

Malaria – Diagnosis and Treatment

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Five species of human malaria

Plasmodium falciparum

Plasmodium vivax

Plasmodium ovale

Plasmodium malariae

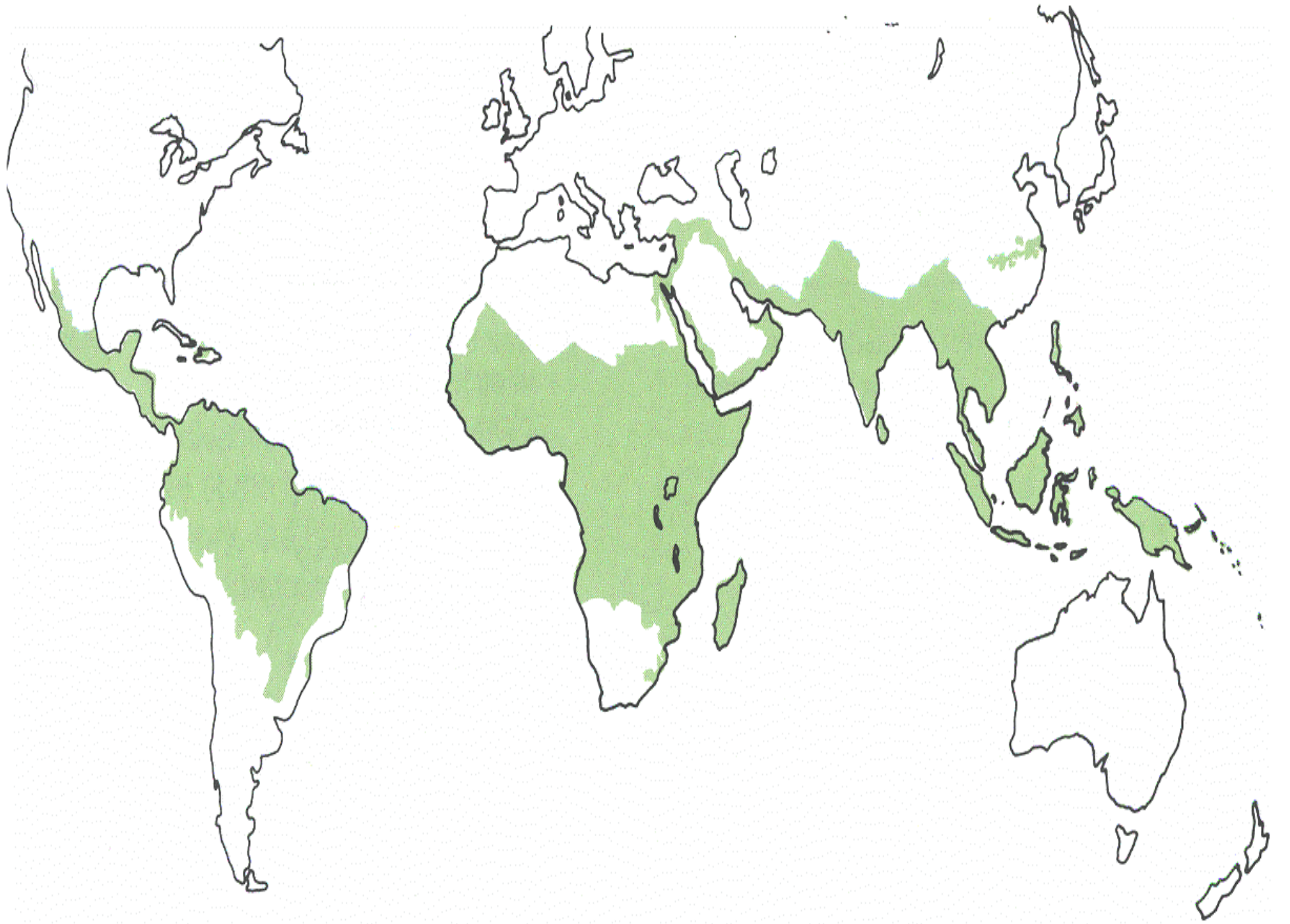
Plasmodium knowlesi

Curable and Preventable

MALARIA

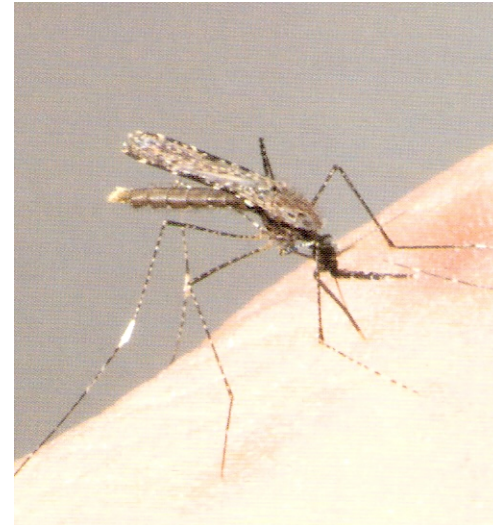
CURRENT WORLD STATUS

- **Malaria is the most important parasitic disease in the world.**
- **Forty percent of the world's population is at risk of malaria infection.**
- **Malaria incidence has increased during the last 2 decades, and it is now estimated that about 270 million people are infected annually, resulting in 2.7 million deaths.**
- **In patients with severe and complicated malaria, the mortality rate is between 20 and 50%**



MALARIA TRANSMISSION

- **Mosquito bites**
- **Blood transfusion**
- **Transplacental**
- **Iatrogenic**

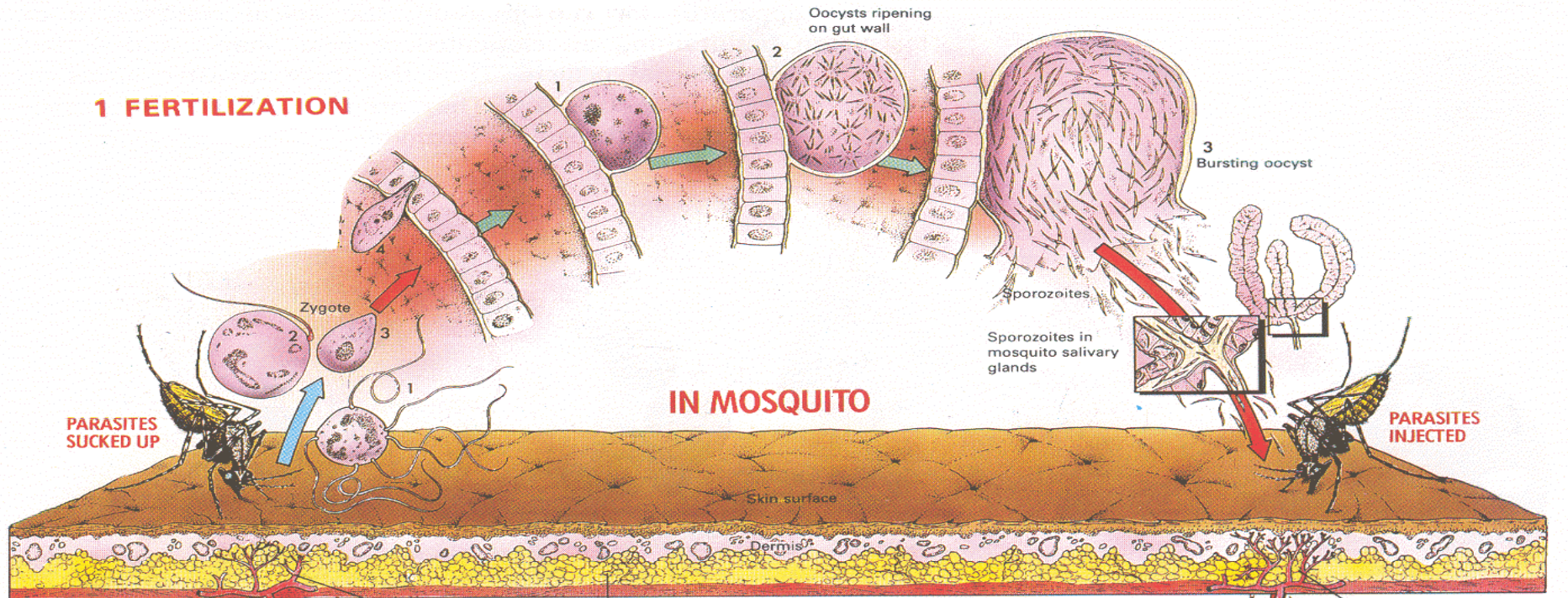


OUTCOME OF MOSQUITO MALARIA INFECTIOUS BITES IN SUB-SAHARAN AFRICA

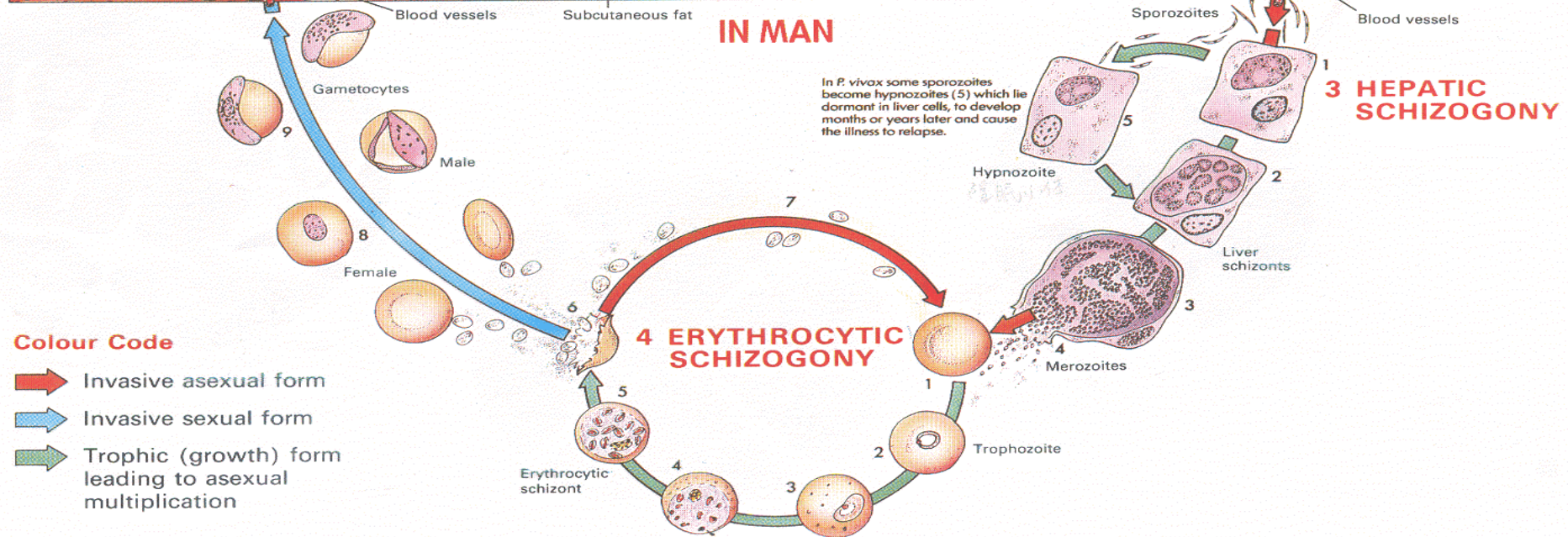
• EVENT	• FREQUENCY
• Infectious bites	• 400
• Patient infected	• 200
• Clinical malaria	• 100
• Severe malaria	• 2
• Death	• 1

2 SPOROGONY

1 FERTILIZATION



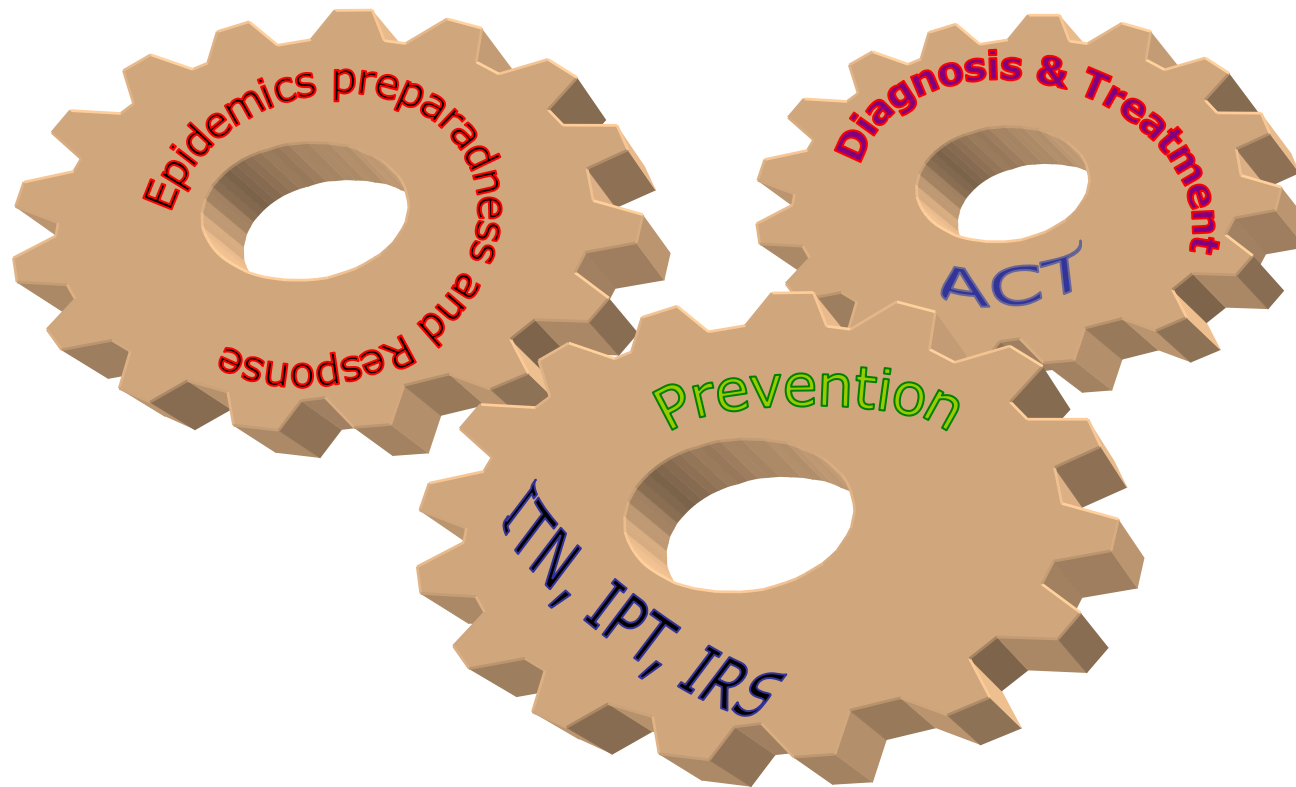
IN MAN



BIOLOGICAL COMPARISON OF THE TYPES OF MALARIA

Feature	Falciparum	Vivax	Ovale	Malariae
Exoerythrocyte (days)	6-7	8	9	14-16
Merozoites in schizont	30,000	10,000	15,000	15,000
Incubation period (days)	12(8-25)	14(8-27) months-years	15(9-17) months-years	15-30 months-years
Erythrocyte (hours)	48	48	48	72
Parasitemia (cum) Percentage	50,000-500,000 up to 2,500,000 up to 60%	20,000-50,000 <2%	9,000-30,000 <2%	6,000-20,000 <1%
Infected RBC	all stages	reticulocytes	reticulocytes	seniles
Relapse	no	yes	yes	no
Recrudescence	yes	no	no	yes?

Malaria Control Strategies



Malaria Case Management



"...Prompt accurate diagnosis and early treatment of malaria is the key to effective disease management."



CLINICAL MANIFESTATIONS



MALARIA – COMMON SYMPTOMS

NON-SPECIFIC

- **FEVER**
- **CHILLS**
- **HEADACHE**
- **NAUSEA, VOMITING**
- **DIARRHEA**
- **LOW BACK PAIN**
- **ARTHRALGIA, MYALGIA**
- **ANOREXIA**
- **FATIGUE**

Table 1. Signs and symptoms in European travelers and immigrants with falciparum malaria.

Sign or symptom	Immigrants (<i>n</i> = 790)	European travelers (<i>n</i> = 869)
Fever	603 (76.3)	704 (81)
Headache	388 (49.1)	432 (49.7)
Fatigue	189 (23.9)	302 (34.8)
Myalgia, arthralgia	136 (17.2)	202 (23.2)
Diarrhea	77 (9.7)	121 (13.9)
Vomiting	96 (12.2)	104 (11.9)
Respiratory complaints	21 (2.7)	30 (3.5)
Neurological complaints	10 (1.3)	22 (2.5)
Skin affections	10 (1.3)	11 (1.3)
Otitis	56 (7.1)	8 (0.9)
Other	157 (19.9)	153 (17.6)
None	49 (6.2)	0

NOTE. Data are no. (%) of patients. Multiple entries are possible.

