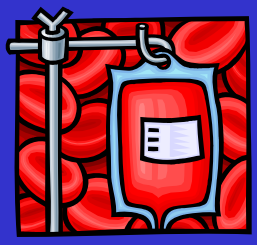
Biomedical Parasitology

Clinical Parasitology

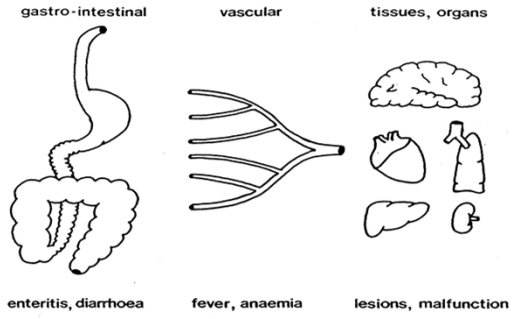
(Haematology)



Prof Peter O'Donoghue

1

Parasites in body compartments



2

Haemo-parasites

<u>protozoa</u>

- flagellates - trypanosomes
- apicomplexa - haemogregarines
  - haemosporidia
  - piroplasms

<u>helminths</u>

- nematodes - filarial worms
- trematodes - blood flukes

All utilize haematophagous insects as vectors

3

Clinical haematology

Blood

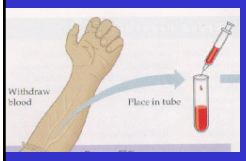
- oxygen transport
- nutrient supply
- metabolic waste disposal

Haematology = study of blood

- for disturbances in:
  - cellular characters (red/white blood cells)
  - subcellular characters (platelets/bodies/haem)
  - acellular characters (protein, fibrinogen, etc)
- for presence of parasites

4

Blood composition



<u>without anticoagulant</u>

serum

clot (cells + fibrinogen)

<u>with anticoagulant</u>

plasma (serum + fibrinogen)

white blood cells

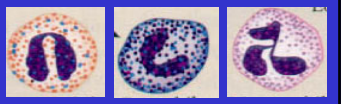
red blood cells

5

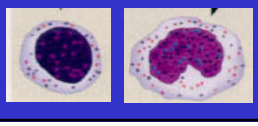
BLOOD CELLS

circulating cells divided into:

- erythrocytes (red blood cells) 
- leucocytes (white blood cells)
  - granular
    - eosinophils
    - basophils
    - neutrophils



  - agranular
    - lymphocytes
    - monocytes



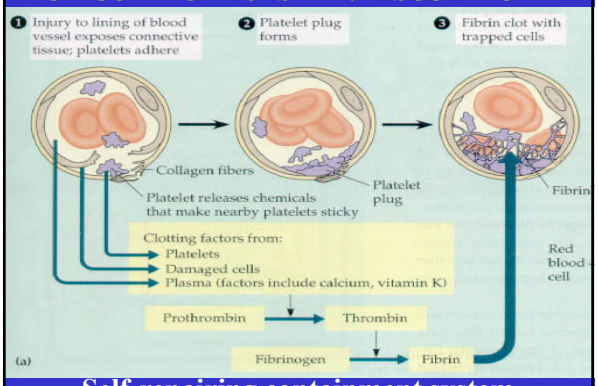
6

PLASMA CONSTITUENTS

• water	solvent
• ions	Na, K, Ca, Mg, Cl, HCO_3 (osmotic balance, pH buffering, regulation of membrane permeability)
• proteins	albumin (osmotic balance, pH) fibrinogen (clotting) gamma-globulins (humoral immunity)
• gases	O_2 and CO_2 (respiration)
• nutrients	glucose, fatty acids, vitamins, etc
• metabolic waste	urea, lactic acid, etc
• hormones	various

7

CIRCULATORY SYSTEM/VASCULATURE



Self-repairing containment system

8

ERYTHROCYTE CHARACTERS

- **size** (anisocytosis, macrocytic, microcytic)
- **shape** (poikilocytes, leptocytes, spherocytes, target cells, acanthocytes, spherocytes, schistocytes)
- **distribution** (single, Rouleau, agglutination)
- **colour** (normochromic, hypochromic, polychromasia)
- **abnormal structures**
  (Howell-Jolly, Heinz, Pappenheimer bodies, basophilic stippling, nucleated red cells, inclusion bodies, nuclear fragmentation, parasites)

9

Clinical Haematology - erythrocytes

- **RBC concentration (millions per µl)**
- **PCV = packed cell volume = haematocrit (%)**
- **RBC diameter (µm)**
- **MCV = mean corpuscular volume (fl)**
- **Hb = haemoglobin (g/dl)**
- **MCH = mean corpuscular Hb (pg)**
- **MCHC = mean corpuscular Hb concentration**
- **ESR = erythrocyte sedimentation rate (mm/h)**
- **reticulocytes (%)**
- **nucleated erythrocytes (%)**
- **fragility test (% saline → haemolysis)**

