Diagnosis and Clinical Complications

David Sullivan, MD

Classical Malaria

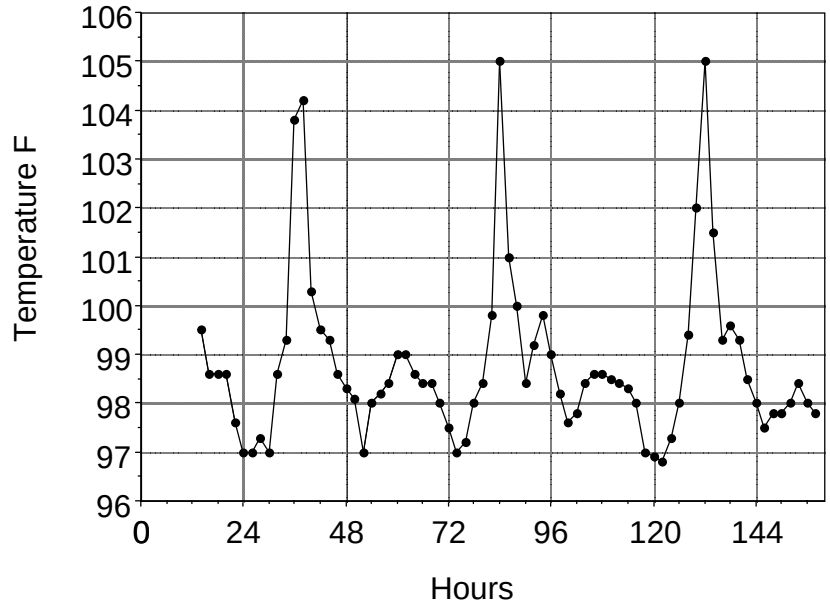
- Fever
- Splenomegaly
- Anemia

Hippocrates, 5th Century BC

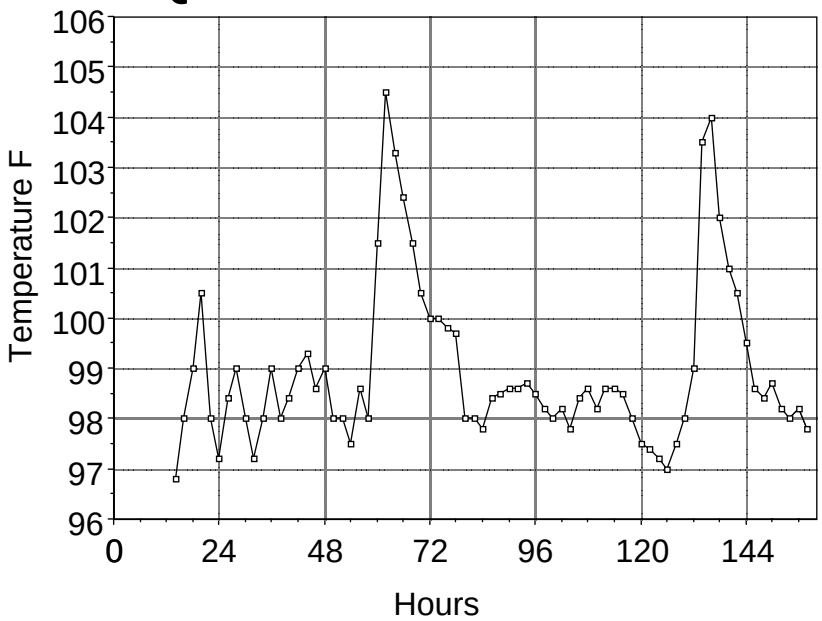
Comparison of Malaria Fever Curves

Adapted from Thayer and Hewetson
Johns Hopkins Hosp Reports V 1895 p. 3-224

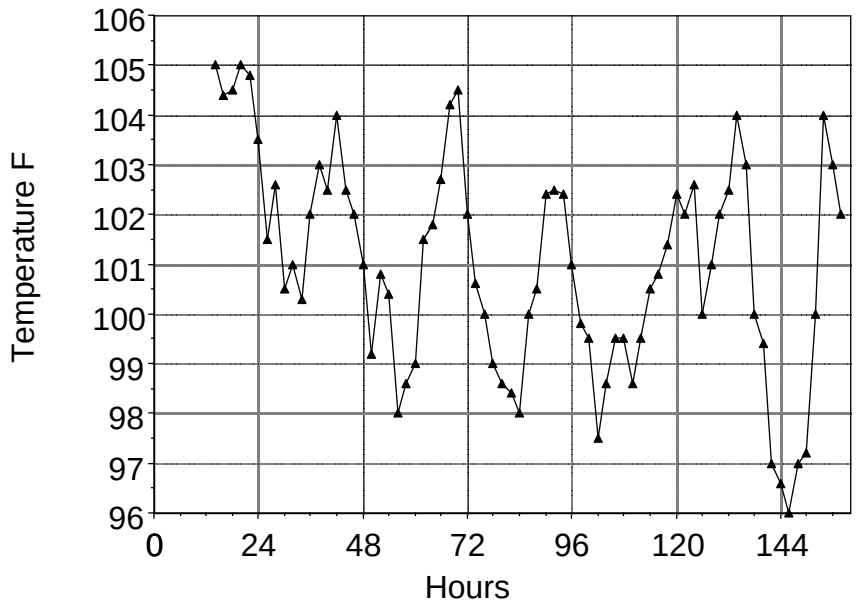
"Tertian" *P. vivax*



"Quartan" *P. malariae*



"Aestivo-autumnal "Quotidian" *P. falciparum*



Diagnosis Based on Clinical Features

Advantages

Cheap

Fast

CDC/ Dr. Lyle Conrad

Disadvantages

Lack of precision

Over-treatment

Axial temperature is not a good indicator of malaria infection in children under holoendemic conditions, as often less than 10% of infections are associated with fever.

Inaccuracies of Clinical Diagnosis

- Malaria is difficult to diagnose clinically
- In studies > 70% of +ve diagnoses are non-parasitemic
- Beware statistics based on clinical reports

Diagnosis Based on Microscopy

Advantages

Gold standard

Quantitative

Useful for other diseases

Disadvantages

Time consuming

Relies upon good microscopes, reagents, and trained technicians

Useful Web Sites for Training in Blood Film Analysis

This site is presented by the Division of Laboratory Medicine at Royal Perth Hospital.
http://www.rph.wa.gov.au/malaria.html

- Dr. Richard Davis AM PhD MSc FAACB FIBMS MASM
- Mr. Graham Icke MSc CBiol FIBiol FIBMS Grad Dip Bus

CDC site
http://www.dpd.cdc.gov/dpdx/HTML/Malaria.asp?body=Frames/M-R/Malaria/body_Malariadiagfind2.htm

