MALARIA-2 : LABORATORY DIAGNOSIS

AIMS

- Diagnostic
- Monitoring response to the treatment
- Drug resistant malaria
- To investigate Complications of malaria

METHODS

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

COLLECTION OF BLOOD

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

Peripheral smear examination

1. Preparation

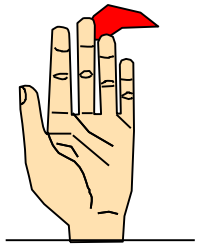
2. Staining

3. Observation

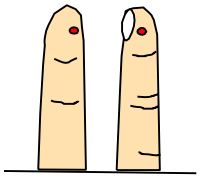
Peripheral Blood Smear → Thick smear
Thin smear

- Prepare smears as soon as possible after collecting venous blood to avoid
  - Changes in parasite morphology
  - Staining characteristics
- Take care to avoid fixing the thick smear
  - Risk of fixing thick when thin is fixed with methanol if both smears on same slide
  - Let alcohol on finger dry to avoid fixing thick

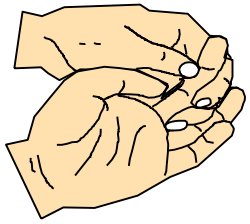
Collection of Blood Smears



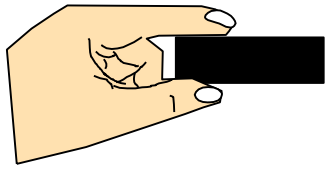
1.
The second or third finger is usually selected and cleaned.



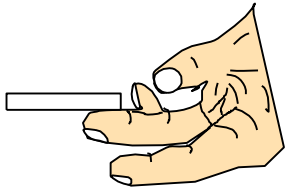
2.
Puncture at the side of the ball of the finger.



3.
Gently squeeze toward the puncture site.

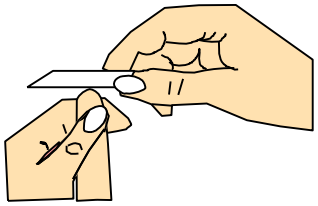


4.
Slide must always be grasped by its edges.

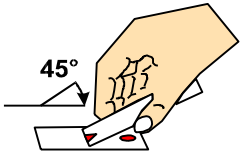


5.
Touch the drop of blood to the slide from below.

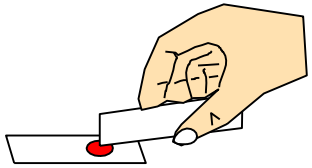
Preparing thick and thin films



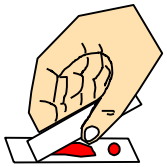
1.
Touch one drop of blood to a clean slide.



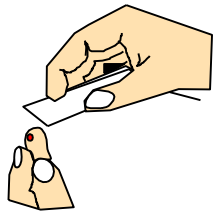
4.
Carry the drop of blood to the first slide and hold at 45 degree angle.



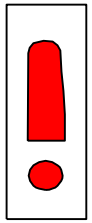
2.
Spread the first drop to make a 1 cm circle.



