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Malaria Diagnosis through Conventional Methods: A Comprehensive Review

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ABSTRACT

The incidence of drug-resistant parasite strains is rising, there is an apparent increase in the number of cases of malaria worldwide, and in a small number of cases and there has been a dramatic rise in international migration and travel. Over 1 million people die from malaria every year; 90% of these deaths are in African children, who live in tropical and subtropical regions. Although there are now efficient techniques to treat malaria, the disease is nevertheless becoming more prevalent for a variety of reasons. The therapy and control of malaria in this emergency circumstance depends on quick and accurate diagnostic techniques. Accurate diagnosis is essential in guiding appropriate treatment and improving patient outcomes. In recent years, advancements in technology have led to the development of new diagnostic tools, including genetic testing and molecular imaging, which have improved the accuracy and efficiency of diagnosis. However, clinical diagnosis remains a complex and challenging process, requiring careful consideration of patient history, symptoms, and test results. This abstract highlight the Leishman stain, Gram stain, and field A and B stain.

Keywords: Malaria, Diagnostic, Leishman stain, Gram stain, Parasite

INTRODUCTION

In medical diagnosis, stains are vital in identifying and characterizing various microorganisms and cells. These stains provide crucial information about the

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morphology, composition, and behavior of cells and organisms, enabling clinicians to make accurate diagnoses and develop effective treatment plans. Some of the most commonly used stains in medical diagnosis include the Leishman stain, gram stain, and Field A and B stain. Continued research and innovation in this field will undoubtedly lead to the development of new and improved stains and techniques [1].

The malaria parasite, *Plasmodium spp.*, undergoes complex morphological changes during its lifecycle, which includes stages in both the mosquito vector and human host. The morphology of the parasite varies depending on the stage and the *Plasmodium* species (e.g., *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*). [2] Here's an overview of the main morphological stages within the human host:

1. Sporozoite Stage

- **Appearance**: Slender, elongated, and motile.
- **Location**: Injected by the mosquito into the human bloodstream and migrates to the liver.
- **Role**: Infects liver cells (hepatocytes), marking the beginning of the human phase.

2. Liver Stage (Exoerythrocytic Schizogony)

- **Trophozoite Stage**: After invading hepatocytes, sporozoites mature into trophozoites.
- **Schizont Stage**: Trophozoites divide and develop into large structures called schizonts, which contain thousands of merozoites.
- **Appearance**: Schizonts appear as large, circular cells filled with small merozoites.
- **Role**: Released merozoites enter the bloodstream and begin the erythrocytic (blood) phase.

3. Erythrocytic (Blood) Stage

- **Ring Form (Early Trophozoite)**:
    - **Appearance**: Thin, ring-shaped structure with a central vacuole and a nucleus resembling a ring.
    - **Location**: Inside red blood cells (RBCs).
    - **Role**: Initial growth stage within RBCs after merozoites invade.
- **Trophozoite Stage (Mature)**:

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  - **Appearance**: Enlarged with a denser cytoplasm and an expanded nucleus.
  - **Role**: Actively feeds on hemoglobin within RBCs, producing hemozoin pigment.

- **Schizont Stage**:
  - **Appearance**: Large, segmented cell containing multiple merozoites (up to 24 in *P. falciparum*).
  - **Role**: Releases merozoites upon rupturing, which then infect new RBCs.

- **Gametocyte Stage**:
  - **Appearance**: Banana- or crescent-shaped in *P. falciparum*; round or oval in other species.
  - **Role**: Sexual stage ingested by mosquitoes, leading to the sexual cycle in the vector.

Each *Plasmodium* species shows slight morphological differences, especially in the ring and gametocyte stages. These differences are crucial for species identification in microscopic diagnosis.

The **Leishman stain** is a type of Romanowsky stain that is commonly used to identify and diagnose blood parasites, particularly useful for identifying intracellular parasites in blood and bone marrow samples. This stain is made up of a mixture of eosin and methylene blue dyes, which react with different components of the cell to produce distinct color patterns. [3]

The **gram stain** is another commonly used stain in medical diagnosis, particularly in the identification of bacterial infections. This stain performed using crystal violet, iodine, and safranin dyes to create a difference between gram +ve and gram-ve bacteria [4]. Gram +ve bacteria seems purple in color when we see in the microscope, while gram -ve bacteria appear red or pink.[3-4] The gram stain is particularly useful in guiding the choice of antibiotic therapy, as gram-positive and gram-negative bacteria behave different towards antibiotics.

The **Field A and Field B** is a type of stain used to diagnose malaria; a parasitic disease causedby the Plasmodium parasite. This stain performed using a mixture of Giemsa and phosphate buffer to produce a specific pH and ionic strength, which enhances the staining of the parasite in blood sample. [5] The field A and B stain is particularly useful for identifying different species of the Plasmodium parasite, which have distinct morphological characterstic.

