

A NEW LABORATORY TECHNIQUE FOR MALARIA DIAGNOSIS

Pages with reference to book, From 207 To 208

Dear Madam,

Malaria persists to be endemic in our population and remains a major source of morbidity and to some extent mortality. Most laboratories diagnose malaria infection by direct blood smears either on thin and or thick films¹. Probability of diagnosis depends on the degree of parasitaemia, technical expertise and time taken for screening blood films. However, malaria escapes detection by these methods. Since newer techniques of Indirect Immuno-fluorescence² and indirect haemagglutination³ are expensive and beyond the technical scope of most laboratories, we have developed a new method which can be performed in all laboratories in Pakistan and which gives high rate of detection. This technique capitalizes the method of Boyum⁴ for the separation and purification of mononuclear cells from whole blood. Lymphocyte purified by this method are contaminated by erythrocytes with any form of inclusion bodies e.g., reticulocytes, normoblasts.

We assumed that erythrocytes parasitized by plasmodium species e.g., ring forms, Schizonts and gametocytes will also separate with lymphocytes. This assumption proved correct and formed the basis of this technique. The method enabled us to concentrate and purify plasmodium parasitized erythrocytes from 2ml of blood to a final volume of 0.02ml. Briefly describing the method, 2ml of heparinised venous blood and 1ml of serum is taken from the patient and the heparinised blood diluted with 2ml of normal saline.

This 4ml of diluted blood is carefully layered by a Pasteur pipette on 5ml of lymphocyte separation medium "Lymphoprep" (Sigma) in a conical 10ml glass

The layered blood is centrifuged at 2500 rpm for twenty minutes in a swing out or angled centrifuge. Lymphocyte and parasitized erythrocyte separate at the interface, bulk of erythrocytes and polymorphs sediment to the bottom while platelets remain suspended in the diluted plasma. Plasma is removed and the layer of cells at the interface is carefully aspirated avoiding taking of Lymphoprep. Aspirated layer is quickly washed twice in normal saline centrifuging each time at 2500 rpm for 5 minutes. The final pellet obtained is suspended in one drop of autologous serum taken initially from the patient. Slides are made in the usual manner and stained by Romanosky stains⁶. The results were calculated in terms of %. Erythrocytes parasitized by ring forms, Schizonts and gametocytes when slides were observed under Oil Immulsion lens and a total of 100 fields observed at each screening. In 20 cases where ring forms were observed, % RBC's parasitaemia ranged from 0 in some cases to 15% in others by direct smear, while this increased to 20 to 90% by concentration method. In 10 cases where Schizonts were also observed, the % RBC's with

Schizonts seen in direct smear was 0 to 0.2% and this increased to 1 to 5% by concentration method. In 12 cases of Plasmodium falciparum infection where gametocytes were observed, the % of parasitized RBC's in direct smear was 0 to 0.1% increasing to 2-10% by our method. Of the total 42 cases studied, 4 (9.5%) were negative by direct smear and become positive for malaria by concentration method.

