Automated Detection of Malaria Parasite in Blood Smear Image

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***Abstract*—Malaria is a serious disease that has caused millions of deaths around the world. According to the World Health Organization (WHO), about 610,000 people died from malaria in 2024. Malaria spreads to humans through the bite of infected mosquitoes that carry Plasmodium parasites. The disease causes high fever with shivering, chills, and headaches, which greatly weaken the body. Children and elderly people are at a higher risk of death from malaria. One major reason for the high death rate is that the parasites in the blood can usually be detected only about 7 days after a mosquito bite. Treatment with antimalarial medicines can begin only after confirming the presence of the parasite. Sometimes, this delay in treatment can become life-threatening. To overcome these limitations, the development of automated diagnostic systems has become essential for the rapid and reliable detection of malaria. Such systems can help reduce the false negative rate and support early diagnosis, which is critical for effective treatment and disease management. Traditionally, malaria detection was done using a blood smear test. In this method, a small blood sample is placed on a glass slide and examined under a microscope to detect malaria parasites. This method requires skilled laboratory technicians and careful observation. Nowadays, computer-aided techniques are used to detect malaria parasites in blood smear images. These systems analyze microscopic images using image processing and machine learning methods. Computer-assisted techniques help reduce human errors and improve the accuracy of diagnosis.**

Malaria can also be detected using the rapid card method, commonly known as the Rapid Diagnostic Test (RDT). In this method, a small drop of blood is placed on a test card, and the result appears within a few minutes. It is a quick and simple method that does not require a microscope. The proposed study has been carried out to identify malaria parasites in microscopic blood smear images using computer-assisted techniques to improve early and accurate diagnosis.

To evaluate the performance of the proposed algorithm, experiments were conducted using the National Institutes of Health (NIH) Malaria dataset, and the results were compared with those of existing methods. The proposed approach achieved a detection accuracy of more than 91%, demonstrating superior performance over comparable techniques. These findings indicate that the developed algorithm is reliable and can serve as a valuable support tool for pathologists and haematologists in the accurate detection of malaria parasites.

I. INTRODUCTION

Malaria is one of the most common and deadly diseases in the world. Pregnant women and young children are at greater risk of getting seriously ill from malaria. In many developing countries, this disease causes a large number of deaths and health problems.

According to the World Malaria Report published in December 2025, progress in controlling deadly diseases like malaria has slowed down because of the COVID-19 pandemic. The pandemic has made prevention, testing, and treatment services more difficult in many countries.

In 2024, about 282 million malaria cases were estimated in 85 malaria-affected countries, including French Guiana. This was higher than the number of cases reported in 2024, and most of the increase occurred in African countries. Six countries — Nigeria, the Democratic Republic of the Congo, Uganda, Mozambique, Angola, and Burkina Faso — together accounted for a large share of malaria cases worldwide. India also carried a significant burden of malaria cases. The World Health Organisation (WHO) report released in December 2025 stated that hundreds of millions of people are affected by malaria every year.

Since 2024, several countries have reported an increase in malaria-related deaths. The WHO's global malaria strategy did not meet some of its 2020 targets,

and without strong and immediate action, the 2030 goals may not be achieved. Recent studies also show that malaria has caused more deaths among young children in the past two decades than earlier estimates suggested.

The current situation is very serious. Malaria could increase again in Africa if quick action is not taken. In many countries where malaria cases are already high, health services for malaria have been badly affected due to several problems such as COVID-19, Ebola outbreaks, floods, and other humanitarian emergencies. In addition, resistance to antimalarial drugs has become a major concern. therefore, developing new technologies and increasing spending on research and development are essential to eliminating malaria. In last year, about US$ 3.3 billion was spent worldwide on malaria control, but this amount needs to increase to more than three times over the next ten years to properly carry out the global malaria elimination plan.

Malaria has affected humans for thousands of years. This study aims to create a new tool that can automatically detect the malaria parasite using image processing and neural network–based classification. This technology could help save many lives and contribute to the goal of a malaria-free world.

II. HISTORY OF MALARIA

Malaria has been known since ancient times. Old medical writings and religious texts from Chinese, Asian, and Indian civilizations describe people suffering from seasonal fevers with chills and shivering, which are common symptoms of malaria.

The word *malaria* comes from Italian. It means “bad air.” In Latin, *mal* means bad and *aria* means air. In the past, people believed malaria was caused by breathing unhealthy air from marshy or wet land.

In 1880, a French army doctor working in Algeria discovered the malaria parasite in the red blood cells of patients. Later, in 1886, the Italian scientist Camillo Golgi studied how the parasite grows and multiplies inside red blood cells.

In 1891, Russian scientist Romanowsky developed a staining method that helped scientists clearly see malaria parasites under a microscope in blood samples. Between 1886 and 1890, three species of malaria parasites that infect humans were identified: Plasmodium malariae, Plasmodium falciparum, and Plasmodium vivax. In 1922, a fourth species, Plasmodium ovale, was discovered.

A major breakthrough came in 1897, when Sir Ronald Ross, a British doctor working in Secunderabad, India, discovered that malaria is transmitted by the bite of infected mosquitoes. In his honor, World Mosquito Day is celebrated every year on August 20. He received the Nobel Prize in 1902 for his important discovery and continued working on malaria research for many years.

Since then, many scientists and organisations around the world have worked to control and eliminate malaria. One major effort is led by the Roll Back Malaria (RBM) Partnership, which was created to reduce the global burden of malaria. Another important organisation, the Centers for Disease Control and Prevention (CDC), has also played a key role in reducing malaria in both developed and developing countries.

Malaria is caused by a parasite called *Plasmodium*. This parasite infects red blood cells (RBCs), also known as erythrocytes. Four main species of Plasmodium infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. Among these, *P. falciparum* and *P. vivax* are the most common. *P. falciparum* is the most dangerous and is responsible for most severe malaria cases and deaths.

Malaria spreads from an infected person to a healthy person through the bite of a female *Anopheles* mosquito, which is the main carrier (vector) of the disease. When a mosquito bites an infected person, it takes in blood that contains the malaria parasite. The parasite then develops and multiplies inside the mosquito. When this infected mosquito bites another person, the parasite enters the new person’s bloodstream and causes infection.

The spread of malaria depends greatly on the number of mosquitoes in an area. Mosquito populations are affected by climate factors such as rainfall, temperature, and humidity. In many tropical regions, malaria is seasonal and often increases during the monsoon season. Malaria outbreaks (epidemics) can happen when people with little or no immunity move into areas where malaria is common, or when environmental conditions suddenly favour mosquito breeding.

Anopheles mosquitoes need water to lay their eggs, so they are usually found near stagnant water like ponds, swamps, and puddles. They grow well in warm, tropical climates. Studies using mathematical models have shown that climate change can affect the spread of malaria by increasing the areas where mosquitoes can survive. Rising global temperatures may increase mosquito populations, especially *Anopheles* mosquitoes. This could lead to the return of malaria transmission in places that are currently malaria-free, including some parts of Australia and the United States.

Many diseases have symptoms similar to malaria. During the incubation period, which usually lasts from 8 to 14 days, malaria often causes flu-like symptoms such as fever, chills, headache, and body aches. The length of this period can vary depending on the species of the malaria parasite.

