1.1 Introduction

Malaria is one of the leading cases of more morbidity in sub-Saharan Africa. There are approximately 1.000.000 deaths worldwide per year, the majority in young children. Many of these death are preventable, the challenges to be met in reducing this burden are those of prevention, improving access and encouraging early presentation to health care services, and increasing the availability and affordability of effective anmti-malarial therapies. In Sudan and regionally in east Africa,there is great concern regarding the adequacy of current anti-malarial protocol. It is widely accepted that level of chloroquine resistant in Sudan demand an alternative to this therapy.

Recent review evidence of anti-malarial resistance in Sudan produced from national conference in Khartoum in October 2003 also presented as yet unpublished data suggested resistance to sulphodoxine –pyrimethamine uncomplicated malaria compinecholorquine with SP. The federal ministry (arteminsion based combination therapy) as first line treatment for uncomplicated malaria (proposed regime: artesunate plus fansider), together with the introduction of this new and highly efficient treatment regime.

A proper diagnosis of malaria must be ensured for each individual patient before receiving (ACT) treatment.

The problem of malaria is compounded by increasing epidemics in Sudan as well asincreasing drugs and insecticides. The challenge for Sudan is to control this disease within a comprehensive and integrated health sector development program

1.2 Classification of malaria parasite:

The malaria parasite belong to the genus *Plasmodium*. The important species of human malaria are *Plasmodium falciparum*(larverania) which causes malignant tertian malaria, *Plasmodium vivax and Plasmodium ovale*causes benign tertian

malaria, and *Plasmodium malariae*which causes benign quartan(Zaman and Keong 1982).

1.3 Geographical distribution:

P.falciparum is widely prevalent in many tropical area of the world and transmission is rare or unlikely in temperate regions. *P.malaria* and *P.ovale*are much less common than *Plasmodium vivax*and *Plasmodium falciparum. P.ovale*is seen mainly in Africa although few cases have been reported from Asia (Zaman and Keong 1982).

1.4 Transmission of malaria parasite:

Malaria infection can occasionally be transferred directly from one person to another by blood transfusion, accidental inoculation or cross placenta. However, transmission usually depends on an insect vector, in which the parasite spends several weeks undergoing the sexual part of its life cycle (Gill and Beaching 2004).

1.5 Life cycle of malaria parasite:

Malaria parasite is transmitted by the bite of an infected female anopheline mosquito,the infective stage is the sporozoite, a microscopic spindle shape cell which is in mosquito saliva.

Thousands of sporozoite may be injected in a single bite. The sporozoite disappear from the blood within 30 minutes, and the successful ones enter the liver cell. The process by which the malaria parasite multiply asexually is called schizogony, whether it take place in hepatocyte or in an erythrocyte. Inside the liver cell, the sporozoite divides by asexual fission to form a cyst like structure called a pre-erythrocytic (PE) schizont, which contains thousands of merozoites. Each merozoite consists of a small mass of nuclear chromatin within a tiny sphere of cytoplasm. When the PE mature,it ruptures and liberatesmerozite. These, now enter the blood stream to penetrate red cell.

The time between the bite of the infected mosquito and the appearance of merozoite ranges between 7 and 30 day in *P.falciparum*(usually around 10 days), and longer in the other species. It may be very long in the cases of *P.vivax*and *P.ovale*,(many months or even more than a year) (Gill and Beeching 2004).

This dormant stage of parasite is the hypnotize. Merozoites released into the blood stream from hepatic PEschizont, attach themselves to red cell by means of surface receptors.

The parasite then penetrates the red cell and resides in avacuole which is derived from the red cell surface. Here, it begins the process off erythrocyte schizongy. Schizongy occurs in the circulating blood in the cases of *P.vivax*, *P.ovale* and *P. malariae*, so in all these infections, schizonts are commonly seen in the peripheral blood. In*P. falciparum*, schizongy takes place in capillaries of internal organs, so, only trophozoite are detected in peripheral blood.

Parasite antigens are capable of linking to receptors expressed on the endothelial cells lining of the capillaries in various tissues and organs of the body.

The resulting cytoadherence of parasitized erythrocytes to endothelial surface leads to the gathering or sequestration of large numbers of mature parasite in deep tissues. The periodicity of schizongy characteristically coincides with the paroxysms of fever and this leads to the traditional names of different types of human malaria.

Some of the merozoites entering red cell do not develop into schizont, but develop more slowly into solid looking parasite called gametocyte. These may persist in the circulation for many weeks without destroying the red cell containing them, and these are the form infective to the mosquito. In each species of malaria, the gametocyte differentiate in to male and female. When the female mosquito swallows the gametocyte in her blood meal, they develop further in her stomach, the gametocyte rapidly develop to produce

spermatozoan –like microgamete, and the female gametocyte become the egg like macrogamete (Gill and Beeching, 2004).

1.6 Clinical feature and pathology:

Malaria is a curable disease if diagnosed and treated correctly. it result in a wide variety of symptoms ranging from not apparent to very mild symptoms to severe disease and even death according to parasite species and level of parasitemia. Malaria disease can be categorized as uncomplicated or sever.

1.7 Uncomplicated malaria:

The classical, but rarely, observed symptoms consist of:

Acold stage(sensation of cold and shivering)

Hot stage(fever,headache, back and joint pain)

Sweat stage (sweat,fail oftemperature, tiredness)

More commonly, the patient presents with combination of following symptom: fever, chills, sweats, headache, nausea and vomiting, body aches and general malaise.

1.8 Sever malaria:

Occurs when *P.falciparum* infection is complicated with serious organ failureorabnormalitiesinpatient's blood or metabolism .The manifestations include: cerebral malaria,severe anemia, black water fever,diarrhea and vomiting, pulmonary oedema and renal failure.

1.9 Pathogenesis:

The symptoms of malaria illness vary, but majority of the patients have fever. The periodicity of schizogony characteristically coincides with paroxysms of fever and this leads to the traditional names of the different types of human malaria. Tertian malaria fever every third day, (*P. vivax and P. ovale*). Sub tertian malaria fever slightly more often than every third day (*P. falciparum*). Quartan malaria, fever every fourth day (*P. malariae*) *P. falciparum* malaria was sometimes called malignant tertian malaria, because of its much greater lethal potential than other types of tertian malaria. Other common symptoms include headache, back pain, chills, increased sweating,

myalgia, nausea, vomiting and diarrhea. The complication of the disease can rapidly progress especially in *P. falciparum* if untreated (Miller *et al,* 2002).

Many patients have lost their lives from *P. falciparum* malaria because they never develop the periodic fever that their doctors wrongly believed to be invariable.

Clinical illness is caused by erythrocyte stage of the parasite. No disease is associated with sporozoite and the developing liver stage of the parasite (Miller *etal,* 2002).

The first symptoms and signs of malaria are associated with the rupture of erythrocytes when erythrocytic stage schizont ruptures. This release of parasites materials presumably triggers a host immune reponse. The cytokines, reactive oxygen intermediate, and other cellular products released during the immune response play a permanent role on pathogenesis, and are probably responsible for fever, chills, sweats, weakness, and other symptoms. In *P. falciparum* malaria, infected erythrocyte adheres to the endothelium of the capillaries and post capillaries venules, leading to obstruction of the microcirculation and local tissue anoxia. The severity of malaria associated anemia tends to be related to the degree of parasitemia, the pathogenesis of this anemia appears to be multifactorial, haemolysis or pahgocytosis of parasitized erythrocytes and infected erythrocytes are the most important factors, and phagcytosis of uninfected erythrocyteand auto-immunhaemolyticanemia has also been implicated, massive intravascular haemolysis leading to haemoglobinuria and renal failure is referred to as black water fever, infection with *P. falciparum* can build up to a level not obtained with the other three species and because of the physiology characteristics of red blood cells infected with *P. falciparum*, may lead to localized capillary obstruction, decrease blood flow, tissue hypoxia, infarction and death, chronic *P. falciparum* infection in children may result in immune complex deposition on glomerular cell, leading to nephrotic syndrome (Markell*et al,* 1999).

1.9.1 Anemia:

Anemia in *P. falciparum* malaria is mainly due to the mechanical destruction of parasitized red cells, Parasitized red cells also lose their normal shape and are destroyed in the spleen. The production of red cells in the bone marrow is also reduced and there is a slow reticulocyte response (Markell*et al*, 1999).

