DEVELOPMENT OF STAINING WRIGHT-GIEMSA STAIN BY DIP QUICK METHOD

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Abstract:
Microscopy continues to be widely regarded as the preferred method for malaria diagnosis on a global scale, particularly in regions where high-quality Giemsa stain is available. Numerous Giemsa stain products have inundated the market in Ghana, potentially without the necessary standardization. In this study we report on the development method to make efficiency of Giemsa stains used for malaria detection. Sample was collected from Loei hospital, Thailand and analyst by using Wright-Giemsa stain dip quick methods, compare with normal methods. The ligand in question exhibits specificity towards the phosphate groups of DNA and selectively binds to regions characterized by a high prevalence of adenine-thymine base pairing.The Development of staining Wright-Giemsa stain by Dip quick method suitable for urgent dyeing or in the field trip. Dip quick stain for Wright-Giemsa stain to reduce time.

Keywords: Dip Quick Methods, Malaria Test, staining methods and Wright-Giemsa stain

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1. Introduction:

Malaria Microscopy (MM) continues to be widely regarded as the most reliable and widely accepted method for diagnosing malaria on a worldwide scale. It helps to detect the presence of malaria parasites from peripheral blood smears [1-3]. The effectiveness of microscopy is reduced in instances where the parasitaemia is at a low level. Therefore, the successful execution of this task necessitates the presence of competent individuals and a well prepared slide. The Giemsa and Leishman stains are frequently employed in haematology laboratories as Romanowsky stains. Romanowsky stains are commonly favoured because to their ability to selectively stain granules in leukocytes[4-5]. Giemsa stain is widely regarded as an effective method for visualising the presence of malaria and other blood parasites. Additionally, it is commonly employed as a standard stain for visualising the various components of blood in a peripheral blood smear. In order to achieve high-quality microscopy results, it is imperative to utilise highquality staining techniques for the preparation of high-quality smears. In Ghana, both commercial and public laboratory facilities commonly acquire Giemsa stock powder or pre-made Giemsa stock solution, from which a working solution is regularly created for utilisation inside these laboratories. Regrettably, the Giemsa stains available in the commercial market are sourced from several producers, resulting in a wide range of variations in terms of quality, potency, and performance.[6-8] There is speculation on the potential impact of the quality of Giemsa stains available in the market on the misdiagnosis of malaria and the diversity observed in malaria diagnosis between different laboratories. Frequently, this situation might have significant consequences for the patient, as they may be referred to more advanced healthcare facilities due to the inability to make a diagnosis.[7-11] The adoption of the new malaria diagnosis protocol by the National Malaria control program, which recommends parasite counting as a best practice for malaria diagnosis, has raised concerns regarding the issues associated with various grades of Giemsa stains available in the market. Therefore, it is imperative to comprehensively assess the quantitative implications of the diverse origins and manufacturers of Giemsa stains in relation to malaria diagnosis within our laboratory facilities[12-15]. The objective of this study was to examine the staining techniques using different Giemsa stains in the selected laboratory facilities and to determine the elements that may influence the accuracy of malaria diagnosis.

2. Materials and Methods

2.1 All chemicals and reagents used in this research are of analytical grade.

2.2 Detection methods

The research was conducted in Loei Hospital in the Muang region of Loei Province, Thailand. The present investigation involved the collection of blood samples from successive febrile patients admitted to the hospital, utilizing EDTA as an anticoagulant. To prevent any potential morphological changes in parasites due to storage, the samples were promptly treated. A sample of 10 μL EDTA blood was then utilized for the simultaneous fabrication of thick smear slides, one colored according to Giemsa method. The thin smear was fixed by immersing it in a covered staining jar filled with anhydrous methanol for a duration of 5 seconds. Subsequently, the slides were allowed to dry naturally. The Giemsa working solution was diluted at a ratio of 1:30 with phosphate buffered water at a pH of 7.2. A new filtered working solution was utilized on a daily basis. The slides bearing the thick and thin smear were subsequently immersed in a Giemsa staining solution for a duration of 10 seconds. Subsequently, the slides were subjected to a gentle washing procedure using tap water, followed by air drying, in preparation for microscopic evaluation. Once all the samples have been completed, the numbers 1-20 will be randomly written on the slides and allocated to 2 senior medical technicians specializing in Clinical Hematology at Loei Hospital. Their task will be to assess the efficacy of color staining in the study.

