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Use of buffy coat thick films in detecting malaria parasites in patients with negative conventional thick films

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ABSTRACT

Objective: To determine the frequency of malaria parasite detection from the buffy coat blood films by using capillary tube in falciparum malaria patients with negative conventional thick films. **Methods:** Thirty six uncomplicated falciparum malaria patients confirmed by conventional thick and thin films were included in the study. The patients were treated with artemisinin combination therapy at Hospital for Tropical Diseases, Bangkok, Thailand for 28 day. Fingerpricks for conventional blood films were conducted every 6 hours until negative parasitemia, then daily fingerpricks for parasite checks were conducted until the patients were discharged from hospital. Blood samples were also concurrently collected in 3 heparinized capillary tubes at the same time of fingerpricks for conventional blood films when the prior parasitemia was negative on thin films and parasitemia was lower than 50 parasites/200 white blood cells by thick film. The first negative conventional thick films were compared with buffy coat thick films for parasite identification. **Results:** Out of 36 patients with thick films showing negative for asexual forms of parasites, buffy coat films could detect remaining 10 patients (27.8%) with asexual forms of *Plasmodium falciparum*. **Conclusions:** The study shows that buffy coat thick films are useful and can detect malarial parasites in 27.8% of patients whose conventional thick films show negative parasitemia.

1. Introduction

Malaria remains one of the world's most important parasitic infections. The current social, economic, and medical impact of malaria in tropical underdeveloped settings is immense. The impact of malaria morbidity and mortality continues to increase across malaria risk areas. The actual number of clinical cases of malaria and its impact are probably underestimated by current surveillance approach. Malaria patients usually present with nonspecific symptoms[1], *e.g.* irregular fever, chills, headache, nausea, vomiting, malaise. Malaria is so common in any people who visit malarious area and malaria should be considered until proved otherwise. World Health Organization[2] recommended prompt parasitological confirmation by microscopy or alternatively rapid diagnostic tests (RDTs) in all patients suspected of malaria before treatment is started and treatment solely on the basis of clinical suspicion should only be considered when a parasitological diagnosis is not accessible. Although microscopy by thin and thick blood films is the gold standard of malaria diagnosis[3], the risk of false negative microscopy is higher if the patient has received recent doses of antimalarial drugs. The choice between RDTs and microscopy depends on local circumstances, *e.g.* the skills available[4]. However, a major drawback of RDTs is more expensive and the sensitivities and specificities are variable. In the diagnosis of severe malaria cases, microscopy is a preferred option. It provides the diagnosis of malaria and is also useful in assessing other important parameters in a severely ill patients. The other rapid modality in malaria diagnosis is fluorescent staining of quantitative buffy coat (QBC)[5], however it

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needs fluorescent microscope or special filter which is not affordable in many hospitals regarding the cost. Therefore, the objective of the study was to determine the frequency of malaria parasite detection from the buffy coat blood films by using capillary tube in falciparum malaria patients with negative conventional thick films.

2. Materials and methods

The study was conducted at Hospital for Tropical Diseases, Bangkok, Thailand. This study was approved by Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand. Thirty six uncomplicated falciparum malaria patients were included in the study. Inclusion criteria were as follows: 1) either male or female; 2) admitted to hospital for 28 days of follow-up after antimalarial treatment; 3) age>15 years; 4) on admission day, pre-treatment, microscopically confirmed positive for asexual stage of *Plasmodium falciparum* parasites by Giemsa stained thick and thin films; 5) received artemisinin combination therapy (ACT) without primaquine after confirmed diagnosis; 6) had no history of antimalarial therapy during 1 month prior admission; and 7) provided fingerprick blood samples, before admission and every 6 hours after ACT treatment until thick films showed negative for parasites, then thick films were conducted daily until discharged from the hospital. If the patients had reappearance of parasitemia, fingerprick blood films were repeated again every 6 hours. Conventional thick and thin blood films were conducted for each fingerprick sample.

Blood samples were also concurrently collected in 3 heparinized capillary (hematocrit) tubes at the same time of fingerpricks for conventional blood films when the prior parasitemia was negative on thin films and parasitemia was lower than 50 parasites/200 white blood cells by thick films. One end of the tubes was immediately sealed by plasticine. The capillary tubes were centrifuged in the microcentrifuge at 1 200 rpm for 5 minutes. The tube was cut by glass ampoule cutter 1 mm below the buffy coat at the top of red blood cell column (Figure 1). The sediment column of blood including buffy coat and subbuffy coat red blood cells was knocked onto a slide and smeared as the same method for thick blood film with Giemsa stain. Plasma column was left in capillary tube. Three thick buffy coat films from 3 capillary tubes of each patient were conducted on a slide (Figure 1). Both conventional blood films and buffy coat thick films were examined by one malaria microscopy expert (Chatnapa Duangdee). Conventional blood film was considered negative after 200 oil immersion microscopic fields were examined whereas buffy coat film was considered negative after all oil immersion microscopic fields were examined. We excluded mixed malarial infection or non-falciparum malaria infection.

