MALARIA : LABORATORY DIAGNOSIS

Blackwater fever

- Malarial haemoglobinuria is some time associated with falciparum malaria, particularly in patients who have experienced repeated infections & inadequate quinine therapy
- Auto antibodies against RBCs

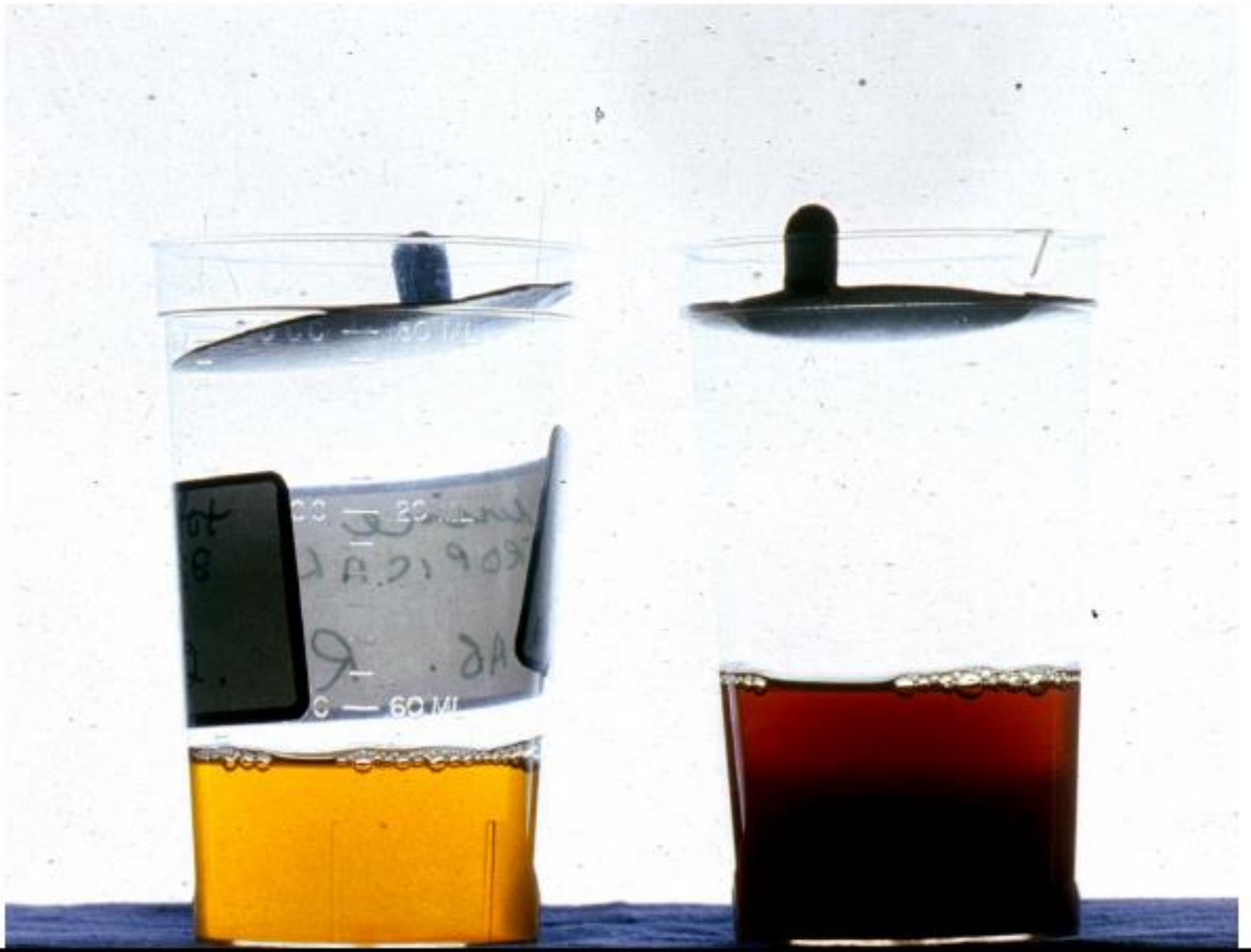
  ↓

  I/V haemolysis – hemoglobinemia & hemoglobinuria

- Parasites are not detected in blood during & just after the attack but may reappear after an week of acute attack

- Fever with rigor, aching pain in loins, bilious vomiting, icterus, haemoglobinuria, circulatory collapse, ARF.
- Urine – red to dark red (port-wine / cola)

  - acidic



Laboratory diagnosis of malaria

METHODS

- MICROSCOPIC
  - Light microscopy (PBS examination)
  - Fluorescent microscopy
  - Quantitative Buffy coat (QBC)
- NON MICROSCOPIC
  - Antigen detection (Rapid immunodiagnostic strip test)
  - Antibody detection
- CULTURE

Peripheral smear examination

1. Preparation

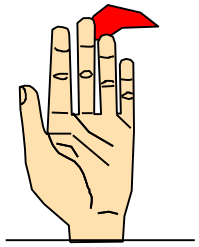
2. Staining

3. Observation

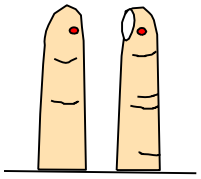
COLLECTION OF BLOOD

- ✓ Capillary blood - finger prick / heal prick / ear lobule
- ✓ Venous blood - EDTA

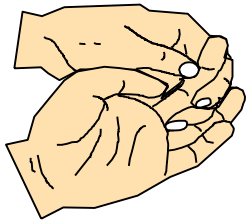
Collection of Blood Smears



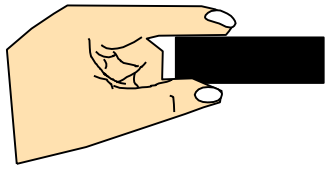
1.
The second or third finger is usually selected and cleaned.



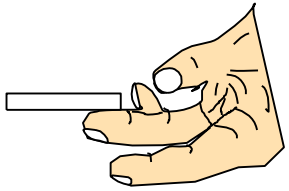
2.
Puncture at the side of the ball of the finger.



3.
Gently squeeze toward the puncture site.

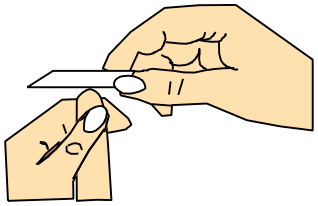


4.
Slide must always be grasped by its edges.

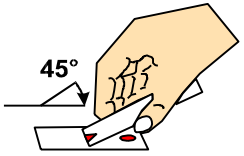


5.
Touch the drop of blood to the slide from below.

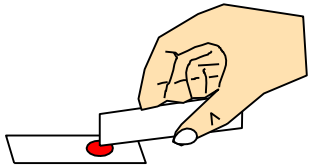
Preparing thick and thin films



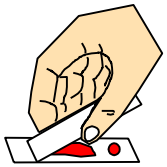
1.
Touch one drop of blood to a clean slide.



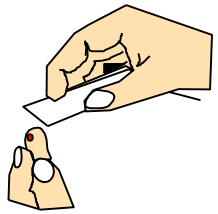
4.
Carry the drop of blood to the first slide and hold at 45 degree angle.



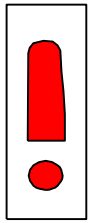
2.
Spread the first drop to make a 1 cm circle.