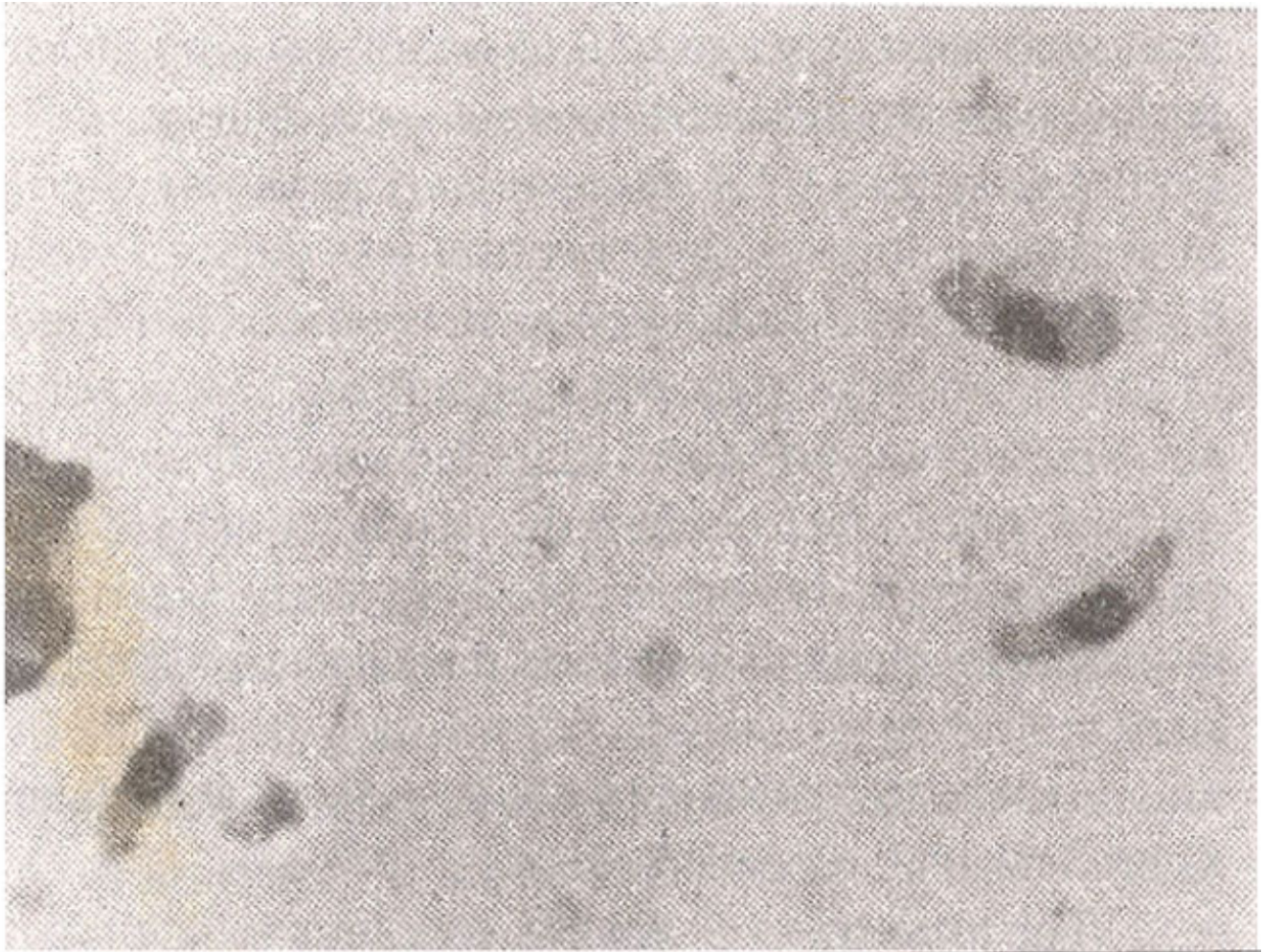


Figure 1 (A). Showing three gametocyte of plasmodium falciparum (Photograph taken under oil immersion lens).

