

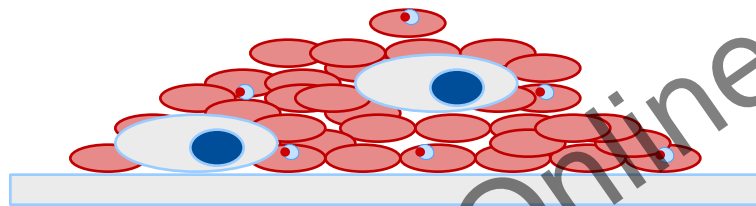
Laboratory diagnosis of Malaria – handout 1

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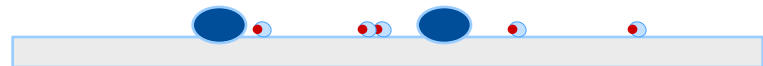
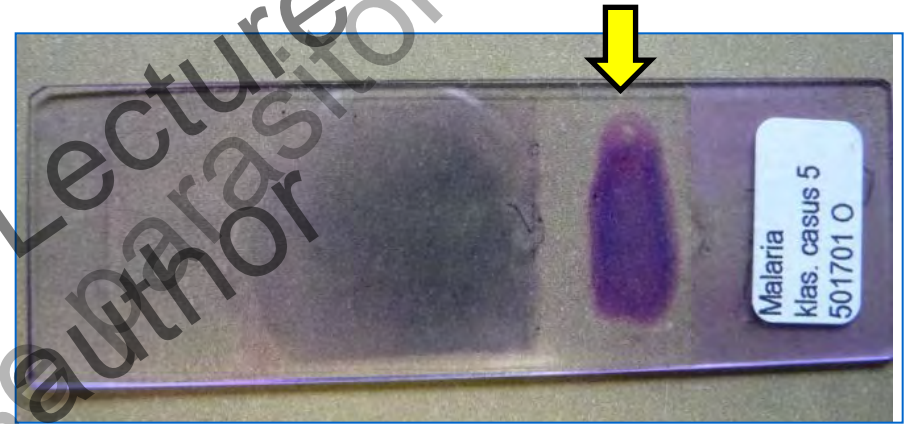
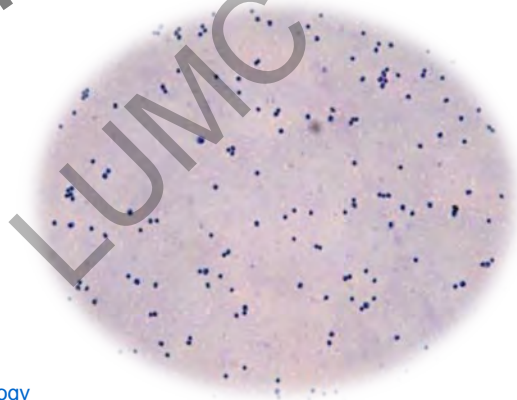
LvanLieshout@Lumc.nl

Microscopy – thick blood smear



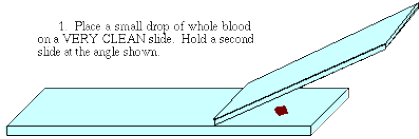
haemolysis RBC + staining

*healthy
blood*

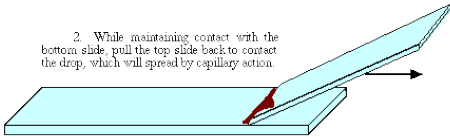


Microscopy – thin blood smear

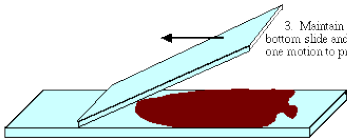
1. Place a small drop of whole blood on a VERY CLEAN slide. Hold a second slide at the angle shown.



2. While maintaining contact with the bottom slide, pull the top slide back to contact the drop, which will spread by capillary action.



