Proceedings of the 28th IEEE
EMBS Annual International Conference
New York City, USA, Aug 30-Sept 3, 2006

Making Medical Devices Accessible: A Drug-Resistant Malaria Detection System

Tina Smilkstein

Abstract— **People living in areas of the world that are affected by disease, famine, or poverty could have their lives drastically improved by currently available electronic technologies, but the cost, complexity and/or limited operating environments of devices which employ these technologies can make it impossible or impractical for many of those in-need populations to actually acquire and make use of them. The barriers to acquisition and use are understandable. Most medical device companies are located in the wealthier countries, as are their clientele, and, to these companies, taking technological advances and either creating new products, or make their pre-existing products more powerful is the logical business and scientific progression. As such, devices seen in production that would be useful are often hospital grade with high-accuracy and varying amounts of adjustability so as to allow them to be used in performing a variety of functions. But it is exactly this accuracy and flexibility that makes them too expensive and complex to easily be acquired and used in environments that have limited financial and skill resources. In this paper, as an example of how to make presently inaccessible technology accessible, a method of detecting drug-resistant malaria strains in laboratories is analyzed, barriers to use of such a system in a poorer, malaria affected region are looked at, and a device is designed and a prototype built that is simple and affordable. Such a system not only allows collection of epidemiological information, but also empowers doctors and researchers to investigate new drugs without expensive laboratories.**

I. INTRODUCTION

Devices for diagnosing illness and gathering epidemiological information are used almost without thought in developed countries. The large number of tests and the accuracy with which those tests can be done enables doctors and researchers to identify, quantify, and discover. But, in regions that lack skilled labor and financial and networking resources, access to quality laboratory equipment is the exception, not the norm. This is a vicious cycle because in many of these regions the lack of these resources is caused by the very disease that having these resources, and therefore the ability to acquire and use proper lab equipment, could solve. Companies employing new or improved technologies build ever more complicated and accurate machines which enable ever more amazing diagnostics and discoveries, but, in the process, make these machines less and less accessible to populations that could greatly benefit from their use. This paper discusses the development of the electronics for a drug-resistant malaria detection system, a system inspired by the unfilled need of a cheap, robust, and simple strain identification device for communities that are direly affected by the treatable disease, malaria.

T. Smilkstein completed this as a graduate student at the University of California at Berkeley, Department of Electrical Engineering and Computer Science. As of F'06: Department of Electrical and Computer Engineering, University of Missouri - Columbia, 349 Engineering Building West, Columbia, Missouri, 65211-2300, USA SmilksteinT@Missouri.edu

Case	Drug	Resistance strains
Drug Sensitive Malaria	Chloroquine	Resistant strains exists (80%)
Drug Sensitive Malaria	Pyrimethamine Sulfadoxine	Resistant strains increasing
Drug-Resistant Malaria	Quinine	More toxic than chloroquinine
Drug-Resistant Malaria	Quinine + Doxycycline	Combination Therapy, expensive
Drug-Resistant Malaria	Mefloquine	Resistant strains exists
Drug-Resistant Malaria	Artesunate	For severe P. falciparum
Hepatic schizont drug	Primaquine	Resistant strains exists
Gametocytes drug	Chloroquine	Resistant strains exists

TABLE I
DRUGS AND THEIR EFFECTIVENESS.

II. IMPORTANCE OF DRUG-RESISTANT MALARIA DETECTION SYSTEM

Africa is presently the continent hardest hit socially and economically by malaria. Each year Africa sees approximately 300-500 million cases and 1.5 to 2.7 million deaths, and, astoundingly, more than 90% of these deaths are in children under 5 years of age [1] [2]. There are many people working to reduce the human and socio-economic costs of malaria through better prevention and treatment methods, but as often happens in low income areas, if prevention costs money, it is forgone and the burden falls on the people involved in treatment. One of the largest challenges to those trying to provide treatment is the appearance of drug-resistant malarial strains, as shown in Table I. Chloroquine is one of the most popular drugs, but now chloroquine resistance is exhibited in 80% of the malaria strains seen. When chloroquine can not be used, other drugs must be used, many of which are more expensive, more toxic, or have a more complicated treatment schedule. Strains of malaria have also emerged that display resistance to the replacement drugs for chloroquine (see mefloquine).

