruit.com/product/301)
- Sheet bandpass filters for excitation wavelength (use fluorescein in prototype) (https://goknight.com/lee-filters-778-quarter-new-colour-blue-fluorescent-sleeve/?sku=778-36-T8&gclid=Cj0KCQiA1-3yBRCmARIsAN7B4H3fnnJs17H9ahLIMujjkx977DJnWmSCN5xO5rUCJEQ_RzUoSs5MflAaAsXWEALw_wcB)
- Sample tubes
- fluorescein

Software

- SolidWorks
- Arduino IDE
- Android or iPhone IDE (depending on the progress of the project)