



## COMPARATIVE STUDY OF QBC, PERIPHERAL SMEAR AND RAPID MALARIA DIPSTICK METHOD IN DIAGNOSIS OF MALARIA

### Medical Microbiology

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### ABSTRACT

**BACKGROUND:** Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. It is preventable and curable. The estimated number of malaria deaths stood at 409 000 in 2019.

**MATERIAL AND METHOD:** 100 samples were included in the study, which further processed for rapid card and peripheral blood smear and QBC diagnostic test for diagnosis of malaria.

**RESULT AND OBSERVATION:** The diagnosis of malaria. Out of total 100 cases thick smear detected 73 positives compared to 70 by thin smear, 72 by dipstick, and 69 by QBC. Thick smear proved to be better than the other methods. While dipstick closely followed thick smear, thin smear and QBC had a back stage in comparison. QBC had the disadvantage of difficulty in species identification.

**CONCLUSION:** Thick smear proved to be better than the other methods. But it shows the disadvantage of difficulty in species identification, rather than dipstick test.

### KEYWORDS

PBS, QBC, Dipstick, Malaria

#### BACKGROUND:-

Malaria is a major public health problem and cause of suffering and premature death in tropical and subtropical countries. In many endemic areas it is becoming increasingly difficult to control because of the resistance of the parasite to antimalarial drugs and the failure of vector control measures.

#### Malaria Global Situation:

- Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. It is preventable and curable.
- In 2019, there were an estimated 229 million cases of malaria worldwide.
- The estimated number of malaria deaths stood at 409 000 in 2019.
- Children aged under 5 years are the most vulnerable group affected by malaria; in 2019, they accounted for 67% (274 000) of all malaria deaths worldwide.
- The WHO African Region carries a disproportionately high share of the global malaria burden. In 2019, the region was home to 94% of malaria cases and deaths.
- Total funding for malaria control and elimination reached an estimated US\$ 3 billion in 2019. Contributions from governments of endemic countries amounted to US\$ 900 million, representing 31% of total funding.
- Study Contradicts NVBDCP and WHO Data:** A study conducted by teams from the office of the Registrar General of India, Centre for Global Health Research at St Michael's Hospital and University of Toronto, Canada, published in *The Lancet* on 20 November 2010 has reported that malaria causes 205 000 malaria deaths per year in India before age 70 years (55 000 in early childhood, 30 000 at ages 5—14 years, 120 000 at ages 15—69 years) with a 1.8% cumulative probability of death from malaria before age 70 years. The report says that 90 per cent of the deaths were recorded in rural areas, of which 86 per cent occurred at home without any medical attention. It also found that Orissa reported the highest number of deaths — 50,000, followed by Chhattisgarh, Jharkhand and Assam. The study, which began in 2002, covered 6,671 areas, each with about 200 households.[9-11] However, WHO has rebutted these estimates.[12]
- Yet other study on the global malaria mortality between 1980 and 2010 by Murray et al, published in *The Lancet* in Feb 2012, estimated the malaria mortality in India in 2010 at 46,800.[13]
- According to the estimates of a 16-member committee set up by the National Vector Borne Disease Control Programme (NVBDCP) to assess India's actual malaria death burden, the total annual number of cases in India may be about 9.7 million, with about 30,014 – 48,660 deaths (40,297 on an average).[14]
- Another paper estimates the malaria burden in India at 180 million, with as many as 90 million cases of *P. falciparum* malaria per year.[15]

#### 1. MATERIAL AND METHOD:

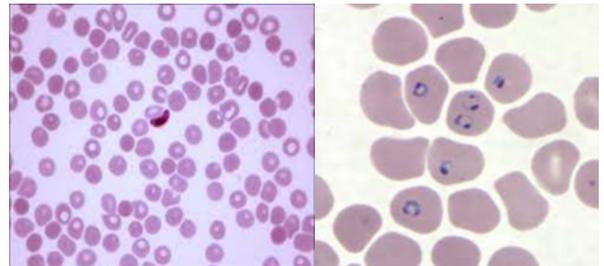
**Study design:** The present study is carried out in this institute in 2019-2021.

#### Specimen:

A total of 100 blood samples of suspected cases of malaria were collected from patients attending the outpatient department and admitted in medical wards.

#### PS for MP:

Both thin and thick smears are prepared.



Gametocyte of *P. falciparum* Ring forms of *P. falciparum*

#### Thin film field's staining technique:

- Place the slide on a staining rack and cover the methanol fixed thin film with approximately 0.5 ml of diluted Field's Stain B.
- Add immediately an equal volume of Field's stain A and mix with the diluted Field's stain B. Leave to stain for 1 minute.
- Wash off the stain with clean water. Wipe the back of the slide clean and place it in a draining rack for the film to air dry.