2.3 Scoring Result

The efficacy of the treatment 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, such as the pigmentation of the cytoplasmic nucleus in red blood cells. This encompasses the incorporation of slide background colours and the utilisation of inclusion styles as follow Table 1.

Table 1 Score the Wright-Giemsa stain and Dip quick stain.

Score	Description
1	Both Colorless with polynucleus, mononucleus, red blood cells, platelets
2	Undistinguished some types of blood cells and being too dark or too light The ground between the blood cells is colored.
3	Able to distinguish different types of blood cells with polynucleus, mononucleus, red blood cells, platelets but the color is too light or too dark
4	Able to distinguish different types of blood cells with polynucleus, mononucleus, red blood cells, platelets
5	Completely colored according to the specified standards polynucleus, mononucleus, red blood cells, platelets

3.Result and Discussion

3.1 Analysist Tab Water Quality

Results of data analysis of the study of the quality of tap water at Loei Hospital in survey, the water has been collected from hematology laboratory.

3.1.1 pH Analysist

The results of the study of the pH value found that the pH value was not much different for the months of January, February, and March 2023, the result show that the pH value is around 7-8 that show on Figure 1.

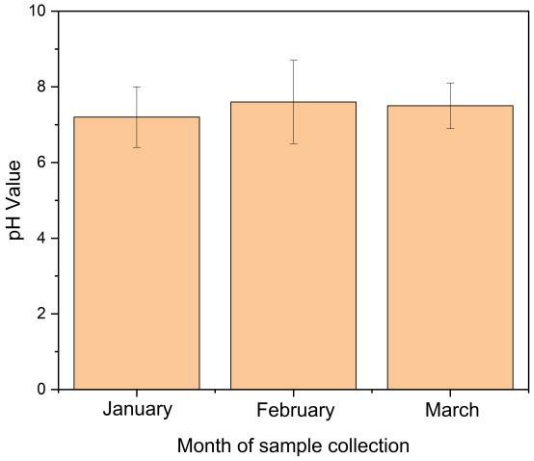

Figure 1. pH value of tab water

3.1.2 Turbidity value of water

The results of the study of water turbidity revealed that the turbidity of tap water at Loei hospitals. There was an increase in average turbidity values in all 3 months of sample collection, turbidity values of 4.2 NTU, 4.6 NTU, and 5.1 NTU, respectively (Figure 2)

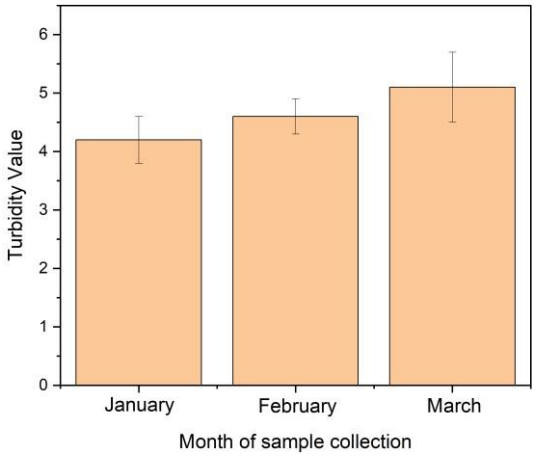

Figure 2. Turbidity Value

3.1.3 Total amount of water soluble substances TDS

Figure 3 shwos the results of the study of total dissolved substances (TDS) is a study of the amount of solids that water solubles were found for all solid solutions collected during January until March, the amount of water-soluble substances increased by 868 milligrams per liter to 1048 milligrams per liter.

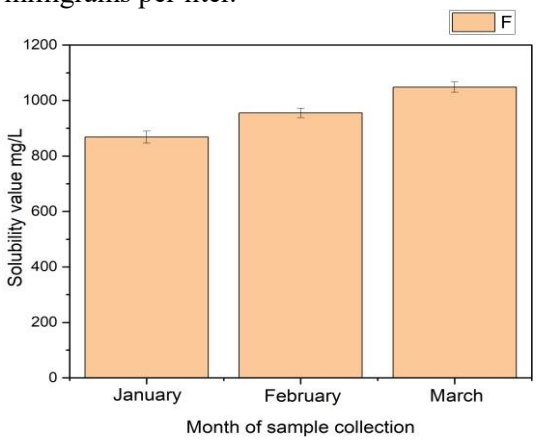

Figure 3. Total Amount solubility value

3.2 Comparison Dip quick stain Wright-Giemsa with standard methods

From comparing the mean and standard deviation with both staining techniques, it can be seen that the dip quick stain method uses a hospital tap water, is show result that this methods can be alternative

