

## **Develop an Inexpensive (and Cost-Effective) Diagnostic Test That Could Be Deployed in Countries with Little Scientific Research Infrastructure**

### **WORKING GROUP DESCRIPTION**

#### **Background**

Tropical parasitic infections such as malaria, leishmaniasis, and trypanosomiasis, are responsible for millions of deaths per year. Most deaths occur in children or young adults in developing countries. Malaria was eliminated in some regions of the world in the 1960s but remains in Africa, Central and South America, and Asia in strains resistant to chloroquine and other inexpensive and commonly available drugs. Mosquitoes are also developing resistance to common insecticides (Mabey et al., 2004; [http://www.gatesfoundation.org/GlobalHealth/Pri\\_Diseases/Malaria/default.htm](http://www.gatesfoundation.org/GlobalHealth/Pri_Diseases/Malaria/default.htm) (2/2/06)).

Point-of-care (POC) diagnostic tests exist for infectious diseases such as malaria. They use immunochromatography to detect antigens or antibodies in a dipstick or lateral-flow format. Companies are manufacturing rapid diagnostic tests for malaria but most have not been carefully evaluated and the performance typically falls below the expected level. The accepted standard for malaria diagnosis remains the evaluation of Giemsa-stained blood smears by light microscopy, which is labor intensive, slow, and requires trained personnel. More widespread availability of simple and accurate dipstick tests for malaria would alleviate a great burden of disease in Africa (Mabey et al., 2004).

#### **The Problem**

Diagnostic tests in developing countries need to be widely accessible, inexpensive, and simple to use.

- Determine the constraints necessary to make diagnostic tests widely available, considering such factors as length of test, follow-up visits, supply of reagents, electricity, equipment required, trained technologists, cost, and so on.
  - What sensitivities and specificities are required in diagnostic tests to serve large populations?
  - Can you take advantage of genomic methods to identify different strains of the disease and use that information to diagnose and ultimately treat individuals on a case-by-case basis and in a cost-effective manner?
  - Design a diagnostic device that combines all the desired features you identify in earlier steps. Provide a scenario of how this device can be deployed and used effectively in developing countries.
  - How can nanotechnology and new rapid diagnostic methods for other targets be adapted to diagnose malaria species, drug resistant mutations, and vaccine resistant polymorphisms in malaria-endemic countries?

If desired, the working group can expand the topic to tropical diseases other than malaria—visceral leishmaniasis, African trypanosomiasis, Chagas' disease (South American trypanosomiasis), and so on.

### Initial References

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### WORKING GROUP SUMMARY

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### Summary

Imagine having to travel dozens of miles from your village or town to reach a health clinic when you get sick. When you finally arrive, you have to stand in line until the doctor is ready to see you. You might then spend another few hours awaiting your test results. Depending on the number of other patients there, you may have to stay the night. And because each clinic provides a limited selection of tests, you could end up repeating this process over several days. This scenario is real for people in parts of Africa and other countries where medical resources are sometimes scarce but where infectious diseases like malaria, tuberculosis, and HIV are rampant. According to the Centers for Disease Control, 39.4 million people—mainly in Africa and Asia—were living with HIV at the end of 2004. Tuberculosis, another infectious disease common in developing countries, racked up 2.4 million cases last year. Malaria affected between 350 and 500 million people, 70 percent of whom live in Africa.

With these scenarios and statistics in mind, our working group approached our task: to design a portable, inexpensive test for malaria or other infectious diseases that is easy to administer and that will deliver fast results—within approximately 30 minutes. The test should take into account different disease strains and examine possible drug resistance.

Our group was a motley crew: among them a few geneticists, a chemical engineer, an AIDS expert, a social and behavioral scientist, an infectious disease guru, an electrical engineer, a science journal editor, an evolutionary biologist, and a pharmacology expert. Individually, no one was equipped to devise a diagnostic test with all the properties we wanted. But together, this diverse group of imaginative scientists

took only eight intense hours to develop a product that is now in the beginning stages of the patenting process.

Of course, creating this product involved a winding road of brainstorming, questioning, and debate. The major issues on day 1: What disease should we test for (we were charged with malaria but had the option of choosing something else)? How should we tackle sample preparation, assuming those using our device would have limited access to electricity, refrigeration, reagents, and additional equipment? Should we create a protein- or DNA-based test? Could the sensitivity and specificity of our device surpass that of current tests? And how would we incorporate nanotechnology?

We spent a large chunk of our first meeting—and even part of the second—deciding which disease we should target. Initially, we hoped to combine HIV, malaria, and tuberculosis tests on a single platform. But inexpensive, portable HIV diagnostics already exist, and tuberculosis tests involve messy sputum samples requiring more preparation than blood samples.