If proper treatment is not given, or if the parasite has become resistant to available medicines, the infection can become severe very quickly and may be life-threatening. Malaria parasites destroy red blood cells, which leads to anemia. They can also block small blood vessels, reducing blood flow to important organs like the brain, which can cause serious complications.

The human body has some natural ability to fight malaria. People with less than 10% of their red blood cells infected often have a better chance of survival. However, when more than 10% of red blood cells are infected (a condition called high parasitaemia), the risk of death increases, and blood transfusion may be required.

Even though many people are still at risk, malaria is both preventable and curable. Between 2001 and 2015, the number of malaria cases worldwide decreased significantly, and deaths from malaria were nearly cut in half. Medicines such as quinine and other antimalarial drugs have been effective in treating *Plasmodium falciparum* malaria. Research studies have also shown that certain drugs can work against malaria parasites in experimental models.

This shows that malaria can be treated successfully if it is diagnosed correctly and early. Therefore, accurate and timely diagnosis is very important for effective treatment and saving lives.

Taxonomy of the malarial parasite Plasmodium

Phylum	Protozoa
Subphylum	Sporozoa
Class	Telosporea
Order	Eucoccidia
Suborder	Haemosporidia
Family	Plasmodiidae
Genus	Plasmodium
Species	falciparum (vivax, ovale and malariae)

Malaria Parasite species

Malaria is a mosquito-borne disease caused by protozoan parasites of the genus *Plasmodium*. The disease is transmitted to humans by the bite of infected female *Anopheles* mosquitoes, which act as vectors.

After entering the human body, the parasite infects red blood cells (RBCs), also called erythrocytes. The destruction of RBCs leads to the common symptoms of malaria, such as fever, chills, and anaemia.

Five species of Plasmodium infect humans:

1. *Plasmodium falciparum*
2. *Plasmodium vivax*
3. *Plasmodium ovale*
4. *Plasmodium malariae*
5. *Plasmodium knowlesi*

The first four species are more common in humans. *P. knowlesi* was previously believed to infect only monkeys, but human cases were first reported in 2008. It can sometimes be confused with *P. malariae* under microscopic examination.

Among all species, *P. falciparum* is the most dangerous and causes severe malaria, while *P. Vivax* is the most widespread species.

All *Plasmodium* species have similar life cycle stages in the human body, although their morphology may vary.

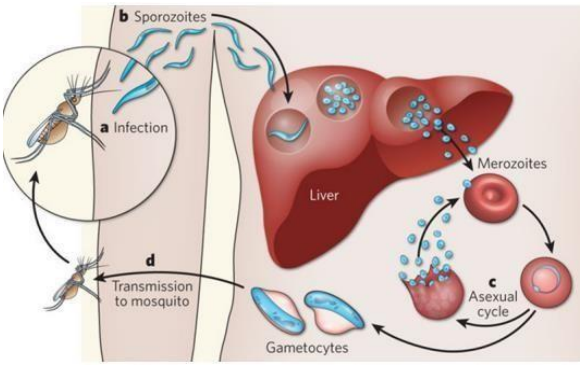
Malaria Parasite Life Cycle

Both a vertebrate host and a mosquito are necessary for the life cycle of the malaria parasite to flourish. In following Figure 1 shows a female Anopheles mosquito that spreads malaria to humans by feeding an infected vertebrate host and releasing sporozoites into that host.

Figure 1- Female Anopheles mosquito taking a blood Sample

Highly mobile reproductive organisms, Sporozoites migrate through the circulatory system to infect the liver's hepatocytes. Schizogony, an asexual reproduction process, results in the formation of thousands of ―merozoites‖ (haploid forms) per hepatocyte until the membrane finally ruptures.
After invading erythrocytes (Red Blood Cells, RBCs), merozoites pass through three main developmental stages: ring stage, trophozoite stage, and schizont stage. Merozoites enter RBCs to avoid destruction by leukocytes (White Blood Cells/WBCs). Inside the RBCs, they obtain nutrients by digesting haemoglobin.

The first intra-erythrocytic stage is the ring stage. Over several hours, the ring form grows and develops into a larger trophozoite. The trophozoite then matures into a schizont, during which asexual reproduction occurs. The mature schizont contains approximately 8 to 24 daughter merozoites. Eventually, the infected erythrocyte ruptures, releasing these merozoites into the bloodstream. Each released merozoite can invade a new RBC and repeat the asexual replication cycle. In severe *Plasmodium falciparum* infections, schizonts may sometimes be observed in peripheral blood. The repeated destruction of erythrocytes during this cycle is responsible for the characteristic symptoms of malaria.
During the erythrocytic stage, the malaria parasite digests hemoglobin and detoxifies the toxic by-products by converting them into hemozoin, an insoluble crystalline brown pigment. Although hemozoin is produced throughout the erythrocytic cycle, it becomes clearly visible mainly in the late trophozoite and schizont stages.



Gametocytes represent the sexual forms of the parasite (male and female) present within human RBCs. They are essential for transmission and contribute to the widespread distribution of malaria and the rapid spread of drug-resistant strains. When a female *Anopheles* mosquito takes a blood meal from an infected person, it ingests these gametocytes. Inside the mosquito's stomach, the male and female gametocytes fuse (fertilization) and undergo meiosis, followed by multiple divisions to form oocysts. Each oocyst produces hundreds of sporozoites. These sporozoites migrate to the mosquito's salivary glands and are transmitted to another human host during the next blood meal, thereby continuing the malaria life cycle.

Malaria Parasite stage
Under a microscope, visual forms become crucial as the life cycle of the parasite species differs in terms of development. The chronological sequence of the discrete group of phases classified as ring, schizont, gametocyte, and trophozoite is shown in Figure 2.
Early stages of Plasmodium falciparum in marginal blood are typically thought to have been present in less than twenty-four hours in non-severe malaria cases. In the extreme scenario, every step is visible in the peripheral blood. The parasites' adhesion to capillaries inside the various organs caused the trophozoite of infected RBCs to fade from the minimal blood circulation; this process is known in medicine as sequester.