Methodology

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1. Leishman Stain

Leishman stain is a type of stain used in the field of microbiology for staining blood smears and other biological samples. It is named after its inventor, the British physician William Boog Leishman, who developed the stain in the early 20th century.

Preparation:

Leishman stain is a mixture of two dyes, eosin and methylene blue, which are dissolved in a mixture of methanol and glycerol. [3] The stain is prepared by adding thedyes to the solvent in specific proportions, and the resulting solution is filtered to remove any impurities. The final solution is a purple-colored liquid that can be stored for several months. Leishman stain solution are shown in figure 1.

Fig no 1. Leishman stain solution

Working:

The eosin in the Leishman stain is a red-colored acidic dye that binds to basic structures in the sample, such as the cytoplasmic granules of white blood cells. The methylene blue is a blue-colored basic dye that binds to acidic structures, such as the nuclei of cells. Together, these dyes provide a contrast that allows for the visualization of various structures in the sample.

Procedure:

To use Leishman stain, a small amount of the sample, typically a blood smear, is placed on a glass slide and make it to dry. [6] The slide is then immersed in the Leishman stain solution for several minutes, depending on the desired intensity of staining. After staining, the slide is rinsed with distilled water to remove extra stain and then make it to air dry. The left slide can be viewed under a microscope, where the different structures inthe sample can be visualized.

Result: -The Leishman stain is particularly useful for identifying and studying the morphology of white blood cells, including lymphocytes, monocytes, and granulocytes. After staining with Leishman stain, the cells are typically observed under a microscope. The stain differentially stains the various components of the cells, allowing for their identification and analysis. Specifically, the stain will color

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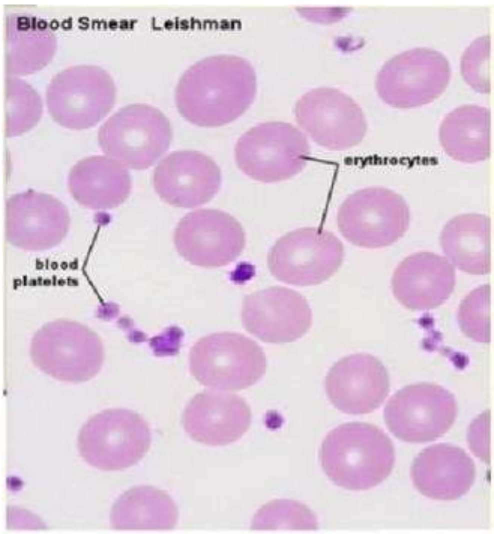
the cell nuclie in purple or blue, and the cytoplasm and other cell components a pink or red color are shown in figure 2.

Figure 2. Appearance of various blood cells after Leishman Staining

The result of the Leishman stain will depend on the specific cells or microorganisms being examined, as well as the specific protocol used for staining However, in general, the stain allows for the visualization and identification of cells and microorganisms, which can aid in the diagnosis and treatment of various diseases and conditions.



Red blood cells	Red to yellowish red.
Neutrophils	Dark purple nuclei, pale pink cytoplasm, reddish-lilac small granules.
Eosinophils	Blue nuclei, pale pink cytoplasm, red to orange-red large granules.
Basophils	Purple to dark blue nucleus, dark purple cytoplasm, dark large granules.
Lymphocytes	Dark purple to deep bluish purple nuclei, sky blue cytoplasm.
Platelets	Violet.
Parasites (e.g. Malaria)	Dark blue.

Figure 3. Result of Leishman Stain

One of the advantages of Leishman stain is its ability to stain both the nucleus and cytoplasm of cells. This allows for the identification of different types of white blood cells, which can be important in diagnosing various diseases. For example, neutrophils, which are the most abundant type of white blood cell, have a distinctive multi-lobed nucleus that is easily identified with Leishman stain. [3] Other types of white blood cells, such as lymphocytes and monocytes, have round nuclei and can be differentiated based on their size and the amount of cytoplasm visible. Result of Leishman Stain of different type of blood cell are shown in figure 3.

In addition to its use in blood smears, Leishman stain can also be used to stain other types of samples, such as bone marrow aspirates, lymph node biopsies, and fine- needle aspirates of solid tumors. The stain is particularly useful in the diagnosis of infectious diseases, such as malaria, which can be identified by the presence of characteristic parasites in the blood.