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



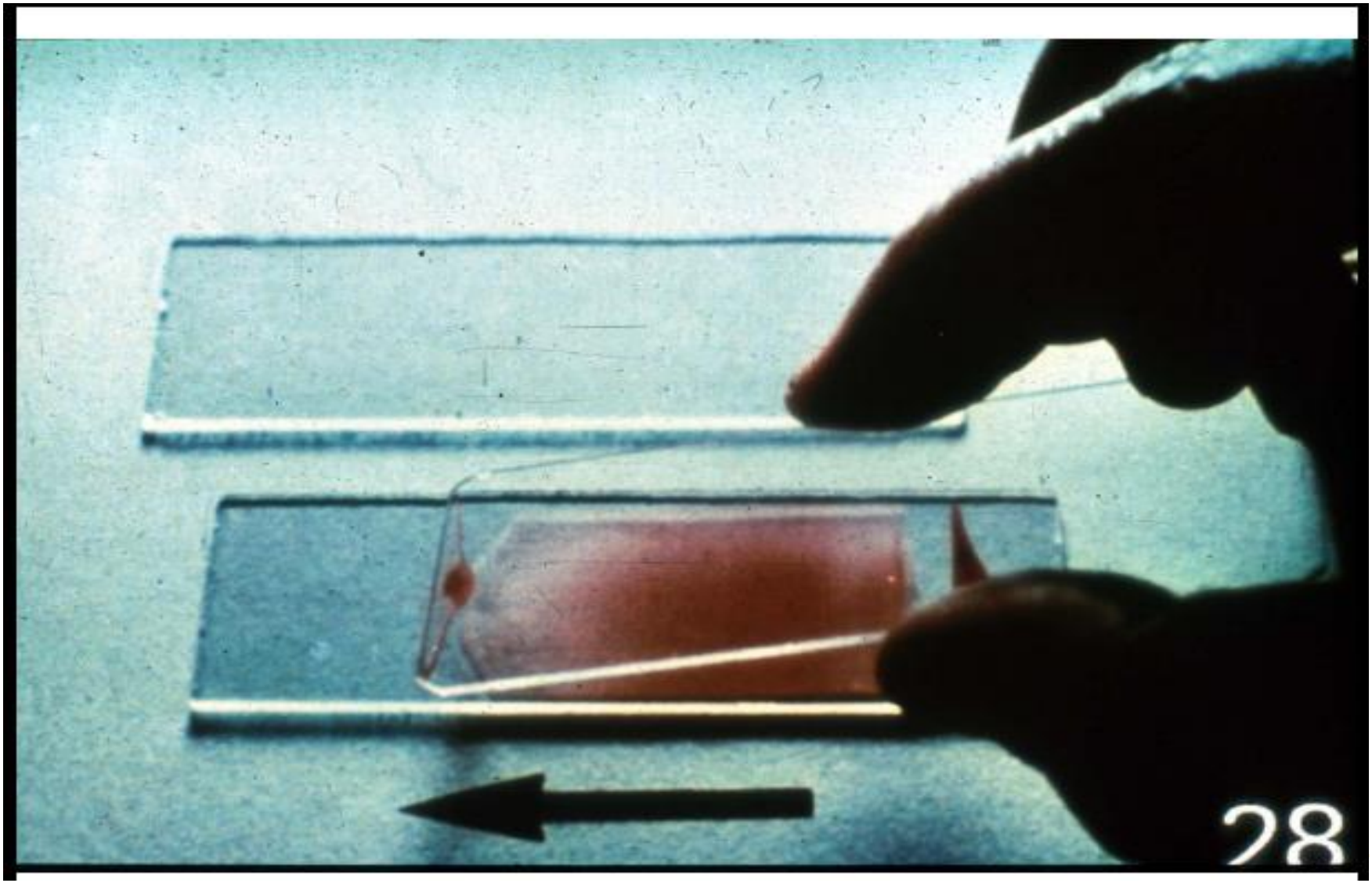
3.
Touch a fresh drop of blood to the edge of another slide.



