Laboratory diagnosis of Malaria – handout 1

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Microscopy – thick blood smear

- Haemolysis RBC + staining
- Healthy blood
Microscopy – thin blood smear

1. Place a small drop of whole blood on a slides and close it using a coverslip.
2. Make a smear with the slide and stain it with a fixative and a stain.
3. Healthy blood should appear in the smear.

fixation + staining

healthy blood
Preparation of slides

Staining:

- chromatin dots (nucleus): deep red
- cytoplasm: blue
- effect on RBC (size, shape, dots; optimal pH 7.2)
- pigment (haemozoin crystal from digested haemoglobin)

Staining procedure (Romanowsky stains):

- Giemsa (best colours, not so fast)
- Field’s stain (thick smear, fast, more difficult to read)
- Diff-Quick (thin smear, fast, more difficult to read)
Preparation of slides

- Prepare 2x thick and 2x thin at least

- Standardization???
  - Use a SOP
Preparation of slides

- Prepare 2x thick and 2x thin at least
- Standardization???
- Examination – use SOP
  - Thick smear 100 (200) fields (≈3-15 minutes)
    - Positive (+ species?)
    - No parasites found
  - Thin smear ??
    - Species (+ counting?)
EDTA of capillary blood?

The use of anticoagulated (EDTA) blood is discouraged because:

- smears require longer time to dry
- thick smears tend to flake from the slide
- stain quality is affected, stippling of infected RBC’s may not be visible
- parasite forms may be distorted and may lyse
- RBC’s may become crenated and look fimbriated

If it is necessary to use EDTA for collection, slides should be made as soon as possible (less than 2 hours after collection) in order to reduce distortion of the parasites and RBC’s. These effects can compromise correct species identification.

- Capillary or vena puncture??
## Comparison thin and thick smear

<table>
<thead>
<tr>
<th></th>
<th>Thin smear</th>
<th>Thick smear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>fixated</td>
<td>haemolysed</td>
</tr>
<tr>
<td>Parasite morphology</td>
<td>unchanged</td>
<td>changed</td>
</tr>
<tr>
<td>Effective volume</td>
<td>1 µl</td>
<td>3-5 µl</td>
</tr>
<tr>
<td>Average thickness</td>
<td>0.0025 mm</td>
<td>0.06 – 0.09 mm</td>
</tr>
<tr>
<td>Volume in 100 fields</td>
<td>±0.01 µl</td>
<td>± 0.25 µl</td>
</tr>
<tr>
<td>Optimal sensitivity</td>
<td>200 p/µl (1500 p/µl)</td>
<td>5-10 p/µl (50-200 p/µl)</td>
</tr>
<tr>
<td>Loss leuko’s (due to staining)</td>
<td>none</td>
<td>up to 8%</td>
</tr>
<tr>
<td>Loss parasites (due to staining)</td>
<td>none</td>
<td>up to 20%</td>
</tr>
</tbody>
</table>
P. falciparum

- marginal form
- ring form
- double dotted rings
- ring form
- young trophozoite
- trophozoite
- early schizont
- schizont
- mature schizont
- female gametocyte
- male gametocyte
P. vivax

ring form  mature ring form  trophozoite
trophozoite  early schizont  schizont  mature schizont
developing gametocyte  female gametocyte  male gametocyte
P. ovale

- young ring
- older ring
- comet form
- 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
Remember

- No parasites seen is not equal to negative
- 2nd technician(?)
- Repeat!
- Parasite counts for all *P. falciparum*
  - Stages?
  - Gametocystes?
  - Pigment?
- 2-5% *P. falciparum* potentially life threatening
- Rapid Diagnostic Test (RDTs) may be an aid
Malaria rapid diagnostic tests

Antigens

- histidine rich protein (HRP2) – *P. falciparum*
  - *Plasmodium*-specific aldolase
  - *Plasmodium* lactate dehydrogenase (pLDH) – Pf / pan
Quantitative Buffy Coat Method

- Acridine-orange
Counting of parasites

- *P. falciparum*
- Thin smear (% RBC)
- Thick smear (p/µl)
- Grid may help
- 2 technicians
- Report separately asexual stages and gametocytes
% infected RBC

- RBC single layers, free
- Count average number of RBC/field
- Examine > 1000 RBC (40 fields of 250 RBCs)
  - different areas/slides
- % infected RBCs
  - multiple infected n=1
  - do not/separately count gametocytes
- If <0.1% also use thick smear

- To recalculate into p/µl
  - 4-6 x 10^6 RBC/µl
p/WBC in thick smear

- 10-12 WBC/field
- Examine > (200) 500 WBC
  - different areas/slides
- Calculate parasites per (500) WBC
  - do not count/separately gametocytes
- To recalculate into p/µl
  - \( \approx 6 \times 10^3 \) WBC/µl
Example

Thin smear:
- 40 fields examined with 250 RBC
- 60 infected RBCs counted
- $60/10,000 = 0.6\%$
- $0.6\% \times 6 \times 10^6 \text{RBC/\mu l} = 36,000 \text{p/\mu l}$

Thick smear:
- 20 fields examined with 10 WBC
- 4 parasites counted
- $4/200 = 2\text{p/100 WBC}$
- $0.02 \times 6 \times 10^3 \text{leuco’s/\mu l} = 120\text{p/\mu l}$