#### Table-1 Results for malaria thin film:

S.N.	Characteristics of malaria parasite	Color
1.	Chromatin of parasite	Dark red
2.	Cytoplasm of parasite	Blue
3.	Schüffner's dots	Red Mauer's dots (clefts) are appear red mauve
4.	Malaria pigment	Brown black Red
5.	Red cells	Grey to pale mauve pink
6.	Reticulocytes	Grey blue
7.	Nuclei of neutrophils	Dark purple
8.	Cytoplasm of mononuclear Cells	Blue gray

#### Thick film field's staining technique

- Holding the slide with the dried thick film facing downwards, dip the slide in to Field's Stain A for 5 sec. Drain off the excess stain by touching a corner of the slide against the side of the container.

- Wash gently for about 5 seconds in clean water. Drain off the excess water.
- Dip the slide into Field's Stain B for 3 seconds. Drain off the excess stain.
- Wash gently in clean water. Wipe the back of the slide clean and place it upright in a draining rack for the film to air dry.

**Note:** If after staining, the whole of the film appears yellow brown (usually a sign that too much blood has been used), too blue or too pink, do not attempt to examine it. Restrain it. (dipping in field's stain A for 1 second, field's stain B for 1 second).

**Table. 2 Results for malaria thick film:**

S.N.	Characteristics of malaria parasite	Color
1.	Chromatin	Dark red
2.	Cytoplasm	blue mauve
3.	Schüffner's dots	
4.	P.Vivax	Pale red
5.	P.ovale	Pale red
6.	Malaria pigment	Yellow brown or Black brown
7.	Nuclei of small lymphocytes	Dark purple
8.	Nuclei of neutrophils	Dark Purple
9.	Granules of eosinophils	Red
10.	Cytoplasm of mononuclear cells	Blue grey
11.	Reticulum of reticulocytes	blue grey stippling

**Collection of specimen:**

Capillary blood by finger prick method and/or venous blood collected in the EDTA bulb.

**Capillary blood method:**

By using sterile technique, the lobe of finger is cleaned using a swab moistened with spirit and the area is allowed to dry. With help of a sterile lancet, the finger is pricked and squeezed gently to obtain enough blood.

**Venous Blood Method:**

By using aseptic precautions, tourniquet is tied over the arm. The area of cubital fossa or any other site is cleaned with spirit and allowed to dry. About 2-3 ml of blood is collected in EDTA bulb with the help of 5 ml disposable syringe and 23 mm gauge needle. The bulb is gently shaken to mix the blood with anticoagulant.

**Procedure:**

Every sample is tested by the following 3 methods:

**Malaria rapid card test**

**QBC capillary method.**

Quantitative Buffy Coat is another direct and rapid test for the diagnosis of malaria. It is based on **acridine orange staining of centrifuged peripheral blood samples in a microhematocrit tube (QBC)** and examination under UV light source (fluorescence microscopy).

**RESULTS AND OBSERVATION:**

**Table - 3 Comparison Between Three Methods For Positive And Negative**

Investigation	Total Case	Positive	Negative
Ps thick	100	73	27
Ps thin	100	70	30
Dip stick	100	72	28
QBC	100	69	31

**Table-3** shows comparison between peripheral smear, Dip stick and QBC in the diagnosis of malaria. Out of total 100 cases thick smear detected 73 positives compared to 70 by thin smear, 72 by dipstick, and 69 by QBC. Thick smear proved to be better than the other methods. While dipstick closely followed thick smear, thin smear and QBC had a back stage in comparison. QBC had the disadvantage of difficulty in species identification.

**Table - 4 Comparison Between Three Methods For Species Identification**

Investigation	Pv	Pf	Mix	Total Positive	Total Negative	Non specific	Total
Ps thin	12	50	8	70	30	-	100

Ps thick	13	51	9	73	27	-	100
Dip stick	10	59	-	69	31	-	100
QBC	10	59	-	69	31	-	100

**Table-4** shows comparison between the different methods in species identification. Thick and thin smears succeeded in identifying maximum of P.vivax infections. Where as dip stick succeeded in identifying maximum of P.falciparum infections. Cases of P. falciparum might be missed in peripheral smear, but will be caught in dip stick when a positive band for P. falciparum appears. Also when there is confusion as to which species the rings belong to, dip stick band helps in clearing the doubt. QBC enjoys a back seat even here as it fails to identify species based on rings alone. Presence of schizont gamete confirm the occurrence of P. vivax and P.falciparum respectively. But in specimens in which only ring forms are seen, QBC fails to identify the species.

**CONCLUSION**

The present study and various other studies bring out various facts about diagnosis of malaria. Peripheral smear has stood the test of time and remains the Gold Standard in diagnosis of malaria.

Out of thin and thick smears, thick smear can identify more number of positive cases as larger volume of blood is taken compared to thin smear. Thus its sensitivity is higher than other methods.

Although thick smear has higher sensitivity, inappropriate method can lead to deformation of the morphology of parasite causing difficulty in species identification. Debris and other cellular material can also confuse with rings. Schizonts of P. vivax and gametes of P. falciparum may deform creating confusion.

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- Mixed infections can be detected in peripheral smear as morphological forms of both P. vivax and P. falciparum can be identified. But here Dip stick and QBC fail Dip stick will give the same result for P. falciparum and mixed infections, showing 2 bands besides the control band. QBC, if only ring forms are seen will fail to confirm if it is P. vivax or P. falciparum or mixed infection.