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Malaria Tests-DNA PCR and Quantitative Buffy Coat (QBC)

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Introduction

Malaria, considered a tropical disease, poses a significant threat to public health especially in Sub-Saharan Africa [1]. It is a blood borne disease caused by a protozoan parasite called *plasmodium* [1,2]. Being blood borne, the malaria parasite (*Plasmodium*) can be found in the blood of infected persons with possible transmission to others either through blood transfusion or vector bite [2]. The malaria vector is the female anopheles' mosquito which bites an infected person, picks the *plasmodium* and transmits it on bite of another individual. Many measures are adopted all over the world to control malaria. Notable among others include:

- Treatment of infected cases with drugs such as any of: Chloroquine phosphate (Most preferred), primaquine phosphate, atovaquone-proguanil (Malarone), Quinine sulfate (Qualaquine) with deoxycycline or the use of Artemisinin-based combination therapies (ACTs) (e.g., combination of artemether-lumefantrine (coartem) and artesunate-mefloquineuse [4]
- Use of some plants and plant parts as remedies [1,3].
- Use of mosquito nets and destruction of vectors with insecticides [1,4].

The right blood test for malaria is necessary for the following reasons:

I. Detection of the malaria parasite in the blood and possibly the type,
II. Whether it is resistant to drugs already or not.
III. Prevention of complication risks and death.
IV. Administration of the right treatment [4,5].

These and many more form the basis for discussing the DNA PCR and Quantitative Buffy Coat (QBC) tests for malaria parasites in this paper.

The Malaria Parasite (*Plasmodium*)

The malaria parasite called plasmodium is of different species namely:

I. *Plasmodium vivax.*
II. *Plasmodium malariae*
III. *Plasmodium falciparum*
IV. *Plasmodium ovale*
V. *Plasmodium knowlesi* [1]

Plasmodium falciparum has been reported the most prevalent specie in Sub-Saharan Africa as well as major cause of malarial deaths globally [6]. The development of malaria takes a series of stages from introduction of infective stage called sporozoites into humans through female anopheles mosquito bites in a blood meal to the disease stage called merozoites. These merozoites develop into gametocytes which can be taken by bite of mosquito where development continues to form sporozoites. The development of sporozoites in man to gametocytes and continuation in the mosquitoes' guts to infective sporozoites is called the malarial life cycle. These developmental stages occur in humans and the female anopheles' mosquito respectively. Therefore, humans and the female anopheles' mosquito in this case are term hosts. The cycle is also classified into two stages according to host involved. These are:

I. Sporagonic cycle in mosquitoes where sexual reproduction occurs.
II. Erythrocytic cycle in humans for asexual reproduction.

The sporagonic cycle begins when the female anopheles mosquito ingests the mature stage called gametocytes from infected humans during a blood meal. The gametocytes which are the sexual stage consists of both male and female gametes which mate in the gut of the mosquito developing what is called an oocysts then into sporozoites.

The malaria cycle continues in humans when the female anopheles' mosquito with mature sporozoites feeds on human blood. This is the unset of the Erythrocytic cycle. The sporozoites are introduced into the blood where they enter the red blood cells (erythrocytes1). They are transported to the liver via blood circulation then enter liver cells called hepatocytes. In the liver, asexual reproduction takes place producing schizonts. These develop into merozoits which are released into the blood on rupture of hepatocytes. This is the stage revealing clinical signs and symptoms of malaria such as vomiting and chills. It is worthy of note that the release of merozoites into the blood does not empty the liver merozoites rather leaves behind what is called reserved merozoites. Merozoites develop into gametocytes the formation of reserved merozoites in humans poses a significant threat to the control of malaria worldwide. This is due to the fact that reserved merozoites do not present symptoms of malaria but subsequent exposure of the host to stress of any kind including hunger can trigger further release of reserved merozoite into the blood to cause malaria without mosquito bite [7,8].

