EFFICACY OF AUTOMATED HEMATOLOGY ANALYSER SYSMEX XN1000 IN DETECTION OF MALARIA

By

DR. AMBICA C

Dissertation submitted to the

B.LD.E. University, Vijayapur, Karnataka

In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the Guidance of

DR. SUREKHA B.HIPPARGI

Professor, Department of Pathology

B.L.D.E. UNIVERSITY'S, SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPUR, KARNATAKA.

2017

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"EFFICACY OF AUTOMATED HEMATOLOGY ANALYSER SYSMEX XN1000 IN DETECTION OF MALARIA"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. SUREKHA B.HIPPARGI** Professor, Department of Pathology B.L.D.E.U's Shri B.M.Patil Medical College, Hospital & Research centre, Vijayapur, Karnataka.

Date: **Dr. AMBICA C**

Place: Vijayapur

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"EFFICACY OF AUTOMATED HEMATOLOGY ANALYSER SYSMEX XN1000 IN DETECTION OF MALARIA"** is a bonafide research work done by **Dr. AMBICA C** in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapur

Dr. SUREKHA B.HIPPARGI
Professor, Department of Pathology,
B.L.D.E. University's
Shri B.M.Patil Medical College,
Hospiatl & Research Centre,
Vijayapur, Karnataka

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

ENDORSEMENT BY HEAD OF DEPARTMENT

This is to certify that the dissertation entitled **"EFFICACY OF AUTOMATED HEMATOLOGY ANALYSER SYSMEX XN1000 IN DETECTION OF MALARIA"** is a bonafide research work done by **Dr. AMBICA C** in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapur

Dr. B.R.YELIKAR
Professor and Head
Department of Pathology,
B.L.D.E University's
Shri B.M.Patil Medical College,
Hospital & Research Centre,
Vijayapur, Karnataka

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

ENDORSEMENT BY PRINCIPAL /

HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"EFFICACY OF AUTOMATED HEMATOLOGY ANALYSER SYSMEX XN1000 IN DETECTION OF MALARIA"** is a bonafide research work done by **Dr. AMBICA C** in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapur

Dr. S.P.GUGGARIGOUDAR
Principal,
B.L.D.E University's
Shri B.M.Patil Medical College,
Hospital & Research Centre,
Vijayapur, Karnataka

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the B.L.D.E. University, Karnataka shall have the rights to preserve, use and disseminate this dissertation / thesis in print or electronic format for academic / research purpose.

Date: **Dr. AMBICA C**

Place: Vijayapur

ACKNOWLEDGEMENT

First and foremost, I thank God, the Almighty, for His blessings throughout my life and in accomplishing the present study successfully.

I am deeply indebted to my **parents, Mr. Somnath C and Mrs. Vinodini C** for their constant encouragement, love and moral support who made me what I am today.

I would like to express my sincere and deepest gratitude to my teacher and guide **Dr. SUREKHA B. HIPPARGI, Professor, Department of Pathology**, for providing invaluable guidance, suggestions and precise approach throughout the course of my study that has been a valuable part of my experience.

I am equally grateful to **Dr. B.R.Yelikar, Professor and H.O.D, Department of Pathology** for his valuable suggestions, constant support and critical appreciation in pursuit of my study.

I am privileged to have an approachable and supportive staff. I am thankful to **Dr. S.U. Arakeri** Prof**, Dr. R.M. Potekar** Prof, **Dr. Mahesh H. Karigoudar** Prof, **Dr. Prakash M. Patil** Assoc Prof, **Dr. Girija S. Patil** Assoc Prof, **Dr. Vijayalaxmi S. Patil** Asst. Prof**, Dr. Anita P. Javalgi** Asst prof, **Dr. Savitri M. Nerune** Asst prof, **Dr. Mamatha K.** Asst Prof and **Dr. Sneha J.** Asst Prof for their supervision, concern and positive feedback at all steps of this work.

Words are not enough to express my love and affection to my husband, **Dr. Ramesh P**, who has been a constant source of inspiration and encouragement, understanding and supporting me at every stage which made this period less intense.

I also thank my brother **Shravan Kumar C** for his support and help in accomplishing the present work.

My sincere thanks to all my batch mates, seniors and juniors who have helped me during my work.

I am very grateful to all the **technical staff of the Central laboratory**, and non-teaching staff of Department of Pathology, who have helped me during this work.

Last but not the least**,** my sincere gratitude to all my study subjects whose cooperation has contributed to this study.

Date: **Dr. AMBICA C**

Place: Vijayapur

LIST OF ABBREVATIONS USED

WHO	World Health Organization
PSE	Peripheral smear examination
QBC	Quantitative buffy coat
CBC	Complete blood count
ICT	Immunochromatographic test
RDT	Rapid diagnostic test
PCR	Polymerase chain reaction
ACC	Automated blood cell counter
WBC-DIFF	White blood cell-Differential
RBC	Red blood cell
P.vivax	Plasmodium vivax
P.falciparum	Plasmodium falciparum
TNF	Tumor necrosis factor
HT	Host targeting
PEXEL	Plasmodial export element
HZ	Hemozoin
HNE	4-hydroxynonenal
15-S-HETE	15-(s)-hydroxyeicosatetraenoic acid
WBC	White blood cell
EDTA	Ethylene diamine tetra acetic acid
FCM	Flowcytometry
AHA	Automated hematology analysers
MHC	Major histocompatibility complex
PCEs	Purple coded events
VCS	Volume-Conductance-Scatter
SD	Standard Deviation
PPV	Positive predictive value

NPV	Negative predictive value
RLALS	Rotated low angle light scatter
AL2	Axial light
RUMALS	Rotated upper median angle light scatter
nRBC	Nucleated red blood cell
WBC-BASO	White blood cell-Basophil
K3EDTA	Tripotassium Ethylene diamine tetraacetic acid
FSC	Forward scatter
SSC	Side scatter
SFL	Side flourescence
DIFF	Differential
WDF	WBC Differential
WNR	White cell-nucleated red blood cell
WPC	White cell precursor
PLT-F	Flourescent Platelet
NEC	Neutrophil-Eosinophil cluster
N	Number
YRS	Years
TP	True positive
FP	False positive
TN	True negative
FN	False negative
PS	Peripheral smear
HB	Hemoglobin
PLT	Platelet
NEU-EOS	Neutrophil-Eosinophil

ABSTRACT

INTRODUCTION

Malaria is an important infectious disease causing significant public health problem and affecting millions of people every year. It is a parasitic disease caused by Plasmodium species. Classical clinical features of malaria include cyclical fever, associated with chills and rigors, body ache, malaise and other non-specific systemic symptoms. Timely diagnosis and treatment of malaria cases reduces the morbidity and mortality associated with it. The gold standard for diagnosis of malaria is the microscopic examination of the stained blood films which is usually labour demanding and time consuming. To overcome these problems and to achieve maximum sensitivity and specificity in the diagnosis of malaria, several alternate methods became popular like the quantitave buffy coat, serological methods and molecular techniques. The major disadvantage of all these methods including the microscopic diagnosis is the clinical request for malaria. During the last few decades, automated hematology analysers came in to the picture in presumptive diagnosis of malaria during CBC analysis, even without an extra request for malaria. These analysers generate several scatter plot and histogram abnormalities by which malaria can be suspected.

OBJECTIVE

To analyze the efficacy of Sysmex XN1000 hematology analyser in detection of malaria by using WBC-DIFF scatter plots.

MATERIALS AND METHODS

A prospective cross-sectional study was carried out in 146 patients from both out-patient and in-patient departments, referred to the Department of Pathology in BLDE University's Shri B.M.Patil Medical College, Hospital and Research centre,

Vijayapur in whom peripheral smear for detection of malarial parasite was requested and satisfying the inclusion and exclusion criteria.

All the blood samples of cases were collected in K3 EDTA vacutainers and processed on Sysmex XN1000 hematology analyser which is based on the principle of fluorescence flow cytometry. The WBC – DIFF scatter plots of these samples were analysed for abnormalities. Leishman stained peripheral smears were prepared and screened for malarial parasite and correlated with WBC-DIFF scatter plots.

RESULTS

Using the WBC-DIFF scatter plot abnormalities, the sensitivity and specificity were 84.6 and 91.5% respectively, considering peripheral smear as the gold standard. Further, the positive predictive value was 84.6% and negative predictive value was 91.5% with an accuracy of 89.0%.

CONCLUSION

Analysis of specific patterns of WBC scatter plots is a rapid process which requires less expertization and can be an important adjuvant diagnostic tool in detection of malaria even in the absence of clinical request. So, it is recommended for the pathologists to review the WBC scatter plots on a routine basis to pick up malarial infection.

KEY WORDS: Malaria, automated hematology analysers, WBC-DIFF scatter plots

TABLE OF CONTENTS

Sl. No.	Contents	Page No
1.	INTRODUCTION	1
2.	OBJECTIVE	4
3.	REVIEW OF LITERATURE	5
4.	MATERIALS AND METHODS	20
5.	RESULTS	29
6.	DISCUSSION	46
7.	CONCLUSION	53
8.	SUMMARY	54
9.	BIBLIOGRAPHY	56
10.	ANNEXURES	62
11.	MASTER CHART	68

LIST OF TABLES

Table No.	Title of table	Page No.
1.	Advantages and disadvantages of conventional microscopic diagnosis of malaria	11
2.	Advantages and disadvantages of automated cell counters in detection of malaria	12
3.	Distribution of cases by age (years)	29
4.	Distribution of cases by sex	30
5.	Distribution of malaria cases diagnosed by peripheral smear	31
6.	Distribution of cases showing WBC-DIFF scatter plot abnormalities on Sysmex XN1000	32
7.	Distribution of cases diagnosed by peripheral smear and showing abnormal WBC-DIFF scatter plot on Sysmex XN1000	33
8.	Species categorization by peripheral smear examination	34
9.	Distribution of cases diagnosed by peripheral smear based on morphological forms	35
10.	Distribution of malaria positive cases diagnosed by peripheral smear and showing abnormalities on WBC-DIFF scatter plot with respect to species	37
11.	Distribution of WBC-DIFF scatter plot abnormalities on Sysmex XN1000 hematology analyser	38
12.	Correlation of WBC-DIFF scatter plot abnormalities with peripheral smear diagnosis	40

13.	Sensitivity analysis of Sysmex XN1000 with comparison to peripheral smear as gold standard	42
14.	Comparison of age, hemoglobin, WBC and platelet counts between malaria positive and negative cases	43
15.	Distribution of abnormalities on scatter plot with respect to species categorization by PSE	44
16.	Comparison of results of various studies with the present study	47
17.	Comparison of various abnormalities of the scatter plot on Sysmex XN1000 with other studies	49
18.	Malaria positive cases showing no abnormality on scatter plot	50

LIST OF FIGURES

Figure No.	Title of table	Page No.
1.	Automated hematology analyser Sysmex XN1000	21
2.	Peripheral smear showing various morphological forms of Plasmodium vivax (Leishman stain 1000x)	25
3.	Peripheral smear showing various morphological forms of Plasmodium falciparum (Leishman stain 1000x)	27
4.	Various scatter plot abnormalities on Sysmex XN1000 hematology analyser	28
5.	Pie chart distribution of cases by age (years)	29
6.	Pie chart distribution of cases by sex	30
7.	Pie chart distribution of malaria cases diagnosed by peripheral smear	31
8.	Pie chart distribution of cases showing WBC-DIFF scatter plot abnormalities on Sysmex XN1000	32
9.	Bar graph showing distribution of cases diagnosed by peripheral smear and showing abnormal WBC-DIFF scatter plot on Sysmex XN1000	33
10.	Pie chart showing species categorization by peripheral smear examination	34
11.	Bar graph showing distribution of cases diagnosed by peripheral smear based on morphological forms	36
12.	Bar graph presentation showing distribution of malaria positive	37

	cases diagnosed by peripheral smear and showing abnormalities on WBC-DIFF scatter plot with respect to species	
13.	Bar graph showing distribution of WBC-DIFF scatter plot abnormalities on Sysmex XN1000 hematology analyser	39
14.	Bar graph presentation showing correlation of WBC-DIFF scatter plot abnormalities with peripheral smear diagnosis	41
15.	Pyramid presentation of sensitivity analysis of Sysmex XN1000 with comparison to peripheral smear as gold standard	42
16.	Bar graph showing comparison of age, hemoglobin, WBC and platelet counts between malaria positive and negative cases	43
17.	Bar graph showing distribution of abnormalities on scatter plot with respect to species categorization by PSE	45

INTRODUCTION

Malaria is one of the most important parasitic diseases ever known to mankind and represents a major global health problem.[1-3] The disease is also called as the "King of Diseases".[4] It is caused by a parasite of the genus Plasmodium and is transmitted by the bite of an infected female *Anopheles* mosquito (malaria vector). Malaria is a highly prevalent disease in tropical and subtropical regions affecting 200-300 million people every year.[1,4] According to the World Health Organization (WHO) world malaria report 2015, the estimated number of malaria cases were 214 million resulting in about 4.38 lakh deaths.[5]

Worldwide, the diagnostic skills and facilities to detect malaria vary enormously and is a challenge to all the laboratory personnel, especially in developing countries like India due to high workload, limited resources and affordability.[6,7] Diagnosis is generally based on a combination of clinical history, travel history and laboratory tests.[8]

The typical presentation includes recurrent paroxysms of fever, chills and rigor with temperatures as high as 105° to 106°F (40.5° to 41°C) associated with malaise, headache and vomiting[8,9,10] Other non-specific symptoms include arthralgia, myalgia, chest pain or diarrhoea.[9] Lack of clinical suspicion is the most important factor in the misdiagnosis of malaria which may be responsible for most of the deaths from malaria infection in the industrialized countries.[11]

Microscopic diagnosis requires special training and expertise in the field of parasitology. Manual peripheral smear examination (PSE) for malarial parasite by light microscopy is considered as the gold standard method for diagnosis of malaria infection, but it is labour intensive and time-consuming.[1-4,6-8] Several studies have shown that laboratory missed diagnosis of malaria is not uncommon which may be

due to lack of proficiency or limited resources.[10] To overcome these factors, several alternative diagnostic methods like the quantitative buffy coat (QBC), immunochromatographic (ICT) rapid diagnostic tests (RDTs) and detection by polymerase chain reaction (PCR) have emerged with increased sensitivity.[10] Even these come with disadvantages of being expensive and inaccessible. However, all these tests rely on clinical suspicion and a request.[2,3]

Complete blood count (CBC) is a routinely performed laboratory test in the workup of febrile patients.[10] The automated CBCs generated by automated hematology analysers detect nonspecific changes, such as anemia and thrombocytopenia, which can be associated with other conditions. Therefore, these changes by themselves are not specific to trigger malaria diagnosis.[11]

During the last 2 decades, automated blood cell counters (ACC) have shown a formidable technological progress. So, in addition to the traditional parameters of the CBC, newer versions of automated analysers are able to provide much more information, like the white blood cell-Differential (WBC-DIFF) scatter plots and flaggings by which malaria can be suspected.[3,8]

In the present era of multispeciality hospitals having a high workload, large numbers of CBCs are requested for a common symptom of fever. So, it is important to distinguish the origin of fever, as a prompt and accurate diagnosis would reduce adverse outcome associated with malaria.[3] Therefore, there is a need for reliable automated hematology analysers which helps in presumptive diagnosis of malarial infection

- Even in the absence of clinical suspicion
- Without requiring additional reagents

There is a call for rapid, sensitive and cost-effective screening method on all febrile samples suspicious of malaria, particularly in endemic areas. Hence the present study is undertaken to assess the performance of hematology analyser Sysmex XN1000 in the diagnosis of malaria, as the analyser gives quick results, needs less technical expertise and can be done with routine blood investigations without any additional cost even in the absence of a clinical request.

OBJECTIVE OF THE STUDY

To analyze the efficacy of Sysmex XN1000 hematology analyser in detection of malaria by using WBC-DIFF scatter plots.

REVIEW OF LITERATURE

History

Allusions to what was almost certainly malaria occur in a Chinese document from about 2700 BC, clay tablets from Mesopotamia since 2000 BC, Egyptian papyri from 1570 BC and Hindu literature like the Atharva Veda and Charaka Samhita as far back as the sixth century BC.[12] The word malaria comes from the Italian word mal'aria meaning 'spoiled air' although this has been controversial.[12] In 1880, discovery of the parasites in the blood of malaria patients by Charles Louis Alphonse Laveran, which he called Oscillaria malariae lead to the understanding of malaria parasites for which he was awarded Nobel Prize in Medicine in 1907.[12]

The accidental discovery of a methylene blue eosin stain by Dimitri Leonidovitch Romanowsky in 1891 is one of the most significant technical advances in parasitology.[12] Romanowsky's stains colour the nucleus of the parasite red and the cytoplasm blue enabling their easy identification.[12]

Ronald Ross demonstrated oocysts in the gut of female Anopheline mosquito in India and proved that mosquito was the vector for malaria.[12] Based on the observations of erythrocytic stages of the parasite, Golgi between 1885-1886 differentiated between tertian (48 hour periodicity) and quartan (72 hour periodicity) malaria and in 1889-1890 Golgi and Marchiafava together added the differences between mild and severe Spring malaria (benign tertian) and Summer-Autumn (malignant tertian) malaria respectively.[12]

By 1890, it was known that three species of the parasite were responsible for malaria with specific properties causing benign tertian (Haemamoeba vivax), malignant tertian (Laverania malariae) and quartan (Haemamoeba malariae) malaria which are now called respectively as Plasmodium vivax, Plasmodium falciparum and

Plasmodium malariae.[12] In 1922, John Stephens discovered the fourth species, Plasmodium ovale. Plasmodium knowlesi, a newer species was discovered in 1932.[12]

HEMATOLOGICAL ABNORMALITIES IN MALARIA

RED BLOOD CELL (RBC)

RBCs are the primary targets for Plasmodium species. Anemia is common in malaria, causing morbidity and mortality, especially in children and pregnant women.[9,13-17] It is usually of the normocytic normochromic type with a variable degree of onset and rapidity.[9,15] The pathophysiology of anemia associated with malaria is multifactorial and complex, dependent on the properties of host and parasite.[15,16] It is due to hemolysis of both infected as well as uninfected erythrocytes and inability of the bone marrow to replace hemolysed RBCs due to inadequate erythroid response.[16] P.vivax has more predilection for reticulocytes. In contrast, P.falciparum has a relatively lesser predilection for young RBCs and significant ability to infect older RBCs.[16,17]

Factors contributing to anemia in malaria include[3,9,14,18-20]

Accelerated RBC destruction

- Direct destruction of red cells by the parasite.
- Reduced deformability of infected RBCs and destruction by splenic macrophages.
- Macrophage-mediated destruction of infected RBCs in the bone marrow and liver sinusoids.
- Destruction of non-parasitized cells by immune mechanisms.
- Destruction of non-parasitized cells by hypersplenism and hyperactive macrophages.