1.10 Immunity against malaria:

Susceptility to malaria infection and disease is regulated by innate and acquired factors. Innate factors include sickle cell trait (which is the cause of sickle cell anemia) developed as balanced polymorphism to protect against serious *P. falciparum* infection, although individuals with sickle cell anemia or sickle cell trait are easily infected with malaria parasites as normal individual, they rarely exhibit malaria disease because *P. falciparum* develops poorly in their erythrocytes. The virtual absence of *P.vivax* in many infected areas in Africa is explained by the fact that most black people do not have duffy blood group antigens, which apparently function as erythrocyte surface receptors for *P. vivax*merozoite. Malaria parasites do no develop well in ovalocytes, and is has been suggested that ovalocytosis, which is quite common in some malarious areas such as New Guinea, may reduce the incidence of malaria (Good *et al*, 1993). Some investigators have suggested that glucose-6- phosphate dehydrogenase deficiency as well as a number of other haemogolbinopathies (including the thalassaemia and hemoglobin F) also protect against malaria infection, but the evidence for these associations is less compelling.

Acquired immunity can also protect against malaria infection and the development of the malaria disease. In malarious areas, both the prevalence and the severity of malaria infection decrease with age. However, in contrast to many viral infections, multiple infections with malaria do not confer long lasting, sterile protective immunity.

Virtually, all adults in malarious areas suffer repeated malaria infection. Individuals who are repeatedly exposed to malaria develop antibodies against many sporozoite, liver-stage, blood stage and sexual stage malaria antigens.

It is thought that antibodies acting against sporozoite, liver-stage and blood stage organism are responsible for the decreased susceptibility to malaria infection and disease seen in adults in malaria, and the antibodies against the sexual stage of plasmodia may reduce malaria transmission. Additional work also suggests that naturally acquired immunity includes the release of cytokines that act against all stages of the parasites, and also cytotoxic T-cell response directly at liver stage of the parasites (Hoffman, 1992).

Acquired antibody mediated immunity is apparently transferred from mother to fetus across the placenta. This passively transferred immunity is also within 6 to 9 months. Pregnant women particularly primigravidas, are more susceptible to malaria infection (Chin, 2001).

1.11 Diagnosis of malaria:

1.11.1 Direct diagnosis:

The specific diagnosis of malaria is made by examining the blood by making a film, drying and staining it. The thin film shows the undistorted parasite within red cell. It is of most use in detailed study of parasite morphology and species identification.

Its disadvantage, is that, it requires a prolonged search to detect a low parasitaemia, so its sensitivity is low. A patient may have fever resulting from *P.falciparum* and yet no parasite is detected by searching the thin film.

The thick film, in which the cells are lysed and stained, allows more red cells to be examined at a time, but, it has the disadvantage that the parasites in the lysed red cells are distorted and the specific features of identification are lost. However, in experienced hand, the thick film is the best method to use for answering the question, does the patient have malaria? (Gill and Beaching 2004).

1.11.2 Serodiagnosis:

Serodiagnosis of malaria depends on finding specific antibodies, and most methods in common use are incapable of distinguishing between antibodies to different species of parasites.

Antibodies may be detectable for several years attack of malaria. The main use of serodiagnosis is in excluding malaria in patients suffering from recurrent bouts of fever.

Serology may also be used in surveys as an approximate measure of exposure of population to malaria. The most used serological technique is indirect fluorescent antibody test (IFAT) (Gill and Beaching, 2004).

1.11.3 Other methods of diagnosis:

Many techniques for identifying malaria parasites are being developed. The quantitative buffy coat (QBC) technique makes use of the fact that parasitized erythrocytes have different gravity from unparasitized red cells and can therefore be looked in a particular segment of blood (Gill and Beaching, 2004).

An alternative test has been developed particularly for *P. falciparum* infection, for example, the immune-chromatographic test (ICT) for detection of circulating histidine-rich protein 2 (HRP2), a specific *P. falciparum* antigen. This test has been shown to be specific, quick and easy to conduct, but the issue of cost remains a concern in developing countries (Torrens et al, 1999). The sensitivity of ICT for detection of *P. falciparum* (HRP2) in whole blood is almost 100%. It is quick, easy, to perform, but could be used only for detection. The disadvantage of ICT is that it cannot determine the stage of parasite and density of infection. In addition, the antigen (HRP2) can persist in blood for several weeks after treatment.

The antigen detection by parasight TM-F dip stick method for the diagnosis of *P. falciparum*, appears highly acceptable. Level of sensitivity and specificity almost equal to those of microscopy (the standard method of malaria diagnosis). The method is based on the detection of soluble *P. falciparum* antigen histidine rich protein 2 (HRP2) in the circulation. As reported by many workers, the test has several advantages over microscopy in endemic areas, which may have implications on disease control. This includes ease and speed of performance and detection of persistent antigenaemia after drug cure speed of an infection (Kodisinghe*et al*, 1997).

The need for better malaria diagnosis is clearly evident, one rapid malaria test, optimal, has recently been introduced. The optimal test is a rapid 15- minute's test that detects and differentiates *falaciparum* from non *falaciparum* malaria. It is based on the detection of parasite lactate dehydrogenase (PLDH) enzyme. On field trials of optimal, the workers have concluded that it is an excellent diagnosis tool and the ability to monitor the results of patient anti-malarial treatment. The value of diagnostic test for patient diagnosis of malaria and follow up testing is clearly evident (Palamer*et al*, 1999).

Molecular biological techniques have been widely introduced in various fields of malaria research. The techniques are based on analyzing several different regions of malaria genome (Arez*et al*, 1999).

Polymerase chain reaction (PCR) is a powerful technology with high sensitivity and specificity. During the past few years, it has become a major diagnostic technique. It is valuable for detection of parasites present at low concentration in blood or serum samples (Beck et al, 1997). It is an advanced, very sensitive and specific technique, but it needs experience and well trained personnel in addition to a well-equipped laboratory, so its use is limited at the present time.

1.12 Misdiagnosis in malaria:

Thus in view of the above mentioned information, diagnosis of malaria is still based mainly on the conventional methods i.e. microscopic diagnosis, but the question about the reliability of microscopy of malaria has been repeatedly raised by clinicians, and this is due to controversial results obtained so often from different laboratories.

These false results are supposed to arise due to many factors, including usage of bad microscopes, low quality staining solutions, unsuitable immersion oils, spending insufficient time examining blood films by untrained laboratorians. Furthermore, extension of the medical laboratory services to rural areas for increasing population coverage has been achieved in some parts of Sudan, but it is unplanned and without provision of the needed requirements for that (Rafa, 2001).

Thus, for reliable results, a quality assurance is needed in order to improve the laboratory performance. With the spread of drug resistance, it is becoming increasingly important to confirm microscopically a diagnosis of malaria (Cheesbrough, 1987).

The goal of global malaria control strategy is to prevent mortality and reduce morbidity and social and economic losses, through the progressive improve and strengthening of local and national malaria capabilities. The four basic technical elements of the global strategy are:

1- To provide early diagnosis and prompt treatment.
2- To detect and prevent epidemics.
3- To plan and implement selective and sustainable preventive measures including vector control.
4- To strengthen local capacities in basic and applied research to permit and prompt the regular assessment of a country malaria situation in

particularthe ecological, social and economic determinants of the disease (WHO, 2002).

Sudan had contributed and endorsed the global strategy for malaria control, which constitutes part of its national plan for the control of malaria. Thus ensuring early diagnosis and prompt treatment, implementation of selective preventive measures early detection activities through improvement of the information system and implementation of operational research results are considered as the main aims of the national plan for malaria control. Is has been observed that unless quickly diagnosed and treated promptly, the clinical picture of malaria deteriorates and will result in grave outcome.

Hence, the process of early diagnosis and prompt treatment is the corner stone in the global strategy of malaria control (National Malaria Administration, 1998). Thus, the implementation of this strategy could not be possible unless there are highly qualified medical laboratories from which a reliable and convincing results are obtained.

Quality assurance is the overall term used to describe the steps and procedures need to be taken in order to ensue reliability of the results because standardization and adequate quality control of procedures will ensure that medical staff receive reliable information for treating patients and health authorities receive reliable data for evaluating malaria control measures (Cheesbourgh, 1998), and with the spread of *P. falciparum* resistance to drugs and the increasing difficulty in controlling *falciparum* malaria in some areas, it is important to diagnose malaria accurately and to treat it correctly. Also, an accurate diagnosis of *vivax* and *ovale* malaria is required to ensure that the patient receives treatment for both the primary attack and against the relapsing forms of the parasites.

