

Technical Note

Evaluation of “Cyscope”, a novel fluorescence-based microscopy technique for the detection of malaria

Lovemore Gwanzura^{1,2}, Justin Mayini¹, Katherine Mabhanga³, Joseph Chipinduro³, Kansomba Mashamba³, Menard Mutenherwa⁴, Susan L. Mutambu³, Junior Mutsvangwa¹, Peter R. Mason^{1,2}

¹*Biomedical Research and Training Institute, Harare, Zimbabwe*

²*University of Zimbabwe College of Health Sciences, Harare, Zimbabwe*

³*National Institute of Health Research, Harare*

⁴*Partec GmbH, Munster, Germany*

Abstract

Introduction: This study was designed to compare the detection of malaria parasites in peripheral blood smears using the Cyscope malaria rapid fluorescent microscopic technique and light microscopy of Giemsa-stained smears.

Methodology: A total of 295 blood smears were collected from patients of all age groups presenting with clinical signs and symptoms of malaria to 10 City Health Clinics in Harare. For each patient two blood films were prepared. Microscopic examination was done independently in two laboratories, with one performing the Giemsa stain and the other the Cyscope method. After the tests were completed, the results were then matched and recorded without any alterations.

Results: An equal number of men and women were malaria positive and their ages ranged from five to 66 years. Concordance in the detection of parasites (positive or negative) was 98.6% (291/295). In all four cases of discordance, malaria parasites were detected using the Cyscope but not with conventional microscopy. The Cyscope gave a 100% sensitivity and a specificity of 98.6%.

Conclusion: The Cyscope may be a valuable addition to diagnostics of malaria in resource-limited settings such as Zimbabwe.

Key words: malaria; fluorescence-based microscopy technique; diagnosis; resource-limited settings

J Infect Dev Ctries 2011; 6(2):212-215.

(Received 28 April 2011 – Accepted 09 May 2011)

Copyright © 2012 Gwanzura *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Malaria, an infectious disease primarily caused by *Plasmodium falciparum*, is estimated to cause over 300 million cases of acute illness annually resulting in over one million deaths. More than 80% of cases are reported from sub-Saharan Africa where the disease may account for as much as 40% of public health expenditure, and more than half of outpatient visits and inpatient admissions [1,2]. In Zimbabwe malaria transmission occurs in 40 of the 59 administrative districts, resulting in more than half the population being at risk. Nationwide about 12% of outpatient clinic visits are for malaria, though in areas of endemic and seasonal malaria this figure may be much higher. While most cases are uncomplicated and respond well to appropriate treatment, malaria is recorded as the cause of death in 8% of cases [3]. Of the five million people estimated

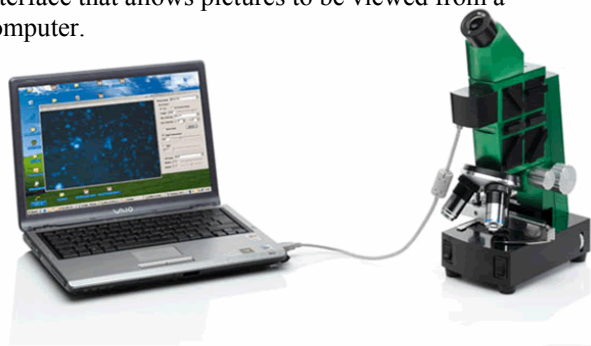
to be at risk in Zimbabwe, about 200,000 are pregnant women, and 900,000 are children under five years of age - the two groups who are particularly at risk of severe or fatal infections.

To prevent serious complications and death, treatment must be given promptly. In much of Africa treatment is given on the basis of clinical symptoms alone, though some would argue that this leads to inappropriate use of anti-malarials, which is both expensive and may contribute to the emergence of resistance [4-6]. Prompt and accurate diagnosis is the corner stone of effective malaria management and control, but limited access to effective laboratory diagnosis and eventually treatment in areas where malaria is endemic reduce the effectiveness of such strategies [7]. The traditional approach to malaria diagnosis is microscopic examination of Giemsa-stained peripheral blood smears. While a number of

newer techniques have been developed, such as rapid antigen detection assays and nucleic acid amplification tests, these have limited value in community-based clinics in rural areas where most cases of malaria infection are seen [8-10]. Microscopy therefore remains the principal means of diagnosis.