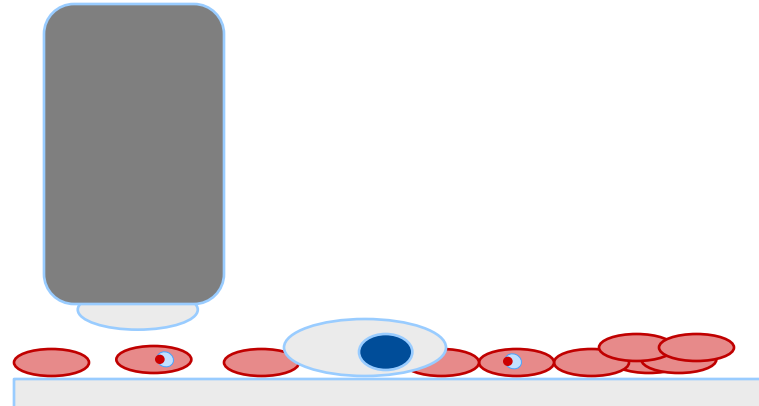
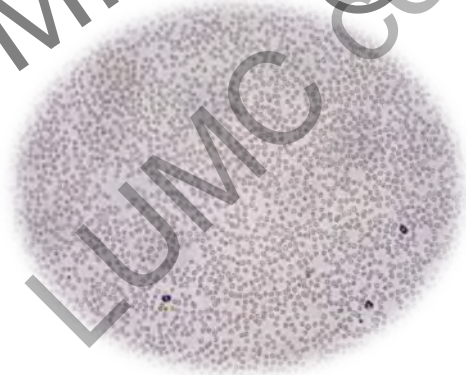
3. Maintain firm contact with the bottom slide and push the top slide in one motion to produce the smear.



fixation + staining



healthy
blood



Preparation of slides

Staining:

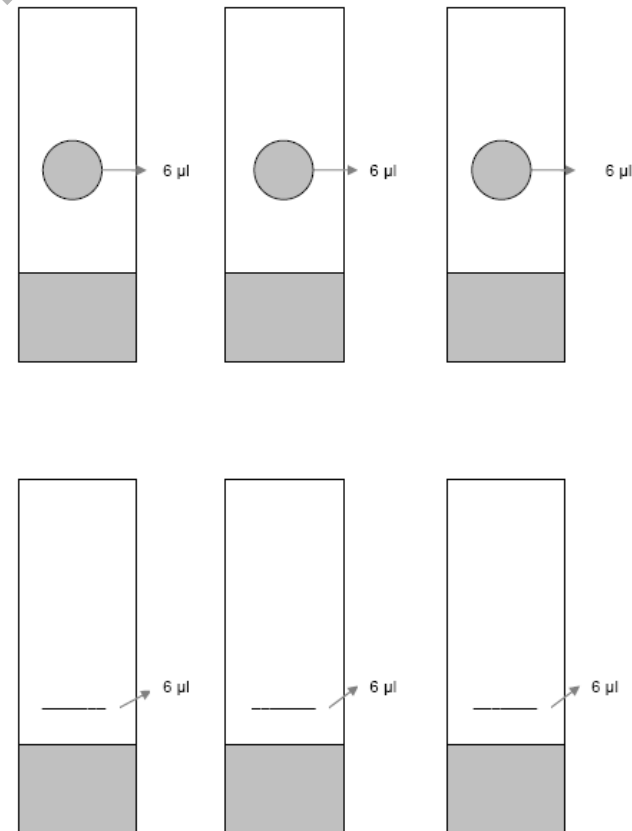
- chromatin dots (nucleus): deep red
- cytoplasm: blue
- effect on RBC (size, shape, dots; optimal pH 7.2)
- pigment (haemozoin crystal from digested haemoglobin)

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 (\cong 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

	Thin smear	Thick smear
Red blood cells	fixated	haemolysed
Parasite morphology	unchanged	changed
Effective volume	1 μl	3-5 μl
Average thickness	0.0025 mm	0.06 – 0.09 mm
Volume in 100 fields	$\pm 0.01 \mu\text{l}$	$\pm 0.25 \mu\text{l}$
Optimal sensitivity	200 p/ μl (1500 p/ μl)	5-10 p/ μl (50-200 p/ μl)
Loss leuko's (due to staining)	none	up to 8%
Loss parasites (due to staining)	none	up to 20%