Decreased RBC production

- Release of inflammatory cytokines such as tumor necrosis factor (TNF) leading to bone marrow suppression.
- Reduction in erythropoietin production.
- Dyserythropoiesis.

Over the last few years, studies were done on malarial parasite ligand induced remodelling of erythrocytes and their role in destruction of mature erythrocytes.[18] It was found that the parasite alters the RBC membrane by changing its transport properties, exposing cryptic surface antigens and inserting new parasite-derived proteins all of which increase their antigenic property rendering them rigid and less deformable.[13]

Parasite entry into the erythrocytes is critical to the establishment of blood stage infection which is mediated by parasite proteins that reside on the surface of the merozoite, in its apical organelles which adhere to erythrocytes.[18] Thus in principle, the vast array of ligands by the parasite could potentially interact with targets on the host erythrocyte.[18]

The growing malarial parasite within the RBC progressively consumes and degrades intracellular proteins, principally hemoglobin. The potentially noxious heme is detoxified by lipid mediated crystallization to biologically inert hemozoin (HZ).[13] Hemozoin released after the lysis of RBCs is more heterogeneous and is phagocytosed by the cells of reticulo-endothelial system.[19]

Further, the breakdown products of HZ such as 4-hydroxynonenal (HNE) and 15-(s)-hydroxyeicosatetraenoic acid (15-S-HETE) interfere with growth of erythroid progenitors and maturation of dendritic cells respectively.[19,20] These data support the

hypothesis that during malaria infection, bone marrow macrophages play a part in the inhibition of erythropoiesis indirectly or directly by oxidative stress.[19]

Studies also support the role of HZ in inhibition of erythropoiesis, slow down of cell cycle and inhibition of crucial receptors.[20]

WHITE BLOOD CELL (WBC)

The white blood cell count in malaria is usually within normal limits but may be increased in severe infection. Other changes include a leucoerythroblastic response, monocytosis and a reactive eosinophilia during the recovery phase. Neutrophil activation, indicated by raised leucocyte elastase levels, may be evident in severe malaria.[15,21] Leukopenia is common in nonimmune individuals.[3]

PLATELET

Thrombocytopenia is a hallmark of malaria.[3,21,22] It is due to increased splenic clearance and reduction in life span of platelets and is associated with increased platelet turnover and raised thrombopoietin levels.[3,15,21] It is commoner in P.vivax malaria as compared to P.falciparum malaria.[22] The mechanism is not fully known but it is believed that the P.vivax has a direct lytic effect on platelets, which may be mediated both immunologically and non-immunologically.[22]

BONE MARROW CHANGES IN MALARIA

Bone marrow in patients with repeated infection with malaria is slate gray because of deposition of phagocytosed hemozoin.[22] The cellularity is usually hypocellular. It can be either normocellular or hypercellular.[22] Normoblastic erythropoiesis with features of dyserythropoiesis and normal iron stores is noted.[15,22] The number of megakaryocytes is increased showing phagocytosis of parasitized RBCs and WBCs.[22] Neutrophils and macrophages also show ingested hemozoin with an increase in lymphocyte and plasma cell populations.[15,22]

DIAGNOSIS

Precise diagnosis on time is crucial in effective management of malaria.[4,23] In many malaria-endemic countries, the lack of resources and inadequate or absence of quality control measures are the most important barriers to reliable and timely diagnosis, hindering effective malaria control.[23] The diagnosis of malaria involve identification of the Plasmodium parasites or its antigens in the blood sample of the patient.[4] The accuracy of malaria diagnosis depends on the type of species, parasitic index, host immunity and clinical context and phase in which the method of diagnosis is applied.[24]

LABORATORY DIAGNOSIS

The non-specific and overlapping signs and symptoms of malaria may result in over-treatment of malaria or under-treatment of other diseases or in misdiagnosis of malaria.[4] So the confirmation of malaria diagnosis by laboratory diagnosis is essential.

In the laboratory, malaria can be diagnosed by using different techniques like the conventional microscopy by peripheral smear examination and several alternative methods like the QBC method, RDT, serological techniques, molecular diagnostic methods such as PCR and automated blood cell counters.[4,8]

MICROSCOPIC DIAGNOSIS:

Malaria is conventionally diagnosed by light microscopic examination of stained thick and thin blood films using Giemsa, Wright, Field or Leishman's stain.[1-4,6-8,25-29] Blood obtained by earlobe or finger prick is the ideal sample because of the

greater density of developed trophozoites or schizonts in blood from this capillary-rich area.[25]

Blood obtained by venipuncture collected in heparin or ethylene diamine tetraaceti cacid (EDTA) coated tubes can be used if used shortly after being drawn to prevent alteration in the morphology of WBCs and malaria parasites.[25] Thick blood film is used for screening of malaria parasite as it allows larger volumes of blood to be examined than the thin film.[15] The thick blood film is 20-40 times more sensitive and is much better than the thin film in detection of low levels of parasitemia and reappearance of circulating parasites during infection, recrudescence or relapse.[13,25] The disadvantage is that the lysis of the RBCs can make the process of scanning for parasites more difficult which can be overcome by experience in finding the parasites among the WBCs and platelets.[25]

Because of the fixed monolayer of RBC; morphological identification of the particular species is much easier in the thin blood film providing greater specificity than the thick-film examination.[25]

Table 1: Advantages and disadvantages of conventional microscopic diagnosis of malaria[1,4,6,7,15,27-31]

ADVANTAGES	DISADVANTAGES
Simple to use	Labour intensive
Economical	Time consuming
Readily accessible	Less sensitive
Species categorization is possible	Require expertise and trained health care workers
Assess parasite density	Inter-observer variability
	A negative film does not exclude malaria: at least three films taken during episodes of fever should be examined
	Parasites, particularly P.falciparum gametocytes may be washed off the slide during staining
	Bulk staining of slides may result in transfer of parasites between the slides

The alternative methods in the diagnosis of malaria have their own advantages and disadvantages, the most common being highly sensitive and expensive respectively.[4]

AUTOMATED BLOOD CELL COUNTERS AND FLOWCYTOMETRY (FCM)

Automated analysers are used worldwide in laboratories for routine hematological analysis.[2,24] The utility of automated blood cell counters and FCM for detecting malarial infection have been reported in the literature and these analysers are available all over the developed countries and their use is growing in the presumptive diagnosis of malaria in developing countries.[2,3,10,24,29,32]

The principle is based on the detection of hemozoin, which is produced when the intra-erythrocytic malarial parasites digest host hemoglobin leading to

crystallization of the released toxic heme into hemozoin. This hemozoin is phagocytosed by the granulocytes (monocytes and neutrophils). Hemozoin within the phagocytes can be detected by depolarization of laser light due to its birefringent property, as cells pass through a flow cytometer channel.[2,4,7,24,32]

Table 2: Advantages and disadvantages of automated cell counters in detection of malaria[4,26,33]

ADVANTAGES	DISADVANTAGES
Potential to diagnose clinically unsuspected malaria cases	Require trained technicians
Rapid	False positive results
Sensitive	

The various analysers which have been studied to aid in the diagnosis of malaria include the Abbott Cell-Dyn analysers, the Coulter analysers and the Sysmex analysers.

Until the early 1990s, many unreliable malaria associated changes in the CBC were described including abnormal extra peaks in the WBC histograms of a Coulter® MaxM analyser and pseudoreticulocytosis in a Sysmex R-1000.[10]

In 1993, a study done on Technicon H1® analyser on 18 patients with malaria and 52 healthy controls found that malaria-infected patients had ≥3% (range 3.3%-20.9%) of 'large unstained cells' suggesting their likely use in screening of malaria.[10]

Unfortunately, all these changes may also appear with other pathologies leading to low specificity and being less accurate for malaria detection.[10]

CELL-DYN ANALYSERS

The Cell-Dyn 3500 was the first automated hematology analyser (AHA) that allowed the detection of malaria during routine investigation by complete blood cell analysis[3,10,24,29,31-36] Since then, various Cell-Dyn analysers were developed and studied to detect HZ pigment in malaria.

It is based on impedance detection (Coulter Principle) which uses scattered laser light of leukocytes at four different angles, called the multiple-angle polarized scatter separation for WBC analysis to generate a WBC differential. The appearance of monocytes (purple-coded events, PCEs) in the eosinophil area (green coded events) is a sign of phagocytosed HZ ensuing in the diagnosis of malaria.[10,11,30-38]

Mendelow BV *et al*[36], in 1999 published their study comprising 224 samples referred specifically for malaria. Out of 224 samples, 95 were malaria positive by microscopy and/or immunological methods, and 129 were malaria negative. All the samples were processed on the Abbott Cell-Dyn 3500 cell counter and they concluded that the feasibility of counter in diagnosing malaria is a novel approach whose main advantage is the potential to make an unexpected diagnosis as a part of screening CBC. They also found a great variation in the sensitivities in malaria detection between Black African (90%) and White (43%) subjects. They stated that the probable possibilities for these differences could be due to polymorphisms in red cell antigens, major histocompatibility complex (MHC) determinants or the TNF sequence variations, all of which play a role in the pathogenesis of the disease.

Hanscheid T *et al*[34] in 1999 did a study in Portugal to assess the performance of Abbott CD3500 analyser and determined its usefulness for the diagnosis of imported malaria with a sensitivity of 95% and specificity of 88%.

In 2002, a study published by Wever PC *et al*[37] on detection of imported malaria with Cell-Dyn 4000 hematology analyser proposed that the analyser may contribute to the diagnosis of imported malaria in centers where technical expertise is lacking. They also suggested that the racial differences in sensitivities may reflect socioeconomic or cultural variations in access to health services and, therefore, in duration of symptoms before presentation. An additional finding in their study is the presence of pseudoreticulocytosis, exclusively in patients with P.falciparum infection with parasitemia of >0.5%. The P.vivax infected reticulocytes are counted as true reticulocytes by the analyser and therefore, pseudoreticulocytosis was not observed in P.vivax infection.

Another study on Abbott Cell-Dyn 4000 analyser by Suh IB *et al*[31] in 2002 for detection and therapeutic monitoring of P.vivax malaria stated that this is a sensitive method for detecting residual parasites in recovering patients. They also suggested that the analyser could detect intact parasite after in-vitro removal of WBCs in addition to the detection of leukocyte-ingested hemozoin pigment.

de Langen AJ *et al*[35] in 2002 did a study on Cell-Dyn 3700 ACC in Northern Namibia and concluded that the automated detection of malaria is a useful diagnostic tool in semi-rural area. In low-risk malaria season, the test can be used for diagnosing malaria because of high sensitivity and in the high-risk season, the test can be used to exclude malaria because of high specificity. The major limitation of their study is the inconsistency of the dot count with repetition of the test, resulting in a change of diagnosis in 14% of cases.

Josephine FP *et al*[32] conducted a study to evaluate the performance of Abbott Cell-Dyn 4000 analyser and found out that in addition to detecting the presence of malaria, the analyser can produce different depolarization patterns for P.vivax and

P.falciparum respectively. This could be due to the different cellular location of the hemozoin pigment in blood cells among different species of Plasmodium.

Ali SF *et al*[30] did a study to detect malaria by automated analyser Abbott Cell-Dyn 3700 AHA and compared with microscopy in a total of 250 suspected malaria cases. They found an overall sensitivity and specificity of 92.5% and 97.3% respectively. They also suggested that following an antimalarial treatment and parasite clearance, hemozoin pigment persists during the convalescent phase resulting in false positive cases. Similarly false negative cases occur in the early periods of infection where production and accumulation of hemozoin lag behind parasite proliferation.

The major limitation of the Cell-Dyn analysers is the high false positive rates due to persistence of circulating haemozoin-laden neutrophils and monocytes.[3,10,39-41]

COULTER ANALYSERS

Coulter analysers use Volume-Conductance-Scatter (VCS) technology to obtain 'positional parameters' of all WBCs.[6,7,10,33] These analysers measure

- Impedance for cell volume
- Radiofrequency conductivity for internal structure and nuclear characteristics
- Flowcytometry-based helium-neon laser light scatter analysis for cellular granularity, nuclear lobularity and cell surface structure

Studies show that malaria results in the recruitment and activation of circulating blood monocytes and lymphocytes with an increase in their size and number.[6,7,10,33] These are reflected as the heterogeneity in the volume of these cells by the scattergrams along with infected RBC's which are depicted in the non-WBC areas of the scattergram called ghost areas.[33]

Study done by Fourcade C *et al* using Coulter GEN-S hematology analyser determined lymphocyte volume standard deviation (SD) and monocyte volume SD and proposed a discriminant factor called "malaria factor" based on the differences in SD volumes of the lymphocyte and monocyte populations, to differentiate between malaria positive and malaria negative samples with an optimal cut-off value of 5.1 for a sensitivity of 96.9% and specificity of 82.5%.[6,7,10,33]

Further, Briggs *et al*[6] calculated a malaria factor of 3.7 with sensitivity and specificity of 98% and 94% respectively. By using the malaria factor ≥3.7, the absence of a WBC peak, platelet count >150 × 10^3/µL (150 × 10^9/L), eosinophil percentage >0.15%, SD volume of monocytes <23.2, and mean volume of monocytes <180, the positive predictive value (PPV) was 70% and the negative predictive value (NPV) 99.7% in detecting malaria.

Lee *et al*[28] in their study on Beckman-Coulter DxH 800™ between 2009 and 2011 proposed that P.vivax malaria samples showed characteristic, aggregated, round to upright, spindle shaped low rotated low angle light scatter (RLALS)/low axial light (AL2)/low rotated upper median angle light scatter (RUMALS) and other signals on the nucleated RBC (nRBC) plots. Further, the follow-up post treatment samples revealed disappearance of these signals. They concluded that DxH 800™ analyser provides sensitive and specific, easily recognisable P.vivax signals on routine CBC, without additional reagents or special procedures.

A study done by Shastry K *et al*[7] on Beckman-Coulter series LH 750 and 755™ found sensitivity and specificity of 97% and 89% respectively at malaria factor cut-off value of >3.4. Moreover, the relationship between variations in platelet count was inversely proportional among malaria positive and negative cases in their study.

Singh A *et al*[33] analysed the performance of Beckman-Coulter LH750 during 2013 to 2014 in a total of 200 clinically malaria suspected cases and correlated with the hematological abnormalities and scattergrams. Abnormal scattergrams were observed in all the cases of malaria. Additional abnormal WBC histogram peaks were noted in 96% cases demonstrating a peak at the threshold of histogram and differences in the patterns of these histograms and scattergrams were also noted in P.vivax and P.falciparum cases helping in differentiating the species. Limitations of the study included lack of correlation between parasitemia and hematological parameters and failure to analyse abnormal graphical changes in mixed malaria infections.

SYSMEX ANALYSERS

Routinely studied Sysmex analysers for malaria diagnosis are Sysmex XE-2100, XS-800i and XT-2000i.[3,8,24,29,42] These analysers work on the principle of flowcytometry in which flow cells scatter a beam of laser light focussed on them displaying various scattergrams.

Several studies reported the occurrence of pseudoeosinophilia in malaria cases. Pseudoeosinophilia is caused when the neutrophils containing malaria pigment are speciously counted as eosinophils because of increased granularity and plotted in the eosinophil area. Pseudoeosinophilia is reported when the difference between manual and automated differential count is >5%.[2,3,24,29,43]

Study done by Huh HJ *et al*[44] during 2006 to 2007 on Sysmex XE2100 demonstrated that samples with pseudoeosinophilia or abnormal WBC scattergrams showed significantly higher parasite counts than the samples without pseudoeosinophilia or an abnormal WBC scattergram.

Yoo JH *et al*[43] between 2006 and 2008 conducted a study in 1801 patients and concluded that the analyser is capable of detecting specific abnormalities like pseudoeosinophilia and abnormal WBC scattergrams in the blood of patients with unexpected malaria.

Mohapatra S *et al*[8] in 2010 conducted a study to compare the efficiency of hematology analyser Sysmex XE2100 with other conventional methods in detection of malarial infection in 430 cases. They concluded that although, the sensitivity of the analyser is found lesser than QBC and ICT, is comparable with the microscopy. They also proposed that normalization of scattergrams correlated with microscopic parasite negativity. The main limitation of the study is that the analyser has not been evaluated for malaria detection in endemic areas.

Jain M *et al*[3] between 2010 and 2012 conducted a study on 80 patients to determine the usefulness of ACC Sysmex XE2100 in detection of malaria in a cancer set-up in suspected cases of malaria and stated that the analyser provides significantly valuable diagnostic parameters in detecting acute P.vivax malarial infection but is not very useful in acute P.falciparum infection.

Adlekha S *et al*[24] conducted a study during 2010 to 2012 on Sysmex XS-800i analyser and correlated spurious eosinophilia with Plasmodium infection diagnosis. Using pseudoeosinophilia they found sensitivity of 61.5% and specificity of 100% and concluded that the analyser can indicate specific abnormalities even in the absence of signs and symptoms of malaria. They also observed decreased space between neutrophil and eosinophil clusters in WBC scattergram in all the cases of pseudoeosinophilia.

Study done by Sharma S *et al*[42] in 2011 on Sysmex XT-2000i found that the sensitivity in detection of malaria is increased when both abnormal WBC

scattergrams and hematological findings are considered together than only WBC scattergrams alone.

