

Development of the Dip Quick Wright-Giemsa Staining Methods for Improving Simplicity and Reducing Cost in Malaria Diagnosis

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ABSTRACT

Microscopy continues to be the benchmark for malaria diagnosis, however, the method seems not only complicate, but also time- and cost-consuming. This study presents the modified Wright-Giemsa staining technique by employing hospital tap water instead a commercial buffer, together with dip quick procedure. Comparative evaluation by 28 medical technicians of Loei Hospital, Thailand, for examining of 48,885 cases indicated more potential than the normally used method in malaria diagnosis and also other hematological identifications of blood cell types, such as red blood cells, leukocytes, and platelets. The developing dip quick method preserved time saving for 5 minutes per sample and 50% decrease of expenses, which were significant for applying as potentially alternative choice in urgent diagnostic situations wilderness areas.

Keywords: Wright-Giemsa staining, Dip quick method, Malaria diagnosis

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Introduction

Malaria remains a significant global health issue. Particularly, the majority of malaria cases and deaths occur in tropical and subtropical areas such as Africa and Southeast Asia regions [1-3]. Generally, malaria in humans is caused by infections of the *Plasmodium* parasites. The disease is primarily transmitted to humans through the bite of an infected female mosquito. Thus, malaria diagnosis is confirmed basically through laboratory tests on a blood sample to identify the presence of the malaria parasites. The timely and precise diagnostic methods are essential for controlling of epidemic and also successful treatment. Among the available diagnostic tools, light microscopy continues to be considered as the gold standard method, because of high specificity and sensitivity to quantify parasitemia [4-5]. In brief, the stepwise procedures including blood smear preparation, staining and microscopic examining are respectively proceeded. The steps of blood sample collection and blood smear preparation are usually accomplished by the trained technicians. For staining, Giemsa and Leishman staining methods, which used the Romanowsky-type stains such as eosin Y and azure B, are frequently applied in clinical laboratories, because the significant contrast between blood cell components and parasites cells are observed clearly [6-9]. In addition, there is a modified Wright-Giemsa stain, based on the principle of acidic-basic dyes combination, which improved the potential of intracellular parasites identification, based on the stronger affinity between dyes and DNA-rich regions [10-13]. Commonly, the staining process is taken for approximately 30 min. Finally, the stained blood smear slide is then visualized under the microscope for identifying parasitic cells.

However, the microscopy's sensitivity is not constant and can be a major limitation, particularly in certain scenarios including low parasitemia and also variable threshold. The method struggles to detect very low parasite densities, which is common in asymptomatic carriers, individuals with partial immunity, or patients who have received a prior dose of antimalarial drugs. These missed cases can act as reservoirs for continued transmission. The minimum number of parasites per microliter of blood that a microscopist can reliably detect varies greatly. While an expert might detect as few as 5 to 50 parasites/ μL . The other drawbacks can be time- and cost-consuming because of particular requirements including specific reagents, and time taking which are dramatically essential for the accurate diagnosis. The particular stains and buffered water (which are normally commercially available and costly) are essentially required for the standard method for better sensitivity and selectivity of detection [14-17]. In addition, the staining process is time-consuming for approximately over 10 min per slide. These are not appropriate especially for the rapid diagnosis in the large hospital which must identify a lot of both normal and emergency cases [18-22].

The ongoing global fight against malaria drives a continuous trend in diagnostic development, emphasizing feasibility, simplicity, cost-effectiveness, and time-effectiveness [23-24]. Researchers and public health initiatives are striving to innovate new tools and refine existing ones to overcome the challenges

posed by diverse epidemiological settings and evolving parasite characteristics. The goal is to ensure that accurate malaria diagnosis is accessible to everyone, everywhere, ultimately contributing to the ambitious targets of malaria elimination. Therefore, this study focuses on the development of a Dip Quick staining method, which based on Wright-Giemsa staining using tap water for improving of simplicity and also reducing cost and time. The developing method would be intended for applying to fieldwork, emergency and also wilderness areas [25-27]. In addition, the effectiveness of the developing method for malaria and other general hematological applications were additionally evaluated by the medical technicians of Loei Hospital, Thailand, and also comprehensively compared with the standard method.