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

OBSERVATION

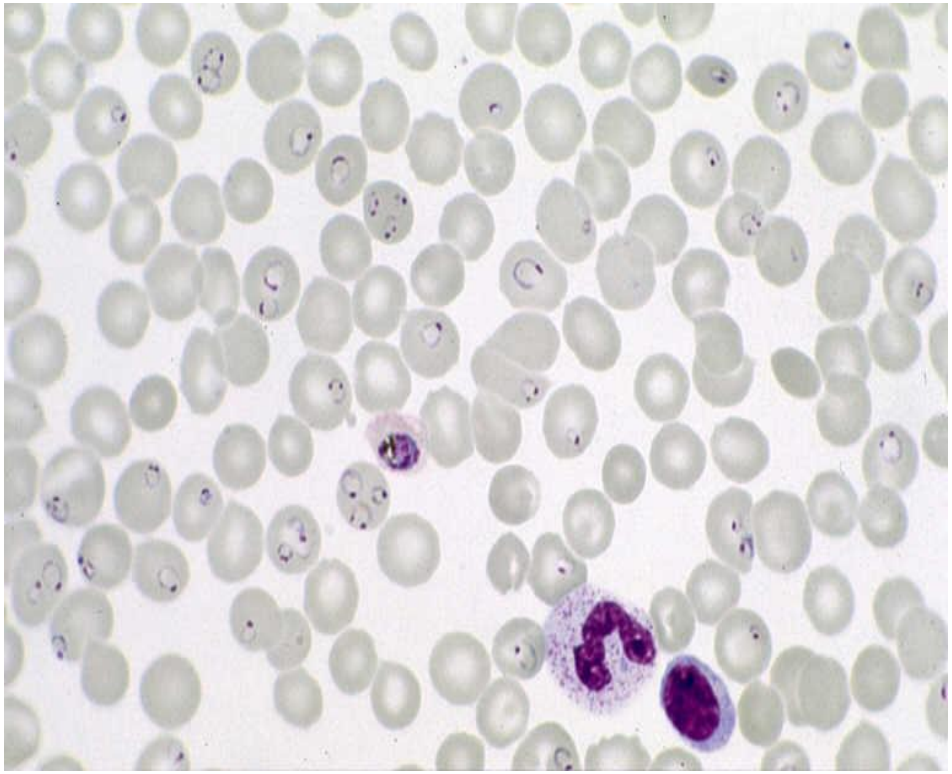
- RBC – size, shape

  - number of parasite / RBC

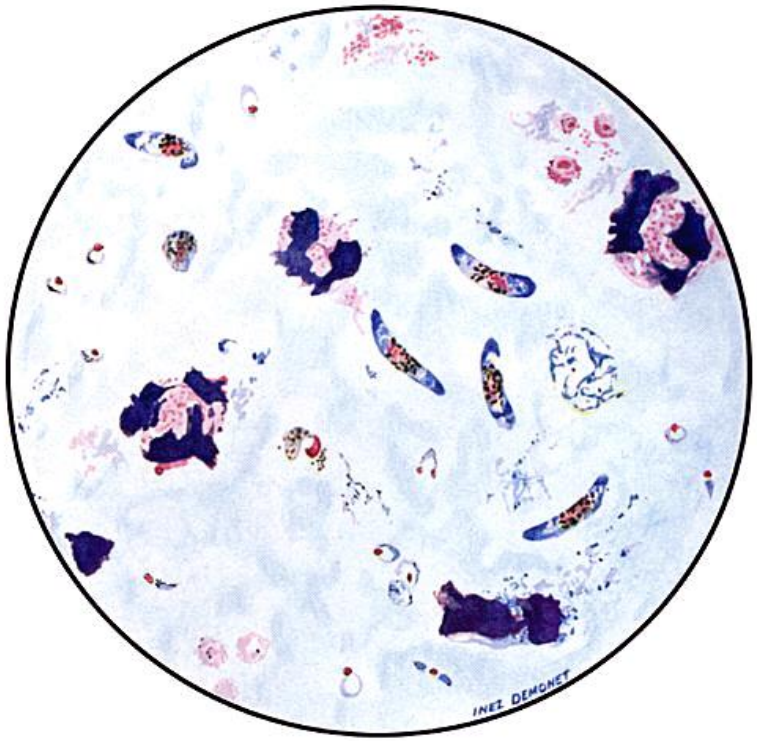
- Identification of species

Ring form – size, cytoplasm, nucleus, location, number

Thin smear



Thick smear



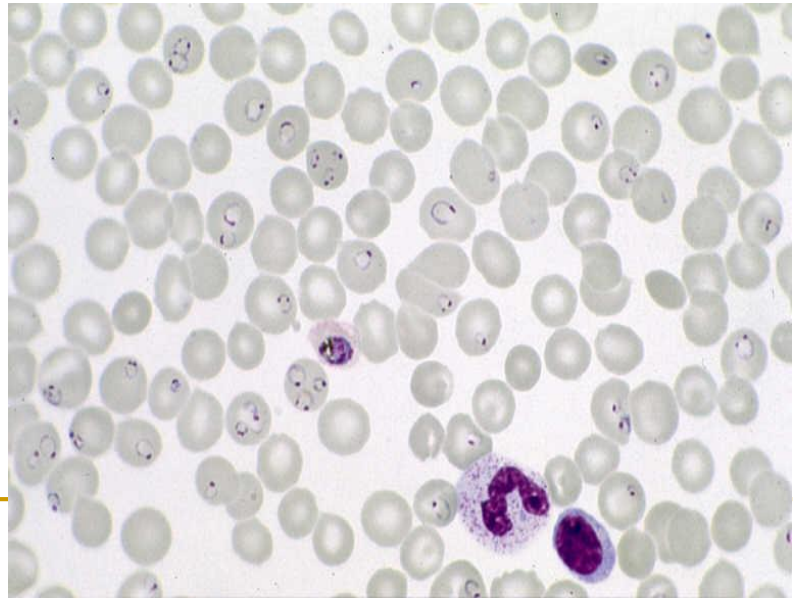
Thin smear

Advantages

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

Disadvantages

- Fixation of smear
- Low parasitaemia
- Less sensitive



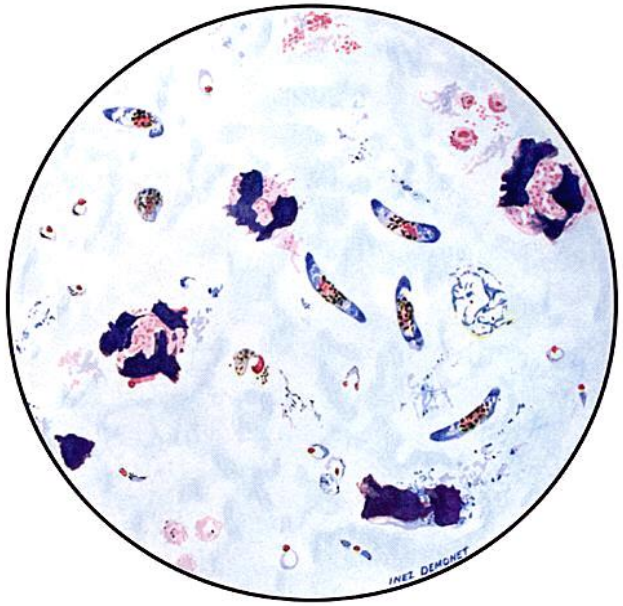
Thick smear

Advantages