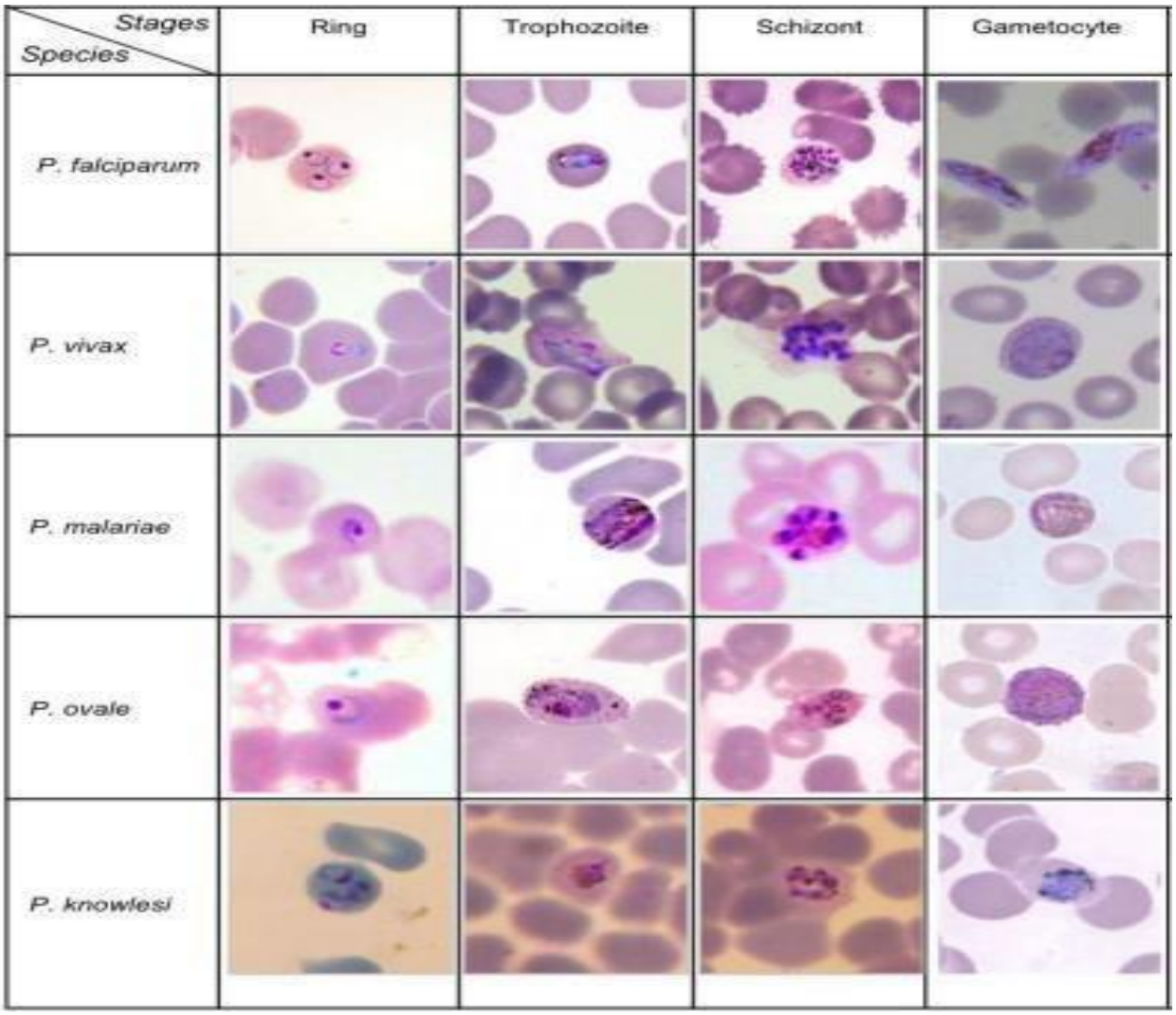


Figure 2 - Species of Malaria plasmodium- life phases in thin blood-film

When capillaries become blocked by freshly infected cells, subsequent mature parasite phases, including schizonts and trophozoites, are visible in the peripheral blood and are characterised as severe infection, which is associated with a poor prognosis.

One or two tiny chromatin spots and visible cytoplasm are seen in the ring stages of P. falciparum. No matter what, the contaminated blood cells do not become larger. Multiple infections are still necessary given the circumstances. Identification of P. Falciparum trophozoites over peripheral blood smears is highly atypical because the mature trophozoites have a denser structure than the younger rings and may have a brown, rounded interior pigment similar to that of malaria.

Peripheral blood abnormalities can also be observed in *Plasmodium falciparum* infections. The schizont stage of *P. falciparum* shows clumped dark brown pigment in the center, with about 2 to 32 nuclei present. The gametocytes of *P. falciparum* are sausage-shaped (crescent-shaped) and can be seen in a blood smear about one week after infection. The chromatin may appear as a single mass, a bulky form, or scattered within the cell. These variations may represent different developmental stages of malaria parasites.

In infections caused by *Plasmodium malariae*, repeated infections are uncommon. In *Plasmodium ovale*, the infected red blood cells become enlarged, oval in shape, and may show fimbriated (tufted) edges. The trophozoites appear amoeba-like and contain larger parasites along with malarial pigment.

Plasmodium vivax infects a wider range of red blood cells compared to some other species. Infected red blood cells usually appear enlarged, which differs from certain other malaria infections.

The erythrocytic (red blood cell) cycle of Plasmodium knowlesi lasts about 24 hours, which is shorter than the 48-hour cycle of Plasmodium falciparum and the 72-hour cycle of Plasmodium malariae. Because of these different cycle durations, the stages seen in peripheral blood correspond to the specific parasite involved.

Plasmodium knowlesi may look similar to Plasmodium malariae under the microscope. However, like Plasmodium falciparum, the cytoplasm of the trophozoites in *P. knowlesi* can appear irregular. Different stages of parasite development may also be seen within a single red blood cell. The trophozoites sometimes form band-like shapes and contain malarial pigment.

The pre-erythrocytic (Tissue) stage of the infection in humans begins when an infected female mosquito bites a human, injecting the sporozoites—infected parasite forms found in the mosquito salivary gland—into human capillaries. Sporozoites inside liver cells undergo a spindle-shaped to round transformation during the erythrocytic phases of all four species of Plasmodium, as shown in Figure.

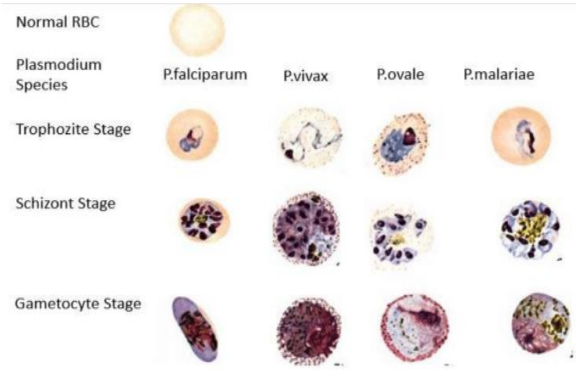


Figure 3 - Malaria Parasite stages

The variations in the shape of infected RBC will be observed andidentified in different stages of malarial parasite infections that will help give the proper treatment. The visual properties of the RBCs in the form of particular dots show diverse species as presented in Table 1.

Table 1 - Identification & Types of Malaria

Size	Inflated	No change	Not Inflated	Inflated
Shape	Amoeboid	Round	Round	Oval
Dote	Minorred dots	Majorred dots	Fewsmall dots	Minorred dots

Component Of Blood

The components of blood should be explained in greater detail to give the reader appropriate prior information given malaria's presence in the systemic circulation. For the transfer of oxygen and nutrients to and the removal of metabolic waste from cells, blood is a vital fluid. Cells are suspended in a clear-to-yellow liquid that is mostly water, but also plasma proteins and cell nutrients. RBCs, WBCs, and platelets make up the three main cell types in the plasma.

1. Red Blood Cells

Red blood cells (RBCs) are responsible for transporting oxygen from the lungs to the tissues of the body. They circulate in the bloodstream for approximately 120 days. After completing their life span, old or damaged RBCs are removed from circulation mainly by the spleen and liver.

The major component of RBCs is hemoglobin, a metalloprotein that plays a vital role in oxygen transport. Hemoglobin gives red blood cells their red color and enables them to carry oxygen efficiently. Each hemoglobin molecule is composed of four subunits. Each subunit contains a globin protein chain and a heme group. The heme group includes an iron (Fe^{2+}) atom at its center.

