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Malaria Laboratory Diagnostic Approaches

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Abstract: *Malaria is an infection caused by plasmodium transmitted bu infected female anopheles mosquitoes to humans especially in this part of the world. Malaria infection has remained endemic and challenging in control and eradication despite all the measures adopted by World Health Organisation.The environmental and genetic factors have contributed immensely to the uncontrolled spread of the infection. A lot of methods are adopted in the diagnosis of malaria infection for proper treatment of the patients. In all these methods Microscopy has remained a reference method for malaria diagnosis but there are other methods which are discussed in this paper. Those involved in the diagnosis should pay attention to the clinical features of the patients and the differential features of different species of plasmodium and their stages for effective treatment. Early diagnosis remains the best option in the treatment of malaria.*

I. Introduction

The traditional and conventional method of diagnosis is light microscopy. It involves preparation of thin and thick blood films and staining with field's Wright's or Giemsa stains. The thin film enables the determination of parasite density and identification of species and has a better specificity compared to thick films. The thick film is more sensitive, enables detection of parasitaemia at lower levels, and during recrudescence or relapse of infection (Moody *et al*., 2000). However, preparation of blood film is laborious, demands expertise and is subject to observer interpretation at varying levels of parasitaemia. The latter affects its sensitivity through microscopy is less sensitive compared to newer methods, it remains the acceptable gold standard because of the need to stick to a consistent format considering low resource e settings. Microscopy has an accuracy of 70 -75% (Moody *et al.*, 2000).

The need for improvement in the diagnostic accuracy of light microscopy in the detection of parasites led to the use of fluorescent dyes and concentrated thick film. This affords a specificity of $\geq$ 93% for infections with p.flaciparum and ability to recognize the small ring forms. The major advantages of using PCR -based technique are the ability of detect malaria parasites in patients with low levels of parasitaemia and identify them to the species level. PCR- based methods are particularly useful for studies on strain variation, mutations, and studies of parasite genes involved in drug resistance (Redd *et al.*, 1996).Traditional malaria diagnosis is presumptive. its specificity is about 21% when diagnosis is based on fever alone and 42% when used in combination with nail bed pallor and splenomegaly (Hopkins et al 2009). Thus about 80% of cases are treated blindly with implication for drug wastage and potential drug resistance. The cost implication of this is enormous (Amexo *et al.*, 2004).

II. Fluorescence microscopy

The fluorescence microscopy is based on the phenomenal that contain material emit energy detectable as visible light when irradiated with light of a specific wavelength. The sample can either be fluorescing in tits natural form like chlorophyll and some minerals or treated with fluorescing chemicals (Moody *et al.*,2002). In malaria diagnosis, many methods have been developed based on this technique. Some of these methods are the quantitative Buff- coat (QBC) methods which is available as a commercial kit, the Kawamoto alcidine – Orange (KAO) process the Benzothiocarboxypuvine (BCP) procedure and the parsec rapid malaria test.

Test performance of the fluorescent microscopy Quantitative Buffy coat (QBC) method the QBC method uses Alcidine Orange (AO) al the fluorochrome to stain the nucleic acids of any malarial parasites in the sample. Although, AO is a very intense fluorescent stain, it is non- specific and stains nucleic acids from all cell types (Moody *et al.*, 2002).

III. KAO and BCP method

While the kawamoto method uses AO as the fluorochroure to stain the nucleic acids of any malarial parasites in the samples, the BCP is used for the BCP method. Both methods are rapid even through the KAO is simpler. The BCP can be applied directly to a lysed blood suspension or to an un fixed but dry thick blood film and stairs the nucleic acid of viable p. falciparum parasites intensely and has a reported sensitivity and specificity of >95% for p. falciparum (Moody *et al.*,2002). The sensitivity of AO staining with parasite levels of <100 parasites/ml has been reported to range from 41.7% - 93% (Lowe *et al.*, 1996) and specificity of AO staining for *P.vivax* infections appears to be about 52% whereas that for *P. faliciparum* infections is around 93%. Both methods cannot distinguish between the various plasmodium species.

IV. Partec rapid malarial test

The test is done using the parteccyscope. It is an innovative microscope that uses both uv fluorescence light and transmitted light simultaneously or in separate and integrates the most available generation of powerful light emitting diode (LED) light sources. it is battery-operated and mobile, designed for several hours of use completely independent from any regular power supply. The parteccyscope is perfectly suited for all applications in light and fluorescence microscopy and has ready- prepared and ready- to- use test slides which carry the dried – in reagents (DAPI).

V. Molecular techniques

Molecular techniques such as polymerase chain reaction (PCR) and Nucleic Acid Sequence – Based Am plication (NASBA) have been developed in the molecular diagnosis of malarial. Since 1990, several experimental assays have been reported that use various primers, extraction and detection techniques Quantitative - nucleic acid Sequence - Based Am plication (QU-NASBA) can detect parasites al level as low as 0,02 parasites/al blood and allows for pr4ecise quantification of the parasites load over a range of 20 – 108 parasites/al blood . The need for a more sensitive and time – efficient assays has led to the development of molecular assays involving real time PCR. Real time PCR assays have the potential to detect low levels of parasitaemia, identify mixed infections, and allow for precise differentiation of species, via melting curve analysis.