Mubeen KH *et al*[29] published their study in 2014, to evaluate the usefulness of Sysmex XE2100 and XT2000i hematology analysers in presumptive diagnosis of malaria in 2610 cases and concluded that the WBC-Basophil (WBC/BASO) scattergram abnormalities had a high sensitivity and positive predictive values in the presumptive diagnosis of Plasmodium vivax malaria when combined with thrombocytopenia.

Study done by Shariff MH *et al*[2] in 2015 on Sysmex XE-2100 in malaria diagnosis by abnormal scattergrams found an overall sensitivity and specificity of 76.88% and 67.86% respectively.

MATERIALS AND METHODS

SOURCE OF DATA:

Patients from both out-patient and in-patient departments, referred to the Department of Pathology in B.L.D.E. University's Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapur in whom peripheral smear for detection of malarial parasite was requested.

Study Period – 1st December 2014 to 30thJune 2016.

INCLUSION CRITERIA:

- All the blood samples in which peripheral smear for detection of malarial parasite was requested.

EXCLUSION CRITERIA:

- Blood samples showing platelet clumps and nucleated RBCs on Sysmex XN1000 hematology analyser were excluded.

METHOD OF COLLECTION OF DATA:

A prospective cross-sectional study was carried out in the blood samples of cases satisfying the inclusion and exclusion criteria. Under aseptic precautions, 2ml of venous blood sample was collected from the antecubital vein and immediately mixed in K3 EDTA (Tripotassium EDTA) vacutainers by gentle inversion. The samples were analysed within 1 hour of collection. These samples were processed on Sysmex XN1000 automated hematology analyser and analysed for WBC – DIFF scatter plot (WDF) abnormalities to detect malaria. Procedures for quality control and quality assurance were followed during the entire period of this study.

Thin blood smears were then prepared for all the samples included in the study, stained by Leishman stain and examined for malarial parasite. The result was correlated with WBC-DIFF scatter plots.

Sysmex XN1000

FIGURE 1: AUTOMATED HEMATOLOGY ANALYSER SYSMEX XN1000

Sysmex XN1000 is a six-part automated hematology analyser which works on the principle of flowcytometry. It uses laser flowcytometry for counting of blood

cells. Depending on the cellular properties different intensities of signals are collected and scattergrams of respective measuring channels are generated which are used for classification of cells and flagging of abnormal population. The semiconductor red-diode laser light passing through the blood sample is scattered in three different aspects to find different leukocyte population[2,3,8,10,24,29]

- Forward scatter (FSC) measures cell size
- Side-scatter (SSC) determines granularity of the internal structure
- Side-fluorescence (SFL) provides information about the nuclear content

The various channels include white cell nucleated channel (WNR), white cell differential channel (WDF), white cell precursor channel (WPC) and fluorescent platelet channel (PLT-F).

WDF CHANNEL

This channel is primarily for classifying WBCs and displays groups of lymphocytes, monocytes, neutrophils with basophils, eosinophils and debris. By flowcytometry method, a two-dimensional scattergram is plotted with SSC representing X-axis and SFL representing the Y-axis.

Eosinophils are more granular and have less nuclear material compared to the neutrophils. Hence in the normal DIFF scatter plot, they are placed to the right of neutrophils.[2]

Birefringent HZ pigment is capable of scattering laser light and hence shows abnormal scattergrams.[2] Depending on the size, nucleus and the content of the pigment in the parasite; their appearance is noted in the WBC-DIFF scatter plot in the area of neutrophil and eosinophil resulting in various abnormalities in this channel.[2]

The various abnormalities in the WBC-DIFF scatter plot include[2,3,8,10,24,29,43]

- Abnormal blue coded events below the neutrophil - eosinophil cluster (NE cluster) in the RBC – ghost region

The reason for this abnormality could be due to the presence of extracellular pigment and RBC lysis which are reflected in RBC ghost region and causing its right shift.[42] Further, the infected RBCs and reticulocytes have significant increase in the nucleic acid content which appear in the RBC ghost area as blue coded events.[3]

- Merging of the neutrophil – eosinophil cluster / decreased space between NE cluster
- Double neutrophil cluster
- Double eosinophil cluster

These abnormalities are because of the birefringent HZ containing neutrophils which appear in the eosinophil area due to increase in side scatter. These pigment containing neutrophils are falsely counted as eosinophils forming double eosinophil or double neutrophil cluster.[2,3] Another possibility could be due to the fusion of the blue coded events with the eosinophil or neutrophil cluster.[3]

- Irregular neutrophil cluster
- Gray zone
- Combination of above patterns

STASTICAL ANALYSIS

Tabulation of data was done using Microsoft Excel software. Statistical analysis of the data was done using

- Sensitivity
- Specificity
- Positive predictive value
- Negative predictive value

- Mean +/- SD
- Percentages and Diagrams
- Tables
- Chi-square test

A p value of <0.05 was considered statistically significant.

FIGURE 2: PERIPHERAL SMEAR SHOWING VARIOUS MORPHOLOGICAL FORMS OF PLASMODIUM VIVAX (LEISHMAN STAIN 1000X)

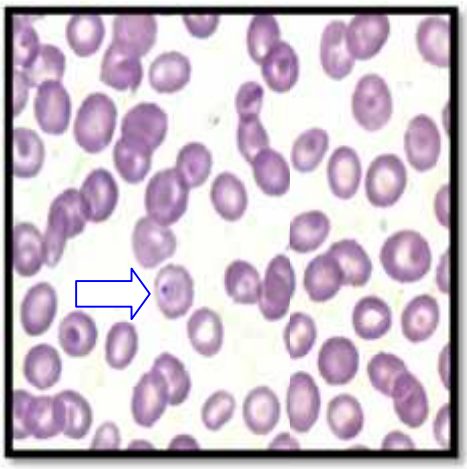


Figure 2a: Peripheral smear showing ring form of P.vivax (Leishman stain, 1000X)

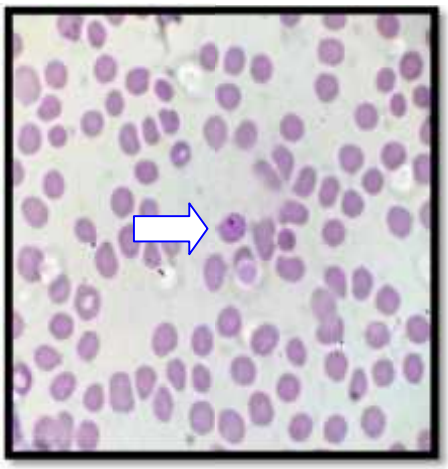


Figure 2b: Peripheral smear showing ring form of P.vivax (Leishman stain, 1000X)

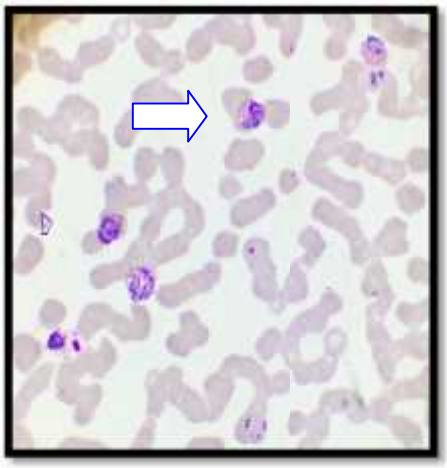


Figure 2c: Peripheral smear showing trophozoite form of P.vivax (Leishman stain, 1000X)

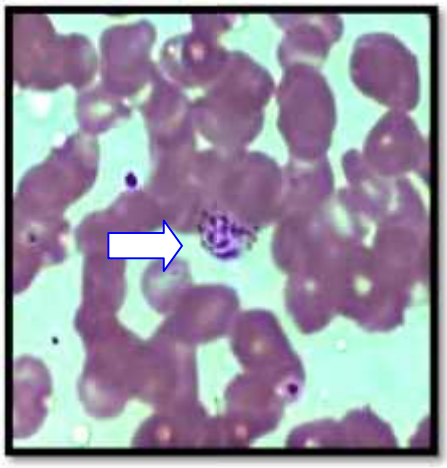


Figure 2d: Peripheral smear showing schizont form of P.vivax (Leishman stain, 1000X)

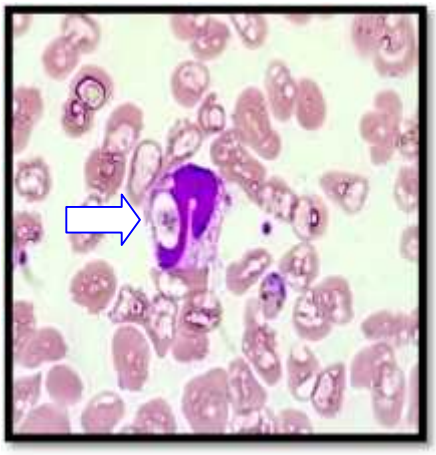


Figure 2e: Peripheral smear showing phagocytosis of schizont form of P.vivax by neutrophil (Leishman stain, 1000X)

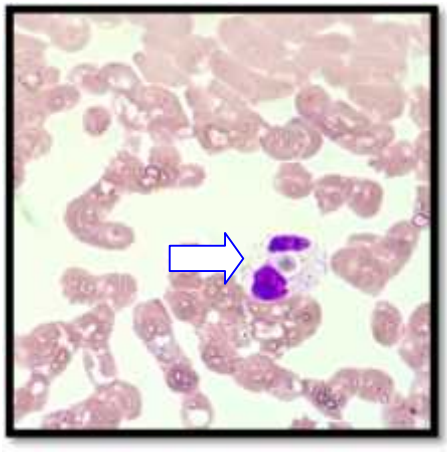


Figure 2f: Peripheral smear showing phagocytosis of hemozoin pigment by neutrophil (Leishman stain, 1000X)

FIGURE 3: PERIPHERAL SMEAR SHOWING VARIOUS MORPHOLOGICAL FORMS OF PLASMODIUM FALCIPARUM (LEISHMAN STAIN 1000X)

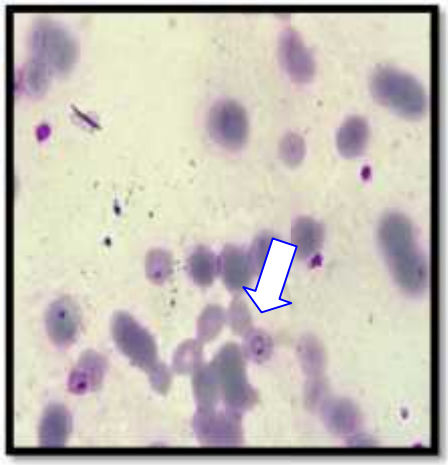


Figure 3a: Peripheral smear showing ring form of P.falciparum (Leishman stain, 1000X)

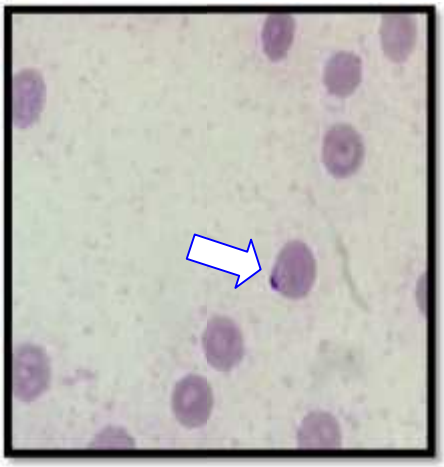


Figure 3b: Peripheral smear showing accole form of P.falciparum (Leishman stain, 1000X)

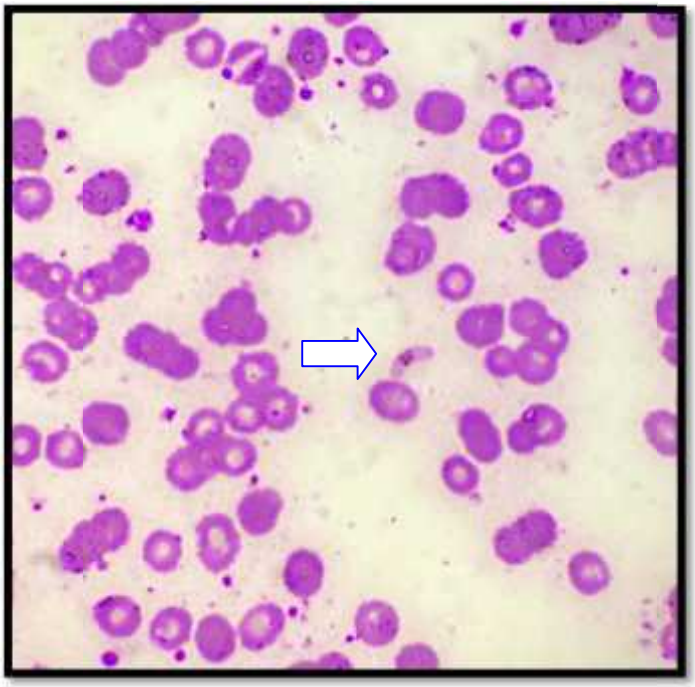


Figure 3c: Peripheral smear showing gametocyte of P.falciparum (Leishman stain, X1000)

FIGURE 4: VARIOUS SCATTER PLOT ABNORMALITIES ON SYSMEX XN1000 HEMATOLOGY ANALYSER

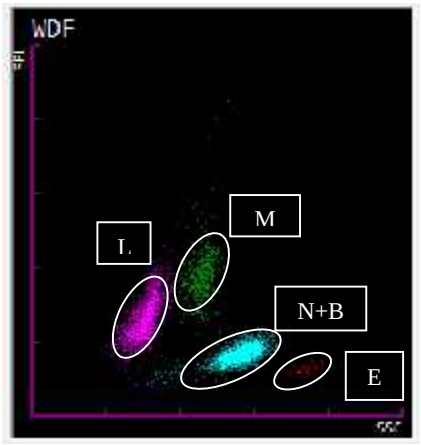


Figure 4a: Normal scatter plot (L-Lymphocyte, M-Monocyte, N+B-Neutrophil and Basophil, E-Eosinophil).

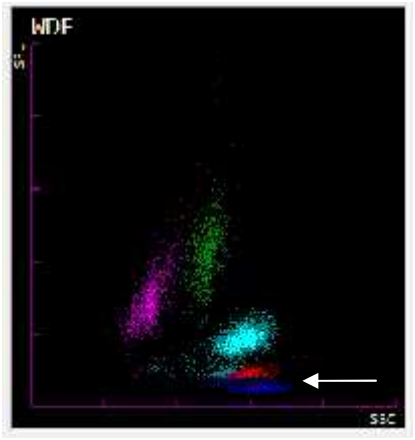


Figure 4b: Abnormal events (Blue coded events) below NE cluster

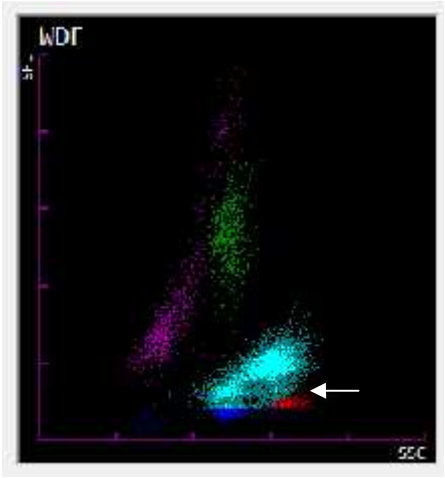


Figure 4c: Decreased space between NE cluster

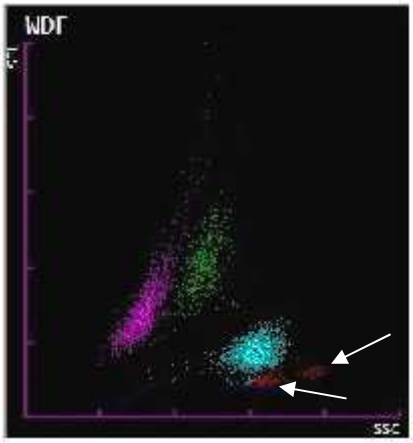


Figure 4d: Double eosinophil cluster

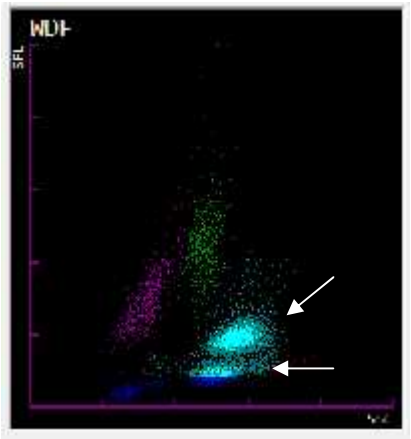


Figure 4e: Double neutrophil cluster

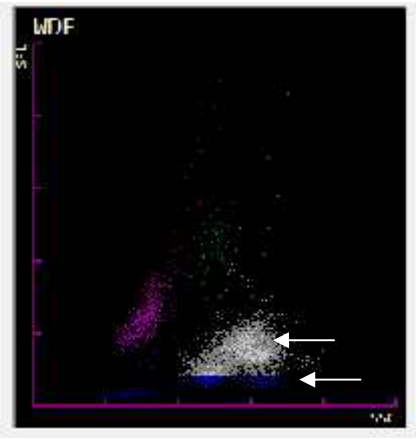


Figure 4f: Graying with abnormal blue coded events

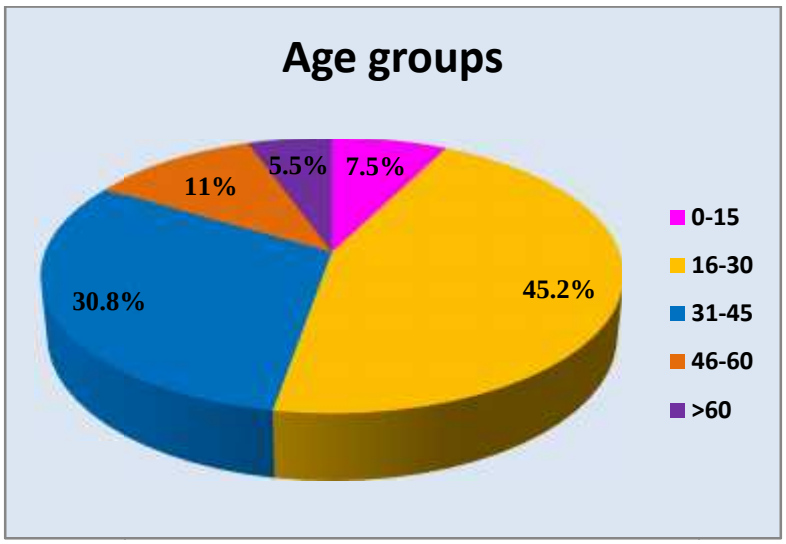
RESULTS

A total of 146 blood samples from patients in whom peripheral smear for diagnosis of malarial parasite was requested and which satisfied the inclusion and exclusion criteria were analyzed.