Oxygen binds to the iron atom in the heme group in a loose and reversible manner. This reversible binding allows red blood cells to pick up oxygen in the lungs and release it in the tissues where it is required. Therefore, hemoglobin enables RBCs to transport oxygen effectively throughout the body.

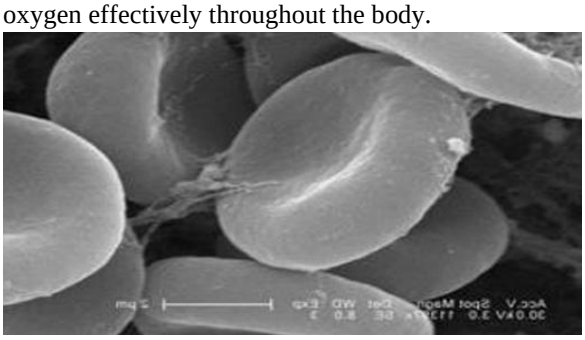


Figure 4 - Electron Microscopy Of RBC

The above Fig.4 illustrates Cells can pass across narrow capillary beds because of the plasma membrane's shaped and compliance. Improved plasma membrane oxygen can be achieved by utilizing a biconcave shape. To maximize haemoglobin content, RBCs also evacuate their nucleus during erythropoiesis, however, immature cells may retain reticular material for a short time while in circulation.

2. White Blood Cells

White blood cells (WBCs), also called leukocytes, are responsible for immunity and help the body fight infections and diseases. They play an important role in protecting the body against pathogens such as bacteria, viruses, and parasites.

There are five main types of WBCs in normal blood:

- Neutrophils (40–75%) – First line of defence; destroy pathogens by phagocytosis.
- Lymphocytes (20–45%) – Responsible for adaptive immunity and antibody production.
- Monocytes (3–11%) – Develop into macrophages and engulf pathogens.
- Eosinophils (0–7%) – Fight parasitic infections and are involved in allergies.
- Basophils (0–1%) – Involved in inflammatory and allergic responses.

In malaria, once the parasite enters a red blood cell (RBC), it becomes less visible to the immune system because mature RBCs do not actively participate in immune signaling.

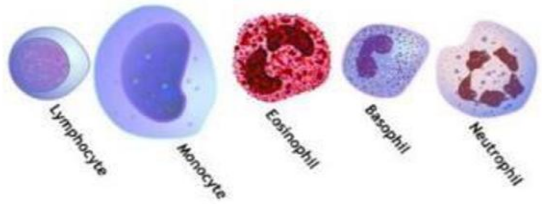


Figure 5 - White Blood cells

3. Platelets

Platelets are megakaryocyte-derived cell fragments that aid in hemostasis and blood clotting. A Wright-stained microscopic picture is shown in Figure – 6, a standard laboratory stain for the examination of blood smears.

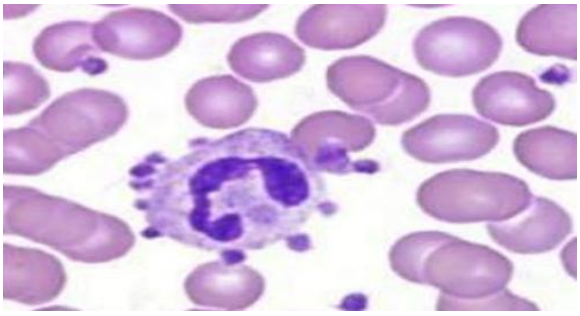


Figure 6- Platelets Observed from Wright stain

Blood Film Image

In normal practice, blood films are viewed under a microscope, and decisions regarding the presence of malaria are made immediately. The slides (blood films) may be stored for future reference, but no images are taken of the blood films. For automatic systems, images of the blood films must be acquired. The images must be of sufficient resolution for the image analysis methods to distinguish between erythrocytes, parasites, thrombocytes, leukocytes, and artifacts. On the other hand, a sufficiently large portion of the blood film must be image to examine sufficient amounts of blood to reach statistically sound decisions. Together, these two requirements dictate that hundreds of images are needed per blood film.

1. Image Acquisition

When hundreds of images are required, manual selection of imaging fields is not practical. Therefore, a dedicated microscope equipped with a suitable camera is generally used to scan a fixed rectangular region of the blood smear. However, the blood smear is not completely uniform. Some captured images may contain no useful information, while others may show an excessively dense accumulation of stained material. As a result, not all images are suitable for diagnosis, and those that are not useful must be discarded. In some cases, fields are selected automatically using additional artificial intelligence techniques.

Modern microscopes are equipped with a fixed illuminator to produce coloured images of blood samples. However, if the controller moves the glass slide too quickly, the camera may fail to capture the images properly. Consequently, the images may appear bluish or show variations in illumination due to differences in angle during capture.

2. Thin Blood Film Image

Given Figure-7 (a, b, c, and d) shows background, erythrocytes, thrombocytes, and potentially leukocytes on thin blood films from malaria-free subjects. Figure (a) depicts an image from a positive slide containing infected erythrocytes (Ip). Figure (b) shows an image from a normal slide. Figure (c) shows an image containing a leukocyte (Lu) and figure (d) shows an image containing a phagocyte (Pg). Thrombocytes are located outside of erythrocytes. Meanwhile, images of thin blood films from malaria-infected subjects also contain background, erythrocytes, and thrombocytes, with the difference that some erythrocytes contain parasites which is shown in Figures (a) and (d).

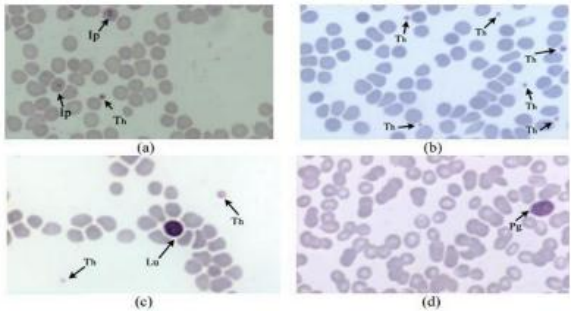


Figure 7- Original Image from Blood Film

A well-prepared thin blood film comprises a single layer of erythrocytes.

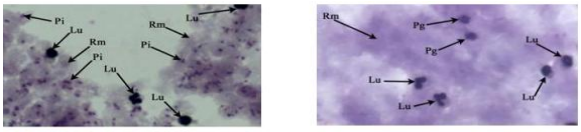
Erythrocytes are visible and dominant in the sense that the total area of the image (number of pixels) associated with erythrocytes is greater than the area of the image of all other components, except possibly the background. In general, thrombocytes occupy the smallest percentage of the total area. This is in line with the fact that the number of thrombocytes is lower than the number of erythrocytes and they are smaller in size.

Leukocytes are much less frequent than either erythrocytes or thrombocytes and are not necessarily present in every thin blood film image. The maximum intensity value is seen in leukocytes, followed by malaria parasites (if present). The intensity profile of malaria parasites is substantially larger than that of erythrocytes, even though the size of a malaria parasite is much less than that of an erythrocyte. When a parasite is found within an erythrocyte (which occurs frequently), the parasite's intensity profile is visible over the erythrocyte's profile. Thrombocytes typically have the lowest intensity histogram above background intensity, whereas erythrocytes have a significantly different intensity. Thin blood film images may show thrombocytes on top of an erythrocyte, making it appear as though they are within the erythrocyte.