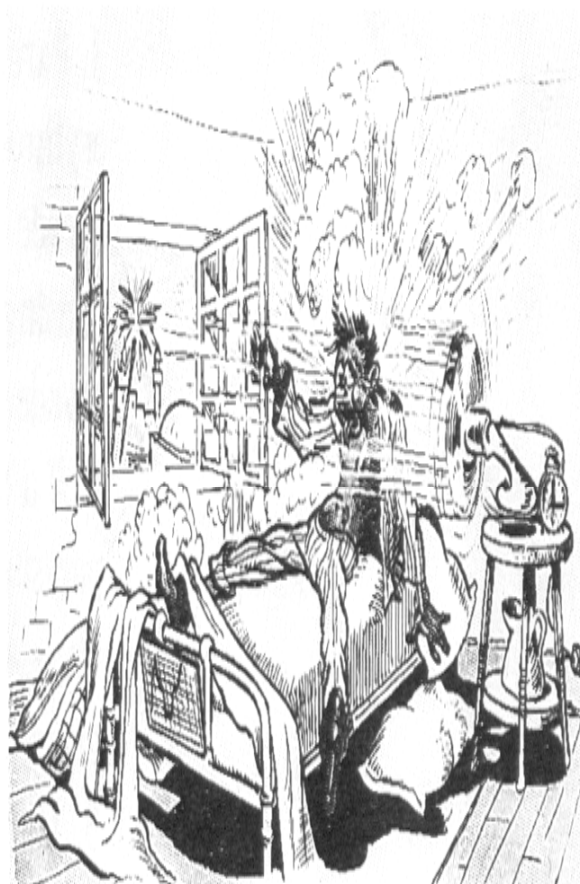
On average, up to 11,000 patients per year in European Union countries, with the number of patients with falciparum malaria estimated at about 8,000 per year

CID 34:572-576, 2002

Malaria stages of the disease



The cold stage



The hot stage



The sweating stage

MALARIAL SYMPTOMS AND TYPICAL MIS-DIAGNOSIS

Organs most affected	Main symptoms/signs	Typical mis-diagnosis
Gastrointestinal tract	Vomiting, diarrhea	Gastric flu, cholera, infectious diarrhea
Brain	Delirium, coma, convulsions	Encephalitis, meningitis, status epilepticus, hypoglycemic coma
Kidneys	Renal failure	Nephritis
Liver	Jaundice, fever	Bacterial and viral hepatitis
Lungs	Pulmonary edema	Pneumonia, heart failure

Severe malaria – cerebral malaria



Severe Malaria

- **Clinical Manifestation:**
- **Impaired consciousness**
- **Respiratory distress (acidotic breathing)**
- **Multiple convulsions**
- **Circulatory collapse**
- **Pulmonary edema (radiological)**
- **Abnormal bleeding**
- **Jaundice**
- **Hemoglobinuria**
- **Laboratory Test:**
- **Severe anemia**
- **Hypoglycemia**
- **Acidosis**
- **Renal impairment**
- **Hyperlactatemia**
- **Hyperparasitemia**

Parasitemia and clinical correlates

Parasitemia	Parasites /μl	Remarks
0.0001-0.0004%	5-20	Sensitivity of thick blood film
0.002%	100	Patients may have symptoms below this level, where malaria is seasonal
0.2%	10,000	Level above which immunes show symptoms
2%	100,000	Maximum parasitemia of <i>P.v.</i> and <i>P.o.</i>

Parasitemia and clinical correlates

Parasitemia	Parasites/ μ l	Remarks
2-5%	100,000-250,00	Hyperparasitemia/severe malaria*, increased mortality
10%	500,000	Exchange transfusion may be considered/ high mortality

*WHO criteria for severe malaria are parasitemia $> 100,000 /\mu$ l and severe anaemia (haemoglobin < 5 g/l).

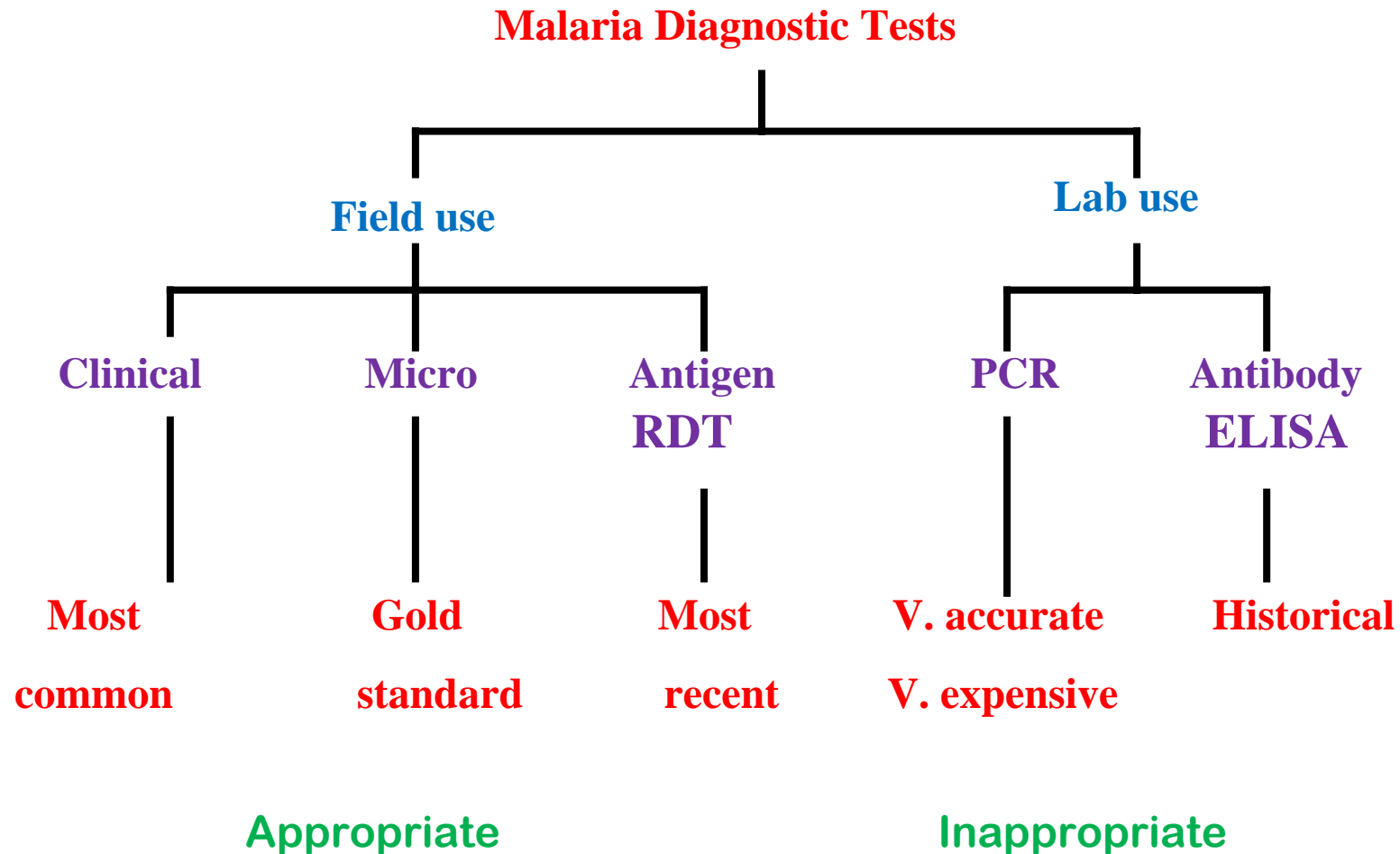
Prognosis is poor if $> 20\%$ parasites are pigment containing trophozoites and schizonts (more mature forms) and/or if $> 5\%$ of neutrophils contain visible pigment.

Hänscheid T. (1999) Diagnosis of malaria: a review of alternatives to conventional microscopy. *Clin Lab. Haem.* 21, 235-245.

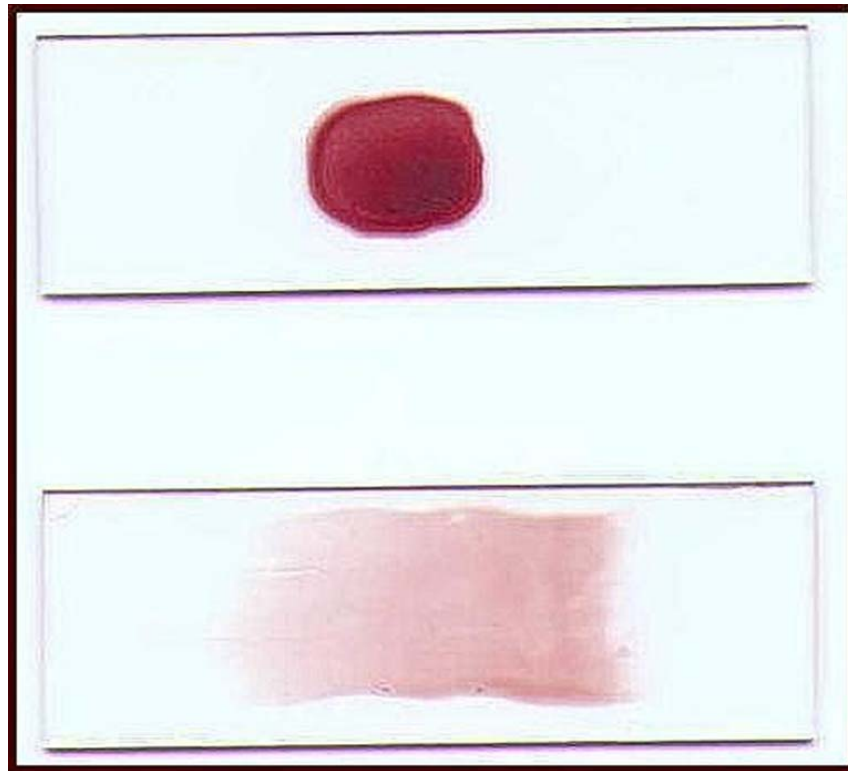
Malaria Diagnosis

- **Symptom-based (clinical) diagnosis**
- **Light microscopy**
- **Quantitative Buffy Coat (QBC) method**
- **Rapid diagnostic tests (antigen capture)**
- **Flow cytometry**
- **PCR-based molecular detection methods**
- **Loop-Mediated Isothermal Amplification**

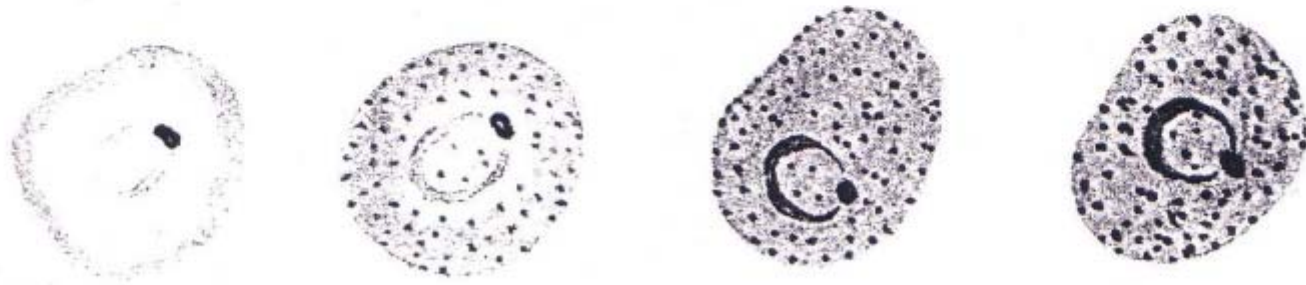
Malaria diagnosis:



Gold Standard Method



pH 7.2

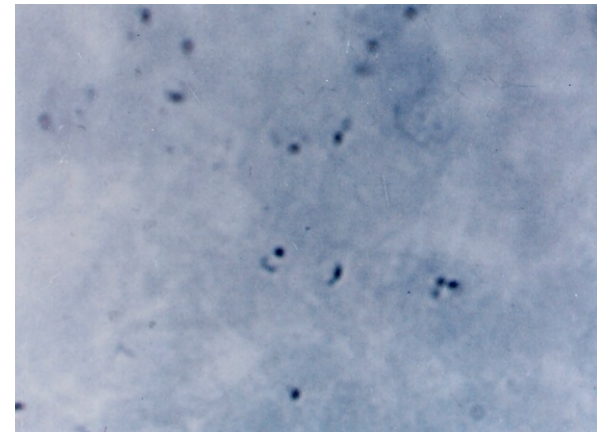


pH ET COLORATION

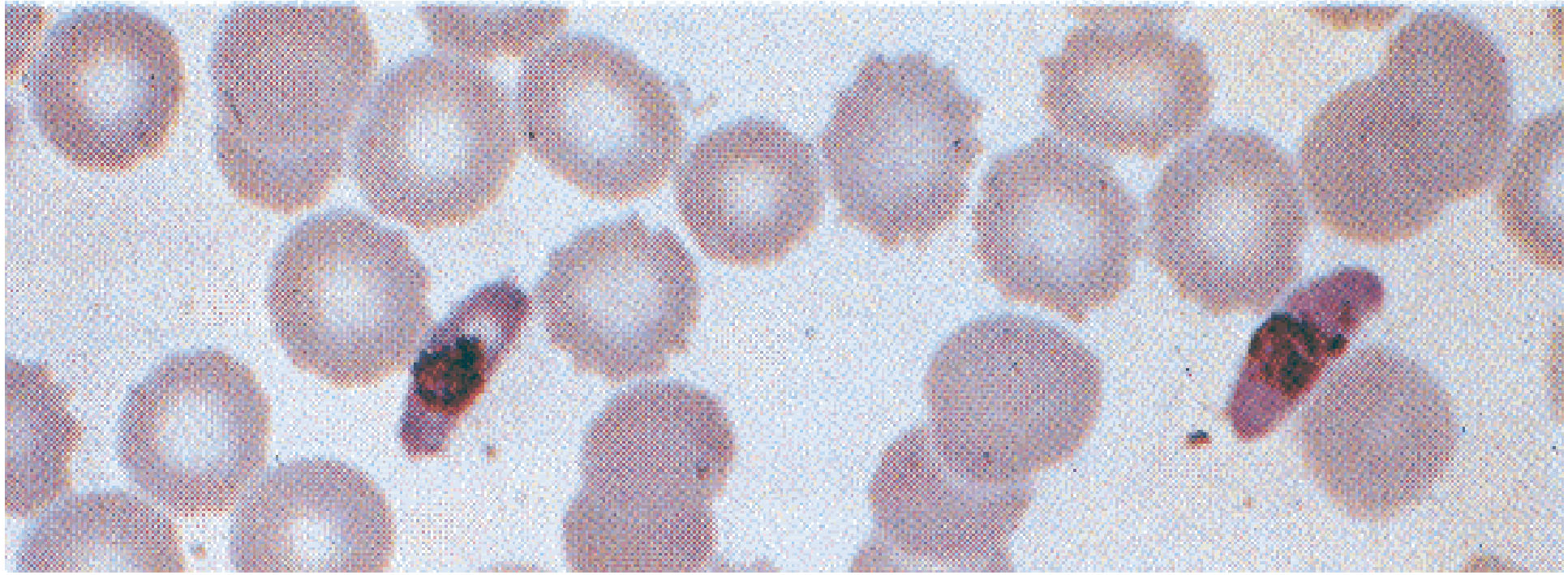
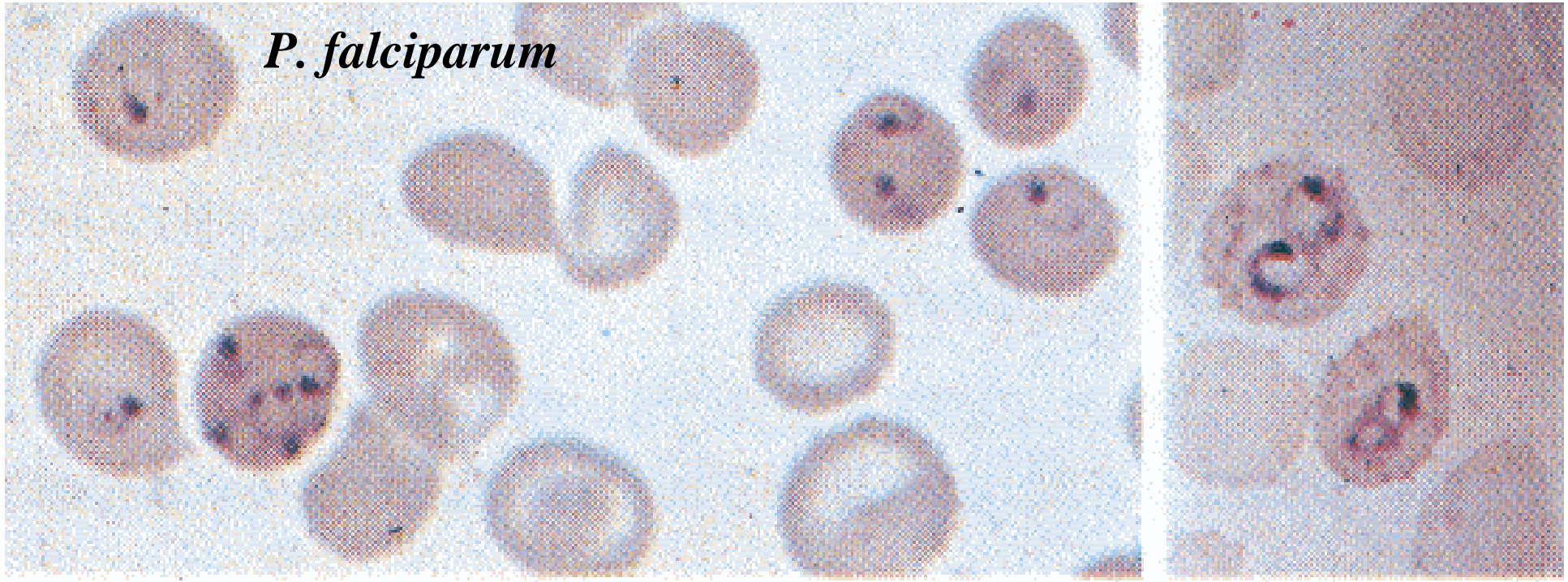
Thick Blood Smear

Examination of a thick blood film should be the first step since this has the advantage of concentrating the parasites by 20 fold in comparison to a thin film, although the parasites may appear distorted making species identification difficult.

