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

- 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.

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

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

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

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

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**

**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

<table>
<thead>
<tr>
<th>Feature</th>
<th><em>P. falciparum</em></th>
<th><em>P. vivax</em></th>
<th><em>P. ovale</em></th>
<th><em>P. malariae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Enlarged infected RBC</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected RBC shape</td>
<td>round</td>
<td>round, distorted</td>
<td>oval, fimbriated</td>
<td>round</td>
</tr>
<tr>
<td>Stippling infected RBC</td>
<td>Mauer clefts</td>
<td>Schuffner spots</td>
<td>Schuffner spots</td>
<td>none</td>
</tr>
<tr>
<td>Trophozoite shape</td>
<td>small ring, appliquă</td>
<td>large ring, amoeboid</td>
<td>large ring, compact</td>
<td>small ring, compact</td>
</tr>
<tr>
<td>Chromatin dot</td>
<td>often double</td>
<td>single</td>
<td>large</td>
<td>single</td>
</tr>
<tr>
<td>Mature schizont</td>
<td>rare, 12-30 merozoites</td>
<td>12-24 merozoites</td>
<td>4-12 merozoites</td>
<td>6-12 merozoites</td>
</tr>
<tr>
<td>Gametocyte</td>
<td>crescent shape</td>
<td>large, round</td>
<td>large, round</td>
<td>compact, round</td>
</tr>
</tbody>
</table>
## Species Differentiation on Thick Films

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<th><em>P. malariae</em></th>
</tr>
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<tr>
<td>Uniform trophozoites</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragmented trophozoites</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compact trophozoites</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pigmented trophozoites</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Irregular cytoplasm</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stippling (“RBC ghosts”)</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizonts visible</td>
<td>very rarely</td>
<td>often</td>
<td>often</td>
<td>often</td>
</tr>
<tr>
<td>Gametocytes visible</td>
<td>occasionally</td>
<td>usually</td>
<td>usually</td>
<td>usually</td>
</tr>
</tbody>
</table>
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
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
Drug resistance is increasing
Chemoprophylaxis can prevent infection
Great importance of personal protective measures
Regard and manage malaria as medical emergency
Questions?