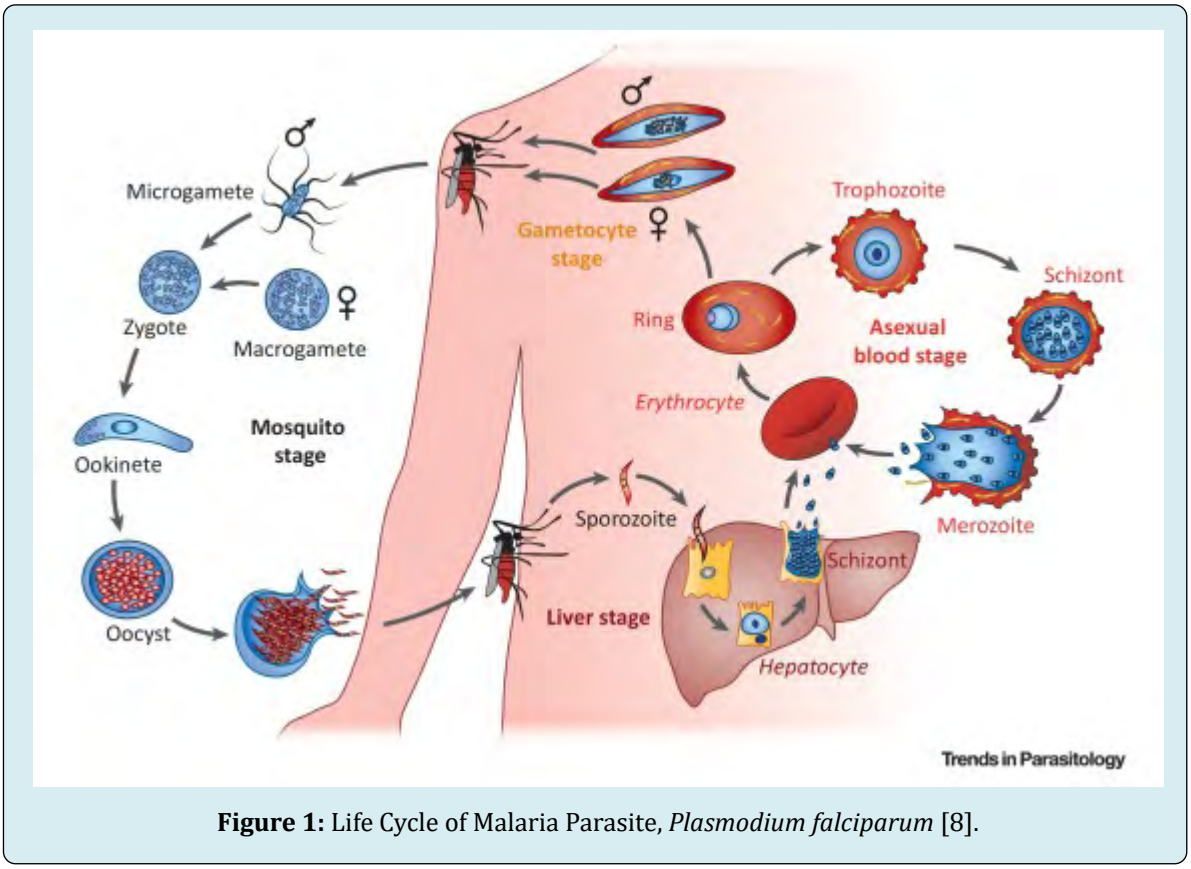

Figure 1: Life Cycle of Malaria Parasite, *Plasmodium falciparum* [8].

Why DNA PCR and Quantitative Buffy Coat (QBC) Tests for Malaria?

The right blood test for malaria diagnosis is necessary for effective treatment against the disease. While the Giemsa stain and Rapid Diagnostic Test (RDT) are useful in identifying the presence of malaria parasites{primary malaria screening) among symptomatic and asymptomatic persons, DNA PCR is done for symptomatic patients that have been screened positive to identify the particular responsible *plasmodium* specie DNA as well as any resistant gene[4,8]. This has become necessary over the thick and thin smear microscopic examination due to the tendency of the parasite morphology to alter as a result of prior exposure to antimalarial drugs, effects of Ethelene diamine tetraacetate(EDTA) contained in blood sample bottles, as well as other related factors as these will make species identification difficult [8,9]. This test is not useful for primary screening for asymptomatic patients. Furthermore, DNA PCR is more sensitive than microscopy and gives a reliable result in the event of few parasites in the blood (low parasitemia) [1,4,8,10].

