Malaria
Advanced Diagnostics & Vaccine Development Initiative

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Diagnostic methods available for malaria

- Microscopic examination of blood smears
- Detection of parasite specific antigens (Rapid detection tests)
- Molecular diagnostic methods to detect parasite DNA (Nucleic acid based amplification methods)

WHO Guidelines for Diagnosis of Malaria

- Early and accurate diagnosis of malaria is essential for effective disease management and malaria surveillance.
- High-quality malaria diagnosis is important in all settings as misdiagnosis can result in significant morbidity and mortality.
- WHO recommends prompt malaria diagnosis either by microscopy or malaria rapid diagnostic test (RDT) in all patients with suspected malaria before treatment is administered.
- Diagnostic testing improves the management of all patients with febrile illnesses, and may also help to reduce the emergence and spread of drug resistance by reserving antimalarials for those who actually have the disease.
- Treatment solely on the basis of clinical suspicion should only be considered when a parasitological diagnosis is not accessible.

Malaria diagnosis in low transmission

- RDTs and microscopy are the primary diagnostic tools for confirmation and management of cases of suspected clinical malaria in all epidemiological situations
- RDTs and microscopy are appropriate for routine malaria surveillance (of clinical cases) in most malaria-endemic settings.
- Generally, use of highly sensitive diagnostic tools like Nucleic acid amplification (NAA) techniques should be considered only in low-transmission settings.
- Use of NAA methods in malaria programmes should be considered for epidemiological research and surveys to map sub-microscopic infections in low-transmission areas.
- NAA methods might also be used for identifying places for special interventions in elimination settings.

Specimen Collection

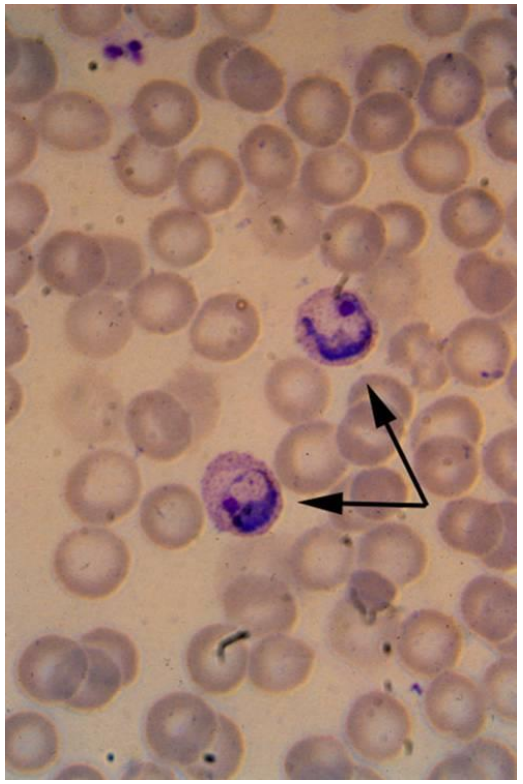
- Ideally, blood can be collected by finger prick for microscopy
  - If other tests being performed, can use venipuncture
  - EDTA is preferred as the anticoagulant as heparin may lead to morphological distortion
- Smears should be prepared and stained within an hour of drawing the specimen.
  - Alterations in morphology may occur if delayed.

Other Information to Collect

- Travel history (Time between bite & symptoms)
  - Help suggest likelihood of infection
    - 9 to 14 days for *P. falciparum, P. vivax, and P. ovale* (or months for vivax and ovale)
    - Up to 40 days for *P. malariae*
    - After one month, probably not *P. falciparum*
- History of prophylaxis or treatment of malaria
- History of transfusion or shared needles
- History of malaria in person (relapse?)
- Knowledge of fever pattern

Microscopy – The Gold Standard

- Benchmark diagnostic standard for over 100 years.
- In expert hands: highly sensitive, specific.
- Results provide a wealth of clinically important data.
- Stained slide serves as a permanent record.



Microscopy

- Thick film considered “gold standard” for detection of parasites
- Expected sensitivity that can be achieved by an experienced microscopist for the examination of the thick blood film procedure is about 50 parasites/µl of blood.
- Thin film considered “gold standard” in species identification
- Smear examinations should be under oil immersion
- Negatives should not be reported until 200 oil immersion fields have been examined

Microscopy Limitations

- Microscopy skills may be lacking in areas not routinely doing malaria evaluations
  - Smear preparation, staining
  - Interpretation
- Mixed infections - can be difficult to diagnose.
- Low parasitemia - can be difficult to diagnose.
  - detectable range: 0.001% parasitaemia
- Hands on time is very high.