TABLE 3: DISTRIBUTION OF CASES BY AGE (YRS)

Age groups (Years)	N	PERCENTAGE (%)
0-15	11	7.5
16-30	66	45.2
31-45	45	30.8
46-60	16	11
>60	8	5.5
Total	146	100

FIGURE 5: PIE CHART DISTRIBUTION OF CASES BY AGE (YRS)

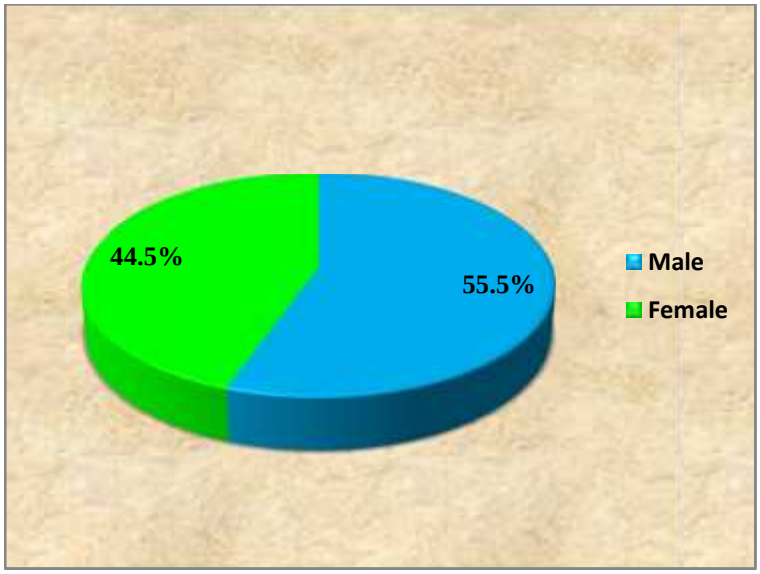


In the present study, the age ranged from 2-85 years. The majority of the patients were between 16-30 years of age comprising 45.2% of cases.

TABLE 4: DISTRIBUTION OF CASES BY SEX

SEX	N	PERCENTAGE (%)
Male	81	55.5
Female	65	44.5
Total	146	100.0

FIGURE 6: PIE CHART DISTRIBUTION OF CASES BY SEX

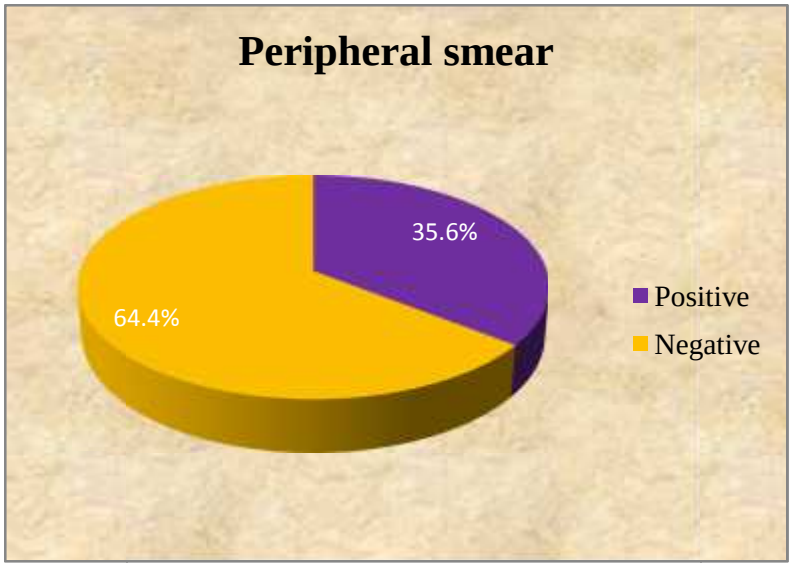


In the present study, the total number of males was 81 (55.5%) and the total number of females was 65 (44.5%).

TABLE 5: DISTRIBUTION OF MALARIA CASES DIAGNOSED BY PERIPHERAL SMEAR (PS)

PERIPHERAL SMEAR	N	PERCENTAGE (%)
Positive	52	35.6
Negative	94	64.4
Total	146	100

FIGURE 7: PIE CHART DISTRIBUTION OF MALARIA CASES DIAGNOSED BY PERIPHERAL SMEAR

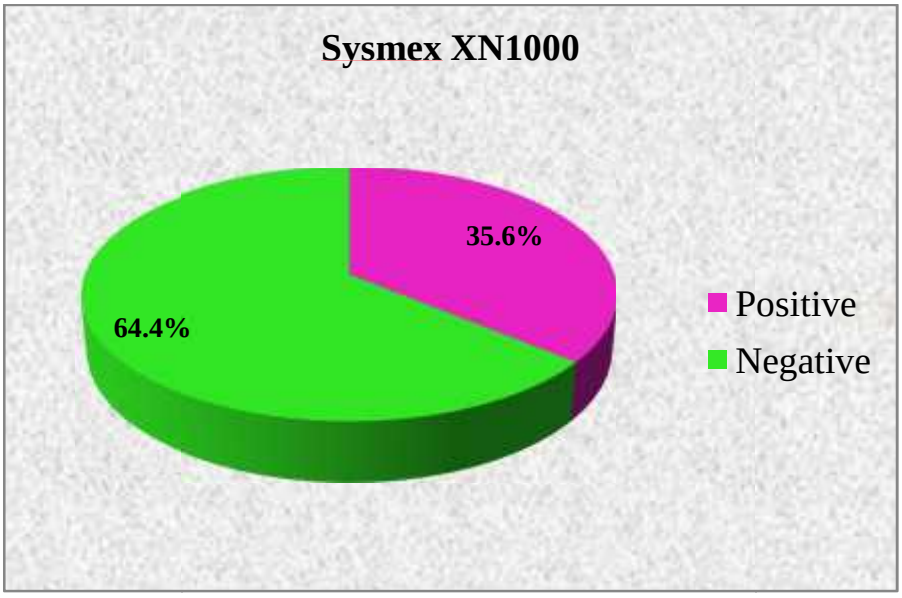


Out of 146 malaria suspicious cases, 52 cases (35.6%) were positive and 94 cases (64.4%) were negative for malaria on peripheral smear examination.

TABLE 6: DISTRIBUTION OF CASES SHOWING WBC- DIFF SCATTER PLOT ABNORMALITIES ON SYSMEX XN1000

SCATTER PLOT	N	PERCENTAGE (%)
Positive	52	35.6
Negative	94	64.4
Total	146	100

FIGURE 8: PIE CHART DISTRIBUTION OF CASES SHOWING WBC- DIFF SCATTER PLOT ABNORMALITIES ON SYSMEX XN1000



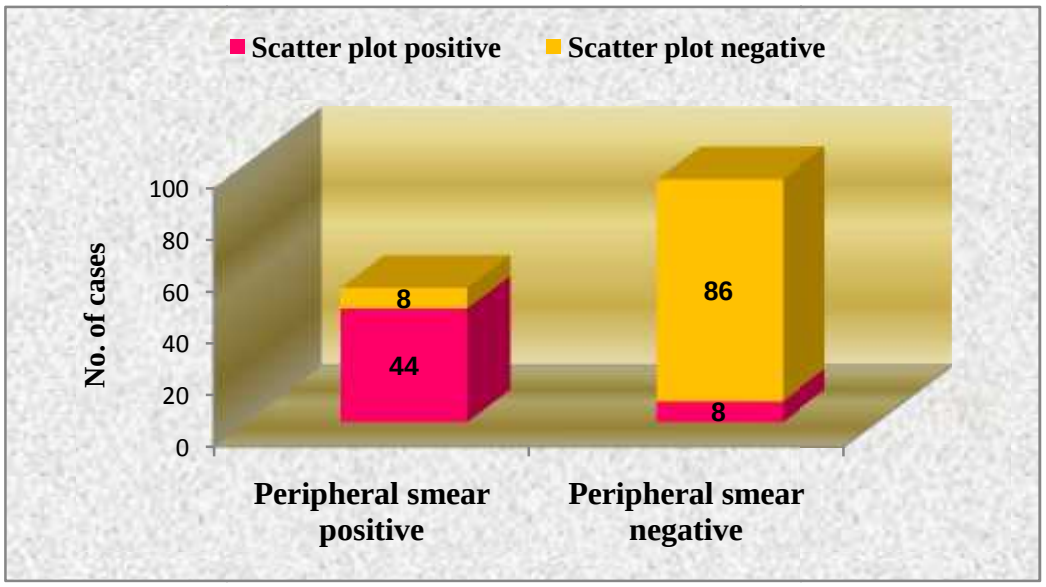
Out of the 146 cases, 52 (35.6%) were showing WBC-DIFF scatter plot abnormalities on Sysmex XN1000 hematology analyser.

TABLE 7: DISTRIBUTION OF CASES DIAGNOSED BY PERIPHERAL SMEAR AND SHOWING ABNORMAL WBC-DIFF SCATTER PLOT ON SYSMEX XN1000

TOTAL NUMBER OF CASES = 146					p value
PERIPHERAL SMEAR	POSITIVE		NEGATIVE		
	52		94		
SYSMEX XN1000	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	<0.001*
	44	8	8	86	

*significantly different at 5% level of significance

FIGURE 9: BAR GRAPH SHOWING DISTRIBUTION OF CASES DIAGNOSED BY PERIPHERAL SMEAR AND SHOWING ABNORMAL WBC-DIFF SCATTER PLOT ON SYSMEX XN1000

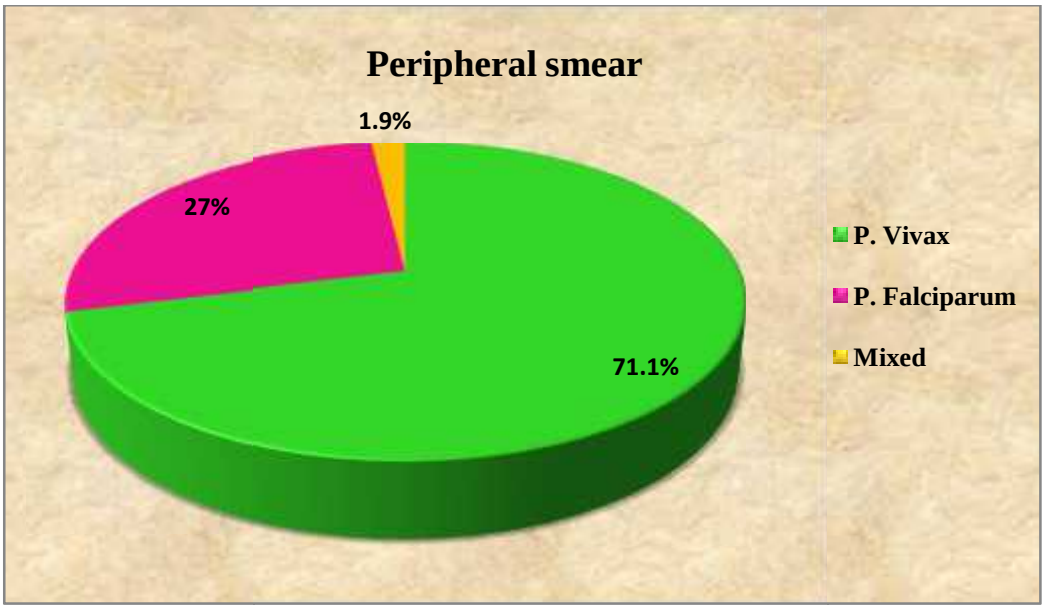


Among 52 positive cases diagnosed by peripheral smear, 44 cases were showing scatter plot abnormalities (true positive – TP) and 8 were negative (false negative – FN). Out of the 94 negative cases on peripheral smear, 8 were showing positive changes on scatter plot (false positive – FP) and the remaining 86 cases were negative for scatter plot abnormalities (true negative – TN).

TABLE 8: SPECIES CATEGORIZATION BY PERIPHERAL SMEAR EXAMINATION

PERIPHERAL SMEAR	N	PERCENTAGE (%)
P. Vivax	37	71.1
P. Falciparum	14	27
Mixed	1	1.9
Total	52	100

FIGURE 10: PIE CHART SHOWING SPECIES CATEGORIZATION BY PERIPHERAL SMEAR EXAMINATION

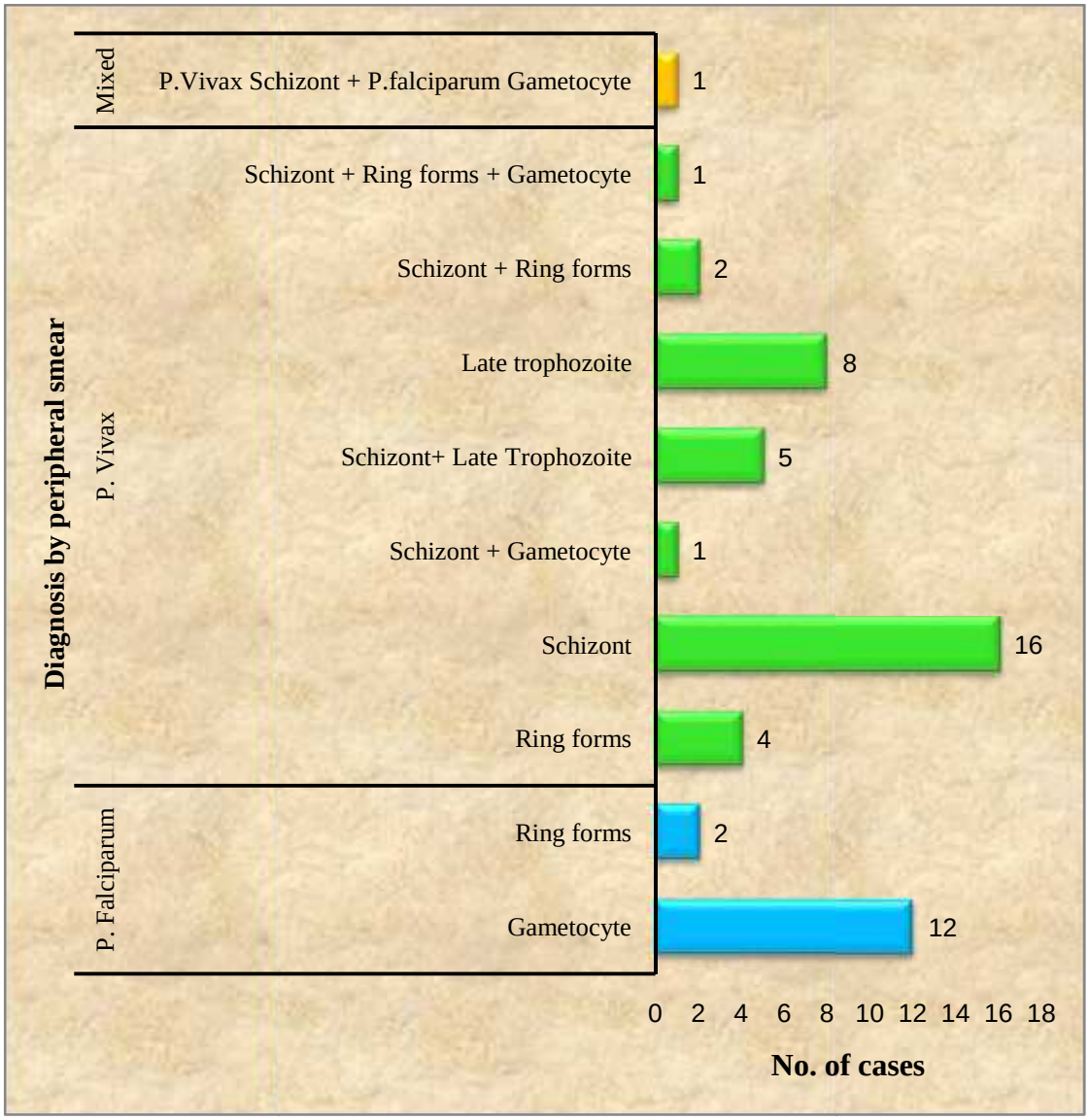


Out of the 52 positive cases, 37 (71.1%) were P.vivax species, 14 cases (27%) were P.falciparum and one case (1.9%) was mixed infection (P.vivax and P.falciparum).

TABLE 9: DISTRIBUTION OF CASES DIAGNOSED BY PERIPHERAL SMEAR BASED ON MORPHOLOGICAL FORMS

Peripheral Smear		N	Percentage (%)
P. vivax	Ring forms	4	7.7
	Schizont	16	30.8
	Schizont + Gametocyte	1	1.9
	Schizont + LateTrophozoite	5	9.6
	Late trophozoite	8	15.4
	Schizont + Ring forms	2	3.8
	Schizont + Ring forms + Gametocyte	1	1.9
P. falciparum	Gametocyte	12	23.0
	Ring forms	2	3.8
Mixed	P.vivax Schizont + P.falciparum Gametocyte	1	1.9
Total		52	100

FIGURE 11: BAR GRAPH SHOWING DISTRIBUTION OF CASES DIAGNOSED BY PERIPHERAL SMEAR BASED ON MORPHOLOGICAL FORMS



Out of the 52 cases diagnosed by peripheral smear, P.vivax comprised 4 (7.7%) ring forms, 16 (30.8%) schizonts, 8 (15.4%) late trophozoites and 9 (17.2%) combined forms. Among P.falciparum, out of 14 positive cases, 12 (23%) were gametocytes and 2 (3.8%) were ring forms. One case of mixed infection (1.9%) showed schizont of P.vivax and gametocyte of P.falciparum.