On the other hand, the Quantitative Buffy Coat (QBC) test is done to detect malaria parasites based on micro-centrifugation, fluorescence and density gradient of infected erythrocytes [11]. It is necessary in the case of unclear or uncertain negative results from smear microscopy but not a replacement, hence enables early detection of malaria

parasites [12.]. It is good but has a limitation of: time consumption, labor intensive and relatively high cost [11,12].

How Malaria DNA PCR and Quantitative Buffy Coat (QBC) Tests for Malaria Parasites are Done

Deoxyribonucleic Acid (DNA) Polymerase Chain Reaction (PCR) also known as (DNA PCR) involves the use of PCR machine to detect and differentiate *plasmodium* species. The process begins by blood sample collection (about 4Ml) into EDTA bottles. This is followed by extraction of DNA from the EDTA anti- coagulated whole blood and testing using PCR on the light cycler 2.0 instrument with specific primers and fluorescence resonance energy transfer (FRET) probes [4,9]. A specific primer set corresponding to 18S RNA can be used to magnify target sequence. One pair of FRET hybridization probe can be design for a particular specie over a region with base-pair mismatch. This allows differentiation of *plasmodium* species by melting curve analysis with a second probe specific to another specie. This test produces reliable results for specie identification and differentiation but does not differentiate between residual nucleic acid which likely persist after several treatments [4,13].

The Quantitative Buffy Coat (QBC) test consists of a capillary tube coated inside with acridine orange stain (microhematocrit tube). After collecting blood into the tube, it is centrifuged thereby allowing the components of the blood and parasites to separate by a density gradient. The malaria parasites become concentrated in different cell layers. These make detection of the cells easier and faster. A plastic float is form expanding and diminishing the buffy coat as well as the upper red blood cell to few cells layer thick. This increases sensitivity. The Acridine Orange binds to DNA and RNA. Any Malaria parasite present binds to the Acridine Orange at the nucleus and Cytoplasm and fluoresces with green and red colors which, when excited to a frequency of 460-nm allow detection of plasmodium morphology through fluorescent microscopy (observation under blue-violet light through a microscope (Figure 2) [12,14].

