

**An Overview of Malaria  
& its  
Laboratory Detection**



**Therese Driscoll**

**AMNCH**

**2003**

## Some relevant statistics from WHO

- § Malaria occurs in over 100 countries
- § 40% of the world's population is at risk
- § WHO estimates 300-500 million cases/year
- § > 1 million deaths/year
- § 90% of deaths occur in Africa, south of the Sahara, mostly among children.
- § In Africa, a child dies from Malaria every 30 seconds.

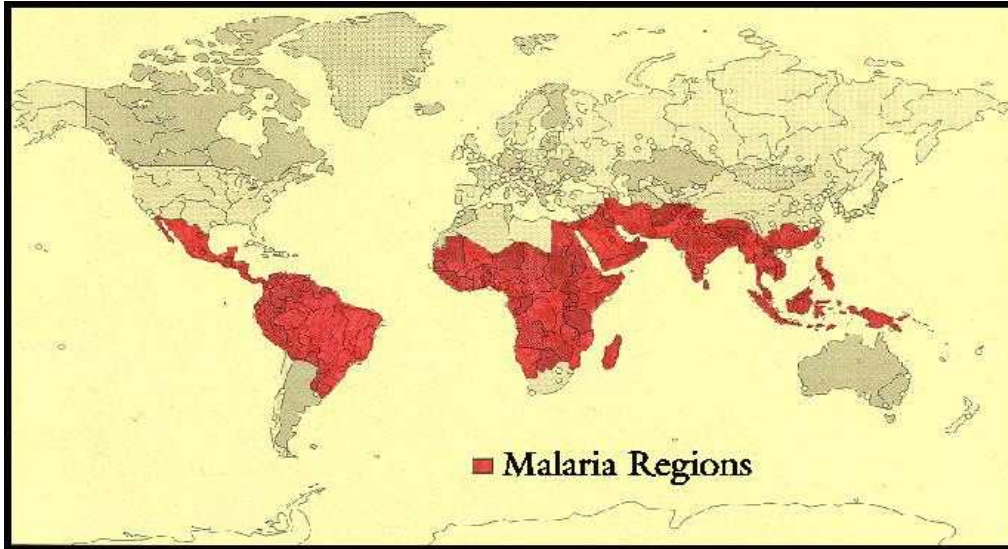
# History:

- § Prehistoric man is thought to have suffered from Malaria
- § It originated in Africa & accompanied human migration to the Mediterranean shores, India & SE Asia.
- § Name comes from the Italian for bad air: Mal-aria
- § The last documented epidemic of malaria in Britain was from 1917 to 1919 in Kent & Sussex
- § Although Australia was declared a Malaria free zone in 1981, 9 cases of locally acquired malaria have been documented in the region since then.

# History cont'd:

- § 1948 : All stages in the life cycle were identified
- § Quinine , an alkaloid derived from the bark of the Cinchona tree has been in use for more than 300 years.  
V. toxic side effects
- § Chloroquin introduced after WW2-less toxic, but development of resistant strains
- § Much research for new drugs with emphasis on combination therapy and search for a vaccine.
- § 2002 Genome of mosquito & malarial parasite sequenced.

# Malaria Endemic Regions



# Malaria & Haemoglobinopathies

- § Infected AS cells sickle more readily than uninfected cells leading to enhanced clearance of infected cells in RES
- § CC cells do not support parasite growth as the cells resist bursting
- § SC cells do not support parasite growth
- § EE & AE red cells are phagocytosed more readily than AA red cells
- § Cells of Thalassemia & G6PD deficiency do not support division of P.f due to their sensitivity to oxidant stress

# Distribution of malarial species endemic to man

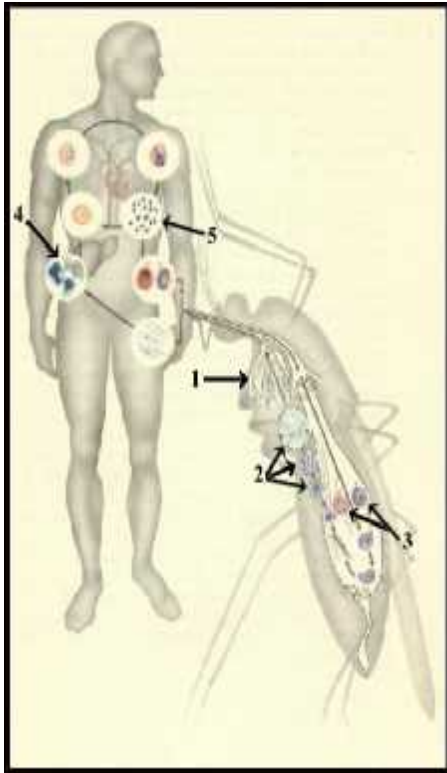
- § 4 species of malaria endemic to man.
- § *P. Falciparum* predominates in Africa, New Guinea & Haiti
- § *P. Vivax* most common in Indian Subcontinent & Central America
- § Pf & Pv codominate in Asia & S.America
- § *P. Malariae* found in all endemic areas especially Sub Saharan Africa
- § *P. Ovale* is unusual outside Africa.