TABLE 10: DISTRIBUTION OF MALARIA POSITIVE CASES DIAGNOSED BY PERIPHERAL SMEAR AND SHOWING ABNORMALITIES ON WBC-DIFF SCATTER PLOT WITH RESPECT TO SPECIES

TOTAL NUMBER OF POSITIVE CASES = 52							
Peripheral smear	P.vivax		P.falciparum		Mixed		p value
	37		14		1		
Scatter plot	Positive	Negative	Positive	Negative	Positive	Negative	<0.001*
	32	5	11	3	1	0	

*significantly different at 5% level of significance

FIGURE 12: BAR GRAPH PRESENTATION SHOWING DISTRIBUTION OF CASES DIAGNOSED BY PERIPHERAL SMEAR AND ABNORMALITIES ON WBC-DIFF SCATTER PLOT WITH RESPECT TO SPECIES

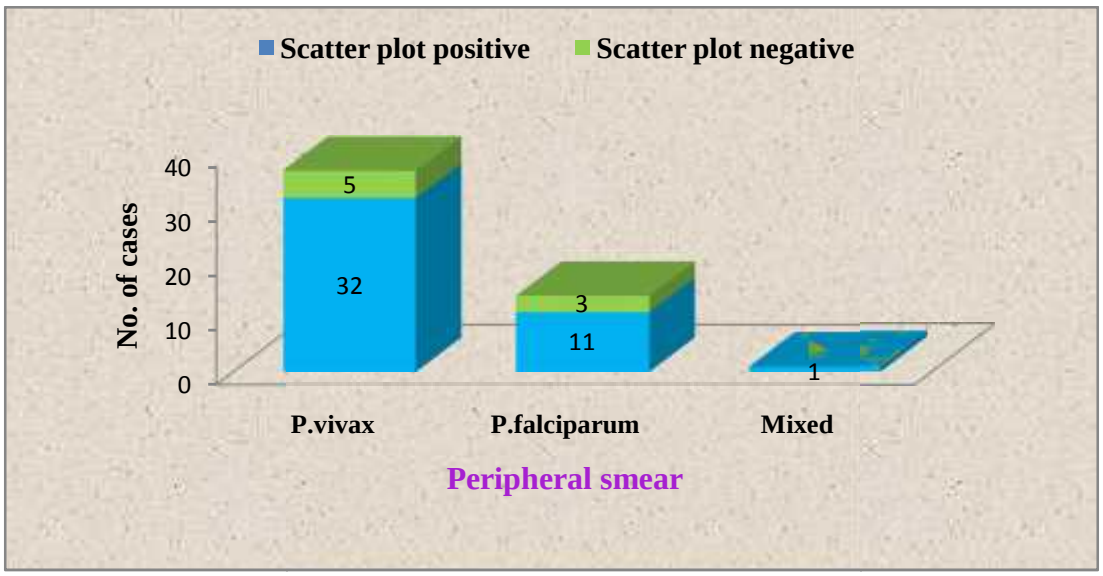
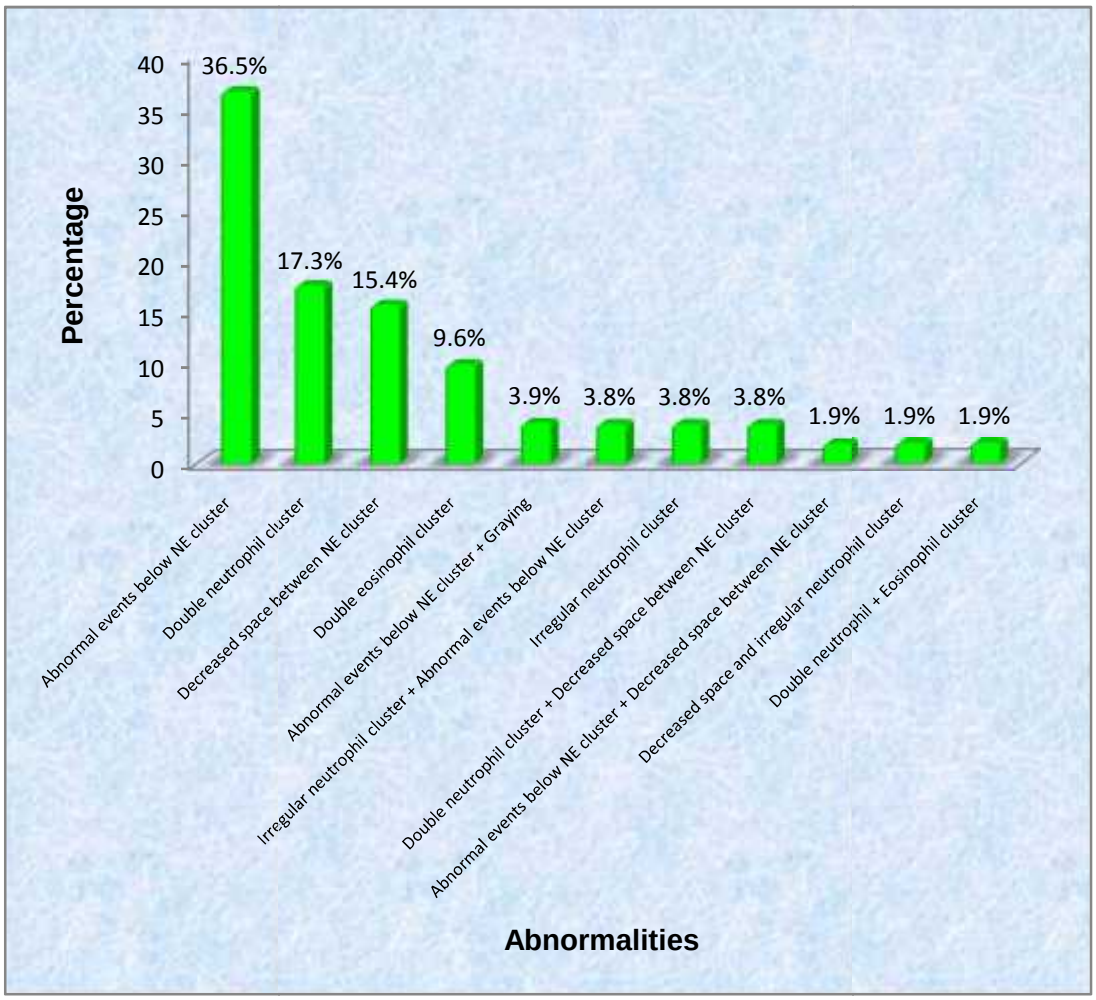


TABLE 11: DISTRIBUTION OF WBC-DIFF SCATTER PLOT ABNORMALITIES ON SYSMEX XN1000 HEMATOLOGY ANALYSER

ABNORMALITIES	N	Percentage (%)
Abnormal events below NE cluster	19	36.5
Double neutrophil cluster	9	17.3
Decreased space between NE cluster	8	15.4
Double eosinophil cluster	5	9.6
Irregular neutrophil cluster	2	3.8
Abnormal events below NE cluster + Graying	2	3.9
Irregular neutrophil cluster + Abnormal events below NE cluster	2	3.8
Double neutrophil cluster + Decreased space between NE cluster	2	3.8
Abnormal events below NE cluster + Decreased space between NE cluster	1	1.9
Decreased space and irregular neutrophil cluster	1	1.9
Double neutrophil + Eosinophil cluster	1	1.9
Total	52	100

FIGURE 13: BAR GRAPH SHOWING DISTRIBUTION OF WBC-DIFF SCATTER PLOT ABNORMALITIES ON SYSMEX XN1000 HEMATOLOGY ANALYSER



The most common abnormality in the present study was abnormal blue coded events below the NE cluster (36.5%) followed by double neutrophil cluster (17.3%).

TABLE 12: CORRELATION OF WBC-DIFF SCATTER PLOT

ABNORMALITIES WITH PERIPHERAL SMEAR DIAGNOSIS

ABNORMALITIES ON SCATTER PLOT	**POSITIVE ON PS**	**NEGATIVE ON PS**
Abnormal events below NE cluster	18	1
Abnormal events below NE cluster + Graying	2	0
Abnormal events below NE cluster + Decreased space between NE cluster	1	0
Decreased space and irregular neutrophil cluster	0	1
Decreased space between NE cluster	6	2
Double eosinophil cluster	4	1
Double neutrophil cluster	8	1
Double neutrophil cluster + Decreased space between NE cluster	2	0
Double neutrophil + Eosinophil cluster	1	0
Irregular neutrophil cluster + Abnormal events below NE cluster	2	0
Irregular neutrophil cluster	0	2
Total	44	8

FIGURE 14: BAR GRAPH PRESENTATION SHOWING CORRELATION OF WBC-DIFF SCATTER PLOT ABNORMALITIES WITH PERIPHERAL SMEAR DIAGNOSIS

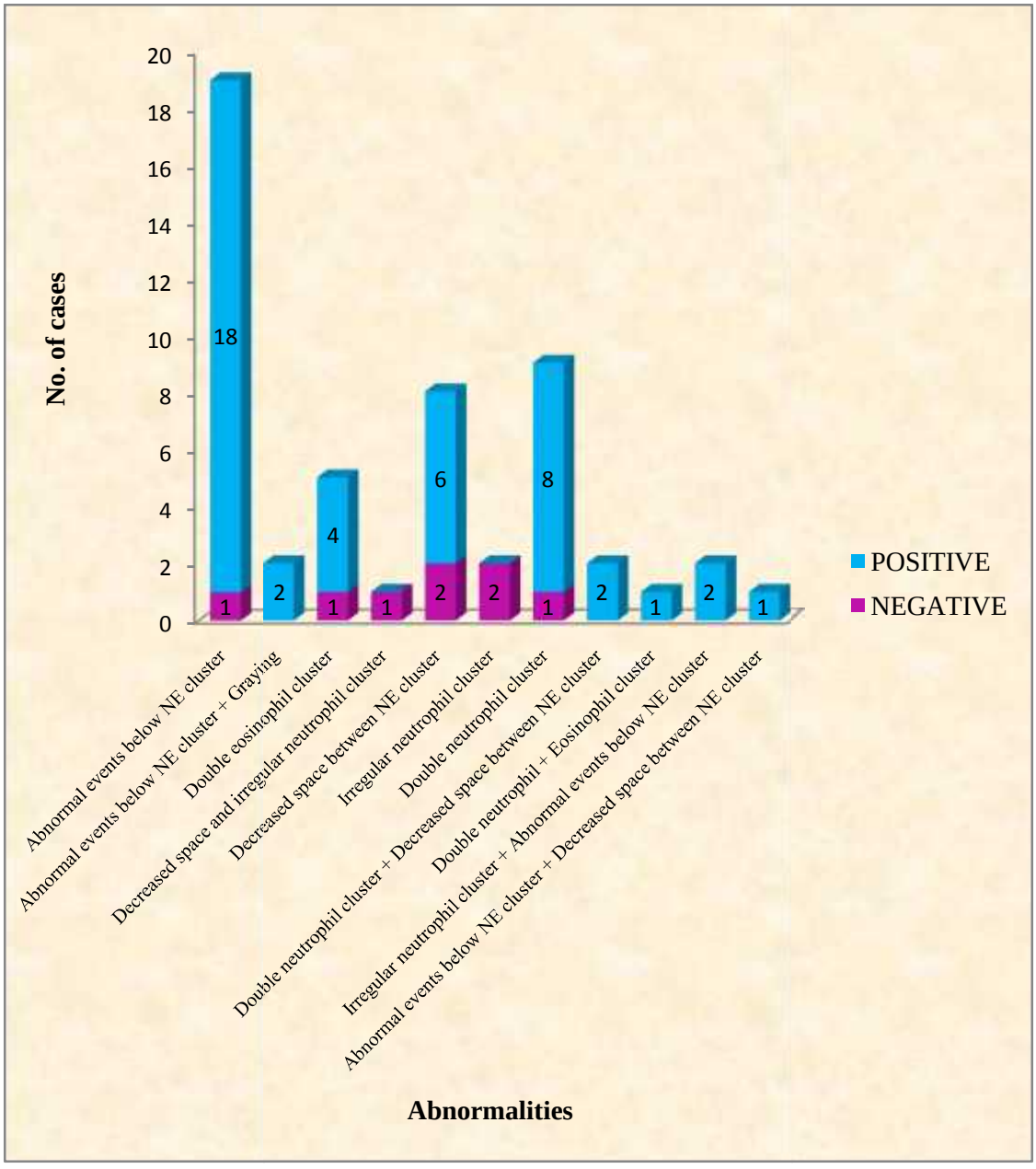
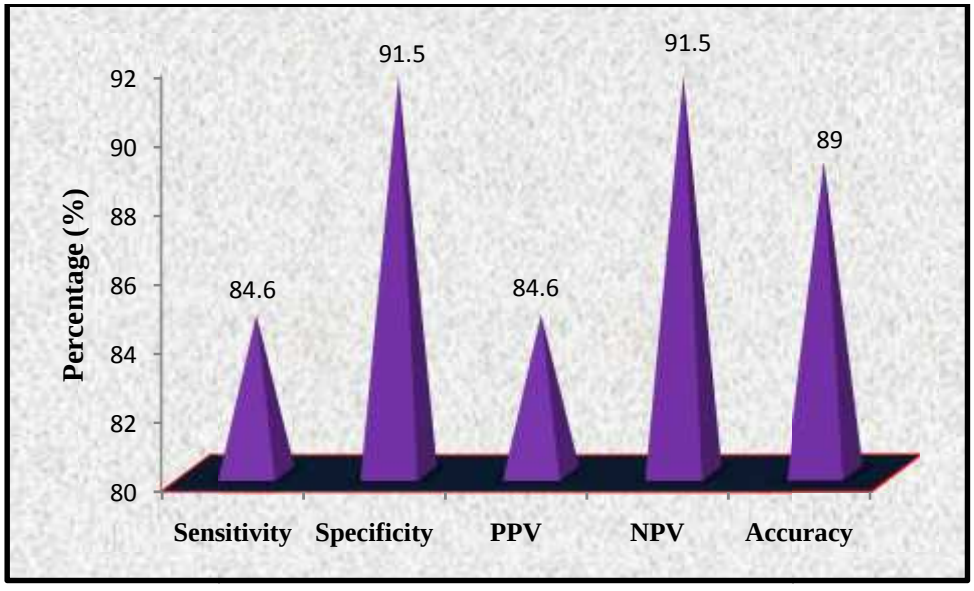


TABLE 13: SENSITIVITY ANALYSIS OF SYSMEX XN1000 WITH COMPARISON TO PERIPHERAL SMEAR AS GOLD STANDARD

Truth table:

TP (No. of cases)	44
FN (No. of cases)	8
FP (No. of cases)	8
TN (No. of cases)	86
Sensitivity (%)	84.6
Specificity (%)	91.5
PPV (%)	84.6
NPV (%)	91.5
Accuracy (%)	89.0

FIGURE 15: PYRAMID PRESENTATION OF SENSITIVITY AND SPECIFICITY ANALYSIS OF SYSMEX XN1000



In the present study, the sensitivity and specificity of Sysmex XN1000 in detection of malaria was 84.6% and 91.5% respectively considering peripheral smear as the gold standard. The accuracy of the analyser in the diagnosis of malaria was 89.0%. Furthermore, the positive predictive value (PPV) was 84.6% and the negative predictive value was 91.5%.

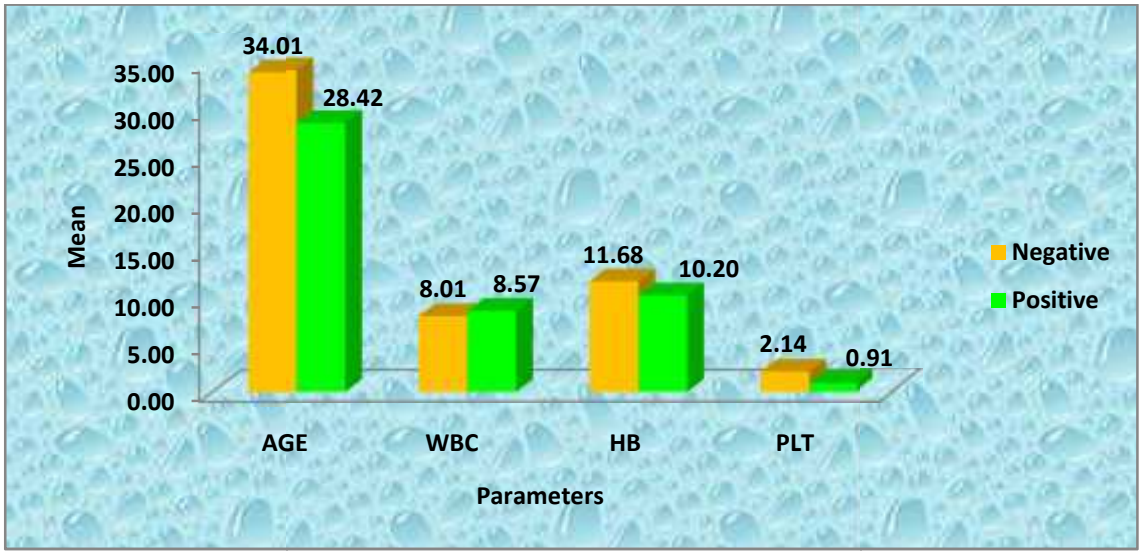
OTHER FINDINGS

TABLE 14: COMPARISON OFAGE, HEMOGLOBIN, WBC AND PLATELET COUNTS BETWEEN MALARIA POSITIVE AND NEGATIVE CASES

Parameters	Negative		Positive		p value
	Mean	SD	Mean	SD	
AGE (Y)	34.0	17.1	28.4	14.1	
WBC (Cells/mm^3)	8010.9	4543.7	8569.4	7341.2	0.571
HB (g/dl)	11.7	3.2	10.2	2.7	0.006*
PLT (Lakhs/ mm^3)	2.1	1.1	0.9	0.8	<0.001*

*significantly different at 5% level of significance

FIGURE 16: BAR GRAPH SHOWING COMPARISON OFAGE, HEMOGLOBIN, WBC AND PLATELET COUNTS BETWEEN MALARIA POSITIVE AND NEGATIVE CASES