10

LEUCOCYTE CHARACTERS

- **abundance**
- **types**
- **abnormalities**
  (nuclear degeneration; hyper/hypo-segmentation; toxic changes such as azurophilic granules, vacuolation, foaminess, basophilia, Dohle bodies; giant bizarre forms)
- **cytoplasmic inclusions**
  (phagosomes; inclusions; bacteria; parasites)

11

Clinical Haematology - leucocytes

- **total WBC (no./µl)**
- **differential count (% types)**
  (lymphocytes, monocytes, neutrophils, basophils, eosinophils)
- **differential absolute count (no./µl)**
- **platelets = thrombocytes (no./µl)**

12

Impact of parasites

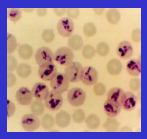
- range of protozoa parasitize RBC &/or WBC
- multiply and released by destroying host cells
- causing range of haematological abnormalities
- compromise blood function (gas, nutrients, ..)
- may disturb blood delivery (vascular changes)

- burden quantitated as % parasitaemia

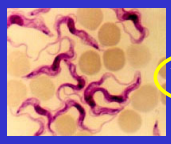
13

Haemoparasites

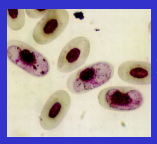
intra-erythrocytic stages | exo-erythrocytic stages



piroplasms



trypanosomes



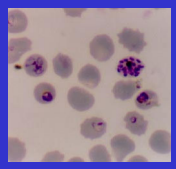
haemosporidia

microfilaria

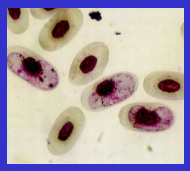
14

Intra-cellular parasites

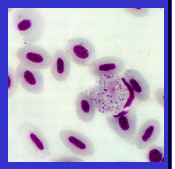
Plasmodium



Haemoproteus

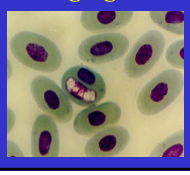


Leucocytozoon

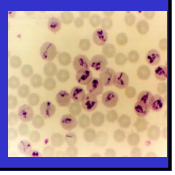


Hepatozoon

Haemogregarina



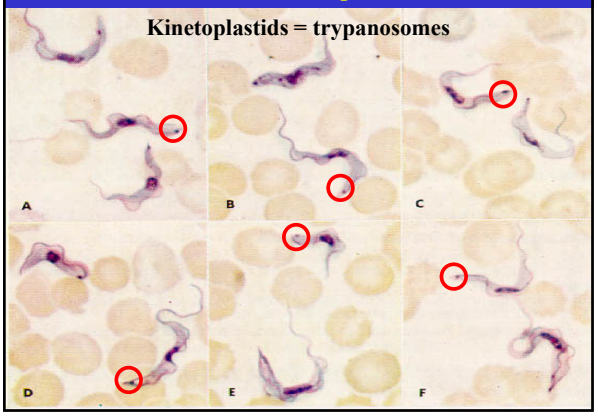
Babesia



15

Extra-cellular parasites

Kinetoplastids = trypanosomes



16

Extra-cellular parasites

Heartworm microfilariae

17

Diagnostic haemo-parasitology

<u>Direct</u> demonstration of parasites

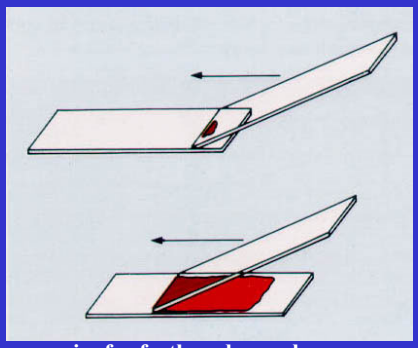
- microscopy - poor sensitivity
- *in vitro* culture - limited availability
- *in vivo* inoculation - host specificity

<u>Indirect</u> demonstration

- symptomatology - nonspecific signs
- clinical parameters - nonspecificity
- serology - cross-reactivity

18

Most microscopic techniques rely on thin blood smear preparation

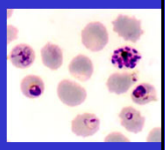


aim for feathered monolayer

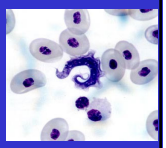
19

Staining smears

Romanowsky stains
- basophilic nuclei
- polychromatic granules



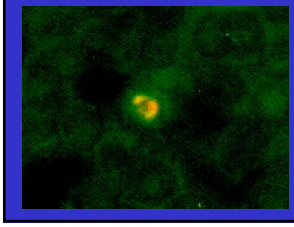
Variants
- Diff-Quik - good for host cells
- Wright - good for host cells
- Giemsa - good for parasites
- Leishman - good for tryps

