Diagnostic Parasitology: Blood and Other Body Fluids

Blaine A. Mathison BS, M(ASCP)
Scientist III, Institute for Clinical and Experimental Pathology,
ARUP Laboratories, Salt Lake City, UT
Adjunct Instructor, Department of Pathology, University of Utah,
Salt Lake City, UT

APHL LabCON 2023
Atlanta, GA
March 12, 2023

Disclosures and Conflicts of Interest

- Major (related to this talk)
  - None
- Other
  - American Society for Microbiology (speaker/honoraria; Editorial Board, *Journal of Clinical Microbiology*)
  - Infectious Diseases Society of America (speaker/honoraria)
  - Seegene (speaker/honoraria)
  - Techcyte Inc. (collaborator)
  - Apacor (collaborator)

What can one expect to find on a blood film?

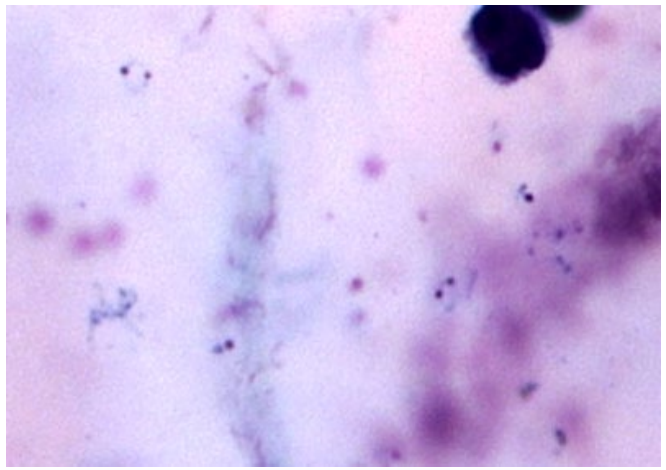
- *Plasmodium* species (malaria)
- *Babesia* species (babesiosis)
- *Trypanosoma* species (Chagas Disease; African sleeping sickness)
- Microfilariae (loiasis, lymphatic filariasis, mansonellosis)
- *Borrelia* spirochetes (relapsing fever)
- *Ehrlichia*/*Anaplasma* morulae
- Intracellular yeast

Types of Blood Films

- Thick Films
  - detection
- Thin Films
  - identification
- Concentration and filtration methods to enhance diagnosis
  - Knott's concentration, Nucleopore filtration (microfilariae)
  - Buffy coat (trypanosomes)

Overview – Thick Film

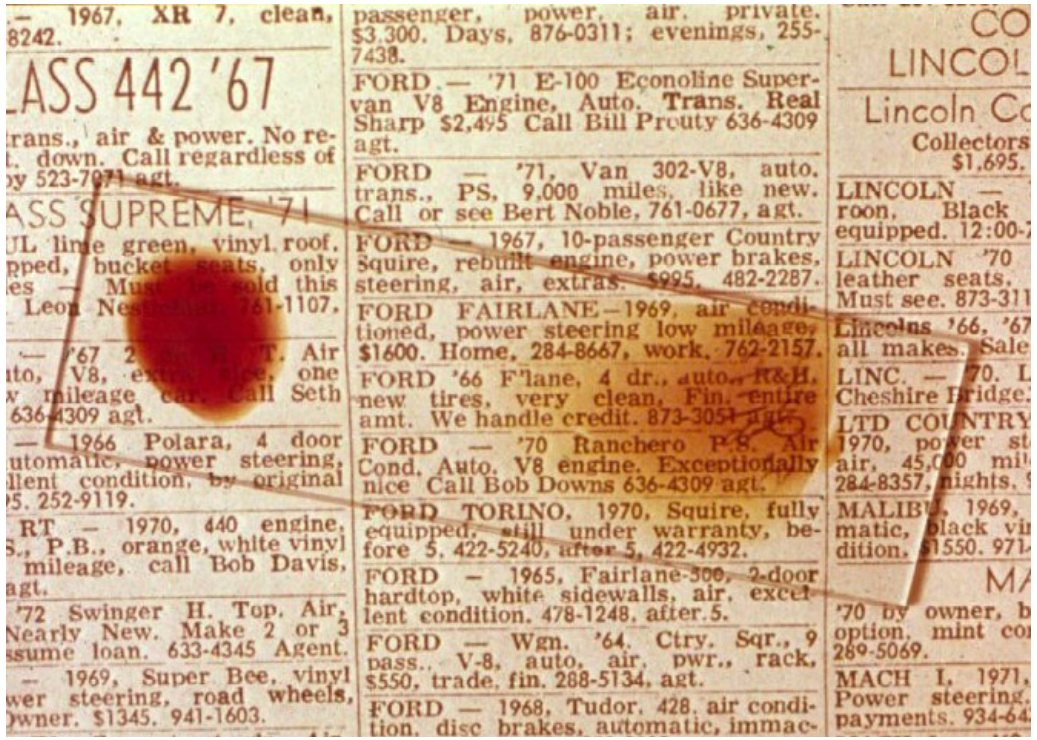
- Made by placing a 2-3 drops of blood on a slide and allowing it to dry
- THE BLOOD IS NOT FIXED IN METHANOL
- When completely dry, it is placed in the malaria stain
- The hypotonic nature of the stain lyses the RBC's, releasing the intracellular parasites