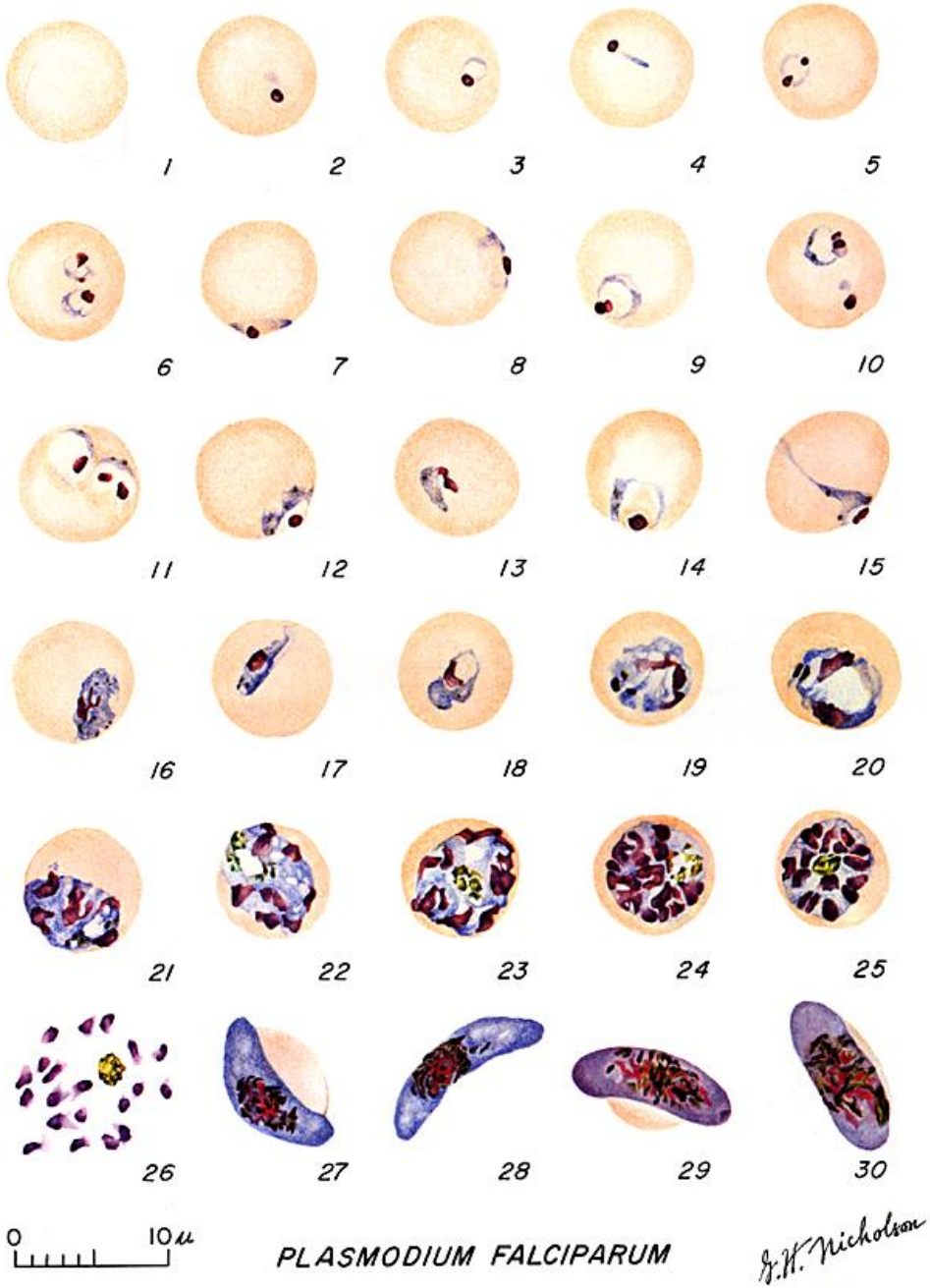
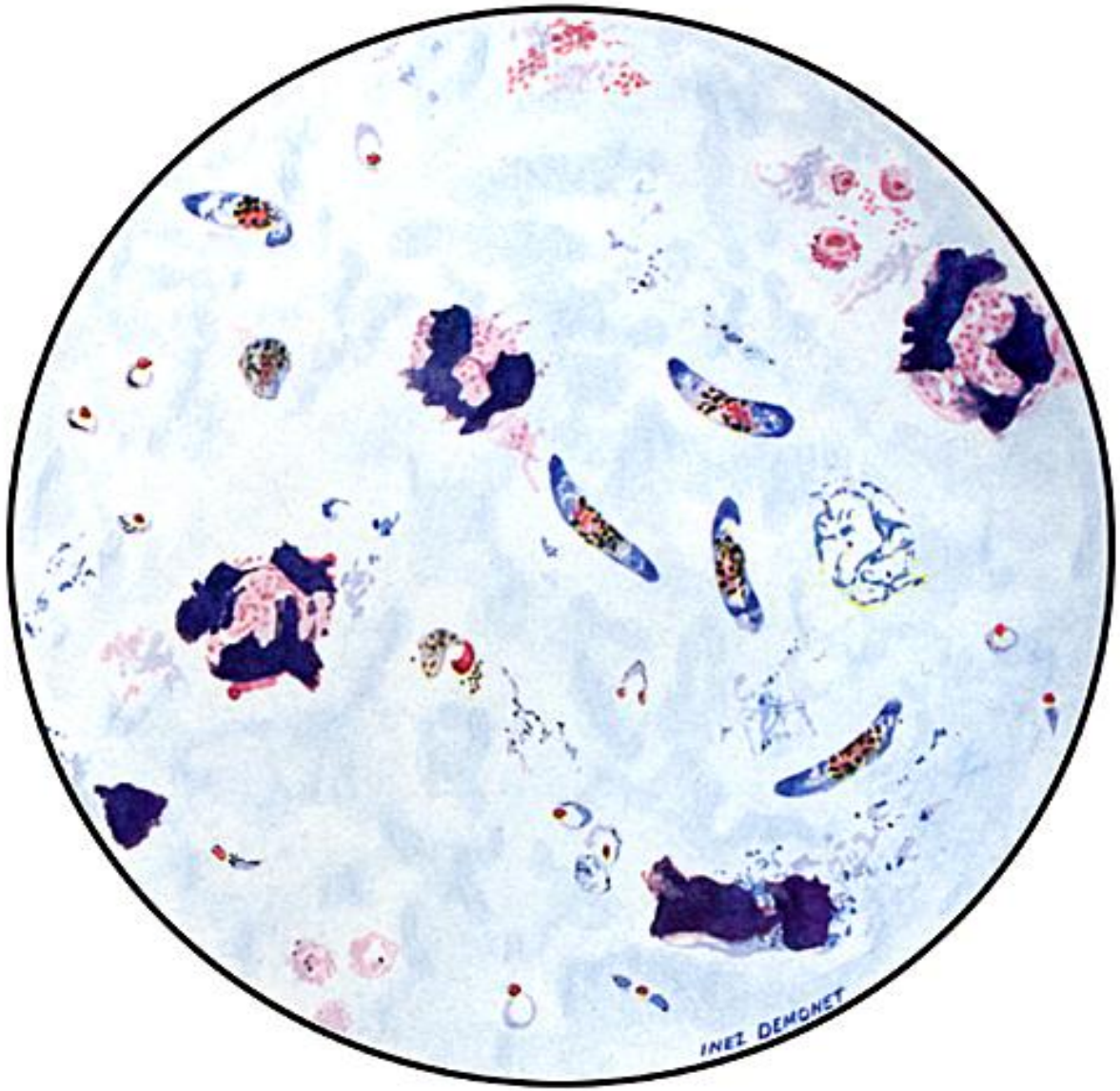


Fig. 1: Normal red cell
Figs. 2-18: Trophozoites (among these, **Figs. 2-10** correspond to ring-stage trophozoites)
Figs. 19-26: Schizonts (**Fig. 26** is a ruptured schizont)
Figs. 27 & 28: Mature macrogametocytes
Figs. 29& 30: Mature microgametocytes (male).

Illustrations from: Coatney GR, Collins WE, Warren M, Contacos PG The Primate Malarias./\ U.S. Department of Health, Education and Welfare, Bethesda, 1971.

P. falciparum Thick film



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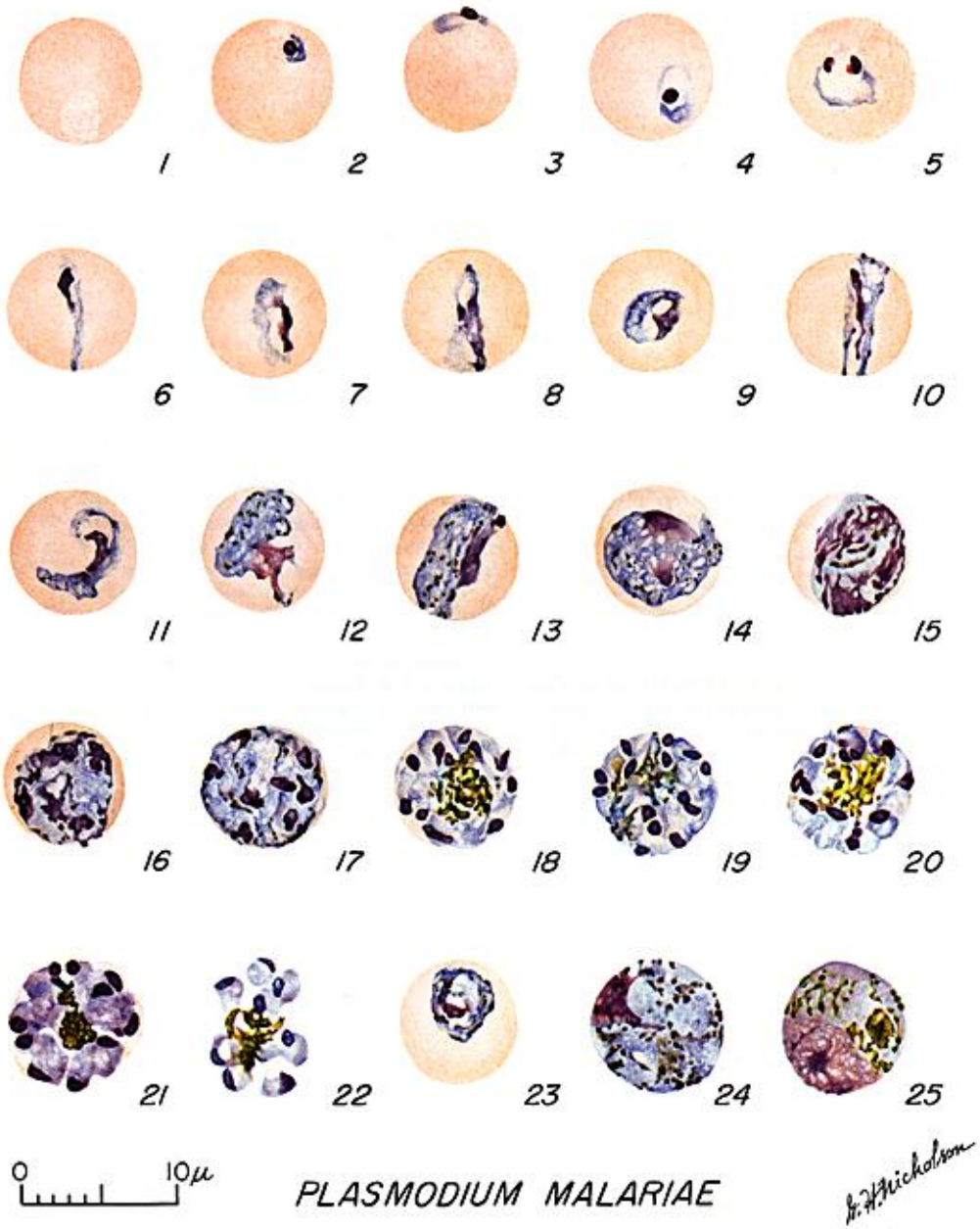
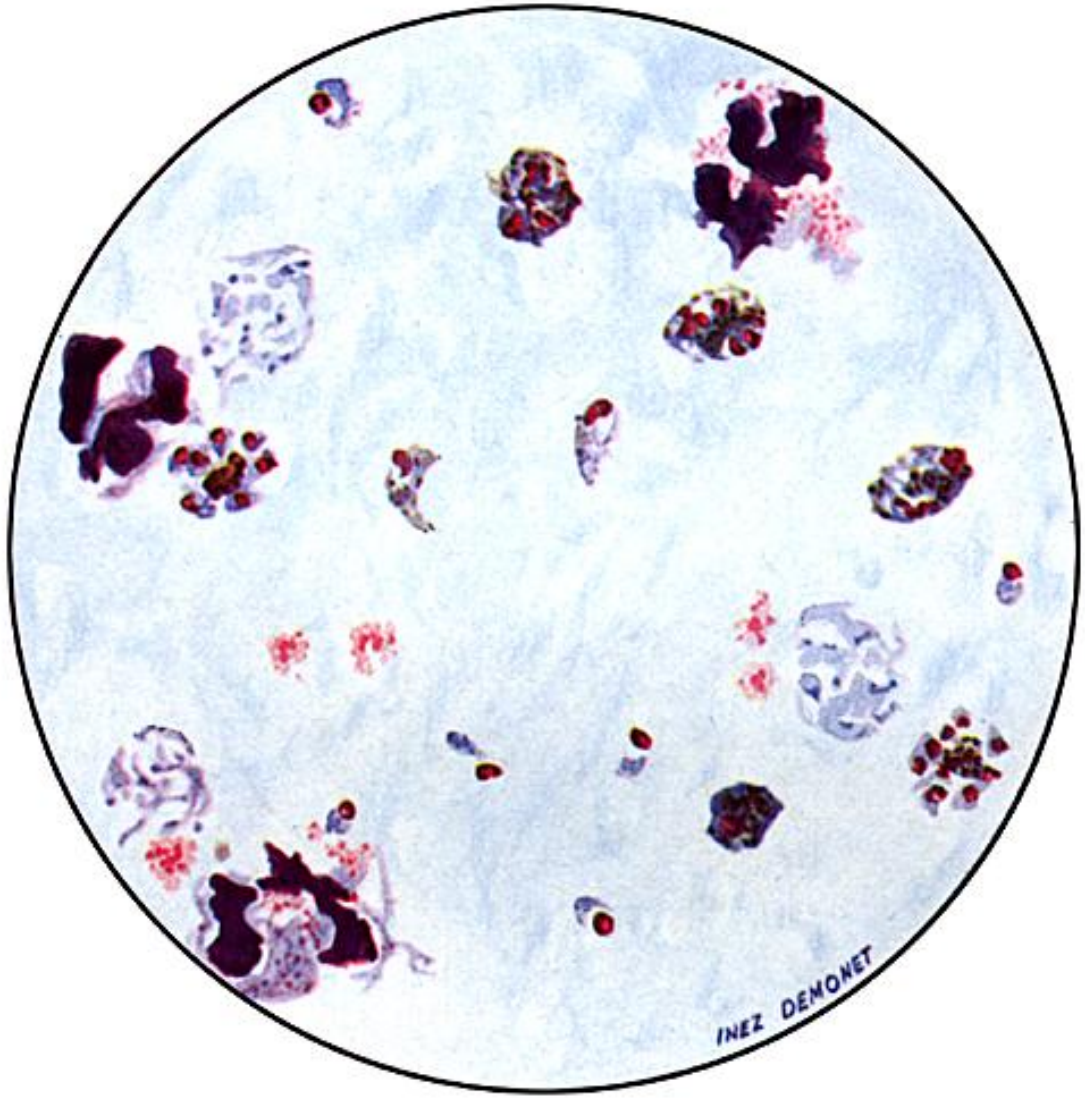