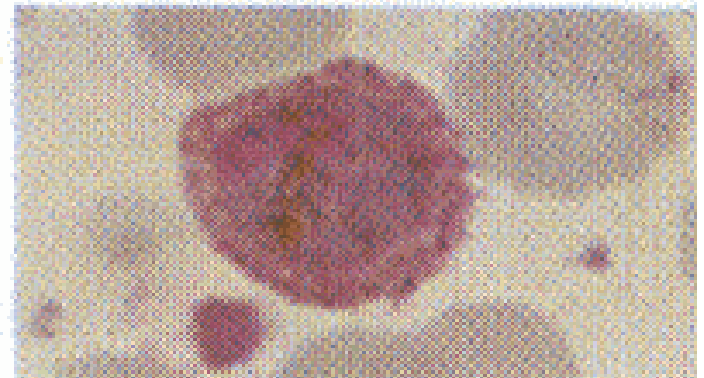
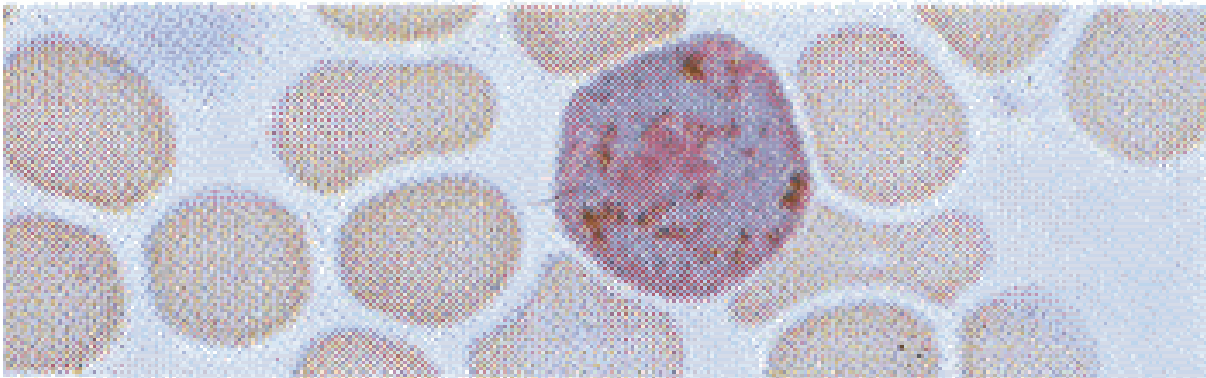
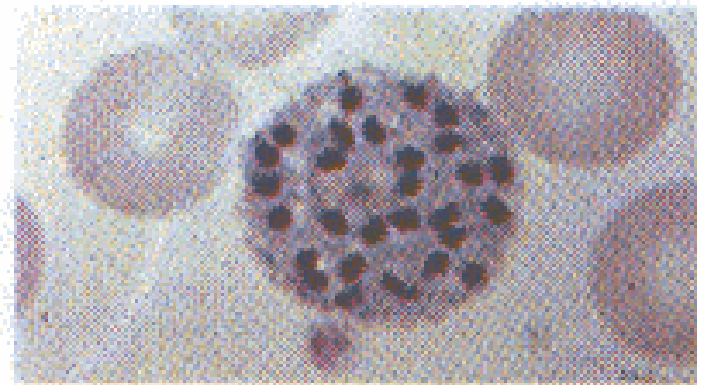
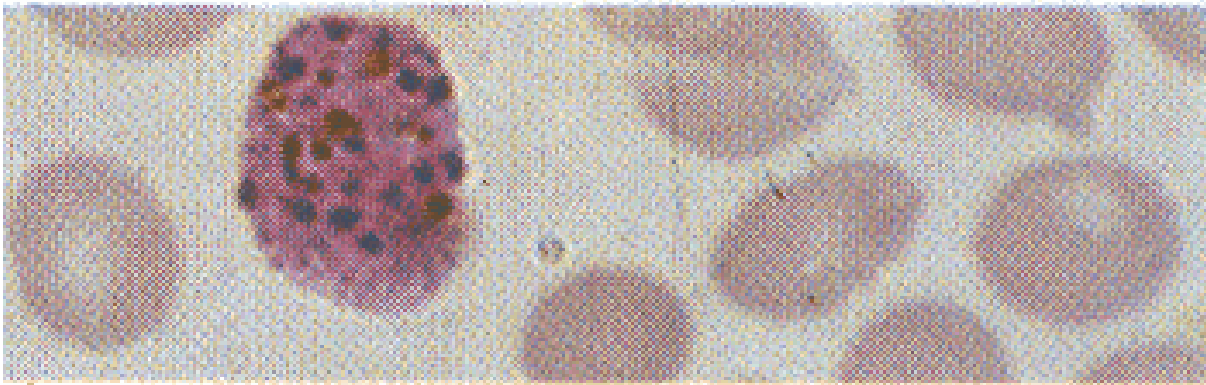
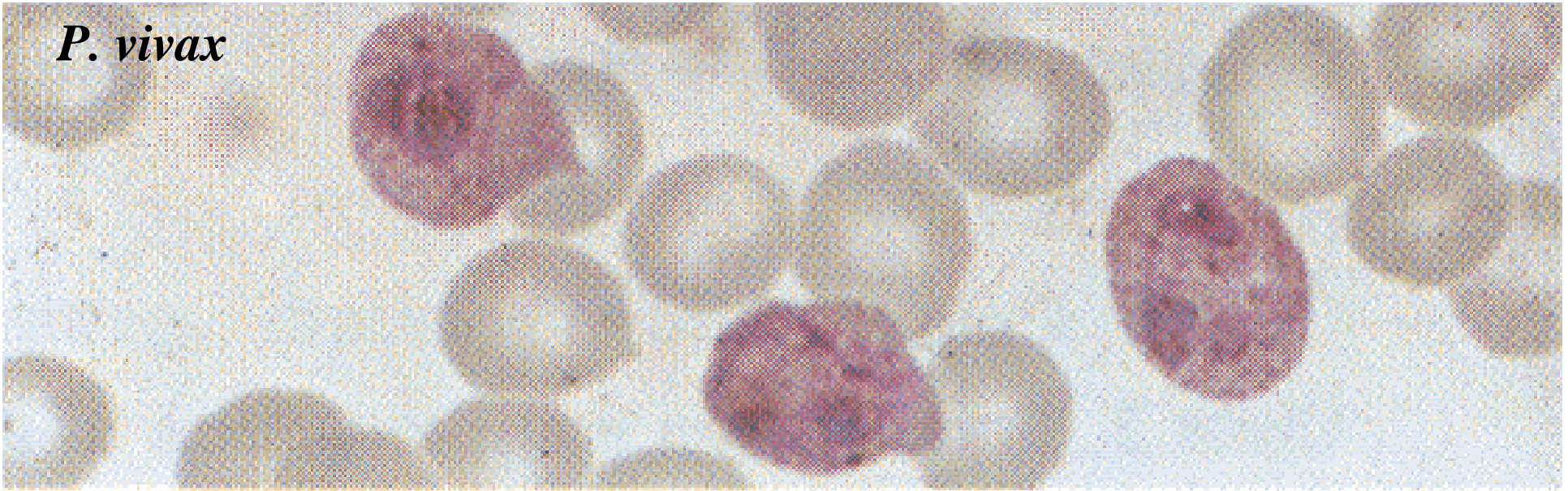
If parasites are seen then the species should be confirmed by the examination of a thin film. Ideally blood should be collected when the patient's temperature is rising.



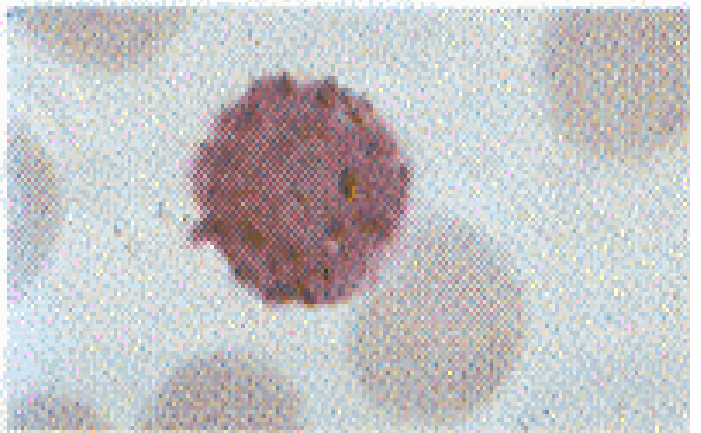
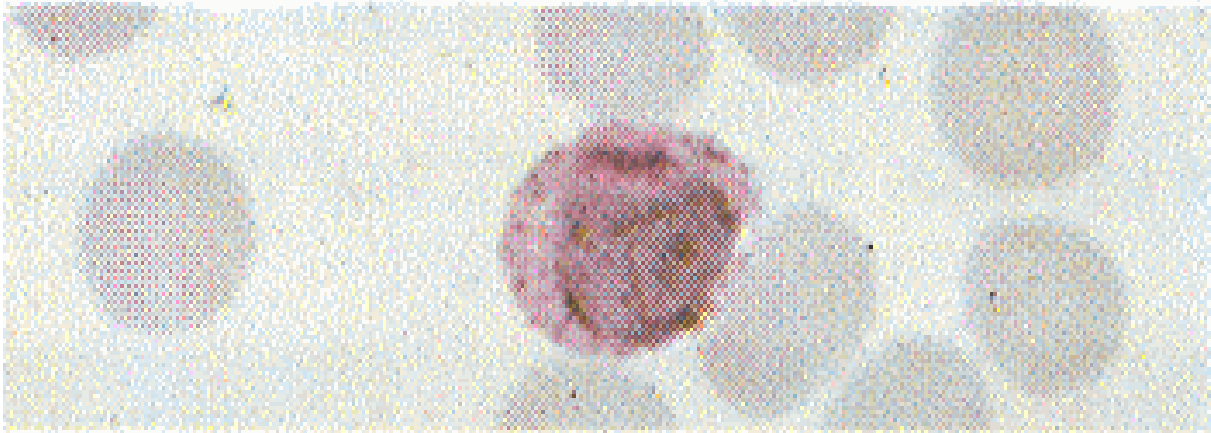
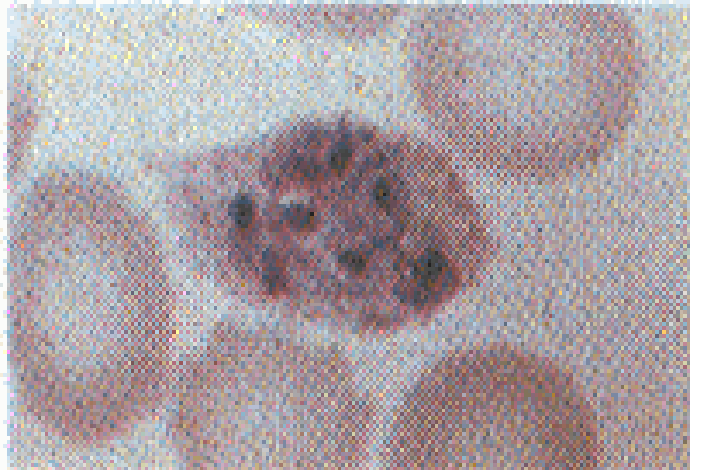
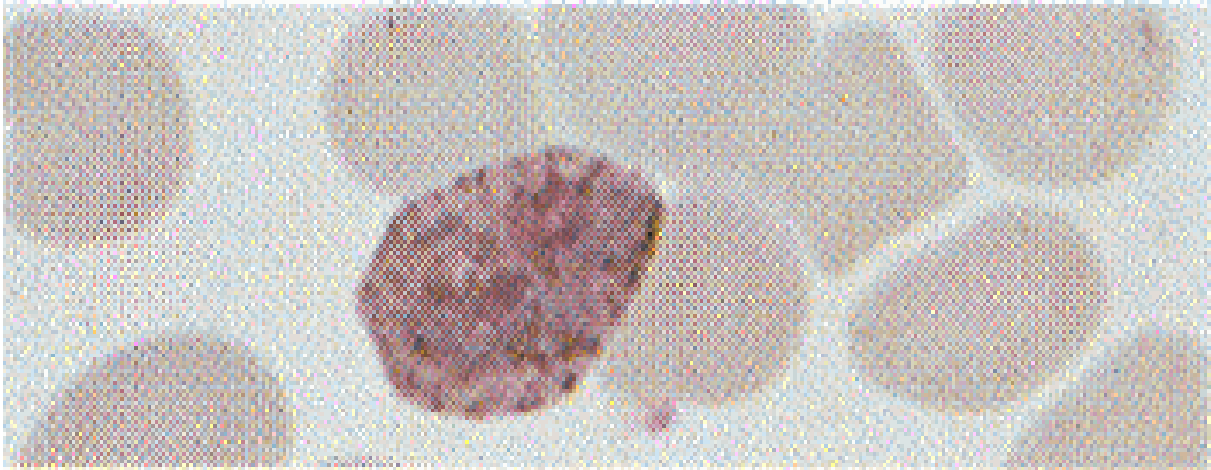
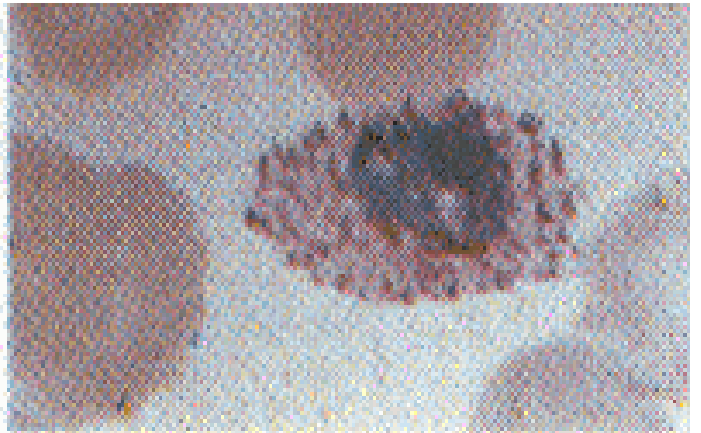
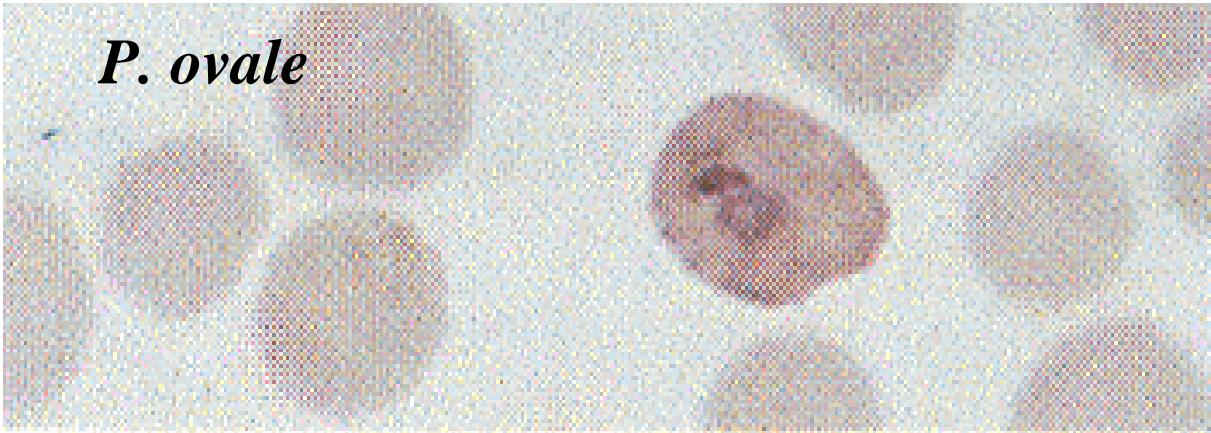
P. falciparum