- More sensitive
- Rapid detection of parasites
- No fixation of smear
- Low parasitaemia
- Larger volume of blood can be assessed as RBCs are lysed

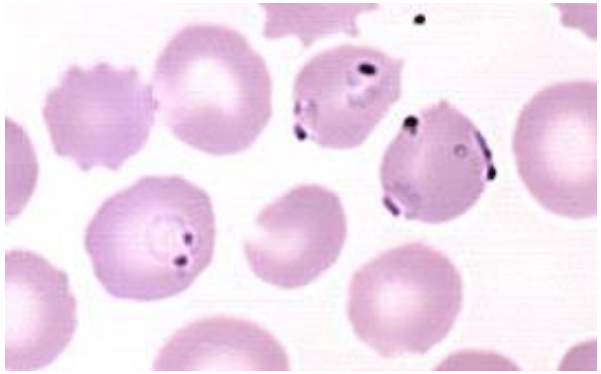
Disadvantages

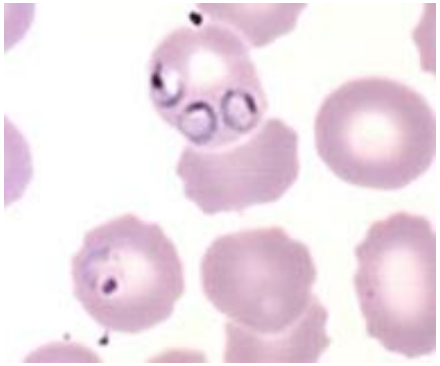
- Intra RBC morphology of parasite can not be seen
- Cannot confirms Plasmodium spp.



Plasmodium falciparum

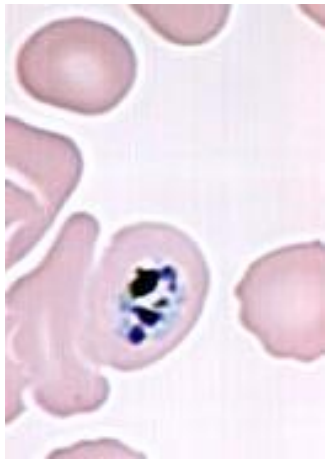
Infected erythrocytes: normal size





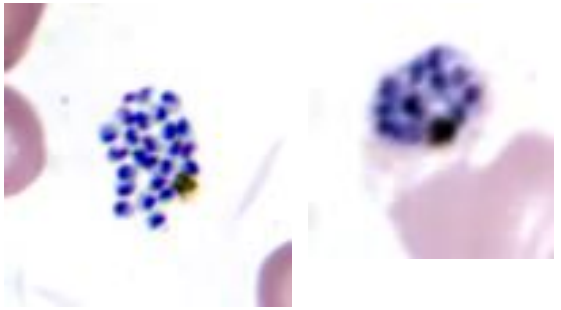
Rings: double chromatin dots; appliqué forms; multiple infections in same red cell