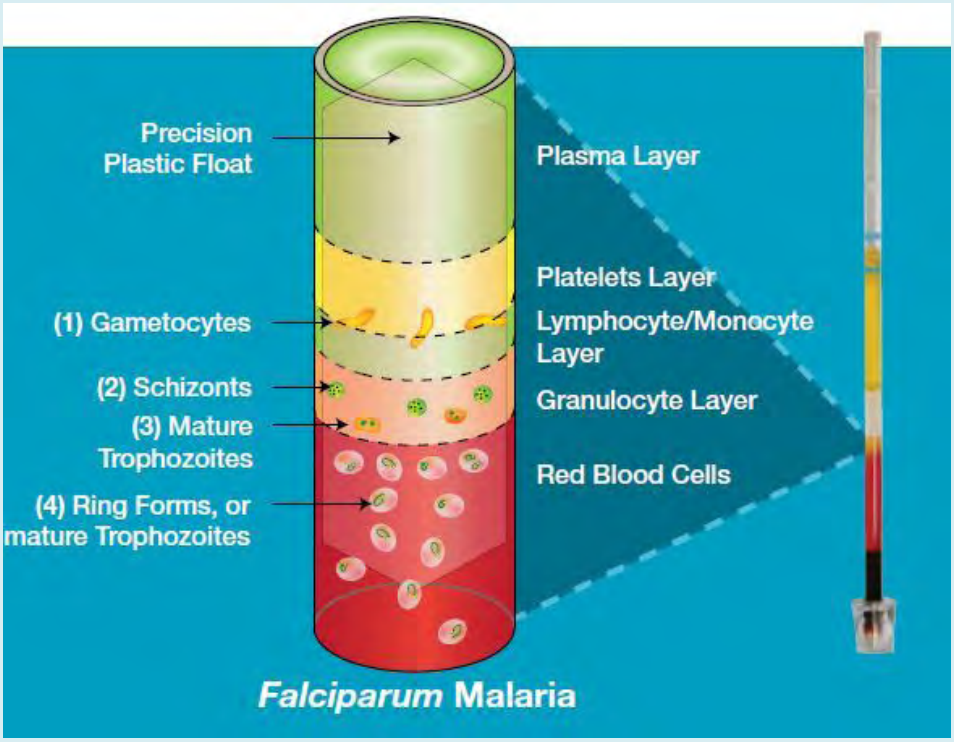

Figure 2: Quantitative Buffy Coat (QBC) Test for *Plasmodium falciparum* [15].

The Malaria QBC test procedure can be summarized as follows:

- Blood sample collection can be done in either a capillary tube finger-prick or a phlebotomy in EDTA containing vials.
- Take about 4-5Mls of blood into QBC tube by capillary action, then rotate the tube some seconds to ensure dissolution of certain residues contained in the blood.
- A plastic float with a specific gravity is inserted midway between that of plasma and red blood cells inside an acridine orange-coated capillary tube.
- Centrifuge the tube for about 5 minutes.

- The blood components and malaria parasites separate and concentrate in different layers immediately after centrifugation. These separations are based on density gradients.
- The float settles on top of the centrifuged packed red cells due to its density. Its large size aids in the expansion of the separated cell layers.
- The centrifuged QBC Malaria test is inserted into a Paraviewer with the tube positioned such that closure end extends over the depressed area held.
- The area around the float beneath the buffy coat can be examined under oil immersion.
- The respective cells within the area can be examined by fluorescent microscopy after excitement at 460nm to detect parasites .As in the sample Figure 2 above, the malaria parasites staining green (DNA) while RNA Orange under blue-violet light Figure 2 [9,14,15].

Possible Outcomes of Malaria DNA PCR and Quantitative Buffy Coat (QBC) Tests

The DNA PCR test for malaria reveals species DNA and differentiate them from others even in the event of scanty parasites in the blood sample (low parasitemia). It can also show pre-formed resistant genes and serves as a confirmatory test for specie identification [9]. Quantitative Buffy Coat (QBC) reveals species differentiation, hence other species concentrates during centrifugation but with different characteristics [15].

Malaria DNA PCR and Quantitative Buffy Coat (QBC) Tests Specificity and Sensitivity.

Malaria DNA PCR lower limit for detection can be as low as 10 to 52 DNA target copies per microliter of whole blood. This suggests a high level of sensitivity. The test is target specific for DNA of the parasites. It confirms the identification of exact specie even in the presence of mixed infections [10,13]. Although the Quantitative Buffy Coat(QBC) tests is specie specific and sensitive, the sensitivity is contributed by the thinning of the float in the test procedure and may not be as high as in DNA PCR malaria test [12-16].

Conclusion and Call to Order

Malaria has cause several deaths worldwide. The right and effective diagnosis enables correct and right treatments. Antigenic variation is the ability of the parasite to alter its antigenicity (recognition as foreign in the body) to avoid elimination by immunization or antibodies in the body. The formation of reserved merozoites in the liver as well as antigenic variation exhibited by the *plasmodium* parasites have been major threats against successful control of malaria worldwide. No stable vaccine has been established globally due to the phenomenon of antigenic variation. Vaccine initially useful becomes ineffective once the *plasmodium* parasite alters its antigenic structure.

Therefore, the right tests suggest the right treatments, see a Medical Doctor when sick and avoid self-medication to prevent drug abuse and misuse which leads to drug resistance. Keep your environments clean, construct good drainages to prevent stagnant water that attracts mosquitos around the home. Use insecticides where necessary to destroy the vector (mosquitoes) and get mosquito nets for yourself and family.

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