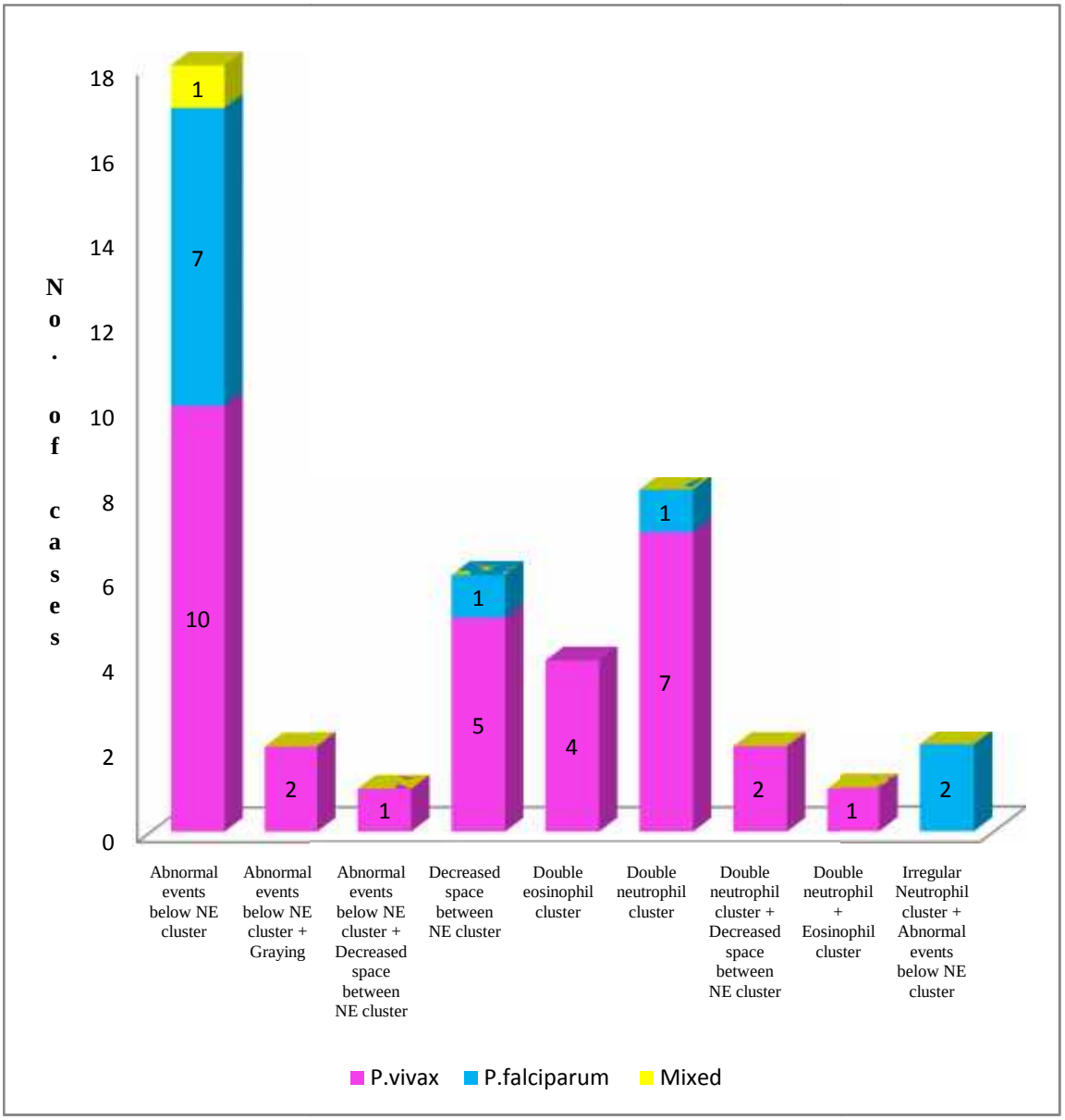


The hemoglobin (p=0.006) and platelet (p=<0.001) values showed significant differences between malaria positive and malaria negative cases.

TABLE 15: DISTRIBUTION OF ABNORMALITIES ON SCATTER PLOT WITH RESPECT TO SPECIES CATEGORIZATION BY PSE

ABNORMALITIES	**P.vivax**	**P.falciparum**	**Mixed**
Abnormal events below NE cluster	10	7	1
Abnormal events below NE cluster + Graying	2	0	0
Abnormal events below NE cluster + Decreased space between NE cluster	1	0	0
Decreased space between NE cluster	5	1	0
Double eosinophil cluster	4	0	0
Double neutrophil cluster	7	1	0
Double neutrophil cluster + Decreased space between NE cluster	2	0	0
Double neutrophil + Eosinophil cluster	1	0	0
Irregular Neutrophil cluster + Abnormal events below NE cluster	0	2	0
Total	32	11	1

FIGURE 17: BAR GRAPH SHOWING DISTRIBUTION OF ABNORMALITIES ON SCATTER PLOT WITH RESPECT TO SPECIES DIAGNOSED BY PSE



The most common WBC-DIFF scatter plot abnormality in both P.vivax and P.falciparum infections were abnormal blue coded events below NE cluster which comprised 10 out of 32 cases in P.vivax and 7 out of 11 cases of P.falciparum malaria respectively.

DISCUSSION

Routine CBC analysis is a preliminary investigation of any febrile case. Diagnosis of malaria by peripheral smear examination is the universally accepted paradigm but the limitations of this method led to the development of several new techniques that simplify and speed up malaria diagnosis with increased sensitivity.

There is an emerging interest in the presumptive diagnosis of malaria by WBC scatter plots and histograms during routine CBC analysis using automated hematology analysers. Consequently, this advanced diagnostic tool has been validated by various studies and is considered important particularly in cases where clinical request is not sent.

In the present study, efficacy of Sysmex XN1000 hematology analyser in detection of malaria which is based on WBC-DIFF scatter plots was assessed in 146 suspicious cases of malaria with an accuracy of 89%.

TABLE 16: COMPARISON OF RESULTS OF VARIOUS STUDIES WITH THE PRESENT STUDY

	Present study	**Sharma S *et al*[42]**	**Shariff MH *et al*[2]**	**Jain M *et al*[3]**	**Mohapatra S *et al*[8]**	**Yoo JH *et al*[43]**	**Huh HJ *et al*[44]**	**Shastry KI *et al*[7]**	**Singh A *et al*[33]**	**Lee HK *et al*[28]**	**Hanscheid T *et al*[34]**	**Baig MA[45]**	**de Langen AJ[35]**	**Josephine FP *et al*[32]**	**Suh IB *et al*[31]**	**Ali SF *et al*[30]**	**Mendelow *et al*[36]**
Analyser	Sysmex XN1000	Sysmex XT2000i	Sysmex XE-2100	Sysmex XE-2100	Sysmex XE-2100	Sysmex XE-2100	Sysmex XE-2100	LH 750 & LH 785™	LH 750	DxH 800™	Cell-Dyn 3500	Cell-Dyn Ruby	Cell-Dyn 3700	Cell-Dyn 4000	Cell-Dyn 4000	Cell-Dyn 3700	Cell-Dyn 3500
PS positive	52/146	148/2251	201/5601	39/80	49/430	413/1801	144/487	90/310	200/200	52/1761	57/174	175/275	90/208	16/889	68/168	37/250	95/224
Analyser positive	52/146	233/2251	179/5601	32/80	52/430	65/1801	75/487	109/310	200/200	52/1761	66/174	171/275	84/208	16/889	62/168	43/250	73/224
Sensitivity	84.6%	83.7%	76.8%	82%	74.2%	15.7%	52.1%	97%	100%	100%	95%	94.9%	93%	100%	91.%	92.5%	72%
Specificity	91.5%	94.8%	67.8%	100%	91.1%	99.7%	100%	89%	100%	100%	88%	95%	97%	100%	100%	97.3%	96%

The sensitivity (84.6%) and specificity (91.5%) of the present study correlated well with the other studies done on Sysmex series analysers, especially with the studies done by Sharma *et al*[42] and Jain M *et al*.[3]

The differences in the kinetics of pigment-containing WBCs among different populations depend on the host immunity factors attributing to variations in the results among various studies.[44,46,47]

The disparity in the results could also be due to the differences in the working principle of various analysers and also the discrepancies in sample size in the respective studies.

A study done by Singh A *et al*[33] by using Beckman Coulter LH-750 observed differences in the patterns of WBC histograms apart from abnormal WBC scattergrams in P.vivax and P.falciparum cases and stated that these graphical patterns can help in differentiating the malaria species.

Josephine FP *et al*[32] and Scott CS *et al*[38] separately conducted studies on Cell-Dyn 4000 and found out that different plasmodium species show different depolarization patterns. P.vivax showed abnormal depolarizing green or black color-coded events with absence of depolarizing purple events in the NEU-EOS plot and a distinct cluster with a low size signature in the EOS I plot. Few random purple and green events in the NEU-EOS and EOS I plots were seen in P.falciparum infection. P.malariae infection showed green, black or blue color-coded random depolarization events in the NEU-EOS and EOS I plots.

TABLE 17: COMPARISON OF VARIOUS ABNORMALITIES OF THE SCATTER PLOT ON SYSMEX XN1000 WITH OTHER STUDIES

	Extended neutrophil/ decreased space between NEC	Double neutrophil	Irregular neutrophil cluster	Double eosinophil	Grey zone	Combination patterns	Prominent blue coded events
Present study (%)	8 (15.4%)	9 (17.3%)	2 (3.8)	5 (9.6%)	-	9 (17.3%)	19 (36.5%)
Mohapatra S *et al*[8] (%)	29 (58%)	1 (2%)	-	5 (10%)	7 (14%)	8 (16%)	-
Shariff MH *et al*[2] (%)	97 (54.2%)	39 (21.8%)	-	-	-	21 (11.7%)	-
Adlekha S *et al*[24] (%)	24 (100%)	-	-	-	-	-	-

In the present study, the most common WBC-DIFF scatter plot abnormality was prominent blue coded events (abnormal events) below NE cluster (36.5%). The next common abnormalities include the double neutrophil cluster and combination patterns (each comprising 17.3%) followed by decreased space between the neutrophil and eosinophil cluster (15.4%).

These findings are in contrast with the other studies wherein the most common abnormality was extended neutrophil with decreased space between the neutrophil and eosinophil cluster.

Study done by Jain M *et al*[3] also noted the occurrence of the abnormal neutrophil cluster similar to the present study. The four of the eight cases of P.falciparum showed abnormal WBC-DIFF channel in the form of prominent blue coded events below the NE cluster.

In a study done by Sharma S *et al*[42] on Sysmex XT2000i, apart from the above discussed WBC-DIFF scatter plot abnormalities, double lymphocyte population was also noted. Out of 129 positive scatter plot abnormalities in malaria positive cases, one showed double lymphocyte population (0.78%) in their study. They also noted abnormal blue coded events below the NE cluster comprising 32.4%.

False negative analysis

In the present study, out of 52 malaria cases, 8 cases were not showing any WBC-DIFF scatter plot abnormalities. Four out of these eight cases showed early ring forms on peripheral smear examination. In the remaining four cases, two showed occasional schizonts of P.vivax, one was gametocytes of P.falciparum and the other case showed schizonts and trophozoites forms of P.vivax.

Table 18: Malaria positive cases showing no abnormality on scatter plot

SPECIES	MORPHOLOGICAL FORM	NUMBER
Plasmodium vivax	Ring forms	2
	Schizonts	2
	Schizonts + Trophozoites	1
Plasmodium falciparum	Ring forms	2
	Gametocytes	1

In a study by Jain M *et al*[3] on Sysmex XE-2100 automated cell counter, three out of 31 cases of P.vivax and four out of eight cases of P.falciparum showed only

early ring forms which were negative for the WBC-DIFF scatter plot abnormalities. They stated that the scatter plot abnormalities were not linked to the parasitic index but to the presence of schizonts, trophozoites and ring forms. Late trophozoites, schizonts and gametocytes positively correlated with WBC-DIFF scatter plot abnormalities in comparison to ring forms.[3]

This could be attributed to the lesser amount of the hemozoin pigment in the ring forms compared to the schizonts and gametocytes. Therefore, more the amount of hemozoin pigment within the cells, greater is the chance of scattering the birefringent HZ by the laser light and producing abnormal WBC-DIFF scatter plot.

Similar findings were observed by Shastry K *et al*[7] on Beckman-Coulter series LH750 and 785^{TM} wherein 4.4% of false negative rate was seen in cases of P.vivax infection, one of which was due to a low event and the rest were due to random error. No false negative cases were noted with P.falciparum infection.

False positive analysis

In our study, out of the 52 cases of abnormal WBC-DIFF scatter plots, 8 cases were negative for malaria by peripheral smear examination. The reason for this false positivity could be due to contamination of the blood sample or presence of other pathological conditions.

Study done by Baig MA[45] on Cell-Dyn Ruby hematology analyser stated that sulphonamide - derived medications form insoluble granules interfere with the light scattering property of the analyser giving false positive results.

In a study by Mubeen KH[29] abnormal blue coded events were observed in three cases which were negative for malaria on peripheral smear. Two of these cases were hemolytic disease of newborn and one case had thalassemia.

False positive cases of 10.5% in a study done by Shastry K *et al*[7] on Beckman-Coulter series LH750 and 785^{TM} were associated with bacterial or viral infection, due to increase in the volumes of lymphocytes and monocytes.

Finally, it is worth mentioning that abnormalities in the DIFF scatter plot and WBC counts of the Sysmex XE-2100 were also seen in samples with Candida species.[10]

- **Re-evaluation of peripheral smear**

In the present study, the cases which were negative on the peripheral smear examination but positive on the scatter plot were re-evaluated for malarial parasite by manual method and eventually one case was found to be positive for malaria. Hence, it is advised for all the pathologists to be very careful in dealing with such cases before the final report is given as negative for malarial parasite.

Similar findings were observed by Jain M *et al*[3], wherein ten cases were incidentally diagnosed from the scatter plot findings which were confirmed by peripheral smear examination.

Further, study by Mubeen KH *et al*[29] also found five cases which were initially negative for malaria by peripheral smear but showing scattergram abnormality were diagnosed as positive for malaria after repeat smear examination.

CONCLUSION

Analysis of specific patterns of WBC scatter plots is a rapid and easy process which requires less expertization and can be used as an adjunct to peripheral smear in detection of malaria. Without the knowledge of these scatter plots, the pathologist might miss the parasite by routine manual examination of smear. By correlating the scatter plot abnormalities with the peripheral smear examination, the hematopathologist can review the slide again even if the parasite is missed in the initial screening. So it is recommended for the pathologists to analyze the WBC scatter plots on a routine basis to pick up malarial infection even in the absence of a explicit clinical request.

The major benefit is that this approach is achieved from an unmodified technology which is designed for a different application of CBC evaluation; that is to say that the scatter plot based detection of malaria can be carried out without any changes in the settings of the analyser.

It would be even more helpful if the manufacturers come up with software modification within the analysers to generate malaria specific suspect flagging or interpretive message which can highlight abnormal scatter plot events offering an additional merit to these analysers and thereby increasing the sensitivity.

SUMMARY

- This study was undertaken in B.L.D.E. University's Shri B.M.Patil Medical College, Hospital and Research center, Vijayapur, Karnataka to study the efficacy of Sysmex XN1000 automated hematology analyser in detection of malaria.
- A total of 146 malaria suspicious cases were included in the study.
- Sysmex XN1000 is a flowcytometry based automated hematology analyser capable of suspecting malaria by generating WBC scatter plots which show specific abnormalities in different areas of the scatter plot.
- The malaria positive cases on the peripheral smear examination were 52 out of 146 cases. Out of these 52 cases, abnormal WBC-DIFF scatter plots on Sysmex XN1000 were noted in 44 cases.
- The most common WBC-DIFF scatter plot abnormality was the abnormal blue coded events below the neutrophil-eosinophil (NE) cluster comprising 36.5%.
- In the present study, Sysmex XN1000 automated hematology analyser could detect malaria with a sensitivity and specificity of 84.6% and 91.5% respectively.
- The present study also noted that there was a significant difference in the hemoglobin value and the platelet count between the malaria positive and malaria negative cases (p value <0.05).
- Therefore, automated hematology analysers help in early diagnosis of malaria thereby reducing the mortality and morbidity.

LIMITATIONS

- The inability of the Sysmex XN1000 to detect ring forms of the parasite.
- Use of Sysmex XN1000 analyser in detection of malaria is only an adjunct to manual method and all the cases showing abnormal scatter plot should be confirmed by peripheral smear examination or other conventional methods.

BIBLIOGRAPHY

1) Rodulfo H, De Donato M, Mora R, González L, Contreras CE. Comparison of the diagnosis of malaria by microscopy, immunochromatography and PCR in endemic areas of Venezuela. Braz J Med Biol Res 2007;40:535-43.
2) Shariff MH, Muzamil Dar A, Vidya P. Malaria diagnosis by abnormal scattergrams in automated hematology analyzer. Int J Pharm Biol Sci 2016;6:55-9.
3) Jain M, Gupta S, Jain J, Grover RK. Usefulness of automated cell counter in detection of malaria in a cancer set up-Our experience. Indian J Pathol Microbiol 2012;55:467-73.
4) Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria diagnosis: A brief review. Korean J Parasitol 2009;47:93-102.
5) WHO: World malaria report 2015. Geneva, World Health Organization, 2015. [Accessed 1st August 2016]. Available from: URL; http://www.who.int/malaria/publications/world-malaria-report-2015/en/.
6) Briggs C, Costa AD, Freeman L, Aucamp I, Ngubeni B, Machin S. Development of an automated malaria discriminant factor using VCS technology. Am J Clin Pathol 2006;126:691-8.
7) Shastry IK, Nayak DM, Manohar C, Belurkar SV, Mohanty A. Malaria detection by automation: The Manipal experience. Sch J App Med Sci 2015;3:1778-82.
8) Mohapatra S, Samantaray JC, Arulselvi S, Panda J, Munot K, Saxena R. Automated detection of malaria with haematology analyzer Sysmex XE-2100. Indian J Med Sci 2011;65:26-31.

