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Comparison of Paracheck Pf® test with conventional light microscopy for the diagnosis of malaria in Ethiopia

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ABSTRACT

Objective: To assess the accuracy of Paracheck Pf® in reference to the conventional light microscopy. **Methods:** A total of 400 patients visiting Awash, Methara and Ziway malaria centers were simultaneously screened with both light microscopy and Paracheck Pf® for the presence of *Plasmodium falciparum* (*P. falciparum*) malaria. **Results:** Of the 190 samples that were negative by light microscope, the Paracheck Pf® showed 11 false positive and 179 true negative results, and from a total of 210 samples positive by light microscope, Paracheck Pf® accurately diagnosed 200 true malaria cases. Taking the light microscopy as a standard test for malaria, the sensitivity, specificity, positive predictive value and negative predictive value of Paracheck Pf® is 95.2% [cofidence interval (CI)=92.4-97.1], 94.2% (CI=91.1-96.3), 94.8% (CI=92.0-96.7) and 94.7% (CI=91.6-96.8), respectively. **Conclusions:** Paracheck Pf® showed good sensitivity and specificity for the diagnosis of *P. falciparum* malaria, and fulfill the world health organization (WHO) recommendation that requires the sensitivity of rapid diagnostic tests (RDTs) to be greater than 95%. Therefore, Paracheck Pf® can be used as an alternative to the Giemsa stain light microscopy in resource poor set ups.

1. Introduction

Malaria is caused by a parasite called *Plasmodium*, which is transmitted via the bites of infected female *Anopheles* mosquitoes. It is one of the major tropical diseases adversely affecting the health of the peoples and the economic development of many developing countries[1]. Each year, between 300-500 million malaria cases and up to 3 million deaths occur throughout the world, Africa accounting for more than 90% of the burden[2-4]. In Ethiopia, malaria remains the leading public health problem where an estimated 68% of the population lives in malarious areas and 75 % of the total land mass is regarded as malarious[5].

Employing an integrated and comprehensive approach that includes early case detection, selective vector control, epidemic management and control, environmental management and personal protection through the use of insecticide-treated bed nets (ITBs) are the main malaria control strategies in Ethiopia[6,7]. Despite recent efforts to control the disease in the country, malaria is still the leading cause of mortality and morbidity in Ethiopia[5].

Although there are several factors that hinder effective malaria control in Ethiopia, absence of reliable method of diagnosis is the major one. In Ethiopia, malaria parasite has been diagnosed by the use of Giemsa stained microscopy. However, since parasitological diagnosis is not accessible in some rural areas of the country diagnosis of cases is accomplished through clinical diagnosis[6]. Clinical diagnosis may result in misdiagnosis (presumptive treatment of all fevers as malaria) and inappropriate use of malaria drugs[8,9]. Furthermore, poor diagnostic standards such as the lack of enough skilled microscopists and inadequate or absence of quality control systems continue to hinder effective malaria control in the country. This shows that the development of a more rapid, sensitive, easy and specific diagnostic method could substantially improve malaria control in Ethiopia.

Several investigations have been conducted to assess the accuracy of different rapid diagnostic tests (RDTs) in reference to the conventional light microscopy, and these studies have reported contradictory results. While some

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studies reported a good performance of RDTs, other studies clearly indicated poor performance of RDTs[10-13]. Therefore, the contradictory reports on the operational characteristics RDTs prompted investigation of the situation in the malaria endemic localities in Ethiopia.

2. Materials and methods

2.1. Study site

The present study was carried out in three Ethiopian malaria endemic localities (Awash, Metehara and Ziway) to assess the accuracy of Paracheck Pf® in reference to the conventional light microscopy. Malaria is seasonal in the study areas with a frequent occurrence of epidemics, often from September to December, following the heavy rainfall season. There is one malaria centre in each region where people with symptoms suggestive of malaria obtain free services for malaria diagnosis and treatment.

2.2. Study population

A total of 400 patients visiting Awash, Methara and Ziway malaria centers between November 2008 and January 2009 were included in the study. Malaria patients who had received anti-malarial treatment within 48 hours prior to confirmation of their malaria and patients co-infected with *Plasmodium falciparum* (*P. falciparum*) and other species of *Plasmodium* parasite were excluded from the study. Patients critically ill and unable to respond for the interview were also excluded from the study.

2.3. Giemsa-stained blood film

Finger-prick samples were collected and placed in grease-free clean glass slid. Each blood smear was stained with Giemsa and examined immediately under the oil immersion microscope objective by two experienced laboratory technicians. The technicians were not told about the health and other status of the study participants. In cases where the results were discordant, a third expert reader was used. The results of the third expert reader were considered the final result.