With the emergence of resistant strains, decisions on treatments by healthcare workers become more complicated. When a proven treatment fails, it must be determined what treatment would actually work. This information is important for both the presenting patient as well as for future patients

infected with the same strain. Tests to determine the strain's drug resistance may be done in a properly equipped laboratory, but, if there are no appropriate laboratory facilities available, often the only method possible to determine resistance is a 'clinical diagnosis', or, simply to try all available treatments until one is found that works. Once this 'clinical diagnosis' is done and an effective drug has been found, that particular healthcare provider will have better knowledge of the treatment for the strains existing in his or her own region, but unfortunately, because communication between regions is often nonexistent, this information is not passed to nearby healthcare providers. When a strain is carried to a new area, the same treatment determination process may have to be repeated. A network which allows each healthcare provider to post and access information on the strains seen in and around their area is very important and would allow for quicker and better treatments, but the first step in making such a system more efficient and accurate is to provide the healthcare workers with a simple tool which would allow them to gather drug resistance information themselves, without having to use patients as test cases.

Methods that have been previously used to identify drug-resistant strains of malaria include in vivo clinical tests, real-time PCR to detect mutations, isotopic microtest, and radioisotopic assays. In vivo testing, or 'clinical diagnosis', of resistant strains rely on clinical observations, which are often not highly accurate. For example, though a particular drug may be appropriate and effective when administered at the correct schedule and dosage, a treatment may fail because it requires inadequate amounts of the drug to be administered. This may show a treatment failure and the drug, though valuable, may be rejected. Real-time PCR is another method used for identifying drug resistance that is more direct. It is used to determine the presence of mutations that are likely to confer resistance. But this method is expensive and requires more complicated lab technique and would not be appropriate for regions without facilities and skilled lab workers. The traditional method is microscopy [4]; a technician simply counts the number of parasites in a given quantity of blood treated with a variety of antimalarial drugs. This is undesirable because it is time-consuming, may not provide required accuracy, and requires having and using a microscope. Radioisotopic assays are also used in the context of testing for drug resistance, and are based on the quantification of radioactive substances taken up by the parasites during their growth in the presence of drug. These assays are expensive, difficult to perform, and involve radioactive products which require extra precaution. These methods require trained staff and investment in equipment beyond what many of the areas most needing this technology can supply or finance.

III. DETECTION METHODOLOGY

The detection method examined in this research was developed by Riscoe lab, Experimental Chemotherapy, Research Service, Portland VA Medical Center and uses the same method as traditional microscopy in preparing treatment of a malaria infected blood sample up to the point directly before determination of quantity of parasite. To determine if a drug is effective against a strain of malaria is to see if it continues to multiply after the drug has been introduced. Simply, if the parasites in an infected blood sample stop multiplying or multiply at a slower rate once a drug has been introduced, then the drug has had some effect on the parasites. If there is no change in the multiplication rate of the parasites, then it is clear the drug is not effective. To be able to tell if a sample's parasites have multiplied, the sample must be diluted and divided into multiple containers. A 96-well plate is used in the prototype discussed in this article.

Once the sample has been divided, some of the wells are prepared as control wells. Two types of control wells are used in the Riscoe lab assay, an upper bound sample which is not treated with any drug and allows the malaria protozoa to multiply freely, and a lower bound sample, one where all parasites are killed so that there is a reference for the number of parasites existing in the original sample. The rest of the wells are given varying amounts of the drug to be tested, and then the plate is put into an incubator for approximately 72 hours to allow the parasites to multiply. Depending on the effectiveness of the drug being tested, the amount of parasite after incubation should be somewhere between the two control wells.