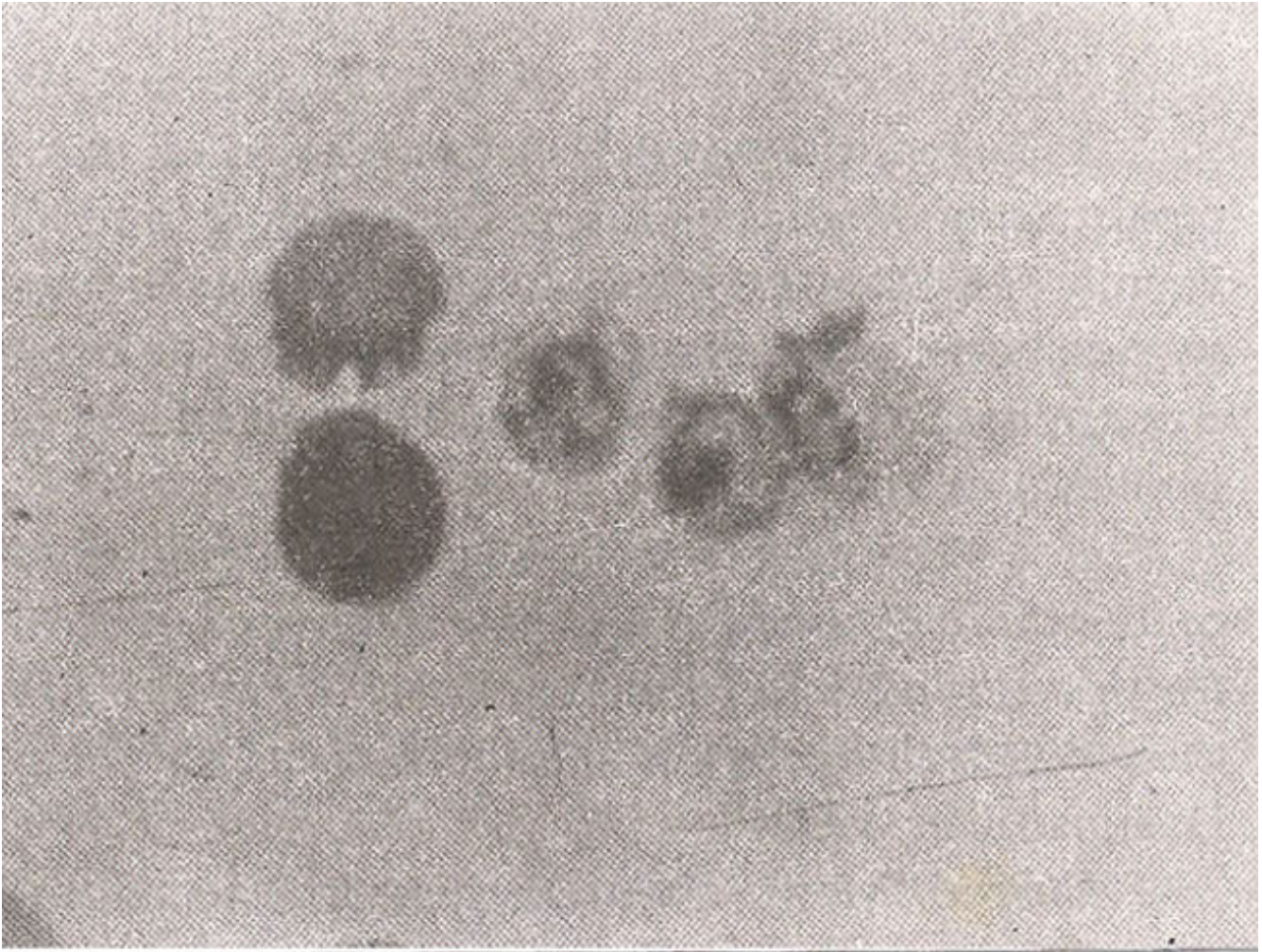


Figure 1 (B). Showing 3 shizonts (Photograph taken under oil immersion lens).

Figure shows our results pictorially.

The advantage of our technique is that it is economical to perform, needs no special equipment and increases the presence of parasitized cells on smears by 5 to 500 fold. Hence detection of malarial parasites becomes easy and many cases with low parasitaemia who are not diagnosed can now be detected by our method. We recommend that in cases of suspected malaria if results -by direct smear are negative, this method may be.