Traditional microscopy using Giemsa-stained blood smears and high-power light microscopes has a number of problems. Giemsa stain is unstable at high temperatures and so has to be freshly prepared; the technique is labour intensive and time-consuming; and low parasite numbers, below 20 parasites/ μl of blood, may be missed. The number of slides that can be examined without undue strain is limited, and tired microscopists may be even more likely to miss occasional parasites in a Giemsa-stained smear [7]. Alternative staining techniques using fluorescent stains have been described, and have the advantage of allowing rapid scanning of slides at lower magnification that both reduces microscopist fatigue and increases rates of detection where the parasitaemia is low [11]. The fluorescent dye SYBR-green 1 has been shown to be the most useful in the detection of malaria parasites [12]. Until recently, however, the need for a special microscope with UV light limited the value of such techniques. Recently a portable fluorescence microscope was developed in Germany ("Cyscope", Partec GmbH, Munster, Germany). The microscope is capable of both fluorescent and transmitted light operation, and incorporates powerful high-efficiency light-emitting diodes (LED) as light sources. The microscope is battery-powered and portable and can be used independently of mains power for many hours. A built-in camera interface enables images of the slides to be taken for further investigation by image analysis software if desired (Figure 1).

Figure 1. The Partec Cyscope comes with built in camera interface that allows pictures to be viewed from a computer.



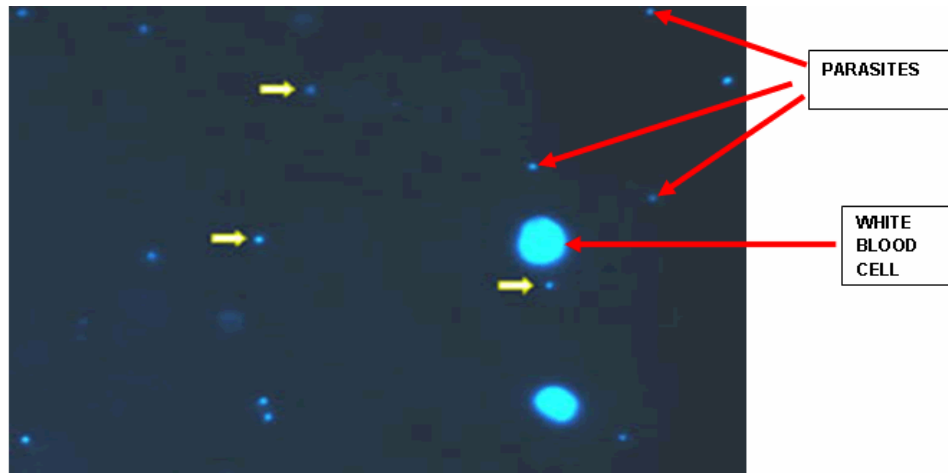
Slides that are pre-coated with fluorescent stains can be used in combination with the Cyscope to provide a rapid, affordable and practical alternative to traditional methods of parasite detection. In this paper we compare the rate of detection of malaria parasites in blood films examined using the Cyscope method and using standard Giemsa-stained blood film microscopy.

Methodology

Blood samples were collected from 330 patients (48% male, 52% female, ages two years to > 60 years) seen at 10 polyclinics in Harare during June to October 2007. All patients had self-presented to the clinic with symptoms consistent with a clinical diagnosis of malaria (mostly fever, headache and malaise) and consented verbally to the collection and use of blood specimens for detection of malaria parasites. Blood was collected into EDTA-coated tubes and kept in a cooler box away from direct light before being transported to the laboratory. Blood specimens were used to prepare thick smears (5 μl blood smeared into a 1cm diameter circle and allowed to air dry) that were stained (unfixed) for 30 minutes with diluted Giemsa using the standard operating procedure of the PHLS. Thick smears were initially examined at x400 magnification for microfilariae. At least 50 fields were then examined at 1,000x magnification (using oil-immersion) for malaria parasites.

A 10 μl sample of the same blood specimen was applied to the surface of the pre-coated slides supplied by the manufacturer. The blood was distributed evenly over the slide, incubated for one minute at room temperature and a cover slip was applied before examination using the Cyscope microscope. Using the normal white-light LED and 20X objective lenses, the focus was adjusted to clearly show red blood cells. The light source was then changed to UV-light to detect fluorescing objects – white blood cells and parasites – which showed as bright blue light against a dark background as shown in Figure 2.

The blood film examinations were performed at the Public Health Laboratory (Giemsa-stain) and the laboratory of the BRTI (Cyscope) without either laboratory knowing the results of their examinations. Only when all the slides had been examined were the results of the independent examinations combined for analysis.

Figure 2. A typical malaria positive slide under the Cyscope showing malaria parasites and white blood cells

Results

Of the 330 samples collected 295 were suitable for examination by both methods, and six (2%) were positive by Giemsa compared with 10 (3%) that were positive by Cyscope. This meant that concordant results (either positive or negative by both tests) were found in 291 (99%) specimens. In all four cases of discordance, parasites were found using Cyscope but not using Giemsa, and subsequent re-examination of the Giemsa-stained slides confirmed the presence of low numbers of parasites. With such small numbers of positive specimens in this urban sample, measures of sensitivity (100%) and specificity (98%) may not be particularly reliable, especially as the “false positives” were subsequently shown on closer examination to in fact be true positives.