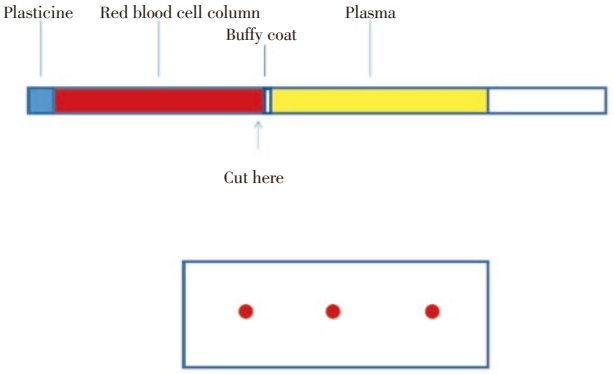

Figure 1. Components of blood in capillary tube after centrifugation (above), and 3 buffy coat thick smears from 3 capillary tubes of each patient taken on a glass slide (below).

3. Results

Table 1 showed that out of 36 patients, 24 patients (66.7%) (group A) had no asexual parasite and 12 patients (33.3%) (group B) had only gametocytes found in conventional thick films. Out of 24 patients in group A, buffy coat films showed 5 patients (20.8%) had ring forms (with or without gametocytes). Buffy coat films could detect 3 patients having gametocytes (12.5%, with negative parasitemia in group A). In group B (n=12), buffy coat films detected ring forms and gametocytes in 5 patients (41.7%). After combining the results of both groups A and B (n=36), buffy coat films could detect remaining 10 patients (n=5 in group A and n=5 in group B; 27.8%) with ring forms (with or without gametocytes) whose conventional thick films showed negative parasitemia or only gametocytemia.

Table 1
Comparative results of conventional thick film and buffy coat films (n=36).

Conventional thick films		Buffy coat films	
Result	No. of patients	Result	No. of patients
Group A: Negative parasitemia*	24	Rings forms only	3
		Ring forms and gametocytes	2
		Gametocytes only	3
		Negative parasitemia*	16
Group B: Gametocytes only	12	Ring forms and gametocytes	5

*: No asexual or sexual (gametocyte) parasite found by Giemsa stain.

4. Discussion

Malaria parasite diagnosis by microscopy needs experience particularly if the patients have very low parasitemia. Thick film is more sensitive than thin film since it uses more blood for examination. Microscopy can detect parasites as low as 10 parasites/μL by experienced microscopist. In this study, adding fingerprick buffy coat film could detect parasites in negative conventional thick film. Buffy coat film gave 27.8% frequency in detecting asexual parasites (ring forms) in negative conventional thick-film patients for only asexual parasites; and 20.8% frequency in negative conventional thick-film patients for both asexual and sexual (gametocyte) parasites, respectively.

Earlier study[6] showed that malaria parasites could be concentrated using centrifugation of blood in capillary tube coated with EDTA and acridine orange. The principle of QBC was that infected red blood cells appeared to be less dense than uninfected ones, and concentrate primary within the zone at the interface, a small 1-2 mm region near the top of RBC column, *i.e.* the buffy layer[7]. These parasites fluorescenced green and orange objects because of the uptake of dye. QBC was more sensitive to detect malarial parasites than conventional thick film particularly when parasitemia was low. However, QBC could not accurately differentiate between different *Plasmodium* species[8-10].

In this study we used ordinary heparinized capillary tube since it was widely available in many health facilities in rural areas. Unlike earlier study[11], this study used ampoule cutter which was more accessible than Adam's pier. Tube cutting by ampoule cutter caused no problem in the procedure in contrast to earlier report[12]. Unlike QBC method for malaria detection, our method needed no fluorescent microscopy. This study showing higher frequency in malaria detection by buffy coat film than negative conventional thick film might be due to 1) parasites were concentrated at RBC layer closed to buffy coat and 2) using 3 thick smears from 3 capillary tubes rather than 1 conventional thick smear might give higher probability to find parasites. Centrifused blood film was found to be superior to conventional blood film since it included both QBC and blood smear principles. Buffy coat thick film might be easier to examine than buffy coat thin film[6] since red blood cells in buffy coat thin film were dense due to blood centrifugation.

In febrile patients who had malaria infection risk, finding of asexual forms confirmedly indicates malarial disease, whereas finding gametocytes might alert clinician to consider malaria in the differential diagnosis, although sexual forms cause no symptom. In some malaria patients having such low parasitemia, asexual forms may not be seen whereas only gametocytes are seen in blood films.

This study showed that buffy coat film by capillary tubes was useful, easy to perform, affordable, and could detect malaria parasites in 27.8% of patients whose conventional thick films showed negative result for malarial disease. Buffy coat film might be additional tool to routine blood film when the patients were suspected of falciparum malaria and parasites were not found by conventional thick film. However, the study included rather small sample size, and it took more time for examining 3 additional buffy coat films. Further study with larger sample size and other malaria species, validation of the study, and determining the longest duration that buffy coat films can detect parasites when conventional thick films show negative parasitemia are required before buffy coat film can be implemented in general practice setting.

Conflict of interest statement

We declare that we have no conflict of interest.

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