# Modes of Transmission to Man



- § Most commonly from the bite of the female Anopheles mosquito in a malaria endemic region
- § Several cases of Airport malaria have been documented.
- § Blood Transfusion
- § Transplacentally from mother to baby

# Life cycle of the parasite



§ 1 Sporozoites in the salivary gland

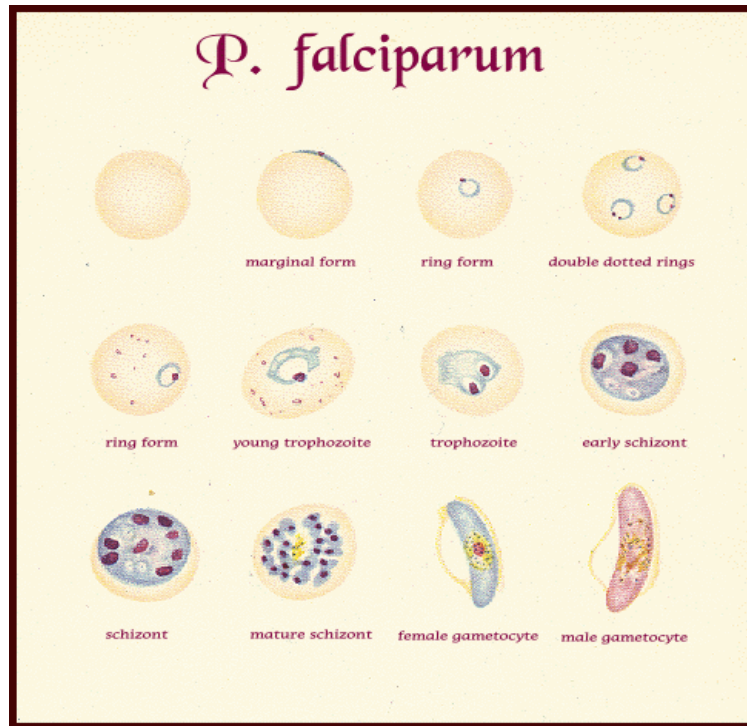
§ 2 Oocysts in the stomach wall

§ 3 M & F sporozoites

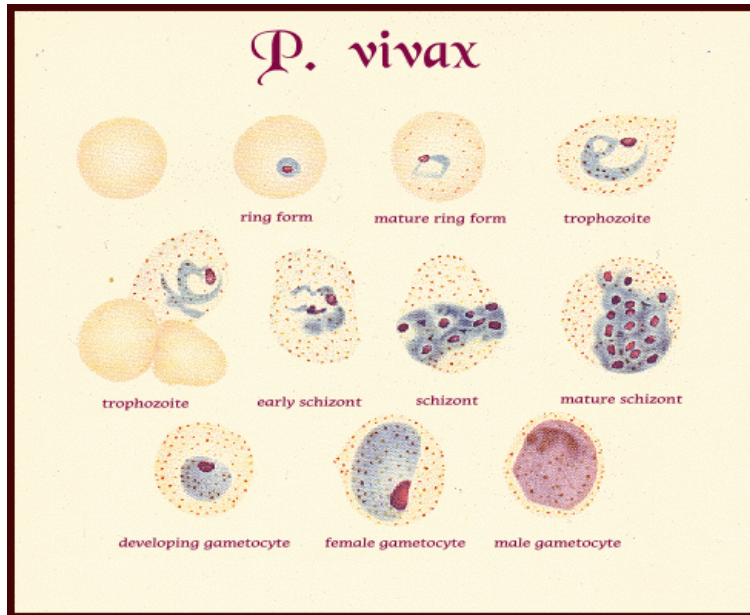
§ 4 Liver stages

§ 5 Release of merozoites from the liver. These then enter the red cells where both sexual & asexual stages develop