Thus, misdiagnosis may result in lives loss because severe untreated infections can be life-threatening, economic loss because of the use of expensive unnecessary drugs, development of drug resistance because of incorrect use of antimalarial drugs. This incorrect use of anti-malaria drugs may also lead to development of chemoprophylaxis which is undesirable in areas of stable malaria transmission because is delays the development of naturally acquired immunity (Cheesbourgh, 1998).

1.13 Global epidemiology of malaria:

The *Palsmodium*has a life cycle divided between a human host and insect vectors, 60 species of the females of the genus *Anopheles* are able to transmit malaria. The mosquitoes survive in warm, humid climate where pools of water provide perfect breeding grounds. It proliferates where awareness is low and where health care systems are inadequately developed. The global outlook for malarial infection is worsening. Currently, 40% of the world's population resides in malaria-prone areas, there are execs of one million deaths, the majority of whom are young children (WHO, 2002)

1.14 Malaria situations in Sudan:

The prevalence of malaria for every 10000 of the population was observed to be decreasing. In 1999, it was 167 and in 2004 it was 5 (National malaria administration,2004), prevalence rate of malaria in Khartoum state was shown to be .1% (Salin and Harbi, 2005). Malaria infection constituted 33.8% from the total diseases in the state for the year 1999, 31.9 of the total in patient and 19.5 of the total deaths in the state (Ministry of health, annual report, 1999). Hemiedan et al(2004-2005) conducted some investigations on Anopheles in eastern Sudan. They also aimed to investigate the morbidity pattern of malaria in the area and to

establish adequate base line data for evaluation of the effectiveness of various preventive measures, including future vaccines.

1.15 Epidemiology of malaria in Sudan:

Epidemiology of malaria in Sudan depends upon any factors including the system of irrigation, floods and seasonal laborers for cultivations and displaced people from war affected areas.

1.15.1 Northern and Nile river states:

In these states, repeated epidemics of malaria were reported in the largest irrigated schemes like EL Zeidab, ELSalaim and Borgaig.

1.151.2 Khartoum state:

Khartoumis considered as potentially high risk area because of its population density, displaced people, rains and floods and poor environmental condition.

1.15.3 Gezira, Sinnar and White Nile states:

In these states, there are the biggest irrigated schemes (Gezira, Managil and Rahad). The biggest sugar cane industries (Sennar,Kenana, Asalayia and El Geneid)and rice irrigated scheme which is located in the White Nilestate.

The average annual rain fall in these states is high, with floods from the Blue Nile and White Nile Rivers.

1.15.4 Gadarif. Kassala and Blue nile states:

It is the largest rain irrigated area in Sudan. These states are bordering Eritrea and Ethiopia and thousands laborers come to this area annually, in addition to displaced people and refugees, transmission is intensive during and after the rainy seasons.

1.15.5 The Western states:

Transmission is mainly seasonal following the rainy season.

1.16 Objective

1.16.1 General objective:

To evaluate the misdiagnosis of malaria in Dongola city laboratories.

1.16.2 Specific objectives:

1-To evaluate the results of microscopic malaria diagnosis as regards the frequencies of the false positive and false negative results.

2- To investigate the possible factors that influence the results in different laboratories in Dongola city such as:

_ General condition of the laboratories.

_ Condition of the slides.

_ Stain used and staining procedure.

_ Quality of the microscope.

_ Quality of the immersion oil.

_ Qualification and training of working staff.

3- To find out feasible solution for the problems related to misdiagnosis of malaria parasite microscopically.

Chapter two

2.1 Materials and methods

2.2 Study design:

It is a cross sectional study.

2.3 Study area:

The study was conducted in Dongolacity, in the northern state, which is located 530 KM north of Khartoum.

2.4 Study population:

The study was carried out on 11 different medical laboratories which comprised the following:

- Three governmental laboratories
-Three nongovernmental laboratories
-Five private laboratories

2.5 Sample collection:

500 blood sample were collected and blood film were made and examined by workers at the above mentioned laboratories ,follow up and confirmation of the result were made first by myself (taking blood sample and making additional thick and thin film blood film) then blood film made was sent to the administration of laboratory in Dongola for further confirmation

2.6 Data collection (Appendix):

Questioneeaire was designed to collect data to evaluate laboratory performance which included the following:

General condition of laboratory. This included cleanness dust and flies, intensity of light and supply of water.

Availability of adequate space.

General condition of slide as to they were clean, grease free, scratch free and clean labeling.

Sampling technique and quality of blood film

Time of staining and changing working solution

Type of stain used which included:

1. Method of preparation of stock stage
2. Amount of constituent used and steps followed to prepare the stain
3. Place of storage of stain
4. Time for staining and for changing work
5. Quality of used immersion oil

Quality and efficiency of microscope by examining:-

a) The mechanical stage
b) The source and intensity of light
c) The lenses
d) The condenser and iris
e) The fine and course adjustment

Qualification and training experience of working personnel.

2.7Methodology:

2.7.1 Preparation of blood film:

For each patient, anew greasefree and scratch free slide was used for preparation of blood film.

Immediately, after collection of blood film by the laboratorian, the same finger prick was squeezed gently and three drops of blood for the thick blood film and a small one for the thin blood film were obtained.

The thin blood film was spread immediately using a smooth edged spreader and the three drops of blood were gently mixed covering an area with a diameter of 1 cm.

Using black lead pencil, the slide was labeled with patient's name and number of the thin blood film.

The slide was placed to dry in horizontal position over night, then the thin blood film was fixed with methanol.

2.7.2 Preparation of Giemsa stain

2.7.2.1 Giemsa stain stock solution:

Giemsa powder 3.8 gm(Azure 11 cosin 3.0 gm +azure 11 0.8)Methanol 250 ml

Glycerol 250 ml

Giemsa powder was weighted and transferred to adry bottle of 500 ml capacity which contains about 50 glass beads.

Using a clean and dry measuring cylinder, 250 ml of methanol were added to the stain, and mixed well, then 250 ml of glycerol were added to solution, and mixed thoroughly.

The bottle stain was placed in water bath in 50-60 c*forone hour then, it was stored in dark brown bottle at room temperature at dark place. For better dissolving of stain, the bottle with stainwas shaken 4 times every day for 5 days.

The stock solution can be kept in room temperature in well stoppered bottle for year or more.

2.7.3 Preparation of buffered water:

For staining blood film for malaria, it essential to have buffered water with PH=7.2 to dilute the stock solution of Giemsastain.

The buffered solution was prepared by dissolving buffer tablets in one liter of distilled water. Each buffer tablet contains 0.7gm KH2PO2 and 1 gmNa2HPO4.

2.7.4 Staining of blood films for malaria:

The slides were placed back in a staining trough ensuring that all thick blood films were placed to one end upwards.

Three percent working solution of Giemsa stain was prepared by adding 3 ml Giemsastock solution to 97 ml of buffered water. The working solution was gently poured into the trough, until the slide was completely covered. The slide was left in the stain for 30 minutes; clean water was gently poured into

the trough to float off debris on the surface of the stain. The remaining stain was poured off gently and it was rinsed again in clean water and the water was poured off.

The slide was removed, and the back of each slide was wiped with clean gauze. The slide was placed in a slide rack (thick blood films down wards to drain and dry, making sure that the films do not touch the edge of drying rack).

2.7.5 Examination of blood films for malaria:

Stained blood films were examined using a binocular light microscope (Olympus CH20) with oil immersion lens 100x.

2.7.5.1 Examinationof the thick blood film:

When the slide was completely dried, a drop of immersion oil was applied to an area of the thick film that appeared mauve colored (usually around edge). In such area, possible to see best staining of malaria parasite (chromatin stained red and cytoplasm stained blue).

After focus of microscope, an area that was well stained and with the best thickness was examined for malaria parasite (the area selected should show clear or pale colored background and purple-coloured neutrophil nuclei and blue colouredcytoplasm). 100 microscopic fields were examined searching for malaria parasite using 100x and moving systematically from one field to another.

