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Hemozoin detection may provide an inexpensive, sensitive, 1-minute malaria test that could revolutionize malaria screening

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Abstract

Malaria remains widespread throughout the tropics and is a burden to the estimated 3.5 billion people who are exposed annually. The lack of a fast and accurate diagnostic method contributes to preventable malaria deaths and its continued transmission. In many areas diagnosis is made solely based on clinical presentation. Current methods for malaria diagnosis take more than 20 minutes from the time blood is drawn and are frequently inaccurate. The introduction of an accurate malaria diagnostic that can provide a result in less than 1 minute would allow for widespread screening and treatment of endemic populations, and enable regions that have gained a foothold against malaria to prevent its return. Using malaria parasites' waste product, hemozoin, as a biomarker for the presence of malaria could be the tool needed to develop this rapid test.

Keywords

malaria; elimination; rapid diagnostic; RDT; Magneto-Optical Detection; hemozoin

1. Introduction

Though malaria has been eliminated from the United States for 65 years, this disease still kills at least 438 000 people per year worldwide¹ and 95% of these deaths are children under age 5.² As the World Health Organization (WHO), Global Fund, and President's Malaria Initiative have invested billions of dollars to improve access to adequate drug treatment, important issues have arisen, "Who do we give the drugs to and which drugs do we give?" In many countries in Southeast Asia, such as Cambodia and Thailand, where malaria is endemic, there is widespread resistance to every class of antimalarial drug² and while revised formulation and combinations of existing drugs are being used, only two new drug

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alternatives are in development: one is tafenoquine which causes rapid hemolytic anemia in patients with G6PD deficiency and therefore requires additional testing prior to use.^{3,4,5} The WHO and local Ministries of Health suggest that developing countries not provide treatment without a confirmed malaria diagnosis because of fears of resistance, the known side effects of drug treatment, and reduction of precious drug stockpiles, particularly of artemisinin and its derivatives. Clinical centers may prescribe sub-optimal drugs such as chloroquine and sulfadoxine-pyrimethamine because of their low cost and widespread availability, instead of the WHO recommended, but more expensive artemisinin combination therapies that may be in limited supply. Healthcare providers in the field frequently face a difficult dilemma without an accurate diagnosis: choosing between treating those who appear ill absent a positive malaria diagnosis (potentially increasing the chances for resistance) or failing to treat an asymptomatic child who may have malaria in order to avert a preventable death. In reality, antimalarial drugs are available over-the-counter at many drug stores for self-treatment, which further adds to growing concern about drug resistance as does the mass drug administration underway in Cambodia.⁶

Current malaria diagnostic techniques are slow and frequently inaccurate. Providers often rely on clinical signs and symptoms to make the diagnosis rather than waiting an hour for a positive microscopic diagnosis. While rapid diagnostic stick tests (RDTs) have reduced the need for trained microscopists at each clinical center, they are slow (averaging 20 minutes) and may be inaccurate. Although little training is needed to use these devices, there is still a learning curve for accurate diagnosis and some RDTs have reduced sensitivities with species other than *Plasmodium falciparum* and minimal sensitivity below 200 parasites per microliter.⁷ The use of RDTs for widespread malaria screening is hampered by the inability of RDTs to detect lower numbers of parasites, dependence on cool storage (for most useful kits) and the time required for diagnosis. Furthermore, for both microscopy and RDTs, the time needed to perform the test and the high limit of detection are insufficient to identify asymptomatic carriers in a population.

For more than a century, the standard method for detection of malaria has been Giemsa-stained microscopic examination of blood. This straightforward method has the advantage of low cost of materials. However, malaria diagnosis by microscopy can take more than an hour and some regions suffer from a dearth of expert microscopists and inadequate equipment.^{9,10,11} The lack of sufficient numbers of skilled microscopists often prompts presumptive diagnosis and treatment initiation without a diagnostic test in febrile patients exhibiting symptoms of malaria.

2. Shortcomings of Field Malaria Diagnostics

Shortcomings of both the widely used microscopic method and current RDT methods reduce the utility of these techniques to perform widespread population screening. While the need is still great, there is no affordable, accurate, diagnostic for malaria that can be completed in less than 5 minutes from the time blood is drawn until the patient receives a diagnosis (Figure 1). There are currently no available methods to diagnose malaria in the field in less than 20 minutes from the time the blood is drawn. Many groups and funding agencies have focused on non-invasive methods for malaria detection. There have been attempts to use

MRI, microwaves, and high-resolution cameras or lasers shown through the fingernail bed to detect malaria parasites. However, none of these methods has yet to demonstrate efficacy and even the most promising have been unable to translate into usable field-friendly versions, and they remain costly. Furthermore, these non-invasive methods do not detect malaria parasites at the low levels needed for elimination.