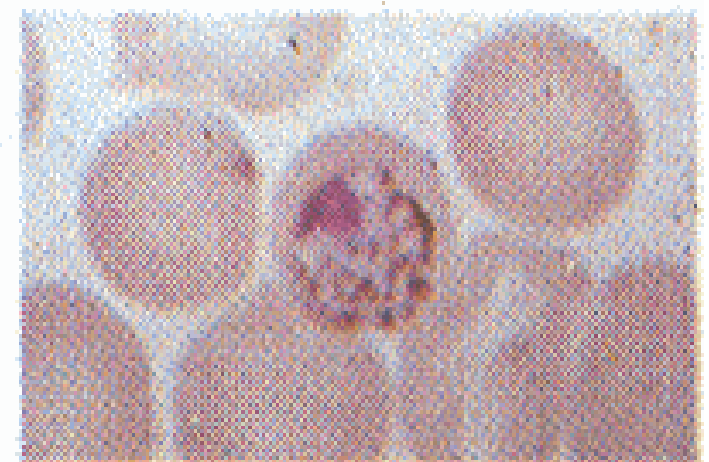
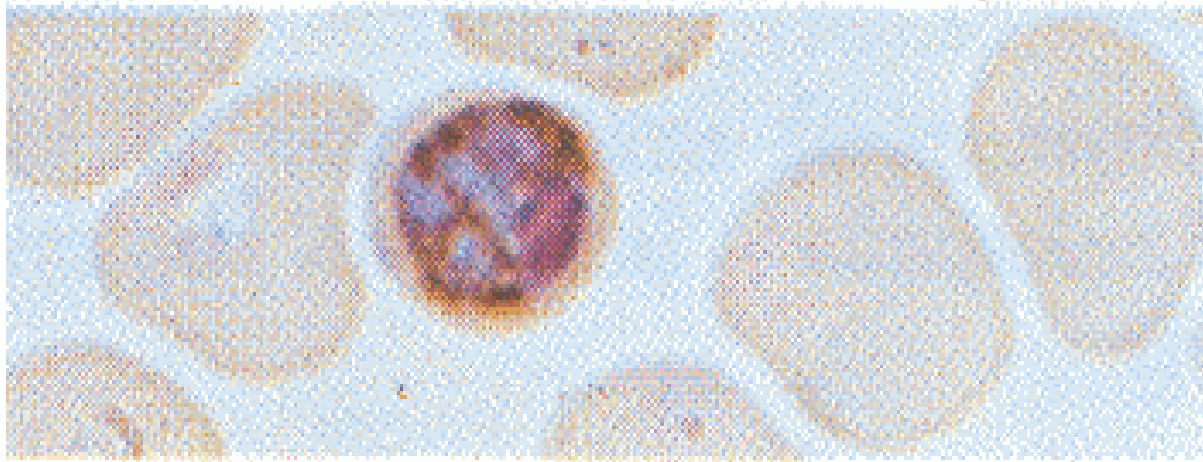
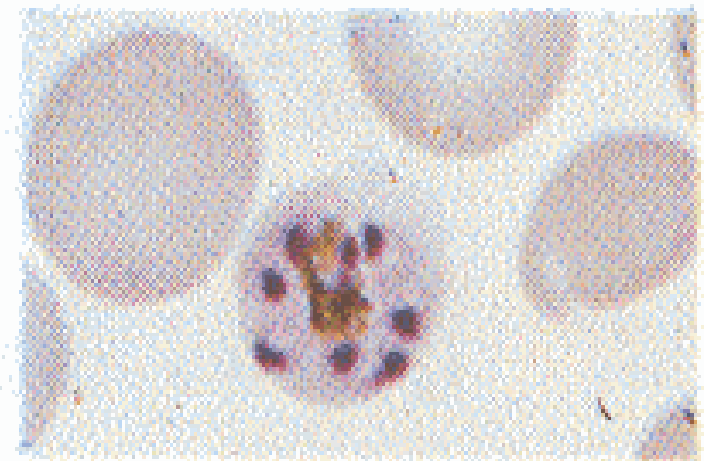
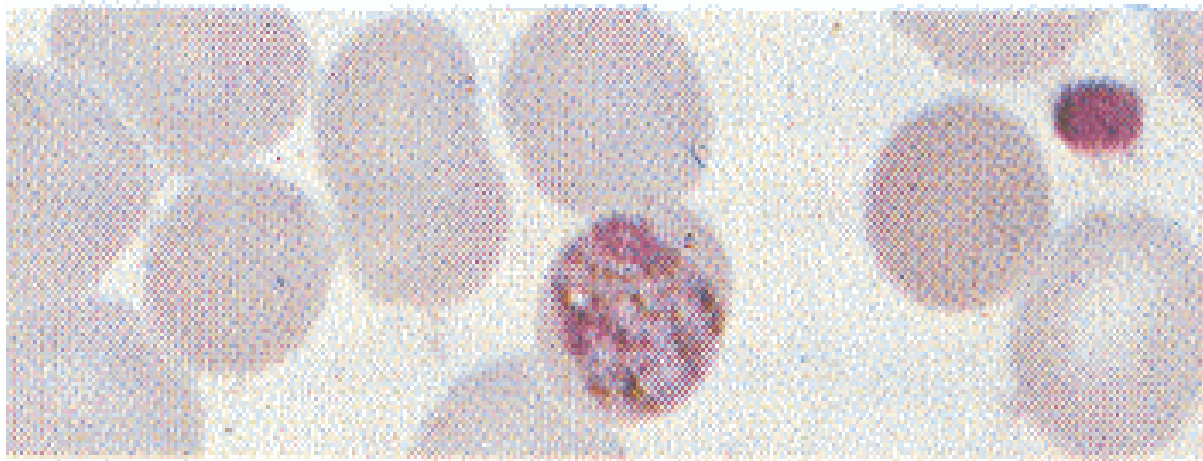
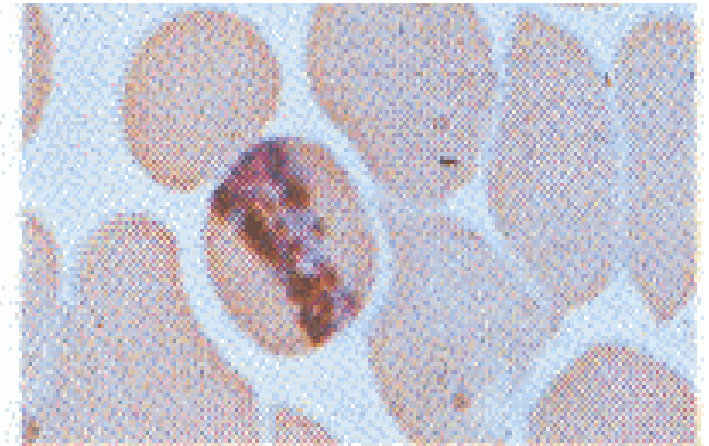
P. vivax

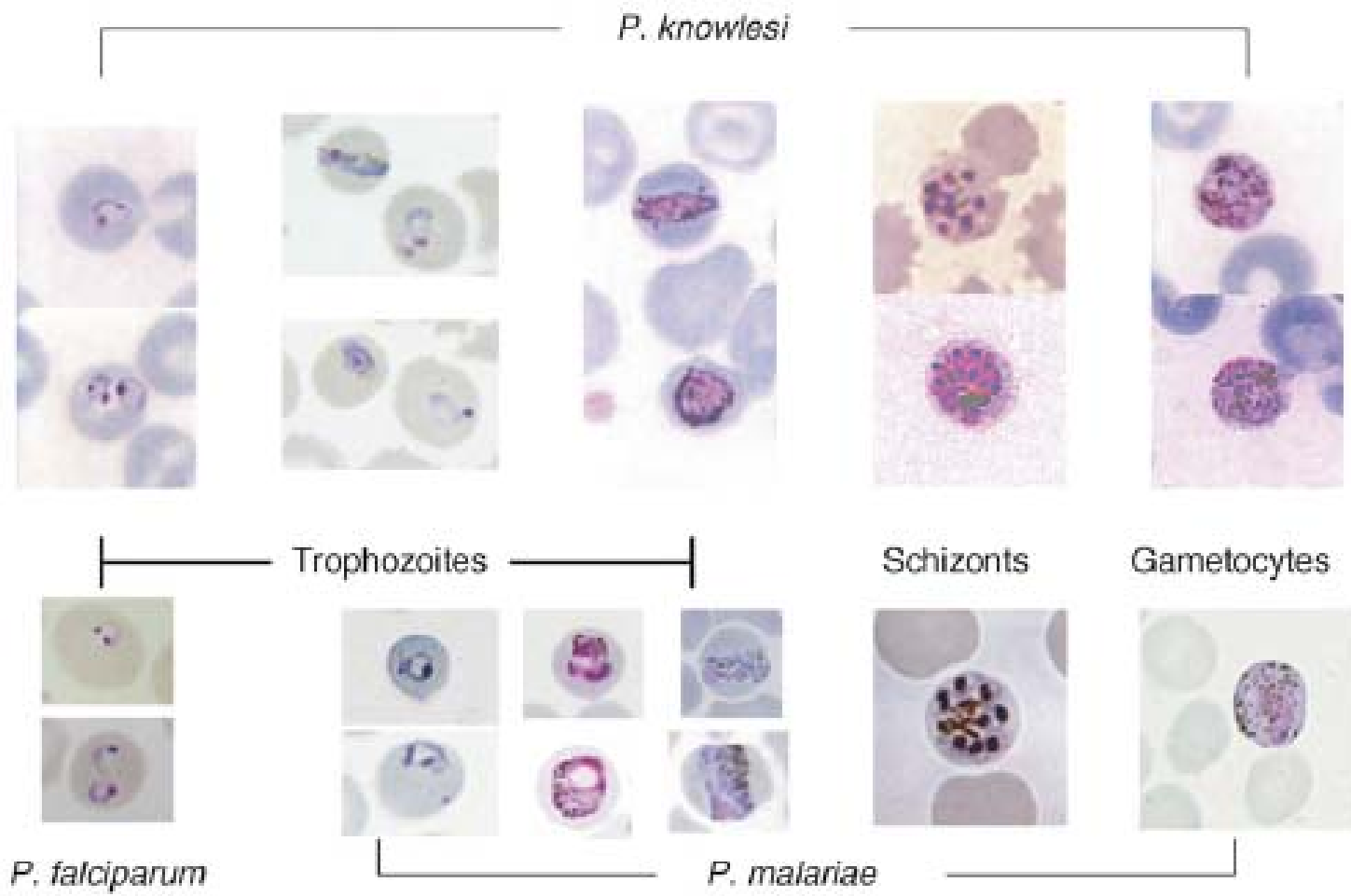


P. ovale



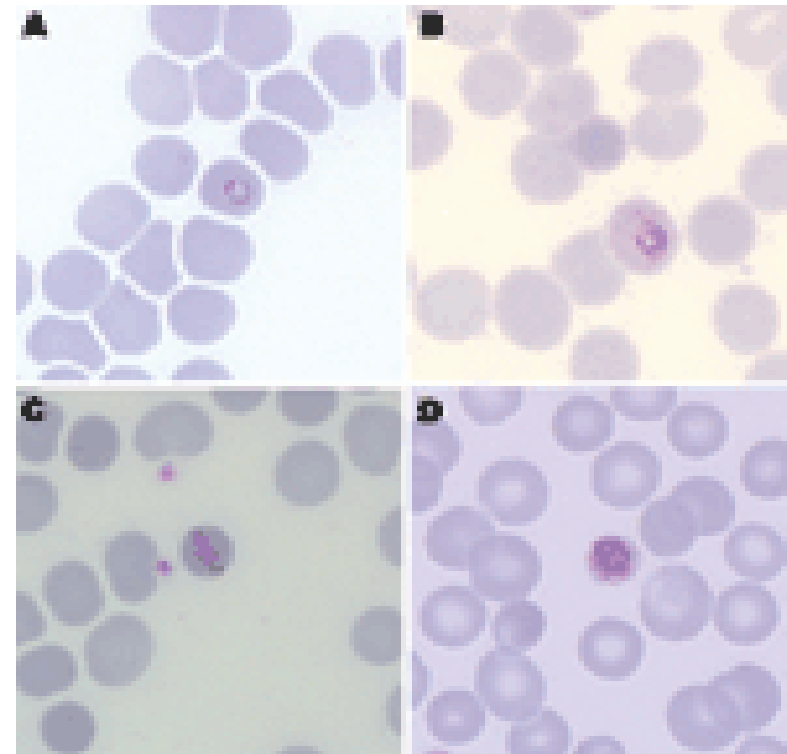
P. malarie

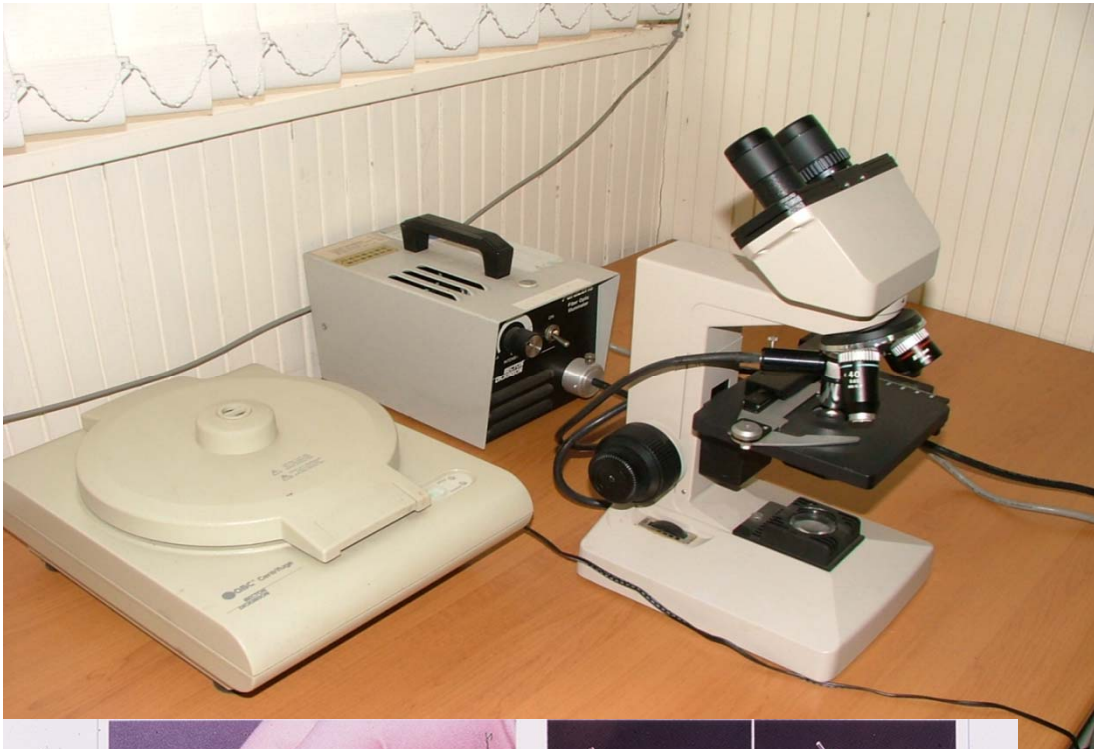




A new species of malaria – *P. knowlesi*

Aspects	Characteristics
Morphology ring	<i>Diff P.falciparum</i>
Morphology mature trophozoite	<i>Diff P. malariae</i>
Clinical	<i>Diff P. falciparum</i>
Phylogenetic	<i>Diff P. vivax</i>
Liver stage	Absence of hypnozoite forms
Asexual cycle	24 hrs
Treatment	Chloroquine



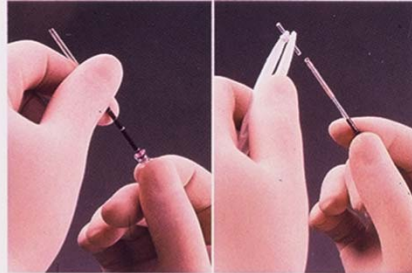


Quantitative Buffy Coat (QBC)



1. Fill tube with blood

15 Seconds



2. Place closure, insert float

10 Seconds



3. Centrifuge

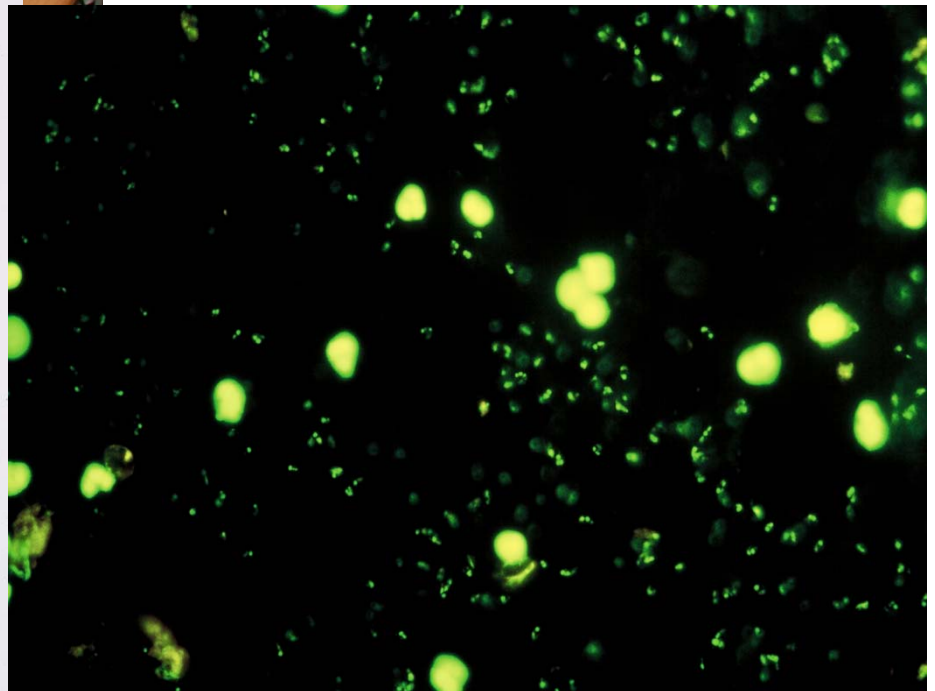
5 Minutes



4. Examine tube

Positive = 5 to 15 Seconds

Negative = 2 to 3 Minutes



Quantitative Buffy Coat (QBC®)