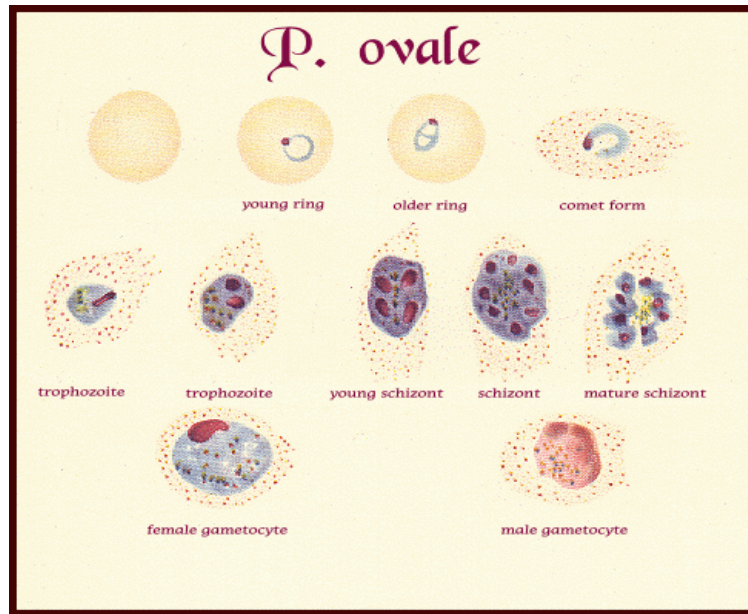
# Blood stages of *P. falciparum*



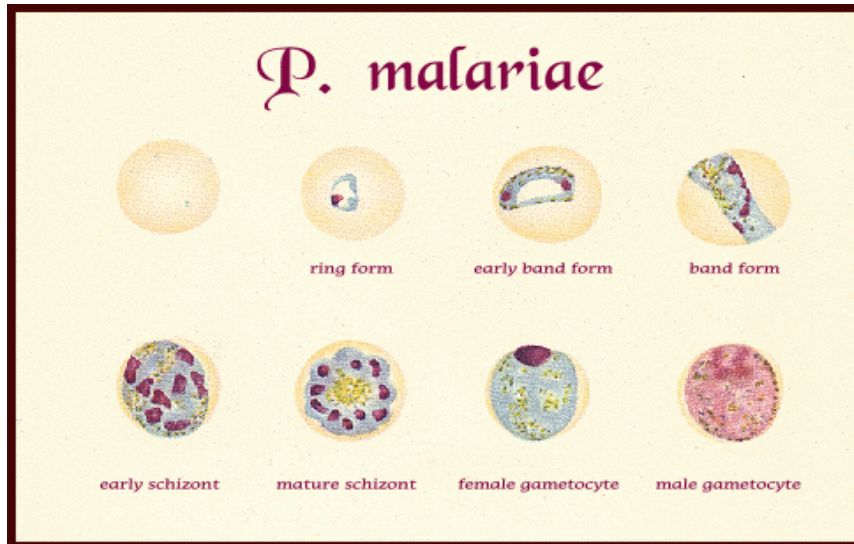
# Blood stages of *P. Vivax*



# Blood stages of P. ovale



# Blood stages of *P. Malariae*



# Pathophysiology of malaria

## § 1. Anaemia

- | Parasite Schizogony
- | Immune haemolysis
- | Clearance of IgG coated red cells from the spleen
- | Maturation defects in the marrow
- | Malarial associated proteins make the red cell less deformable

# Pathophysiology of malaria

## § 2 .Thrombocytopenia

- | Decreased platelet survival time
- | Abnormal megakaryocytes
- | Enhanced splenic uptake or sequestration

# Pathophysiology of malaria

## § 3. Leucopenia

- | Phagocytosed haemozoin & trophozoites may inhibit production of cytokines by monocytes
- | This may play a role in increased cytoadherence, vascular permeability & chemotaxis as well as immunodepression in malaria

# Laboratory Diagnosis of Malaria

## Required Clinical History:

§ Travel History

§ History of malaria

§ Pyrexia within last 24,48,72 hrs

§ Prophylaxis

§ Date of return home

§ If patient is a child:

- Country of birth
- Maternal Hx during pregnancy

# Laboratory Diagnosis of Malaria

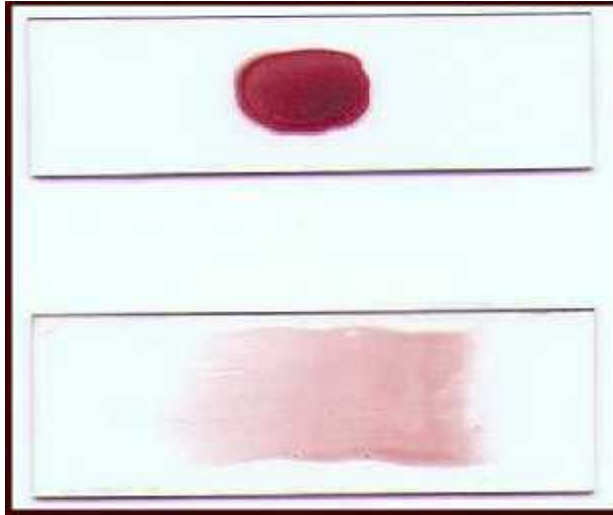
## § Collection of Samples

- | What sample? *EDTA or finger/heel prick*
- | When should I take it? *Optimal time of detection of various stages of malaria is midway between chills. However collection should be performed immediately upon first suspicion of malaria.*
- | For how long? *Up to 3 days*

# Laboratory Tests performed

- § **FBC:** WCC may be low, normal or high
- §           HB: Patient may be initially
- §           dehydrated & later anaemic
- §           Plts: Reduced in 80% of cases of malaria
- § Preparation of thick & thin films
- § Screening kits for malaria

# Staining of thick & thin films



§ Thick films stained unfixed by Field's stain.

§ Thin films stained by Giemsa Stain at pH 7.2

## Preparation & staining of thin films

- § Should be made within 2 hours after blood drawn
- § Air dry
- § Fix in methanol for 1 min
- § Stain with 1/10 dilution of Giemsa for 25 min.  
*Dilution must be made with buffered water at pH 7.2*
- § Wash gently in tap water and dry vertically.
- § Examine using X 100 objective
- § Perform parasite count if parasites detected.

## Preparation and staining of thick films

- § Use same volume of blood as for thin
- § Spread over 1 sq. cm
- § Air dry. If not completely dry, blood will wash off slide.
- § Dip vertically in Field's stain A for 3 sec
- § Wash in tap water
- § Fields B 3 sec
- § Wash in tap water. Drain vertically to dry.