Discussion and Conclusion

This study has demonstrated that the Cyscope is a useful and reliable tool to use in the detection of malaria parasites in blood specimens. The technique used is simple and rapid, slides can be read easily and quickly, and the Cyscope itself is a robust instrument that can be used in resource-limited settings as well as in more sophisticated laboratory settings. In a sample with low rates of parasitaemia there was good concordance between Cyscope examinations and examination of Giemsa-stained blood smears, with the Cyscope detecting a small number of additional positive cases. There is a clear need to further expand investigations of the technique in areas where malaria

transmission is common and a higher percentage of parasitaemic patients would be expected.

In this study there was no attempt to determine the parasite load through quantitative evaluation of parasites.

The limit of detection of parasites using Giemsa-stained smears is about 20 parasites per microlitre of blood, but there are no data available on the limit of detection of parasites by the Cyscope system. Our finding of a number of infections where parasites were detected by Cyscope but not by conventional thick smear microscopy suggests the limit of detection is much lower, but quantitative assays are needed to provide these data. While conventional microscopy may be used to identify parasite species on the basis of morphology of parasites and infected cells, identification to species level is not possible with the Cyscope system because it is only the DNA and not the whole cell that is stained. In areas where one particular species of *Plasmodium* predominates, such as Zimbabwe where > 98% infections are due to *P. falciparum*, this is not a great problem; however, in areas where infections with other species are more common, specific identification may be needed to ensure correct treatment. Furthermore, we have presented no data on the effects of specimen age on the sensitivity of the Cyscope and this may be an issue in areas where specimens are stored before being examined.

The Cyscope may be a valuable addition to diagnostics in resource-limited settings. The evaluation done here concerned only malaria

parasites, but there is potential for adaptation of the technique to other situations where fluorescence microscopy may improve the ability to detect pathogens in clinical material. Further studies are needed using specimens from malaria-endemic areas, using specimens that have been stored for differing periods of time and perhaps also to evaluate the value of the Cyscope system in monitoring responses to treatment. We hope to be able to present these data in the near future.

Acknowledgements

Funding for this study was provided by Biomedical Research Training Institute. We thank Partec Germany and Partec Southern Africa for the kind donation and availing of the Partec Rapid Easy Malaria test kits and the Partec Cyscope Malaria [microscope] which we used to perform the Malaria tests and the screening of the Malaria parasites respectively.

References

1. WHO (2002) The global malaria situation: Current tools for prevention and control. Global Fund to fight AIDS, Tuberculosis and Malaria, 55th World Health Assembly, WHO, A55/INF.DOC./6, 10 May 2002.
2. Global Risk Forum (2009) GRF Fact Sheet on Malaria. Available http://www.grforum.net/userfiles/file/GRF%20Factsheets/11122009_FactSheet_Malaria_en_final.pdf. Accessed 1 February 2012.
3. Ministry of Health and Child Welfare Zimbabwe (2007) Maternal and Perinatal Mortality Study 2007. Available http://www.unicef.org/zimbabwe/ZMPMS_report.pdf. Accessed 1 February 2012.
4. Amexo M, Tolhurst R, Barnish G, Bates I (2004) Malaria misdiagnosis: effects on the poor and vulnerable. *Lancet* 364: 1896-1898.
5. Kallander K, Nsungwa-Sabiiti J, Peterson S (2004) Symptom overlap for malaria and pneumonia—policy implications for home management strategies. *Acta Tropica* 90: 211-214.
6. Snow RW, Craig M, Deichmann U, Marsh K (1999) Estimating the mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bull World Health Organ* 77: 624-640.
7. Makler MT, Palmer CJ, Ager AL (1998) A review of practical techniques for the diagnosis of malaria. *Annals of Tropical Medicine and Parasitology* 92: 492.
8. Reyburn H (2007) Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomized trial. *British Medical Journal* 334: 403-409.
9. World Health Organization (2006) The use of malaria rapid diagnostic tests. Second Edition. Geneva: WHO (WHO-TDR/WHO-WPRO 2006.)
10. Hashizume M, Kondo H, Murakami T, Nakahara S, Lucas ME, Wakai S (2006) Use of rapid diagnostic tests for malaria in an emergency situation after the flood disaster of Mozambique. *Public Health* 120: 444-447.
11. Mendiratta DK, Bhutada K, Narang R, Narang P (2006) Evaluation of different methods for diagnosis of *P. falciparum* malaria. *Indian Journal of Medical Microbiology* 24: 49-51.
12. Guy R, Pennefather P, Crandall I (2007) The use of fluorescence enhancement to improve the microscopic diagnosis of *falciparum* Malaria. *Malaria Journal* 6: 89.

Corresponding author

Prof. Lovemore Gwanzura
BRTI Laboratories
PO Box CY1753
Causeway, Zimbabwe
Telephone: 263 4 735000; Fax: 263 4 73533
Email: gwanzura@mweb.co.zw
URL: www.brti.co.zw

Conflict of interests: No conflict of interests is declared.