2.7.5.2 Examination of the thin blood films:

Thin blood films were used for identification of *Plasmodium* species. When the film was completely dry after staining, a drop of immersion oil applied the lower third of the thin films. Systematic moving from one field to another was followed so as to identify and/ or to confirm the species.

2.8 Data analysis:

Data was analyzed using SPSS program. The chi_squared test was used for difference in proportion. 0.05 % was taken as cut off limit for 95% statistical significance.

Chapter three

3.1 Results

The overall prevalence of malaria in Dongola reported by different labs, from the patients whomwere referred by physicians was found to be 26%. After follow up and re-examination, the overall prevalence rate reported was shown to be 15% (table 1, figure 1). The difference was found to be statistically significant at P =0.00.

The study showed that the general condition of the labs building was suitable in 3 of inspected labs and 8 labs were in an unsuitable condition the percentage of false positive in suitable labs was 59% and in unsuitable labs was 57% (table 2, figure 2).

The result revealed that the number of false positive slides among examined slides constituted 62% in the private labs ,while it constituted 60% and 47% in the non-government and governmental labs respectively (table 3 , figure 3) , the difference between three laboratories was found to be highly significant at P =0.00. However, the number of false negative among the examined slides constituted 88%, 85% and 95% in the governmental, non-governmental and private laboratory respectively (table 4, figure 4) . The difference was found to be statistically significant at P=0.00.

The result revealed that the collection technique was good in 9 laboratories with 42% falsepositive, while it was bad in 2 laboratories and false positive 43%(table 5,figure 5). This difference in percentage was found to be statistically insignificant at P=0.09.

The result showed that the staining technique was good in 6 laboratories and bad in 5 laboratories with false positive constitutein 27% and

66%respectively (table 6, figure 6). The difference was found to be statistically significant at P=0.00.

The result demonstrated that, when using good and efficient microscope, the false positive rate was low36% while it was high 60% when using inefficient microscope(table 7). The difference was found to be statistically significant at P=0.00.

Using good quality immersion oil gave false positive rate of 36% while using bad quality immersion oil resulted in high rate of false positives 60% (table 8). The difference was found to be statistically significant at P=0.00.

The result demonstrated that good smearing revealed 38% false positiverate, while bad smearing resulted in 46% false positive rate (table 9, figure 7). This difference was found to be statistically significant at P=0.00.

The results revealed that the false positives among the university graduates constituted 26%, 58% among the diploma holders and 46% among the mixed staff (university graduates and diploma holders) (table10, figure 8). The difference was found to be statistically significant at P=0.00.

The result revealed that the false positive among trained personnel in malaria diagnosis was 30% while the false positive increased to 51% among those who had no training (table 11). This difference was found statistically significant at P=0.00.

Table (1): The overall Prevalence rate of malaria in Dongola.

Examiner	Number Examined	Number Of Positive Slides	Prevalence
Different laboratories	500	131	26%
Investigator	500	76	15%
P.value	0.000		

Table (2): The effect of general condition of the laboratory building on the examination result.

General condition of laboratories	Number of laboratories	Number of slides	Number of positive slides	Own Result			
				TRUE		FALSE	
					%	Number	%
Suitable	3	194	44	26	59 %	18	41 %
Unsuitable	8	306	87	50	57 %	37	43 %

Table (3): The false positives among the examined slides in different types of laboratories.

Sector	Number of laboratories	Number of slides	Number of positive slides	Own result			
				TRUE		FALSE	
				Number	%	Number	%
Governmental	3	160	32	17	53 %	15	47 %
Non-governmental organization	3	140	30	12	40 %	18	60 %
Private	5	200	69	26	38 %	43	62 %
Total	11	500	131	55	42 %	76	58 %
P.value	0.000						

Table (4): The false negatives among the examined slides in different types of laboratories.

Sector	Number of laboratories	Number of slides	Number of positive slides	Own result			
				TRUE		FALSE	
				Number	%	Number	%
Governmental	3	160	128	15	12 %	113	88 %
Non-governmental organization	5	140	110	16	15 %	94	85 %
Private	8	200	131	7	5 %	124	95 %
Total	16	500	369	38	10 %	331	90 %
P.value	0.000						

Table (5): The effect of the collection technique on the positivity of the result.

Collection technique	Number of laboratories	Number of slides	Number of positive slides	Own result			
				TRUE		FALSE	
				Number	%	Number	%
Good	9	389	103	60	58 %	43	42 %
Bad	2	111	28	16	57 %	12	43 %
P.value		0.09					

Table (6): The effect of the staining technique on the positivity of the result.

Staining technique	Number of laboratories	Number of slides	Number of positive slides	Own result			
				TRUE		FALSE	
				Number	%	Number	%
Good	6	322	79	58	73 %	21	27 %
Bad	5	178	52	18	34 %	34	66 %
P.value		0.000					

Table(7):The effect of the efficiency of the microscope on the positivity of the result.

Efficiency of microscope	Number of laboratories	Number of slides	Number of positive slides	Own result			
				TRUE		FALSE	
				Number	%	Number	%
Good	7	328	96	62	64 %	34	36 %
Not good	4	172	35	14	40 %	21	60 %
P.value		0.000					

Table(8):The effect of the quality of the immersion oil on the positivity of the result.

Quality of immersion oil	Number of laboratories	Number of slides	Number of positive slides	Own result			
				TRUE		FALSE	
				Number	%	Number	%
Good	7	328	96	62	64 %	34	36 %
Not good	4	172	35	14	40 %	21	60 %
P.value		0.000					

Table(9):The effect of smear preparation on the positivity of the result.

Smearing technique	Number of laboratories	Number of slides	Number of positive slides	Own result			
				TRUE		FALSE	
				Number	%	Number	%
Good	6	317	72	44	61 %	28	39 %
Bad	5	183	59	32	54 %	27	46 %
P.value		0.000					

Table(10):The effect of staff qualification on the positivity of the result.

Staff qualification	Number of laboratories	Number of slides	Number of positive slides	Own result			
				TRUE		FALSE	
				Number	%	Number	%
University graduate	4	154	42	31	74 %	11	26 %
Diploma	1	39	24	10	42 %	14	58 %
Mixed staff	6	307	65	35	54 %	30	46 %
P.value			0.000				

Table(11):The effect of training in malaria diagnosis on the positivity of the result .

Training	Number of laboratories	Number of slides	Number of positive slides	Own result			
				TRUE		FALSE	
				Number	%	Number	%
Trained	23	326	56	39	70 %	17	30 %
Not Trained	11	174	75	37	49 %	38	51 %
P.value		0.000					

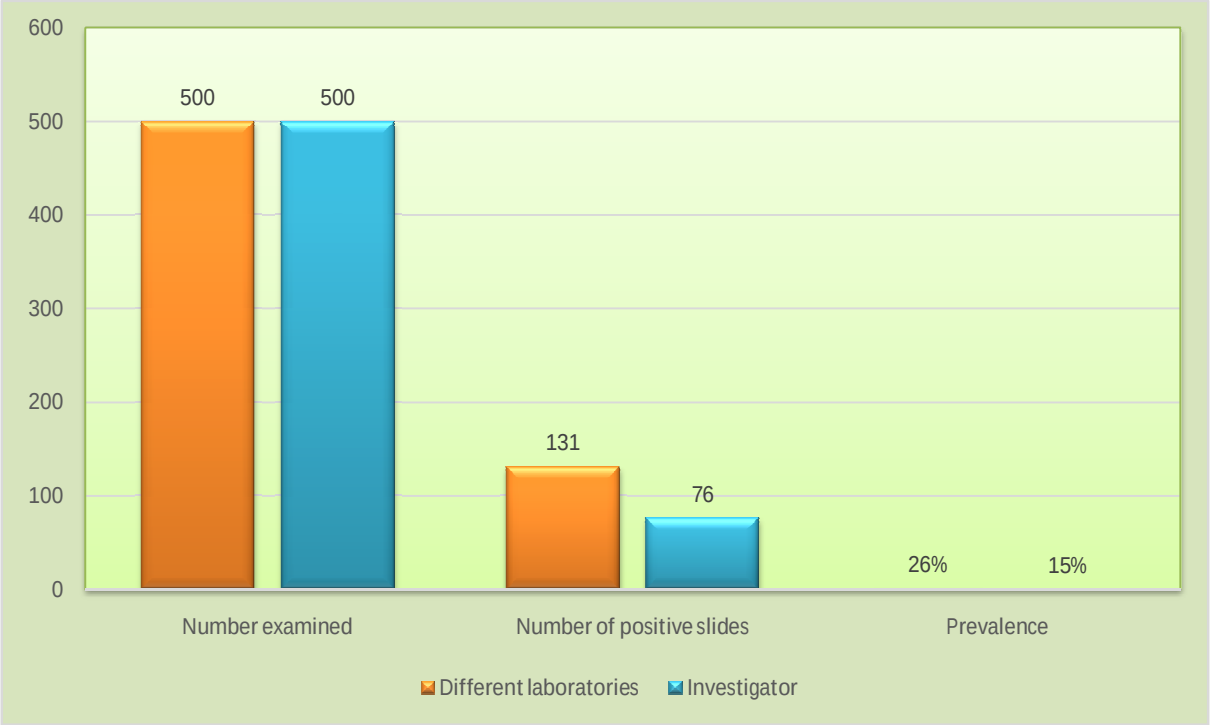

Figure (1): The overall prevalence rate of malaria in Dongola.