Slide courtesy of Dr. Bobbi Pritt, Mayo Clinic

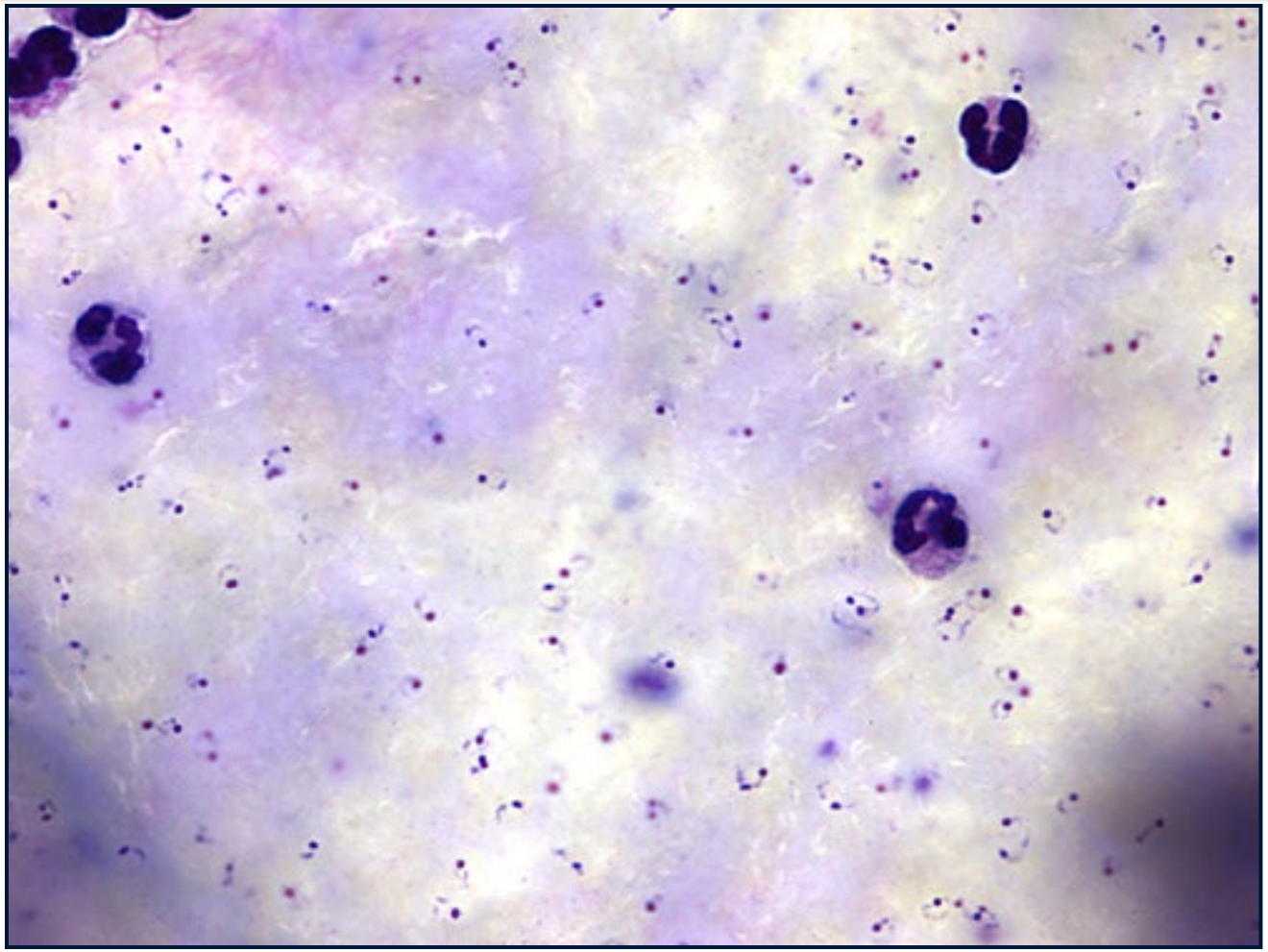
Tips for making a good thick film

- Thick as possible, while still being able to read newsprint through it