Test of performance of real time PCR

Real time PCR is much easier to perform because it offers the option of using single probes instead of multiple probes with complex procedures. it is less time consuming since results interpretation is not gel based but rather on melting curve analysis which takes less time with 100% sensitivity and specificity. Real time PCR also prevents carry over contamination as products are not reopened for gel based electrophoresis on the other hand, the thermal cyclers, premiers, probes etc used for the amplification processes are very expensive and therefore

cannot be used for distinct hospitals and malarial endemic areas where they are needed most. It also require much expertise and experience which are not available in these endemic areas.

VI. Flow cytometry

Flow cytometry carries some potential as an alternative tool for malaria diagnosis. Whereas this method appears to be too expensive for malaria – endemic countries, it might be of great value in affluent countries where flow cytometric blood cell differentiation is used routinely in hematology laboratories.. Automated hematology analyzers have demonstrated unexpected abnormalities in differential white blood cell plots and reticulocytes histograms from patients with malaria. Normal monocytes can be discriminated from monocytes that have ingested the malarial breakdown product homozoin because of the ability of homozoin to depolarize laser light used for routine differentiation of eosinophils. Nuclear materials of intraerythroaptic malaria parasites could be discriminated by fluorescent nucleic acid dye used in routine quantifdication of reticulocytes. The presence of infected erythrocytes acids to a distinct fluorescent spike in reticulocyte histograms, referred to as pseueloreticutocytosis.

VII. Mass spectrometry

This is an in vitro method of detecting malarial parasites at sensitivity of 10 parasites/al of blood. it comprises a protocol for clean-up of whole blood sample, followed by direct ultraviolent laser desorption time of flight mass spectrometry. Intension signals are observed from intact ferriprotoporphyrin ix (Haeme), sequested by malaria parasites during their growth in human red blood cells. The haeme group is photoactive and turns out to be easily detectable by direct laser – desorption mass spectrometry. The laser – desorption mass spectrum of the haeme is structure – specific, and the signal intensities are correlated with the sample parasitaemia. Many samples could be prepared in parallel and measurement per sample may not longer than a second.

VIII. Malaria magnetic deposition microscopy (MDM)

In an attempt to overcome some problems inherent to blood smear microscopy, a magnet based approach to concentrate malaria parasites and alignment detection of malaria – infected erythrocytes by microscopy has been developed. This system, malaria magnetic deposition microscopy (MDM), exploits the fact that plasmodium parasites produce a crystalline by – product, haemozoin, from haeme which is be rated during hemoglobin digestion. MDM captures parasitized erythrocytes in a narrow magnetic field and deposits them directly into a small region of a polyester slide, which is immediately ready for fixation and staining. by concentrating parasites MDN increases the sensitivity of diagnosis and decreases the time it takes to read the slide and it the ability to concentrate parasites of all four human malaria parasite species, including efficient capture of *P.faciparum* gametocytes, *P.falciparum*-infested blood samples were enriched 4.0 – fold from a parasiteamia of 2.7% to nearly 100% whilst *P.vivax* infected blood samples were enriched up to 250-fold, from an initial parasitaemia of 0.1% to cluster with 25% infected erythrocytes.

IX. Light microscopy

The accepted laboratory practice for the diagnosis of malaria is the preparation and microscopic examination of blood films stained with Giemsa, wright or fields stain.

Test performance

Giemsa stain

Giemsa microscopy6 is still regarded as the gold standard and the most suitable diagnostic instrument for malaria control because it is expensive to perform, able into differentiate malaria species and quantify parasites. However, microscopy is labour-intensive, time consuming, requires well trained (Reyburn *et al.,* 2004) functional infrastructures plus effective quality control (QC) and quality assurance (QA) (Wongsrichanalai *et*

al., 1991). Microscopic diagnosis has been shown to be sensitive and nonspecific, especially when parasitaemia are low or mixed infectious are present (Ammexo *et al.*, 2004).

Field stain

Field's stain is widely used as a rapid staining technique for thick and thin blood film for the diagnosis of malaria. this is mostly due to the fact that this technique is easy, quick and the stains are commercially prepared, ready for use and malaria of all stages of plasmodium including the Schueffuer's and James's dots of *P.Vivax* and *P.Ovale* respectively.

X. Conclusion

Malaria infection has remained highly endemic in this part of the world. It is a serious public health challenge to the patients and the health care providers. A lot of researches have been going on to stop this deadly disease that has killed many and is still killing persons not minding the age of the patients. No vaccine up till now has been approved for the prevention of malaria infection. The best thing now is early diagnosis that must be accurate, precise and reliable and also timely and prompt treatment. A lot of methods were discussed in this paper even though that the microscopy remained the reference method. More lives will be saved if these methods are adequately and properly utilized.

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