PARASITOLOGY

DIAGNOSIS (Dˣ)

Prof Peter O'Donoghue

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DIAGNOSIS OF WHAT?

- INFECTION (presence of parasites)

  - stage: incubation, latent, pre-patent, patent
  - type: cryptic, occult, ectopic…
  - consequences: asymptomatic, subclinical, clinical

- DISEASE (perturbation in structure/function = pathology

  - onset: fast / slow (acute / chronic)
  - duration: transient / prolonged (acute / chronic)
  - manifestations: symptoms / signs
  - severity: mild to fatal

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Diagnosticians

Clinicians / Practitioners (medical / veterinary)

supported by:
- health-carers (e.g. nurses)
- laboratories (e.g. scientists / technicians)

"Dˣ is central intellectual activity of medicine!"

process to turn data about patient into names of diseases
- serves as a guide to action / intervention (Mˣ, Tˣ)
- helps foretell future (prognosis)

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Dˣ process

Difficult to teach clinical reasoning
- quite intuitive, experiential, reflective…

Nevertheless, begin with basic systematic process:

P	presentation	- main complaint
H	history	- medical, morbid state
D	data	- physical examination
		- laboratory tests

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Knowledge integration

HOST IDENTITY:
- medical
- veterinary

SIGNS OF DISEASE:
- enteric
- vascular
- visceral

SITE OF INFECTION:
- gut
- blood
- tissues

TRANSMISSION CYCLE:
- faecal-oral
- vector-borne
- predator-prey

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Test matrix (samples)

ante-mortem | post-mortem



fomites | fluids | tissues

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Parasitology diagnostic tests

Direct detection of parasites
- macroscopic examination (visible characters)
- microscopy (live/fixed, contrast/stained, fluorescence)
- culture (*in vitro*, *in vivo*, xeno-D^{x})
- imaging (X-ray, ultrasound, CT, MRI)

Indirect indication of disease
- symptomatology (fever, inflammation, wheeze, etc…)

Indirect demonstration of parasite products/host responses
- haematology (FBC, ESR, differential, etc…)
- biochemistry (plasma/serum, liver/muscle/gut, etc…)
- serology (host antibodies, parasite antigens)
- molecular biology (parasite proteins/DNA/RNA)

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Coprology

(working with faeces)

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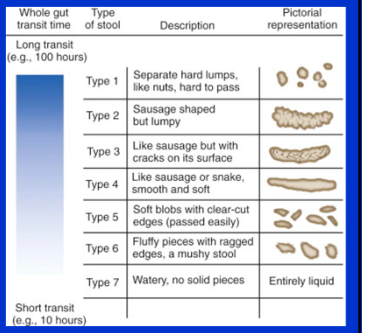
Diarrhoea

- Definition:
  - Imprecise…
  - Fluid stools
  - 3+ bowel movements/day?
  - Increased stool weight?
- Need to consider:
  - frequency, volume, consistency, colour, content, smell



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General Syndromes

- Small intestine diarrhoea (non-inflammatory)
  - leucocytes absent
  - mucus rare
  - blood rare
  - voluminous
  - little pain
  - no fever
- viruses, *Vibrio cholerae, E. coli* (ETEC, EPEC), *Staphylococcus, Bacillus, Clostridium perfringens, Giardia, Cryptosporidium, Isospora, Cyclospora*
- Large intestine diarrhoea(inflammatory)
  - leucocytes present
  - mucus present
  - blood present
  - normal volume
  - severe pain (LLQ)
  - fever may be present
- *Shigella, Salmonella, Yersinia, Campylobacter, Clostridium difficile, E. coli* (EHEC, EIEC), *Aeromonas, Vibrio parahaemolyticus, Entamoeba*

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Diagnostic tests

- macroscopic (characteristics)
- microscopic (cysts, eggs, larvae)
  - permanent stained smears (fixed, stained)
    - trichrome, iron haematoxylin, acid-fast, …
  - wet mount (saline, iodine, methylene blue)
  - concentration techniques
    - sedimentation (formalin-ether, iodine-trichrome)
    - floatation (saturated salt/sugar)(FEC)
- sticky tape test (pinworm)
- endoscopy/colonoscopy
- culture (filter paper, Baermann, …)
- copro-antigen (DFA, EIA, dipstick)
- molecular biology (DNA extraction, PCR)

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Sedimentation / Floatation

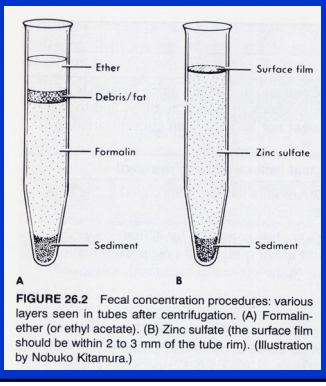

FIGURE 26.2 Fecal concentration procedures: various layers seen in tubes after centrifugation. (A) Formalin-ether (or ethyl acetate). (B) Zinc sulfate (the surface film should be within 2 to 3 mm of the tube rim). (Illustration by Nobuko Kitamura.)