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Leishman stain can also be used to diagnose non-infectious diseases, such as leukemia and lymphoma, which can be identified by abnormal cells in the blood or other tissues.One of the disadvantages of Leishman stain is that it requires a relatively long staining time, typically 5-10 minutes, which can be time-consuming for laboratory technicians. In addition, the stain is not as stable as other Romanowsky stains, such as Wright's stain, and can fade over time. This can be a particular problem in tropical countries, where high temperatures and humidity can accelerate the degradation of the stain.[7]

Despite these limitations, Leishman stain remains a widely used staining technique in microbiology and pathology. Its ability to stain both the nucleus and cytoplasm of cells makes it a versatile tool for identifying different types of cells, while its use in staining blood smears has been critical in the diagnosis of various infectious and non-infectious diseases. Ongoing research is focused on improving the stability and performance of Leishman stain, as well as developing new staining techniques that can provide even greater clarity and detail in biological samples.

2.Field stain A and Field stain B

Field stain A and Field stain B are two commonly used stains in microscopy, especially for the visualization of blood parasites. These stains are often used together as a combination staining technique and are known as the Giemsa stain. [3] Field stain A and Field stain B solution are shown below in figure 4.

Figure 4. Field stain A and B solution

Field stain A:

Field stain A is a solution of eosin Y, which is a red dye, dissolved in methanol. Eosin Y is an acidic dye that binds to basic structures in the cell, such as cytoplasmic proteins, collagen, and erythrocyte membranes. This stain is used as the first step in the Giemsa stain process and stains the erythrocytes and nuclei of the leukocytes pink to red. [3]

Field stain B:

Field stain B is a solution of azure B, which is a blue dye, dissolved in methanol. Azure B is a basic dye that binds to acidic structures in the cell, such as DNA and RNA. This stain is used as the second step in the Giemsa stain process and stains the nuclei of leukocytes blue-purple to violet. [2]

Procedure:

The procedure for staining a slide with Giemsa stain involves the following steps: [8] and shown below in figure 5.

1. Prepare the slide by applying a thin layer of the blood sample on a disinfectant glass slide and make it air dry. The sample should be evenly distributed and not too thick, as this may cause overlapping of cells.

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2. Fix the slide by immersing it in absolute methanol for 1-2 minutes. This will fix the cells to the slide and preserve their morphology.
3. Prepare Field stain A by adding 4 grams of eosin Y to 1 liter of methanol. Mix well until the eosin Y is completely dissolved.
4. Stain the slide with Field stain A for 5-10 minutes.
5. Wash the slide with purified water to remove extra stain.
6. Prepare Field stain B by adding 4 grams of azure B to 1 liter of methanol. Mix well until the azure B is completely dissolved.
7. Stain the slide with Field stain B for 20-30 minutes.
8. Wash the slide with clean water to remove extra stain.
9. Air dry the stain on slide.
10. Examine the slide under a microscope with a 100x objective lens. The erythrocytes and nuclei of the leukocytes should be visible and stained pink to red and blue-purple to violet, respectively

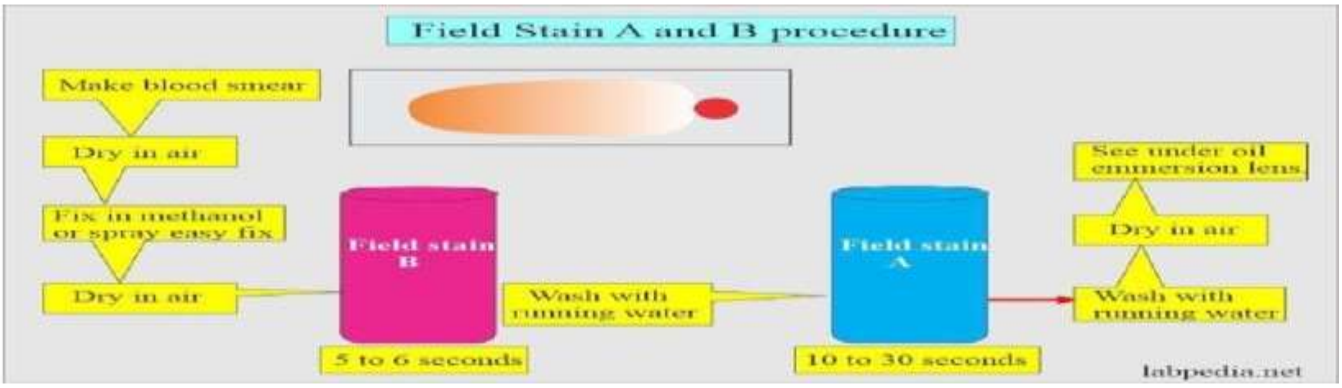

Figure 5. Procedure of Field stain A and B

In conclusion, Field stain A and Field stain B are two critical stains used in the Giemsa staining technique. Field stain A stains erythrocytes and the cytoplasm of leukocytes, while Field stain B stains the nuclei of leukocytes. The combination of these two stains allows for better visualization of blood parasites and other cell structures under a microscope.