- **Fluorescent microscopy after centrifugation**
- **AO-coated capillary is filled with 50-100 μ l blood**
- **Parasites concentrate below the granulocyte layer in tube**
- **May be slightly more sensitive than light microscopy but some reports of 55-84%**

Quantitative Buffy Coat (QBC ®)

- **Useful for screening large numbers of samples**
- **Quick, saves time**
- **Requires centrifuge, special stains**
- **3 main disadvantages**
 - **Species identification and quantification difficult**
 - **High cost of capillaries and equipment**
 - **Can't store capillaries for later reference**

Malaria rapid diagnostic test



Malaria rapid diagnostic test



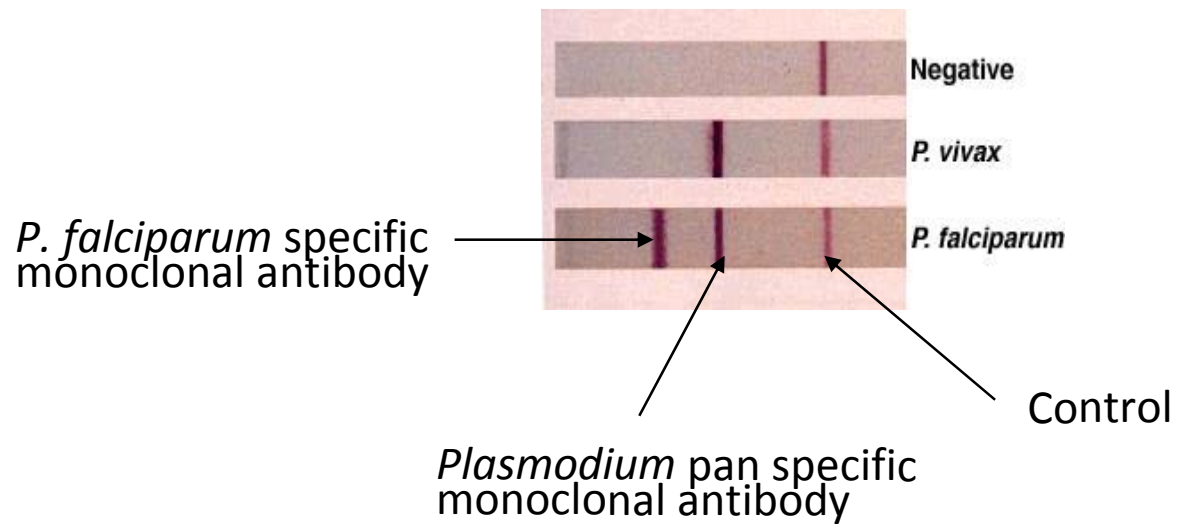
Rapid Diagnostic Test (ICT)



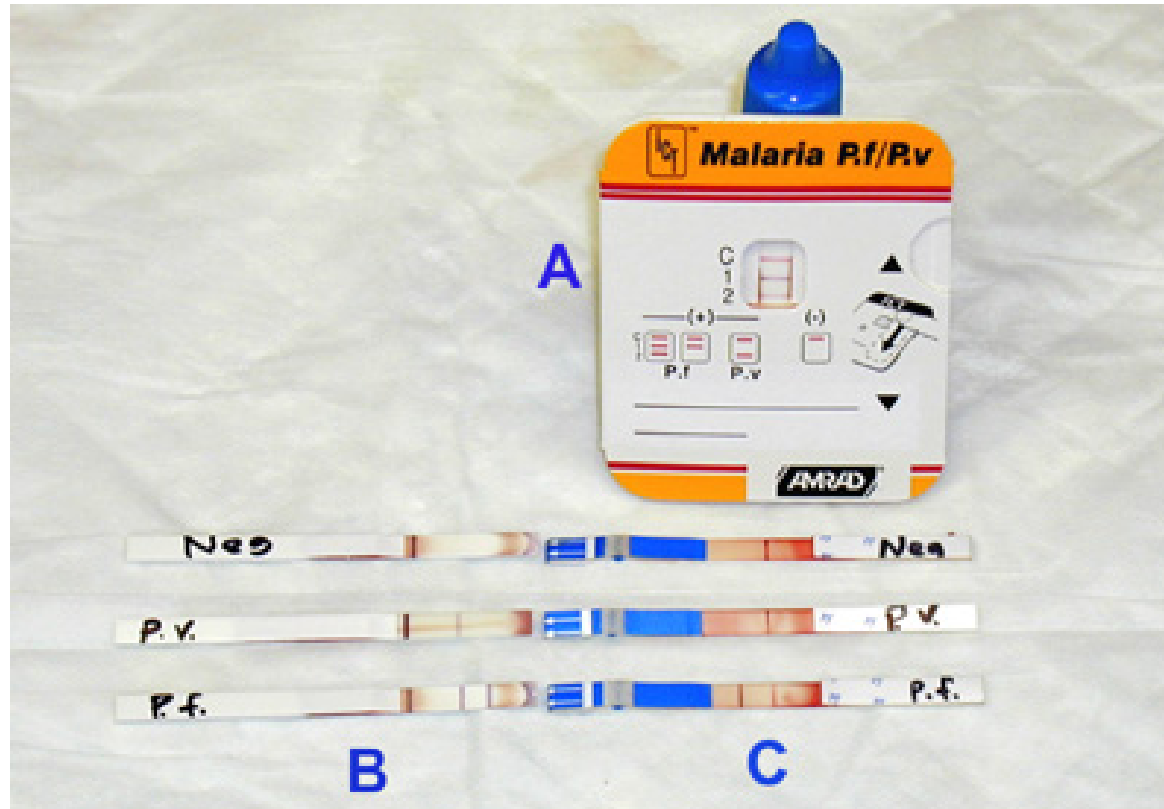
Antigen Detection

Malaria Immunochromatographic Dipstick

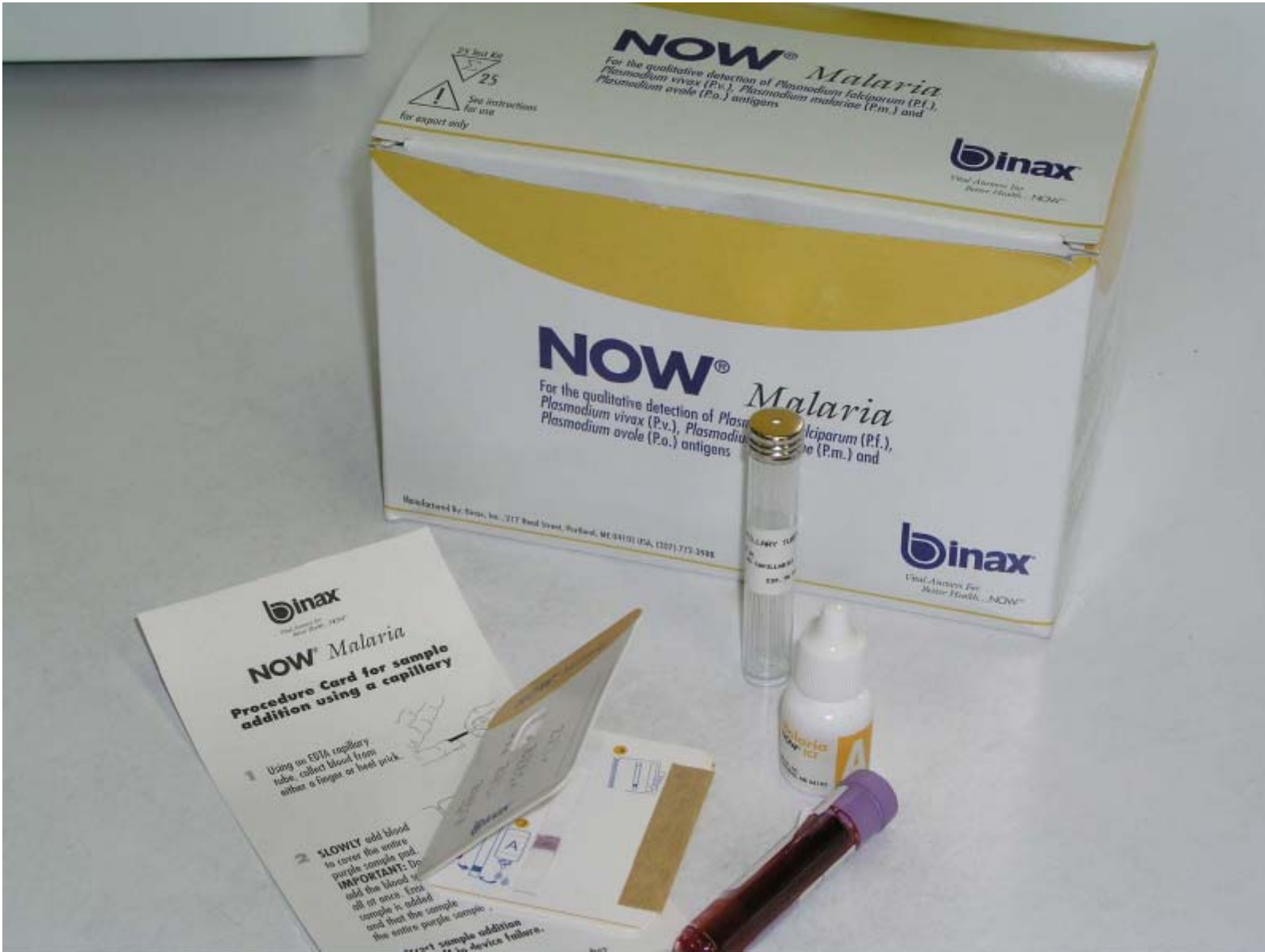
OptiMAL Assay



Detection of *Plasmodium* antigens



- A: HRP-2 (histidine-rich protein 2) (ICT)
- B: pLDH (parasite lactate dehydrogenase)(Flow)
- C: HRP-2 (histidine-rich protein 2) (PATH)



Flowcytometry

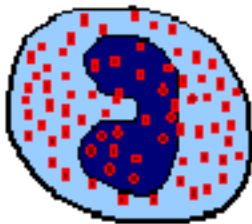
- Cell size
- Cellular granularity



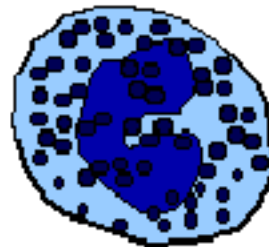
Neutrophil



Erythrocyte



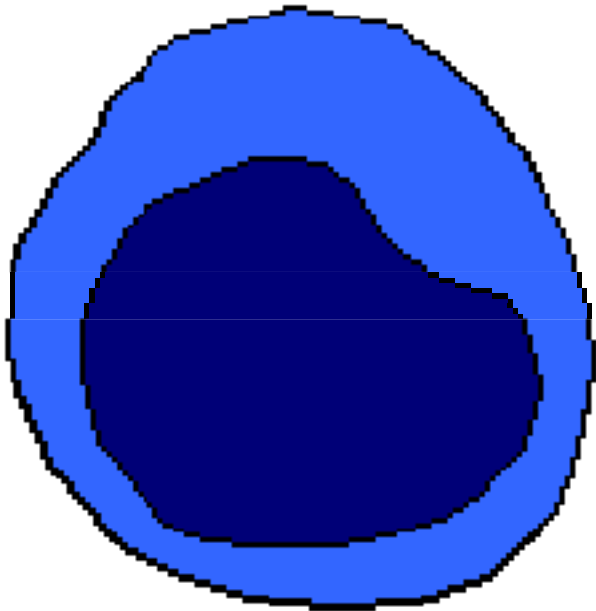
Eosinophil
(red granules)



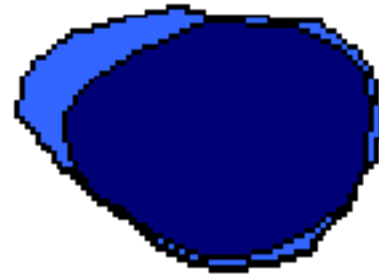
Basophil
(dark granules)

Typical granulocytes (with erythrocyte for size comparison)

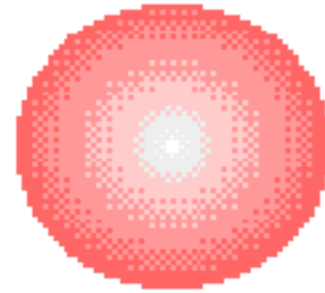
Agranulocytes



Monocyte



Lymphocyte



Erythrocyte

Typical agranulocytes (with erythrocyte for size comparison)

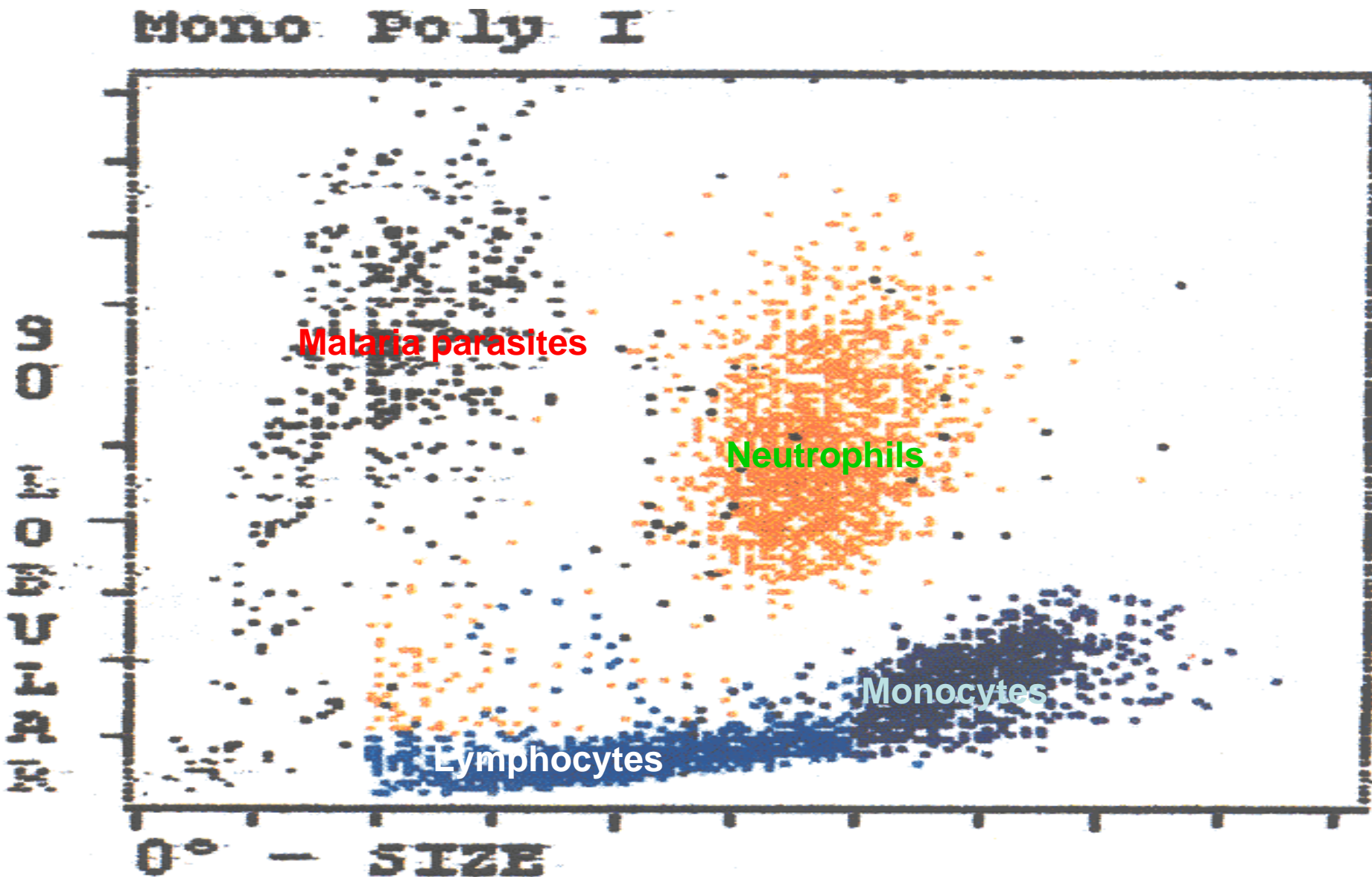
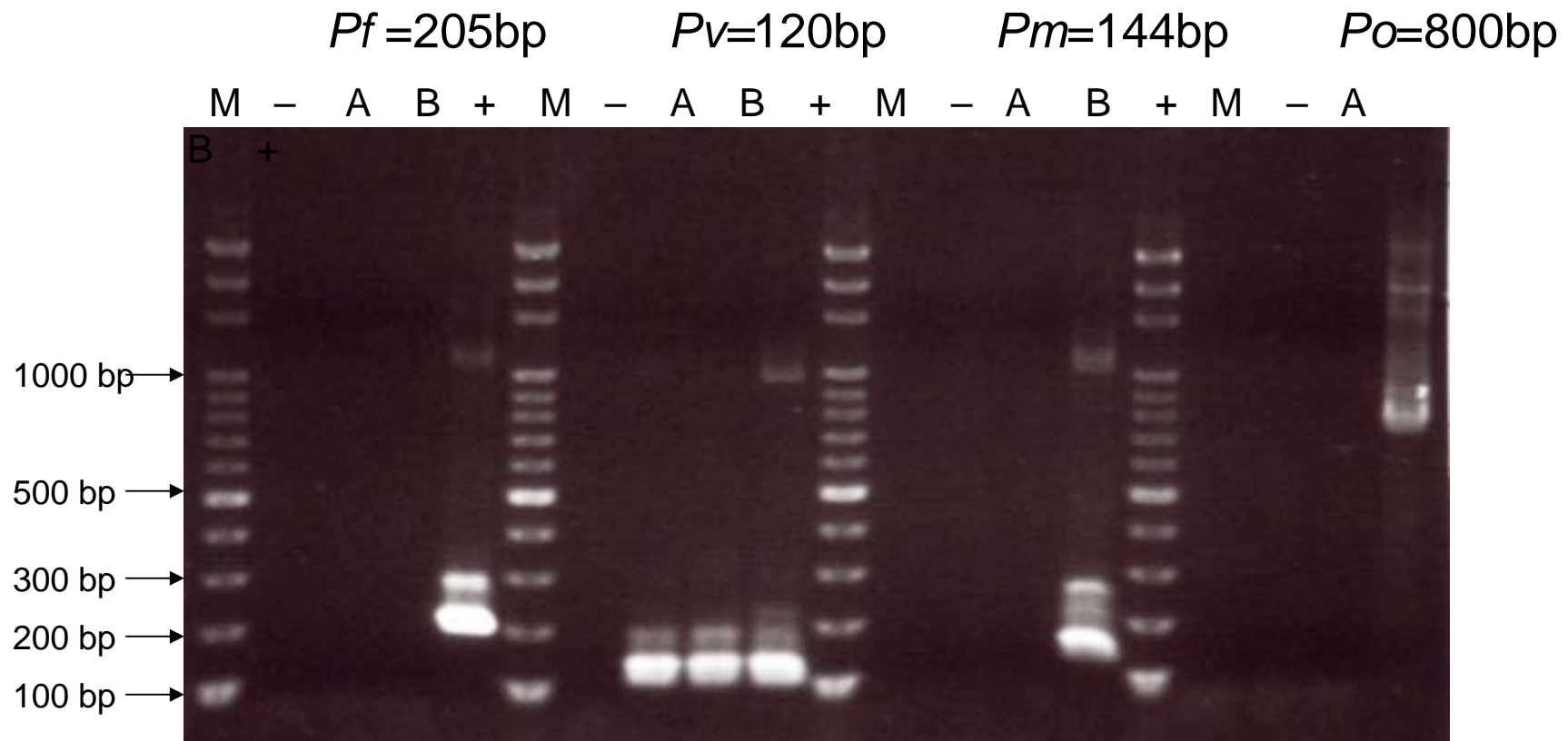