protozoal (oo)cysts
helminth eggs
(filled with E reserves)

heavier than water, thus sediment

buoyant in viscous media, thus float
dep on SG (1.1-1.4)
- $MgSO_4$
- $ZnSO_4$
- sugar/sucrose
- KI

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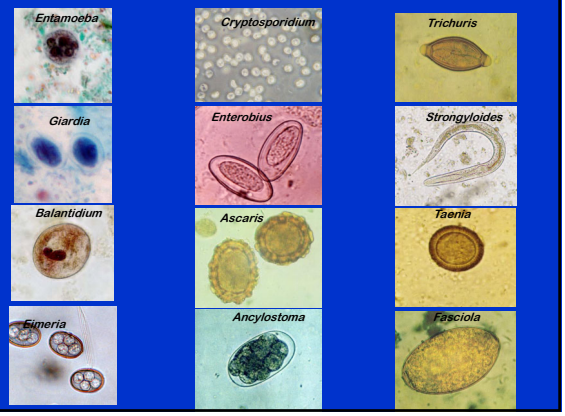
PARASITES IN FAECES



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Haematology

(working with blood)

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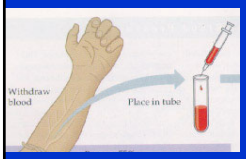
Blood composition



<u>without anticoagulant</u>

serum

clot (cells + fibrinogen)

<u>with anticoagulant</u>

plasma (serum + fibrinogen)
white blood cells
red blood cells

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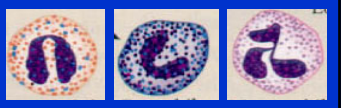
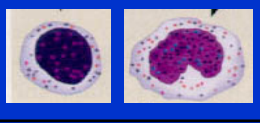
BLOOD CELLS

circulating cells divided into:

- erythrocytes (red blood cells)

- leucocytes (white blood cells)
  - granular
    - eosinophils
    - basophils
    - neutrophils
  - agranular
    - lymphocytes
    - monocytes





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BLOOD EXAMINATION

- wet smear (motile mf, tryps)
- permanent stained blood films
  - thick/thin, Giemsa/haematology
- concentration procedures
  - microhaematocrit centrifugation (buffy coat)
  - Knott's concentration (lyse rbc)
  - membrane filtration (5 um Nucleopore)
  - gradient centrifugation (Hypaque, Ficoll)
- culture (*in vitro, in vivo*)
- immunoserology
  - Ab (CFT, IHAT, IFAT, ELISA, RIA)
  - Ag (immunochromatography, EIA)
- molecular (DNA extraction, PCR amplification)

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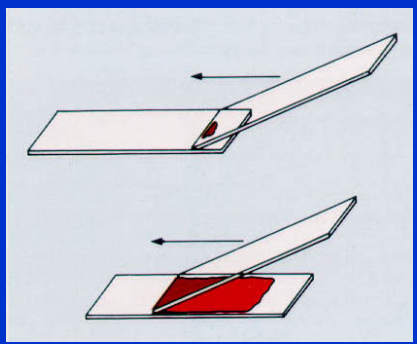
Most microscopic techniques rely on thin blood smear preparation



aim for feathered monolayer

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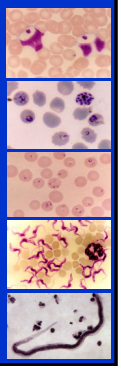
HAEMO-PARASITES

PROTOZOA	meronts	gamonts
Dactylosomatidae	rbc	rbc
Haemogregarinidae	viscera	rbc
Lankesterellidae	RE cells	RE cells
Plasmodiidae	liver	rbc
Haemoproteidae	RE cells	rbc
Leucocytozoidae	RE cells	wbc
Babesiidae	rbc	rbc
Theileridae	wbc	rbc
Trypanosomatidae	extracellular	
NEMATODA		
Onchocercidae	extracellular	



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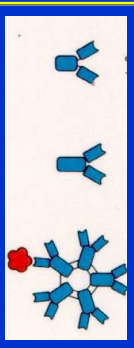
SEROLOGICAL TESTS

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



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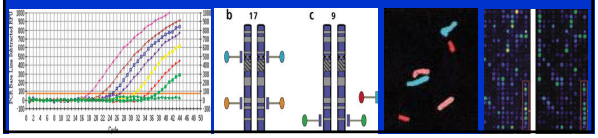
Molecular pathology