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



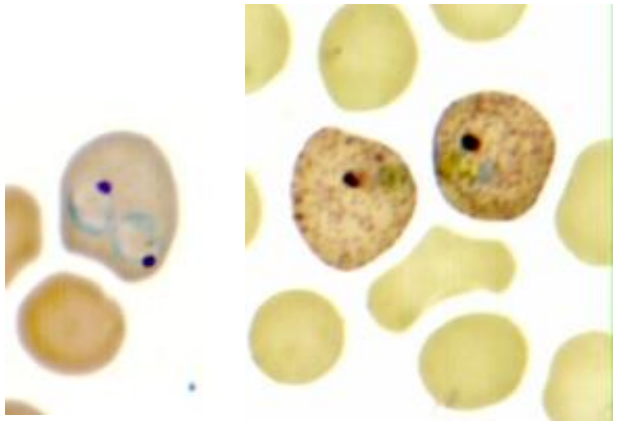
Trophozoites: compact (rarely seen in peripheral blood)

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

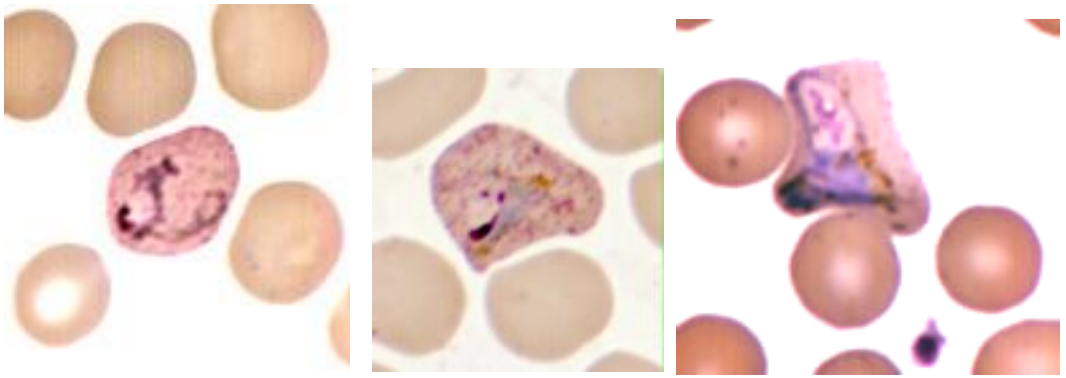


Plasmodium vivax

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

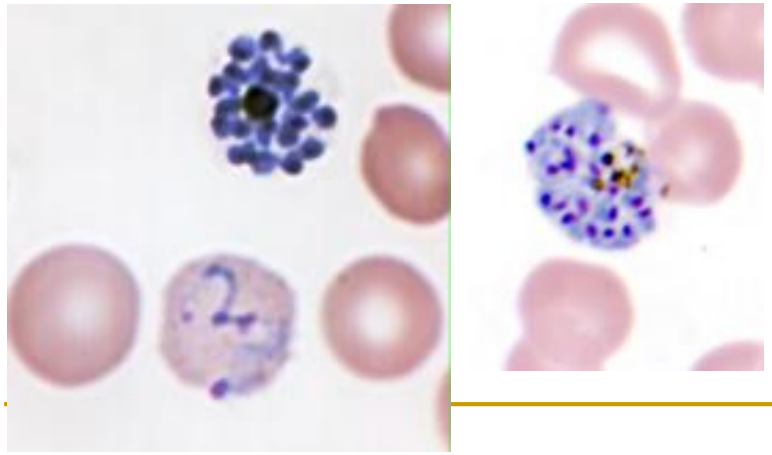


Rings

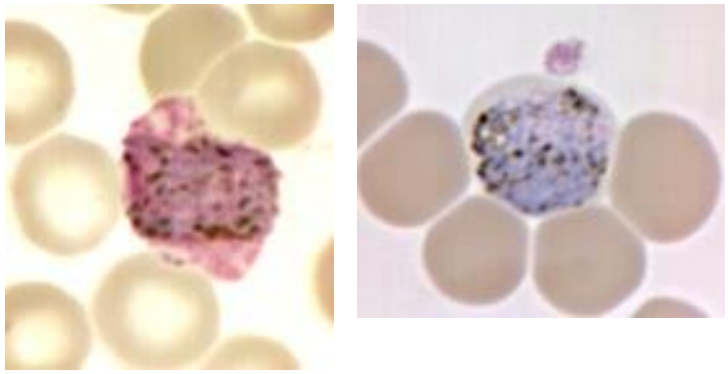


Trophozoites: ameboid; deforms the erythrocyte

Schizonts: 12-24 merozoites



Gametocytes: round-oval



Species Differentiation on Thin Films

Feature	***P. falciparum***	***P. vivax***	***P. ovale***	***P. malariae***
Enlarged infected RBC		+	+	
Infected RBC shape	round	round, distorted	oval, fimbriated	round
Stippling infected RBC	Mauer clefts	Schuffner spots	Schuffner spots	none
Trophozoite shape	small ring, appliqu☺	large ring, amoeboid	large ring, compact	small ring, compact
Chromatin dot	often double	single	large	single
Mature schizont	rare, 12-30 merozoites	12-24 merozoites	4-12 merozoites	6-12 merzoites
Gametocyte	crescent shape	large, round	large, round	compact, round

Species Differentiation on Thick Films

Feature	***P. falciparum***	***P. vivax***	***P. ovale***	***P. malariae***
Uniform trophozoites	+			
Fragmented trophozoites		++	+	
Compact trophozoites			+	+
Pigmented trophozoites				+
Irregular cytoplasm		+	+	
Stippling (“RBC ghosts”)		+	+	
Schizonts visible	very rarely	often	often	often
Gametocytes visible	occasionally	usually	usually	usually

Fluorescent Microscopy

- Modification of light microscopy
- Fluorescent dyes detect RNA and DNA that is contained in parasites
- Nucleic material not normally in mature RBCs
- Kawamoto technique

  - Stain thin film with acridine orange (AO)
  - Requires special equipment – fluorescent microscope
  - Staining itself is cheap
  - Sensitivities around 90%

Quantitative Buffy Coat (QBC)

- Fluorescent microscopy after centrifugation
- AO-coated capillary is filled with 50-100 µl 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 Serology – antibody detection

- Immunologic assays to detect host response
- Antibodies to asexual parasites 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
  - Retrospective confirmation of empirically-treated non-immunes