5.
Pull the drop of blood across the first slide in one motion.

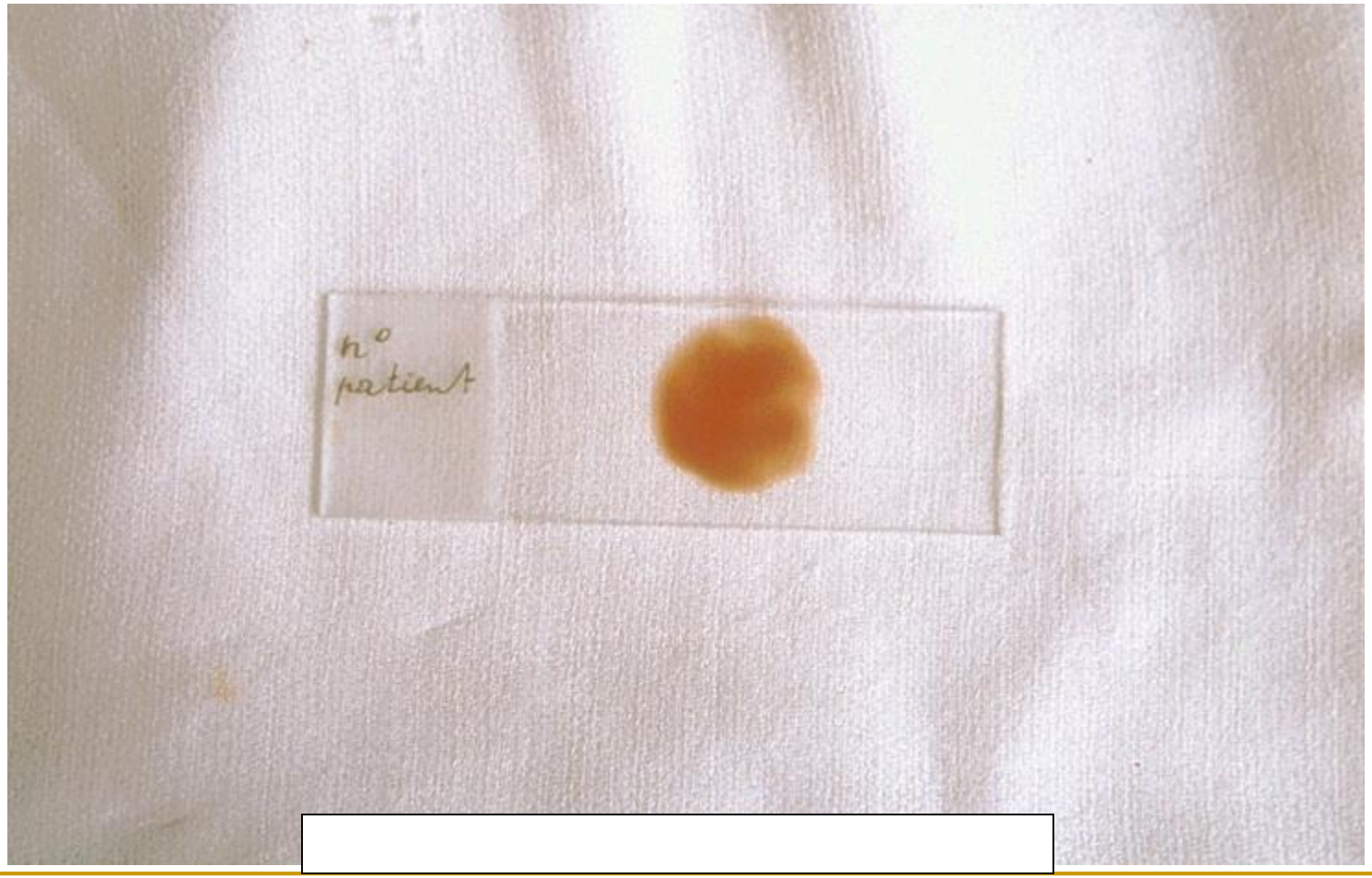


3.
Touch a fresh drop of blood to the edge of another slide.

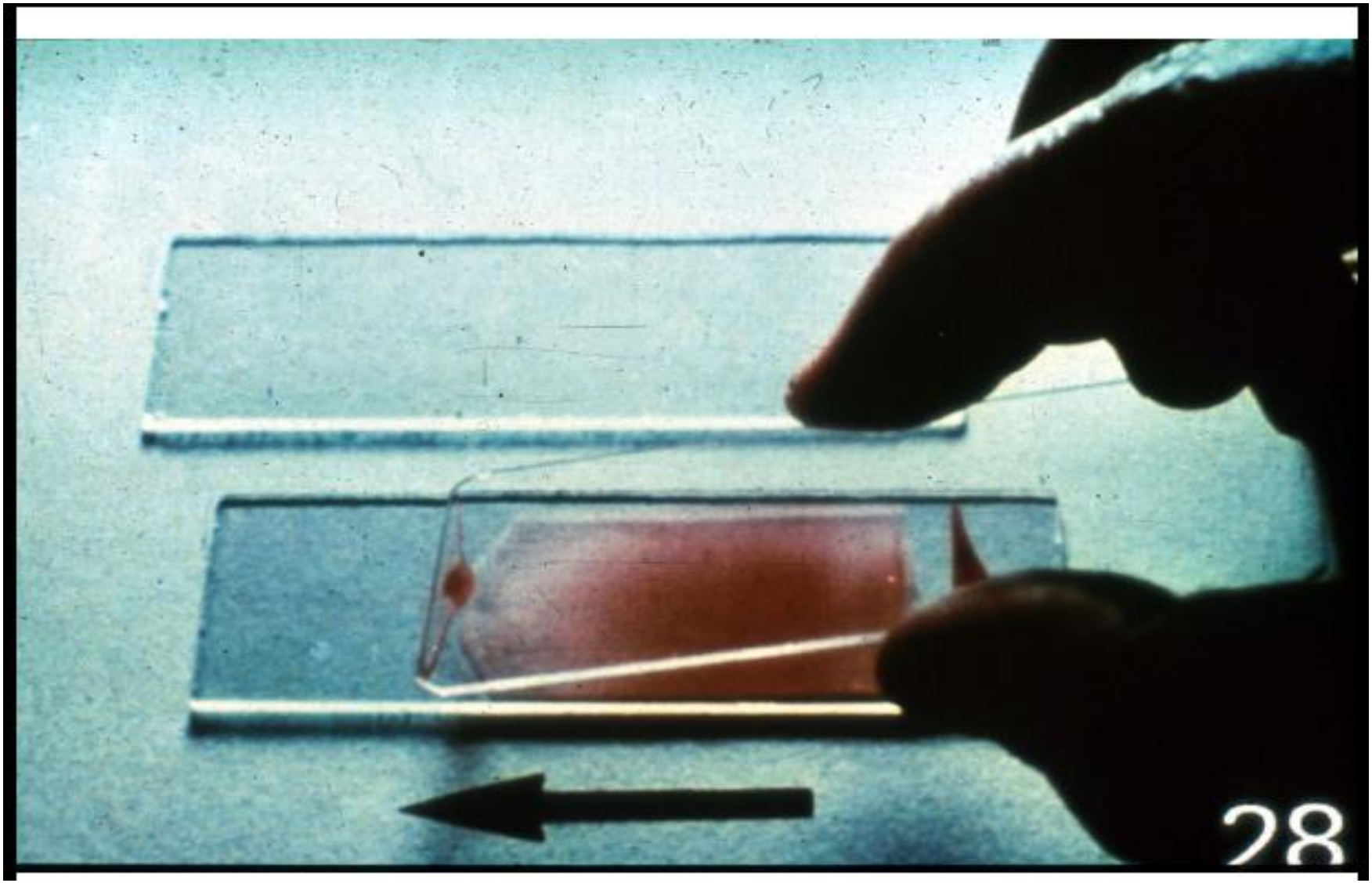


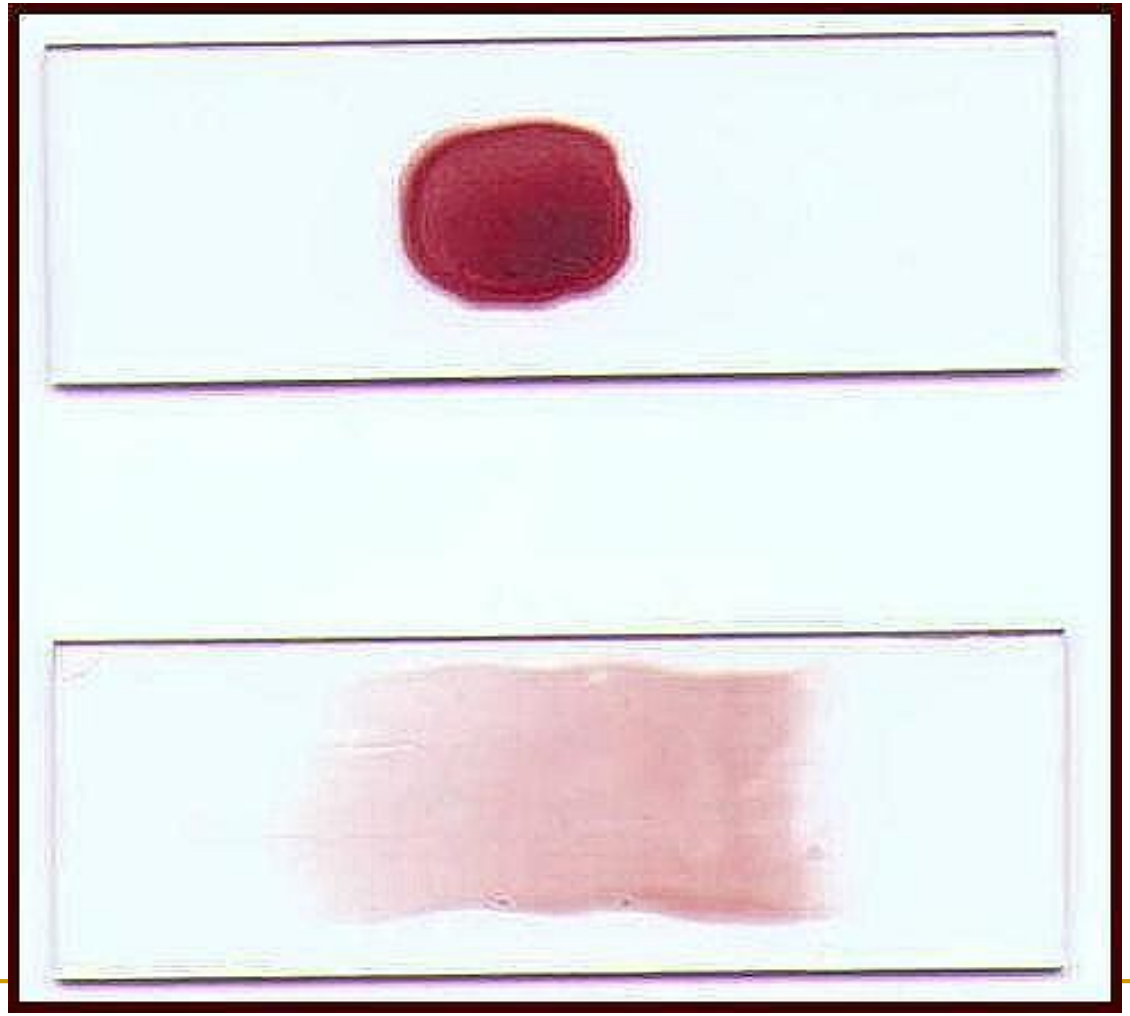
6.
Wait for both to dry before fixing and staining.