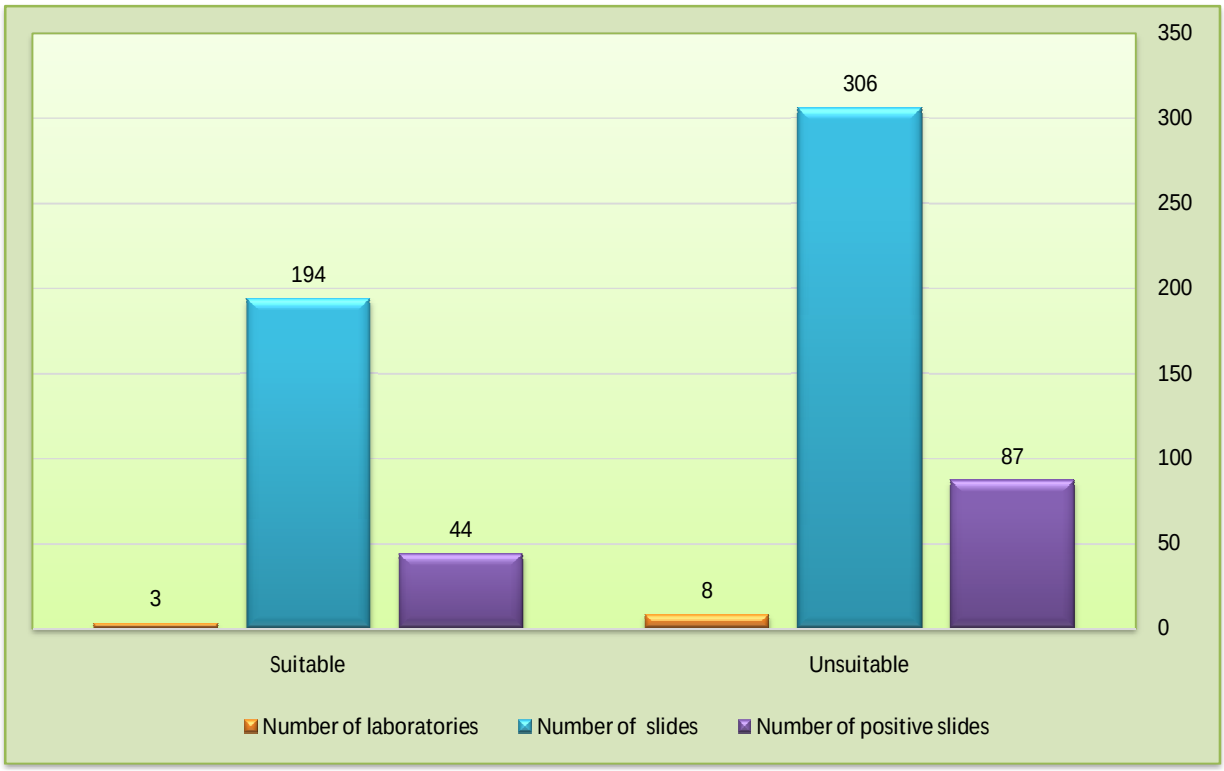

Figure (2): The effect of general condition of the laboratory building on the examination result.

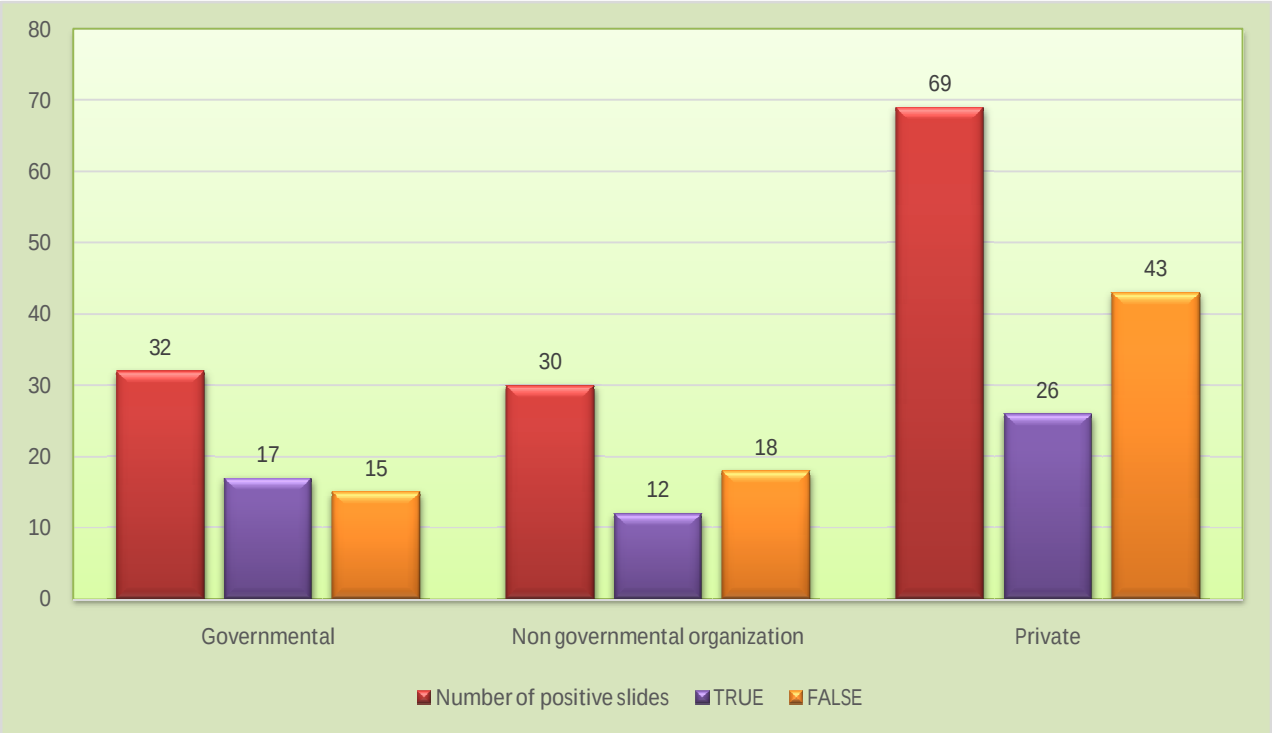

Figure (3): False positives among the examined slides in different types of laboratories.