Fig. 1: Normal red cell
Figs. 2-5: Young trophozoites (rings)
Figs. 6-13: Trophozoites
Figs. 14-22: Schizonts
Fig. 23: Developing gametocyte
Fig. 24: Macrogametocyte (female)
Fig. 25: Microgametocyte (male)

Illustration from: Coatney GR, Collins WE, Warren M, Contacos PG. /\ The Primate Malarias. U.S. Department of Health, Education and Welfare, Bethesda, 1971.

P. malariae Thick film



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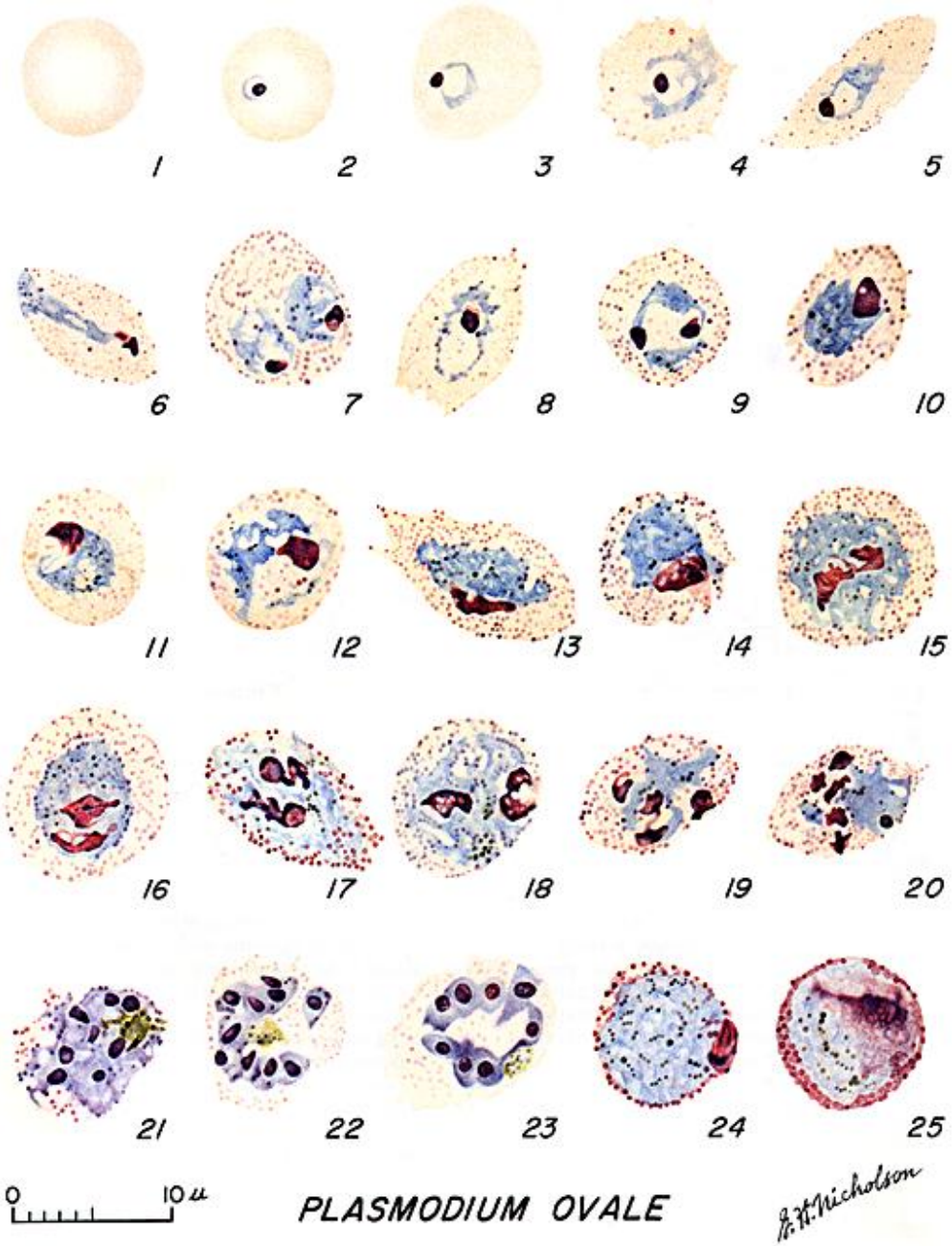
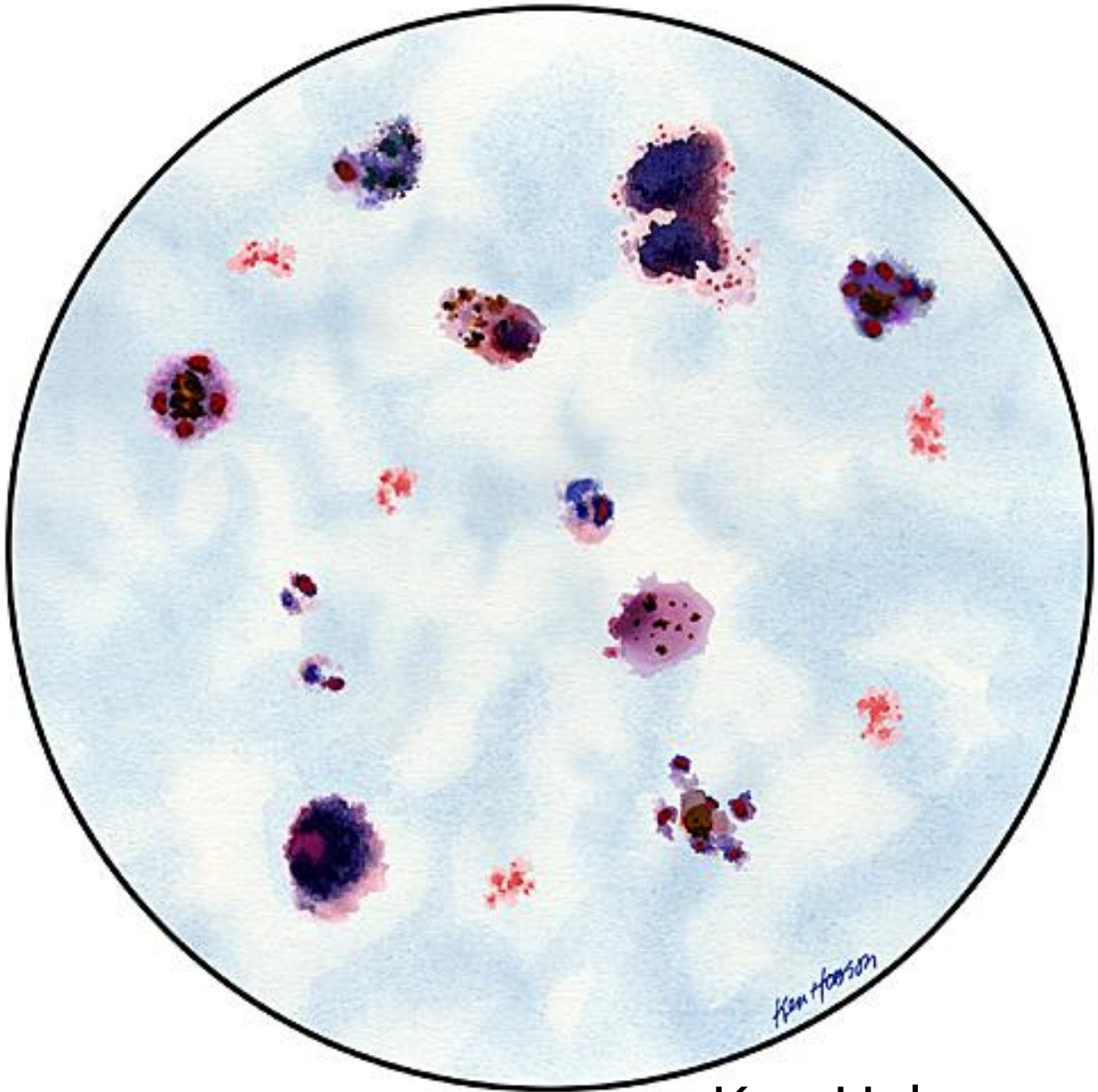


Fig. 1: Normal red cell
Figs. 2-5: Young trophozoites (Rings)
Figs. 6-15: Trophozoites
Figs. 16-23: Schizonts
Fig. 24: Macrogametocytes (female)
Fig. 25: Microgametocyte (male)

Illustration from: Coatney GR, Collins WE, Warren M, Contacos PG. The Primate Malarias. U.S. Department of Health, Education and Welfare, Bethesda, 1971.