Thick Smear



Thin smear





The Romanowsky stains

- Leishman's stain
- Wright's stain
- Giemsa stain
- Field stain
- JSB (Jaswant Sing & Bhattacharji) stain

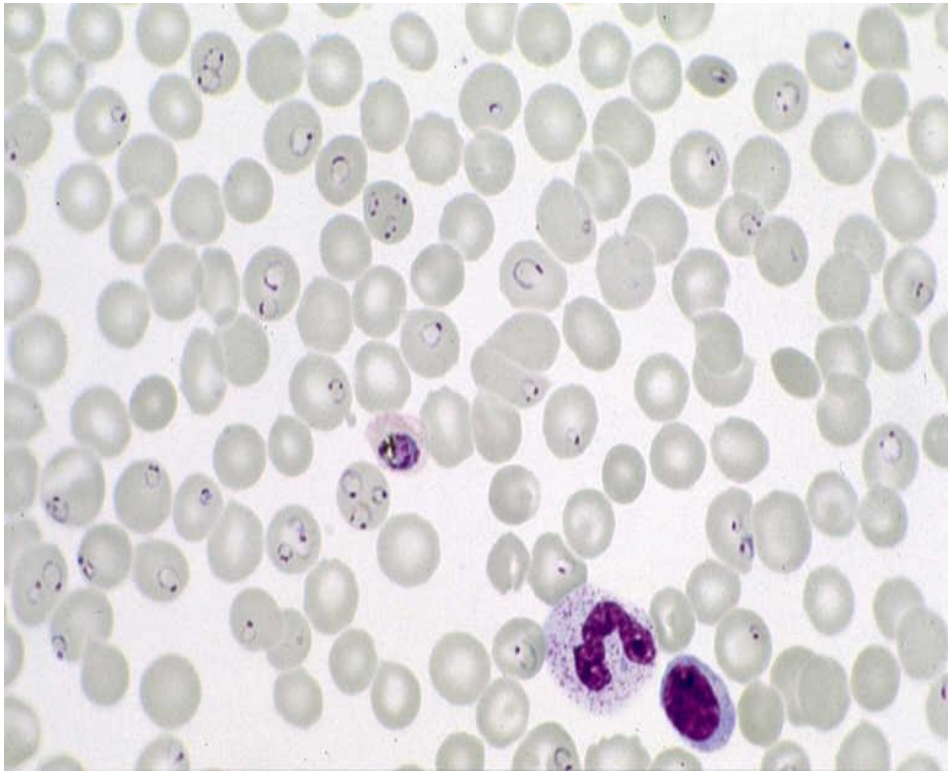
Appearance of PBS – Romanowsky stain

- Cytoplasm of parasite & WBC – Blue
- Nuclei of parasite – Red
- Nuclei of WBC – Purple
- RBC - Pink

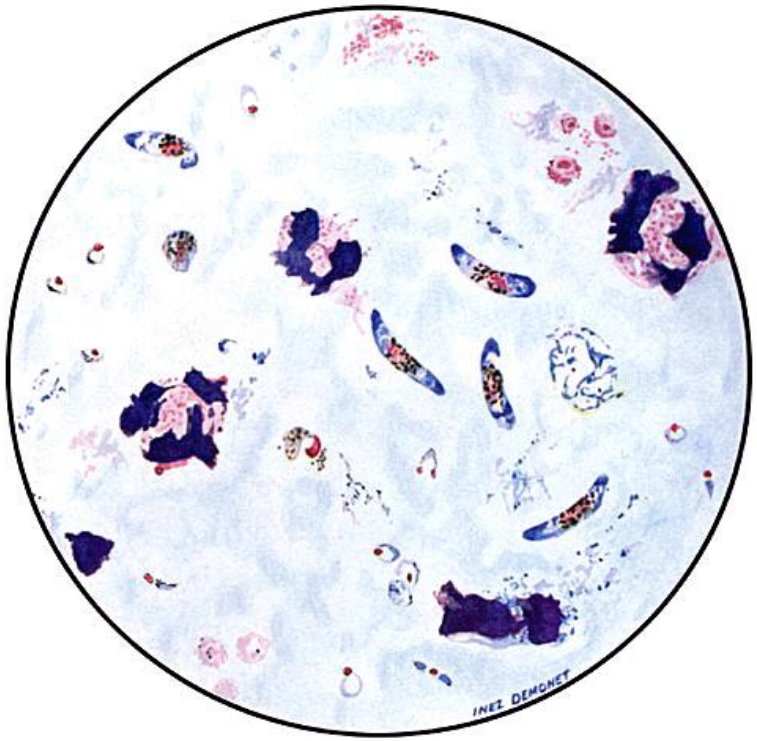
OBSERVATION

- RBC – size, shape
  - number of parasite / RBC
- Identification of species

Thin smear



Thick smear



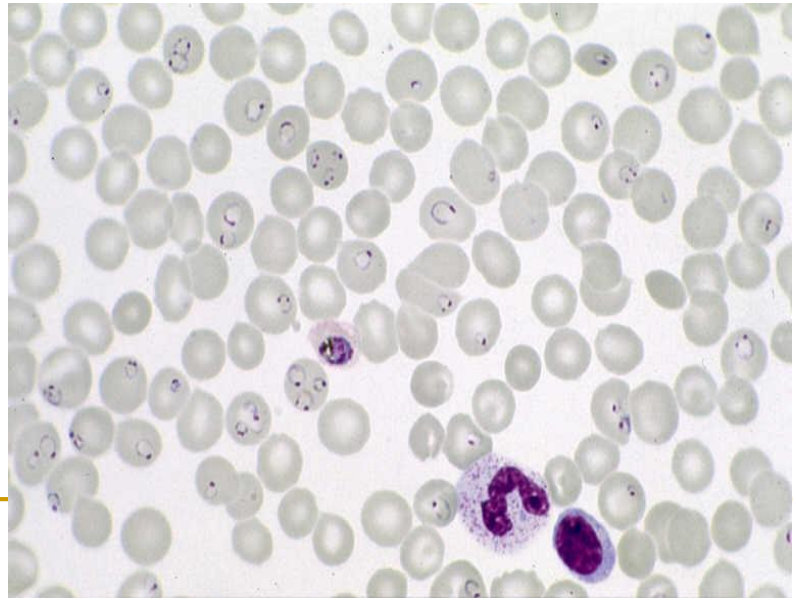
Thin smear

Advantages

- Intra RBC morphology of parasite can be seen
- Species identification
- RBC morphology
- Mixed infection
- % of parasitized RBC
  - Severity
  - Know response to the treatment

Disadvantages

- Fixation of smear
- Low parasitaemia
- Less sensitive



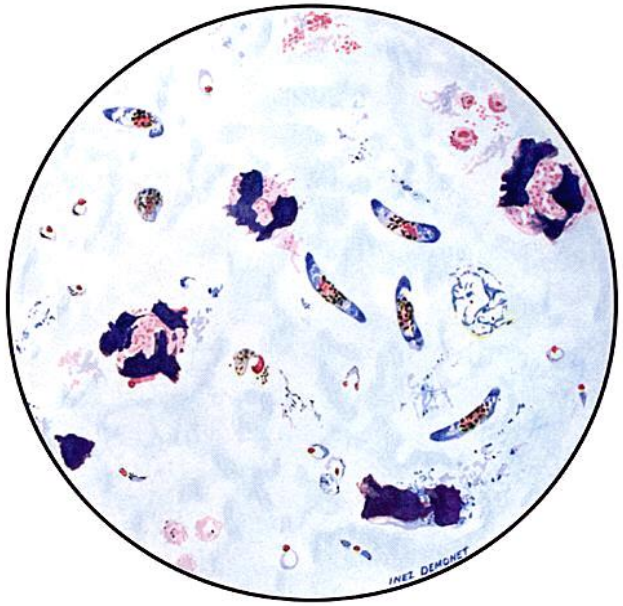
Thick smear

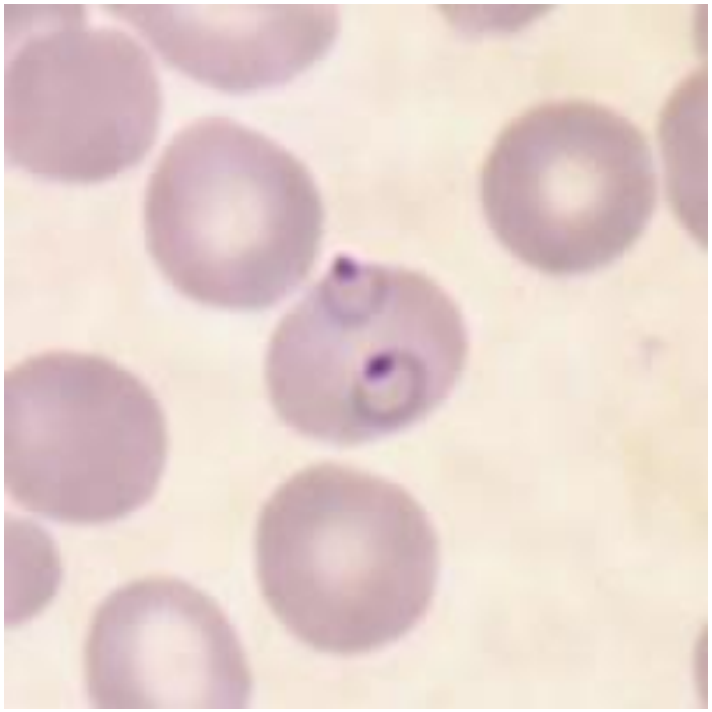
Advantages

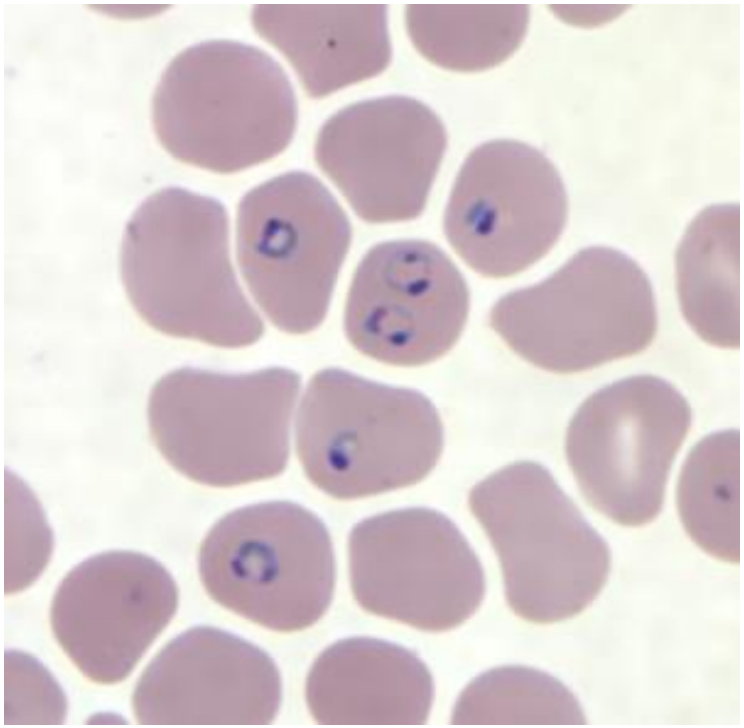
- More sensitive
- Rapid detection of parasites
- No fixation of smear
- Low parasitaemia can be detected