3. Thick Blood Film Image

As erythrocyte diameters increase with the increasing thickness of the blood film, so does the amount of blood represent by an image of a thick blood film, even when the resolution remains the same. However, in thick blood films, erythrocytes have been dissolved so individual erythrocytes are not visible in thick blood film images. The image contains residual erythrocyte membranes, thrombocytes, leukocytes, and possibly artifacts and parasites if the subject is malaria-positive. Because the volume of blood is larger, many more parasites can be seen in a thick blood film image compared to a thin blood film image of the same resolution from the same subject.

Like thin blood film images, leukocytes generally have the highest intensity profile, followed by malaria parasites (if present) and background. However, thick blood film images are significantly different from thin blood film images. Significant differences in thick blood film images are that erythrocytes disappear, and residual erythrocyte membranes result from haemolysis, the process of releasing cytoplasm of erythrocytes into surrounding blood fluid, present in thick blood film images as shown in figure-8 (a) and (b). Pi is the Parasite, Rm is the residual erythrocyte membrane, Lu is the leukocyte and Pg represents phagocytes. Figure-8 (a) is an example of the image, from a positive slide, containing parasite leukocytes. Figure-8 (b) is an example of a image containing leukocytes and phagocytes.



(a)Image containing parasites and Leukocytes

(b) Image containing leukocytes And phagocytes

Figure- 8

The fact that a thick blood film image is only two-dimensional is one of the most significant differences between an image of a thick blood film and a thick blood film as can be seen through a microscope. This means that the images do not allow the user to change focus as is done in practice for direct microscopy.

Therefore, some objects are difficult to interpret because they are out of focus or overlap with other objects.

Malaria Diagnostic Method

Although malaria is a curable disease, it can progress rapidly and may develop into severe or cerebral malaria, especially when caused by *Plasmodium falciparum*. This species is particularly dangerous because it is associated with serious neurological complications. Therefore, timely diagnosis is extremely important to prevent life-threatening outcomes.

The primary step in diagnosing malaria is detecting the presence of the parasite in the blood. In addition to confirming infection, identifying the specific species of the parasite is essential, as different species may require different treatment approaches. Detecting mixed infections (infection with more than one species) is also important for proper management. Furthermore, observing the developmental stage of *P. falciparum* helps in assessing the severity of the disease.

Determination of the parasitemia level (the number of parasites present in the blood) is not only useful for confirming infection but also for evaluating the severity of the disease. It plays a crucial role in monitoring the patient's response to treatment and in detecting possible drug resistance.

Malaria can now be diagnosed using several different methods. Each diagnostic technique has its own advantages and limitations, and no single method is considered completely ideal. The choice of method often depends on available resources and clinical conditions.

The diagnosis of malaria is further complicated by various factors such as cost, durability and stability of diagnostic products, and geographical limitations. In many regions, especially remote or resource-limited areas, these challenges can affect the availability and reliability of proper diagnostic testing.

1. Clinical Diagnostic Method

During a physical examination, malaria is often suspected based on symptoms such as vomiting, fever, headache, abdominal pain, and dizziness. However, these symptoms are not specific to malaria and may overlap with those of other diseases. Because of this similarity, accurate diagnosis requires a high level of medical expertise. In addition, several other life-threatening infections present with similar symptoms, which increases the risk of misdiagnosis. Relying only on clinical signs may therefore lead to incorrect medical prescriptions and inappropriate treatment.

2. Microscopy Method

Among the available diagnostic techniques, light blood film microscopy is still considered the gold standard for malaria diagnosis. In this method, a drop of the patient's blood is placed on a glass slide, stained, and examined under a microscope to detect the presence of malaria parasites. In addition to laboratory diagnosis, patients are often initially evaluated based on symptoms such as vomiting, fever, headache, abdominal pain, and dizziness during physical examination. However, these symptoms overlap with many other diseases, which makes accurate diagnosis challenging and requires considerable medical expertise. Although clinical examination is cost-effective, relying solely on symptoms can result in misdiagnosis. Other serious infections may present with similar symptoms, potentially leading to incorrect medical prescriptions and inappropriate treatment.

Thick Blood Smear and Thin Blood Smear

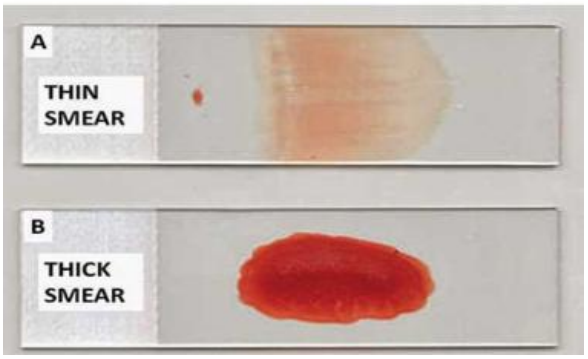


Figure-9

Capillary blood is usually obtained from the fingertip and placed on a clean, high-quality glass slide. To prepare a thin smear, another slide is held at an angle of about 30°–45° and used to spread the blood drop smoothly across the surface, forming a thin film with a feathered tail at one end. Thin smears are mainly used to identify the malaria parasite and to differentiate between various species.

A thick smear is prepared by placing a single drop of blood on a glass slide and spreading it gently into a small circular area without creating a thin layer. A thin

smear generally contains about 1–1.5 μL of blood, whereas a thick smear contains approximately 3–4 μL. Since the thick smear concentrates multiple layers (about 20–30 layers) of blood cells into a smaller area, it increases the chance of detecting parasites when examined by a trained professional.

When the parasite count is low, even as little as 20 parasites per microliter of blood, the thick smear is more effective for rapid detection of malaria.

The main advantages of this method are simple and less expensive. Its most significant advantage is the extensive trained technical personnel required to perform manual diagnosis under a microscope and it takes only 15-30 minutes for each slide diagnosis.

3. Fluorescence Microscopy Method

As an alternative to conventional light microscopy, several diagnostic techniques based on fluorescence microscopy have been developed. In these methods, a special fluorescent dye (fluorochrome) that binds to the nucleic acids of the malaria parasite is used to stain the blood sample. The stained sample is then examined under a fluorescence microscope, which emits light at a specific excitation wavelength, causing the stained parasites to glow and become visible.

The main advantage of fluorescence microscopy over routine light microscopy is that the glowing parasites are easier to detect. This allows the microscopist to identify infected cells more quickly and with less effort. One of the most widely used fluorescence-based methods is the Quantitative Buffy Coat (QBC) technique. In this method, blood is collected in capillary tubes coated with Acridine Orange stain and then centrifuged. Centrifugation separates the blood components into layers, making it easier to examine the layer where parasites are concentrated under a fluorescence microscope. The theoretical detection limit of the QBC method is similar to that of light microscopy. However, some field studies have shown that it may be more sensitive in detecting cases with low parasitemia. Despite this advantage, the method has certain limitations. Acridine Orange is a non-specific stain, meaning it also stains other cell nuclei, which can make it difficult to clearly distinguish malaria parasites.