At this point, the method used by Riscoe labs deviates from the traditional method of determining the amount of parasite in the processed sample. The method used is called Malaria SYBR Green Fluorescence (MSF) Assay [5] and determines the amount of parasite by sensing the intensity of the sample's fluorescence, a fluorescence caused by a dye, SYBR Green I, that upon binding with double-stranded DNA exhibits a large enhancement of fluorescence. One challenge is that, as well as the parasite, the white blood cells in the blood sample also contain double-stranded DNA, and since the fluorescence signal from the malarial DNA may be small compared to the level of fluorescence from the SYBR Green dye binding to white blood cell DNA, the detection device requires a high level of sensitivity. To lessen this problem other dyes have more recently been used that provide larger fluorescence enhancement and therefore require less sensitivity. One of such dyes is SYBR Gold, a dye that may have superior intensity to SYBR Green, and possibly be cheaper. Other dyes, also, are becoming available and may help in cost and intensity of fluorescence. Fluorescing other parts of the protozoa or even byproducts of the malaria parasite are also being looked at and would remove the white-blood cell noise problem. But for sensing any of these dyes or materials the required sample preparation and the detection hardware is essentially the same.

Once the malaria parasites are dyed, the intensity of their fluorescence must be read. At Riscoe labs this is done using a $20,000 table-top microplate spectrofluorometer from Molecular Devices. This is an outstanding machine, but the price and adjustability makes introduction to regions that are most in need of such a system close to impossible. To gain perspective on what $20,000 means to a person living in,

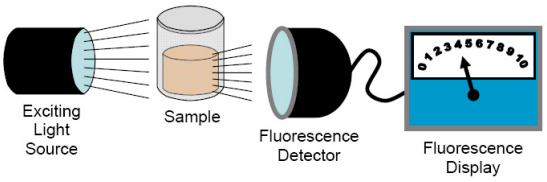

Fig. 1. Basic system diagram.

say, the hard-hit subsaharan region of Africa, note that the average income is less than $1/day for the region, and as such, it would take more than 20,000 man-days to pay for one of these machines locally.

The complexity of operating the device is also a barrier. Needing skilled lab technicians would make use of the machine impossible in most communities heavily affected by malaria. The rest of the sample preparation may be done with prepared plates and a simple incubator, but the use of the spectrofluorometer in the detection system puts the system out of reach for many of those that could really use it.

The goal of this project was to build an inexpensive, robust, and simple device to take the place of the spectrofluorometer in the Riscoe lab detection system.

IV. FLUOROMETER DESIGN

Fluorescence is the light emitted when a material is excited by a light of a different wavelength. Figure 1 shows a basic fluorometer system. The three blocks, the light source, fluorescence sensor, and display will be discussed in this section, as well as the high-level design decisions.

A. High-Level Design Decisions

The Molecular Devices microplate spectrofluorometer has many features including reading from the top or bottom of the sample container (the microplate), bathing the sample with light all across the spectrum to see all points of fluorescence, and giving output as an absolute value of brightness given in luminescence units. The system being targeted here does not need such flexibility nor accuracy. SYBR Green dye was picked as the dye to be used in this prototype, and, as such, the bathing light and the fluoresced light to be sensed become fixed values at $497nm$ and $520nm$ respectively. Also, unlike the Molecular Devices spectrofluorometer, the relative value of brightness is the important information we need from the device, i.e. the relative amount of parasite between samples, not absolute value of brightness. Also, to keep the cost of using this device as low as possible, the plates selected to be used were near the most inexpensive on the market. These plates themselves exhibit fluorescence at wavelengths around $520nm$, so, after comparing results of reading from the top and bottom of the plate, the decision was made to sense fluorescence from the top of the plate, where the device would not have to sense through the plate plastic.