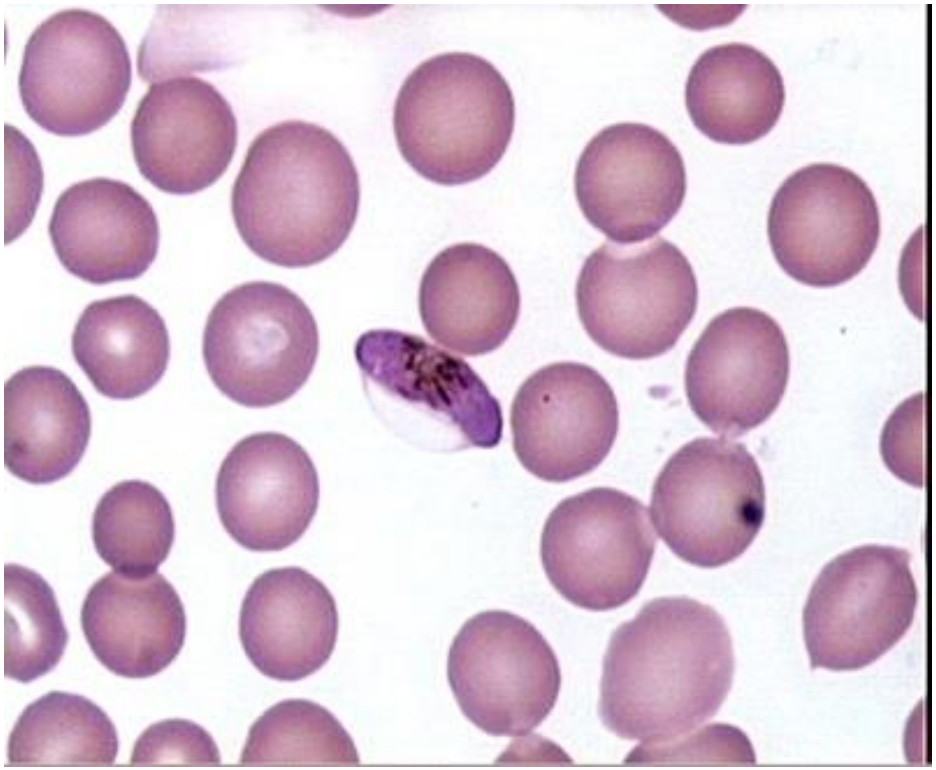
Disadvantages

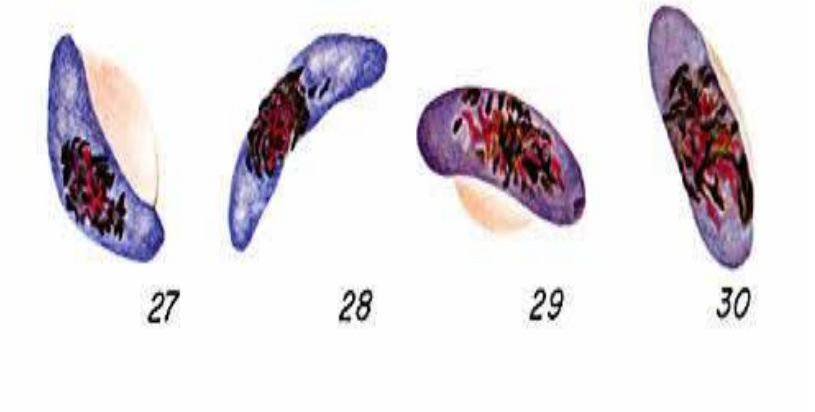
- Intra RBC morphology of parasite can not be seen
- Cannot confirms Plasmodium spp.

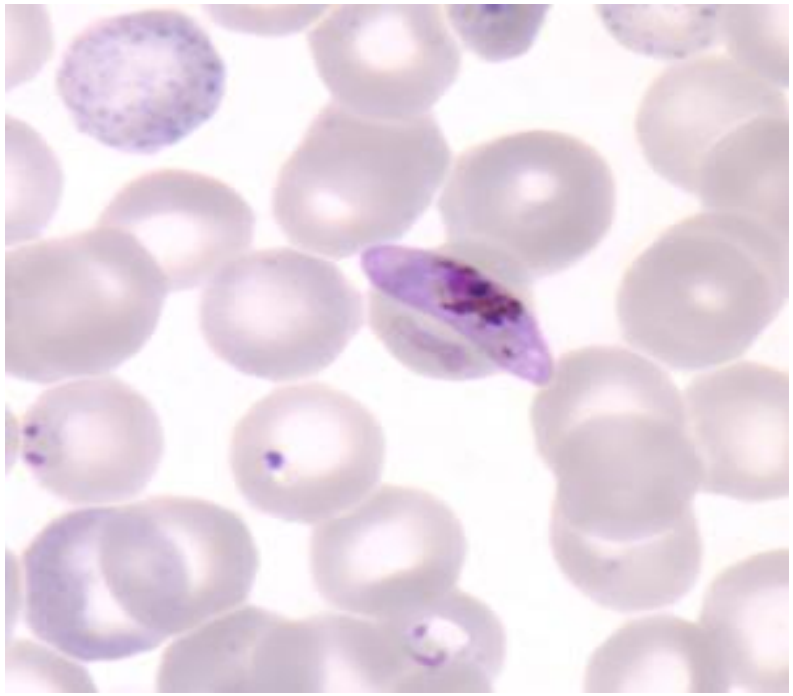






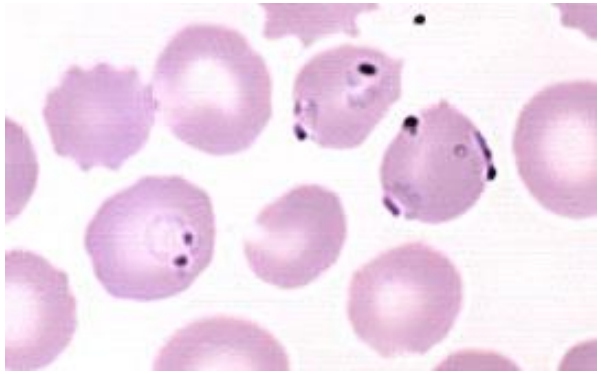


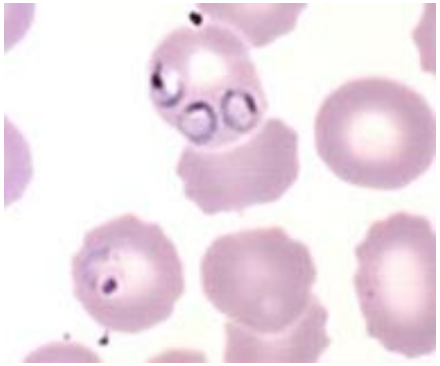

27
28
29
30



Plasmodium falciparum

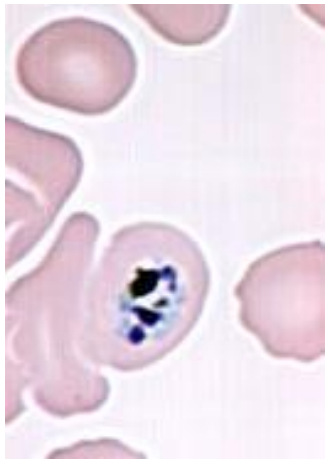
Infected erythrocytes: normal size





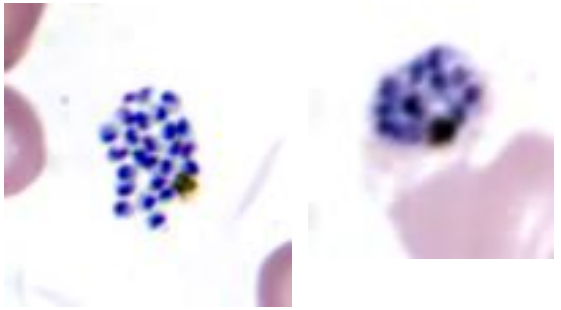
Rings: double chromatin dots; appliqué forms; multiple infections in same red cell