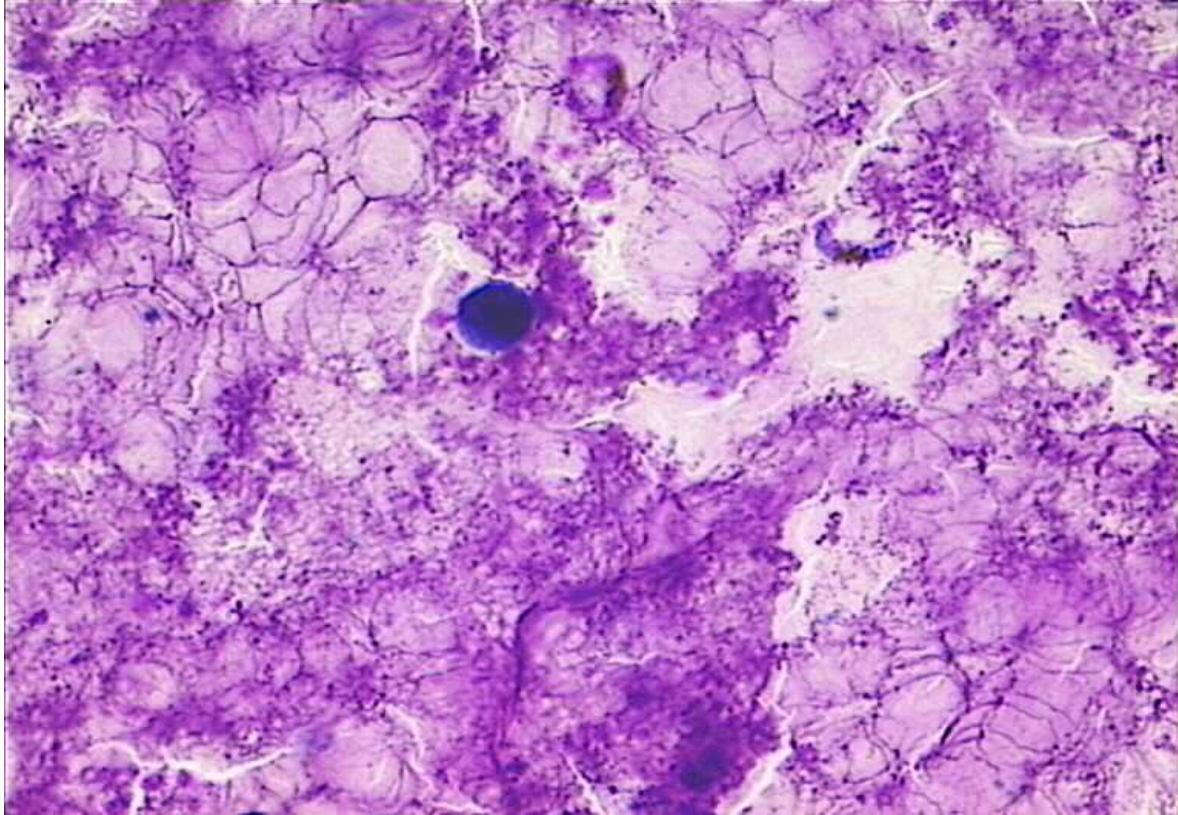
## FAQ's

§ How long should I look at a thick film for?

§ How long should I look at a thin film?

§ What part of a thick film should I examine?

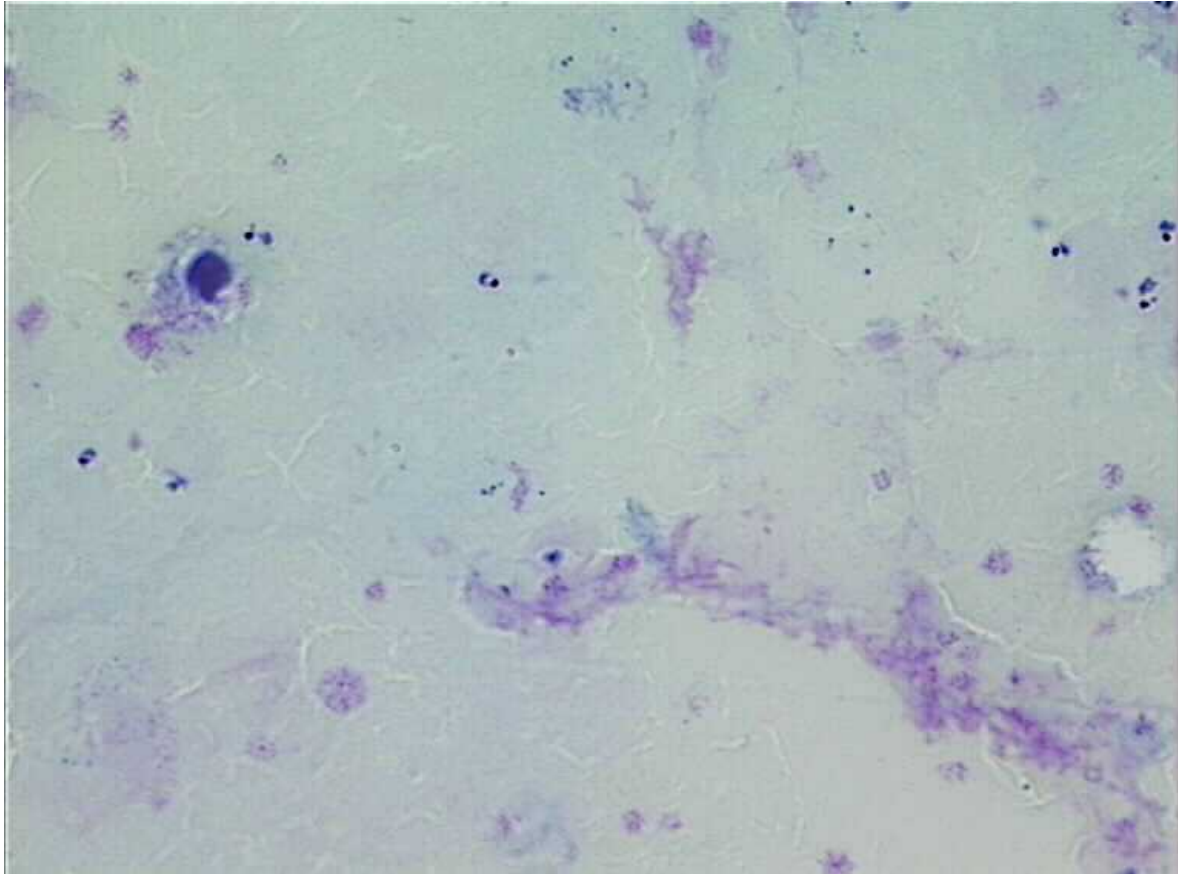
## Bad thick film showing Gametocytes of *P. falciparum*