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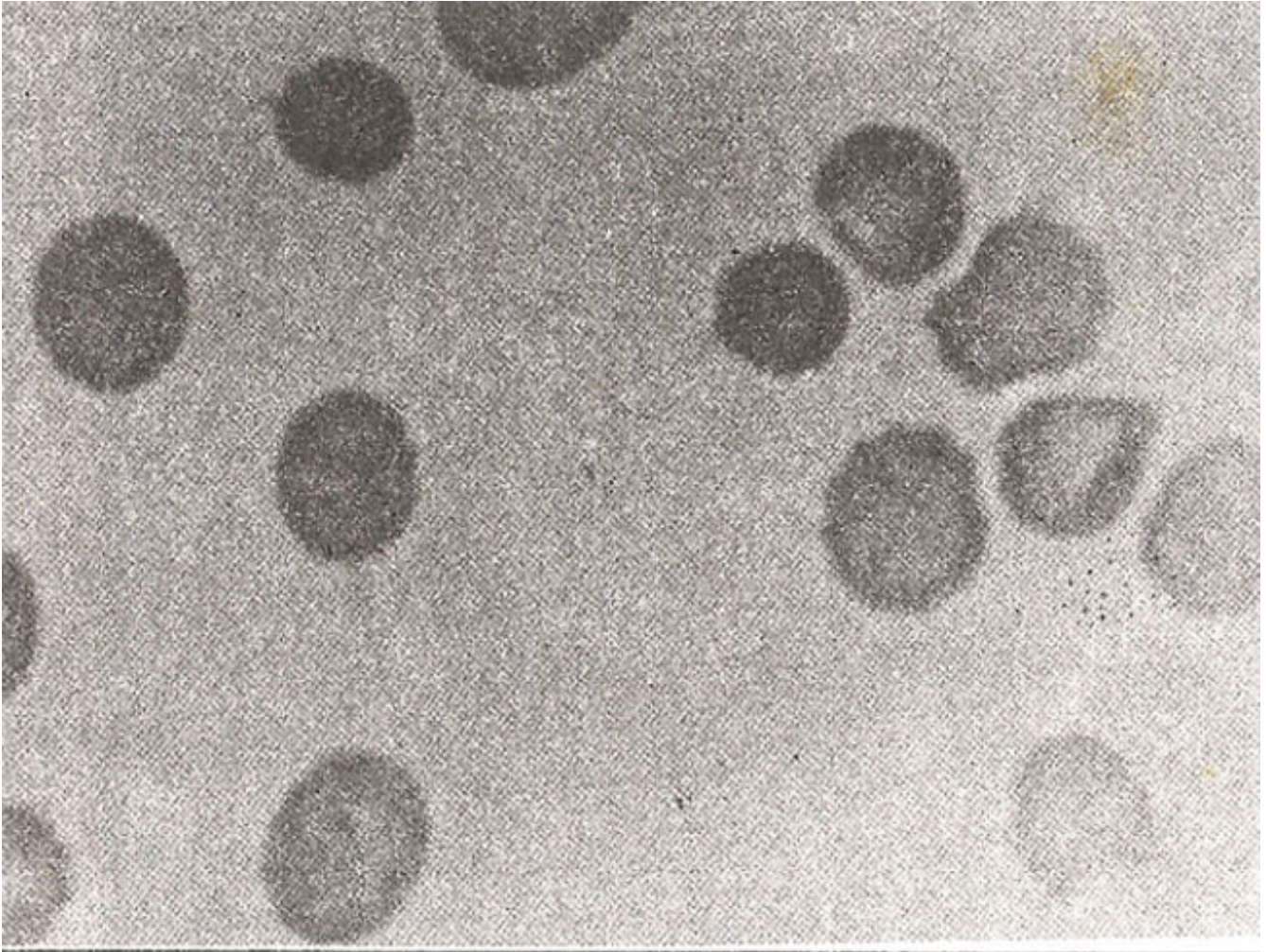


Figure 1 (C). Showing ring forms (Photograph taken under oil immersion lens).

Figure 1 (C). Showing ring forms (Photograph taken under off immersion lens).
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