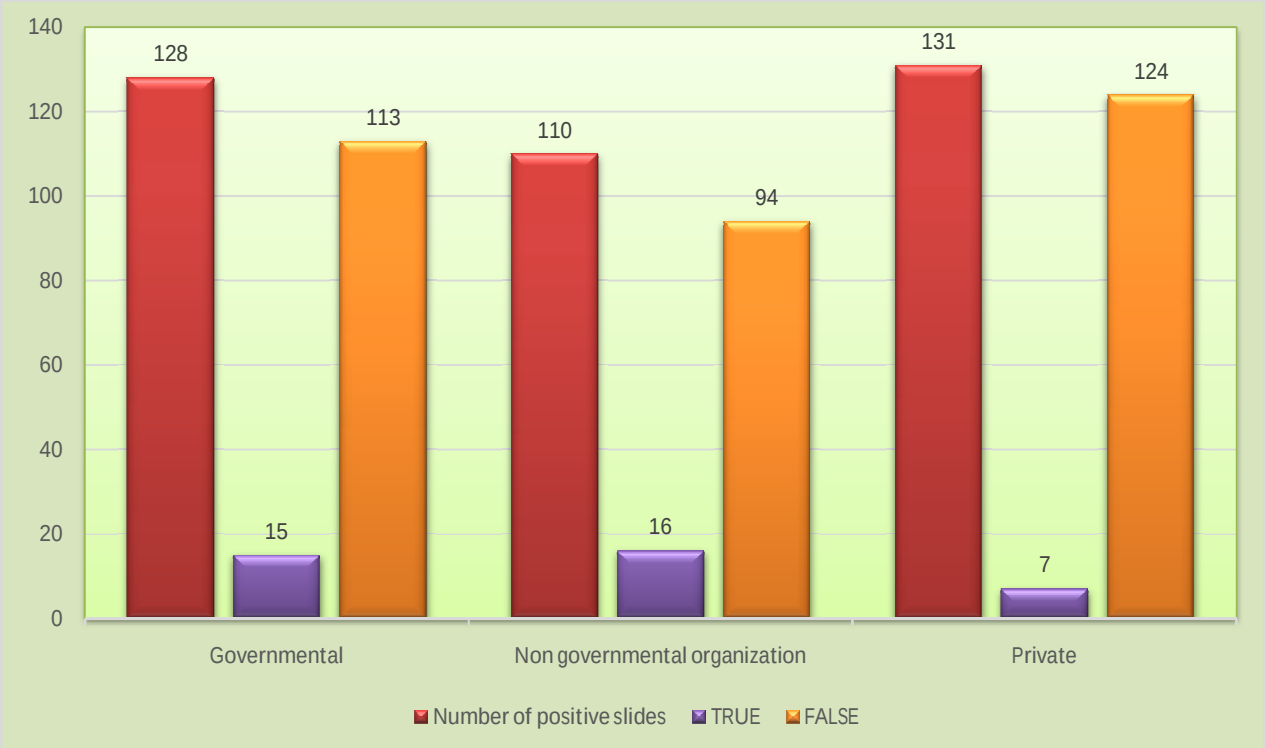

Figure (4): False negatives among the examined slides in different types of laboratories.

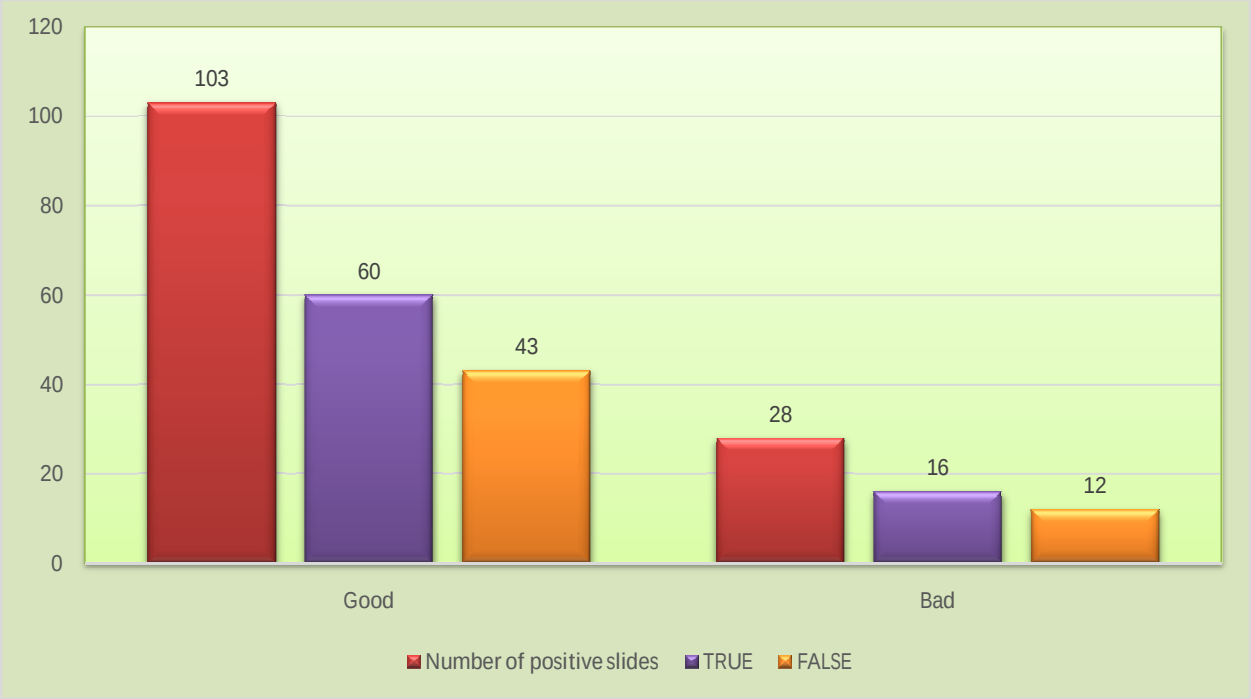

Figure (5): The effect of collection technique on the positivity of the result.

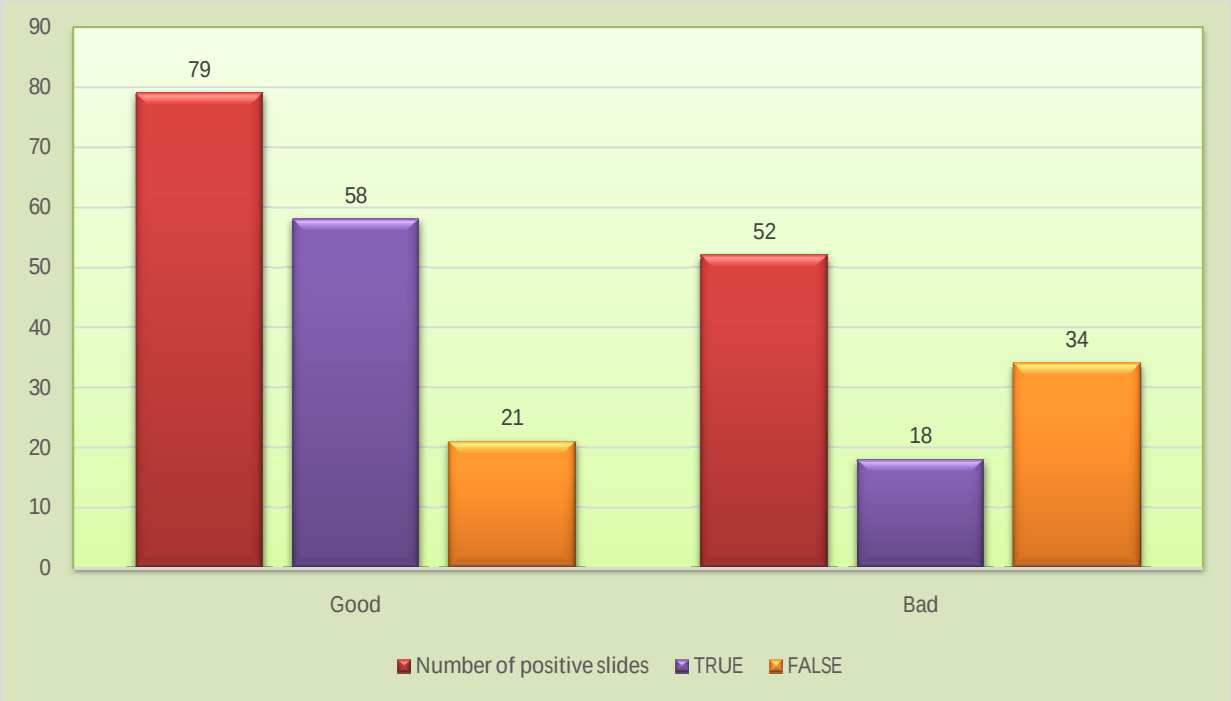

Figure (6): The effect staining technique on the positivity results.

