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Comparative Staining Methods for Microscopic Diagnosis of Malaria

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ABSTRACT

Objectives: The gold standard for laboratory diagnosis of malaria has remained microscopy of blood smears. Present study compared Leishman and Fields stains with Giemsa staining method for detection of malarial parasites in peripheral smear.
Methods: A prospective study was carried out at a tertiary care hospital and included 200 blood specimen from patients, clinically suspected of malaria. Blood (2ml) was collected in EDTA bulb. Peripheral smears were prepared and stained with Leishman and Field stain and compared with Giemsa stain.
Results: Malarial parasites were detected in 132 of 200 patients in peripheral smears. Leishman stain gave 100% sensitivity and specificity. Fields' stain gave 98% sensitivity and 100% specificity and was found to be the most rapid and convenient staining method.
Conclusion: Giemsa and Leishman's stains are the method of choice for staining peripheral smears. However, Fields' stain was found to be the most rapid and convenient method.

KEYWORDS Malaria, Leishman stain, Fields stain, Giemsa stain

Introduction:
Malaria has plagued mankind since ancient times and is still a significant threat to half of the world's population - 3.3 billion people living in 109 countries are at risk of contracting the disease. Estimates suggest that malaria afflicts between 350 and 500 million people every year.[1]

Malaria has been known since ancient times and has been diagnosed based on patient's signs and symptoms. The parasites in the blood were first seen in 1880 by a French army surgeon, Alphonse Laveran.[2,3,4] The discovery that the mosquito acted as a vector was due to the intuition of Patrick Manson. He was unable to undertake this investigation himself and persuaded Ronald Ross, an army surgeon, to carry out the work in India. In 1897, Ross saw what is now known to be the oocysts of *P.falciparum* in an anopheline mosquito.

In 1891, Romanowky introduced staining methods for these parasites.[2,3] Today more than a century later, microscopic detection and identification of Plasmodium species in Giemsa stained blood films remains the gold standard for laboratory diagnosis. The Romanowsky stains are best used to study the structural details of parasites. Several modifications are now available which are easier to use and give better results.

Aim: To compare Leishman and Field's stains for the detection of malaria parasites in peripheral smear with Giemsa staining method.

Materials and methods:
The study was carried out at a tertiary care hospital and included 200 blood specimen from patients clinically suspected of malaria. 3ml of blood was collected as per protocol in an EDTA bulb. Three thick and thin peripheral smears were prepared and stained with Leishman and Fields stain and compared with Giemsa stain as per standard protocol.[5,3] Readymade Giemsa, Leishman, Field's A and B stains from HiMedia Laboratories Pvt. Ltd. were used. Speciation of the plasmodium species was done from the thin peripheral smear. Data were analyzed in SPSS. Proportions were compared using the Chi – square test.

Results:
Of the 200 samples processed in the laboratory, 132 had positive blood films.

Field's stain was negative in 3 cases which were positive by Giemsa and Leishman stain (table 1). Leishman stain gave 100% sensitivity, specificity, PPV and NPV when compared with Giemsa staining. Field's stain gave a lower sensitivity but 100% specificity (table 2). Field's stain was rapid and convenient for both thick and thin smears (table 3)

Discussion:
Three stains were compared; Giemsa, Leishman, and Field's. Giemsa stained thick smear was taken as the gold standard. Thin smear was used for species identification. In present study when Leishman stain was compared with Giemsa, it gave100% sensitivity and specificity (Table 2). Leishman stained thick films are considered to be the gold standard in malaria diagnosis.[6] Giemsa staining is the most commonly used method for both thin and thick films all over the world for the quality of the stain and of greater importance, its stability in tropical climates.[7] Some laboratories prefer Leishman stain as it is alcohol based and simultaneous fixing and staining occurs.[8] The staining quality of Leishman stain is excellent.[8,7] In the tropics care needs to be taken in storing Leishman stain as evaporation of alcohol may concentrate the stain and alter the staining time. The main disadvantage in using Giemsa and Leishman stain is that the staining process is time consuming. This can be overcome by using rapid Field technique.[8] Fields staining is a good method to stain thick films and is extremely quick.[7]

In the present study Field's stain failed to pick up three cases of malaria (Table 1). It had a sensitivity of 97.72 % and a specificity of 100 %. Mendiratta et al when evaluating different methods for diagnosis for *P.falciparum* malaria compared Leishman and Field's stain. Out of the 443 subjects examined for *P.falciparum*18.28% were detected by Leishman stain and 6.32 % by Field's stain. Field's stain missed 53 (65.4%) cases. All smears positive by Field's stain were positive by Leish-

man stain. The sensitivity of Field's stain was found to be low (34.57%).[9] Similarly when Lema et al have compared 5 methods of malaria detection in an outpatient setting which also included staining methods, they observed 82-98 % sensitivity and 85-99% specificity, 67 – 98 % PPV and 97-99% NPV for Giemsa stain. They had 86-98% sensitivity, 94-100 % specificity, 67-98% PPV and 97-99 % NPV for Field's stain. They found that staining for Giemsa took longer. However on the basis of sensitivity, specificity, convenience and cost they thought that Field's stained thick blood film remains the most appropriate method for diagnosis of *P. falciparum* in health facilities.[10]

The present study shows that Leishman stain is comparable to Giemsa in sensitivity and specificity. Fields stain gave 97.72% sensitivity and 100% specificity. But convenience in using the Fields staining method, stability of its reagent in tropical countries, shorter duration of staining method makes it an appropriate method to be used in conditions where a large number of slides need to be stained and interpreted (Table 3). Determining a good staining method which is rapid, cost effective, gives consistent results and can be used both by experts and novices is the key to an effective diagnosis.

Conclusion:

In the present study, Leishman stain gave 100 % sensitivity and specificity when compared to Giemsa stain. Field's stain had a sensitivity of 97.72 % and specificity of 100%. Giemsa and Leishman stain gave excellent results. Although Field's stain showed a slightly decreased sensitivity compared to Leishman and Giemsa stain it was found to be the most rapid method especially when a large number of slides needed to be processed.

TABLE 1: RESULTS OF GIEMSA, LEISHMAN AND FIELD'S STAIN (N=200)

Stain	Positive for malaria	Percentage positivity
Giemsa	132	100
Leishman	132	100
Field's	129	97.72

TABLE 2: COMPARISON OF SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE (PPV), NEGATIVE PREDICTIVE VALUE (NPV) OF LEISHMAN AND FIELD'S STAIN (N=200)

Stain	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Leishman	100	100	100	100
Field's	97.72	100	100	95.77

TABLE 3: COMPARISON OF GIEMSA, LEISHMAN AND FIELDS STAIN

	Giemsa	Leishman	Fields
Fixation of thin smear required	Yes	No	Yes
Dehaemoglobinisation of thick smear required	Yes	Yes	No
Time required for staining	30 minutes	15 minutes	<1minute
Percentage positivity	100	100	97.72

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