  - Will be approx 20-30 cells thick (therefore good for screening)



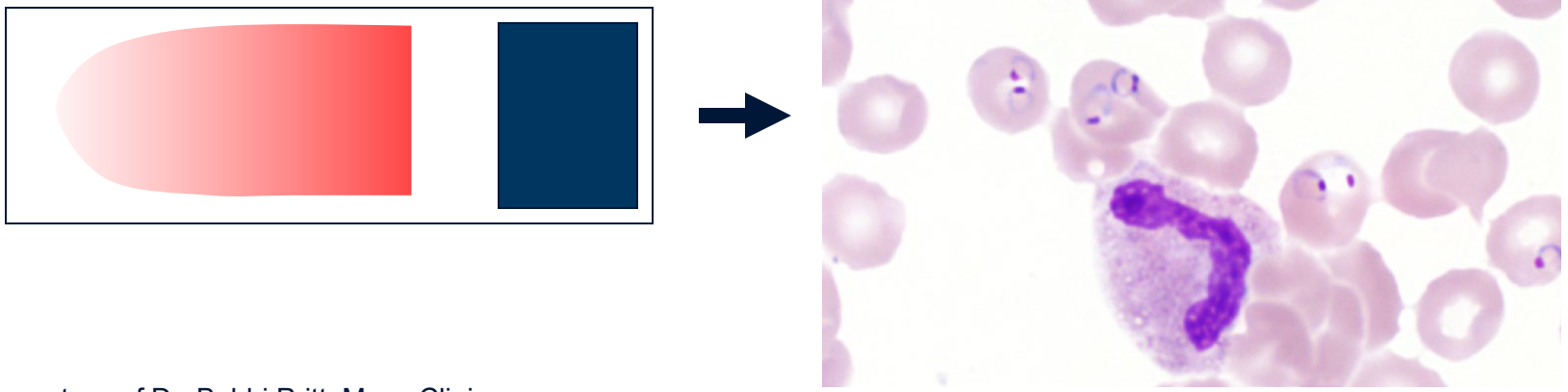
Slide courtesy of Dr. Bobbi Pritt, Mayo Clinic