The test shows high specificity (more than 91%) in infections caused by *Plasmodium falciparum*, but its specificity is much lower for other species when compared to light microscopy. In addition, the technique requires specialized equipment and centrifugation, making it technically demanding. It also does not allow accurate determination of parasite species or precise measurement of parasitemia levels.

4. PCR Method

The molecular diagnostic method, commonly known as Polymerase Chain Reaction (PCR), is used as a confirmatory test for malaria. It is considered one of the most sensitive and specific diagnostic techniques, especially in cases of low parasitemia. PCR is often used in well-equipped laboratories because it provides more accurate results compared to conventional Giemsa-stained microscopy.

PCR works by amplifying the DNA of the malaria parasite. In this technique, the parasite's genetic material is multiplied many times to make it easier to detect. The process involves repeated heating and cooling cycles, known as thermal cycling. During heating, the DNA strands separate, and during cooling, new copies of the DNA are formed. This repeated process allows even a very small amount of parasite DNA to be detected. In laboratory tests, PCR has been able to detect as few as five parasites per milliliter of blood.

However, PCR has several limitations. The procedure is complex, expensive, and time-consuming, often taking more than 24 hours to produce results. It also requires trained technicians and specialized laboratory equipment. Due to these factors, PCR is not widely available in low-resource or rural malaria-endemic areas. Regular quality control and maintenance are also necessary, which further limits its use. As a result, PCR is mainly used for research purposes and for confirmatory diagnosis in advanced healthcare centers and larger hospitals.

5. Rapid Diagnostic Method

Rapid Diagnostic Tests (RDTs) were developed. These tests are designed to provide quick results without the need for complex laboratory equipment. RDTs are based on immunochromatographic techniques that detect specific antigens produced by *Plasmodium* parasites in the blood. RDTs commonly identify three main malaria antigens. These include Histidine-Rich Protein 2 (HRP-2), which is specific to *Plasmodium falciparum*, and Plasmodium lactate dehydrogenase (pLDH), which can help differentiate between *P. falciparum* and *P. vivax*. Certain forms of pLDH (pan-

specific types) can detect all *Plasmodium* species. Another antigen used is Plasmodium aldolase, which is also considered pan-specific. By using different combinations of these antigens, RDTs can detect *P. falciparum*, *P. vivax*, or mixed infections. An RDT typically consists of a nitrocellulose strip. One end of the strip contains dye-labeled antibodies that are specific to the target malaria antigen, while the other end contains a test line with immobilized antibodies.

A small blood sample is collected by finger prick, mixed with a buffer solution, and applied to the strip. If malaria antigens are present in the blood, they bind to the labeled antibodies and move along the strip with the buffer. These complexes accumulate at the test line, forming a visible colored line that indicates a positive result. Several RDT kits approved by the World Health Organization (WHO) are commercially available and have been widely evaluated in both laboratory and field conditions. While many commonly used RDTs can detect only *P. falciparum* or *P. vivax*, some advanced kits can detect multiple species and differentiate between them. Although RDTs provide rapid and convenient results, they offer only a rough estimation of parasitemia, which may sometimes be inferred from the intensity of the test line.

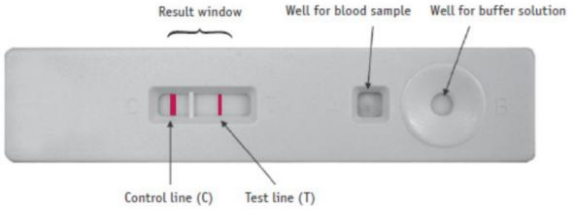


Figure-10 - Malaria Rapid Kit

The main advantage of Rapid Diagnostic Tests (RDTs) is that they are simple to use and do not require specialized training to perform or interpret. Because of their straightforward design, they can be used in primary healthcare settings and even in remote areas with limited medical facilities.

RDTs provide results within 15–30 minutes and do not require electricity, microscopes, or other laboratory equipment. This makes them especially suitable for field use and in resource-limited settings. Due to these advantages, RDTs are widely used in malaria-endemic countries, and it is estimated that nearly half of malaria tests in such regions are performed using RDTs.

Rapid Diagnostic Tests (RDTs) for malaria, although widely used, have several important limitations. One of the major drawbacks is their limited ability to detect all species of *Plasmodium*. Many commonly used RDTs are designed primarily to detect *Plasmodium falciparum* and *Plasmodium vivax*, and may not accurately identify other species or mixed infections.

Another limitation is reduced sensitivity in cases of low parasitemia. When the parasite density in the blood is very low, RDTs may produce false-negative results. In addition, false-positive results may occur because certain malaria antigens, such as HRP-2, can remain in the bloodstream even after successful treatment and clearance of parasites.

RDTs also do not provide an accurate estimation of parasitemia and therefore cannot reliably assess the severity of infection. Unlike microscopy, they do not offer information about the developmental stages of the parasite. Furthermore, the performance of RDTs can be affected by storage conditions, as exposure to high temperature and humidity may reduce their stability and accuracy.

Another important concern is the presence of HRP-2 gene deletions in some strains of *Plasmodium falciparum*, which may lead to false-negative results in HRP-2–based tests. Due to these limitations, RDTs are mainly used for rapid screening, and confirmatory testing with microscopy or molecular methods may be required in certain cases.

6. Automatic Diagnostic Method

Among the various techniques available for malaria detection, automated malaria diagnosis is one of the most recent developments. This method aims to minimize human intervention by digitizing the entire microscopic examination process. It uses a machine vision system that imitates the procedures followed in manual microscopy. An automated diagnostic system consists of both hardware and software components. The hardware includes digital microscopes equipped with features such as autofocus, automated field selection, and computer- or mobile-controlled slide movement. Imaging devices such as slide scanners and camera-integrated digital microscopes are also essential parts of the system.

On the software side, advanced image processing algorithms are required for pre-processing the captured images and for detecting and identifying malaria parasites. These algorithms help in analyzing

blood smear images and determining the presence and extent of infection with improved accuracy and efficiency.

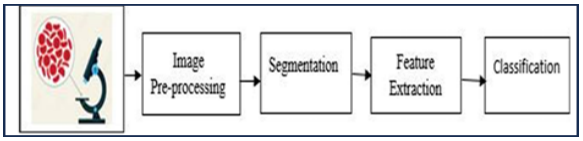


Figure 11- Automatic method of Malaria Diagnostic Process

Traditional automated malaria diagnosis generally consists of four main stages: image pre-processing, cell segmentation, feature extraction (feature selection), and classification of infected and uninfected cells. At each stage, different techniques are applied to improve the accuracy of detection.

In the pre-processing stage, the quality of microscopic blood smear images is enhanced to improve the performance of subsequent steps such as segmentation, feature extraction, and classification. Artifacts such as air bubbles and impurities present in the slides may negatively affect the analysis and lead to incorrect classification. To reduce noise in microscopic images, various filtering techniques are applied. Morphological operations are also used to refine cell boundaries and remove unwanted elements. Methods such as adaptive thresholding and histogram equalization help improve image brightness and contrast. In addition, normalization in the HSV color space can be used to minimize variations in color and contrast.

