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New Strategies for the Diagnosis and Screening of Malaria

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Abstract

Thin and thick blood film microscopy are the "gold standard" for malaria diagnosis. In recent years, there have been important developments in malaria diagnostic tests including fluorescence microscopy of malaria parasites stained with acridine orange, dipstick immunoassays that detect species-specific parasite antigens, and more recently, detection of parasite nucleic acids after amplification by PCR. With some of these methods, sensitivities and specificities approaching and even exceeding those of the thin and thick film can be attained. In particular, PCR-based tests for plasmodium DNA or RNA are more sensitive and specific than other tests for malarial parasites. A specific application for PCR diagnosis of malaria could be blood donor screening. Clinical trials of blood donor screening for malarial parasites by PCR are being conducted, in which pooled donor samples are screened to increase efficiency and reduce costs. Some of the new diagnostic methods may have specific applications in particular settings, depending on the purpose and location of testing, and other factors such as cost, desired sensitivity and specificity, speed and ease of use.

Key words: Malaria;PCR;Blood donation

1. Introduction

There are an estimated 150 million cases of malaria infection annually worldwide, resulting in a significant demand for malaria diagnostic tests. Thin and thick blood film microscopy remain the "gold standard" for malaria diagnosis. The method depends on the visualization of parasites using light microscopy of Giemsa-stained thin and thick blood smears. Although this procedure is cheap and simple, it is labor intensive and requires personnel who are well trained in the morphological differentiation of Plasmodium species for accurate diagnosis. While the thick film shows much greater sensitivity than the thin film, the thin film remains essential for species identification and parasite quantitation.

2. New Diagnostic Tests for Malaria

In recent years, alternative methods to identify malaria infections with varying degrees of specificity and sensitivity have been developed. These include fluorescence microscopy of malaria parasites stained with acridine orange, dipstick immunoassays that detect species-specific parasite antigens, and more recently, detection of parasite nucleic acids after amplification by PCR. With some of these methods, sensitivities and specificities approaching and even exceeding those of the thin and thick film can be attained.

An example of the use of acridine orange fluorescence to detect malarial parasites is the Quantitative Buffy Coat technique (QBC® Becton Dickinson, Cockeysville, MD, USA) [1] method. This uses a custom-designed capillary tube coated with heparin and acridine orange that is filled with the sample to be tested. After centrifugation, parasites stained with acridine orange are viewed by their fluorescence in a region near the buffy coat red cell interface, which is extended by a plastic float in the tube. The QBC® technique has the advantages of being rapid (6-12 min) and easy to interpret, but is inadequate for species identification or quantitation and lacks a permanent record.

Antigen detection tests for P.falciparum such as the ParaSight-F test (Becton Dickinson, Cockeysville, MD, USA) and the Amrad ICT Combination Malaria Test (Amrad Corporation, Richmond, Australia) employ solid phase dipstick technology in antigen capture (antibody sandwich) assays to detect the histidine-rich protein II of P.falciparum. The tests are not quantitative and antigenemia persists for several days after parasite clearance. Another antigen detection test (OptiMAL®, Flow Inc, Oregon, USA) utilizes a dipstick coated with monoclonal antibodies against the intracellular metabolic enzyme, lactate dehyrogenase (LDH) [2]. Differentiation of malaria parasites is based on antigenic differences between parasite LDH isoforms. Since parasite LDH is produced only by live Plasmodium parasites, this test has the ability to differentiate live from dead organisms. In general, antigen detection tests are rapid (10 min), easily transported, easy to perform and require no electricity or elaborate apparatus.

Recently, PCR assays have been developed for the diagnosis of malaria. The PCR method is capable of a very high sensitivity and specificity exceeding that of the thin and thick film [3-5]. Generally, the PCR method may be more appropriate for mass screening rather than for testing individuals, since it requires complex apparatus and a trained operator. In cases where species identification by PCR differs from results obtained by blood film microscopy, PCR results can be verified by sequencing of the amplification products.

The 18S rRNA gene has been used as a DNA target to differentiate plasmodial species by nested PCR [6] and RT-PCR [7]. Other DNA targets such as the circumsporozoite protein (CSP) gene [8-10] have also been investigated as species-specific regions. Tan et al [11] have demonstrated that the extrachromosomal large subunit ribosomal-RNA (LSU-rRNA) gene is extensively conserved within Plasmodium species, and is suitable as a genus-specific DNA target region. Based on this finding, a sensitive and reliable generic- as well as species-specific (mitochondrial DNA) multiplex PCR-based diagnostic assay for malaria has been developed [4].

3. The Role of PCR in Screening Blood Donors for Malarial Parasites

In order to prevent transfusion-transmitted malaria in the United States, Mungai et al [5] have reiterated the need for careful screening of blood donors by interview to exclude recent malaria exposure. In Europe, donor interview with serologic screening has been shown to reduce wastage of blood from donors exposed to malaria [12]. Such measures are consistent with the aim of deferring blood donors in developed countries who have been exposed to malaria, since asymptomatic malaria infection can persist in humans for several years. Malarial parasites are also able to survive in stored blood at 4°C for 7-10 days and to survive cryopreservation in glycerol [13].

In areas that are endemic for malaria, donor interview and serologic screening are futile. In non-endemic regions that are located near endemic areas (“adjacent non-endemic regions”), deferral of potential donors who have travelled to endemic areas has resulted in a significant reduction of the blood supply. A sensitive and efficient laboratory method of screening donors for malarial parasites in adjacent non-endemic regions could therefore reduce the number of donors who have been deferred due to travel to endemic areas. Although there may be a risk of collecting blood from a donor with an asymptomatic, persistent low grade malaria infection, the incidence of such infections in non-endemic regions is generally extremely low. The most appropriate method for the screening of blood donors for malarial parasites could be the detection of parasite nucleic acids by PCR, in view of its high sensitivity and specificity, and ease of automation. Such a strategy would also reduce transfusion-transmitted malaria in endemic areas, owing to the greater sensitivity of PCR detection of malaria in chronic carriers [3].

Very low parasitaemias may be found in immune individuals. Previous studies have found evidence of malaria infection detected only by PCR. In a study carried out in a holoendemic area in Senegal, Bottius et al [3] reported that two-thirds of the inhabitants were positive for malaria by PCR, but not by blood film microscopy. In another study in Singapore [4], PCR could detect very low parasitemias and mixed-species infections that were missed by antigen-detection tests and microscopy. While PCR tests for plasmodium DNA or RNA are more sensitive and specific than other tests for malarial parasites, it remains unclear whether PCR can detect the lowest parasite densities that can cause malaria [5]. The feasibility of blood donor screening for malarial parasites by PCR is being explored in large scale clinical trials in Singapore, in which pooled donor samples are screened, to increase efficiency and to reduce costs. However, PCR screening for malaria undoubtedly requires technical resources that are hurdles for under-developed countries.

4. Conclusion

The newer diagnostic methods for malaria may have specific applications in particular settings, depending on the purpose and location of testing, and other factors such as cost, desired sensitivity and specificity, speed and ease of use. PCR-based methods are a new and powerful method for malaria diagnosis that could be applied to the screening of blood donors for malaria.

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