Rapid Diagnostic tests based on detecting Parasite Antigens

- These tests employ immunochromatographic methods based on the detection of malarial antigens present in peripheral blood.

- Most tests use monoclonal antibodies that detect particular malarial antigens in blood specimens.

- Sensitivity: ~ 100 parasites/microliter blood
- Specificity > 90%

- Generate results within 15 minutes and do not require skilled microscopists.

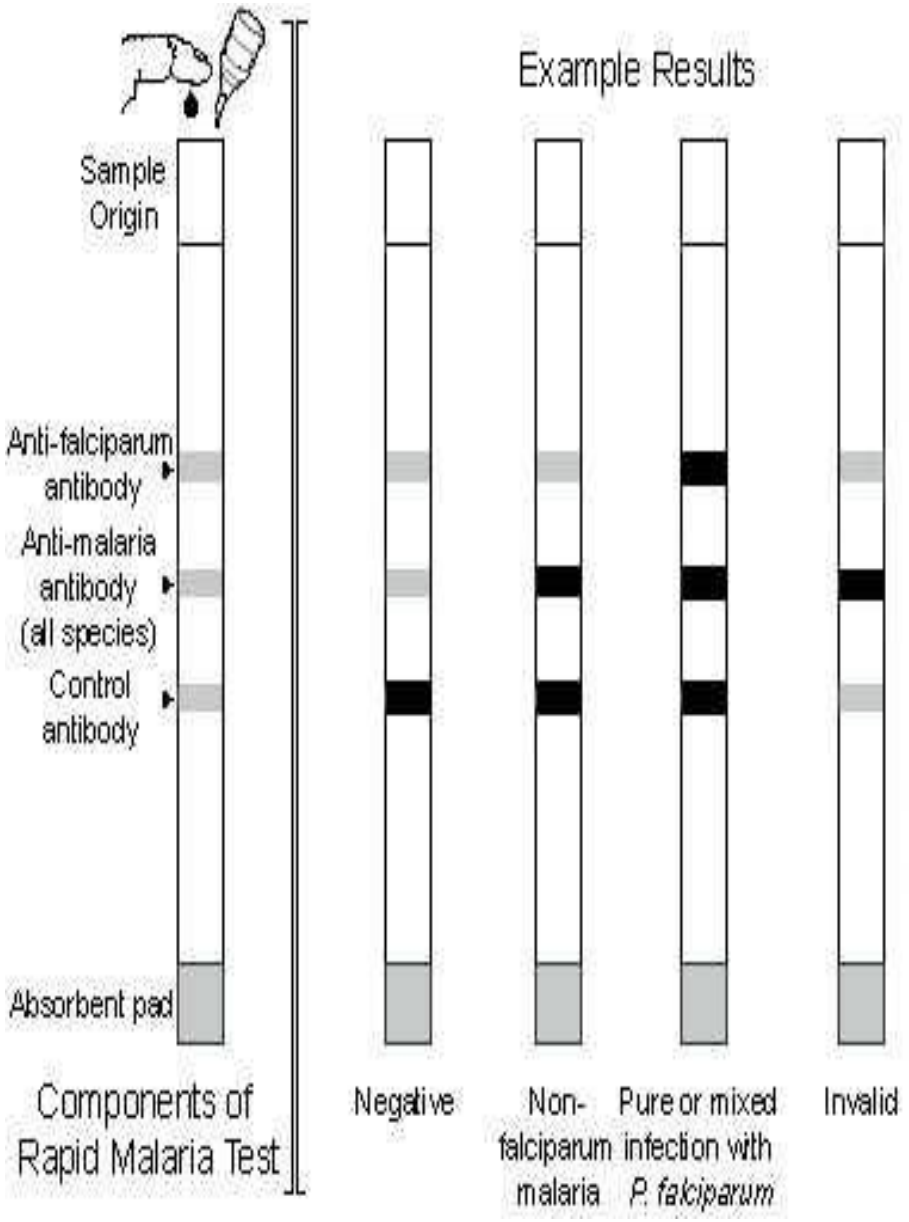
- Tests have been developed that detect antigens including the
  - histidine-rich protein II (HRP-II) of *P. falciparum*
  - Pan-malarial antigens: aldolase
  - Parasite specific lactate dehydrogenase (pLDH).