P. falciparum



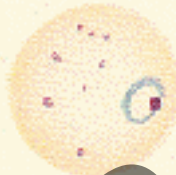
marginal form



ring form



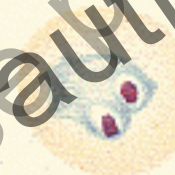
double dotted rings



ring form



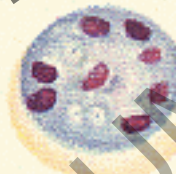
young trophozoite



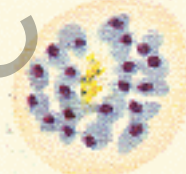
trophozoite



early schizont



schizont



mature schizont

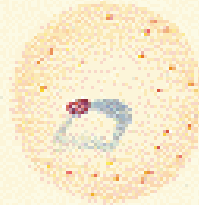
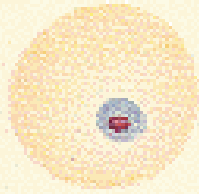
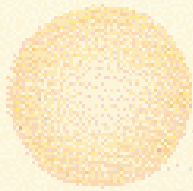


female gametocyte



male gametocyte

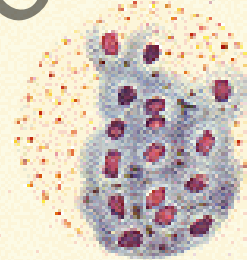
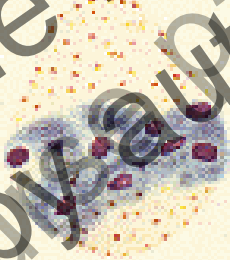
P. vivax



ring form

mature ring form

trophozoite

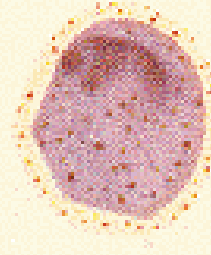
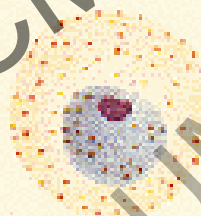


trophozoite

early schizont

schizont

mature schizont

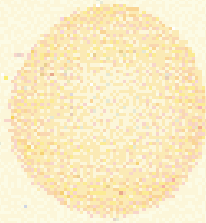


developing gametocyte

female gametocyte

male gametocyte

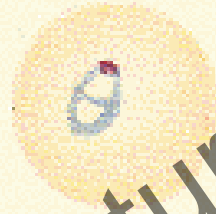
P. ovale



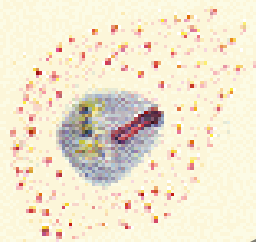
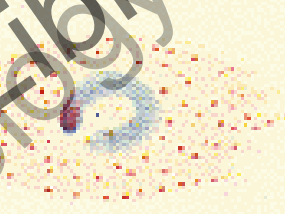
young ring