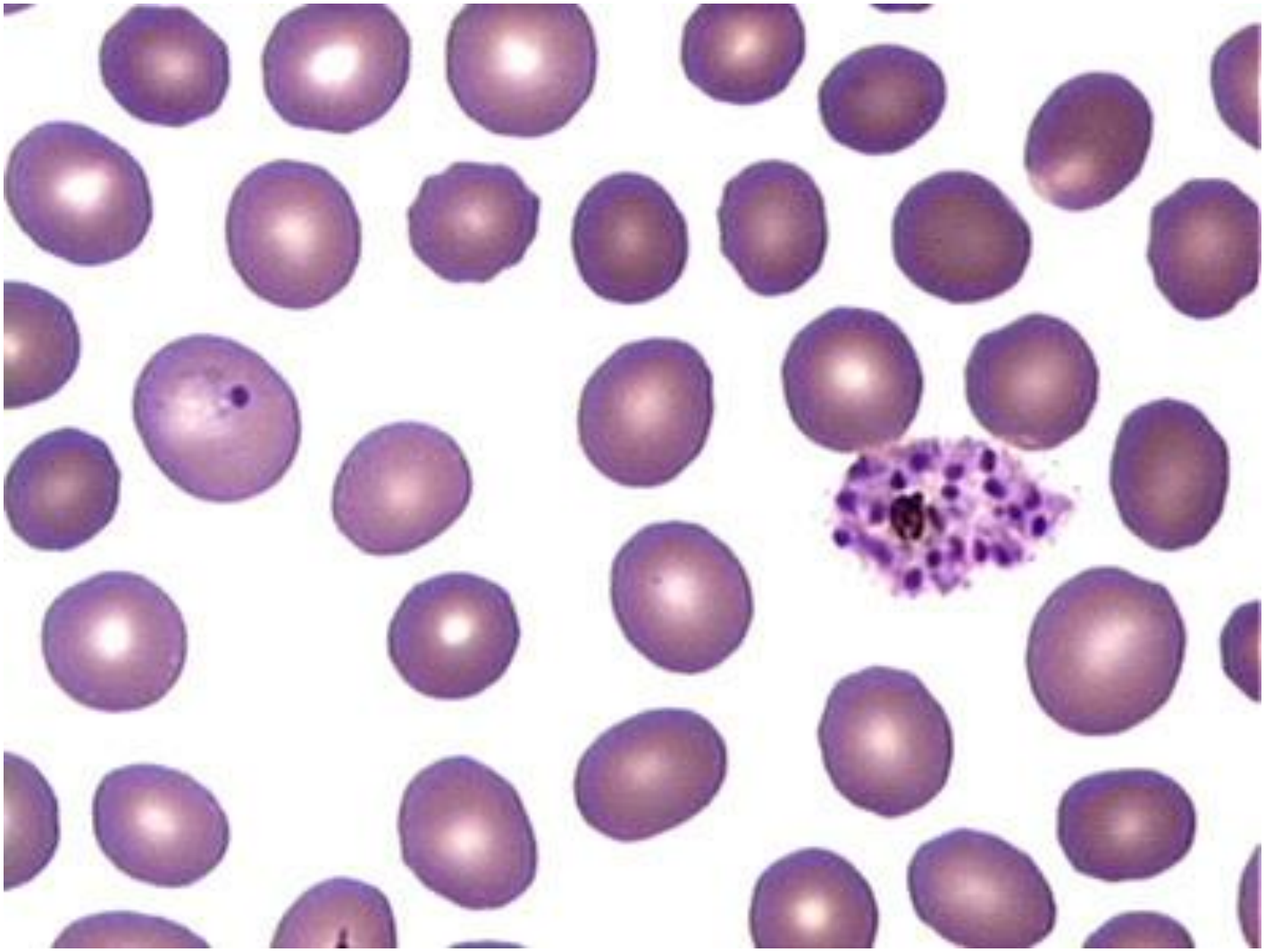
Gametocytes: mature (M)and immature (I) forms (I is rarely seen in peripheral blood)

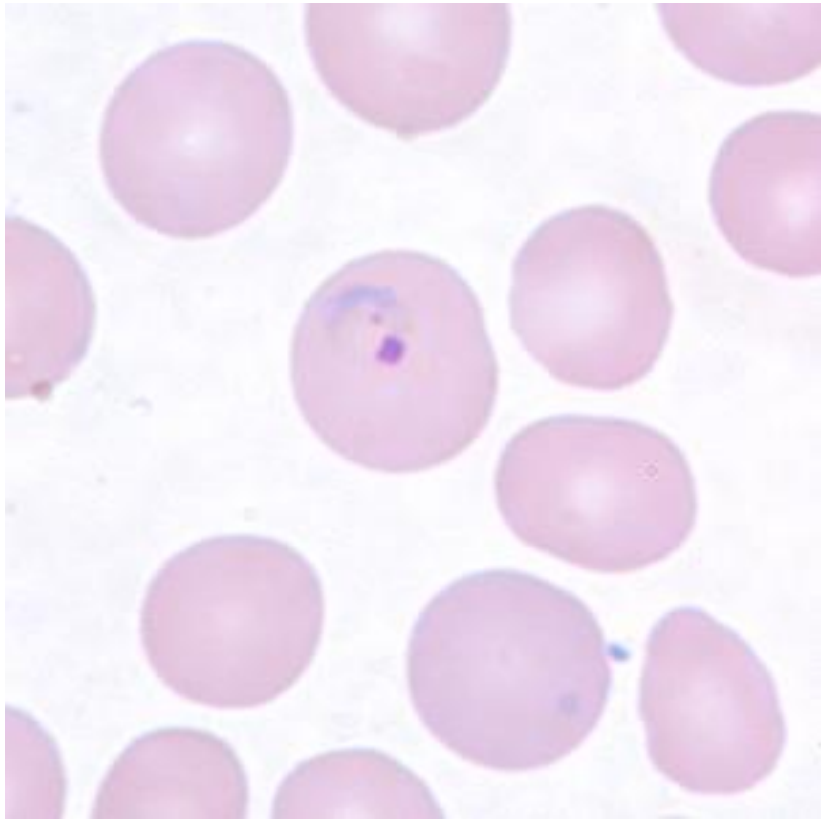


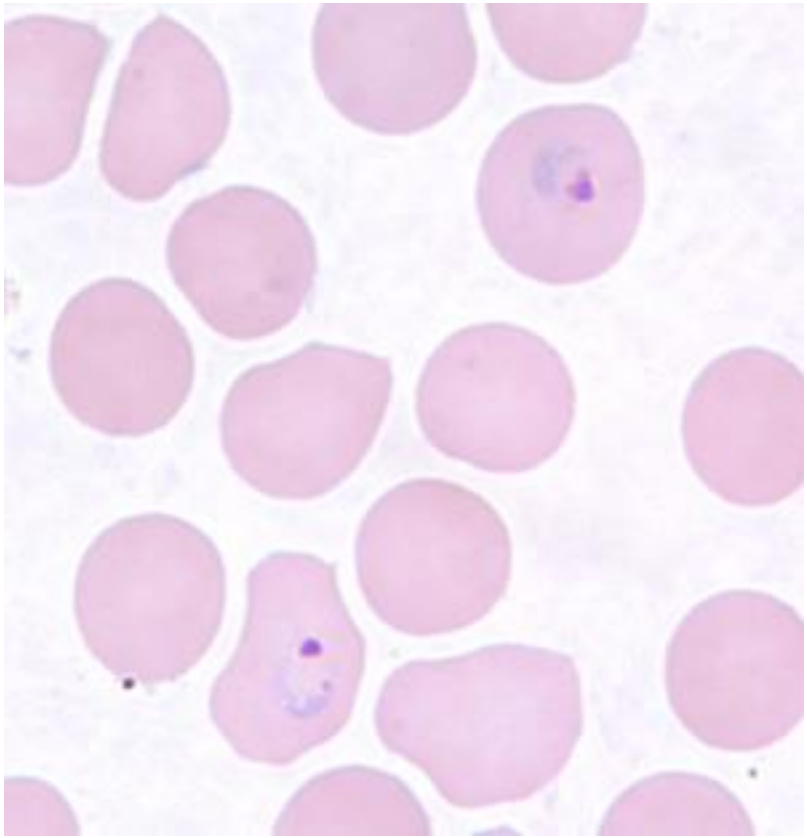
Trophozoites: compact (rarely seen in peripheral blood)

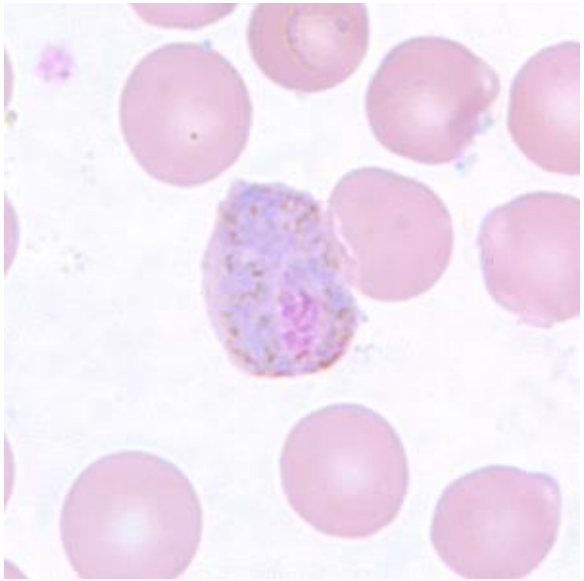
Schizonts: 8-24 merozoites (rarely seen in peripheral blood)