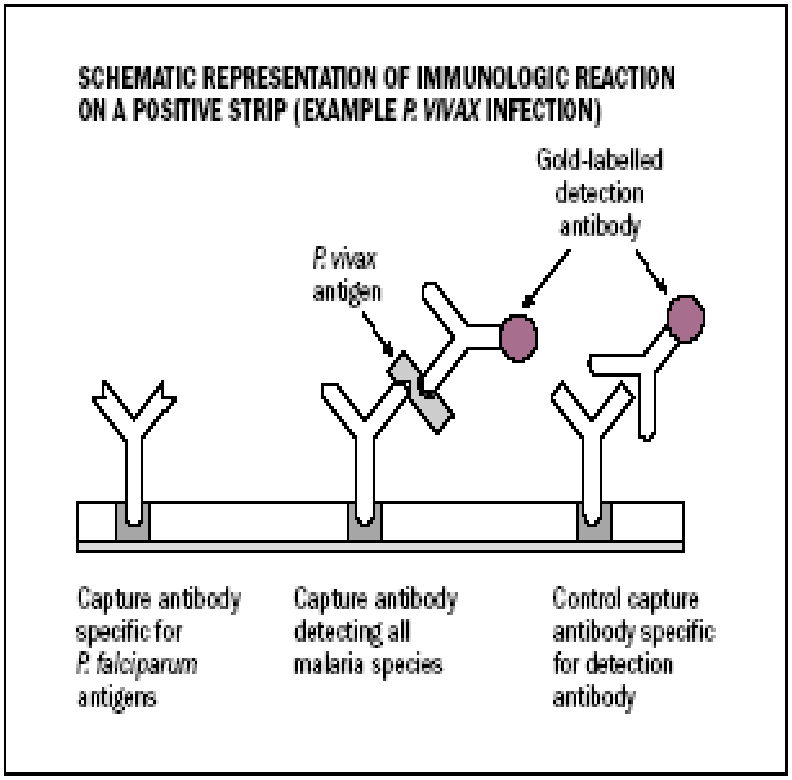
Components of a antigen detection kit

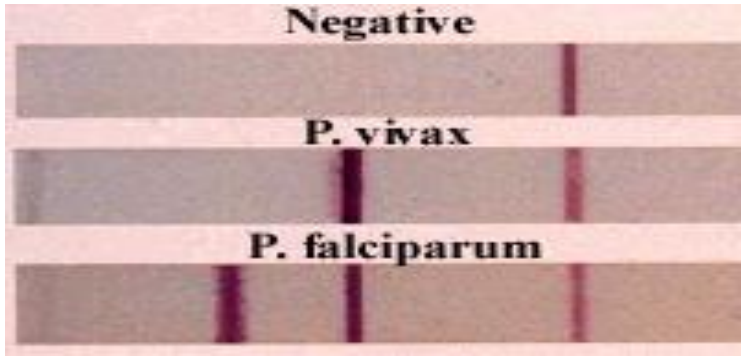
- Control capture monoclonal antibody specific for a labeled detection antibody (often gold labeled)
- Capture antibody specific for and that can detect a malaria antigen present in all *Plasmodium* species (pan malaria antigen: aldolases)
- Capture antibody that specifically detect *P. falciparum* antigen like HRP-II
- Specimen: 2 to 50µL of finger-prick blood, anticoagulated blood, or plasma

Method:

- Specimen is mixed with a buffer solution that contains a haemolysing compound and a specific antibody that is labelled with a visually detectable marker such as colloidal gold.
- Hemolysed specimen is added to the sample pad
- If the target antigen is present in blood, the antigen/detection antibody complex is formed and it migrates up the test strip to be captured by the pre-immobilized capture antibodies specific against the antigens.
- Let it stand for 15 minutes
- A washing buffer is then added to remove the hemoglobin and permit visualization of any coloured lines formed by the immobilized antigen-antibody complexes.