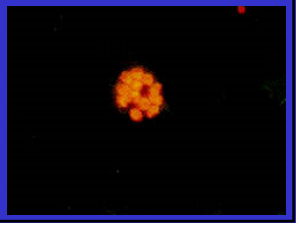


20

Fluorochrome stains

Acridine orange (AO) for malaria microscopy with ultraviolet (UV) light source
UV-excited AO emits photons

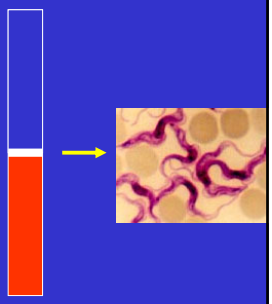




21

Capillary tube centrifugation

- fill haematocrit tube
- plug ends
- centrifuge (10')
- measure PCV
- harvest buffy coat (score and snap)
- examine for motile parasites (tryps/mf)



22

Concentration

Knott's test (for microfilaria)
- clarify blood by hypotonic lysis of RBC (use dilute formalin)
- pellet remaining cells by centrifugation
- visualize by methylene blue staining
- examine wet preparations for mf

23

Summary of diagnostic techniques

Direct detection of intra-erythrocytic stages
- blood smears (thick, thin)
- histochemistry (Romanowsky, fluorochromes)

Direct detection of exo-erythrocytic stages
- capillary tube centrifugation (haematocrit)
- concentration (Knott's test)

Indirect detection - serology

24

Serology

Provides presumptive evidence of infection by demonstration of:

- host antibodies
- parasite antigens

Useful for:

- antemortem diagnosis
- detecting carriers (asymptomatic)
- differentiating acute and chronic infections

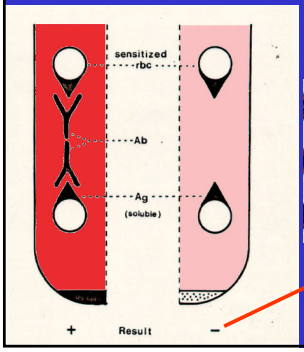
25

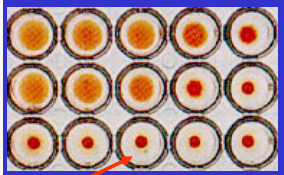
Antigen-Antibody Tests

- precipitin tests
- immunodiffusion
- immunoelectrophoresis
- complement fixation
- agglutination tests
- immunofluorescence
- enzyme immunoassays
- radio immunoassays

26

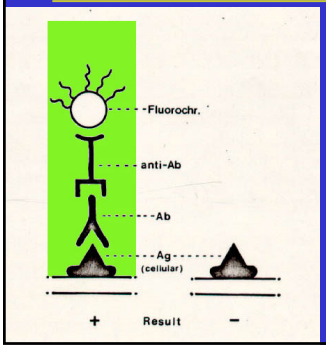
IHAT
Indirect Haem-Agglutination Test

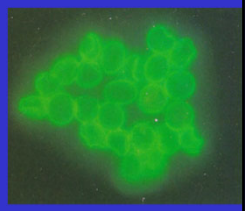




27

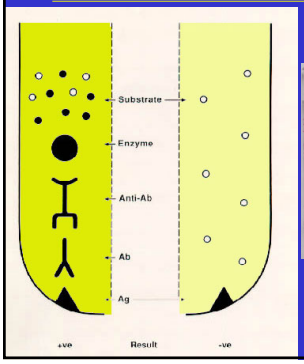
IFAT
Indirect Fluorescent-Antibody Test

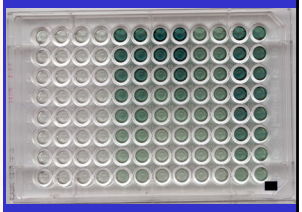




28

ELISA
Enzyme Linked Immuno-Sorbent Assay





29

Ideal characteristics of diagnostic test

- safety consideration
- cost efficient
- time efficient
- long-lived reagents
- ease of performance
- reproducibility
- specificity
- sensitivity
- accuracy

30

EFFICACY OF TEST

SEROLOGY	INFECTION STATUS: infected	INFECTION STATUS: not infected	
positive	A	B	A+B
negative	C	D	C+D
	A+C	B+D	N

ACCURACY = (A+D)/N
SENSITIVITY = A/(A+C)
SPECIFICITY = D/(B+D)

31

Consequences of misdiagnosis

Poor sensitivity
unacceptable number of false negatives

- no treatment → disease progression → death

Poor specificity
unacceptable number of false positives

- unnecessary treatment → side effects → cost

32