P. ovale Thick film



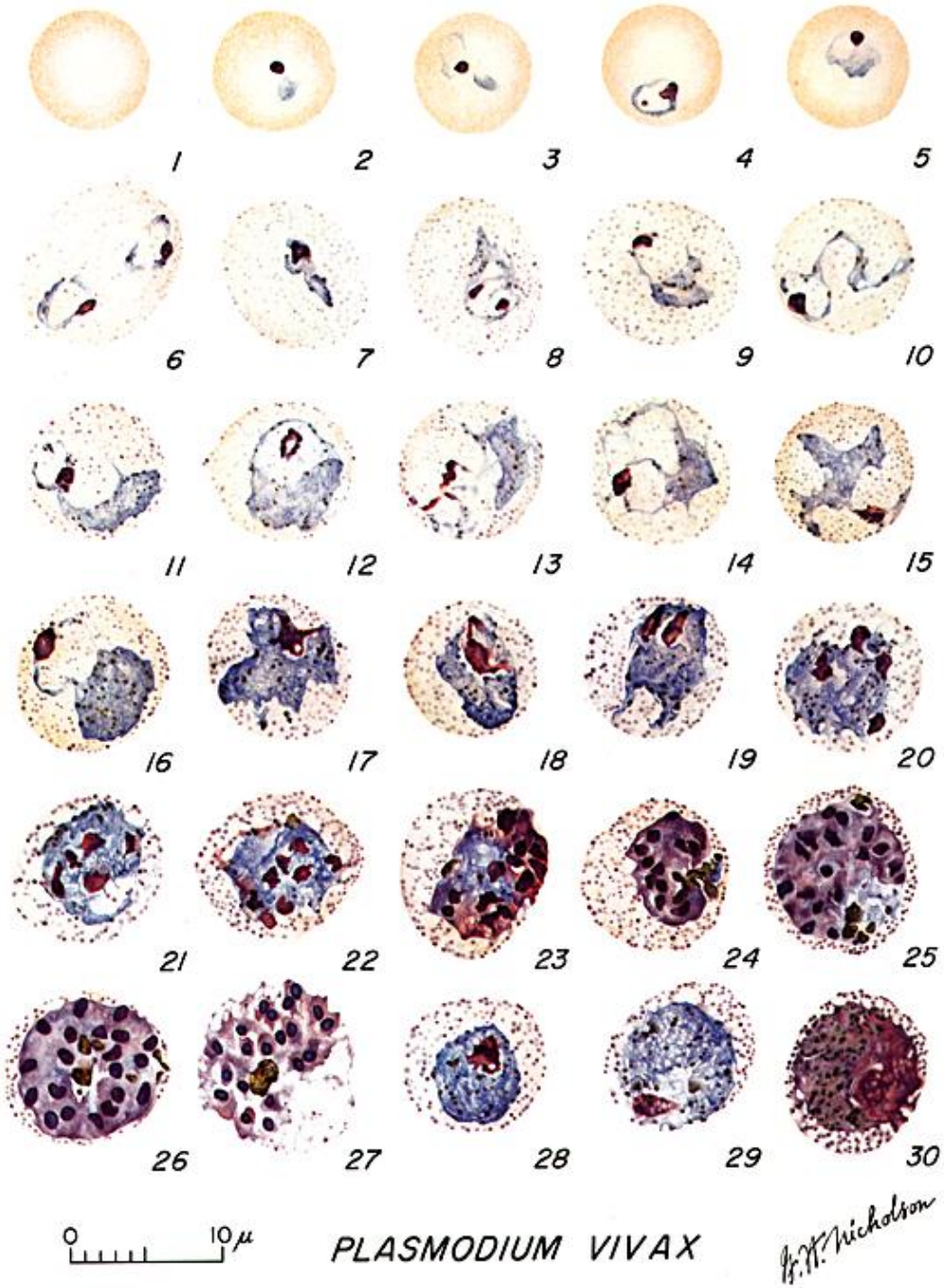


Fig. 1: Normal red cell
Figs. 2-6: Young trophozoites (ring stage parasites)
Figs. 7-18: Trophozoites
Figs. 19-27: Schizonts
Figs. 28 and 29: Macrogametocytes (female)
Fig. 30: Microgametocyte (male)

Illustration from: Coatney GR, Collins WE, Warren M, Contacos PG. The Primate Malarias./\ U.S. Department of Health, Education and Welfare, Bethesda, 1971.

P. vivax Thick Film

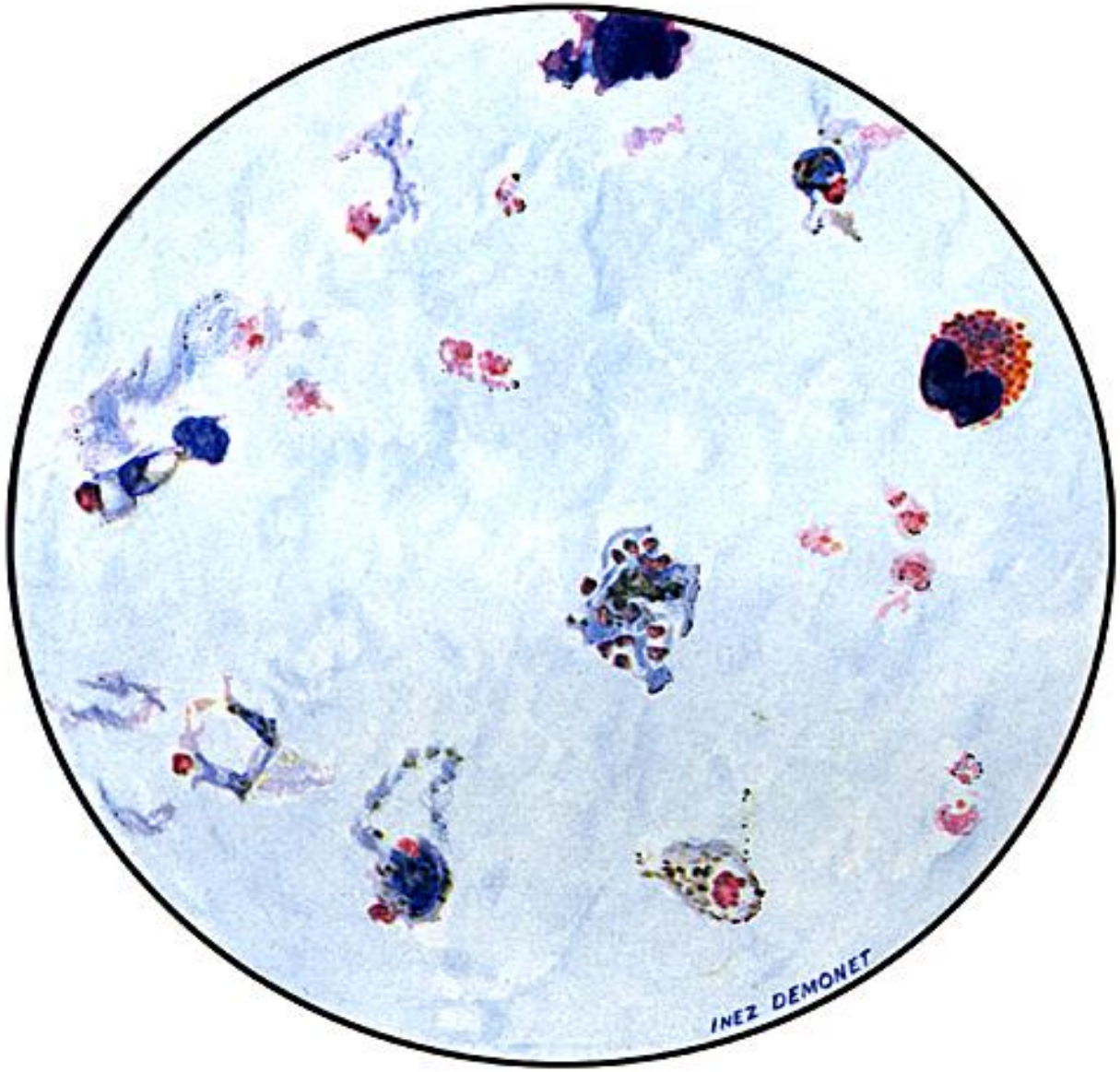
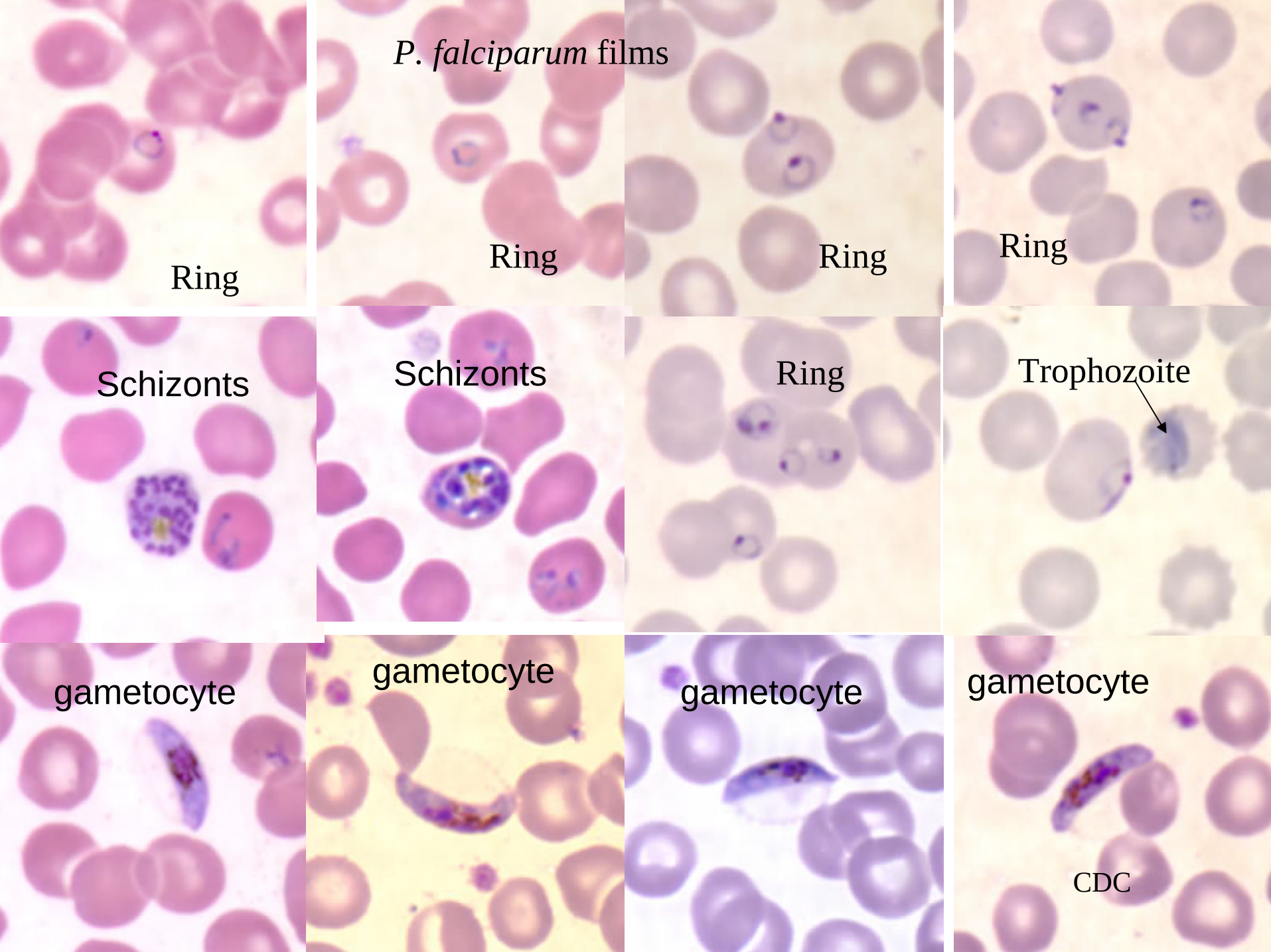