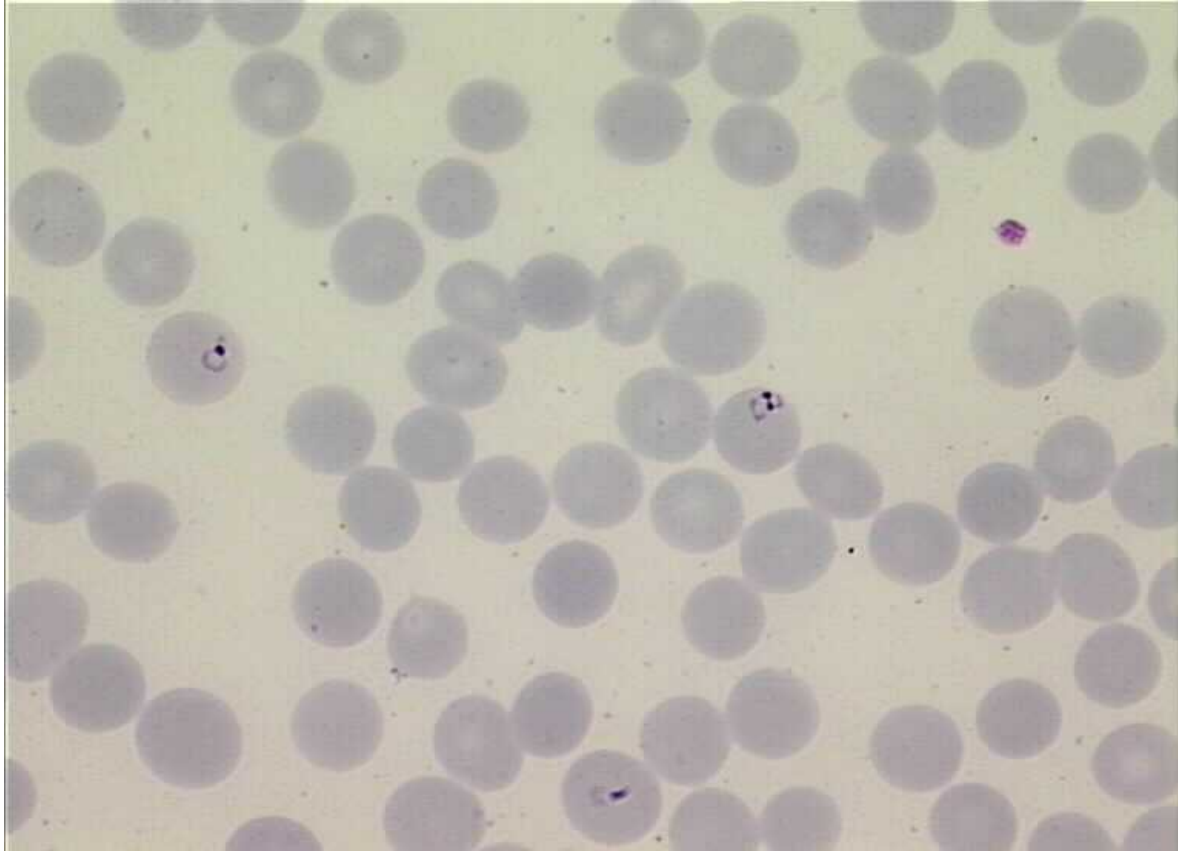
**Same patient, thin film showing trophozoites & gametocytes of P.f**