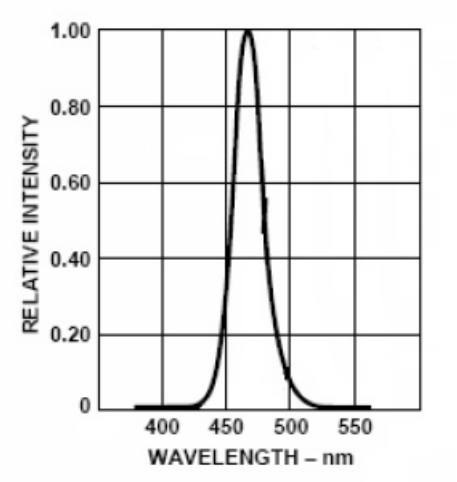

Fig. 2. Relative intensity vs. wavelength of light source.

B. Exciting Light Source

Light source candidates included various types of LEDs and lasers. Lasers had the advantage of being very narrowband around the bathing frequency and therefore not contributing to the light detected at the fluorescence frequency. But availability, complexity of use and cost issues made them less attractive than LEDs. After testing a number of LEDs, it was determined that they gave adequate performance for what the device needed and lasers were removed as a candidate. The LED finally chosen was a blue Agilent Technologies T-13/4 (5 mm) Super Bright Precision Optical Performance InGaN Lamps. The LED used had peak intensity between $472nm$ and $476nm$, but due to its brightness ($3200lcd$ to $4200lcd$), there was still enough light at $497nm$ to excite the sample. Further tests need to be done using a cyan LED (peak intensity at $500nm$) to see if performance is improved or hindered. Cyan would increase intensity at the exciting wavelength of $497nm$ but may also contribute to the light seen at the sensing wavelength and require higher sensitivity in the sensing hardware.

The only drawback found when using LEDs was that, though their peak intensity is around $474nm$, their spectrum is bell-shaped and has tails which extend out the fluorescing wavelength of $520nm$. The extra light at $520nm$ contributes to the background noise and makes detection of the dye fluorescence more difficult. Fig. 2 shows the bell curve of wavelength versus relative intensity for the blue LED (adapted from Agilent HLMP-CB18-TW000 Data Sheet). Though the contribution at $520nm$ is small, a physical $497nm \pm 10nm$ filter was attached to the LED to remove as much background noise as possible. Further investigation suggested that a filter actually may not be needed, but is included in the design discussed here.

C. Fluorescence Sensing

The choice of the hardware for the sensing unit once again hinged on price, robustness, and simplicity. One of the least expensive options available was a simple photodiode with a

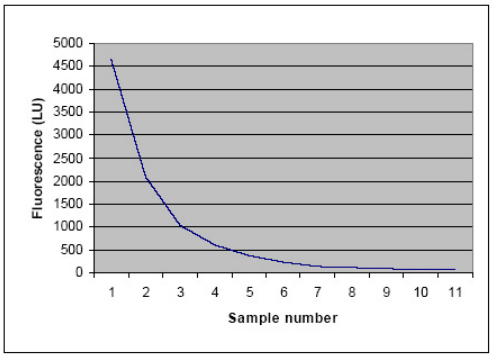

Fig. 3. Documented fluorescence intensity of row of test plate wells.

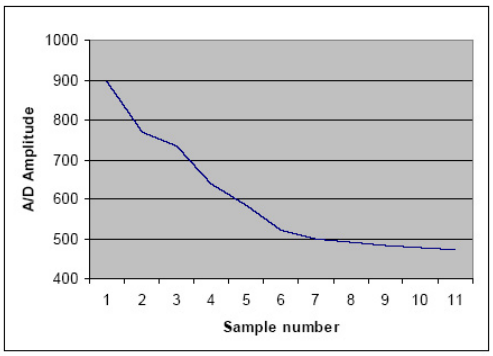

Fig. 4. Prototype reading of fluorescence intensity of row of test plate wells.

physical $520nm$ filter. The worry with that type of system was whether using a filter would reduce the intensity of the signal to the point where it would be impossible for the photodiode to detect small differences between samples. Testing determined that a filter with approximately 75% transmission still allowed enough of the signal through for accurate fluorescence readings down to the differences in intensity desired for the system.