Cell segmentation is a critical step in the automated diagnosis process. In this stage, the processed image is divided into smaller regions representing red blood cells (RBCs), white blood cells (WBCs), and malaria parasites. Various unsupervised techniques, such as histogram-based methods and hole-filling approaches, are used for segmentation. RGB color images may also be utilized for segmentation in moderately bright images. Thresholding techniques in the HSV color space, particularly using the saturation (S) and value (V) channels, are commonly applied to separate cells from the background.

Feature extraction is influenced by the shape, texture, and color of red blood cells. In stained blood smear images, color features are particularly significant. Therefore, the HSV color space and the green channel of the RGB color space are often recommended for extracting meaningful features. Several feature extraction methods may be applied, including Histogram of Oriented Gradients (HOG), Haralick texture features, and Local Binary Patterns (LBP). These features help distinguish between infected and uninfected cells, as well as identify different types of malaria parasites based on color and morphological characteristics.

In the classification stage, various machine learning algorithms are used to identify malaria-infected cells. Techniques such as linear Euclidean distance classifiers combined with Poisson distribution and Gabor filtering have been applied for malaria detection in blood smear images. K-means clustering can also be used for unsupervised identification of infected cells. Supervised classifiers such as Support Vector Machines (SVM) and Artificial Neural Networks (ANN) use normalized RGB color information and texture features that are robust to staining variations for parasite detection.

With the advancement of machine learning, conventional diagnostic methods have been further improved. Deep learning techniques, in particular, eliminate the need for manual feature extraction, as they automatically learn relevant features from image data. Convolutional Neural Networks (CNNs) have become widely used in medical image analysis due to their ability to learn complex patterns directly from images. In this research work, an attempt is made to develop a CNN-based model for the detection of malaria parasites.

Malaria Estimation-

The creation of Confusion Matrix is used to do malaria detection. It offers data on how well the classification system's actual and predicted classifications perform. Error matrix is another term for a confusion matrix. The performance of such kind of system is assessed commonly utilizing available data with the matrix. The confusion matrix aimed at the binary classifier is used in the thesis for performance evaluation. It is observed that all right predictions are situated diagonally of the table; therefore, it is simple to inspect visually the table aimed at estimation of faults since they are depicted by outside diagonal values given in Table.

Actual Class	Predicted Class		
		Yes	No
	Yes	True Positive (TP)	False Positive (FP)
	No	False Negative (FN)	True Negative (TN)

Performance Measures
There are various standard terms defined for a 2-class matrix:

Accuracy (AC): The metric accuracy is the ratio of entire predictions that are precise.

$$\text{Accuracy (AC)} = \frac{TP + TN}{TP + TN + FP + FN} \times 100$$

True Positive (TP):
It is possible to use the metric TP as the number of positive examples in which the classifier is correctly identified.

True Negative (TN):
The Classifier is detected correctly as negative, and the metric TN might represent the number of negative examples that were discovered.

False Positive (FP):
Its metric FP represents the number of positive examples discovered by the classifier, however, in real-time it is negative.
False Negative (FN): The metric FN represents the number of cases in which the classifier detected as negative, but was positive in real-time.
The method always possesses a maximum score of accuracy yet having a maximum score could not provide assurances that the method is established well—the following measures are utilized for better assessment of the classifier for excellent performance.

True Positive Rate (TPR):
The metric TPR is called as Recall. Here, this could be utilized for exhibiting the positive instance percentage that is recognized correctly. The following equation is utilized.

False Positive Rate (FPR):
The metric FPR is called a false-alarm rate. The FPR is the ratio of negative instances that are classified incorrectly as positive. Here, the following equation is utilized.

False Negative Rate (FNR):
The metric FNR is also called a miss rate. The FNR shows the percentage of negative events that were classified as being such. The following equation is utilized for computation.
The better-performing method needs to have a maximum value of TPR that is 1. The low FNR and FPR values are 0 ideally. In reality, it is rare to possess TPR=1 FPR=FNR=0, yet these computations are resourceful for comparing the performance of the manifold method designed for solving a similar issue.
Recall and precision are accuracy-matrix since they could be utilized for characterizing the classification. The precision could be instances percentage marked to be positive, which are also positive.

Summary
Malaria is a treacherous disease caused by the bite of a female mosquito, which 27 destroys the Red Blood Cells of humans. The ratio of infected cells of the parasite to the entire amount of RBCs known as parasitemia can be utilized, as a scale of severity towards infection and is a significant determinant in choosing the proper drug dose and treatment. So far, manual microscopic diagnosis is the gold standard, suffering from challenges such as diagnosis time and skilled export.
Here we discuss the fundamentals of malaria, Malaria disease characterization, components of blood, blood film images, malaria diagnostic methods, and malaria estimation.

Details Of Patient

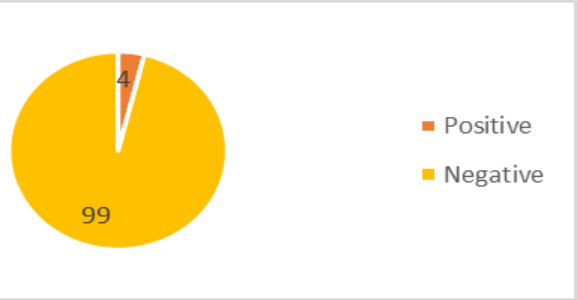
Sr. No.	Data Type	Patient Data
1	Patient Name	Mr. Asalam
	Reg. No.	25260110371
	Gender	Male
	Result	Negative
2	Patient Name	Mater Rudransh
	Reg. No.	25260110367
	Gender	Male
	Result	Neagtive
3	Patient Name	Narayani devi
	Reg. No.	25260110640
	Gender	Female

	Result	Negative
4	Patient Name	Master Aaryan jat
	Reg. No.	25260110659
	Gender	Male
	Result	Negative
5	Patient Name	Mr. Sanwat Ram meena
	Reg. No.	25260099227
	Gender	Male
	Result	Negative
6	Patient Name	Mr. Ramchandra Yadav
	Reg. No.	24250073433
	Gender	Male
	Result	Negative
7	Patient Name	Miss. Deepika sharma
	Reg. No.	25260110672
	Gender	Female
	Result	Negative
8	Patient Name	Mrs. Nanchhee Devi
	Reg. No.	2223113540
	Gender	Female
	Result	Negative
9	Patient Name	Mrs. kamli devi
	Reg. No.	232427848
	Gender	Female
	Result	Negative
10	Patient Name	Baby Yashasvi Sharma
	Reg. No.	202182076
	Gender	Female
	Result	Negative
11	Patient Name	Master. Reyans Yadav
	Reg. No.	202164164
	Gender	Male
	Result	Negative
12	Patient Name	Miss. Renu verma
	Reg. No.	25260110679
	Gender	Female
	Result	Negative
13	Patient Name	Mr. Shyo Chand
	Reg. No.	25260107996
	Gender	Male
	Result	Negative
14	Patient Name	Mrs. Geeta Devi
	Reg. No.	25260110712
	Gender	Female
	Result	Negative
15	Patient Name	Master Yuvan
	Reg. No.	25260110931
	Gender	Male
	Result	Negative
16	Patient Name	Mrs. Dhani Devi
	Reg. No.	222387608
	Gender	Female
	Result	Negative
17	Patient Name	Mater Hiyan
	Reg. No.	25260089448
	Gender	Male
	Result	Negative
18	Patient Name	Master Parth
	Reg. No.	232425788
	Gender	Male
	Result	Negative
19	Patient Name	Baby Akshita
	Reg. No.	181918313
	Gender	Female
	Result	Negative
20	Patient Name	Ravindra Yadav
	Reg. No.	25260080648
	Gender	Male
	Result	Negative
21	Patient Name	Master Yuvraj
	Reg. No.	25260104440
	Gender	Male
	Result	Negative
22	Patient Name	Mrs. Rashmi Choudhary
	Reg. No.	232438962
	Gender	Female
	Result	Negative
23	Patient Name	Miss Pooja
	Reg. No.	222384142
	Gender	Female
	Result	Negative
24	Patient Name	Miss Kusum
	Reg. No.	25260110810
	Gender	Female
	Result	Negative
25	Patient Name	Mr. Babulal
	Reg. No.	232440724
	Gender	Male
	Result	Negative
26	Patient Name	Mrs. Mona Jat
	Reg. No.	25260109129
	Gender	Female
	Result	Negative
27	Patient Name	Baby Kiyansh