- PCR (polymerase chain reaction)
  (incl. RT-PCR, Q-PCR, RAPD, RFLP…)
- FISH (fluorescent *in situ* hybridization)
- SKI (spectral karyotyping imaging)
- DNA microarrays



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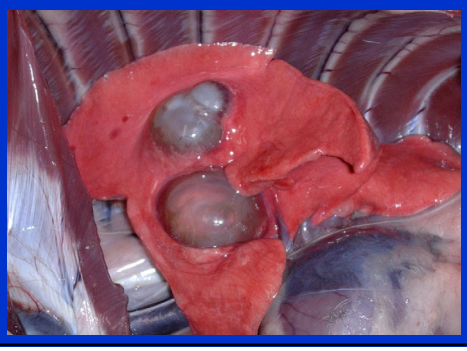
Histology

(working with tissues)



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SAMPLES

Ante-mortem (host alive)
- tissue biopsies
- skin scrapings
- aspirates
- sputum
- swabs

Post-mortem (host dead/euthanized)
- any tissue/organ/fluid
- worm counts (GIN)
- gut digest (immature/hypobiotic)
- lung/perfusion (lungworms)
- brain smears (piroplasms)….

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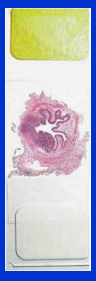
PROCEDURES

- gross pathology (macroscopic)
- histopathology (microscopic)
  - frozen/fixed sections
  - histochemical staining
  - immuno-labelling
- concentration
  - luminal content (counts, …)
  - tissue digest (larvae, arthropods)
- culture (*in vitro, in vivo*)
- molecular (extract DNA, PCR)



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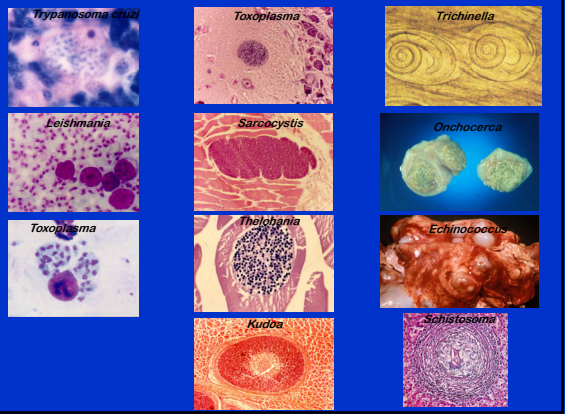
PARASITES IN TISSUES



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Clinical Review

Site	Symptoms	Transmission	PROTOZOA	HELMINTHS	ARTHROPODS
Gut	diarrhoea, blockage, anaemia	faecal-oral	amoebae diplomonads coccidia ciliates	round-, pin-, whip-,thread-, hook-worms tapeworms enteric flukes	-
Blood	anaemia, fever, ischaemia	vector-borne	trypanosomes haemosporidia piroplasms	filiarial worms blood flukes	-
Tissues	lesions dysfunction inflammation	predator-prey	cyst-forming coccidia microspora	hydatids cysticerci liver flukes *Trichinella*	-
- skin	lesions	direct	-	-	flies fleas lice mites ticks

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Ideal characteristics of diagnostic test

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

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Sensitivity

Definitions:
1. the state or quality of being sensitive
2. the smallest concentration of a substance that can be reliably measured by a given analytical method
3. the probability that a person having a disease will be correctly identified by a clinical test

- not how few parasites can be detected (ng/mL, parasitaemia…)
  ⇒ limit of detection (LOD)
- diagnostic test sensitivity is a measure of inclusion (true positive rate)

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Specificity

Definitions:
1. the quality or state of being specific
2. the probability that a person who does not have a disease will be correctly identified by a clinical test

- not how specific a test is for a parasite genus/species/strain/serotype/genotype
  ⇒ test cross-reactivity (presence/absence)
- diagnostic test specificity is a measure of exclusion (true negative rate)

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EFFICACY OF TEST

		INFECTION STATUS: infected	INFECTION STATUS: not infected	
TEST	positive	A true +	B false +	A+B
	negative	C false -	D true -	C+D
		A+C	B+D	N

TEST ACCURACY = (A+D) / N
TEST SENSITIVITY = A / (A+C)
TEST SPECIFICITY = D / (B+D)
POSITIVE PREDICTIVE VALUE = A / (A+B)
NEGATIVE PREDICTIVE VALUE = D / (C+D)

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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

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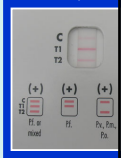
What is acceptable?

