

The reliability of malaria rapid diagnostic tests through the seasons in Madagascar: Could storage conditions affect the efficacy of rapid diagnostic tests?

Malaria is a serious health problem in many areas of the world. The Center for Disease Control (CDC) reported that Africa South of the Sahara and parts of Oceania such as Papua New Guinea are at the greatest risk of malaria transmission [1]. In spite of the extensive efforts being made to reduce the burden of malaria through insecticide-treated mosquito nets, indoor residual spraying, intermittent preventive treatment for pregnant women with sulfadoxine- pyrimethamine, diagnosis with rapid diagnostic tests (RDTs) or microscopy, and treatment with artemisinin-based combination therapy, many countries still have millions of documented cases of malaria each year.

One country in particular that is suffering from the devastating effects of malaria is Madagascar. The CDC reported that malaria is present in all regions of Madagascar and the entire country is considered at risk for the disease [1,2]. A World Bank country status report stated that, “The malaria burden is [also] higher in Madagascar than most of the sub-Saharan Africa” [3]. For 2011, the *World Malaria Report 2012* documented 21,290,000 cases of malaria in Madagascar [4]. In addition, the *World Malaria Report 2011* announced that most African regions decreased malaria cases by greater than 50%, however, Madagascar only decreased malaria cases by less than 25% [5]. Furthermore in 2010, the *Malaria: Burden and Interventions Evidence Overview* published by the UK Department for International Development speculated that nationwide elimination of malaria in Madagascar is not feasible [6].

Since the burden of malaria is declining by a smaller proportion in Madagascar than other areas in the region, it would be useful to study the efficacy of the current procedures in place to control and reduce malaria. One of these intervention methods is the use of RDTs. RDTs are a relatively new method of malaria diagnostics designed for areas where access to microscopy or Polymerase Chain Reaction (PCR) technology is not available. Since the high malaria

transmission season coincides with when RDTs are most susceptible to damage from heat and

moisture, I believe investigating the storage conditions of RDTs could provide insight into why more RDTs have lower specificity statistical measures during the high malaria transmission season.

RDTs are lateral flow antigen-detection tests. If specific malaria parasite antigens are present in the blood sample, the anti-malaria antibodies in the test strip will capture the antigens and the test strip will change colors to produce a stripe. Stripes located in different regions of the test strip indicate which of the different species of the Plasmodium parasite that causes malaria (*P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*) are present in the positive blood sample [7].

Madagascar’s National Malaria Control Program states that, “Malaria diagnosis should be confirmed by microscopy at hospitals and by RDTs at all health centers” [3]. The World Health Organization (WHO) and the President’s Malaria Initiative, an interagency initiative led by the US Agency for International Development, also support this policy. RDTs are a quick, convenient, and low-cost diagnostic method, however they are not as accurate as microscopy or PCR. Three studies comparing the performance of RDTs with the reference method of microscopy evaluated the precision of RDTs through the statistical measures of sensitivity, specificity, PPV, and negative predictive value (NPV). Sensitivity measures a test’s ability to designate an individual with a disease as positive. Specificity measures a test’s ability to designate a healthy person (no disease) as negative. PPV is the proportion of positive test results that are true positives. NPV is the proportion of negative test results that are true negatives.

RDT performance during high (rainy) malaria transmission season

Study	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Bisoffi [8]	94(92-96)	78(72-83)	88(85-91)	88(93-92)
Diarra [9]	92.9(91.5-94.1)	77.2(73.4-80.8)	92(90.5-93.3)	79.4(75.6-82.8)
Ratsimbaoa [10]	90.2(81.7-95.7)	87.2(78.3-93.4)	87.1(78.0-93.4)	90.4(81.9-95.8)

RDT performance during low (dry) malaria transmission season

Study	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Bisoffi [8] Diarra	86(78-92)	90(86-92)	72(63-79)	95(92-97)
[9] Ratsimbaoa	74.9(70.9-78.5)	87.5(84.8-89.9)	81.8(78.0-85.2)	82.3(79.3-85.0)
[10]	93.7(69.8-99.4)	83.3(35.9-99.6)	72.7(49.8-89.3)	93.7(69.8-99.8)