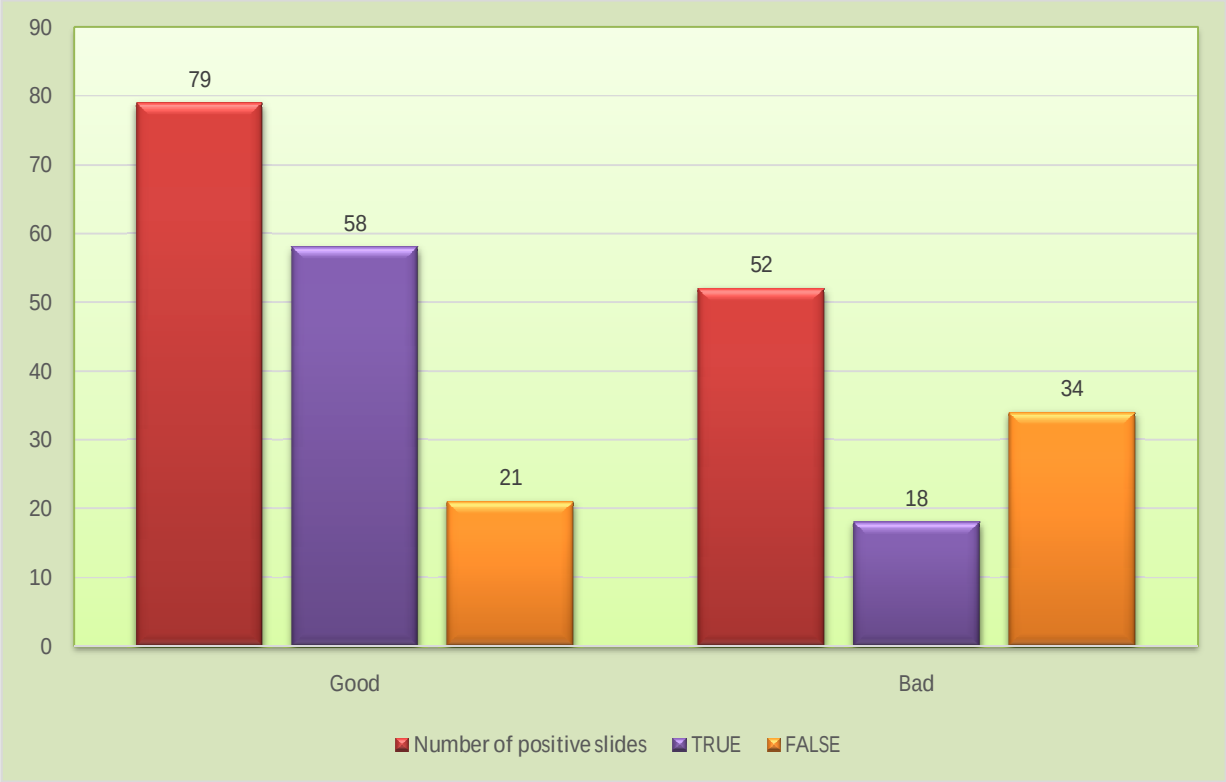

Figure (7): The effect smear preparation on the positivity results.

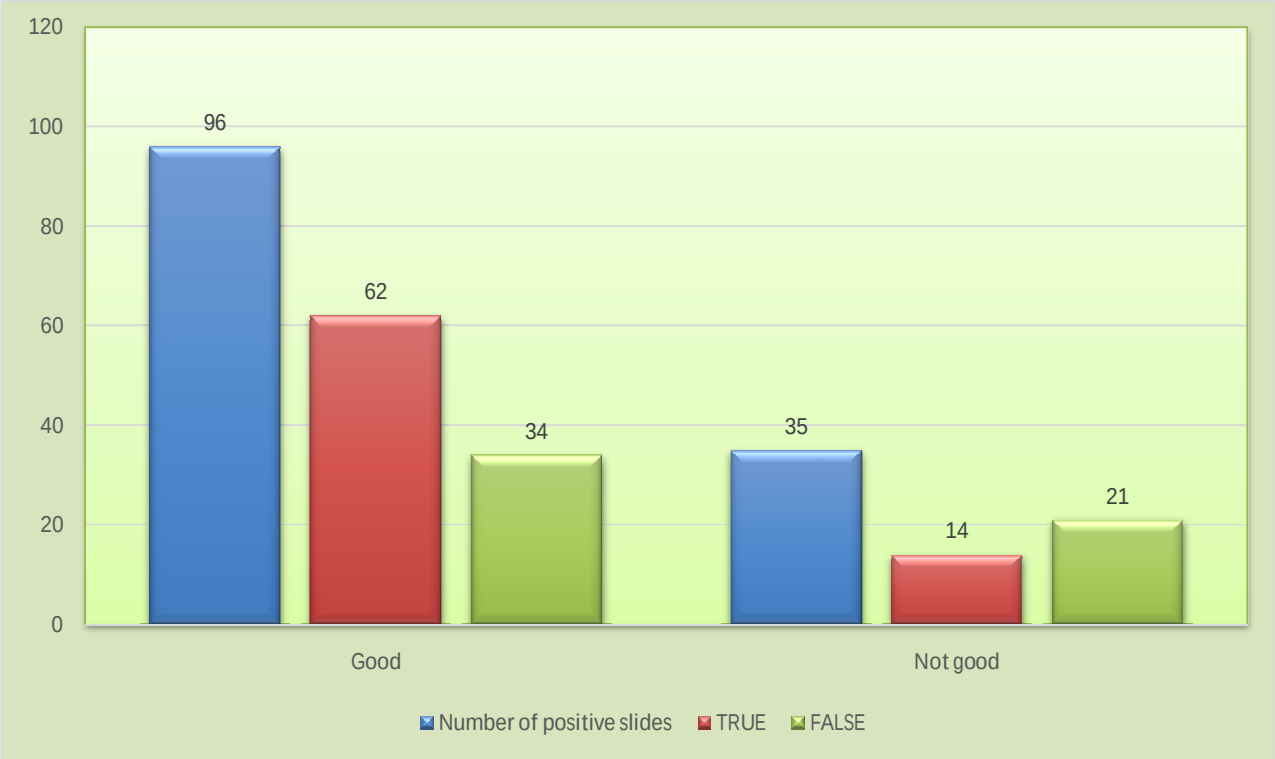

Figure (8): The effect staff qualification on the positivity results.

Figure (9): The effect of training in malaria diagnosis on the positivity of the result.

Chapter four

4.1 Discussion

Microscopic identification of malaria parasite is considered as the main and conventional method of malaria laboratory diagnosis, although considerable efforts have been under taken to develop new laboratory method for diagnosis of malaria parasite. The use of thick blood film examined by light microscope is the most common and reliable method (Kilian*et al,* 2000).

Microscopy, however, has its own biases and limitations. Result will not only depend on the quality of the microscopy , staining , and technique with which blood film is prepared and the parasite are accounted but also on the concentration and motivation of microscopist (Payne,1988).

This study is an attempt to evaluate the reliability of malaria microscope looking through both variation of result and associated quality assurance basics. These basics are general condition of laboratory, general condition of glass slide, qualification and experience of laboratorian.

The study assumption is that any defect in one or more of these basics will consequently affect the reliabitly and accuracy of laboratory results.

The ideal general condition of the laboratory in which the blood film for malaria are examined is to be clean, free from dust and insect and with good intensity of light and ventilation and with adequate available space for performing an organized laboratory routine work.

On evaluation of the effect of the general condition of the laboratory on the microscopist performance, the result showed that with good general condition of the laboratory, the reliability of the result is not equal with the result when the checked laboratory is in bad general condition.

This may be explained by the fact that, the general condition of the laboratory influences in the examination result. This result in line with Rafaa (2001), who reported higher results in laboratory with good general condition.

Slide used in preparing films for malaria have be clean, grease free and scratch free. Significance was observed in result when using glass slides. It was obvious that the false positives in the private laboratories reached 62%. This might probably be due to using unsuitable slide. All slide must be clean and free from grease and scratch. Using unsuitable slide will confuse malaria diagnosis and prevent detachment and washing of thick blood film during staining process. This finding agrees with comments of WHO (1983).

The study has focused on the way in which blood is collected, spread, and dried and if it has any influence on sensitivity on parasite detection. Our result showed that 58% of blood film were truly diagnosed when the sampling technique was done in the right way. Whereas only 57% were truly diagnosed when the sampling technique was done in wrong way. This might be justified by the fact that collecting large amount of blood than that actually required may result in formation of extra thick film which cannot be easilyread. In contrast, small amount of blood is not re representative for all blood constituents. Moreover, a blood film that was not sufficiently dried may be washed out during staining and washing.

One of most important findings was the variation in results due to different quality of stain used.