2.4. Paracheck Pf® test

Approximately 5 μL of blood sample was transferred directly from the finger or toe of the study participants into each sample well and approximately 6 drops of buffer were added. After 15 minutes the results were read as recommended by the manufacturers (Orchid Biomedical System, Verna Goa, India).

2.5. Statistical analysis

The collected data was computerized using excel program, exported and analyzed by SPSS version 16 and JavaStat two way contingency table. Sensitivities, specificities, and positive and negative predictive values were calculated using Giemsa stained microscopy as a gold standard.

2.6. Ethical clearance

Ethical clearance was obtained from the Ethical Review Committee of Department of Biology, Addis Ababa University. Written informed consent was obtained from all study participants and mothers/caretakers of children under 18 who participated in the study after explaining the purpose and objective of the study.

3. Results

During the study period, 400 febrile patients were screened for *P. falciparum* infection with both Paracheck Pf® and Giemsa stained microscopy (Table 1). Of these, 210 were found to be positive and 190 were found to be negative for *P. falciparum* malaria by light microscopy. The Paracheck Pf® detected 10 negative samples that were positive by light microscope. Giemsa stained microscopy detected 200 positive results that were also positive by Paracheck Pf® (Table 1).

Table 1

Paracheck Pf® results compared to the reference Giemsa stained light microscopy.

Test and result		Light microscopy		Tatal
		Positive	Negative	
Paracheck Pf®	Positive	200	11	211
	Negative	10	179	189
Tatal		210	190	400

Of the 190 samples that were negative by light microscopy, the Paracheck Pf® gave 11 false positive results, indicating 94.2% (91.1-96.3) Paracheck Pf® specificity. Furthermore, taking the Giemsa stained microscopy as a standard test for malaria, the sensitivity, positive predictive value (PPV) and negative predictive value (NPV) of Paracheck Pf® is 95.2% [confidence interval (CI)=92.4-97.1], 94.8% (CI=92.0-96.7) and 94.7% (CI= 91.6-96.8), respectively.

4. Discussion

The results of this study have shown that, the sensitivity and specificity of Paracheck Pf® was 95.2% and 94.2%, respectively. However, a relatively high Paracheck Pf® sensitivity was detected in many populations, especially those living in malaria endemic areas[14,15]. Paracheck Pf® showed 100% sensitivity when compared to microscopy, as reported by Swarthout *et al*[14] from amongst children aged 6-59 months in eastern Democratic Republic of Congo. Furthermore, Sharew *et al*[15] examined 668 febrile patients who were identified in Wondo Genet, southern Ethiopia, where *P. falciparum* is endemic and found 99.4% Paracheck Pf® sensitivity. In this study, although Paracheck Pf® is found to have a relatively low sensitivity, it fulfills the WHO recommendation that requires the sensitivity of RDTs to be 95%[16]. Therefore, having this sensitivity Paracheck Pf® can be used as an alternative to light microscopy in resource poor set ups.

In the present study, it was also shown that Paracheck Pf® failed to detect 10 cases, which were positive by microscopy. This is not in consistence with similar study in eastern Democratic Republic of Congo[14], where all samples positive by light microscopy were also positive by Paracheck Pf®. The mechanism that RDTs cause false negative result is not

fully understood. However, a logical explanation provided on deletion or mutation of the histidine-rich protein 2 (HRP-2) gene[17]. Some studies have clearly established the deletion or mutation of HRP-2 in patients with falciparum malaria. Also, this deletion or mutation of HRP-2 is associated with false negative results of RDTs[17]. Therefore, deletion or mutation of HRP-2 may have been responsible for the conflicting reports on false negative results of RDTs.

In this study, it has been observed that Paracheck Pf® detected 11 cases which were negative by microscopy. Individuals living in malaria endemic areas who experience repeated malaria infections develop a degree of immunity that confers some protection from complicated malaria such as parasitemia[18,19]. And this has been linked with the presence of samples with parasite density below the detection threshold for microscopy[19]. However, unlike Giemsa stained light microscopy, Paracheck Pf® detects HRP-2 which could be produced in such low level of parasites[14,19,20]. Therefore, since the present study was conducted in malaria endemic area, the false positive results obtained may be due to the presence of parasite density below the detection threshold for microscopy and detectable level of HRP-2 in some of the study participants.

In conclusion, Paracheck Pf® showed good sensitivity and specificity for the diagnosis of *P. falciparum* malaria, and fulfill the world health organization (WHO) recommendation that requires the sensitivity of rapid diagnostic tests (RDTs) to be greater than 95%. Therefore, Paracheck Pf® can be used as an alternative to the Giemsa stain light microscopy in resource poor set ups.

Conflict of interest statement

We declare that we have no conflict of interest.

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