Methodology

Ethical clearance statement

The study was conducted in accordance with the ethical standards of the institutional and national research committee. The developing protocol was reviewed and approved by the ethics committee of Loei Hospital, Thailand, on 2023 August 24, with approval number EC 023/2566.

Analysis of tap water qualities

The tap water samples were collected differently from two places, including water tank (site 1) and hematological laboratory (site 2) of Loei Hospital. The pH of each water sample was measured directly on-site using a portable pH meter equipped with an automatic temperature compensation probe. The pH meter was calibrated daily using standard buffer solutions (pH 4.01, 7.00, and 10.01) before measurements. Turbidity was determined using a portable turbidimeter calibrated with standard formazin solutions [28]. Total Dissolved Solids (TDS) were measured gravimetrically using a portable TDS meter calibrated with a standard NaCl solution. Total hardness, expressed as milligrams per liter of calcium carbonate (CaCO_3) was determined using the EDTA titrimetric method, with the presence of an Eriochrome Black T indicator and a buffer solution (pH 10.0) [29]. Total coliform and fecal coliform bacteria were enumerated using the membrane filtration (0.45 μm) method by morphology-based counting of total coliforms and fetal coliform colonies [30].

Staining protocol and identification of parasites

Blood samples were obtained from febrile patients who took place at Loei Hospital, Mueang District, Loei Province, Thailand, utilizing EDTA tubes to inhibit clot formation. For guarantee of the precise outcomes and avoid alterations of parasite morphology, all samples were undergone in processing right after collection. A volume of 10 μL from each blood sample was utilized to create both thick and thin blood smears. The thin smears were fixed by immersing them in absolute methanol for 5 seconds, followed by air drying. The Wright-Giemsa working solution was freshly prepared each day by diluting Wright-Giemsa stain

in a 1:30 ratio with phosphate-buffered water (pH 7.2). The slides underwent staining by being immersed in the staining solution for a duration of 10 seconds, after which they were gently rinsed with tap water and allowed to air dry. All stained slides were assigned random labels ranging from 1 to 20 and visualized by 6 experienced medical technicians using microscope of malaria diagnosis and other hematological identifications. All of the analytical grade reagents and chemicals utilized in this study are commercially available.

Scoring method for evaluation of effectiveness

There were 6 medical technicians evaluated the quality of staining, the clarity of various blood cell types, and the appropriateness for diagnosis using a standardized scoring system. The treatment's effectiveness was assessed using a scoring system that measured the extent of red blood cell impairment. Leukocytes and platelets are cellular components that exhibit distinct characteristics, including the pigmentation of the cytoplasmic nucleus observed in red blood cells. This entails the incorporation of slide background colors and the implementation of inclusion styles as specified in Table 1.

Table 1 Score levels and descriptions for evaluation of the Dip quick Wright-Giemsa staining method

Score	Description
1	All cell components were still colorless or not clearly visible
2	Some cell types are stained, but difficultly distinguished, staining cell were more over lighter or darker, and background is rough.
3	Blood cells were identified, but staining is still not optimal—too light or too dark.
4	Blood cells are clearly distinguishable with good staining.
5	Excellent staining quality with clear and complete visibility of all blood components.

Statistical analysis

All data were analyzed using the IBM SPSS Premium software (version 29.0) [31]. The evaluating score are presented as mean $\pm$ standard deviation (SD). Group comparisons were performed using independent *t*-tests for two groups, and a two-tailed *p*-value of <0.01 was considered statistically significant.

Results

Quality of tap water in Loei Hospital

The monthly data (January–March) presented in Table 2 show a steady rise in tap water parameters, including pH (around 7.4–7.8), turbidity (around 3.8–5.1 NTU), total dissolved solids (roughly 850–1050 mg/L), hardness (354–451 mg/L), chloride (8.8–10.1). The observed gradual changes indicate that the water quality evolved to become increasingly alkaline, laden with particulates, and enriched with minerals over time. However, almost determining parameters were still in standard quality. Exceptionally, TDS value of site 1 in February and chloride of site 2 in March that were slightly higher, while the hardness seems more over than the standard limit for around 50–100 mg/L.