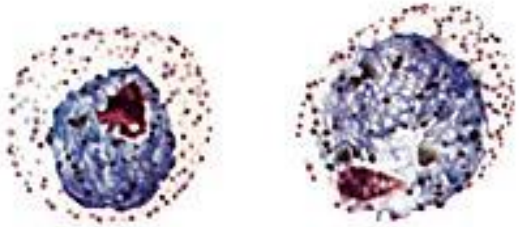






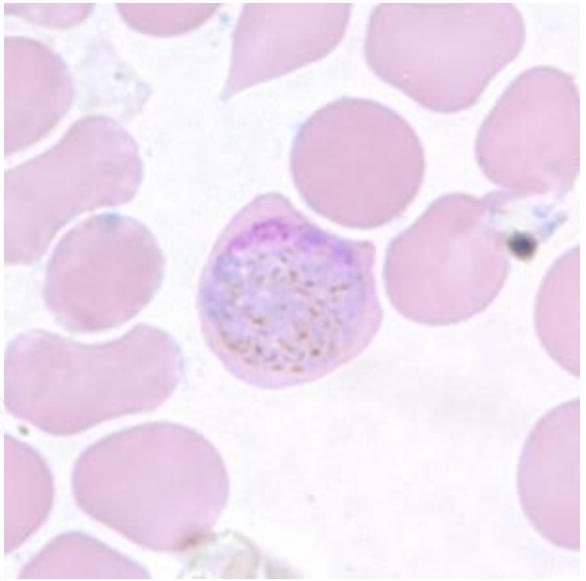






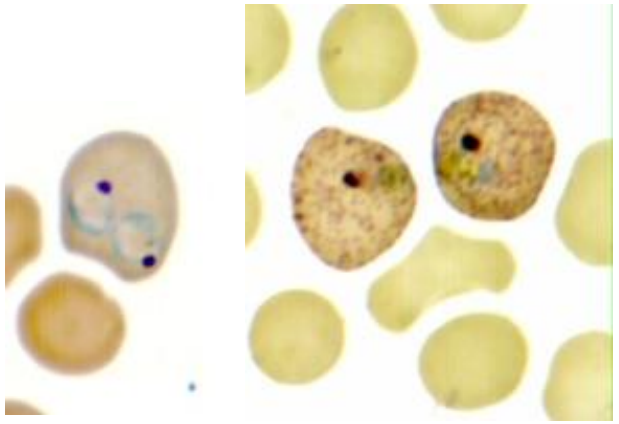
28

29

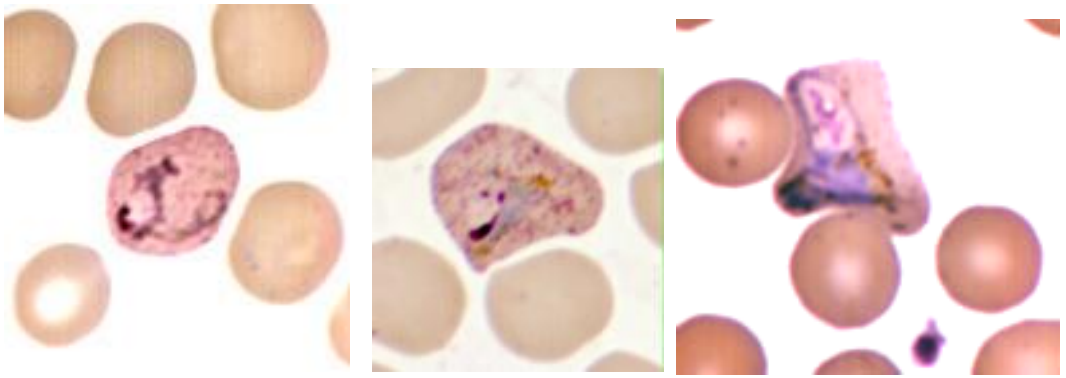


Plasmodium vivax

Infected erythrocytes: enlarged up to 2X; deformed; (Schüffner's dots)

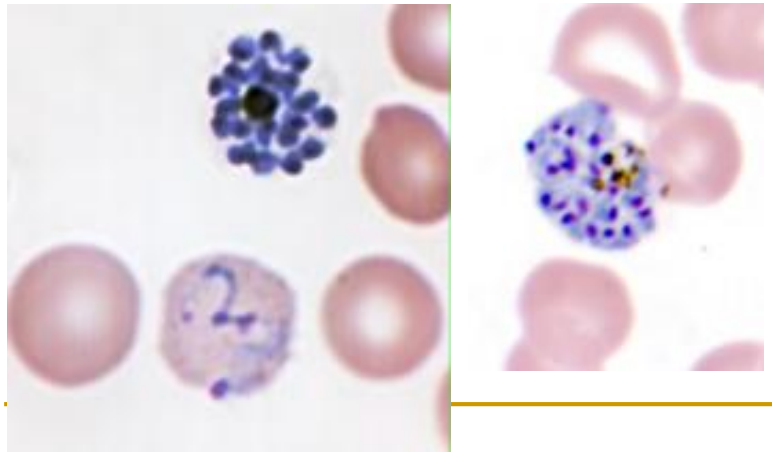


Rings

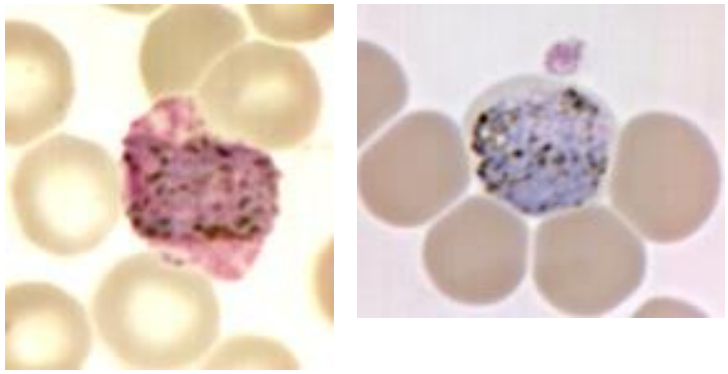


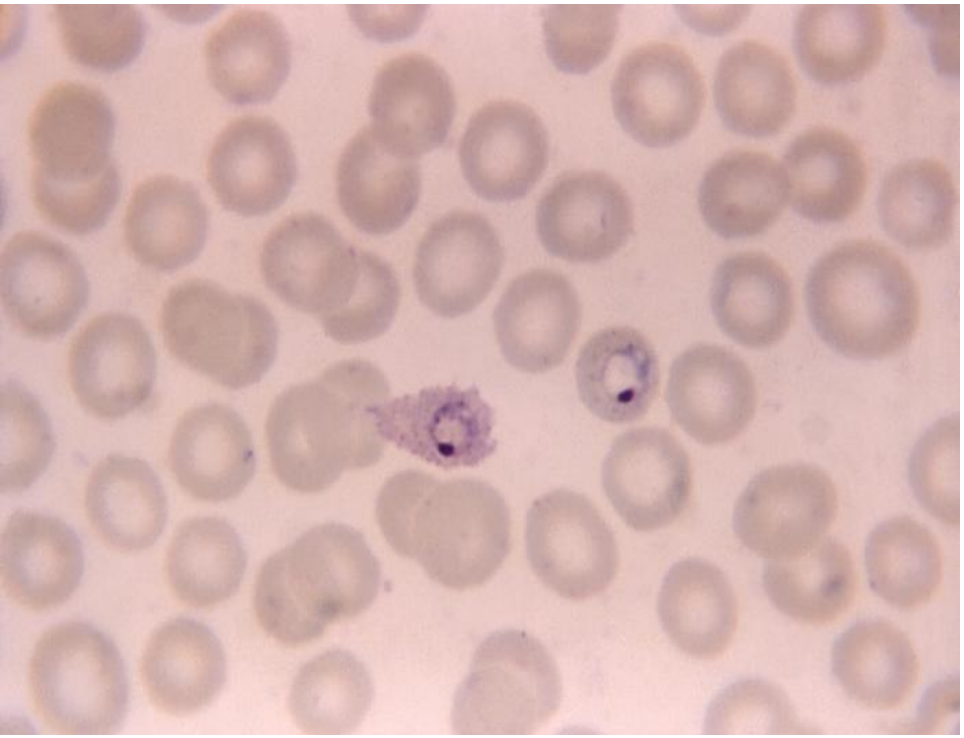
Trophozoites: ameboid; deforms the erythrocyte