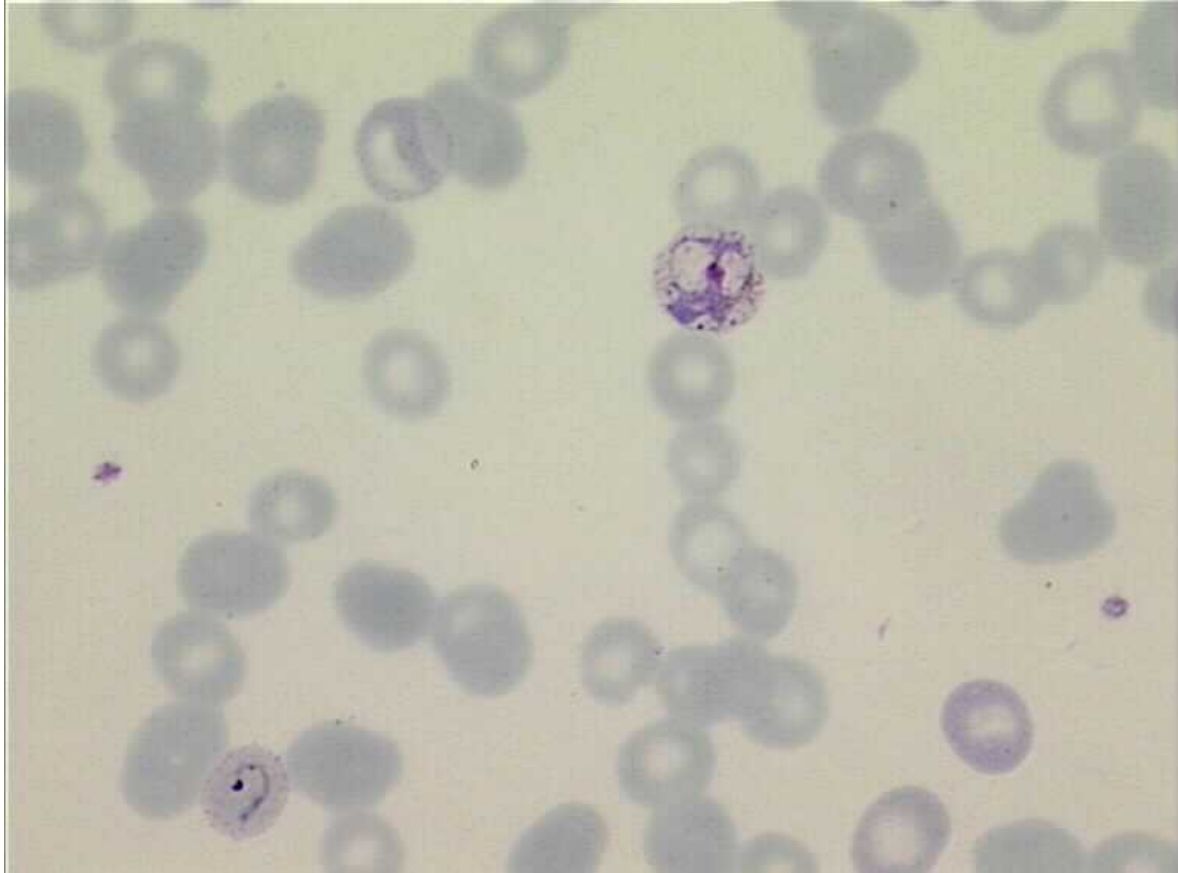
## Well stained thick film, Trophozoites of *P. Falciparum*