The commonly used technique for detecting malaria antigens is the **Rapid Diagnostic Test (RDT)**. RDTs are immunochromatographic tests that quickly detect *Plasmodium* antigens in a blood sample [9]. These tests are popular because they are fast, simple, and require minimal laboratory equipment, making them ideal for use in resource-limited or remote settings. Here's an overview of the main types:

1. **HRP2-Based RDTs:** These tests detect *Plasmodium falciparum*-specific **histidine-rich protein 2 (HRP2)** antigen, which is a specific marker for *P. falciparum*, the most dangerous malaria species. They are sensitive to *P. falciparum* but may not detect non-*P. falciparum* infections.
2. **pLDH-Based RDTs:** RDTs targeting **parasite lactate dehydrogenase (pLDH)** can detect both *P. falciparum* and non-*P. falciparum* species like *P. vivax*, *P. malariae*, and *P. ovale*. These tests can distinguish between species but are generally less

sensitive than HRP2-based RDTs.

3. **Aldolase-Based RDTs:** Some RDTs detect **pan-malarial aldolase**, a general malaria antigen present in all *Plasmodium* species. These tests are used for initial screening and to detect mixed infections.

Result:

Gram +ve bacteria will be seen purple or dark blue when seen using the microscope, whereas Gram-ve bacteria will be seen red or pink. [3-4] The cell morphology and arrangement can also be observed, providing additional information about the bacteria. For example, cocci (spherical cells) may appear in clusters or chains, while bacilli (rod- shaped cells) may be solitary, paired, or arranged in chains.[5] The Gram stain results can provide valuable information about the nature and identity of bacterial cells and help in their identification and classification. Malaria's initial signs and symptoms are extremely vague and changeable. The non-specific nature of the signs and symptoms makes a clinical diagnosis of malaria still difficult. As a result, combining clinical and parasite-based findings can significantly improve the accuracy of malaria diagnosis [10].

CONCLUSION:

Clinical diagnoses are made on the basis of the patient's signs and symptoms as well as physical examination results. Malaria's initial signs and symptoms are extremely vague and changeable. The non-specific nature of the signs and symptoms makes a clinical diagnosis of malaria still difficult. As a result, combining clinical and parasite-based findings can significantly improve the accuracy of malaria diagnosis. By providing a more efficient, time- and money- saving alternative to traditional staining techniques, the Leishman and Giesma staining is a more recent staining approach that is of great assistance in high- throughput haematology facilities. It

improves the nuclear and cytoplasmic differential staining and can be applied to automated blood counters and stainers. As a result, the microscopic analysis of thick and thin blood films serves as the "gold standard" test for identifying parasitemia in the blood and directing the most effective course of action.

Reference:

1. Sato S. Plasmodium—a brief introduction to the parasites causing human malaria and their basic biology. Journal of physiological anthropology. 2021 Jan 7;40(1):1.
2. Sinden RE, Gilles HM. The malaria parasites. InEssential Malariology, 4Ed 2017 Dec 14 (pp. 8-34). CRC Press.
3. Sathpathi S, Mohanty AK, Satpathi P, Mishra SK, Behera PK, Patel G, Dondorp AM. Comparing Leishman and Giemsa staining for the assessment of peripheral blood smear preparations in a malaria-endemic region in India. Malaria journal. 2014 Dec;13:1-5.
4. Gregersen T. Rapid method for distinction of Gram-negative from Gram-positive bacteria. European journal of applied microbiology and biotechnology. 1978 Jun;5:123-7.
5. Halebian S, Harris B, Finegold SM, Rolfe RD. Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. Journal of clinical microbiology. 1981 Mar;13(3):444-8.

6. Holm C, Jespersen L. A flow-cytometric gram-staining technique for milk-associated bacteria. Applied and environmental microbiology. 2003 May;69(5):2857-63.
7. Wittekind DH. Romanowsky-giemsa stains. InConn's Biological Stains 2020 Aug 13 (pp. 303-312). Taylor & Francis.
8. Shalini, S. Comparision of the Peripheral Smear Examination by Leishman Stain, Fluorescent Stain and Immunochromatographic Test in Diagnosis of Malaria. Diss. Rajiv Gandhi University of Health Sciences (India), 2015.
9. Krampa FD, Aniweh Y, Awandare GA, Kanyong P. Recent progress in the development of diagnostic tests for malaria. Diagnostics. 2017 Sep 19;7(3):54.
10. White NJ, Pukrittayakamee S. Clinical malaria in the tropics. Medical journal of Australia. 1993 Aug;159(3):197-203.