Example Results
Sample Origin
Anti-falciparum antibody
Anti-malaria antibody (all species)
Control antibody
Absorbent pad
Components of Rapid Malaria Test
Negative
Non-falciparum malaria
Pure or mixed infection with P. falciparum
Invalid


SCHEMATIC REPRESENTATION OF IMMUNOLOGIC REACTION ON A POSITIVE STRIP (EXAMPLE P. VIVAX INFECTION)
Gold-labelled detection antibody
P. vivax antigen
Capture antibody specific for P. falciparum antigens
Capture antibody detecting all malaria species
Control capture antibody specific for detection antibody


Negative
P. vivax
P. falciparum

HRP-II based testing

- HRP-II is a water soluble protein that is produced by the asexual stages and gametocytes of *P. falciparum.*

- Therefore diagnose only *P. falciparum* malaria.

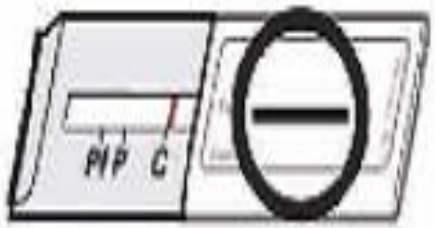
- *It is* shown to remain in blood for at least 28 days after the initiation of antimalarial therapy.

- The PfHRP2 test strips have 2 lines, one for the control detection antibody and the other for the PfHRP2 antigen.

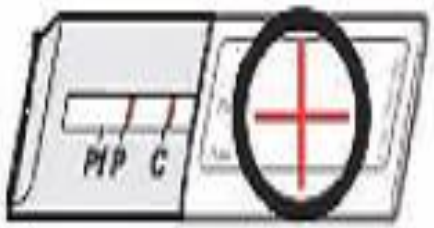
Lactate dehydrogenase based testing

- **Parasite lactate dehydrogenase (pLDH)** is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites and released from the parasite infected erythrocytes.
- In all 4 human malaria species, different isomers of pLDH exist.
- Differentiation of malaria parasites is based on antigenic differences between the pLDH isoforms.
- Since pLDH is produced only by live *Plasmodium* parasites, this test has the ability to differentiate live from dead organisms.
- Example: "Optimal" utilizes a dipstick coated with monoclonal antibodies against the parasite lactate dehydrogenase (pLDH).

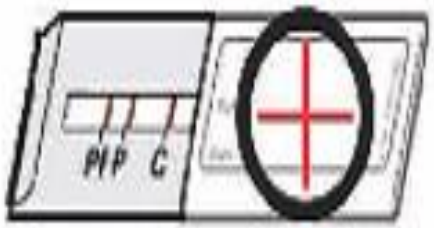
Results



Negative for all types of Malaria



Positive for Plasmodium sp.
(P. vivax / P. ovale / P. malariae)



Positive for Plasmodium falciparum
+/- (P. vivax / P. ovale / P. malariae)

Plasmodium aldolase based testing

- ***Plasmodium* aldolase** is an enzyme of the parasite glycolytic pathway expressed by the blood stages of *P. falciparum* as well as the non-falciparum malaria parasites.

- Monoclonal antibodies against *Plasmodium* aldolase are pan-specific in their reaction and have been used in a combined 'P.f/non P.f' immunochromatographic test that targets the pan malarial antigen along with PfHRP2.

Interpretation of assays with combination of antigens

- Example: The Binax assay – FDA approved kit
- contains both the HRP II (for *P. falciparum*) and aldolase which is a pan-malarial antigen.
- Colour change on the control line and the pan specific line indicates non-falciparum infection.
- Colour change on all the 3 lines indicates the presence of *P. falciparum* infection, either as mono-infection or as a mixed infection with non-falciparum species.
- Mixed infections of *P. falciparum* with the non-falciparum species cannot be differentiated from pure *P. falciparum* infections.

Molecular methods for parasite DNA

PCR based Molecular methods are

- **Highly specific**: PCR methods can give valuable information when difficult morphological problems arise during attempts to identify parasites to the species level.