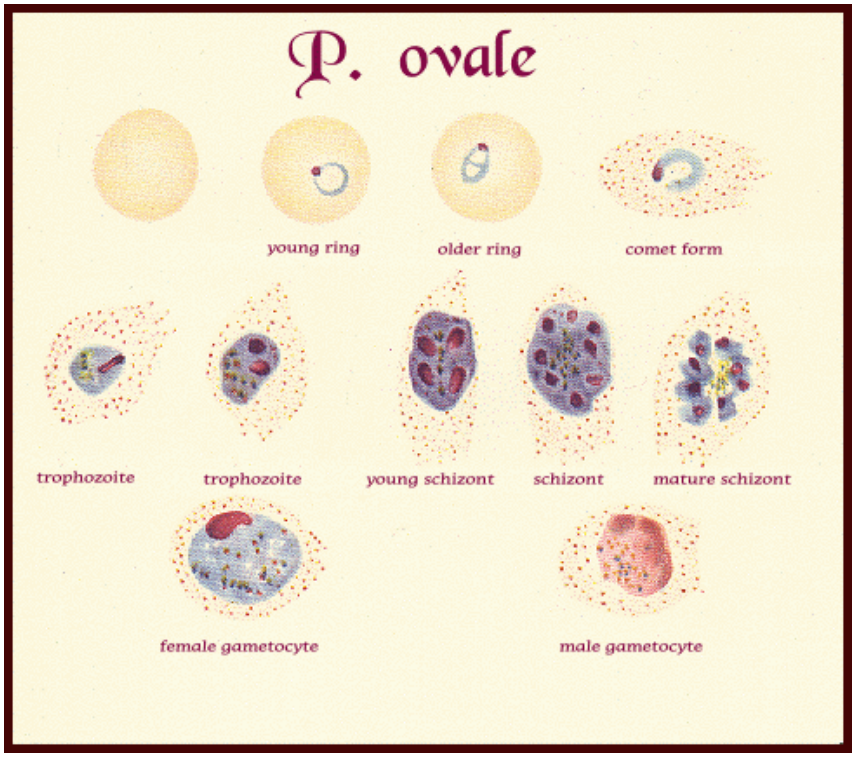
Schizonts: 12-24 merozoites

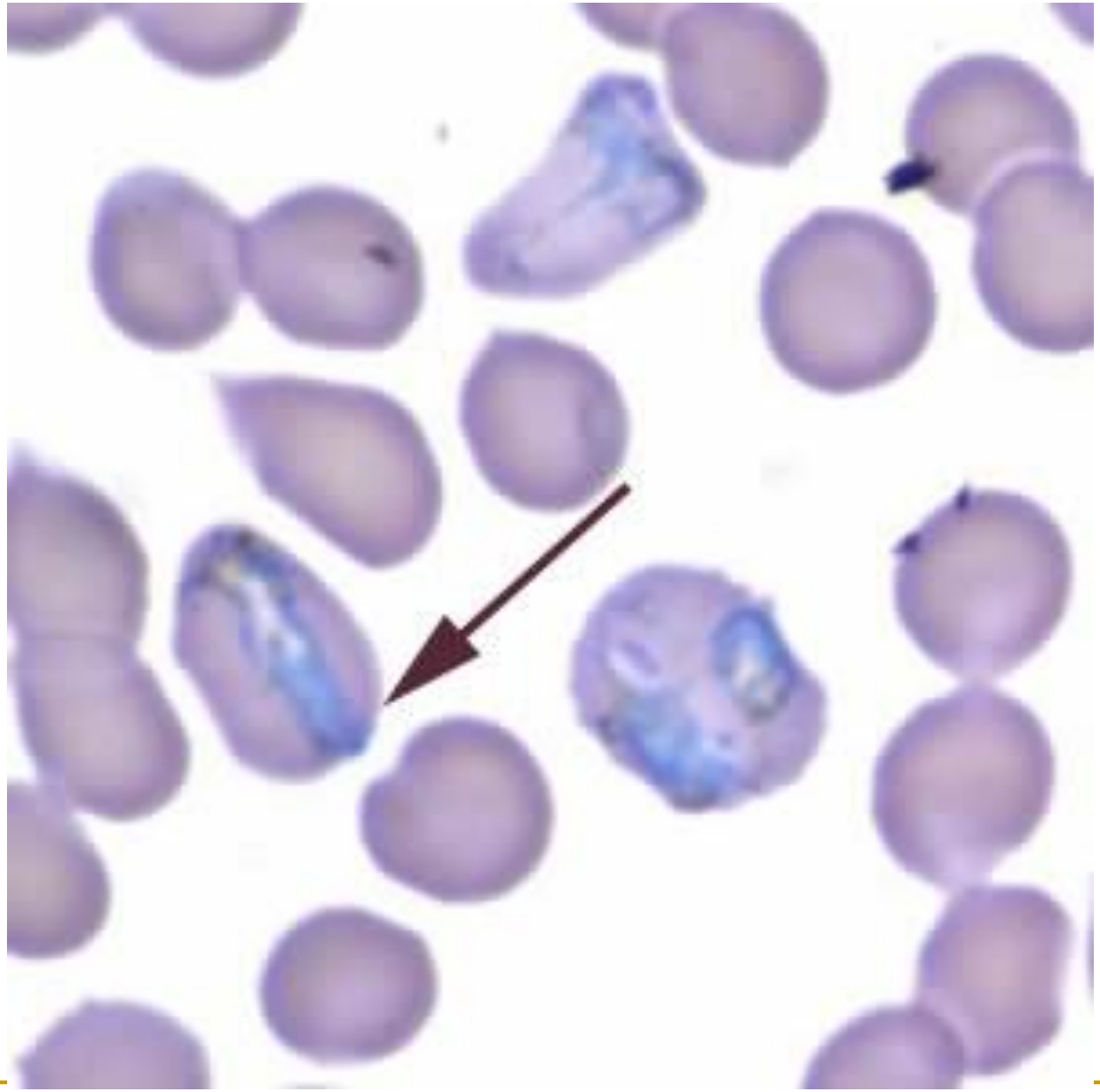


Gametocytes: round-oval

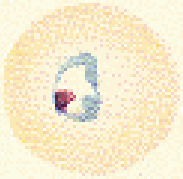





P. ovale
young ring
older ring
comet form
trophozoite
trophozoite
young schizont
schizont
mature schizont
female gametocyte
male gametocyte

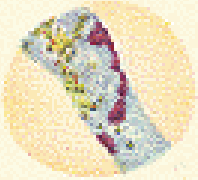


P. malariae



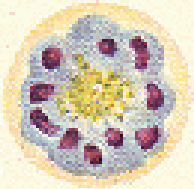
ring form

early band form

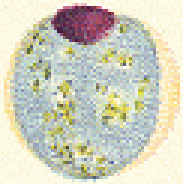


band form

early schizont



mature schizont



female gametocyte

male gametocyte

Grading of smear – Thick smear

1-10	100 OIF	+
1-10	10 OIF	++
1-10	OIF	+++
>10	OIL	++++

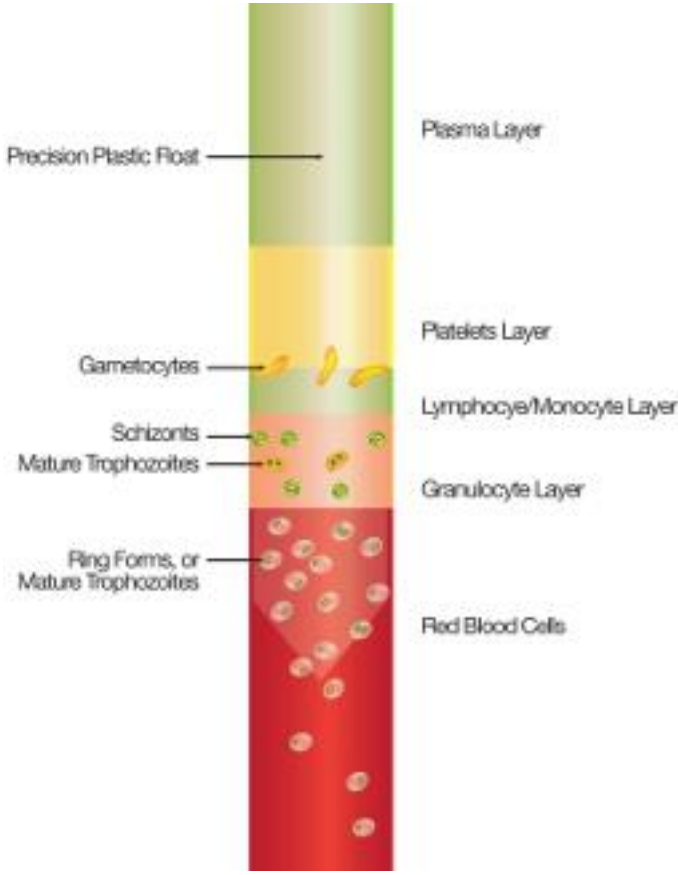
Fluorescent Microscopy

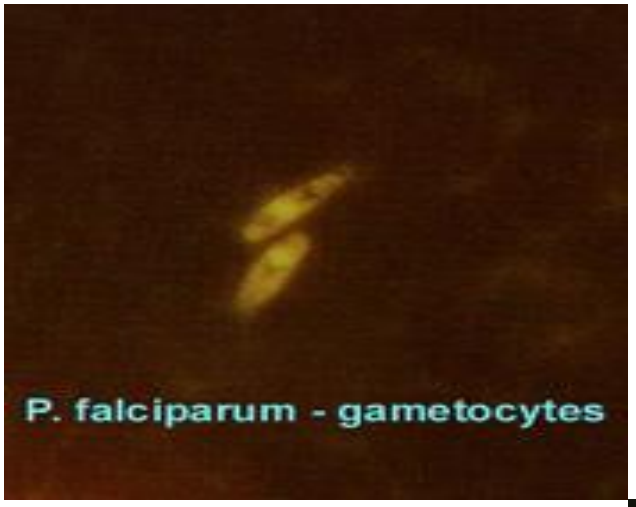
- Benzathiocaroxypurine – A florescent dye Modification of light microscopy
- Detect RNA and DNA that is contained in parasites after penetrating RBC
- Does not stain nuclei of WBC
- Nucleic material not normally in mature RBCs

- Kawamoto technique
  - Stain thin film with acridine orange (AO)
  - Requires special equipment – fluorescent microscope
  - Nuclear DNA – Green , Cytoplasmic RNA –red

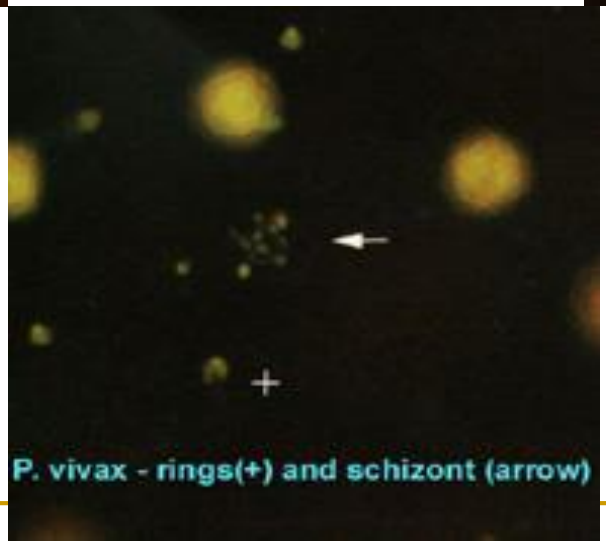
Quantitative Buffy Coat (QBC)

- Fluorescent microscopy after centrifugation
- AO-coated capillary is filled with 50-100 µl blood
- Centrifuge at 12000 rpm for 5 min
- Parasites concentrate below the granulocyte layer in tube
- AO stains DNA – parasites appear as shining stars in dark night


Precision Plastic Float
Plasma Layer
Platelets Layer
Gametocytes
Lymphocye/Monocyte Layer
Schizonts
Mature Trophozoites
Granulocyte Layer
Ring Forms, or
Mature Trophozoites
Red Blood Cells


P. falciparum - gametocytes


P. vivax - gametocyte


P. vivax - rings(+) and schizont (arrow)

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 Serology – antibody detection

- Immunologic assays to detect host response
- Antibodies appear some days after invasion of RBCs and may persist for months
- Positive test indicates past infection
- Not useful for treatment decisions