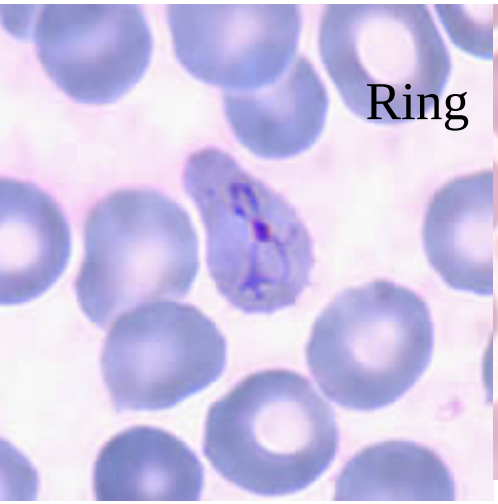
Illustration from: Wilcox A. Manual for the Microscopical Diagnosis of Malaria in Man. U.S. Department of Health, Education and Welfare, Washington, 1960.

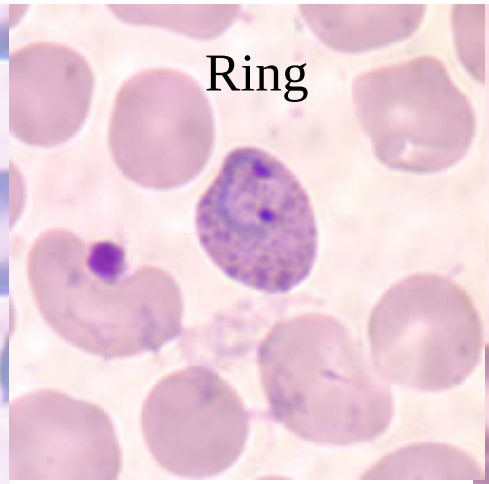
Distinguishing Blood Film Characteristics

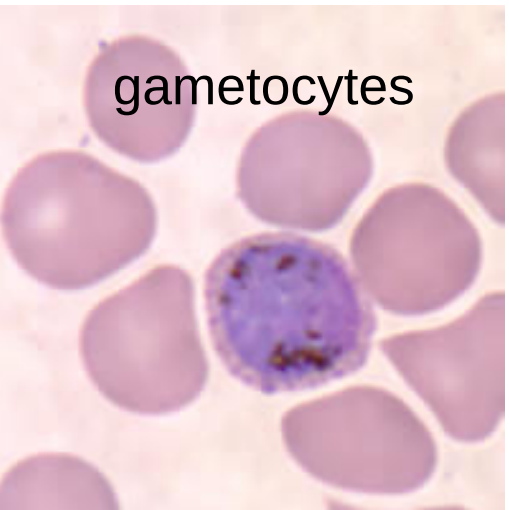
Feature	*P. falciparum*	*P. vivax*	*P.ovale*	*P. malariae*
Red cell size	Normal	Large	Large	Normal
Merozoites in schizont	Up to 32	Up to 16	Up to 8	Up to 8
Rings	Fine, delicate double chromatin dots and applique forms	Large, irregular	Large, irregular	Square or band appearance
RBC cytoplasm	Maurer's dots	Schuffner's dots	Schuffner's dots	
Gametocytes	Sickle or banana shape	Round	Round	Round
Special	Trophozoite and schizonts rare	Amoeboid trophozoites	Comet/oval rbc. Only in Africa	Band form and daisy schizonts

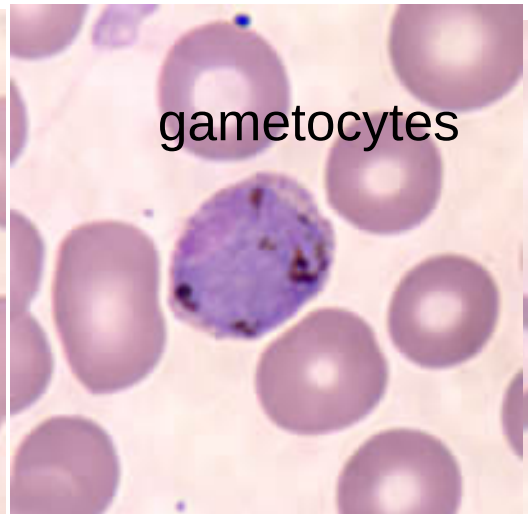

P. falciparum films
Ring
Ring
Ring
Ring
Schizonts
Schizonts
Ring
Trophozoite
gametocyte
gametocyte
gametocyte
gametocyte
CDC

