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Short Communication

Devising a cost-effective method to improve the specificity and sensitivity of malaria detection

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It has been a challenge to increase the concentration of malaria parasites in blood without compromising on specificity. The standard quantitative Buffy coat (QBC) method improves sensitivity at the cost of specificity. It cannot do species identification either. This study aims to improve the specificity of standard QBC method and to enable species identification. The dye was washed off used QBC capillary tubes. Whole blood samples of ten malaria-positive patients were drawn onto the washed QBC capillaries. The float was re-inserted and centrifugation carried out. The capillary tubes were broken at the area where parasite-infested cells were most likely to be seen. Smears were made and stained using the conventional Romanowsky method. Ordinary microscopy was carried out and parasitaemia quantified. Parasites' number was found to be on average ten times higher per oil immersion field compared to the conventional thin smear methods. Species identification was easier than in thick smears. This pilot study modifying the QBC technique of malaria detection demonstrated a ten- fold increase in sensitivity compared to conventional thin smear preparations. This was not at the cost of specificity unlike standard QBC method. Species identification was easier than in thick smears.

Keywords: Malaria detection, QBC method, thin smear technique, thick smear technique, Modified QBC method

Trial registration: Not applicable

INTRODUCTION

Despite advances in medicine, malaria continues to be a global public health challenge. The issue is not confined to endemic areas in developing nations. "The mortality rate from malaria is approximately 0.3% in the US and 0.26% worldwide". Most of the cases in the developed world are reported on those who visited endemic areas. (Daily, J. P., Minuti, A., Khan, N., 2022)

It has always been a challenge to increase the concentration of malaria parasites in blood without compromising specificity. The conventional thick smear method is a good sensitive tool. But the disadvantage is that significant expertise is needed to perform this method.

Alternative methods to concentrate the parasites started as early as the beginning of the 20th century. These methods were based on sedimentation or differential centrifugation (Worth,1964). Bennett found that centrifugation concentrated haemoparasites just below and above the buffy coat (Worth, 1964). These efforts underwent a revolution with the advent of quantitative buffy coat (QBC) analysis (Wardlaw and Levine, 1983). QBC was originally designed "to provide a rapid means of performing a complete blood cell count in a physician's office." (Wardlaw and Levine, 1983).

The main difference between the QBC method and the earlier centrifugal separation efforts was that QBC physically expanded and separated the buffy coat into three distinct layers of granulocytes, non-granulocytes and plateletsin a capillary tube. A cylindrical plastic 'float' is inserted into the capillary tube. The float has a specific gravity in between that of plasma and RBCs. When the blood is centrifuged, the float settles in the buffy coat area. The latter expands around the float due to the limited availability of room inside the capillary. The capillary tube is pre-coated with a fluorescent dye (acridine orange) which is taken up by the nucleoproteins. Micrometric measurements of the separated regions in fluorescent light enable the

quantification of the different cell layers. This same group of researchers observed that blood borne parasites like malarial trophozoites were detectable within the expanded area (Wardlaw and Levine, 1983).

Later, Spielman et al (1988) demonstrated that "red blood cells infected with diverse stages of *Plasmodium falciparum and P. vivax* are lighter than non-infected cells and somewhat heavier than granulocytes; thus, they can readily be detected by direct inspection of UV-illuminated tubes". They found that centrifugation using the QBC capillary tubes for malaria screening enhanced the detection rates by at least eight times compared to the conventional thin film method, considered the gold standard for malaria detection. However, the authors admit that the method could not differentiate between the species of malaria. The other disadvantage is that a microscope with fluorescent attachment is essential here, which presents a difficulty in low-income countries where malaria is prevalent. "When the QBC system is taken to the field away from the air-conditioning and reliable power supplies to the humid and impoverished reality of the malarious tropics, it does not do so well. Confusing fluorescent material precipitates inside the tubes if they are exposed to a very humid condition for more than a few days" (Anon.,1992).