Table 2 Determining qualitative parameters for tap water of Loei Hospital in January–March

Parameters	January		February		March		Standard values
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	
pH	7.4	7.6	7.5	7.7	7.6	7.8	6.5–8.5
Turbidity (NTU)	4.2	3.8	4.6	4.3	5.1	4.9	10
Total Dissolved Solids (mg/L)	868	956	955	978	1048	994	1000
Hardness (mg CaCO ₃ /L)	366	354	412	389	451	411	300
Chloride (mg/L)	8.8	9.6	9.1	9.7	9.6	10.1	10
Coliform (CFU/100 mL)	1.9	1.9	1.9	1.9	1.9	1.9	<2
Fecal coliform (CFU/100 mL)	1.9	1.9	1.9	1.9	1.9	1.9	<2

Characteristics of cell component stained using the Dip Quick technique

The cell components which were stained using the Dip-quick method, demonstrated in Figures 1a–1d, clearly maintained the crisp cellular morphology, sharply defined leukocytes, and minimal background artifacts. While, the standard stained smears (Figures 1e–1h) appeared the slightly less distinct, together with more hazer background and subtle color inconsistency. In addition, platelet and lymphocyte structures resulted from Dip-Quick experiment are more vivid, Figures 1a and 1b, respectively. Nuclear segmentation of neutrophils was also observed clearly (Figures 1c and 1g), and overall cell contrast appears enhanced relative to standard staining (Figures 1e–1h).

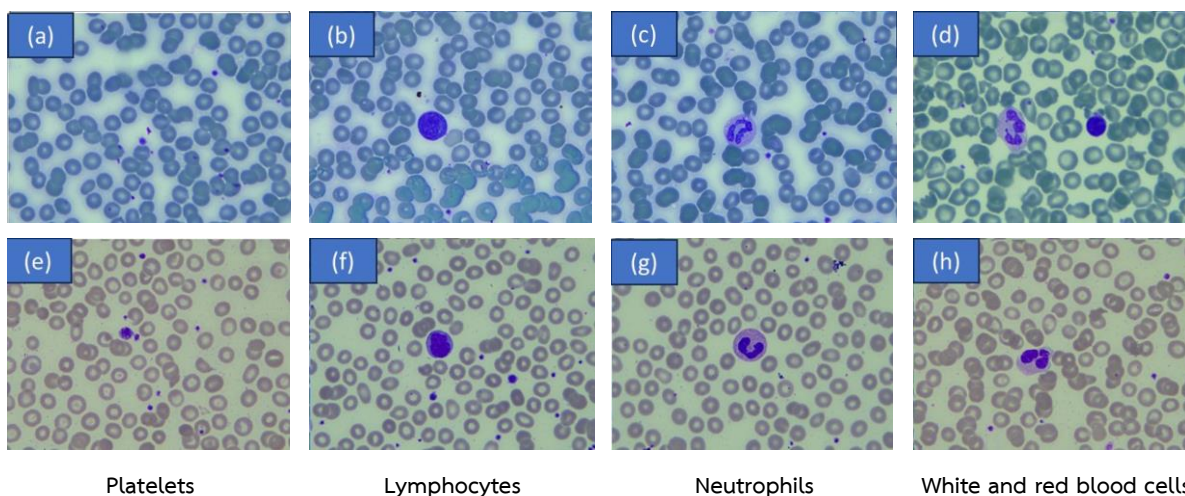


Figure 1 Comparison of microscopic appearances of cell components stained using the Dip Quick method (a-d) and the standard tray method (e-h); including platelets (a and e), lymphocytes (b and f), neutrophils (c and g), together with white and red blood cells (d and h)

Diagnostic effectiveness of the Dip quick and standard methods

The efficiency scores of the Dip quick and standard methods which were evaluated by 6 experienced technicians (repeatedly 25 slides for each technician) demonstrated in Table 3. The evaluating score of the Dip quick method was significantly different to standard method ($p < 0.01$) for all technicians. The Dip quick was more preferred with the higher averaged-score of 4.58 ± 0.07 , than the normally standard method (3.32 ± 0.48). Moreover, there were other examining of the stained slides by 22 medical technicians, who evaluated 48,885 slides for staining quality or diagnostic potential. In which of examining slides, 150 malaria cases had successfully identified, while others cases of hematological applications demonstrated a differentiation of blood cell types, such as red blood cells, leukocytes, and platelets. Assigning scores ranging from 3-4 were often evaluated by those 22 technicians. The significant level of agreement noted between the two raters, with most slides receiving the same scores, indicates a robust consistency in the scoring process. The observing percentage of agreement is at least 80% (around 16–17 of 20 slides rated identically), indicating substantial to nearly perfect reliability, provided that the marginal distributions are comparable.