The low specificity relative to that of microscopy reported in these studies suggests that RDTs have a high type I error rate. In other words, RDTs are more likely to produce false positive results than microscopy. In a recent study on RDT accuracy, Bisoffi et al expressed their concern with RDT false positive results: “The risk involved in false positive results has not been given the attention it deserves” [8]. False positive results are a serious issue because they could lead to over-treatment. If a RDT produced a false positive result, an individual who did not have malaria would be prescribed medication they did not need. As a result, if the same individual contracted malaria in the future the disease may be more difficult to treat because they may have built up a resistance to malaria treatments. If malaria becomes more difficult to treat, the disease will consequently become more difficult to eliminate.

One reason RDTs as diagnostic tools lack the accuracy of microscopy is because RDT performance is affected by temperature. Most RDT manufacturers specify that RDTs must be stored between 4°C and 30°C in the sealed packaging. RDTs must also never be frozen or exposed to extreme heats [11]. “The highest temperatures [in Madagascar] tend to occur in the rainy season” of the year [12]. The rainy season is also the high transmission season for malaria. For this reason, I believe it could be useful to study the storage conditions of RDTs. If RDTs have lower specificity values during the warmest part of the year, improper storage conditions could be affecting the accuracy of RDTs.

In a study performed on RDTs in tropical climates, Jorgensen et al commented on this connection between high temperatures and RDT efficacy:

Excessive temperatures were documented in all storage facilities except the air-conditioned central storeroom in Cambodia ... The temperatures recorded in Cambodia and the Philippines are likely to have a significant impact on performance during the shelf life of some RDTs ... and exposure to such conditions may explain published reports of reduced sensitivity or failure of RDTs that performed well in other studies. [13]

To explore if RDT storage conditions are affecting RDT efficacy, I will evaluate RDT storage conditions by survey. To guide my research of RDT storage conditions, I will use the information published by organizations like WHO for health center staff about proper storage practices and solutions for protecting RDTs against high temperatures and moisture. In *Transporting, Storing and Handling Malaria Rapid Diagnostic Tests in Health Clinics*, WHO illustrates many inexpensive ways to control storage temperatures without air-conditioning or refrigeration. Some examples include planting trees for shade and storing RDTs in in-ground storage [14]. At each health center I visit, I plan to observe and record what storage precautions are being taken to prevent RDTs from becoming less effective with no implicit judgment. In addition, I will record which storage conditions are in violation of the RDT proper storage guidelines put forth by WHO and RDT manufacturers like Core Diagnostics. I will also record the temperature of the RDT storage areas using an electronic temperature monitor (Extech TH10 Temperature USB Datalogger). This device will allow me to take many readings of the temperature and record the data on my computer. I also hope to compare false positive rates to climate in order to ask statistically what the false positive rates are in different climates under different storage conditions if the false positive rates are available.

To carry out my research project, I plan to visit health centers in high malaria transmission areas with hot climates because the RDTs at these health centers will be more susceptible to heat damage. I also want to study the storage conditions of health centers in warm regions so I can analyze how these health centers approach RDT storage differently. For contrast, I will study health centers in cooler regions to observe how storage practices differ in a

region where RDTs are not at as great of a risk for heat damage. The study sites I will visit include Antsiranana, Morondava, Toamasina, and Antananarivo. Antsiranana is an urban area in the northern corner of Madagascar in a hot and sub-humid climate. Morondava is a city located in western Madagascar in a hot and semi-arid climate. Toamasina is an urban area located on the eastern coast of Madagascar in a temperate and perhumid climate. Antananarivo is located in an urban area in the center of Madagascar in a cool and humid climate. Antsiranana, Morondava, and Toamasina have presumed malaria cases per consultant rates of 20 to 30 percent annually. Antananarivo has a presumed malaria cases per consultant rate of less than 10 percent annually [12]. I also incorporated visits to different types of health centers into my research project to compare how access to storage equipment like air-conditioning, ventilation and shelving, and the materials the health center is made of influence RDT storage conditions. Visiting health centers will furthermore allow me to engage with the local culture because at the health centers I will have the opportunity to interview health center staff and engage in their daily activities as much as I am permitted. Moreover, I will visit health centers in urban and rural areas, which will expose me to the different lifestyles in Madagascar.