- **Highly sensitive** (< 5 parasites/µl of blood)

  5-10 pg to 1 ng of parasite DNA can be detected
  ~ 0.0004 - 0.004 % parasitaemia

- Large no. of samples can be screened

Applications of Nucleic Acid Amplification based testing (Molecular Diagnostics) in Malaria

- Malaria surveillance in elimination programmes
- Epidemiology research
  - To study parasite population dynamics in epidemiological investigations (to detect different strains circulating in the parasite reservoir)
- Blood bank screening
- Travel medicine – to track imported cases
- Hunt for sub-patent asymptomatics:
  - sub-microscopic infections with extremely low parasitaemia
- Vaccine and drug studies (Trials)
- Genotyping for resistance
  - To detect the emergence and spread of drug resistant parasites: markers for drug resistance
- To confirm mixed infections with both *P. falciparum* and *P. vivax* so that differential therapy can be given

PCR Targets and Primers

- 18S small subunit rRNA gene target is the most common
  - Moderate copy number
  - Well conserved
  - Able to detect all 4 species of malaria

- Alternatives:
  - Cytochrome *b* gene
  - Mitochondrial genes
  - Var *genes*

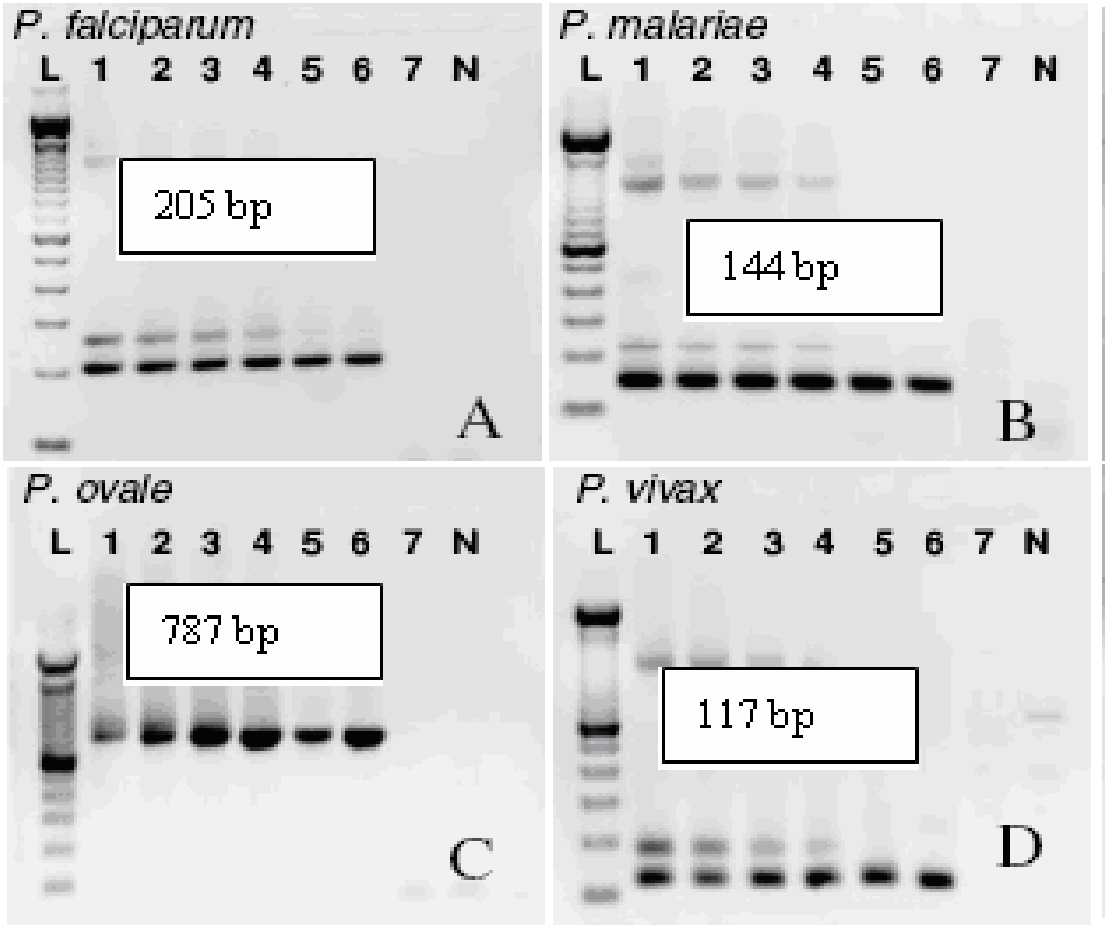
Nested PCR based on 18S ss-RNA genes

- Amplification based on 18S, ss-rRNA (small sub unit rRNA) gene

- Able to detect all 4 human malaria parasites and different PCR fragments are generated for different species:

  * *P. falciparum …. 205 bp fragment*
  * *P. vivax …. 120 bp fragment*
  * *P. malariae …. 144 bp fragment*
  * *P. ovalae …. 800 bp fragment*

Nested amplification of ***Plasmodium ss-rRNA*** **gene**



Important molecular markers to evaluate the emergence of drug resistance

- In *P. falciparum*, 8 point mutations in the transmembrane protein **Pfcrt**, a **chloroquine resistance related transporter** are completely associated with chloquine resistance.