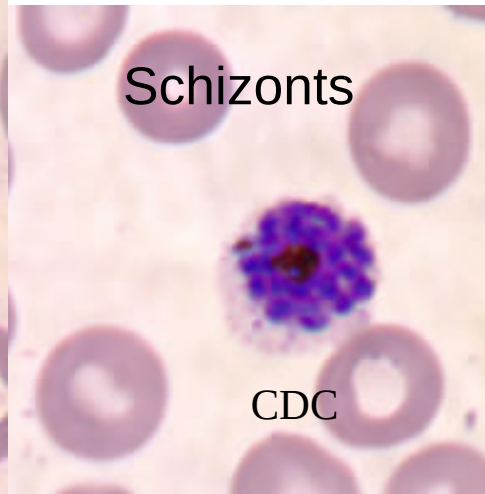
P. vivax Films



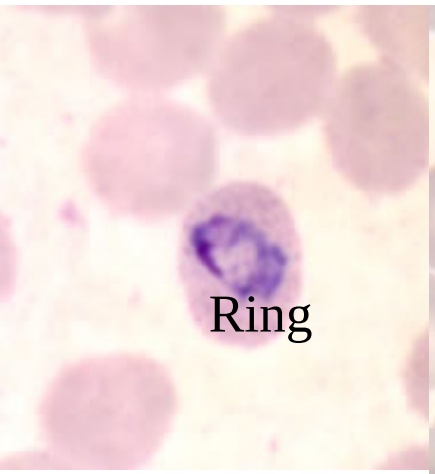


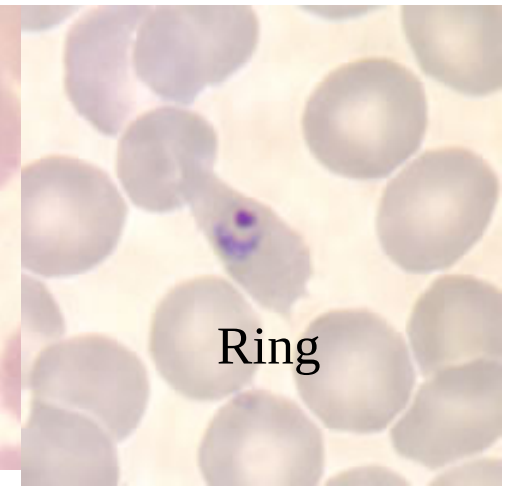


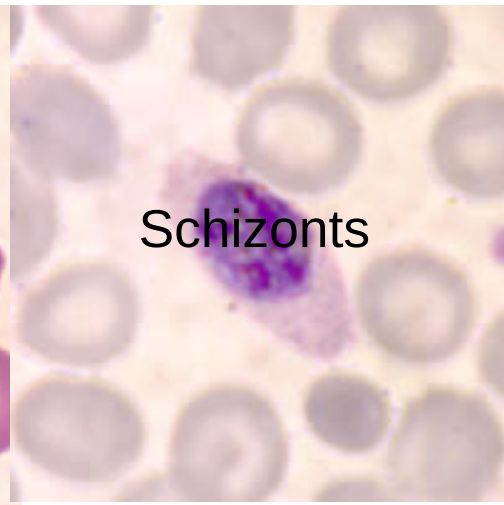


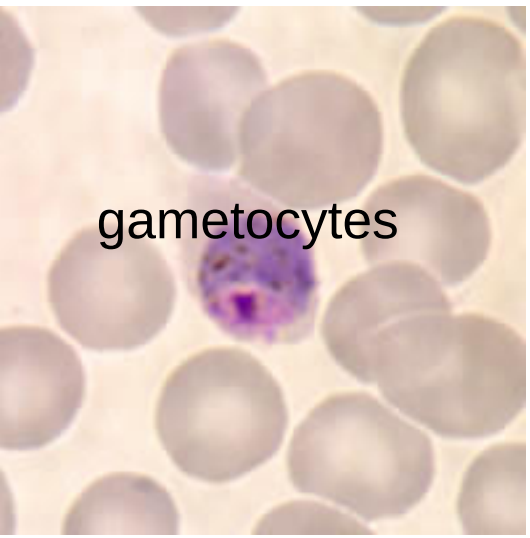


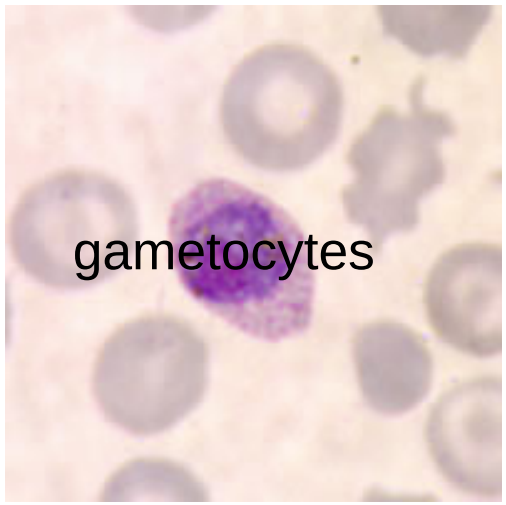
P. ovale Films

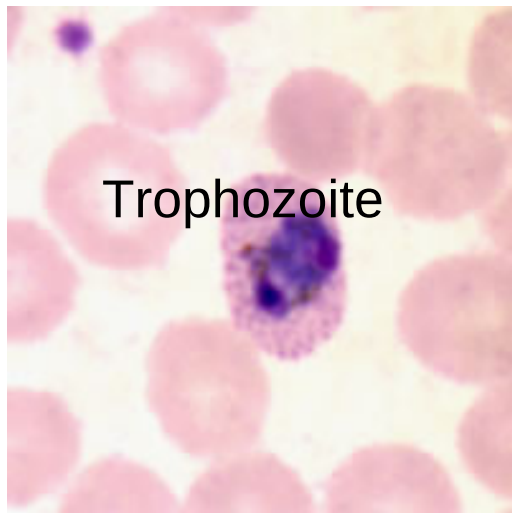






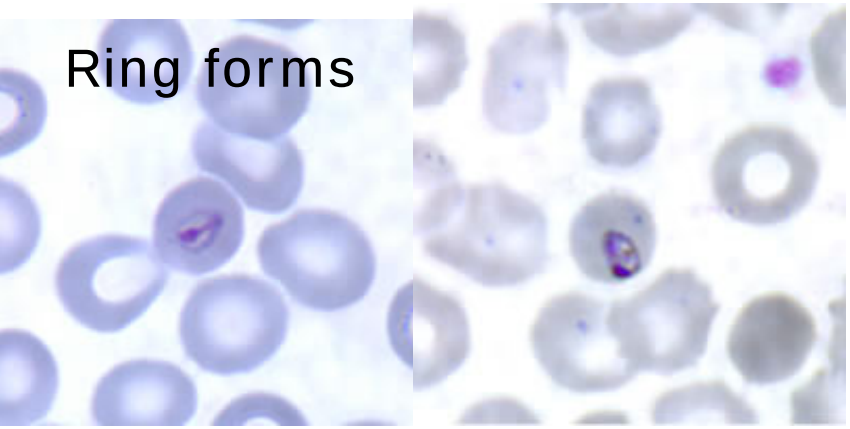


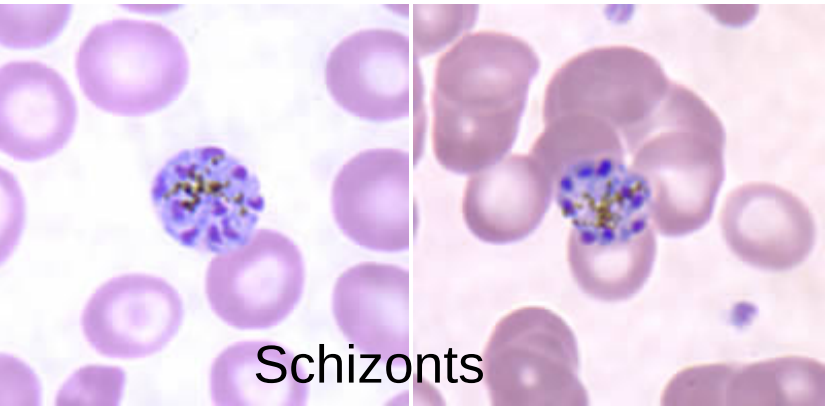


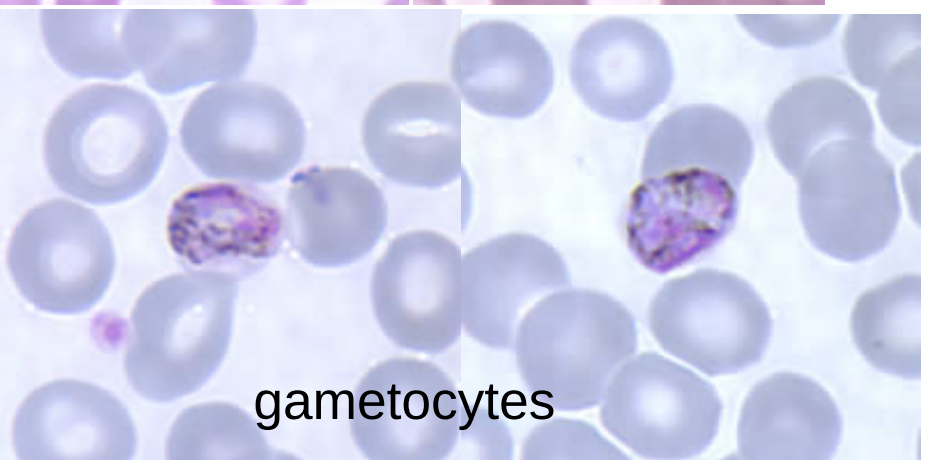


CDC

P. malariae Films







CDC

Rapid Diagnostic Tests

Advantages

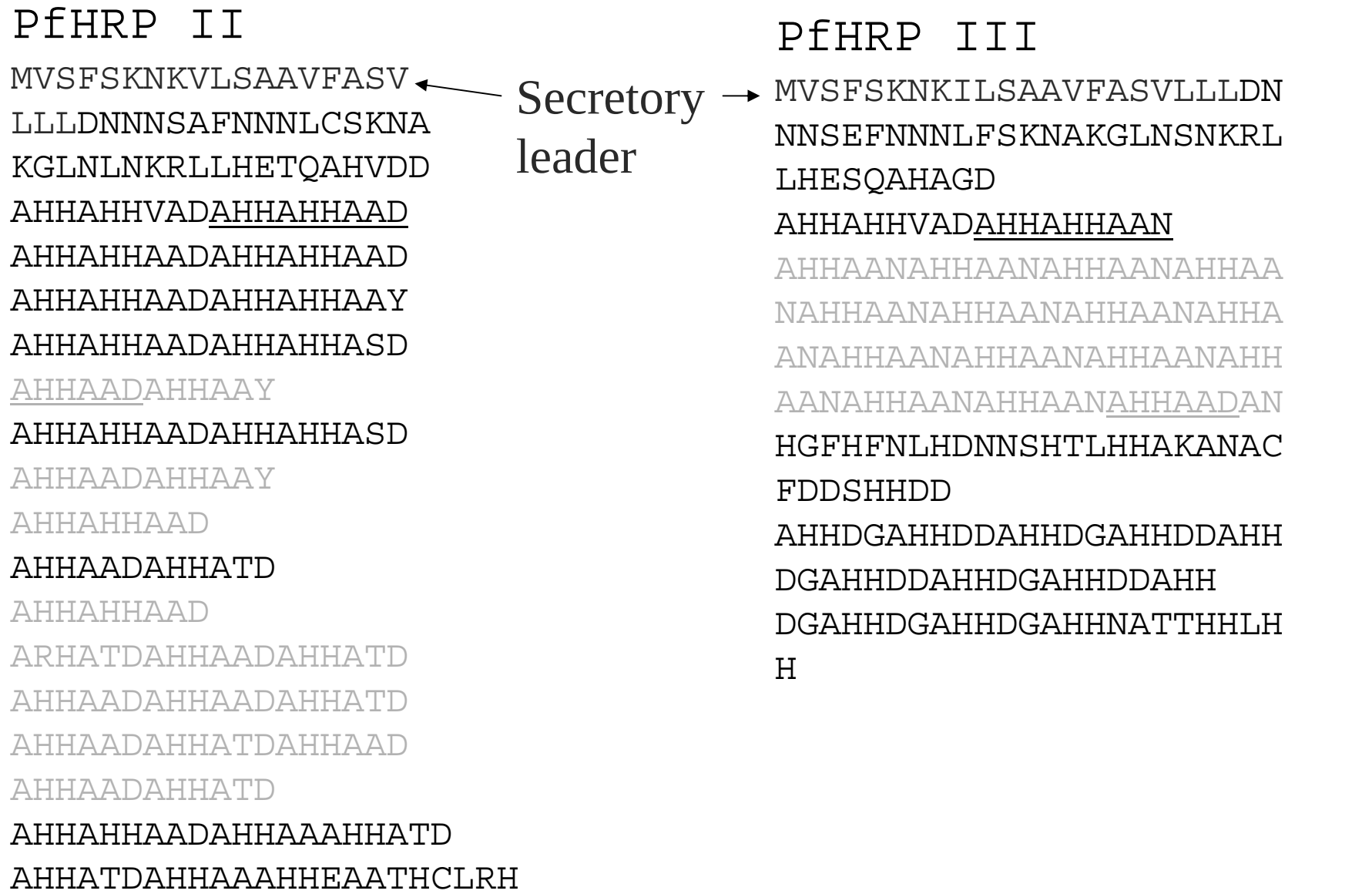
- Sensitive
- Fast
- Simple to perform
- No need for special equipment or electricity