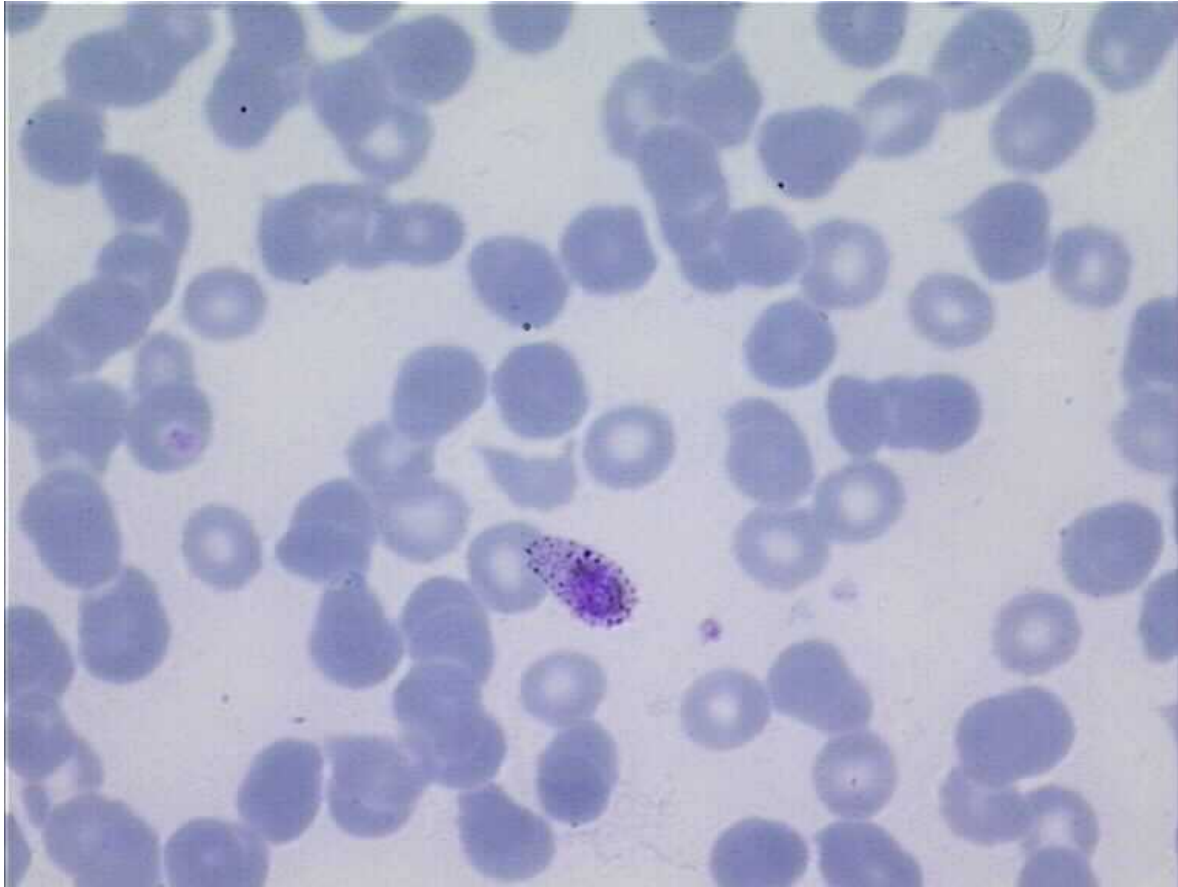
## Trophozoites of *P. falciparum*



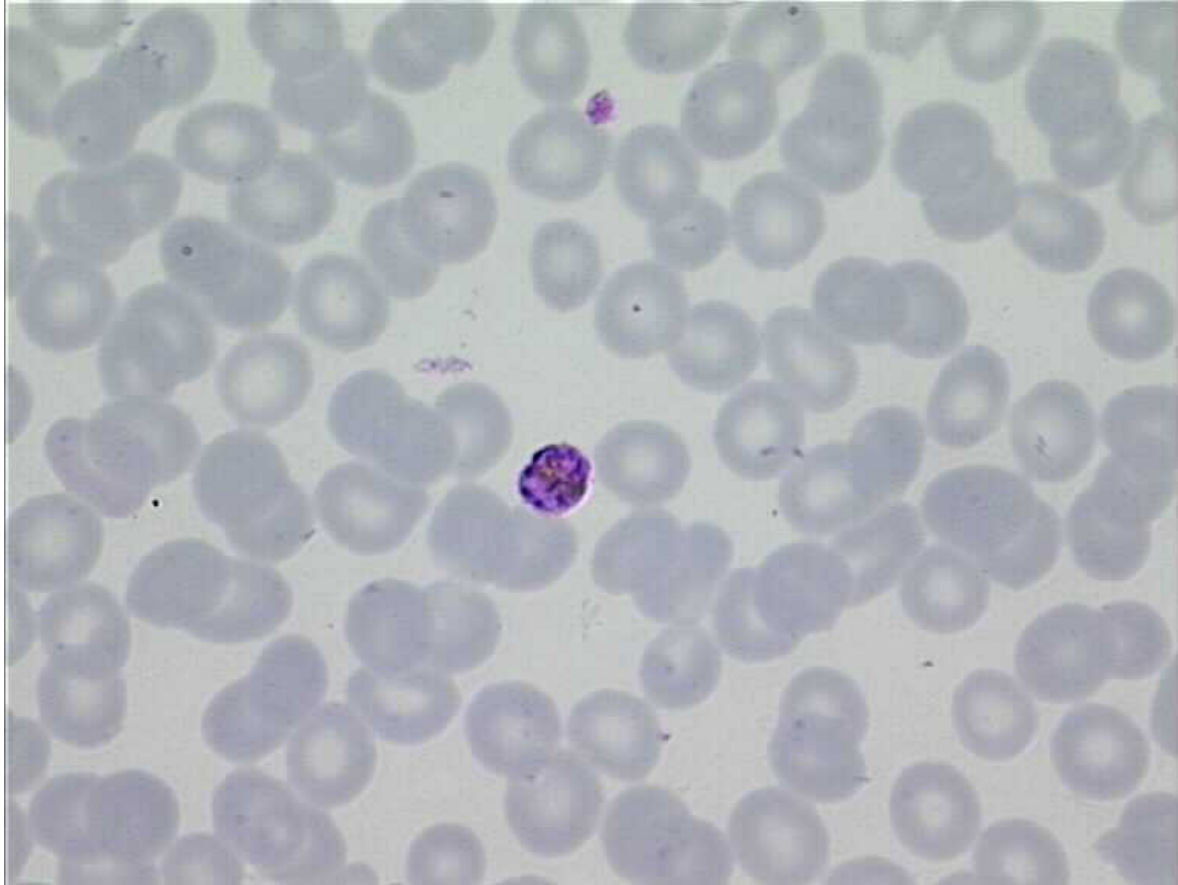
## Trophozoites of *P. Vivax*



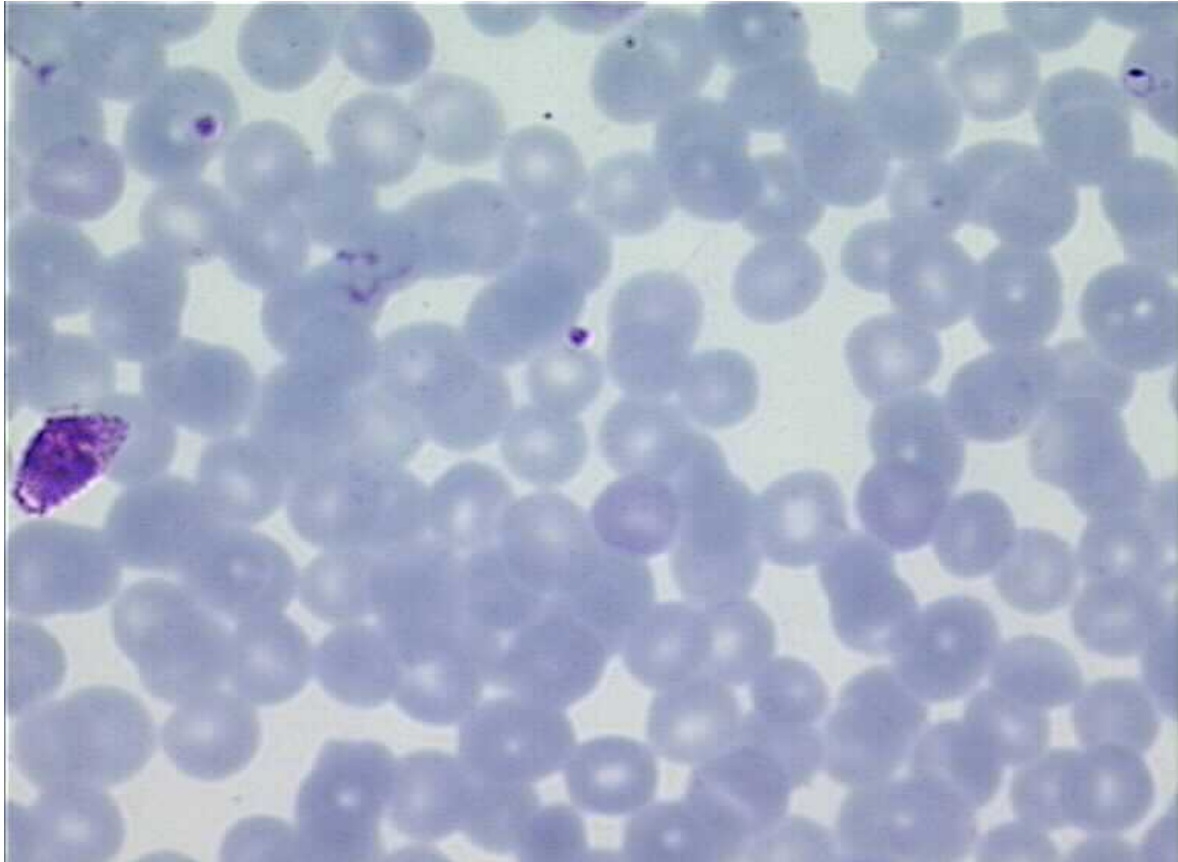
## Trophozoites of P.Ovale



## Schizonts of *P. Malariae*



## Schizont P.Ovale & Trophozoite of *P. Falciparum*



# Rapid diagnostic tests for Malaria Parasites

- § WHO has begun dialogue with scientists, clinicians & manufacturers of RDT's.
- § In its publication “New perspectives in Malaria Diagnosis” it stipulates the following:
  - ▮ **RDTS's should be at least as accurate as results derived from microscopy performed by as average technician under routine field conditions.**
  - ▮ **A sensitivity of > 95 % compared to microscopy should be attained**
  - ▮ **Levels of 100 parasites /ul blood (0.002%) should be detected with a sensitivity of 100%**

# **Rapid diagnostic tests for Malaria Parasites ctd**

- **Quantitative/semiquantitative information essential**
- **Viable/non viable organisms**
- **Predict outcome of treatment or resistance**
  
- Clearly a demanding list.
- Current RDT's include the Optimal IT and ICT Pf/Pv kits.

## Optimal IT

- § The principle of the test is the detection of pLDH , an enzyme found in the glycolytic pathway of Plasmodium spp.using methods that can assay LDH separately from any human LDH present in the sample.
- § Immunochromatography, using gold-labelled pan - specific anti pLDH mabs to capture antigen from blood and separate mabs against P.falciparum-specific and pan species antigens on a cellulose nitrate strip, is the format used for the test device

## Optimal test kit continued.

- § Performance of test has been improved by individual packaging. Safety is improved and effect of humidity on test performance eliminated.
- § Studies have shown that for P.Falciparum a sensitivity of 94% and for P.Vivax a sensitivity of 88% can be claimed .some studies report a higher sensitivity for P.Vivax.