Rapid immunodiagnostic strip test

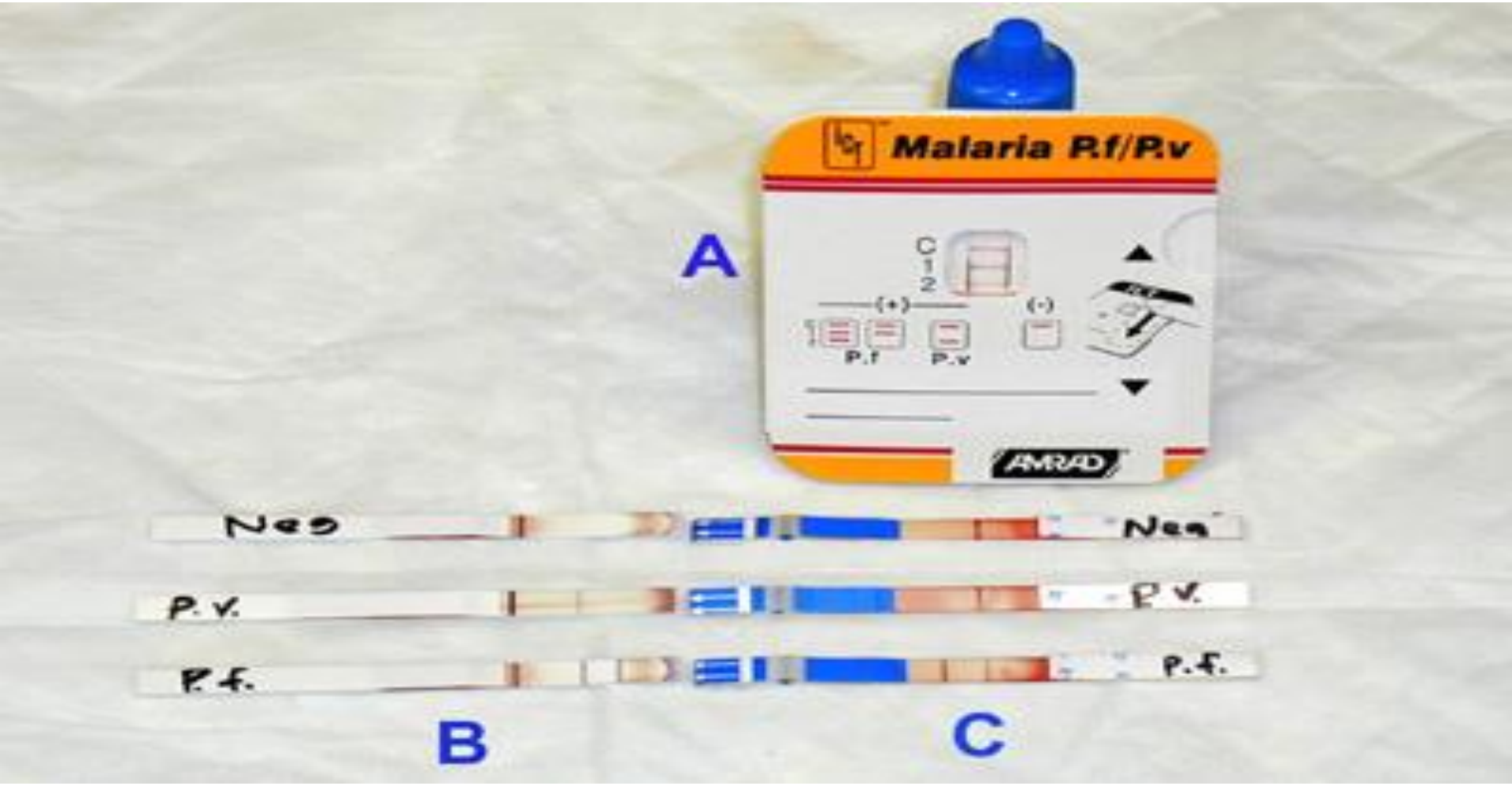
1. Histidine rich protein-2 (HRP-2) detection
   Immunochromatographic test

- Para sight F test
- ICT Malaria PF

2. pLDH test (OptiMAL test)

- reflects presence of viable malarial parasites
- to monitor response to drug
- to detect drug resistant malaria

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)

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**
  - **Proper wearing of uniform**
  - **PERMETHRIN**
  - **Bed nets**
  - **Mosquito repellent coils**
  - **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

  - to reduce severe & complicated manifestations of the disease

  - MSP, PfHRP2, Erythrocyte membrane Ag
- Transmission blocking vaccines

  - to arrest the development of the parasite in the mosquito

Summary

- Mosquito-borne infectious disease
- Tropics, subtropics
- P. falciparum, vivax, ovale, malariae
- Incubation period nearly two weeks
- Cyclic paroxysms
- Fever
- Thick and think blood smears for diagnosis

7/27/2011

ELECTRONIC THESIS/DISSERTATION WORKSHOP

Working Smart to Create Your ETD

Shirley Hancock,

ET/D ReviewManager

Primary Text: ***Guide to the Preparation of Electronic Theses and Dissertations****

Corollary Aids: ***Appendix to the Guide*** **and copy of the PowerPoint slides***

APPENDIX TO THE GUIDE

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* Available at the ET/D web page: http://www.uthsc.edu/grad/TandD/index.php?page=TandD; links also available in the ET/D Workshop site under Workshop Docs

7/27/2011

2

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Summary

- **Drug resistance is increasing**
- **Chemoprophylaxis can prevent infection**
- **Great importance of personal protective measures**
- **Regard and manage malaria as medical emergency**

Questions?