Disadvantages

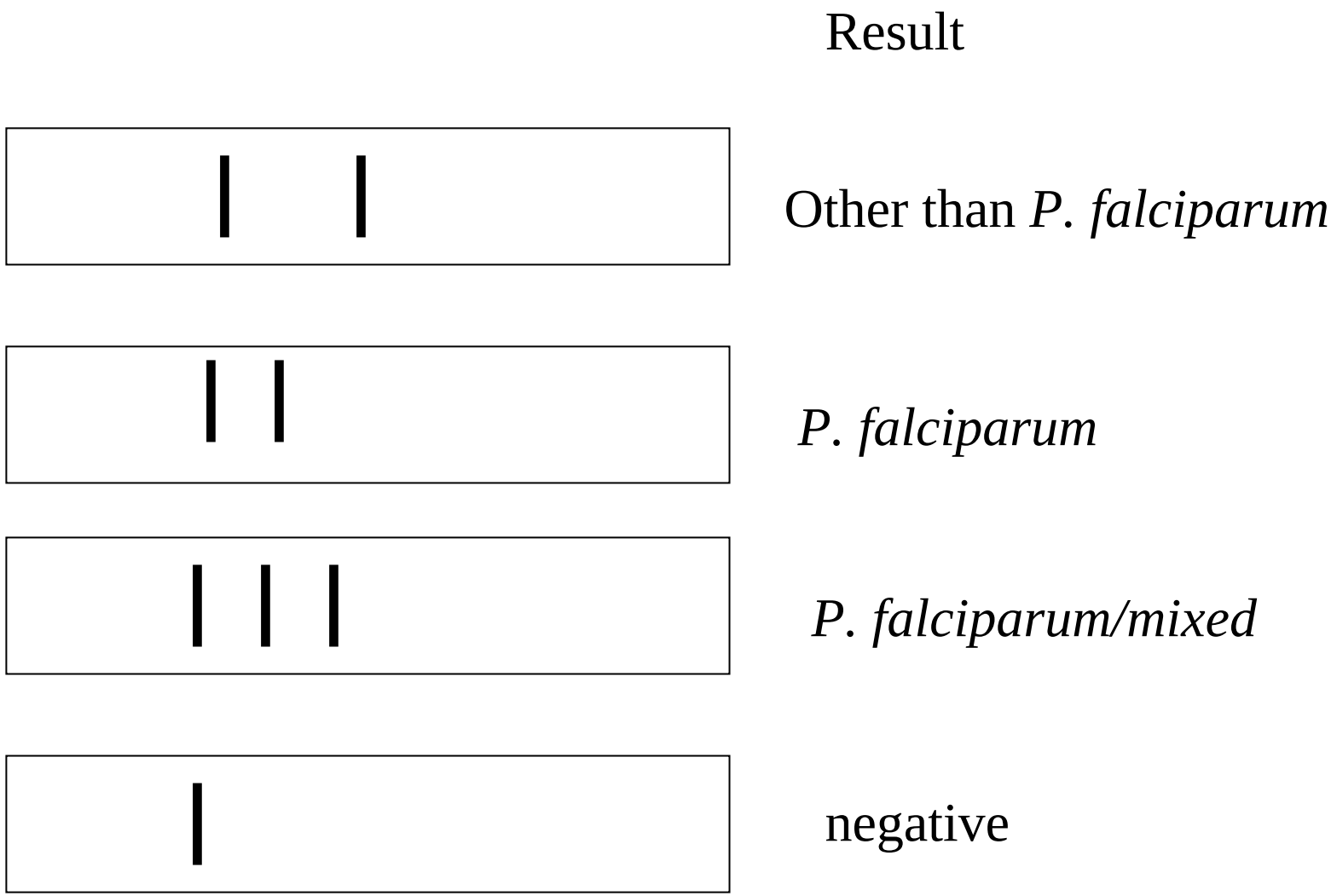
- HRPII:
  - Not suitable for non Pf species
  - Remains positive for 2 weeks after treatment
- Not quantitative
- Expensive (US$0.60-2.50 per test)

Why Target HRP II for Detection?

- Multiple His-Ala repeating regions for antibody epitopes
- Present in infected RBC cytoplasm and parasite digestive vacuole
- Secreted in plasma
- Directly related to parasitemia, parasite biomass, and parasite developmental stage


PfHRP II
MVSFSKNKVLSAAVFASV
LLLDNNNSAFNNNLCSKNA
KGLNLNKRLLHETQAHVDD
AHHAHHVADAHHAHHAAD
AHHAHHAADAHHAHHAAD
AHHAHHAADAHHAHHAAY
AHHAHHAADAHHAHHASD
AHHAADAHHAAY
AHHAHHAADAHHAHHASD
AHHAADAHHAAY
AHHAHHAAD
AHHAADAHHATD
AHHAHHAAD
ARHATDAHHAADAHHATD
AHHAADAHHAADAHHATD
AHHAADAHHATDAHHAAD
AHHAADAHHATD
AHHAHHAADAHHAAAHHATD
AHHATDAHHAAAHHEAATHCLRH
Secretory leader
PfHRP III
MVSFSKNKILSAAVFASVLLLDN
NNSEFNNNLFSKNAKGLNSNKRL
LHESQAHAGD
AHHAHHVADAHHAHHAAN
AHHAANAHHAANAHHAANAHHAA
NAHHAANAHHAANAHHAANAHHA
ANAHHAANAHHAANAHHAANAHH
AANAHHAANAHHAANAHHAADAN
HGFHFNLHDNNSHTLHHAKANAC
FDDSHHDD
AHHDGAHHDDAHHDGAHHDDAHH
DGAHHDDAHHDGAHHDDAHH
DGAHHDGAHHDGAHHNATTHHLH
H

- Aldolase and lactate dehydrogenase enzymes
- Abundant production by parasites
- Aldolase has over 90% identity at amino acid level
- LDH has less and enables species specific monoclonal antibodies
- LDH is basis for Optimal test
- Aldolase is in ICT test as non HRP II band
- Both aldolase and LDH have short half life and go away within 1-2 days of treatment
- HRP II can linger for more than a week


Result
Other than P. falciparum
P. falciparum
P. falciparum/mixed
negative
Control
aldolase
HRP II

RDTs in Africa?
Current situation

Problems

- Asymptomatic parasitaemia
- Expense

Special situations

- Complex emergencies
- Malaria epidemics
- Low transmission settings
- Military
- Travellers

RDTs in Africa
Future options

- Changing cost-benefit
  - Rising drug costs
- Possible uses
  - Confirmation of treatment failure (pLDH)
  - Severe disease in peripheral settings
- BUT…
  - Will RDT diagnosis change clinical practice?
- Need for operational studies

Malaria Rapid Diagnostic Tests

WHO site: http://www.wpro.who.int/sites/rdt

Contains

- Explanation of RDT
- Use of RDT
- Guidelines on purchasing an RDT including an important table that compares good manufacturing practice on known suppliers
- Collections of published reviews and trials
- Collection of publications and committee documents
- Useful links pertaining to malaria diagnosis http://www.wpro.who.int/sites/rdt/links.htm

Clinical Complications of Malaria

P. Falciparum

- Cerebral coma
- Anemia
- Pulmonary edema
- Shock
- Lactic acidosis
- Hypoglycemia
- Tropical splenomegaly
- Pregnancy
  - Maternal Death
  - Stillbirth
  - Low birth weight
  - Anemia