Table 3 Scores of the developing Dip Quick and standard tray methods evaluated by 6 technicians

Technicians	Score (mean $\pm$ SD)	
	Dip Quick method	Standard tray method
1	4.56 $\pm$ 0.51	3.32 $\pm$ 0.48
2	4.64 $\pm$ 0.49	3.24 $\pm$ 0.44
3	4.56 $\pm$ 0.51	3.24 $\pm$ 0.44
4	4.68 $\pm$ 0.48	3.16 $\pm$ 0.37
5	4.56 $\pm$ 0.51	3.12 $\pm$ 0.33
6	4.38 $\pm$ 0.51	3.32 $\pm$ 0.48
Total average	4.58 $\pm$ 0.07	3.23 $\pm$ 0.08

Discussion and conclusion

The different results by two staining methods, a developing Dip quick and standard tray methods, align directly with underlying tap water quality characteristics of pH level, turbidity, and dissolved mineral content fluctuations. When rinse water deviates in pH beyond around 6.7–6.8, it can disrupt the optimal interaction between buffer solutions and dye components azure B, eosin Y, and methylene blue resulting in uneven staining or color shifts. Likewise, elevated turbidity (particles) and increased dissolved solids can deposit on smear surfaces, contributing to background artifacts and reducing image clarity. Thus, images captured from Dip Quick stained samples likely benefited from controlled rinses (possibly using a calibrated buffer or deionized water), producing sharper contrast and minimal haze. In contrast, standard Wright-Giemsa slides if rinsed with tap water exhibiting elevated pH or turbidity may display reduced definition, diffuse backgrounds, or slight staining artifacts, matching observations in panels [32-35]. Therefore, the superior clarity and consistency in Dip Quick images strongly reflect the impact of optimized water conditions during staining, while small but measurable variations in tap water quality (pH, turbidity, solutes) can meaningfully impair outcomes in standard protocols. Maintaining rinse water within specified pH and particulate ranges is therefore essential for achieving reproducible, high-quality Wright-Giemsa staining in both clinical and research settings. Considering the ordered nature of the evaluating scores, all technicians were more preferred for the Dip Quick method, possibly driven from either the more simplicity or the precise diagnostic potential. The difference averaged-evaluating score of approximately 1.4 level reasonably indicated the enough effectiveness of the Dip quick method for utilizing in the clinical laboratory instead of the previous standard method. In addition, other applications for the hematological diagnosing were also successful, which based on the clearer observing stained-cell components by the Wright-Giemsa combination dyes.

In summary, a rapid staining technique was developed by alternative using a hospital tap water instead of a commercial buffered water, and also minimizing the steps involved in mixing the buffer with the dye. The developing protocol saved processing time approximately 5 min per slide. The Wright-Giemsa stain minimizes the necessity for purchasing buffer from a dye sales company. Utilize tap water instead, ensuring that the efficiency of coloring the blood smear adheres to established standards. The benefit for using of tap water to wash instead of a quick dyeing buffer expedites urgent diagnostic work, allowing emergency patients to receive test results promptly. The cost is lower due to the absence of the tray method for color application. The distinction is evident in the deep hue produced by the dip quick dye method, and it is distinctly observable. Effectively distinguishes between different types of white blood cells. The tray method for staining yields a standard pink color, however, it requires significantly more time compared to the dip quick technique. From the examination of blood smear staining. The Wright-Giemsa stain method, specifically the quick dip method, utilizes tap water in place of buffer. This approach demonstrates effective staining and minimizes the reliance on company-provided buffer. However, the variability in tap water quality across different regions can lead to inconsistencies in color performance. A water supply system has been established to regulate the pH level of tap water, ensuring it remains within the range of pH 7 to 8, thereby enhancing the efficiency of the dyeing process.

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