We decided to focus on malaria, because it would require simple blood samples and would allow us to examine all the challenges outlined in our task. Furthermore, an improved malaria test could fulfill a pressing public health need on a large scale. Though malaria disease complex is transmitted by four main malarial plasmodium species, the fastest and cheapest test on the market does not indicate which of those species is present. Microscopy tests do, but require trained professionals. Furthermore, people who take antimalarial drugs on a frequent basis, as is often the case, harbor pathogens that often develop drug resistance. If we could take these factors into account, we would simplify and accelerate the malaria-testing process and lay the foundation for tests of other infectious diseases.

In the end, we constructed a device composed of two parts: (1) a reusable sample platform that includes a battery, display, and a sample docking port and (2) a disposable sample chip on the order of a few square centimeters that inserts into the platform and performs all the testing necessary for analysis using nanotechnology. Each chip is a miniature laboratory that processes and analyzes the samples.

Developing the platform itself was straightforward, but designing the sample chip necessitated all the expertise in the room. Our hope was that we could load a blood sample onto the chip and initiate a series of reactions that would indicate whether the patient has malaria, the species of plasmodium, and whether the malarial strain is resistant to a particular drug. If necessary, we could create two chips: one that would give a “yes or no” diagnosis, and another that would subsequently provide the details for those diagnosed with the disease.

Among the details that challenged us:

1. *Should we employ a protein- or DNA-based test?* We could test for either antiplasmodium antibodies or the DNA of the malaria parasite. The problem with antibody tests is that they don't distinguish between current or past infection. A DNA-based test can: enough organisms are present to diagnose an active infection. Additionally, DNA tests involve simpler sample preparation methods and diagnostic probes.

2. *How large a sample would we need to get accurate results?* Ideally, the patient would provide a blood sample from a simple finger prick. This sample would

adhere to a loop—a ring-shaped device—that would fit securely into the chip. But we debated about how much sample we would need to recognize the parasite. Assuming there are eight to nine plasmodial cells per microliter of blood, 100 microliters would yield about 800 to 900 cells—enough for detection.

3. *How do we separate the DNA from other components in the blood?* We wanted to target the parasite's DNA, so we needed to separate it from other components in the blood, like proteins. To do this we incorporated a filter on the chip itself that would isolate the DNA. Nanotechnology would allow us to pinpoint just the parasitic DNA. We decided to use nanowires—tiny wires smaller than the width of a dust mite. These wires could be equipped with “docks” that attract and anneal only to the parasite DNA. Because nanowires are extremely sensitive, they can detect malarial DNA even if the disease is in the beginning stages and only a few parasites are present.

The result is a “lab on a chip” that would target and examine DNA of malaria parasites. The general procedure is as follows: a blood sample is inserted into the chip, where it rests on top of a small filter. The technician presses a button on the display console to initiate sample processing. A small amount of eluate runs over the top of the filter and carries off the proteins and other waste. A simple vacuum device routes the waste to a miniature “trash bin” on another section of the chip. Another solution unhooks the DNA from the filter and allows it to pass through. Shear forces tear the DNA apart to release the nucleic acids, which are hooked to magnetic tags that route the nucleic acids to the part of the chip outfitted with nanowires. Different nanowires are equipped with different “docking DNA,” corresponding with various malarial strains and drug resistance codes. If parasitic DNA is present, it hooks to the matching nanowire. The outcome of the test is displayed on the device console.

The main benefits of this device over other available tests are that it provides detailed results in a short amount of time. We estimate the process will take about 30 minutes—meaning at least 16 people could be tested in a single day, as opposed to four (assuming that a microscope-based test takes at least two hours). Furthermore, patients infected with drug resistant strains will avoid paying for medicine that will not work and that may exacerbate the drug resistance problem. And because the testing is confined to a disposable chip, it is safe and easy to perform.

After designing the device, we discussed several unknowns:

- How well the device will actually work
- Whether it is too similar to other new devices (some of which were already being developed independently by members of our group)
- The actual cost of each chip (the group estimated about \$5 per chip and \$2,000 for the machine and portal)
- How much user training will be required
- How the chips will be disposed of
- How the device itself will be cleaned and how easily it might get contaminated
- Who will provide medical counseling postdiagnosis
- How the device will be distributed

Group members are currently addressing these questions as they seek project funding and apply for a patent.

By the end of the conference we were imagining a new scenario: a technician outfitted with one or two of these diagnostic devices, simple sample collection equipment, and some sample chips visits a village. After taking a small blood sample from the patient, the technician loads the sample on the chip, presses a button, and an entire nanolab gets to work. Less than an hour later the device provides a detailed diagnosis—even if the patient is in the early stages of the disease and has not started to show symptoms.

Our hope is that this malaria test is just the beginning. If it works, we could design other chips that fit into the same platform to diagnose diseases like tuberculosis and HIV. An entire battery of tests could be performed in an office or in the field. Ultimately, we hope the test will save patients and healthcare providers money and time. Above all, we hope it will save lives.