3. LAMP assay

One diagnostic, loop-mediated isothermal amplification (LAMP) requires extensive training, and uses relatively inexpensive equipment compared with conventional polymerase chain reaction (PCR). However, it requires a laboratory environment as well as access to electricity making it difficult to use in the field. It has higher sensitivity than microscopy but lower specificity, using PCR as a reference standard.^{12,13} Because LAMP amplifies genomic DNA several fold, even very slight contamination during processing may produce false-positive results.¹⁴ Importantly, to be useful for population screening or interventions intended to eliminate disease, LAMP would need higher throughput than is presently available in order to screen larger numbers of samples.^{15,19} The length of time and the cost per test make it a poor choice for screening or as a first line test in most circumstances.

4. Microscopy

Another approach that has attempted to improve the accuracy of microscopic detection of malaria is the use of cell phone based cameras and software to aid the microscopist in the field.¹⁶ The software is designed to identify parasites on a microscope slide or to send pictures of candidate parasites to experts worldwide. This technology may be a useful research tool for improving the differentiation of the malaria species, but the images produced by these cameras are not yet adequate for accurate discrimination, even given the improvements in camera resolution. Although using cell phone technology appears to be attractive to public and private funding agencies, the minimum detection level remains the same as standard microscopy and it not only suffers from the same shortcomings as standard thin smear light microscopy but also is limited by image quality. Time to make the slide is still a factor; only a small number of cells can be observed (~200 cells per field from a thin smear slide) by cell phones and expert microscopists must still be available in real time to review the slides – and when they are not physically present there must be access to the internet or cell signal. The utility of this technology as a diagnostic is so limited that funding to advance its use for population screening should be discouraged.

5. Emerging Technologies

A number of new methods for detecting malaria parasites in blood samples are currently in development.

Novel DNA tests for molecular diagnostics are being developed for portable, point-of-care platforms. For example, one approach, termed the nanorattle-based method, uses a surface-enhanced Raman scattering (SERS) nanoplatform. In the presence of a magnet, magnetic

beads containing capture probes and target sequences and ultrabright SERS nanorattles loaded with reporter probes detect a specific DNA sequence of *Plasmodium falciparum*.¹⁷

Efforts are underway to use depolarized light flow cytometry to diagnose malaria in blood samples.^{18,19} Differential staining of AT vs. GC content and cell size, coupled with the presence of light reflecting hemozoin²⁰ will allow for the diagnosis of malaria and for the identification of the *P. vivax* in blood samples. The addition of fluorescent antibodies will further improve diagnosis. Flow cytometers are becoming more compact and less expensive as developers have responded to mounting pressure to produce low cost machines to measure CD4 levels to track response to HIV treatment.

Magnetic resonance relaxometry (MRR) also may prove to be useful for malaria diagnosis. Although the utility MRR has been hampered by its inability to detect low level parasitemia, recently researchers have surmounted this challenge by the use of microfluidic cell enrichment with a saponin lysis before MRR for rapid, label-free detection of malaria parasites.²¹ Although the MSS approach is still prohibitively expensive for developing countries, the potential of this method to detect low levels of parasitemia is promising.

6. Hemozoin at a Biomarker of Malaria

Several teams are focusing on hemozoin as a biomarker for malaria.^{22,23,24} Given its magnetic and birefringent qualities, hemozoin is a potentially attractive marker. Methods using polarized laser light have improved microscopic detection.²⁵ Mass spectrometers can also detect hemozoin and have been used to identify malaria positive patients who were previously thought to be negative.²⁶ Like cytometers, the price of mass spectrometers has declined but they still cost many tens of thousands of dollars. With trained technicians, mass spectrometers could be used for high throughput screening at a central facility, however the requirement of a central facility makes it difficult to use for eradication efforts. New hand-held spectrometers, currently under development, have the potential to significantly reduce the cost and enhance the point-of-care capability of this method of malaria detection.²⁷ However, questions remain about whether these devices will be sufficiently robust to withstand environmental stresses in developing countries and whether sufficient numbers of trained technicians will be available to operate them. The feasibility of using this technology in field settings remains to be demonstrated.