P. vivax (P. ovale)

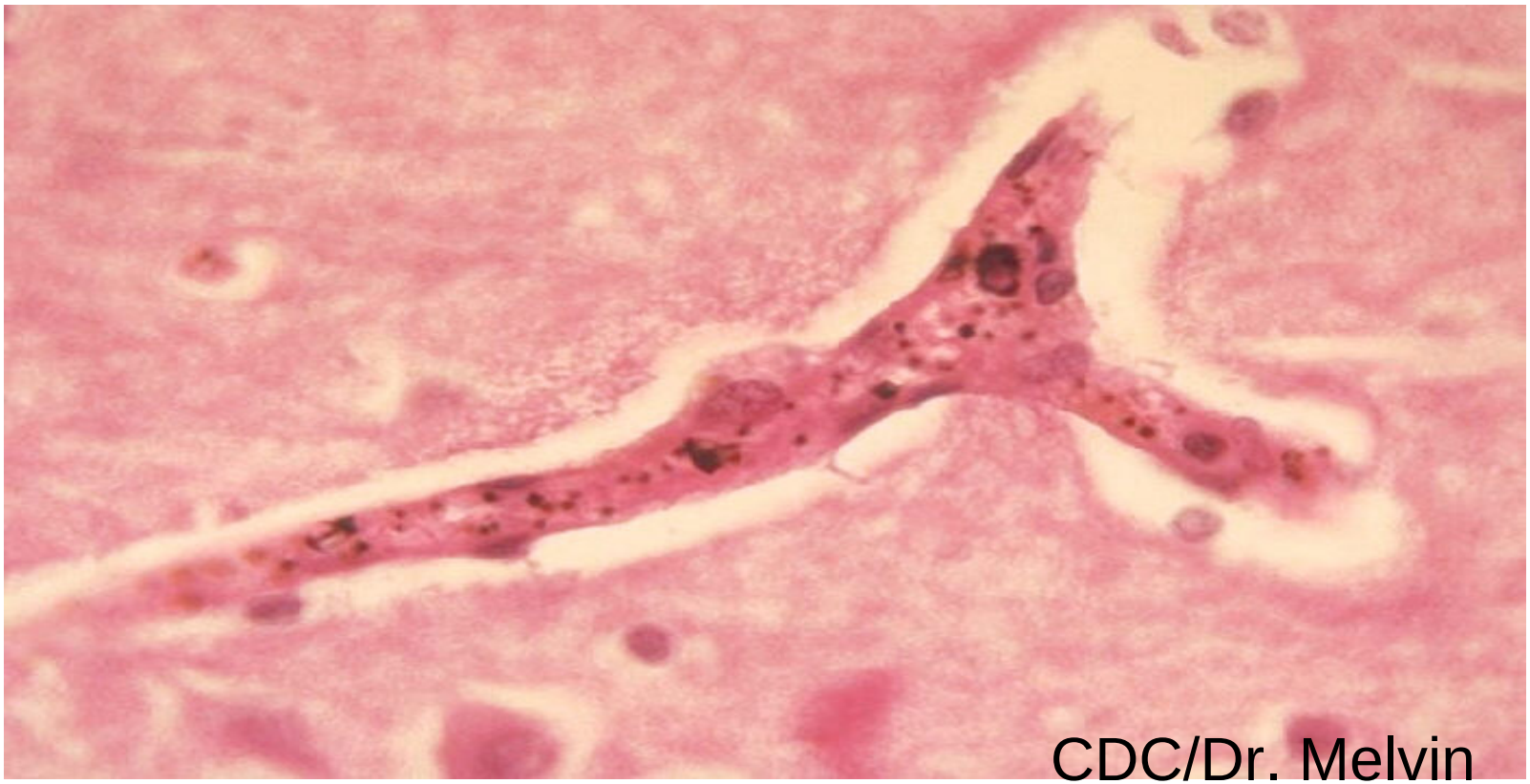
- Splenic rupture
- Anemia (mild)
- Debilitating fevers
- Higher TNF-alpha per parasite

P.malariae

- Immune complex
- Glomurulonephritis leading to nephrotic syndrome

Cerebral Malaria

Adherent parasites release cytokines. In one study 94% of persons with cerebral malaria had adherent parasites compared with 13% of those without change in mental status. Steroids have no effect on mortality, no increase in vascular permeability is observed, anaerobic glycolysis in brain tissue predominates.



CDC/Dr. Melvin

Cerebral Malaria: Signs and Symptoms

- About 90% become comatose before dying
- Gradual impairment or coma following seizure
- Extensor posturing
- Immobile or tossing about

Neurologic Sequelae

- Uncommon in adults or non immunes
- Common in African children
  - Psychosis
  - Extrapyramidal tremor
  - Cranial nerve lesions
  - Polyneuropathy
  - Mononeiritis multiplex
  - Guillain-Barre syndrome
  - Focal epilepsy

Modified Glasgow Scale

Best Verbal Response

- Oriented: 5
- Confused: 4
- Inappropriate Words: 3
- Incomprehensible sounds: 2
- None: 1

Best Motor Response

- Obeys commands: 6
- Localizes pain: 5
- Flexion to pain:
  - Withdrawal: 4
  - Abnormal: 3
- Extension to pain: 2
- None: 1

Blantyre Scale

Eye Movements

- Directed: 1
- Not Directed: 0

Verbal Response

- Appropriate cry: 2
- Moan or inappropriate cry: 1
- None

Best Motor Response

- Localizes painful stimulus: 2
- Withdraws limb from pain: 1
- Non-specific or absent response: 0

Total = 0-5

Unrousable coma <2

Severe Anemia

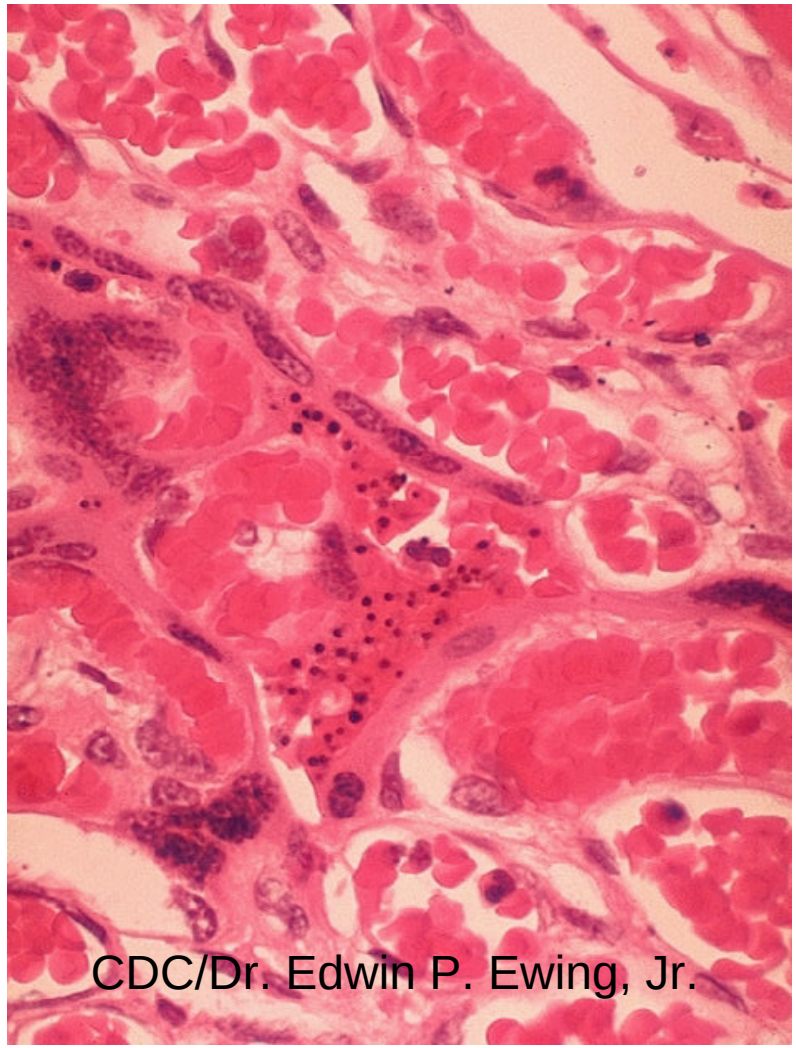
- Not only red blood cell destruction, but also decreased production
- Due to iron deficiency and ineffective erythropoeisis, Rouleaux formation of uninfected erythrocytes increases spleen destruction
- Peak incidence in African children from holoendemic areas between ages of 6 months and 2 years
- Associations with secondary bacterial infections
- Transfusion is life saving

Pathogenesis of Severe Anemia

- Degree of anemia corresponds to duration and severity of parasitemia.
- Parasitemia does not predict risk of death in severe anemia and in Kenya over half of children with severe anemia had less than 10,000 parasites per ul.
- Treated uncomplicated *P. falciparum* malaria will decrease the hematocrit by one seventh.
- Severe anemia kills as hemoglobin falls below 5g/dl
- Mortality rises with tissue hypoxia and metabolic acidosis.
- Another infection can tip to catabolic metabolism.

Placental Malaria

- Unstable epidemiology
  - Maternal death, abortion, stillbirth, premature delivery, low birthweight
- Stable (Holoendemic) epidemiology
  - Clinical symptoms and parasitemia is higher in primigravida
  - Low birthweight
- Non-immunes
  - Higher mortality
  - Progressive anemia
  - Quinine induced hypoglycemia



Primagravida women expose chondroitin sulfate A on placenta endothelial cells to which a new population of *P. falciparum* parasites predominates and causes microvascular sequestration in the placenta, disrupting its function.

Vertical transmission

- Congenital
  - Parasitemic neonate within 7 days of birth
- Blood transfusion
  - *P. malariae*

Pulmonary edema

May develop at any stage of disease

Iatrogenic (presents as patient recovering)

Increased RR, dyspnea, crepitations are first clinical signs

ARDS with normal right heart pressures

CXR

- Bronchopneumonia
- Metabolic acidosis
- ARDS

Tropical Splenomegaly Syndrome

- Also known as Hyperreactive malarial splenomegaly
- Progressive, massive, splenic enlargement
- 80% of some areas of PNG
- Past medical history of repeated attacks of fever or malaria

Tropical Splenomegaly Syndrome

- Abdominal distention, vague dragging sensation, sharp abdminal pains
- Peritonism suggesting perisplenitis
- Cachexia
- Lower leg ulcerations
- NC/NC Anemia with hemolytic episodes
- Very low or undetectable parasitemia

Tropical Splenomegaly Syndrome

- Untreated morality rate is high
- Death due to overwhelming pulmonary or skin infections
- Definition:
  - Gross splenomegaly
  - Elevated IgM (polyclonal)
  - Clinical and immunological response to anti-malarial prophylaxis

Why Is *P. falciparum* So Dangerous?

- Ability to infect all age of RBCs
- Higher multiplication capacity
- Sequestration (cytoadherance and rosetting)
- Capillary leak syndromes
- End organ failure

The Numbers

- 70 kg person has @ 5 liters of blood = 5 x 10^3 ml = 5 x 10^6 µL times 5 x 10^6 RBCs per µL of blood = 2.5 x 101^3 RBCs
- 1% parasitemia = 1 in 100 iRBCs = 2.5 x 1011 parasites = 250 billion parasites
- *P. vivax* invades predominately reticulocytes and so has a built-in ceiling, but *P. falciparum* can invade all ages of RBCs.
- Pyrogenic density *P. falciparum* 10,000/uL nonimmune; 100,000/uL immune; *P. vivax* 100/uL