Figure 1. Scattergram generated by an automated blood-cell analyzer. Orange points, neutrophils; blue points, lymphocytes; purple points, monocytes.

**High sensitivity of detection of human malaria parasites by
the use of nested PCR**

Mol Biochem Parasitol

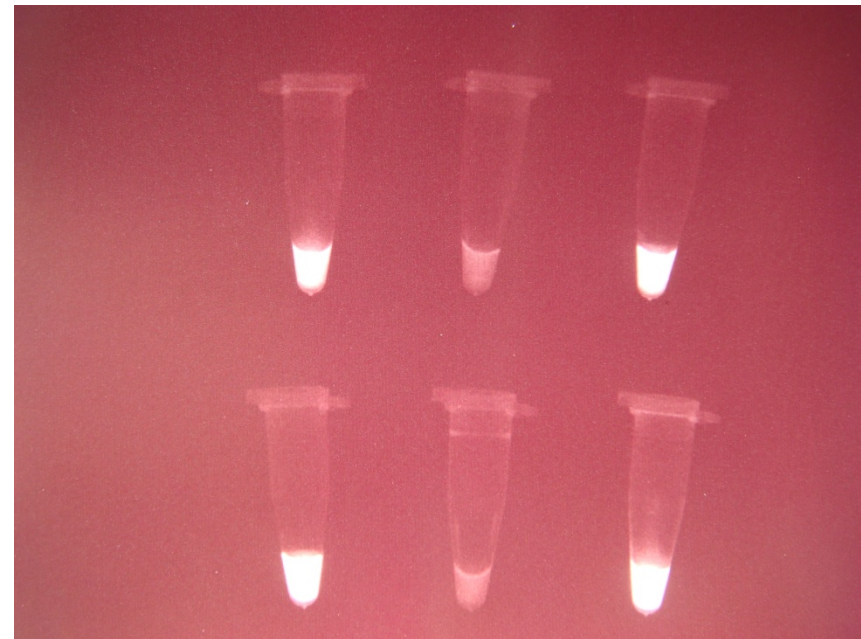
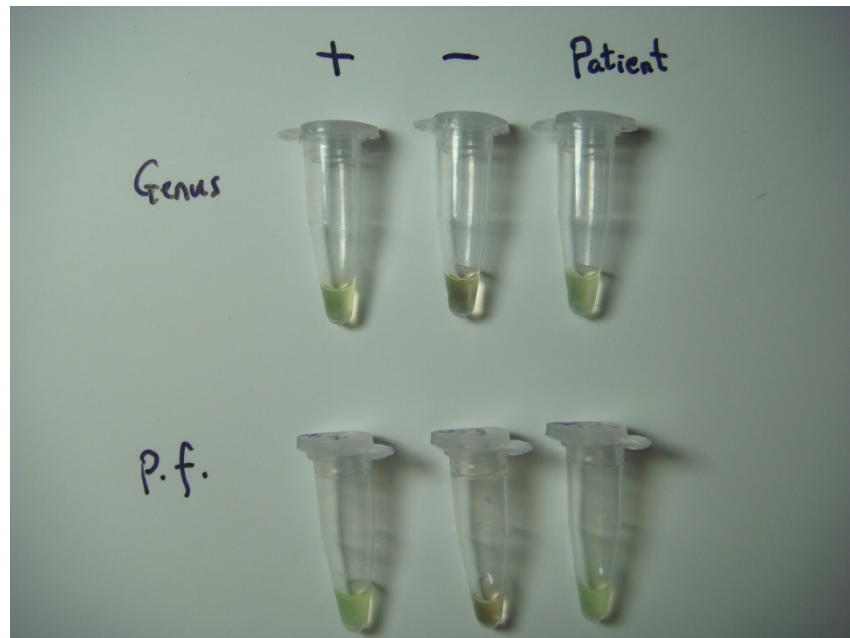
61: 315-20, 1993



M:100 bp DNA ladder - : negative control + : positive control

Samples A and B

Loop-Mediated Isothermal Amplification for Malaria Diagnosis



DIAGNOSTIC MALARIA

- **METHOD**

- Blood thin film
- Blood thick film
- Quantitative buffy coat
- Antibody test
- Antigen capture test
- PCR

- **SENSITIVITY**

- 100/ul (parasitemia 0.002%)
- 10-20/ul
- <10/ul
- ?
- 50/ul
- <1/ul

Diagnosis

	Clinical dx	Microscopy	RDTs
Sensitivity	85%	60-95%	85-95%
Specificity	40%	75-95%	95%
Infrastructure needed	Minimal	High	Moderate
Skill needed	Minimal	High	Moderate
Cost	\$0	\$0.10-0.40	\$0.70-1.00
Current use	High	Moderate	Minimal

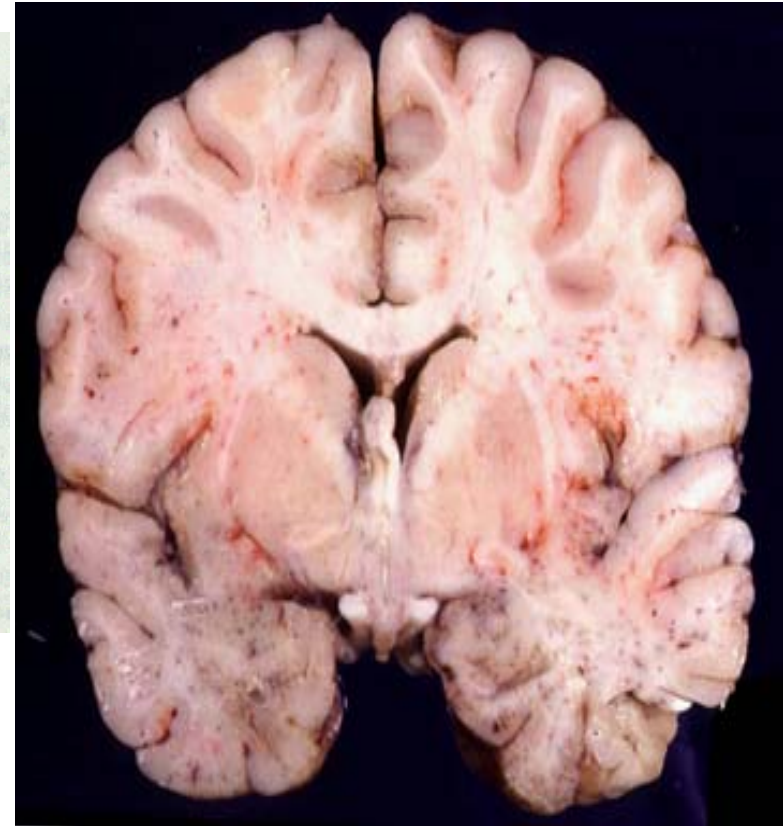
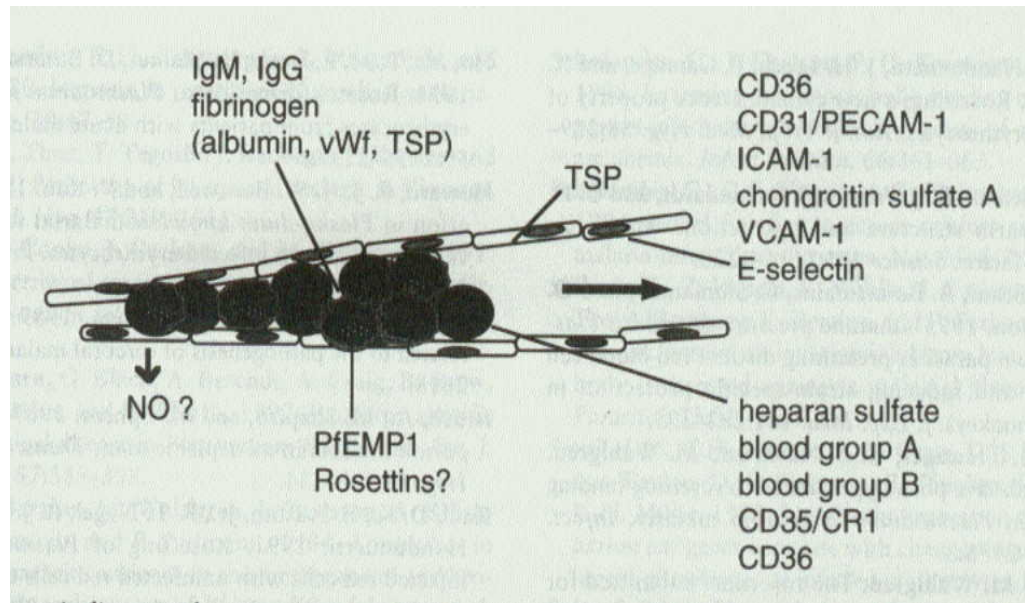
A microscopic image showing a field of cells. In the center, there is a distinct cluster of dark, granular material, possibly representing a pathogen or a specific cellular structure. The surrounding cells are lighter and more diffuse.

**IMMUNE RESPONSE
AND
PATHOGENESIS**

PATHOGENESIS OF MALARIA

- **INVASION OF RED CELLS:** The entire process of invasion is completed within 30 seconds. In falciparum malaria the EBA 175 and/or MSP-1 appear to interact with the red cell sialoglycoproteins (glycophorins), whereas in vivax malaria, the red cell Duffy antigen on the uninfected RBC is involved.
- **CYTOADHERENCE:** The process (sequestration) whereby mature infected cells (PfEMP-1) specifically bind to endothelial cells (CD36, ICAM-1, VCAM-1, E-selectin, thrombospondin, chondroitin sulfate A) in postcapillary venules, preventing destruction through the spleen; localized at sites of reduced oxygen tension favoring parasite growth; and facilitating the invasion of uninfected RBC.
- **ROSETTING:** RBC containing the more mature stages of parasite (PfEMP-1) bind uninfected RBC to their surface (CR1), may involve microcirculatory obstruction.
- **PARASITE TOXINS AND CYTOKINE:** Both GPI-anchored parasite molecules and hemozoin-associated protein are favored candidates. Such malaria toxins lead to the release of TNF- α and IL-1, responsible for fever and other clinical symptoms and signs.

Cerebral Malaria



- 1. High cytokine levels could be toxic on their own.**
- 2. High levels of cytokine also enhance the second process thought to be responsible for cerebral malaria: sequestration of infected RBCs**

Sequestration & cytoadherence

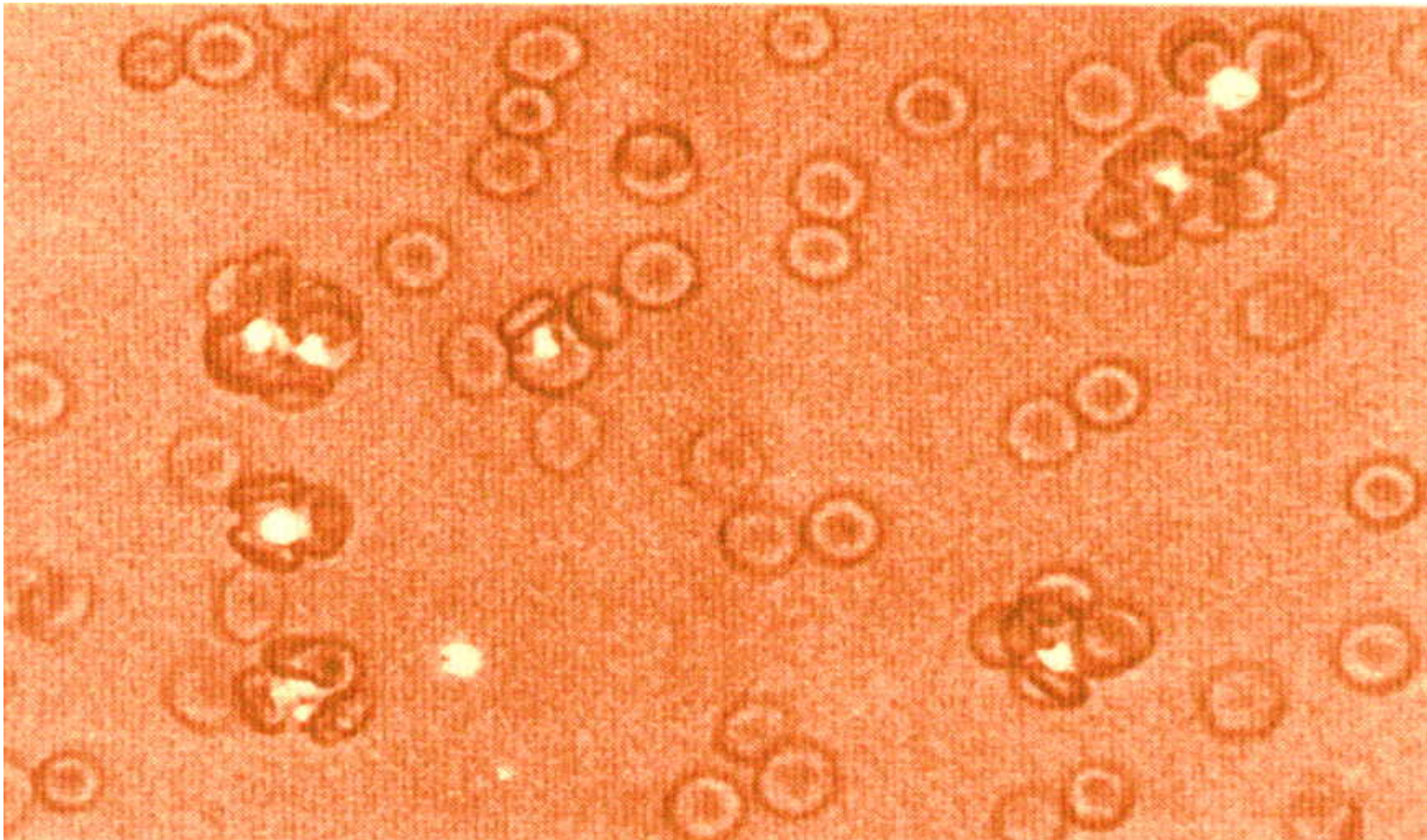


Rosetting (adhesion of infected RBCs to other RBCs) and clumping (adhesion between infected cells) was first observed in in vitro culture

Rosetting was also found in 50% of field isolates and correlated strongly with the severity of the observed disease



Rosetting of *P. falciparum* in vitro



RELAPSE OR RECRUDESCENCE?

Phenomenon	Relapse	Recrudescence
Malaria species	<i>P. vivax</i> <i>P. ovale</i>	<i>P. falciparum</i> <i>P. malariae?</i>
Mechanisms	Hypnozoite (exo-erythrocytic)	Sequestration (endothelial cytoadherence)
Duration	months - years	weeks - months
Clinical setting	Eradication by primaquine	Antigen variation Drug resistance?