- § False negatives found when <100 parasites/ul present.
- § Poor performance for P.Ovale and Malariae possibly attributed to lower parasite numbers and isoenzymes of LDH

## Optimal test kit continued.

- § If the blood is found to contain two different species, one of which is falciparum, the configuration of the test is such that the result should always indicate the presence of P. Falciparum. This will inevitably miss the the other species present, but as P.f is the species that causes the vast majority of deaths from malaria the test species is advantageous as currently designed.
- § As pLDH is only produced by viable parasites, the ability to follow its decline is a useful tool.

## ICT Pf/Pv kit

- § HRP-2 is a water soluble protein produced by asexual stages and young gametocytes of Pf. It is expressed on the RBC membrane surface, and because of its abundance , it was the first antigen to be used to develop an RDT for its detection.
- § Like the Optimal Assay, the principle of immunochromatography is used.
- § 2mabs are employed, one specific for HRP2 of Pf, the other detects a pan specific antigen common to all 4 species.

## **ICT Pv/Pf test kit continued.**

- § Manufacturers claim that the kit has a sensitivity of 100% for Pf and 89% for Pv.
- § Reports in the literature vary widely.
- § False positives can be caused by Rheumatoid Factor.
- § HRP2 has been detected in 27% of patients followed as late as 28 days post treatment.

# Conclusion

- § RDT's are useful, but in order to ensure correct interpretation, their inherent limitations have to be well known.
- § At present a negative RDT cannot be accepted at face value and needs to be confirmed by microscopical examination.
- § Microscopical examination of thick and thin films remains the Gold Standard for Malaria Diagnosis.