The stain was checked for their preparation, storage, and time of staining and duration of changing. The stain used is Giemsa stain. It gives the best staining of malariaparasite. The study showed that the true diagnosis was 73% when stain is good and 34% when stain was of bad quality.

It is recommended that the stains should be changed frequently in a routine clinical laboratory to reduce the risk of infection and carryover of the parasite, and also to assist in thestandardization of staining (Shute 1988).

Using the staining solution for prolonged time without changing, may affect the purity of stain. Bycontinuous dipping of the films and removal of parts of these films inside the solution. These exposed solutions may grow fungi and ciliates. Dust and dirt may also find their way to staining jars.

An efficient microscope is characterized by complete controlled mechanical stage , built in source of illumination with a good intensity of light, perfectly function fine and coarse adjustment knobs, good light directing condenser and iris, scratch free and oil cleaned lenses and the microscope should be well protected from dust and fungal growth.

The microscopes of checked laboratories were examined for their efficiency. Result showed significance between efficient and inefficient microscope. Paradoxally , our result revealed that the more accurate result were detected when the general condition of the microscope was good and false result were likely to be due to the bad general condition of the microscopes used in this laboratory.

The immersion oil used in microscope in order to avoid the bending effect of air on the beam of the light and its limitation on the objective, as mentioned by Cheesbrourph (1998). Whenever possible, the immersion oil recommended by manufacturer of microscope should be used but actually what happens is that some laboratorianusedifferent types of fluids to the immersion oil in order to increase its amount or using alternatives which change the oil refractive index and by turn results in scattering of the light beam and losing the details of the blood films.

The study revealed that malaria parasites are easily detected and identified by trained microscopist. Central laboratory staff should be encouraged, to train and supervise local community health workers in the laboratory techniques required to confirm a diagnosis of malaria. This will help to ensure that malaria is diagnosed correctly and at an early stage (Cheesbrourgh, 1998).

The effect of training on microscopic diagnosing of malaria was evaluated. Considerable variation in the result was observed when blood films were examined by either trained or untrained laborotorian. The study showed that qualified and well trained labrotorian have got abetter performance than untrained labrotorian. This in fact is due to experience gainedduring in-service training.

The diagnosis of malaria is still sometimes difficult because of the sensitivity of microscope screening at low level of parasitemia. In this study, it was clearly observed that the false negative results are more frequently recorded when the parasite count is low.

The checked laboratories in this study were grouped in to three groups governmental,non-governmental and private laboratory. Evaluation of their performance was in accordance with their type. It has been found that governmental laboratory have recorded the best result 53% of true positive result. This result was in agreement with (Rafa, 2001).

Chapter five

5.1 Conclusions:

- Accurate malaria diagnosis requires well trained labrotaroian, good stain, good microscope and high quality immersion oil.
- The use of an efficient microscope and well trained personnel are required for accurate diagnosis of malaria.
- The high percentage of false positive results will lead to unnecessary treatment.
- False result will influence adversely the implementation of an efficient control program.

Chapter six

6.1 Recommendation:

- Quality assurance step must be applied to improve the laboratory performance for microscopical diagnosis of malaria.
- Training program in malaria is very important; supplies for all aspects concerning malaria diagnosis microscopically must be available and easy to take.
- For peripheral laboratories with limited facilities and working staff with limited experience , support and supervision should come from nearest reference laboratory, such support should include the supply of standard solution , stain equipment , and to help those who are performing the laboratory work to have significant knowledge and practical experience .
- A highly qualified team with a check listed should have annual program of visit to peripheral laboratories. If both the external quality assessment scheme and the team visits reported bad results,thelabrotorians must be forbidden from working in microscopic diagnosis of malaria till they receivea good training programs.

Appendix

Sudan University of Science & Technology

College of Graduate Studies

Department Of Parasitology and Medical Entomology

Laboratory number:……Sector :…….

General condition of laboratory	Cleanness
	Space
	Ventilation
	Electricity supply
	Water supply
Collection	Cleaning of finger
	Lancet
	Slide
Smear	Preparation
	Type
	Dry
Stain	Type
	Storage
	Procedure
	Time of working solution
Microscope	Brand
	Mechanical stage
	Lenses
	Oil used
	Source of light
	Coarse
Personnel	Qualification
	Training courses of malaria
Laboratory	Number of false slide
	positive
	Negative

References

-Arez, A.P; Farnert, A; correia, A.T; Bjorkman, A; snounou, G. and Rosanio, V.(1999). Polymerase chain reaction. Transaction of the Royal Society of Tropical Medicine and Hygiene, 93:50-53.

-Beck, H; steiger, s; Genton, B. and Edoh, D. (1997). PCR amplification thin of DNA from malaria parasite on fixed and stained thick and blood films. Transaction of the Royal Society of Tropical Medicine and Hygiene, 91:361-363.

-Cheesbrough, M. (1998). District laboratory practices in tropical countries. 1st Edition. Cambridge shire. UK 246-251.

-Chin, J. (2002). Controlof communicable disease manual 17 the addition, Washington, DC, American public Health Association 100-140.

-Gill, G.V . and Beeching, N.J. (2004). Tropical Medicine. 5th edition Blachwell Science . 26-64.

- Good, M. F, Saul, A.J and Molseculara, S.A.(1993). Immunological consideration in Malaria Vaccine Development Beca Raton, CRC Press, 300-330.

- Himeidan,Y; Elbashir, M.I; El-Rayah, E.A and Adam,I. (2005). Epidemiology of malaria in new Halfa, An irrigated area in eastern Sudan. Eastern Mediterranean Health Journal. 11(3):25-30.

-- Himeidan,Y; Dukeen. M.Y; El-Rayah, E.A and Adam,I. (2005). Anopheles arabiensis: abundance and insecticide resistance in an irrigation area of eastern Sudan .Eastern Mediterranean Health Journal. 10(1/2)167-174.

-Hoffman, S.L.(1992). Diagnosis , treatment and Prevention of Malaria .Med. Clin. North Am 76:1327.

-Kilian, A. H. D, Metzger, W. G , Mutschelknauss, E.J, Kabagambe, G, Langi ,P , Krote, and Sonnenhburg, F.(2000). Reliability of malaria microscopy in epidemiology studies, Tropical medicine and international health 5(1): 3-8.

- Kodisinghe, H.M; Permawansa, S. and Naotunne, T.(1997). The parasite Tm-Fdip stick test .Transaction of the Royal Society of Tropical Medicine and Hygiene, 91:398- 402.

-**Markell, E; John, DT. AndKrotoski, W.A. (1999).** Medical Parasitology. 8th edition, Philadephia, lea and febiger-USA ,90-120.

-Millar ,L.H; Borach, D.L and March, K. (2002). The pathogenic Basic Natural 415 (6872):673-679.

- **Ministry of Health (1999).** Khartoum state annual report .

-**National malaria administration (1998)**

-**National malaria administration (2004)**

-Palamer, C,J; Validum, L; Lino, J; Campa, A;Validum,**C; Markler, M; Cuadrado, R. and Arba, A.(1999).** Field evaluation of the Optimal Rapid Malaria Diagnosis Test during anti-malaria . Transaction of the Royal Society of Tropical Medicine and Hygiene, 93:517-518.

-Payne, D, (1988). Used and limitations of light microscopy for diagnosis of malaria at the primary health care level, Bulletin of the world health organization 66:621-626.

-Rafa, Ibrahim (**2001)**. Causes of false result in microscopic diagnosis of malaria in Khartoum state. M.Sc thesis, Faculty of pharmacy, University of Khartoum .

-Salim .E.A and Harbi S.O.(2005). Malaria administration parasitic investigation for malaria.

- **Shute ,G.T., (1988).** The microscopic diagnosis of malaria, malaria principle and practice of essential malariology, 1:782- 813.

-Torrens, T.K; Walker, J. and stow , N.W.(1999). An assessment of the accuracy of clinical diagnosis of malaria. Transaction of the Royal Society of Tropical Medicine and Hygiene, 93:519-250.

- WHO, (**1983).** Bench aids to human malaria diagnosis .

- WHO,(2002). Second Inter Country Meeting of National Malaria Programme Manager. Muscat, Oman, 24-28 March 2002.

-**Zaman, V. and Keong, L.A. (1982).** Malaria parasite . In: Hand Book of Medical Parasitological,2nd edition, ADIS Health Science Press, 37-46.