CASE MANAGEMENT

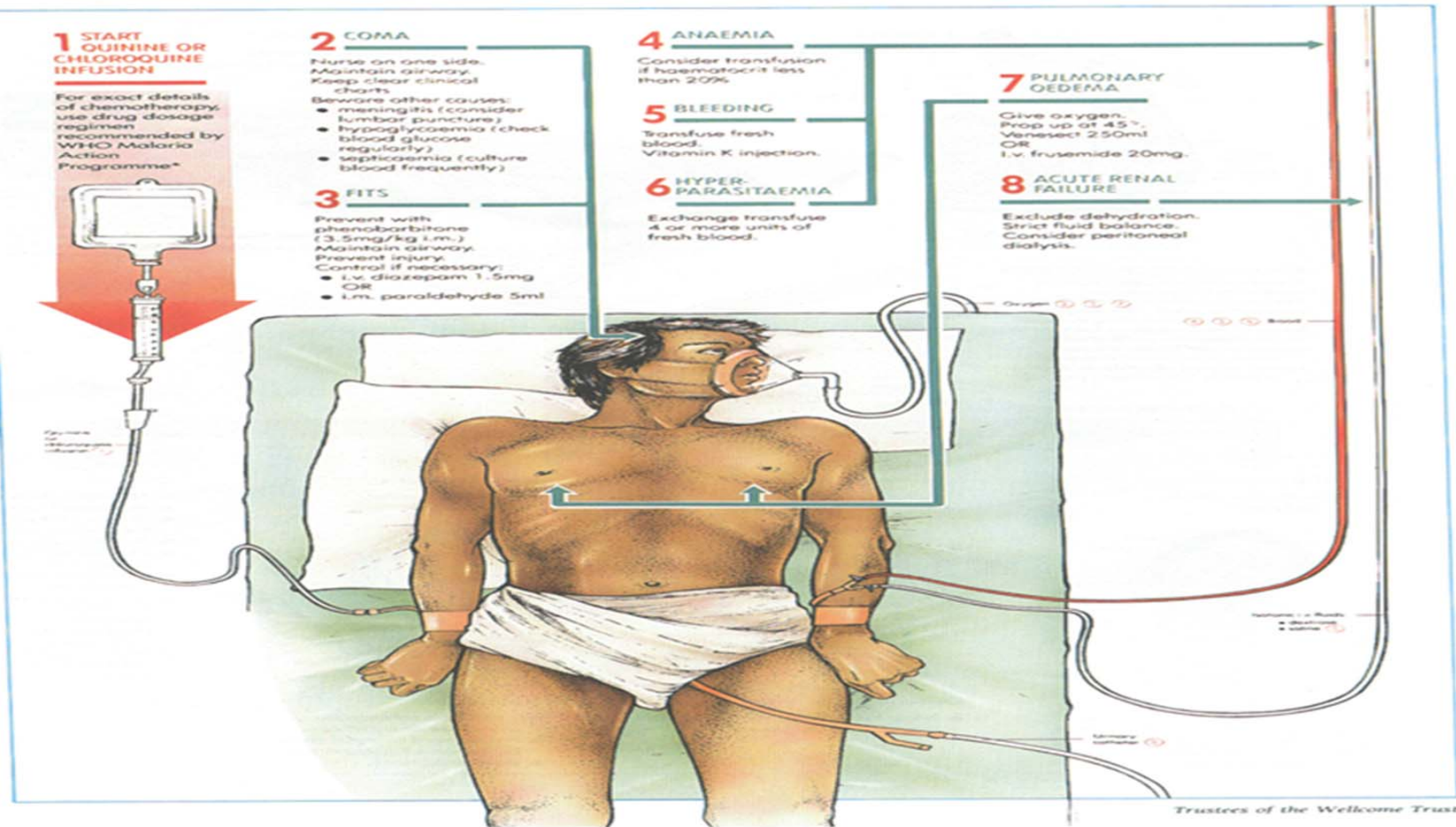


COMMON ERRORS IN THE TREATMENT OF MALARIA

- **Initial blood smear is negative and not repeated**
- **Drug of choice is not readily available**
- **Lack of high index of suspicion of malaria due to**
 - 1. Presentation with fever and GI symptoms**
 - 2. Failure to take an adequate travel history**
 - 3. Lack of knowledge about the incubation period of malaria**

Malaria is preventable and curable but can be fatal if delay

A.3 Intensive care of severe malaria



ASSESSMENT OF MALARIA SEVERITY

- **MILD: Parasitemia < 2% and temperature <39C and patient ambulant, and no complications***
- **SEVERE: Parasitemia > 2% or schizonts** or temperature > 39C or patient non-ambulant, or complications***

*Complications = cerebral involvement, severe anemia, renal failure, pulmonary edema, hypoglycemia, hypovolemia, bleeding, DIC, acidosis, jaundice, et al.

**The presence of schizonts or pre-schizonts on the film may mean:

- (1) The peripheral parasitemia is unrepresentative of the total parasite burden due to sequestration
- (2) A further cycle of replication is imminent

Principles of Treatment

Treatment of malaria depends on the following factors:

- **Type of infection**
- **Severity of infection**
- **Status of the patients**
- **Associated conditions/diseases**

Aims of Treatment

Aims	Causation	Therapy	Drugs
To alleviate symptoms	Symptoms are caused by blood forms of the parasites	Blood schizonticidal drugs	Chloroquine, quinine, PS, artemisinin
To prevent relapses	Relapses are due to hypnozoites of <i>P. vivax</i> / <i>P. ovale</i>	Tissue schizonticidal drugs	Primaquine
To prevent spread	Spread is through the gametocytes	Gametocytocidal drugs	Primaquine for <i>P. faciparum</i> , chloroquine for all other Artemisinin

Common Antimalaria Drugs

- **Chloroquine**
- **Sulfadoxine-pyrimethamine (Fansidar®)**
- **Mefloquine (Lariam®)**
- **Atovaquone-proguanil (Malarone®)**
- **Quinine**
- **Doxycycline**
- **Artemisinin-based combination therapy (ACT)**

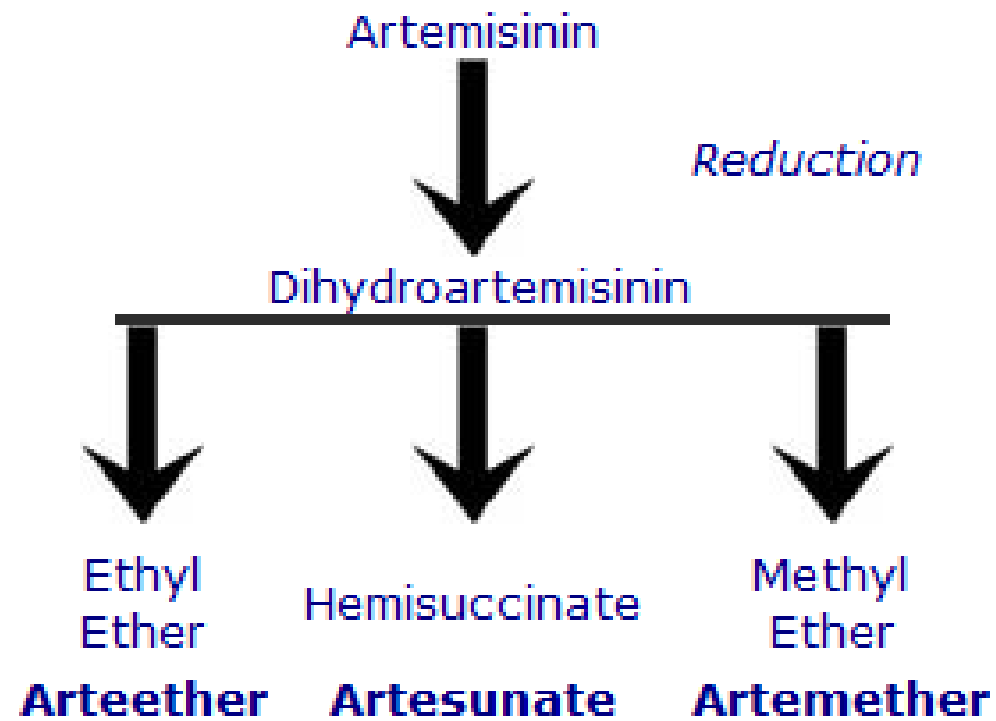
CINCHONA TREE



Artemisia annua - artemisinin



The Artemisinin Derivatives



Artemisinin treatment by dose but never monotherapy

Drug	Artemether	Arteether	Artesunate
Oral for mild cases	4 mg/kg (1st day), followed by 2 mg/kg for 6 days	Nil	4 mg/kg (1st day), followed by 2 mg/kg for 6 days
Parenteral for severe cases	3.2 mg/kg (1st day, IM), followed by 1.6 mg/kg for 6 days	3 mg/kg (IM) for 3 days	2.4 mg/kg (IV), q12 hr, for 24 hrs, followed by qd for 6 days

Artesunate in hepatic or renal failure

- **Artesunate dosages need not be changed because of hepatic or renal failure or concomitant or previous therapy with other medications, including previous therapy with mefloquine, quinine, or quinidine.**
- **There are no known interactions between artesunate and other drugs.**
- **WHO does not recommend artemisinin is applied for monotherapy.**

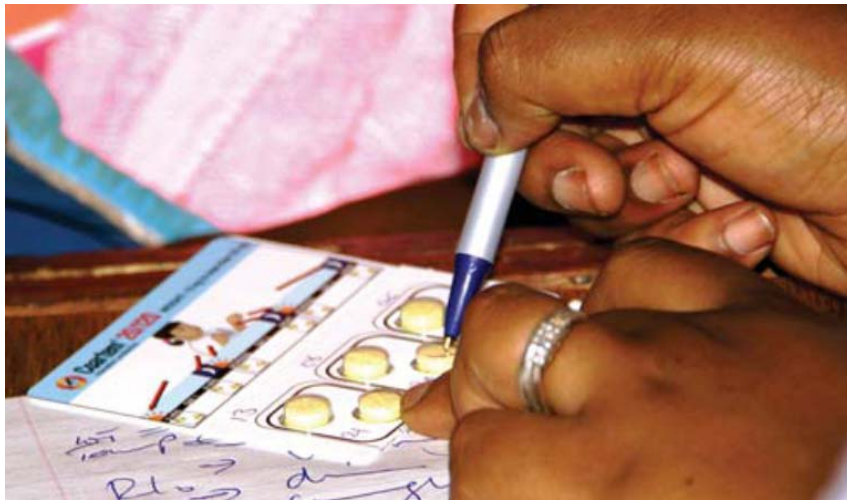
Artemisinin-based combination therapy (ACT)

- **1) Coartem®: artemether (20 mg) + lumefantrine (120 mg) per tablet**
- **2) Winthrop®: artesunate (100 mg) + amodiaquine (270 mg) per tablet**
- **3) Artequick®: artemisinin (80 mg) + piperaquine (400 mg) + primaquine (4 mg) per tablet**
- **4) artesunate plus sulfadoxine/pyrimethamine (in areas where SP efficacy remains high)**
- **5) artesunate plus mefloquine (in areas with low to moderate transmission)**
-

Choosing antimalarials: cost

- Chloroquine \$0.11
- SP (Fansidar) \$0.14
- Quinine \$1.27
- Mefloquine \$2.55
- **Artemether-lumefantrine \$9.12/2.40/1.80**
- Artemether-SP \$2.40
- A-P (Malarone) \$48.00

ACT treatment



Uncomplicated malaria

- For those body weight > 5 Kg –
Coartem® (artemether + lumefantrine)
- For those body weight < 5 Kg –
Winthrop® (artesunate + amodiaquine)

Pregnant women

- 1st trimester – quinine
- 2nd and 2nd trimesters –
Winthrop® (artesunate + amodiaquine)

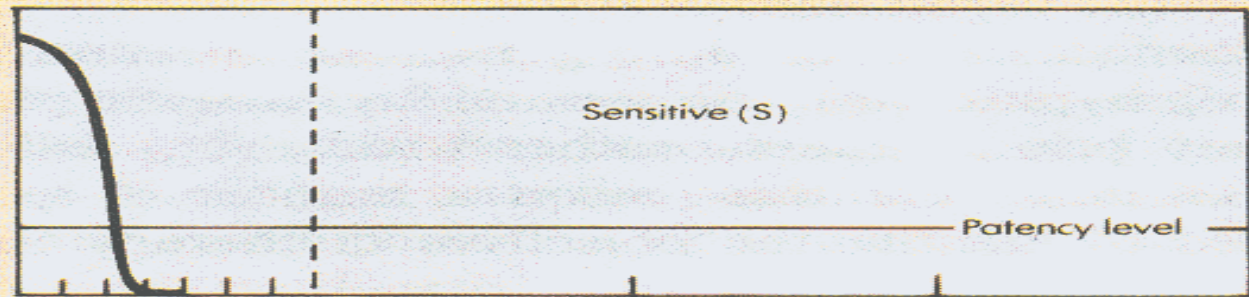
TREATMENT OF MILD MALARIA

- **Oral treatment unless patient is vomiting, in which case treat as in severe malaria**
- **ACT regimens**
- **Quinine 10 mg salt/kg (max 600 mg) 8 hourly, reduce to a 12 hourly regimen if develops severe cinchonism (tinnitus & deafness)**
- **When the patient is better and the parasitemia has cleared, give ONE of the following as a SECOND drug:**
- **Fansidar 3 tablets stat (1 tablet per 20 kg): avoid if a history of sensitivity to sulphonamides and avoid in pregnancy;**
- **Doxycycline 100 mg/day x 14 days (not in pregnancy); or tetracycline 250 mg/day x 7 days (not in pregnancy)**

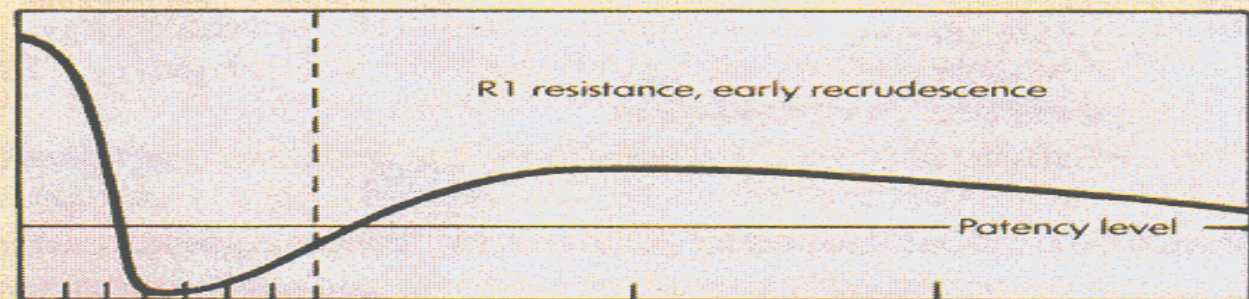
TREATMENT OF SEVERE MALARIA

- Quinine dihydrochloride iv 10 mg/kg (max 600 mg) in 250 ml of normal saline over 4 hr, maintenance dose every 8-12 hr x 10 days
- A **loading dose** of iv quinine **20 mg/kg (max 1400 mg) over 4 hr** should be given if the patient fulfils WHO criteria for severe disease
- Patients who have received a treatment dose of mefloquine within 3 days should not have a loading dose of quinine, and quinine should be used with caution because of risk of arrhythmia
- **12-hr interval iv** quinine usually sufficient, but may be given 8-hr
- Check quinine level immediately before 4th dose
- Quinine dihydrochloride **im** into the anterior thigh: dilute in normal saline to 60 mg/ml, give half the dose into each thigh
- **Convert to oral quinine** 8 hourly when patient is better and can reliably take orally
- Stop quinine and give second drug once parasitemia has cleared
- **Artesunate IV**

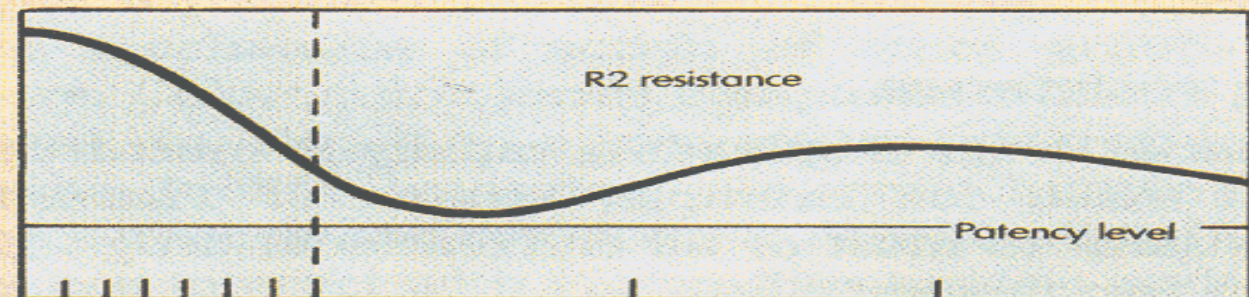
Asexual *P. falciparum* parasitaemia



S: Sensitive. No parasites in the blood at seven days; no recrudescence.



R1: Resistant, stage 1. After initial clearance parasites reappear in the blood after seven or more days.



R2: Resistant, stage 2. The number of parasites in the blood is reduced initially, and rises again after seven or more days.