Malaria Serology – antibody detection

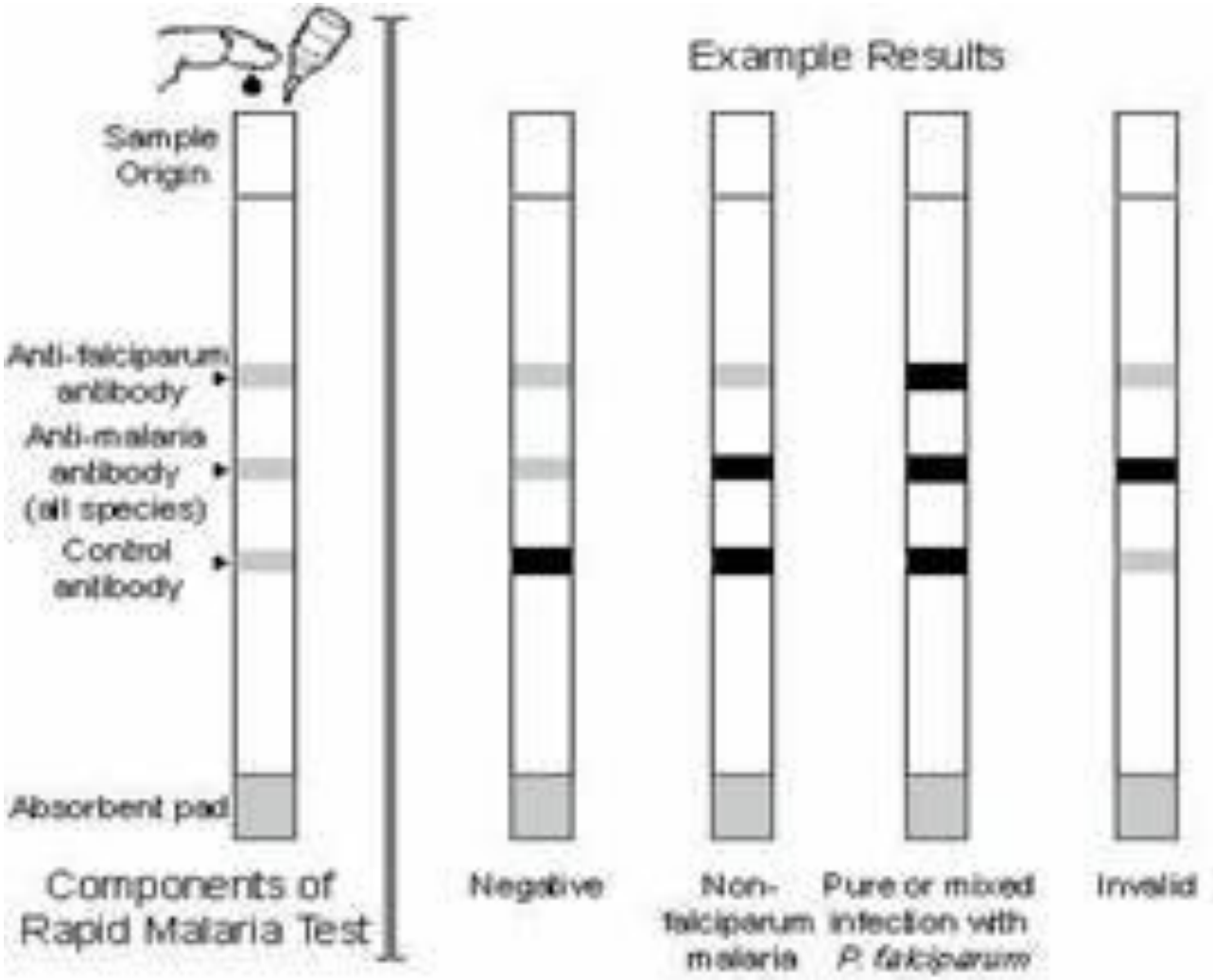
- Valuable epidemiologic tool in some settings
- Useful for
  - Identifying infective donor in transfusion-transmitted malaria
  - Investigating congenital malaria, esp. if mom's smear is negative
  - Diagnosing, or ruling out, tropical splenomegaly syndrome

Antigen detection by Rapid immunodiagnostic strip test

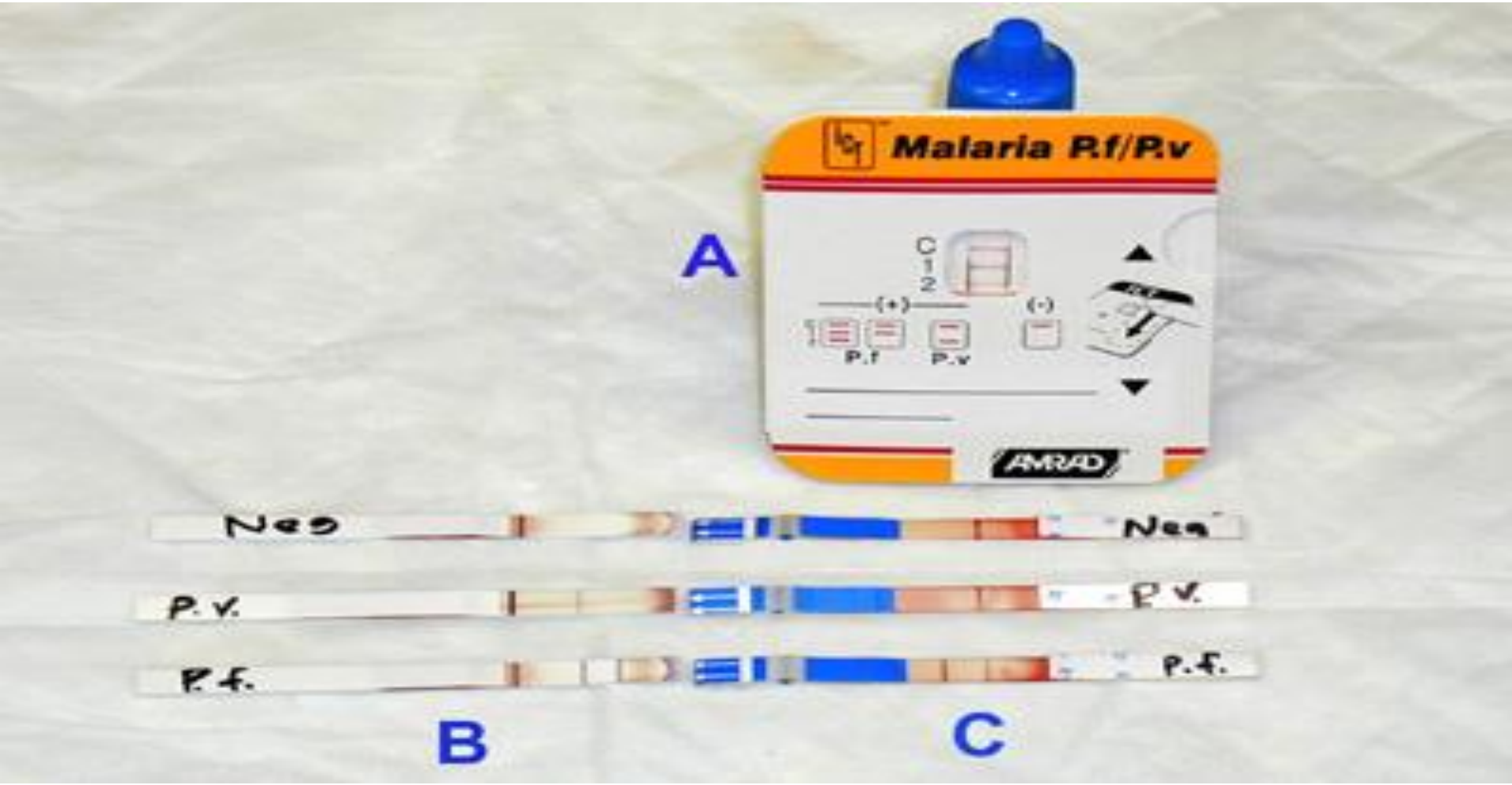
1. Histidine rich protein-2 (HRP-2) detection
   - present only in P.falciparum

- Para sight F test
- ICT Malaria PF

2. pLDH test (OptiMAL test)
   1. Present in all plasmodium species


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

Detection of *Plasmodium* antigens




Malaria
Malaria
Malaria
WWW.B2B-TODAY.COM®

PREVENTION & CONTROL
of
MALARIA

Points Of Attack

1. Attack the parasite in the human host

2. Reduce contact between humans and mosquitoes

3. Decrease mosquito population

Attack The Parasite In The Human Host

- **Treat malaria infections with effective medications**
- **Use prophylactic drugs to prevent illness and/or infection**

Reduce Contact Between Humans And Mosquitoes

- **Personal protective measures**
  - **Screening windows**
  - **Protective clothing**
  - **Bed nets**
  - **Mosquito repellent cream/coils**
    - **PERMETHRIN**
    - **Neem oil**

Decrease Mosquito Population

- Surveillance of mosquito populations
- Identify and eliminate breeding sites
- Proper insecticide application
  - Attack larval stages
  - Attack adult mosquito
- Biological control
  - Gambusia & Guppy fish
  - Bacillus thuringiensis

VACCINES

- Anti-Sporozoite vaccine
- Anti-asexual blood stage vaccines
- Transmission blocking vaccines

Safe & effective vaccine still not available

Summary

- Mosquito-borne infectious disease
- P. falciparum, vivax, ovale, malariae
- Anopheles mosquito - Incubation period nearly two weeks
- Life cycle –
  - Sexual method –
    - Gametocyte, zogote, ookinete,oocyst, sporozoite
  - Asexual method –
    - Sporozoite
    - Pre-erthrycotic schizogony
    - Erythrocytic schizogony
    - Gametogony
- Cyclic paroxysms
- Fever
  - Tertian fever, quartan fever

Summary

- **P.faciparum –**
  - **Cerebral, Algid & Septicemic malaria**
  - **Black-water fever**
- **Thick and think blood smears for diagnosis**
- **Newer techniques : QBC, Ag detection**
- **Chemoprophylaxis can prevent infection**
- **Great importance of personal protective measures**
- **Regard and manage malaria as medical emergency**

Questions?