Another way I will collect data about RDT storage conditions is by requesting physicians, nurses, and health center staff to fill out a survey I will design about RDT storage conditions. My survey will include questions to address how the health center changes its storage practices to account for temperature changes throughout the seasons and general questions about malaria diagnostics and treatment for background knowledge. The survey will be written in English and French. It is my understanding through my contact, Dr. Philip Trangmar, that individuals with a high school education in Madagascar speak French. Therefore, the health center workers should speak French or English. Some sample survey questions include: Do you store RDTs away from direct sunlight? How often do you get shipments of RDTs? How do you protect RDTs against

high temperatures and prevent harmful insects and rodents from entering the area? Through this survey, my goal is to understand general RDT storage conditions, how storage conditions change in response to temperature changes, and if these variables are related to incidences of false positive test results. Through my analysis of the data, I also hope to understand the effect that modifying storage conditions throughout the year has on RDT performance in relation to false positive test results.

To gain valuable background knowledge about the healthcare state of Madagascar, malaria, and RDTs I will volunteer with Mada Clinics for two weeks. Mada Clinics is a non-governmental organization that operates a free health clinic and school in the village of Maventibao near Antsiranana. While volunteering with Mada Clinics, I will work alongside healthcare staff in the health center. On Tuesdays and Fridays, the healthcare staff travels to other villages in the area to provide medical care. During this time, I will record how RDTs are transported in relation to proper transport guidelines put forth by WHO and I will measure the temperature of the RDTs during transport. I will also record the temperature of the RDT storage area in Mada Clinic's health center several times a day for a week to gain a better understanding of how the temperature in the RDT storage area fluctuates throughout the day [15].

I believe volunteering at a health center will provide me with invaluable first-hand knowledge about healthcare in Madagascar, the practicality of using RDTs at health centers where microscopy is not available, and help me understand the role that limited resources have on the functioning of health centers. In addition, Mada Clinics has contacts at hospitals in Diego Suarez and with Population Services International (PSI). With their assistance, I will work alongside an English-speaking physician in a city hospital to observe how healthcare workers with more resources approach malaria diagnostics differently. At this time, I will also record the temperature of the RDT storage area in the hospital. In addition, I will travel with PSI as they

distribute RDTs to rural health centers, measure the temperature of the RDTs during being transport, and record the precautions being taken to reduce the risk of heat damage [15].

Besides the temperature data collection I will perform at health centers in Antananarivo, Morondava, Antsiranana and Toamasina for a series of subsequent statistical comparisons, I have made arrangements to shadow two individuals who are experts in Madagascar's healthcare system. I believe it is important to incorporate shadowing experiences into my research project because these experts have extensive knowledge about malaria RDTs. Therefore, I will be able to learn more about RDT storage practices and malaria in Madagascar from these experts than I could through only studying RDT storage conditions at health centers myself.

I will be shadowing Dr. Arsène Ratsimbaoa, the deputy director of the National Malaria Control Program of the Ministry of Health Madagascar. Dr. Ratsimbaoa is also the project manager of Public Health and Education at the Faculty of Medicine in Antananarivo, the director of the Infectiology Centre Charles Mérieux, and has authored/co-authored many publications including some about malaria RDT performance. The other expert I will be shadowing is Rivonala Razafison. Razafison is a freelance science journalist who has reported on the healthcare state of Madagascar and the challenges its healthcare workers face. I am confident that these shadowing experiences will provide me with insight into the treatment of patients with malaria. Furthermore, these shadowing experiences will allow me to further immerse myself in the local culture and strengthen my understanding of how malaria impacts the everyday life of the Malagasy people because these experts have agreed to allow me to travel with them and shadow their work. I believe these shadowing experiences are essential to strengthening my research project because I will be able to present the data I collect with an understanding of what malaria means to the Malagasy people.

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