methods for fast detection in the urgent cases because Table 2 and Figure 4 shows that all the result from 20 slide show the score more than 3 its mean all the slide able to distinguish different types of blood cells with polynucleus, mononucleus, red blood cells, platelets but the color is too light or too dark. The staining of red blood cells was purple-red, with a dimple in the middle. The staining of nuclei and staining of white blood cells were neutrophil lymphocyte and white blood cells platelet has the correct colour according to standards.

Table 2 shows the scoring results from microscopy.

No. slide	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Staff 1	3	3	4	4	4	3	4	4	4	4	4	3	3	3	4	3	4	4	4	3
Staff 2	4	4	4	4	4	3	3	4	3	4	4	3	4	4	4	3	4	4	4	4

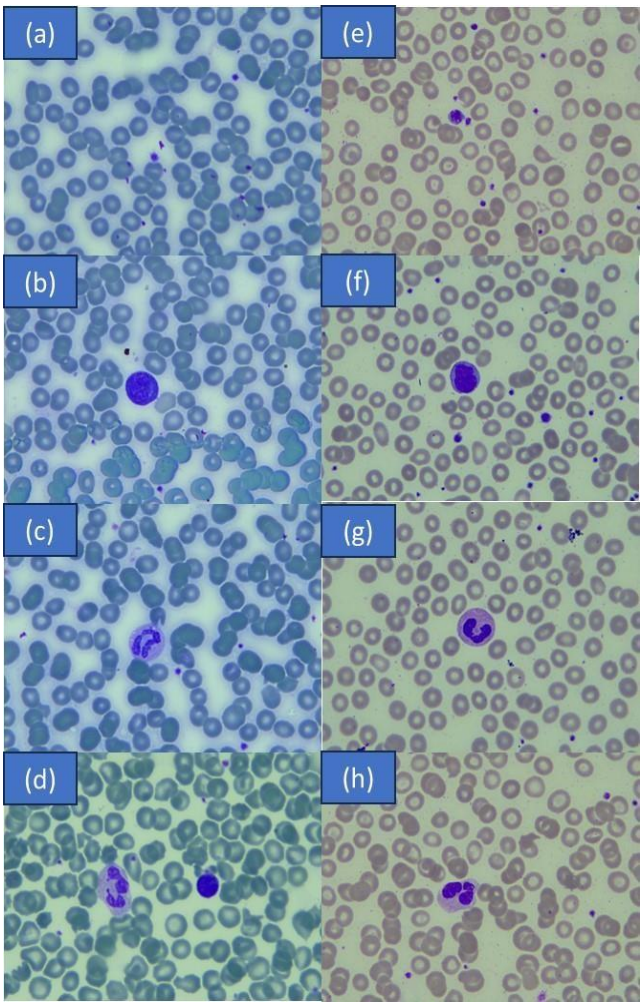

Figure 4. Comparison Photo of dip quick (a) Platelets (b) Lymphocyte (c) Neutrophil (d) White blood cells and red blood cells with standard methods (e) Platelets (f) Lymphocyte (g) Neutrophil (h) White blood cells and red blood cells

4. Conclusion

A quick dip staining method was developed, dip quick stain uses a buffer with hospital tap water, which reduces the steps that require mixing buffer with dye. Wright-Giemsa stain and reduces the use of buffer that must be purchased from a dye sales company. Use tap water instead while still maintaining the efficiency of coloring the blood smear according to standards. The advantage is Quick in dyeing buffer not required use tap water to wash instead makes urgent diagnostic work, emergency patients receive test results quickly. The price is cheaper because the color is not poured like the tray method. The difference is seen in the dark color in the dip quick dye method, but it can be clearly seen. Separates the white blood cell type well. Staining using the Tray method is a standard pink color but still takes a lot more time than dip quick. From the study of blood smear staining. Wright-Giemsa stain method, quick dip method, dip quick stain, using tap water instead of buffer, has effective staining, reduces the use of buffer

from the company, but the quality of tap water in each area is not the same, causing the colors to have different performance. A water supply system has been developed to control the pH level of tap water so that it does not exceed pH 7- 8, which will make dyeing more efficient

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