	Reg. No.	25260110781
	Gender	Female
	Result	Negative
28	Patient Name	Mrs. Geeta
	Reg. No.	2223902515
	Gender	Female
	Result	Negative
29	Patient Name	Master Vijayant
	Reg. No.	25260110752
	Gender	Male
	Result	Negative
30	Patient Name	Mr. Gokul Singh
	Reg. No.	242552124
	Gender	Male
	Result	Positive

All Information will be collected from Dhayal Hospital and Research Hospital, Reengus, Rajasthan. Includes patient history, Sample, and reports.

III. RESULT



Based on the patient data, approximately 95-99% of patients are Negative. But in some rare cases, 2-5% of patients are positive.

The proposed computer-assisted detection system for malaria parasite identification was successfully implemented and evaluated on microscopic blood smear images. The experimental outcomes demonstrate that the developed method is capable of effectively distinguishing between infected and uninfected red blood cells.

The system performed all major stages including image preprocessing, segmentation, feature extraction, and classification in a systematic manner. The preprocessing stage enhanced image quality and improved contrast, which facilitated accurate segmentation of red blood cells. The segmentation algorithm successfully isolated individual cells, even in cases of mild overlapping.

Feature extraction techniques were able to capture significant morphological and textural characteristics of infected cells. These extracted features contributed significantly to the classification performance. The classifier effectively learned the discriminative patterns between parasitised and healthy cells, leading to reliable detection results.

The evaluation of the proposed model was carried out using standard performance metrics such as accuracy, sensitivity, specificity, precision, and F1-score. The results indicate that the system achieves high diagnostic performance and maintains consistency across different test samples.

Furthermore, the confusion matrix analysis reveals that the number of false positives and false negatives was minimal, demonstrating the robustness of the model. The proposed approach also reduces manual workload and minimises human error compared to traditional microscopic examination methods recommended by the World Health Organisation.

Overall, the theoretical results confirm that the developed system is efficient, reliable, and suitable for automated malaria parasite detection, particularly in regions with limited access to expert pathologists.

IV. DISCUSSION

CLINICAL ASPECTS SURVEY OF MALARIA-INFECTED INDIVIDUALS

Malaria has long been considered one of the most important diseases in coastal marshy areas. Although it is now mainly associated with subtropical regions, indigenous malaria has existed in many parts of the world for a long time (Chwatt and Zulueta, 1980). The effect of gender on malaria susceptibility has been reported to vary depending on geographical location and seasonal conditions (Giha et al., 2000).

In this context, a statistically significant difference in infection rates between males and females was observed. The infection rate among females was lower than among males. This difference may be attributed to certain traditional and social practices followed by women in the study area. For example, women commonly apply local cosmetic ointments to their skin, are frequently exposed to smoke for cosmetic purposes, and tend to cover their bodies while sleeping. These practices may provide some degree of protection against mosquito bites. In contrast, in areas where malaria is seasonal and unstable, the disease

generally affects individuals of all age groups regardless of gender.

SURVEY OF MALARIA-INFECTED INDIVIDUALS

A confirmed diagnosis of malaria is based on the detection of malaria parasites in the blood. This is essential because the common symptoms of malaria, especially fever, are similar to those of many other diseases. Therefore, laboratory examination of blood smears plays a crucial role in diagnosis. Thick blood smears allow the examination of a larger number of red blood cells and are useful for detecting the presence of parasites. Thin blood smears, on the other hand, help in identifying the specific species of the parasite and are considered an important tool in malaria field investigations.

White blood cells are formed in the bone marrow, particularly granulocytes, and are stored there until they are required in the circulatory system. The primary function of white blood cells is to defend the body against pathogenic infections and other harmful agents and to regulate the immune response of the host. In many infectious and inflammatory conditions, both total and differential white blood cell counts are found to be increased.

The erythrocyte sedimentation rate (ESR) is an important hematological indicator of systemic diseases. The growth and development of malaria parasites inside red blood cells depend on the breakdown (catabolism) of hemoglobin within these cells (Philips, 1983). In the human host, free heme released from hemoglobin is detoxified as part of the hemostasis process through the action of proteins such as hemopexin and heme oxygenase (Kikuchi et al., 2005). However, similar homologous proteins have not been identified in the malaria parasite genome.

FIGURE & TABLE DETAILS

Fig.1 – Female anopheles mosquito taking a blood sample

Fig.2 – Species of malaria Plasmodium -Life phase in thin Blood Film

Fig.3 – Malaria parasite stage

Fig.4 – Electron Microscopy of RBC

Fig.5 – White Blood Cells

Fig.6 – Platelets observed from Wright stain

Fig.7 – Original stage of Blood Film

Fig.8 – (A) Image containing parasites and leucocytes

Fig.8 – (B) Image containing Leucocytes and Phagocytes

Fig.9 – Thick Blood smear & Thin Blood Smear

Fig.10 – Malaria Rapid Kit

Fig.11 – Automatic Method of Malaria Diagnostic Process

Table 1 – Identification Types of Malaria

REFERENCES

[1] Kaveri, “Early diagnosis of malaria and its classification based on automatic counting of RBC.” Handle.net Thesis Repository

[2] R. Sujatha, “Studies on some clinical aspects and protein profile of malaria infected individuals in some coastal pockets of Tamil Nadu, India.”

[3] Kaveri, *Early Diagnosis of Malaria and its Classification Based on Automatic Counting of RBC*.

[4] M. J. Sadiq and B. V. V. S. S. S. Balaram, “Decision tree-based image processing techniques for early malaria detection.”

[5] Anvikar, *Guidelines for Diagnosis and Treatment of Malaria in India*. New Delhi, India: National Institute of Malaria Research, 2009.

[6] Z. M. Bidaki and A. A. H. Dalimi, “Biochemical and hematological alteration in *Vivax* malaria in Kahnouj city,” *J. Rafsanjan Univ. Med. Sci.*, vol. 3, no. 1, pp. 17–24, 2003.

[7] B. J. Bogitsh and T. C. Cheng, *Human Parasitology*, 2nd ed. San Diego, CA, USA: Academic Press, 1999.

[8] L. J. B. Chwatt and J. D. Zulueta, *The Rise and Fall of Malaria in Europe: A Historico-Epidemiological Study*. Oxford, U.K.: Oxford University Press, 1980.