R3: Resistant, stage 3. The drug has little effect on the number of parasites in the blood.

0 1 2 3 4 5 6 7 14 21 28
Days after start of treatment on Day 0

From WHO Technical Report Series 529 (1973)

PREVENTION OF MALARIA

Lack of a suitable malaria vaccine

Integrated malaria control programme

Insect repellents for personal protection

Chemoprophylaxis

**Nonimmune travelers to an area
where malaria is endemic**

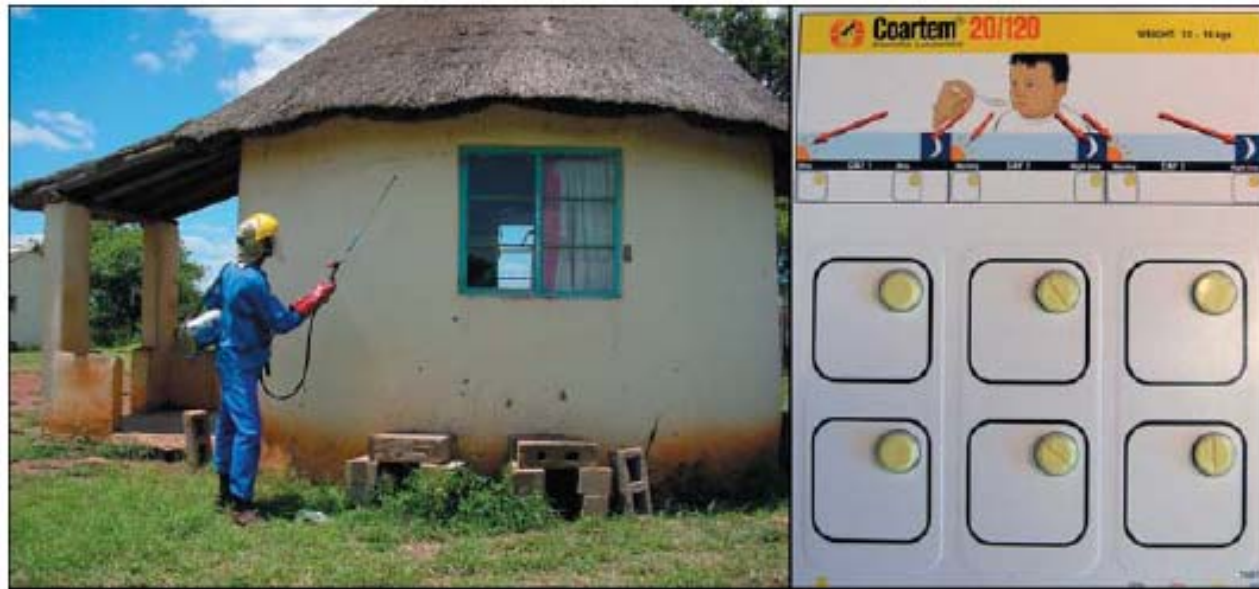
Pregnant women

Children with sickle cell anemia

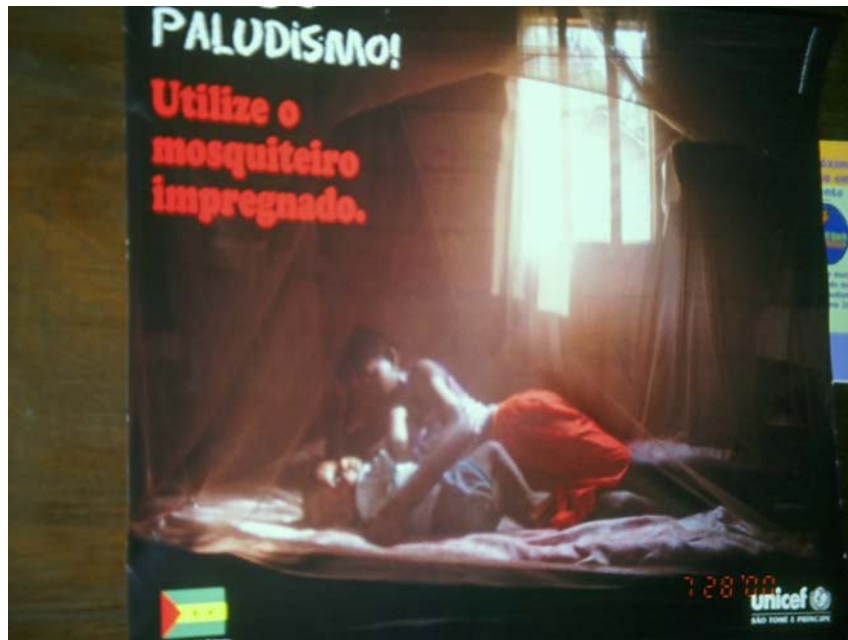
Integrated malaria control

- **Long-lasting insecticide-treated nets (LLINs)**
- **Indoor residual spraying (IRS)**
- **Larviciding**
- **Intermittent preventive treatment (IPT)**
- **Artemisinin-based Combination Therapy (ACT)**
- **Health education**

Rolling Back Malaria 擊退瘧疾



Long-lasting insecticide-treated nets (LLINs)



Intermittent Preventive Treatment (IPT) Fansidar (Pyrimethamine/sulfadoxine)



MALARIA



Marcos Boulos - USP

Mosquito attractants



- **Dark Clothing**
- **Carbon Dioxide (you exhale CO₂)**
- **Lactic Acid (in perspiration fluid)**
- **Floral or Fruity Fragrances (perfumes)**
- **Skin Temperature**
- **Moisture (perspiration)**

Biting times – important for long-term travel

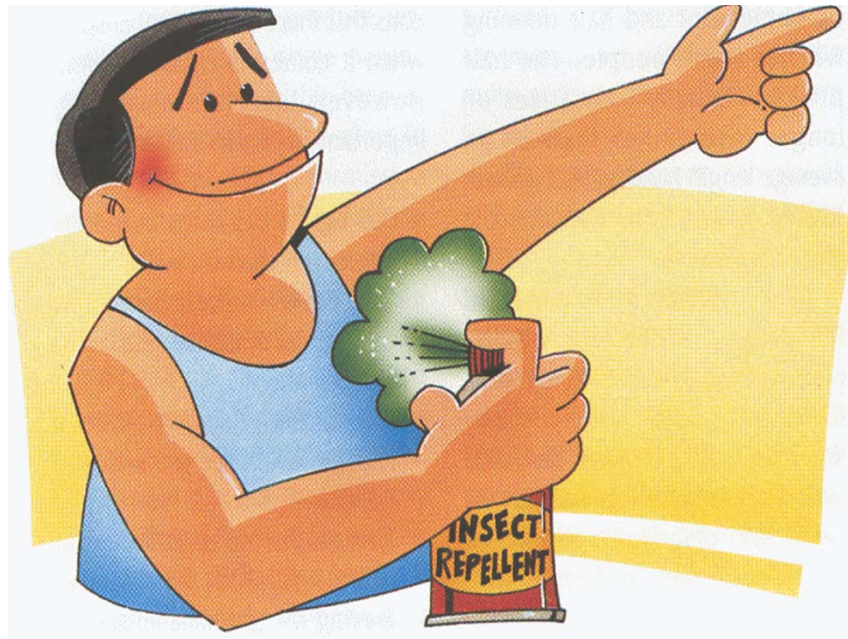
An. gambiae (Africa) –

•late night indoor feeder – peaks at 22:00 and 02:00 – nets (LLINs) ideal

An. darlingi (Amazon) –

•early evening biting – peaks at 20:00 and 22:00 – repellents essential

Choosing the right insect repellent



Choosing The Right Insect Repellent

Good Housekeeping

Helpful Guide Explains:

- New Controlled Release™ technology
- Where each type of repellent works best
- New EPA regulations banning kid's formulas

天然防蚊液 - 香草萃取複方 - 無效



量色 80
蚊子忌避劑，防止蚊蟲叮
咬、預防登革熱、瘧疾、
日本腦炎。
份 薰衣草、天然尤加利、檸
檬香茅、薄荷萃取物。
象 室外活動需要避免蚊蟲叮
咬之大人、小孩。
點 適用於居家、露營、登山
、垂釣、野營、郊遊、庭
園除草等戶外活動及蚊子
出沒場所。
式 直接噴灑於外露衣物之皮
膚表面。

香茅防蚊環 -



Mosquito repellent (DEET)



HOW TO AVOID MOSQUITO BITES

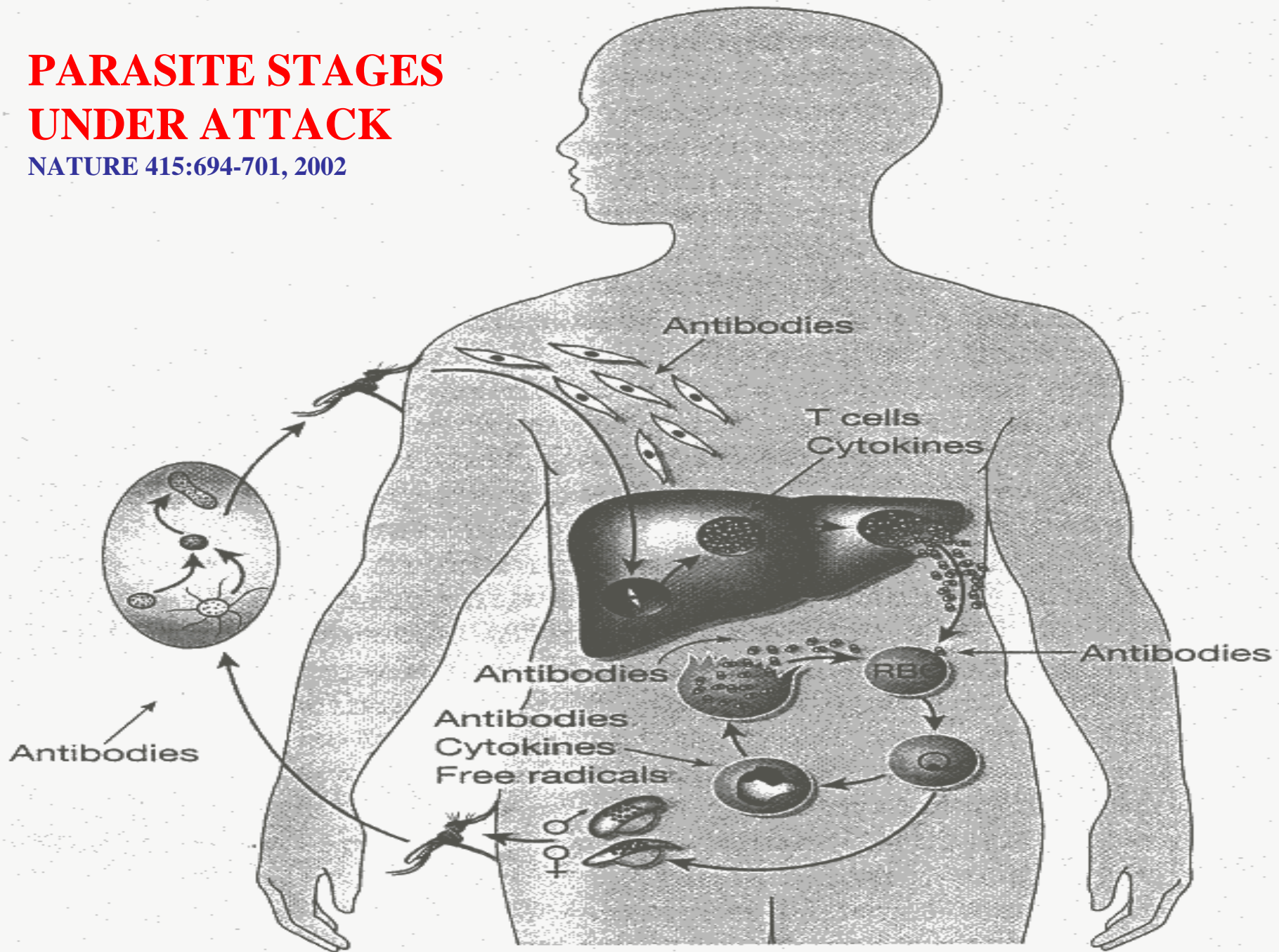
- **Apply insect repellent (containing 20-35% N,N-diethyl-m-toluamide) to exposed skin.**
- **Wear long-sleeved clothing and long pants if you are outdoors at night.**
- **Use a mosquito net (treating with permethrin) over the bed if your bedroom is not air-conditioned or screened.**
- **Spray an insecticide or repellent on clothing, as mosquitoes may bite through thin clothing.**
- **Spray permethrin or a similar insecticide in your bedroom before going to bed.**

MALARIA CHEMOPROPHYLACTIC DRUG DOSAGE

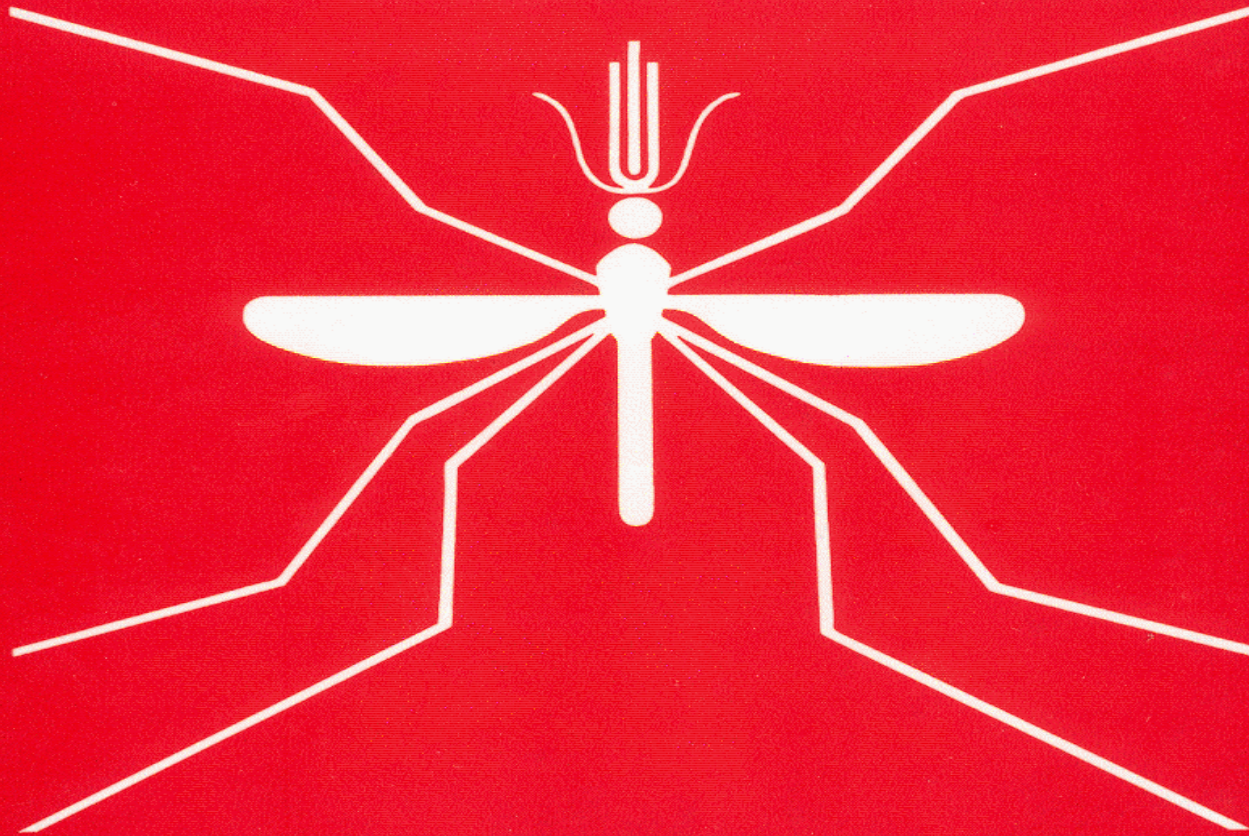
Drug (trade name)	Packaging	Adult dose	Child dose
Atovaquone- proguanil (Malarone)	One tablet containing Atovaquone 250 mg + proguanil 100 mg	1 tablet/day	11-20 kg: ¼ tablet 21-30 kg: ½ tablet 31-40 kg: ¾ tablet > 40 kg: adult dose
Chloroquine phosphate (Aralen)	150 mg base	300 mg base/week	5 mg base/kg/week
Doxycycline (Vibramycin)	100 mg	100 mg/day	2mg/kg/day; not for children < 8 yrs old
Mefloquine (Lariam)	250 mg base	250 mg base/week	5 mg base/kg/week
Primaquine	15 mg base	15 mg base/day for 14 days	0.3 mg base/day for 14 days
Proguanil (Paludrine)	100 mg	200 mg/day	4 mg/kg/day

PARASITE STAGES UNDER ATTACK

NATURE 415:694-701, 2002



MALARIA



**WAITING FOR
THE VACCINE**

Can malaria be eliminated?

Comparison	2000s	1950s
Aim	Elimination	Eradication
Strategies	Integrated	Limited
IRS	Pyrethroid	DDT
Bednets	LLINs	None
Pregnancy /children	IPT	None
Diagnosis	Blood films/RDT/PCR	Blood films
Treatment	ACT	Chloroquine
Chemoprophylactic	Mefloquine/Malarone	Chloroquine
Vaccine	Partial protection?	None