Plasmodium species

Four species of the protozoan genus *Plasmodium* (*P.vivax, P.ovale, P.malariae* and *P.falciparum*) infect humans, and give rise to malaria. One hundred million people are infected annually, and the disease is very widespread in tropical and warm temperate regions where the appropriate anophiline mosquito vectors are found. Malaria is transmitted to humans by sporozoites from mosquito salivary glands. After inoculation, sporozoites enter liver parenchymal cells and undergo asexual multiplication (tissue schizogony) to form merozoite-filled tissue schizonts. Schizont rupture releases merozoites which then invade red cells by means of attachment to specific binding sites. The merozoites divide asexually forming trophozoites which subdivide to form red cell schizonts. The asexual replication cycle then recurs at regular intervals until treatment, acquisition of immunity or death supervenes. With the relapsing malarias, due to *P.vivax* and *P.ovale* merozoites may also be released from long-term persisting liver forms called hypnozoites (which are dormant sporozoite forms); these are responsible for late relapses. Some intraerythrocytic parasites differentiate to form gametocytes, which are taken up into the mosquito stomach when the insect feeds, and the sexual part of the cycle takes place (sporogony). Ultimately sporozoites are formed which migrate through the mosquito body cavity and enter the salivary glands, to complete the parasite life cycle. Blood transfusion and transplacental spread may result in non-vector borne transmission.

Malaria presents most commonly as fever, which is initially irregular in pattern, but which frequently becomes characteristically regular as the host immune response results in synchronisation of the later cycles of multiplication. Febrile episodes may be accompanied by rigors, hypotension, gastrointestinal disturbance and altered consciousness. Patients are often asymptomatic between paroxysms, although hepatosplenomegaly is frequently detectable. Haemolytic anaemia is an important complication, and often results in mild jaundice. Secondary tissue hypoxia may result both from anaemia, but also by blockage of small vessels and may result in seizures and coma, renal failure and ARDS-like responses. The majority of these complications are associated with *P.falciparum* infections.

Diagnosis

Diagnosis of malaria is still most commonly performed by detection and identification of the organisms in a Giemsa-stained thick or thin blood film. Thick films are more sensitive due to the much larger volume of blood per unit area of the slide, but are more difficult to interpret for the inexperienced laboratory. According to the parasite morphology observed, it is possible to diagnose the infecting species. Wright's stain has also been used for the examination of thin films, and some workers have described the use of fluorescent microscopy of acridine orange-stained preparations.

PCR or DNA probe-based methodologies have been described for the specific diagnosis of malaria, but are as yet not widely available, particularly in endemic area. Various ELISA-based systems to detect parasite antigens, such as the histidine-rich protein (HRP2), have been described and are commercially available. Detection of serum antibodies is generally unhelpful diagnostically in the acute situation due to the 3-4 week delay before seroconversion.

References

Krogstad DJ. *Plasmodium* species. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases, 4th edn. New York, Edinburgh, London: Churchill Livingstone. 1995:2415–27.

Makler MT, Gibbins B. Laboratory diagnosis of malaria. Clin Lab Med. 1991;11:941–56.

Plasmodium falciparum
ANTIGEN DETECTION

Manufacturer: Cellabs Pty Ltd
Cat. No./Trade name: KM2/MALARIA CELISA™

SUMMARY

[Well-MAb]–**Ag**–[MAb-HRP]–[TMB]–A_{450}

Assay type: EIA (non-competitive)
Detection: Colorimetric A_{450}
Format: Microtitre well, Ab coated
Sample type: Lysed blood
Sample pre-treatment:
None
Sample volume: 100 µl
Number of tests: 192
Controls - standards run in assay:
Controls: Neg (1), high Pos (1)
Incubation:
1 hr (RT) + 1 hr (RT) + 15 min (RT)
Washes: 2

CONTENTS

Antibodies, antigens, labelled components:
Anti-*P. falciparum* MAb bound to well
Anti-*P. falciparum* MAb HRP conjugated
Substrate: TMB
Controls - standards supplied:
Controls: Neg (1), high Pos (1)
Additional reagents:
None
Special equipment required:
None

INTERPRETATION

Comments on interpretation:
Classification of samples is according to cut-off; no further testing
A positive result suggests a current or very recent infection. Test may remain Pos several days after parasites are no longer detectable in blood films
No. of references: None

NOTES

131414.0

For Research use only and for use as a confirmatory test for diagnostic problems, screening blood transfusion products and travel-related infection

Plasmodium falciparum
ANTIBODY DETECTION

Manufacturer: Cellabs Pty Ltd
Cat. No./Trade name: KM3/MALARIA Ab CELISA™

SUMMARY

[Well-Ag]–**Ab**–[AHIg-HRP]–[TMB]–A_{450}

Assay type: EIA (non-competitive)
Detection: Colorimetric A_{450}
Format: Microtitre well, Ag coated
Sample type: Serum, plasma
Sample pre-treatment:
None
Sample volume: 100 µl
Number of tests: 192
Controls - standards run in assay:
Controls: Neg (1), high Pos (1)
Incubation:
1 hr (37°C) + 1 hr (37°C) + 15 min (RT)
Washes: 2

CONTENTS

Antibodies, antigens, labelled components:
P. falciparum Ag bound to well
Anti-human Ig Ab HRP conjugated
Substrate: TMB
Controls - standards supplied:
Controls: Neg and high Pos
Additional reagents:
None
Special equipment required:
None

INTERPRETATION

Comments on interpretation:
Classification of samples is according to cut-off; no further testing
Serum samples which give values above the cut-off point should be considered as positive for malaria antibody. This suggests that the donor has or has had malaria. It does not imply that the donor is carrying malaria parasites at this particular time
No. of references: 7

NOTES

131781.0

This test system is not designed as a diagnostic method for active clinical malaria and must not be used for this purpose

Plasmodium falciparum
ANTIBODY DETECTION

Manufacturer: bioMerieux
Cat. No./Trade name: 72751/Falciparum-Spot IF

SUMMARY

[Slide-Ag]–**Ab**–[AHIg-FITC]–fluorescence

Assay type: Immunofluorescence assay (indirect)
Detection: Fluorescence microscopy
Format: Slide, Ag coated
Sample type: Serum
Sample pre-treatment:
Absorption of serum with group A1 RBCs to avoid nonspecific fluorescence with RBCs in substrate preparation
Sample volume: 10 µl of 1:20 and 1:40 dilutions
Number of tests: 100
Controls - standards run in assay:
Controls: Neg (1)
Incubation:
30 min (37°C) + 30 min (37°C)
Washes: 2

CONTENTS

Antibodies, antigens, labelled components:
P. falciparum Ag (from human RBCs, group A1) bound to slide
Anti-human Ig Ab (g) FITC conjugated
Substrate:
Controls - standards supplied:
Controls: Neg (PBS)
Additional reagents:
None
Special equipment required:
None

INTERPRETATION

Comments on interpretation:
Positive: intense fluorescence of organisms situated inside infected RBCs
No. of references: 5

NOTES

131281.0