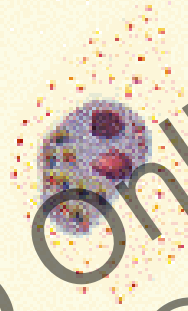
older ring



comet form



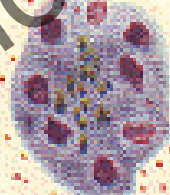
trophozoite



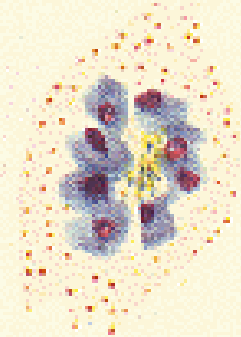
trophozoite



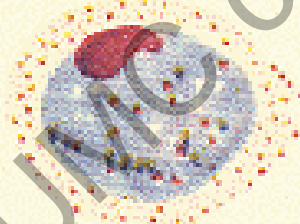
young schizont



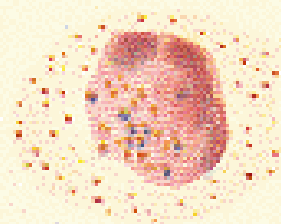
schizont



mature schizont

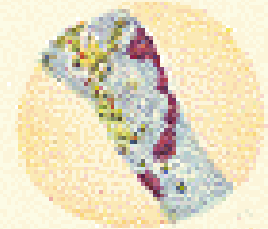


female gametocyte



male gametocyte

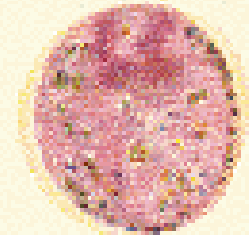
P. malariae



ring form

early band form

band form



early schizont

mature schizont

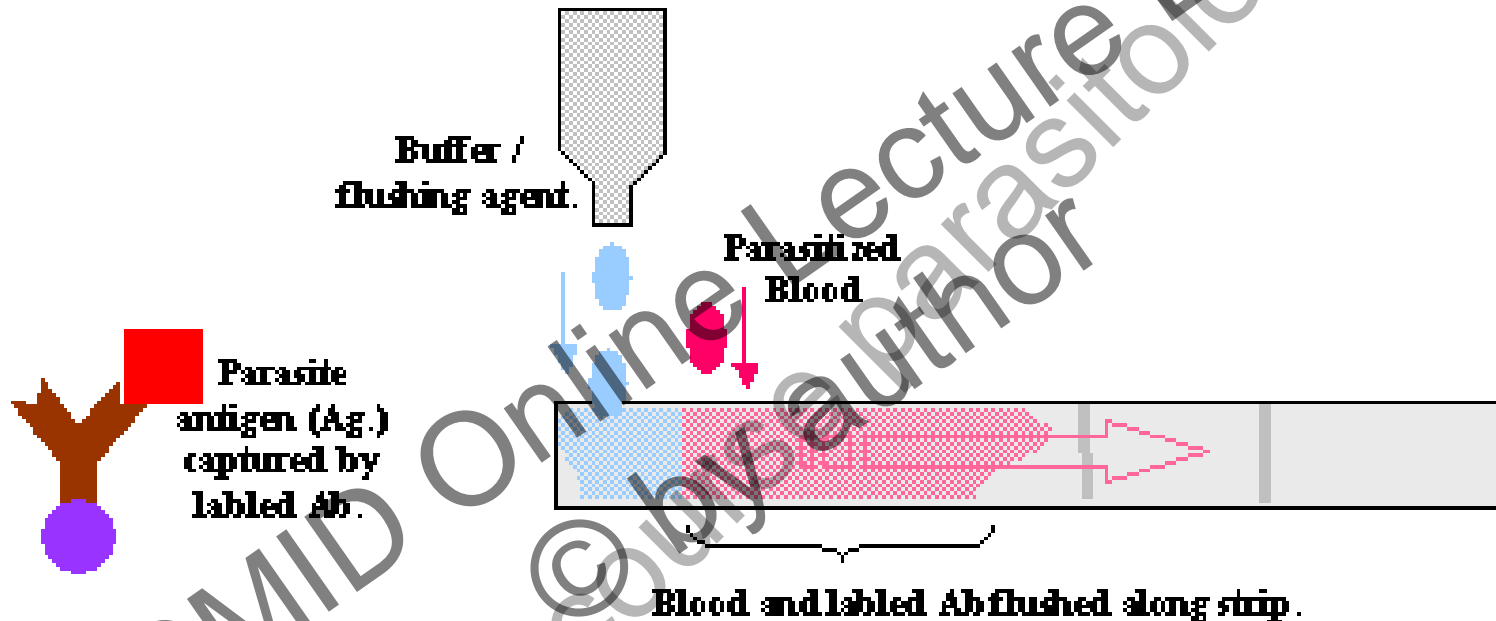
female gametocyte

male gametocyte

Remember