9) Means RT, Glader B. Acquired Nonimmune Hemolytic Disorders. In: Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F, Rodgers GM (Eds). Wintrobe's Clinical Hematology. 13th ed. Philadelphia: Lippincott Williams & Wilkins;2013. p1846-54.

10) Campuzano-Zuluaga G, Hänscheid T, Grobusch MP. Automated haematology analysis to diagnose malaria. Malaria J 2010;9:346-60.

11) Hanscheid T, Pinto BG, Pereira I, Cristino M, Valadas E. Avoiding misdiagnosis of malaria: A novel automated method allows specific diagnosis, even in the absence of clinical suspicion. Emerg Infect Dis. 1999;5:836-7.

12) Cox F. History of the discovery of the malaria parasites and their vectors. Parasites & Vectors 2010;3:5-13.

13) White NJ, Breman JG. Malaria. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J (Eds). Harrison's™ priciples of internal medicine. 18th ed. New York: Mc Graw Hill;2012. p1688-1705.

14) McAdam AJ, Milner DA, Sharpe AH. Infectious Diseases. In: Kumar V, Abbas AK, Aster JC (Eds). Robbins & Cotran Pathologic Basis of Disease. 9th ed. New Delhi: Reed Elsevier India;2014. p390-2.

15) Bates I, Ekem I. Haematological aspects of tropical diseases. In: Hoffbrand AV, Catovsky D, Tuddenham EGV, Green AR (Eds). Postgraduate Haematology. 6th ed. Oxford: Wiley-Blackwell;2011. p956-62.

16) Pathak VA, Ghosh K. Erythropoiesis in Malaria Infections and Factors Modifying the Erythropoietic Response. Anemia 2016;2016:1-8.

17) Douglas NM, Anstey NM, Buffet PA, Poespoprodjo JR, Yeo TW, White NJ *et al*. The anaemia of Plasmodium vivax malaria. Malaria J 2012;11:135.

18) Haldar K, Mohandas N. Malaria, erythrocytic infection, and anemia. Hematology. 2009;87-93.

19) Lamikanra AA, Theron M, Kooij TWA, Roberts DJ. Hemozoin (Malarial Pigment) Directly Promotes Apoptosis of Erythroid Precursors. PLoS ONE 4(12): e8446. doi:10.1371/journal.pone.0008446.

20) Skorokhod O, Caione L, Marrocco T, Migliardi G, Barrera V, Arese P *et al*. Inhibition of erythropoiesis in malaria anemia: role of hemozoin and hemozoin-generated 4-hydroxynonenal. Blood 2010;116:4328-37.

21) Wickramasinghe SN, Abdalla SH. Blood and bone marrow changes in malaria. Baillieares Best Pract Res Clin Haematol 2000;13:277-99.

22) Arshad AR. Thrombocytopenia in Malaria: Can Platelet Counts Differentiate Malaria from Other Infections?. J Coll Physicians Surg Pak 2015;25:31-34.

23) Nkrumah B, Acquah SEK, Ibrahim L, May J, Brattig N, Tannich E et al. Comparative evaluation of two rapid field tests for malaria diagnosis: Partec rapid malaria test® and Binax Now® malaria rapid diagnostic test. BMC Infect Dis 2011;11:143-50.

24) Adlekha S, Jaiswal RM, Chadha T, Singla A. The Correlation of Spurious Eosinophilia in Automated Hematological Analyzer Sysmex XS-800i with Plasmodium Infection Diagnosis. Indian J Med Sci 2011;65:469-75.

25) Moody A. Rapid Diagnostic Tests for Malaria Parasites. Clin Microbiol Rev 2002;15:66-78.

26) Hänscheid T. Current strategies to avoid misdiagnosis of malaria. Clini Microbiol Infect 2003;9:497-504.

27) Schindler HC, Montenegro L, Montenegro R, Carvalho AB, Abath FGC, Jaureguiberry G. Development and optimization of polymerase chain

reaction–based malaria diagnostic methods and their comparison with quantitative buffy coat assay. Am J Trop Med Hyg 2001;65:355-61.

28) Lee HK, Kim SI, Chae H, Kim M, Lim J, Oh EJ et al. Sensitive detection and accurate monitoring of Plasmodium vivax parasites on routine complete blood count using automatic blood cell analyzer (DxH800^{TM}). Int J Lab Hem 2012;34:201-7.

29) Mubeen KH, Devadoss CW, Rangan RA, Gitanjali M, Prasanna S, Sunitha VP. Automated hematology analyzers in diagnosis of Plasmodium vivax malaria: an adjunct to conventional microscopy. Mediterr J Hematol Infect Dis 2014;6: e2014034, DOI: 10.4084/MJHID.2014.034.

30) Ali SF, Jamil S, Akhtar MN, Farooq M. Automated deection of malaria: A comparison with microscopy. Annals 2005;11:208-9.

31) Suh IB, Kim HJ, Kim JY, Lee SW, An SSA, Kim WJ *et al*. Evaluation of the Abbott Cell-Dyn 4000 hematology analyzer for detection and therapeutic monitoring of Plasmodium vivax in the Republic of Korea. Trop Med Int Health 2003;8:1074-81.

32) Josephine FP, Nissapatorn V. Malaria: the value of the automated depolarization analysis. Southeast Asian J Trop Med Public Health 2005;36:68-72.

33) Singh A, Narang V, Sood N, Garg B, Gupta VK. Malaria Diagnosis Using Automated Analysers: A Boon for Hematopathologists in Endemic Areas. J Clin Diag Res 2015;9:5-8.

34) Hanscheid T, Melo-Cristino J, Pinto BG. Automated detection of malaria pigment in white blood cells for the diagnosis of malaria in Portugal. Am J Trop Med Hyg 2001;645:290-2.

35) de Langen AJ, van Dillen J, de Witte P, Mucheto S, Nagelkerke N, Kager P. Automated detection of malaria pigment: feasibility for malaria diagnosing in an area with seasonal malaria in northern Namibia. Trop Med Int Health. 2006;11:809-16.

36) Mendelow BV, Lyons C, Nhlangothi P, Tana M, Munster M, Wypkema E et al. Automated malaria detection by depolarization of laser light. Br J Haematol 1999;104:499-503.

37) Wever PC, Henskens YMC, Kager PA, Dankert J, Gool TV. Detection of Imported Malaria with the Cell-Dyn 4000 Hematology Analyzer. J Clin Microbiol 2002;40:4729-31.

38) Scott C, Zyl DV, Ho E, Meyersfeld D, Ruivo L, Mendelow BV et al. Automated detection of malaria-associated intraleucocytic haemozoin by Cell-Dyn CD4000 depolarization analysis. Clin Lab Haem 2003;25:77-86.

39) Hanscheid T, Pinto B, Cristino JM, Grobusch MP. Malaria diagnosis with the haematology analyser Cell-Dyn 3500^{TM}: What does the instrument detect?. Clin Lab Haem 2000;22:259-61.

40) Hänscheid T, Romão R, Grobusch MP, Amaral T, Melo-Cristino J. Limitation of malaria diagnosis with the Cell-Dyn® analyser: not all haemozoin-containing monocytes are detected or shown. Int Jnl Lab Hem 2011;33:e14-e16.

41) Dromigny J, Jambou R, Scott C, Perrier-Gros-Claude J. Performance evaluation of automated depolarization analysis for detecting clinically unsuspected malaria in endemic countries. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2005;99:430-439.

42) Sharma S, Sethi N, Pujani M, Kushwaha S, Sehgal S. Abnormal WBC scattergram: a clue to the diagnosis of malaria. Hematology. 2013;18:101-5.

43) Yoo JH, Song J, Lee KA, Sun YK, Kim YA, Park TS et al. Short Report: Automated detection of malaria-associated pseudoeosinophilia and abnormal WBC scattergram by the Sysmex XE-2100 haematology analyser: A clinical study with 1801 patients and real-time quantitative PCR analysis in vivax malaria-endemic area. Am J Trop Med Hyg 2010;82:412-4.

44) Huh HJ, Oh GY, Huh JW, Chae SL. Malaria detection with the Sysmex XE-2100 hematology analyzer using pseudoeosinophilia and abnormal WBC scattergram. Ann Hematol 2008;87:755-9.

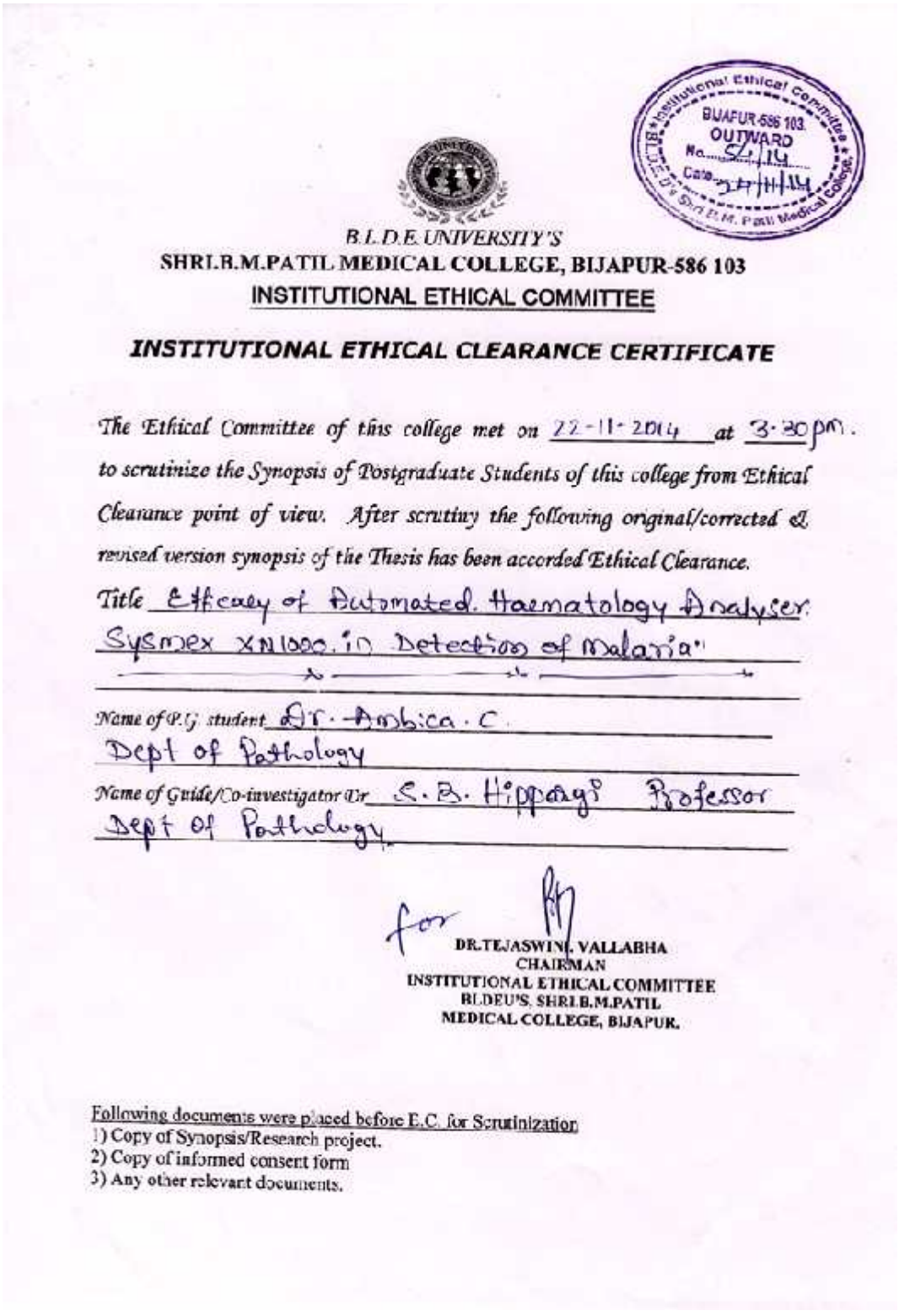
45) Baig MA. Evaluation of false +ve cases & diagnostic accuracy of Abbotts Cell-Dyn Ruby for diagnosis of malaria parasite. Int J sci Res 2015;4:1232-4.

46) Day NPJ, Diep PT, Sinh DX, Loc PP, Chuong LV, Chau TTH et al. Clearance kinetics of parasites and pigment-containing leukocytes in severe malaria. Blood 1996;88:4694-700.

47) Huh J, Jung J, Yoon H, Chung W. Pseudoeosinophilia associated with malaria infection determined in the Sysmex XE-2100 hematology analyzer. Ann Hematol 2005;84:400-2.

ANNEXURE-I

ETHICAL CLEARANCE



Institutional Ethical Committee
BIJAPUR-586 103.
OUTWARD
No. 54/14
Date 24/11/14
B.L.D.E. Shri B.M. Patil Medical College

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 22-11-2014 *at* 3.30 pm. *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title Efficacy of Automated Haematology Analyser Sysmex XN1000. in Detection of Malaria"

Name of P.G. student Dr. Ambica. C
Dept of Pathology

Name of Guide/Co-investigator Dr S. B. Hippargi Professor
Dept of Pathology.

for
DR.TEJASWINI VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE-II

INFORMED CONSENT FOR PARTICIPATION IN

DISSERTATION/RESEARCH

I, the undersigned, ,S/O D/O W/O, _____ aged years, ordinarily resident of do hereby state/declare that Dr _____ of _____ Hosptital has examined me thoroughly on at (place) and it has been explained to me in my own language that I am suffering from _____disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled_____under the guidance of Dr _____ requesting my participation in the study. Apart from routine treatment procedure the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor_____ has also informed me that during conduct of this procedure _____like adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place:

ANNEXURE III

PROFORMA FOR STUDY

Demographic Details:

Name :

Age :

Sex : M/F

RELIGION :

OCCUPATION :

RESIDENCE :

OPD / IPD No. :

Lab. No. /Sample No. :

Chief complaints:

History of present illness:

Past history:

Family history:

General physical examination:

Systemic examination:

- **Cardiovascular system**
- **Respiratory system**
- **Central Nervous System**
- **Per Abdomen Examination**

Clinical diagnosis:

Hematological investigations: (Complete blood count)

Parameters	
WBC	
RBC	
HGB	
HCT	
MCV	
MCH	
MCHC	
PLATELETS	
RDW-SD	
RDW-CV	
PDW	
MPV	
P-LCR	
PCT	
NRBC	
NEUTROPHIL	
LYMPHOCYTE	
MONOCYTE	
EOSINOPHIL	
BASOPHIL	
IG	

PERIPHERAL SMEAR (THIN SMEAR) EXAMINATION:

WBC-DIFF SCATTER PLOT MESSAGE:

KEY TO MASTER CHART

Y	Years
M	Male
F	Female
WBC	White blood cells
HB	Hemoglobin
PLT	Platelets
TN	True negative
TP	True positive
FN	False negative
FP	False positive
NEC	Neutrophil Eosinophil cluster
PL	Plasmodium
PV	Plasmodium vivax
PF	Plasmodium falciparum
S+RF+G	Schizont + Ring form + Gametocyte

MASTER CHART

S.NO	NAME	AGE (Y)	SEX	LAB NO.	PERIPHERAL SMEAR	SCATTER PLOT	ABNORMALITY	IMPRESSION	WBC (cells/mm^3)	HB (g/dl)	PLT (lakhs/mm^3)
1.	Adarsha	25	M	103239	NEGATIVE	NEGATIVE	-	TN	7740	15	2.16
2.	Aditya P	20	M	101668	NEGATIVE	NEGATIVE	-	TN	7900	14.4	3.07
3.	Akkubai	24	F	128089	NEGATIVE	NEGATIVE	-	TN	6480	10.8	3.78
4.	Anil M	36	M	120000	NEGATIVE	POSITIVE	Abnormal Events Below NE Cluster	FP	13230	12	1.93
5.	Anita Tukaram Akkalakot	19	F	28390	NEGATIVE	NEGATIVE	-	TN	6200	8.1	2.91
6.	Ansubai	35	F	21427	NEGATIVE	NEGATIVE	-	TN	9970	11.6	3.89
7.	Ansuya Dejappa Pujari	72	F	19271	NEGATIVE	NEGATIVE	-	TN	5460	9.1	0.26
8.	Arjun Sachin Stavarmath	6	M	18826	NEGATIVE	NEGATIVE	-	TN	18660	10.7	3.79
9.	Ashwini L H	21	F	34511	NEGATIVE	NEGATIVE	-	TN	16740	12.9	2.3
10.	Basayya	66	M	105419	NEGATIVE	NEGATIVE	-	TN	8820	11.9	0.84
11.	Bhavani Madappa Kotanur	4	F	125959	PV SCHIZONT PF GAMETOCYTE	POSITIVE	Abnormal Events Below NE Cluster	TP	9840	10.4	0.2
12.	Bheemaraddi Guranathraddi Hotti	32	M	14548	PL.FALCIPARUM G	POSITIVE	Abnormal Events Below NE Cluster	TP	5590	6	2.46
13.	Bheemshen Yadav	36	M	130865	PL.VIVAX SCHIZONT+TROPHOZOITE	POSITIVE	Double Eosinophil Cluster	TP	5910	15.1	1.62
14.	Bhuvaneshwari Iranagouda Patil	45	F	23983	PL.FALCIPARUM G	POSITIVE	Decreased Space Between NE Cluster	TP	6600	10.4	0.53
15.	C S Bhavikatti	55	M	32724	NEGATIVE	NEGATIVE	-	TN	11910	16.5	1.99
16.	Chandru Ram	30	M	135024	PL.VIVAX SCHIZONT	POSITIVE	Abnormal Events Below NE Cluster	TP	5910	13.3	0.34
17.	Darshan Babu Singe	10	M	120431	PV - S+RF+G	POSITIVE	Double Neutrophil+Eosinophil Cluster	TP	6880	11.4	0.51
18.	Devandra Sangapur	27	M	69310	PL.VIVAX TROPHOZOITE	POSITIVE	Double Eosinophil Cluster	TP	4280	12.5	0.27
19.	Dundamma Shivanagouda Biradar	22	F	52460	PL.VIVAX TROPHOZOITE	POSITIVE	Decreased Space Between NE Cluster	TP	3320	8.4	0.41
20.	Dundegowda	32	M	96697	PL.VIVAX RING FORMS	NEGATIVE	-	FN	9520	13.4	0.72
21.	Gangadhar	40	M	117122	NEGATIVE	NEGATIVE	-	TN	8500	13	2.89
22.	Gangawwa Bheemappa Kakandaki	65	F	29521	NEGATIVE	NEGATIVE	-	TN	17620	9.4	4.4
23.	Geeta	22	F	120339	NEGATIVE	NEGATIVE	-	TN	4430	8.5	1.2
24.	Geeta Bagalkot	45	F	106247	PL.VIVAX RING FORMS	NEGATIVE	-	FN	2760	10.4	1.61
25.	Girish	28	M	12785	PL.VIVAX RING FORMS	POSITIVE	Abnormal Events Below NE Cluster	TP	3780	11.7	0.47
26.	Goutam Ramsaray Ramchandar	32	M	163895	PL.VIVAX SCHIZONT	POSITIVE	Double Neutrophil Cluster	TP	3660	10.3	0.53