Table 5. **Trial I. comparison of prediction of parasitaemia by ICT, slide and reported symptoms in 334 patients, and axillary temperature in 244 patients with parasitaemia, using parasitaemia detected by ICT and/or slide as standard[a]**

Test/symptom	Sensitivity (%)		Specificity (%)		PPV[b] (%)		NPV[c] (%)	
ICT	97.9	(94.5–99.3)[d]	–	–	–	–	97.2	(92.5–99.1)
Slide (restained)	74.9	(68.1–80.7)	–	–	–	–	73.9	(66.9–79.9)
Slide (local)	60.0	(52.7–66.9)	–	–	–	–	64.1	(57.2–66.9)
Fever (F)	95.4	(91.1–97.7)	16.5	(11.0–24.0)	61.6	(55.8–67.1)	71.9	(53.0–85.6)
Chills (C)	86.2	(80.3–90.5)	38.8	(30.8–47.5)	66.4	(60.2–72.1)	66.7	(55.2–76.5)
Headache (H)	79.5	(73.0–84.8)	17.3	(11.6–24.8)	57.4	(51.3–63.3)	37.5	(26.0–50.5)
F + C	82.6	(76.3–87.5)	47.5	(39.0–56.1)	68.8	(62.4–74.6)	66.0	(55.8–75.0)
F + H	74.9	(68.1–80.7)	32.4	(24.8–40.9)	60.8	(54.3–67.0)	47.9	(37.6–58.4)
F + C + H	66.2	(59.0–72.7)	54.7	(46.0–63.1)	67.2	(60.0–73.7)	53.5	(45.0–61.9)
F + (C +/or H)	91.3	(86.2–94.7)	25.2	(18.4–33.4)	63.1	(57.2–68.7)	67.3	(52.8–79.3)
Axilliary temperature ⩾ 37.0 °C	67.5	(58.4–75.5)	45.8	(36.8–55.1)	56.1	(47.7–64.1)	57.9	(47.3–67.8)
Axilliary temperature ⩾ 37.5 °C	35.8	(27.5–45.0)	86.2	(78.7–91.4)	71.0	(57.9–81.4)	58.6	(51.3–65.6)
Axilliary temperature ⩾ 38.0 °C	23.6	(16.6–32.2)	93.3	(86.9–96.9)	78.4	(61.3–89.6)	54.4	(47.3–61.3)

[a] Recently treated patients excluded.
[b] PPV = positive predictive value.
[c] NPV = negative predictive value.
[d] Figures in parentheses are 95% confidence limits.

ICT = immuno-chromato-graphic test)



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What is acceptable?

Malaria Antigen Detection - RDTs

Feature	PfHRP-2 tests	pLDH tests
Sensitivity/ Specificity*	Sensitivity 92-100% Specificity 85- 100%	Sensitivity ***P.f.*** 88-98% ***P.v.*** 89-94% Specificity ***P.f.*** 93-99% ***P.v.*** 99-100%
Commercial cost/test**	Approximately US$ 0.60 –1.00	Approximately US$ 2.50
Commercial products	1) PATH falciparum Malaria IC Strip test – Program for Appropriate Technology in Health 2) MAKROmed™ 3) Orchid ®	1) OptiMAL® - Flow, Inc. 2) Binax NOW ®ICT Malaria - Binax, Inc.

* Compared to microscopy, results from multiple studies
** Varies by size of order and vendor

RDT = rapid diagnostic test

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What is acceptable?

Comparison Test	Sample Size SNAP 3Dx Test/Reference Test +/+	−/+	+/−	−/−	Total	Sample Type	Relative Sensitivity and Specificity 95% Confidence Limit	Kappa Statistic
PetChek® Heartworm	152	3	0	157	312	Serum/ Plasma/ Whole Blood	Sen., 98% (95% CL 94%–100%) Spec., 100% (95% CL 97%–100%)	0.98
Heartworm Necropsy	54	8	0	0	62	Serum/ Plasma	Sen., 87% (95% CL 76%–93%)	N/A
E. canis IFA/Western blot	79	1	0	164	244	Serum	Sen., 99% (95% CL 92%–100%) Spec., 100% (95% CL 97%–100%)	0.99
B. burgdorferi IFA/Western blot	171	14	0	170	355	Serum	Sen., 92% (95% CL 88%–96%) Spec., 100% (95% CL 97%–100%)	0.92

Comparison Test	Sample Size SNAP® *Giardia* Test/Reference Test +/+	−/+	+/−	−/−	Total	Sample Type	Relative Sensitivity and Specificity 95% Confidence Limit	Kappa Statistic
Immunofluorescence microscopy	74	4	1	144	223	Fecal	Sen., 95% (95% CL 87%–98%) Spec., 99% (95% CL 96%–100%)	0.95
Microplate ELISA	75	3	0	145	223	Fecal	Sen., 96% (95% CL 88%–99%) Spec., 100% (95% CL 97%–100%)	0.97

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