D. Fluorescence Display

The results discussed here were obtained through the software supplied with the Analog Devices A/D evaluation board used in this research. The Analog Devices software displayed results to a PC, but the addition of a display directly on the detection device is in the planning, and will, when finished, display results in numerical form and also as a sigmoidal dose response curve.

V. TESTING METHODS AND RESULTS

Riscoe labs supplied a prepared fluorescing plate for testing of the prototype. All wells of the test plate had varying amount of fluorescing material that required an exciting light wavelength of $490nm$ and had a fluorescing wavelength of $510nm$. The fluorescence of the test plate was documented using the Molecular Device spectrofluorometer. The filters of the prototype were changed to match the wavelengths needed for the test plate. Rows of wells were scanned with the prototype system and results were compared.

The documented fluorescence of the test plate for an arbitrary row, is shown in Fig. 3. The same row, read by the prototype is shown in Fig. 4. The information that is important is the relative brightness of the wells, and that was correctly read for all wells examined.

The final device cost $150 to build, assuming approximately $40 for a display to be added later.

VI. CONCLUSIONS AND FUTURE WORK

In this work, the barriers to the use of a drug resistant malaria detection system in malaria-endemic regions of Africa were analyzed. The barriers identified were price, complexity of operation, and operating environment requirements, all of which needed to be below a certain threshhold for a system to be practical for the regions targetted. A prototype was successfully designed and implemented which cost $150, is simple to operate and determine results, and can run on batteries if needed.

The fluorometer technology this prototype employed has many applications such as medical research, clinical diagnostics, environmental testing, bioprocess monitoring/biotechnology, food quality control, pharmacology, and development of new pharmaceuticals. The changing of the LED and filters can make the prototype into any number of fixed wavelength fluorometers.

In the future, I hope that barriers that keep other useful systems from helping people are analyzed and borken down as was done in this project.

VII. ACKNOWLEDGMENTS

The author gratefully acknowledges the companies that generously supplied samples and advice to this project. Analog Devices contributed the ADuC816 (1 MIPS 8052 MCU, 8KB Flash, dual 16-bit ADC and 12-bit DAC) evaluation board and software. Agilent Technologies contributed the InGaN LEDs, and MATRIX supplied a large assortment of 96-well plates. Dr. Martin Smilkstein from Riscoe lab, Experimental Chemotherapy, Research Service, Portland VA Medical Center and a member of WHO/TDR (WHO Special Programme for Research and Training in Tropical Diseases) must be thanked for his patience in helping me understand the biological side of the malaria testing device. Thanks also to Howard Barney of Bayhouse Corporation for tips on building the device.

REFERENCES

[1] World Health Organization. "Investing in health research for development", *Report of the Ad Hoc Committee on Health Research Relating to Future Intervention Options*, Geneva, 1996, Report No.: TDR/GEN/96.1

[2] International Federation of Red Cross and Red Crescent Societies. "Malaria in Africa", http://www.ifrc.org/WHAT/health/archi/fact/fmalar.htm

[3] Wilson PE et al, Real-time PCR methods for monitoring antimalarial drug resistance. *TRENDS in Parasitology*, Jun 2005; 21(6): pp 278-283

[4] Rieckmann et al, Drug Sensitivity of Plasmodium Falciparum: An In-vitro Microtechnique. *Lancet*, Jan. 7, 1978.

[5] Smilkstein et al, Simple and Inexpensive Fluorescence-Based Technique for High-Throughput Antimalarial Drug Screening. *Antimicrobial Agents and Chemotherapy*, May 2004, pp 1803-06.