27.	Gurubai	32	F	108005	NEGATIVE	NEGATIVE	-	TN	11050	13.1	2.57
28.	Gururaj	22	M	101393	NEGATIVE	NEGATIVE	-	TN	5860	17.4	2.69
29.	Hanamanth	45	M	104101	NEGATIVE	NEGATIVE	-	TN	12430	14.8	3.76
30.	Husenma Allisab Mulla	25	F	16969	NEGATIVE	NEGATIVE	-	TN	16890	12.2	1.91
31.	Iranna Desai Hallolli	39	M	18947	NEGATIVE	NEGATIVE	-	TN	4390	3.2	0.26
32.	Jagadish Basappa Unki	28	M	19090	NEGATIVE	NEGATIVE	-	TN	8600	14	2.68
33.	Jagdish	25	M	135944	PL.VIVAX TROPHOZOITE	POSITIVE	Abnormal Events Below NE Cluster	TP	4690	8.3	0.94
34.	Jitendar	20	M	19341	NEGATIVE	NEGATIVE	-	TN	15310	14.9	4.11
35.	Jyoti	16	F	125393	NEGATIVE	NEGATIVE	-	TN	6090	7.5	3.83
36.	Jyoti Panchu Math	30	F	28963	NEGATIVE	NEGATIVE	-	TN	9650	12.3	2.35
37.	Kalappa Siddappa Badiger	44	M	27027	NEGATIVE	NEGATIVE	-	TN	19890	15.6	1.18
38.	Kalavati	28	F	112362	NEGATIVE	NEGATIVE	-	TN	8.38	11	2.32
39.	Kamala	22	F	141242	PL.FALCIPARUM G	POSITIVE	Abnormal Events Below NE Cluster	TP	2440	8.6	2.23
40.	Kamalabai Gurappa Hanchinal	74	F	19880	NEGATIVE	NEGATIVE	-	TN	10850	14	1.8
41.	Kamalaxi	22	F	141239	PL.FALCIPARUM G	POSITIVE	Abnormal Events Below NE Cluster	TP	2250	8.6	2.11
42.	Karunakar	32	M	103997	NEGATIVE	NEGATIVE	-	TN	7320	16.4	1.76
43.	Kasturi Maligeppa Madar	29	F	74148	NEGATIVE	NEGATIVE	-	TN	5710	11.6	1.54
44.	Kaveri	22	F	137095	PL.VIVAX SCHIZONT	POSITIVE	Double Neutrophil Cluster	TP	10290	6.9	0.52
45.	Lakki Kumar	18	M	107411	NEGATIVE	NEGATIVE	-	TN	11030	14.1	4.75
46.	Lakshmibai Chanamalappa Kolar	70	M	11740	NEGATIVE	NEGATIVE	-	TN	13060	8.3	3.07
47.	Laxman	18	M	16881	NEGATIVE	POSITIVE	Decreased Space And Irregular Neutrophil Cluster	FP	35220	12.1	0.22
48.	Laxmi	22	F	102334	NEGATIVE	NEGATIVE	-	TN	24820	10.6	2.07
49.	Laxmi	4	F	116473	NEGATIVE	NEGATIVE	-	TN	3050	1106	0.11
50.	Lingeshwar Sidramayya Niranjanmath	42	M	10429	NEGATIVE	NEGATIVE	-	TN	7820	14.8	2.6
51.	Madev Banasude	60	M	188382	PL.VIVAX SCHIZONT	POSITIVE	Double Neutrophil Cluster	TP	7770	13.5	0.3
52.	Mahadevi	18	F	32781	NEGATIVE	NEGATIVE	-	TN	3550	13.2	1.52
53.	Mahananda Madhu Tevaratti	25	F	192884	PL.FALCIPARUM G	NEGATIVE	-	FN	6810	7.4	0.27
54.	Malakappa Yallappa Talawar	47	M	162937	PL.VIVAX SCHIZONT+TROPHOZOITE	NEGATIVE	-	FN	6270	11.6	0.49
55.	Malappa Biradar	31	M	29055	NEGATIVE	NEGATIVE	-	TN	6840	14.1	0.5
56.	Maliikarjun	16	M	146900	PL.VIVAX TROPHOZOITE	POSITIVE	Abnormal Events Below NE Cluster + Graying	TP	2520	10.7	0.69
57.	Mallikarjun	36	M	128323	NEGATIVE	NEGATIVE	-	TN	8200	8.6	2.78
58.	Mangalabai	35	F	117116	NEGATIVE	NEGATIVE	-	TN	5860	13.1	1.97
59.	Manish	18	M	146979	PL.VIVAX SCHIZONT+TROPHOZOITE	POSITIVE	Double Neutrophil Cluster	TP	3350	10.8	0.64
60.	Md. Hussain	42	M	101	PL.VIVAX SCHIZONT	POSITIVE	Abnormal Events Below NE Cluster	TP	3500	10.5	0.15
61.	Md. Yousuf	20	M	107466	NEGATIVE	NEGATIVE	-	TN	6510	15.1	2.53

62.	Megha Shrinivas Kulakarni	21	F	19029	NEGATIVE	NEGATIVE	-	TN	6040	12.2	1.66
63.	Meghana	52	F	19385	NEGATIVE	NEGATIVE	-	TN	6990	13.4	2.13
64.	Mohamood	32	M	126061	PL.VIVAX TROPHOZOITE	POSITIVE	Double Neutrophil Cluster	TP	4270	14.6	0.34
65.	Mohan	42	M	106861	PL.VIVAX SCHIZONT+RING	POSITIVE	Double Neutrophil Cluster + Decreased Space Between NE Cluster	TP	2660	9.5	0.45
66.	Mohan Bhimappa Irakal	42	M	141841	PV SCHIZONT & RING FORMS	POSITIVE	Abnormal Events Below NE Cluster	TP	2660	9.5	0.45
67.	Motilal Ram Bhihari	22	M	75890	PL.VIVAX SCHIZONT	POSITIVE	Decreased Space Between NE Cluster	TP	9790	15	1.11
68.	Murshid	25	M	131923	PL.VIVAX SCHIZONT+TROPHOZOITE	POSITIVE	Abnormal Events Below NE Cluster	TP	11420	13.9	0.43
69.	Nandangowda	54	M	116514	NEGATIVE	NEGATIVE	-	TN	3020	8.5	2021
70.	Nanibai	70	F	109706	NEGATIVE	NEGATIVE	-	TN	8810	12.2	3.49
71.	Neelamma Tippanna Pote	87	F	17336	NEGATIVE	NEGATIVE	-	TN	6980	12.9	1.85
72.	Nimbabai Kuber Kamble	60	F	19651	NEGATIVE	NEGATIVE	-	TN	5900	9.2	2.19
73.	Ningamma Nagayya Bandivaddar	45	F	31314	NEGATIVE	NEGATIVE	-	TN	15510	5.9	2.45
74.	Panasappa	55	M	100454	NEGATIVE	POSITIVE	Irregular Neutrophil Cluster	FP	15590	9.6	0.73
75.	Panchakashari	53	F	104115	NEGATIVE	NEGATIVE	-	TN	6380	13.7	2.1
76.	Parashuram	4	M	128283	PL.FALCIPARUM G	POSITIVE	Abnormal Events Below NE Cluster	TP	10470	9.6	2.43
77.	Parashuram Madappa Kotanur	2	M	124509	PL.VIVAX TROPHOZOITE	POSITIVE	Abnormal Events Below NE Cluster	TP	27690	3.7	0.83
78.	Parashuram Madappa Kotanur	2	M	124509	PL.FALCIPARUM G	POSITIVE	Irregular N Cluster + Abn Events Below NE Cluster	TP	25840	4	0.8
79.	Pavan Kumar T	22	M	180874	PL.VIVAX SCHIZONTS	POSITIVE	Abnormal Events Below NE Cluster	TP	2560	7.7	0.64
80.	Prabandanachar	32	F	52171	PL.VIVAX TROPHOZOITE	POSITIVE	Decreased Space Between NE Cluster	TP	5890	12.8	1.01
81.	Preetam Madhu Tevaratti	3	M	192875	PL.FALCIPARUM RF	NEGATIVE	-	FN	4070	7.6	0.31
82.	Preeti Vaishwakarma	20	F	30160	NEGATIVE	NEGATIVE	-	TN	8960	12.8	2.48
83.	Priti	26	F	29870	NEGATIVE	POSITIVE	Decreased Space Between NE Cluster	FP	3990	10.6	1.11
84.	Priyanka	23	F	107862	NEGATIVE	NEGATIVE	-	TN	14250	10.2	2.56
85.	Pundalik Awati	44	M	26624	NEGATIVE	NEGATIVE	-	TN	14330	13.9	1.38
86.	Radha B	10	F	125065	PL.FALCIPARUM G	POSITIVE	Abnormal Events Below NE Cluster	TP	13840	7.8	0.3
87.	Raheman Choudri	21	M	18799	NEGATIVE	NEGATIVE	-	TN	7500	13.3	1.72
88.	Raj Kumar Hiremath	28	M	157547	PL.VIVAX SCHIZONT+TROPHOZOITE	POSITIVE	Double Eosinophil Cluster	TP	6980	12.5	0.29
89.	Raju P	32	M	121943	PL.FALCIPARUM G	POSITIVE	Abnormal Events Below NE Cluster	TP	9680	3.7	1.15
90.	Rakesh Kumar	25	M	110816	NEGATIVE	NEGATIVE	-	TN	4410	14.4	0.68
91.	Ramesh	23	M	113704	NEGATIVE	NEGATIVE	-	TN	2330	13.8	0.85
92.	Ramngouda R Kolur	35	M	121662	PL.VIVAX TROPHOZOITE	POSITIVE	Abnormal Events Below NE Cluster	TP	5270	11	0.78
93.	Rani PT	25	F	29756	PL.VIVAX SCHIZONT	NEGATIVE	-	FN	2940	3.2	1
94.	Ratna	55	F	107950	NEGATIVE	NEGATIVE	-	TN	6430	10.7	2.51
95.	Ratnabai Malleshi Gornal	40	F	18832	NEGATIVE	POSITIVE	Irregular Neutrophil Cluster	FP	19460	8.4	2.73
96.	Ravindra	31	M	127967	NEGATIVE	NEGATIVE	-	TN	13730	15.9	2.67

97.	Renuka	25	F	114885	NEGATIVE	NEGATIVE	-	TN	10410	13.9	3.58
98.	Renuka Hosamani	37	F	16912	NEGATIVE	NEGATIVE	-	TN	11320	10	3.62
99.	Roopa	25	F	111460	NEGATIVE	NEGATIVE	-	TN	9560	12.9	3.58
100.	Roopa Basavaraj Malled	20	F	33118	NEGATIVE	NEGATIVE	-	TN	1340	3.6	0.15
101.	S. Kumar	56	M	104790	NEGATIVE	NEGATIVE	-	TN	6640	15.3	1.74
102.	Sagar	34	M	29847	NEGATIVE	NEGATIVE	-	TN	3710	12.4	0.98
103.	Sagar P	32	M	114610	PL.VIVAX SCHIZONT	POSITIVE	Abnormal Events Below NE Cluster	TP	25170	8	2.4
104.	Sahibgowda	60	M	187214	PL.F RING FORMS	NEGATIVE	-	FN	9630	15.3	0.22
105.	Saine	8	M	21360	NEGATIVE	NEGATIVE	-	TN	3820	11.8	2.27
106.	Salama Saleem Bhola	24	F	33145	NEGATIVE	NEGATIVE	-	TN	6410	13.1	1.87
107.	Sameer	21	M	104059	NEGATIVE	NEGATIVE	-	TN	4060	15.6	2.01
108.	Santosh Chawan	18	M	14740	NEGATIVE	NEGATIVE	-	TN	13940	15	2.84
109.	Sarubai Malakappa Biradar	70	F	26077	NEGATIVE	POSITIVE	Decreased Space Between NE Cluster	FP	6530	11.4	1.76
110.	Satish	25	M	122514	PL.VIVAX SCHIZONT	POSITIVE	Decreased Space Between NE Cluster	TP	5260	7.5	0.85
111.	Savita Gurunath Kannur	16	F	32780	NEGATIVE	NEGATIVE	-	TN	8180	2.1	2.28
112.	Shaila Dhanappa Burukule	36	F	17878	NEGATIVE	NEGATIVE	-	TN	2590	5	0.37
113.	Shailaja	25	F	29858	NEGATIVE	NEGATIVE	-	TN	2050	10.1	0.99
114.	Shailaja	41	F	101577	NEGATIVE	NEGATIVE	-	TN	11360	13	4.3
115.	Shailaja	43	F	102267	NEGATIVE	NEGATIVE	-	TN	9390	12	3.96
116.	Shantappa Golappa Ganiger	49	M	18927	NEGATIVE	NEGATIVE	-	TN	13780	9.7	3.01
117.	Sharath	26	M	30171	PL.FALCIPARUM G	POSITIVE	Irregular N Cluster + Abn Events Below NE Cluster	TP	5480	11.5	0.66
118.	Shashank Mallikarjun Kalasad	8	M	21183	NEGATIVE	NEGATIVE	-	TN	8490	11	3.4
119.	Shekarappa	52	M	29876	PL.FALCIPARUM G	POSITIVE	Double Neutrophil Cluster	TP	5350	11.5	0.35
120.	Shivanand	20	M	29158	PL.VIVAX SCHIZONT	POSITIVE	Decreased Space Between NE Cluster	TP	7530	8.3	1.01
121.	Shivappa	36	M	108373	NEGATIVE	NEGATIVE	-	TN	1620	6.4	0.47
122.	Shivaputra Nagappa Yalameli	40	M	29411	NEGATIVE	NEGATIVE	-	TN	5210	15.1	1.52
123.	Shobha	25	F	116319	NEGATIVE	NEGATIVE	-	TN	5210	8.6	2.7
124.	Shruti	20	F	102373	NEGATIVE	NEGATIVE	-	TN	5960	11.1	2.58
125.	Shweta	53	F	101756	NEGATIVE	NEGATIVE	-	TN	4910	11.3	2.48
126.	Siddamma Sharanu Mandewal	28	F	22609	NEGATIVE	NEGATIVE	-	TN	6720	11.8	2.2
127.	Siddappa Pujari	26	M	32595	NEGATIVE	NEGATIVE	-	TN	5600	16.7	2.69
128.	Siddaragowda	41	M	107402	NEGATIVE	NEGATIVE	-	TN	5590	13.1	1.74
129.	Sidlingappa	34	M	456	PL.VIVAX SCHIZONT+G	POSITIVE	Double Neutrophil Cluster	TP	5490	12.9	0.66
130.	Subash	55	M	16854	NEGATIVE	NEGATIVE	-	TN	5940	15.6	2.63
131.	Subhash	34	M	13346	PL.VIVAX RING FORMS	POSITIVE	Abnormal Events Below NE Cluster + Graying	TP	13610	8.2	0.17

132.	Sulochana	45	F	26103	NEGATIVE	NEGATIVE	-	TN	6800	11.6	3.25
133.	Sulochana	46	F	112904	PL.VIVAX SCHIZONT	POSITIVE	Abnormal Events Below NE Cluster	TP	5330	9.6	0.62
134.	Sunanda	35	F	34478	NEGATIVE	NEGATIVE	-	TN	5870	12.2	1.56
135.	Sunil	16	M	117113	NEGATIVE	NEGATIVE	-	TN	1730	14	1.14
136.	Suresh	32	M	29771	NEGATIVE	POSITIVE	Double Neutrophil Cluster	FP	23310	10.3	3.94
137.	Susheel	26	M	91926	PL.VIVAX SCHIZONT	POSITIVE	Abnormal Events Below NE Cluster	TP	3780	11.2	0.5
138.	Sushilabai Laxaman Hugar	45	F	142098	PL.VIVAX SCHIZONT	NEGATIVE	-	FN	4510	7.2	0.5
139.	Sushma K	19	F	101747	PL.FALCIPARUM G	POSITIVE	Abnormal Events Below NE Cluster	TP	2760	8	0.61
140.	Swati	16	F	104118	NEGATIVE	NEGATIVE	-	TN	5140	5.9	2.58
141.	Swetha	16	F	106296	NEGATIVE	NEGATIVE	-	TN	4200	9.6	1.19
142.	Usha	17	F	105163	NEGATIVE	POSITIVE	Double Eosinophil Cluster	FP	7070	12.3	0.68
143.	Vidyadar Revanasidda Udchan	21	M	28396	NEGATIVE	NEGATIVE	-	TN	15050	12.9	2.26
144.	Vishwanath	17	M	134660	PL.VIVAX SCHIZONT	POSITIVE	Double Neutrophil Cluster	TP	3100	11.9	0.15
145.	Yallawwa	42	F	16667	NEGATIVE	NEGATIVE	-	TN	7130	13	2.71
146.	Zaheer	24	M	154574	PL.VIVAX SCHIZONT	POSITIVE	Double Eosinophil Cluster	TP	6750	12.5	0.26