Aim

To devise a technique to eliminate the disadvantages of the standard QBC method of malaria detection so specificity is improved and species identification is possible.

MATERIALS AND METHODS

The dye was washed off used QBC capillary tubes. The QBC capillaries were thoroughly cleaned and dried. The floats were preserved. Blood samples of ten malaria-positive patients that had been collected in EDTA tubes were drawn onto the washed QBC capillaries. The float was re-inserted and centrifugation carried out as recommended by the QBC manufacturers. The capillary tubes were broken with the aid of a diamond pencil at the area where parasite-infested cells were most likely to be seen, i.e., just below the buffy coat area. Smears were made onto a slide with this material and stained using the conventional Romanowsky method of staining. Ordinary microscopy was carried out and parasitaemia quantified as number per oil immersion field. The results were compared with the conventional thin smears. Five samples from healthy, non-infected individuals were treated similarly to serve as negative controls.

RESULTS AND DISCUSSION

Parasites' number was found to be on an average ten
Parasites' number was found to be on an average ten times higher per oil immersion field compared to the conventional methods (See Figure 1). This improvement in sensitivity (over conventional thins smears) is comparable to that by standard QBC method. Negative controls yielded valid outcomes.

The standard QBC techniques had the disadvantage of positive interference from bacterial presence. The modified QBC method was unaffected by bacterial presence as this method does not rely on the taking up of fluorescent dye by the nucleoproteins. Species identification is not possible in standard QBC method. Though it was not as good as conventional thin smear preparations, modified QBC method enabled species identification easier compared to conventional thick smears.

An area of concern is that in this experiment, diamond pencil was used to cut the capillary. This poses a health and safety risk to the operator. This can be overcome with further improvisation and automation.

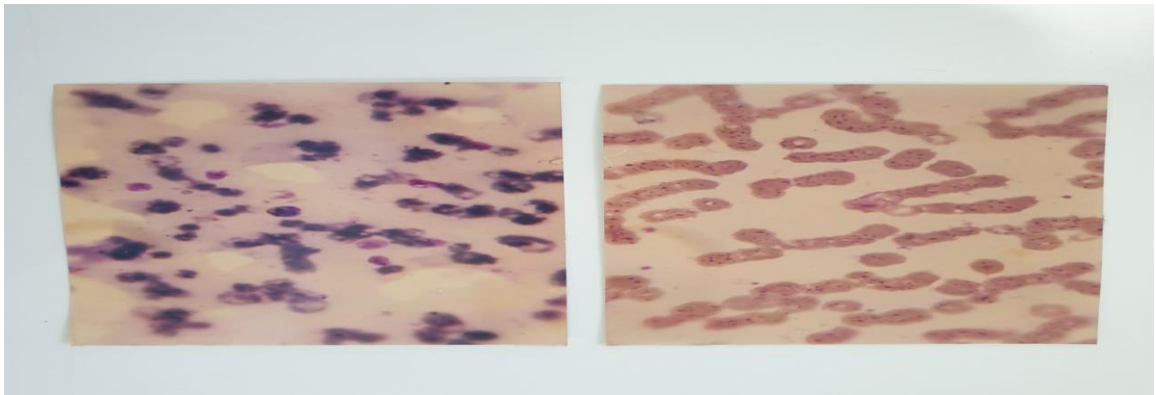

Figure 1 The image on the left is that of the modified technique and on the right is of the conventional thin smear of the same sample.

CONCLUSION

This pilot study modifying the QBC technique of malaria detection demonstrated aten- fold increase in sensitivity compared to conventional thin smear preparations. This was not at the cost of specificity unlike conventional thick

smear technique or the standard QBC technique. The method holds promise as a useful tool in screening for malaria in endemic areas where resources are limited. Larger study involving more samples is required to further validate the results. An easy method (preferably automated) to cut the capillary tube thereby eliminating the risk of infection to the operator would make the method significantly more user-friendly.

Declarations:

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