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

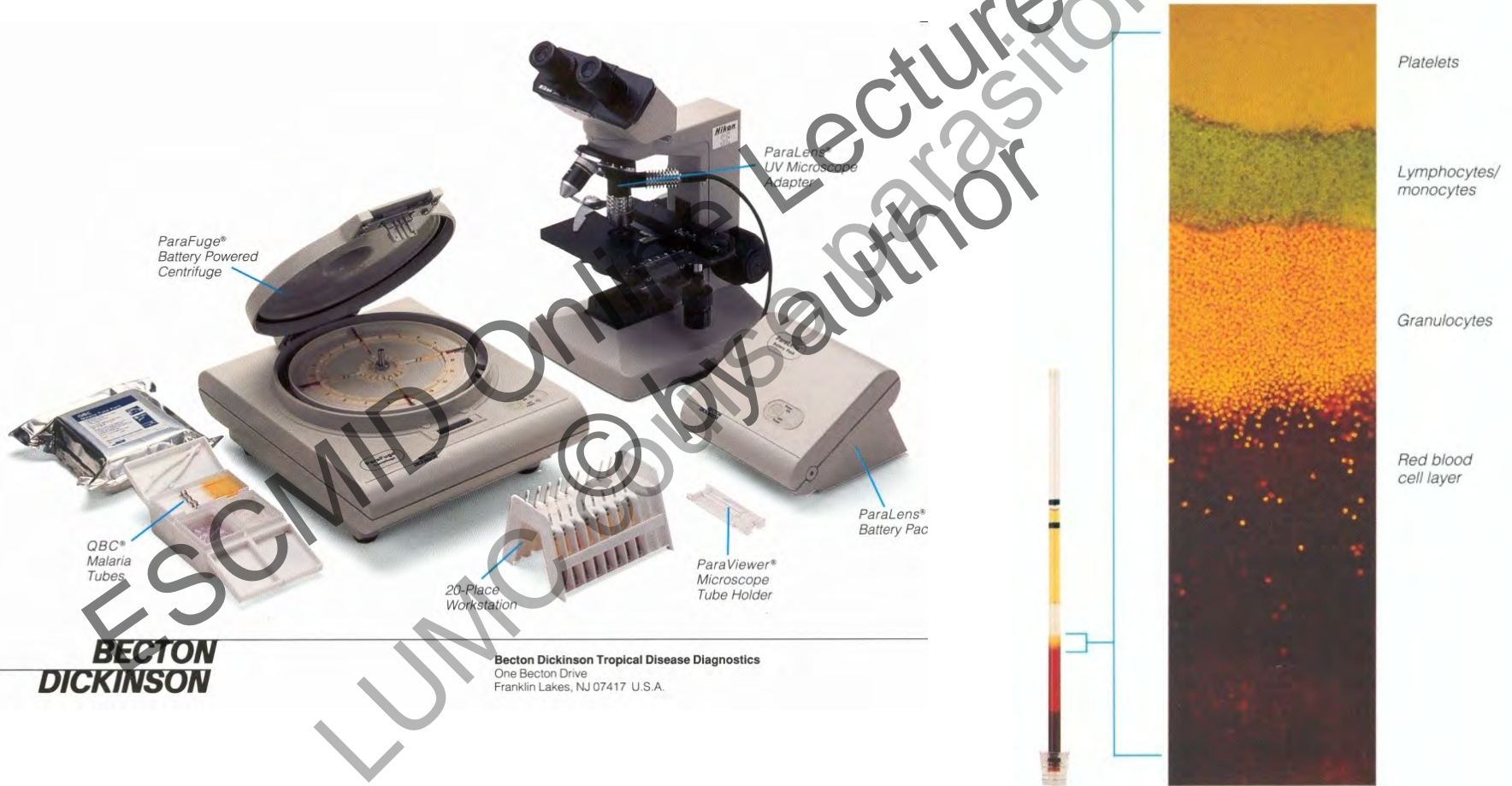
Malaria rapid diagnostic tests



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/ μ l)
- 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/ μ l
 - $4-6 \times 10^6$ RBC/ μ l

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/ μ l
 - $\cong 6 \times 10^3$ WBC/ μ l

Example

Thin smear:

- 40 fields examined with 250 RBC
- 60 infected RBCs counted
- $60/10,000 = 0.6\%$
- $0.6\% * 6 \times 10^6 \text{ RBC}/\mu\text{l} = 36.000 \text{ p}/\mu\text{l}$

Thick smear:

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