- PCR diagnostic assays coupled with Pfcrt mutation detection available as surveillance tools for drug resistance.

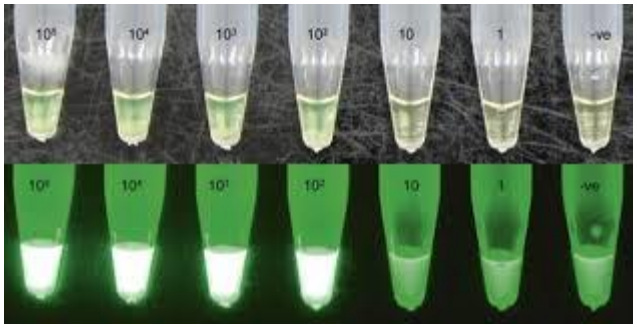
- In *P. falciparum* resistance to Pyrimethamine is associated with point mutations in **Dihydrofolate reductase (dhfr)** enzyme and sulfadoxine resistance is linked to point mutations in **Dihydrofolate Pteroase Synthetase (dhps) enzyme.**

- PCR and probes that are highly predictive of treatment failure for SP are now available.

LAMP method as a diagnostic tool

Loop-mediated isothermal amplification (LAMP)

- Based on amplification of nucleic acid by isothermal method.
- Like PCR, LAMP amplifies a specific section of DNA.
- Unlike PCR, LAMP does not require a thermocycler, the reaction takes place at a constant temperature.
- This process uses multiple different primers and a DNA polymerase
- LAMP has a sensitivity of about 5 parasites per microliter.
- LAMP can detect malaria DNA for from dried blood spots, which are commonly used to collect and test for malaria in active surveillance.
- Target genes: mitochondrial genes of parasites (genus specific targets)
- Results determined by turbidity or fluorescence.
- Results within 60 min.
- Appropriate for use in the field.



Comparison of Diagnostic Platforms

Assay	Limit of detection	Time	Speciation	Cost
Microscopy	50 parasites/µL	20 min per slide	Yes	Rs. 250.00
RDT	>100 parasites/µL	20 min	Pf and non-Pf	Rs. 1500.00
Lab based PCR	< 5 parasites/µL	3 hrs	Yes	Rs. 4000.00
LAMP	5 parasites/µL	2 hrs	No	Rs. 750.00

Malaria Vaccine Development

- The current global malaria vaccine portfolio consists of around 65 vaccine candidates, including 41 in the preclinical trials and clinical trial stages.

- In addition, GlaxoSmithKline Vaccines' RTS,S is currently in Phase 3 clinical development—making it the first malaria vaccine candidate to advance this far.

Malaria Vaccine Development

- Goals:
  - to prevent infection
  - Abolish severe pathological manifestations
  - reduce transmission
  - Ultimately eradication of malaria

- For endemic populations ---- a vaccine that reduces mortality and pathogenesis highly relevant

  (prevent life-threatening infection by reducing parasite burden or by neutralizing pathogenic mechanisms, while allowing the development and maintenance of immunity by continuous natural boosting)

- For non-immune individuals (who have never been to or infrequently exposed to malaria) --- complete prevention of infection is necessary.

Different Approaches

1. Anti-parasite vaccines (based on different stages)

- Pre-erythrocytic vaccines (liver stages)
- Asexual blood stage vaccines
- Transmission blocking vaccines

2. Anti-disease vaccines:

Pathological reactions or the “disease” is initiated by malaria toxins/immune mediators - cytokines.

Anti-disease vaccines operate against parasite toxins

eg of targets: Cytokines (TNF)

Glycosyl-phosphatidyl-inositol (GPI) derived from parasite membrane proteins

Objectives

- Describe the methods available for diagnosis of malaria.
- Outline the components and different approaches for malaria rapid detection testing based on antigen detection.
- Describe the DNA based testing platforms available for the diagnosis of malaria.
- Compare the application of different methods and their suitability for each application.
- Outline the goals of a potential malaria vaccine in terms of target population.
- Describe different approaches for malaria vaccine development.