Other teams are using magnets to align the byproduct of hemoglobin digestion, hemozoin, in a magnetic field so that they block an amount of polarized light that is proportional to the concentration of hemozoin in the sample.^{28,29} When exposed to a strong magnetic field, the hemozoin particles align perpendicular to a strong magnetic field.³⁰ Because the hemozoin particles are birefringent they reduce the amount of transmitted polarized laser light from passing through a sample.³¹ The benefits of these methods are the low level of user training required, simple sample preparation, and the speed (<1 minute) with which malaria may be diagnosed.³² Additionally, this hemozoin biomarker is cleared from the blood stream within nine days³³ as opposed to several months for HRP2 protein upon which most RDTs are based.³⁴ In the past, using hemozoin as a detection method was considered less effective, but it was not because hemozoin was an unsatisfactory biomarker. Instead, early efforts were

hampered by poor implementation and inadequate understanding about the particle and its levels in the blood stream. For example, Delahunt et al. considered hemozoin is a poor biomarker. They used dark field microscopy to detect hemozoin particles but only looked at 1500 red blood cells before making the call about whether the sample was positive or negative. After looking at samples from 23 patients they declared the technology a failure. More modern and emerging technologies however, use far improved optical techniques to search for hemozoin particles in 10 million red blood cells or more and have shown much better sensitivity.³¹

7. Conclusions

A rapid (<5 min), inexpensive, point-of-care malaria diagnostic technique is desperately needed to minimize the underreported malaria cases in distant rural villages and to make accurate diagnosis and to identify asymptomatic carriers and allow for adequate treatment to reduce or eliminate reservoirs for malaria.

8. Expert commentary

In some regions, as much as half the population may be asymptomatic carriers of malaria⁸ which is a part of the malaria transmission equation which is all but ignored because of the lack of an appropriate diagnostic method. However, It is only through widespread screening of whole, at risk, populations that we can identify and treat carriers and ultimately achieve localized malaria eradication. A faster and less costly diagnostic method would enable mass screening, ensure timely treatment, and permit monitoring the effectiveness of treatment over time. More screening to identify cases and asymptomatic carriers and faster treatment would not only serve to reduce malaria-related morbidity and mortality but also would interrupt transmission, saving even more lives in the future. Hemozoin as a biomarker of the disease appears to have the potential to meet these needs.

9. Five-year view

There are efforts underway to develop methods detect hemozoin noninvasively, using transdermal optical excitation and acoustic detection of vapor nanobubbles around intraparasite hemozoin.³⁵ To date, the results of this approach have only been reported in a single patient and questions have been raised about the study method, in terms of the reliability of the diagnosis and the rationale for failing to determine parasitemia at the time of the device test rather than hours before and after.³⁶

Future goals in rapid malaria testing include not only rapid assessment of parasite species but also the rapid assessment of the drug resistance status of parasites, finding and quantifying G6PD-Deficiency to further advance toward personalized medicine, and extending the utility of existing drugs until new ones enter the market.

Over the next five years as these faster methods move from the bench to the field, we will be able to screen populations for the first time in real time, treat them in real time and screen multiple times per year at an even lower cost. Though they may be in the market along with

the existing standards of microscopy and RDTs, these new capabilities offer the best chance to interrupt malaria transmission in endemic areas and herald a new era of malaria control.

10. Key issues

- Malaria is still an enormous problem in many parts of the world.
- Current malaria diagnostics are very slow, often expensive, and inaccurate which limits their utility.
- The 214 million cases of malaria reported underestimates disease prevalence because it only represents patients who have been diagnosed at a government run clinic or facility.
- Lack of identification of asymptomatic malaria carriers is hampering malaria elimination.
- To rid the world of malaria will require an affordable method able to discern whether a person has malaria in <1 minute. This is the only way we can logistically and economically afford to effectively screen entire populations and travelers.
- The hardest person to diagnose and treat for any infectious disease is the last one.

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Figure 1. Speed, Cost, and Minimum Level of Parasite Detection by Method

These graphs show the time it takes to run the procedure, cost for running each sample, and the minimum amount of parasites each method can detect.^{37,38} The accuracy of each method and startup costs vary widely within each method and therefore are not shown. Malaria detection methods run the gamut from very inexpensive and inaccurate (i.e., thin smear microscopy) to the highly accurate but extremely slow (i.e., PCR). Thin smear microscopy is only effective for speciation of malaria parasites once a positive sample has been found. Flow cytometric methods have improved and offer the potential to screen for multiple types of infections at once, but presently they require expensive machinery and highly skilled technicians. RDT dipstick tests eliminate the need for highly trained personnel at the expense of cost and accuracy. RDTs are ineffective below 200 parasites per microliter. Magneto-Optical detection methods currently in development have the potential to be fast (<1 minute), inexpensive, and highly sensitive for <5 parasites per microliter. Magnetic extraction methods such as MACS columns³⁹ or Magnetic Deposition Method⁴⁰ are useful for research purposes but at over \$10 per sample with processing times of more than one hour, they are not feasible for malaria screening and therefore are not included in this comparison.