Thick Films



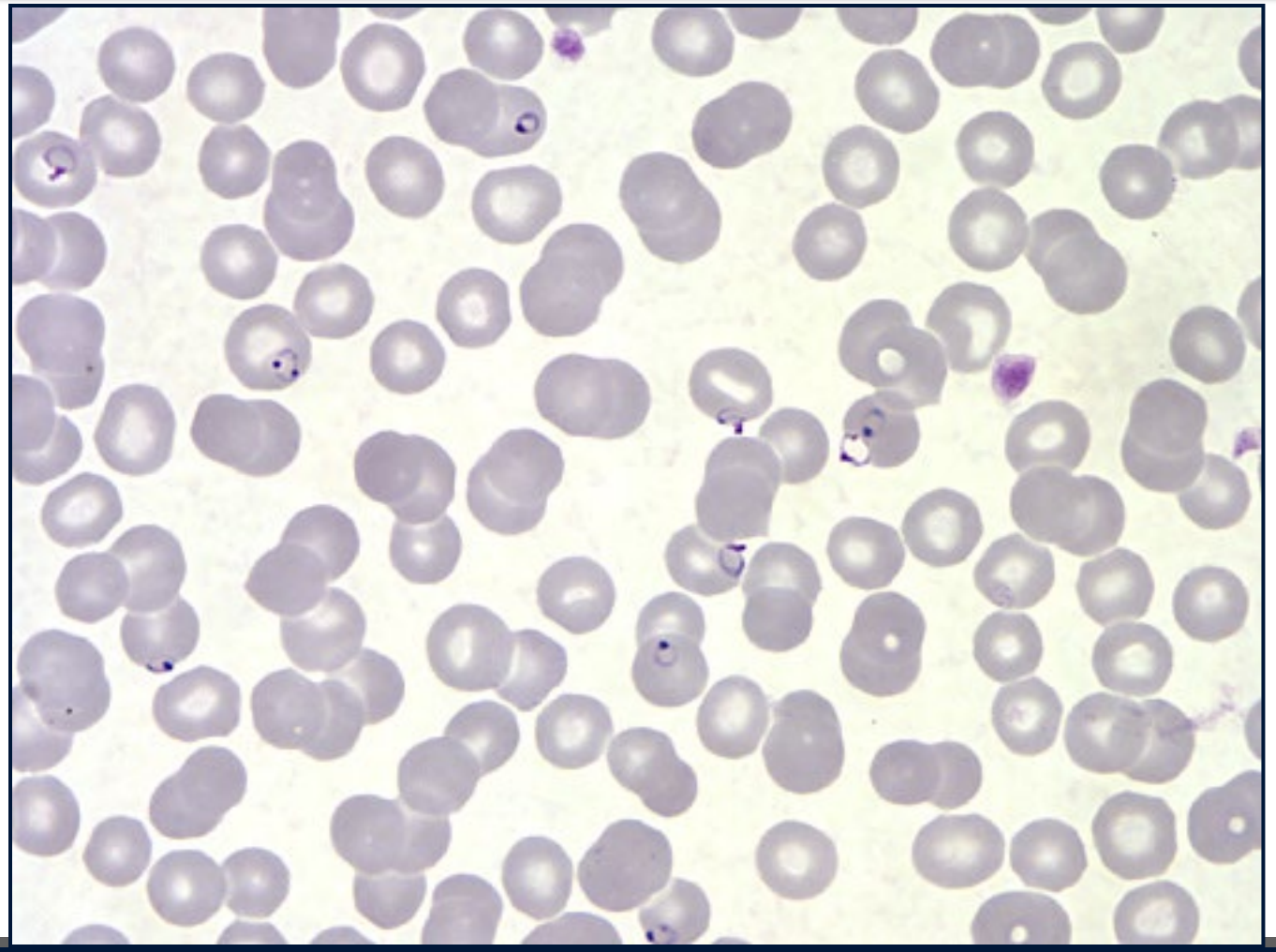
Overview – Thin Film

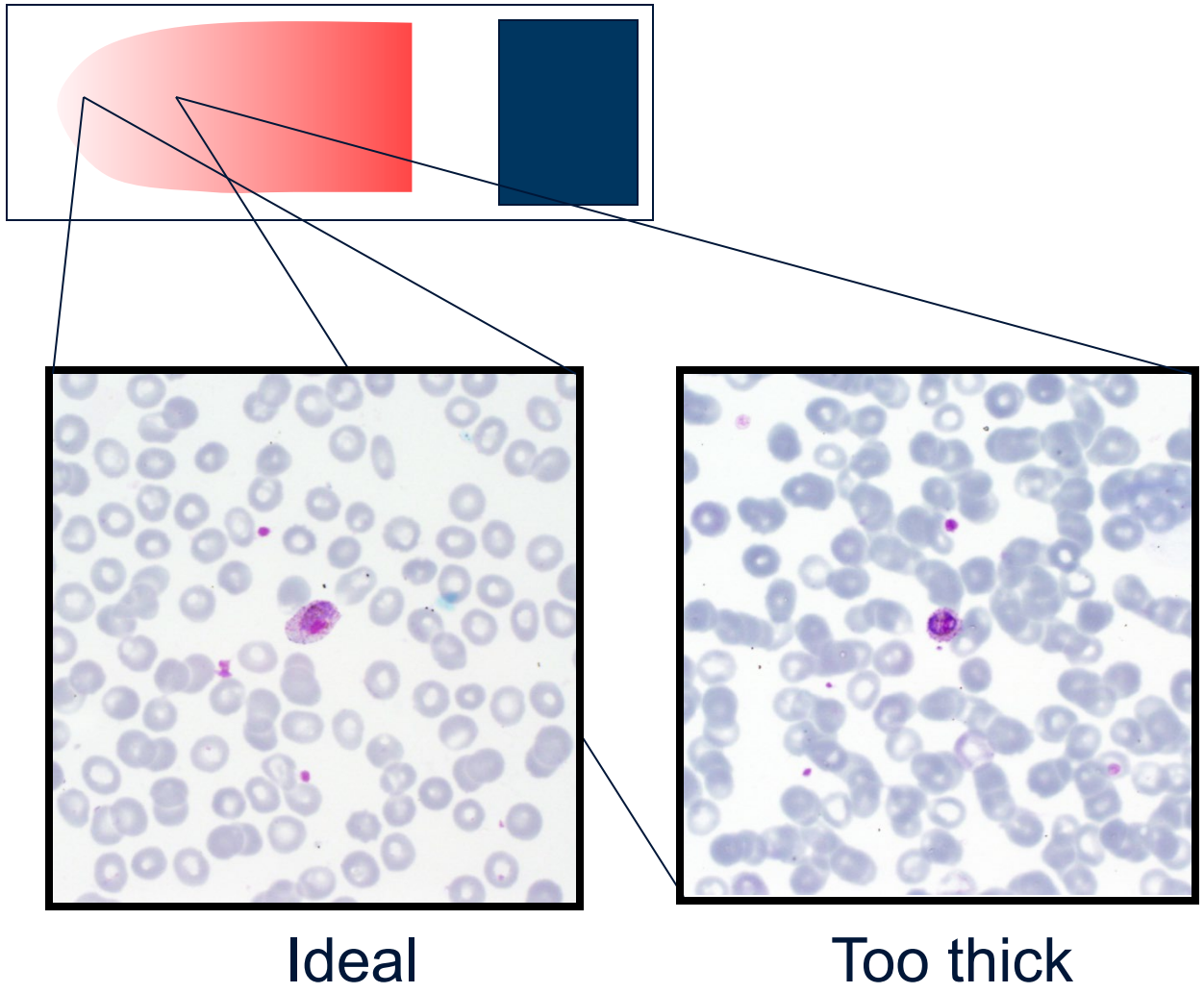
- Made in the same manner as a hematology smear
- Fixed in methanol prior to staining
- Morphology of RBC's remain intact, along with the intracellular parasites



Slide courtesy of Dr. Bobbi Pritt, Mayo Clinic

Thin Films





Slide courtesy of Dr. Bobbi Pritt, Mayo Clinic

Staining

- Giemsa (pH 7.2)
- Wright or Wright-Giemsa
  - Some features (Schüffner's stippling, Maurer's clefts) may not be visible
- Rapid Stains
  - Good for field work

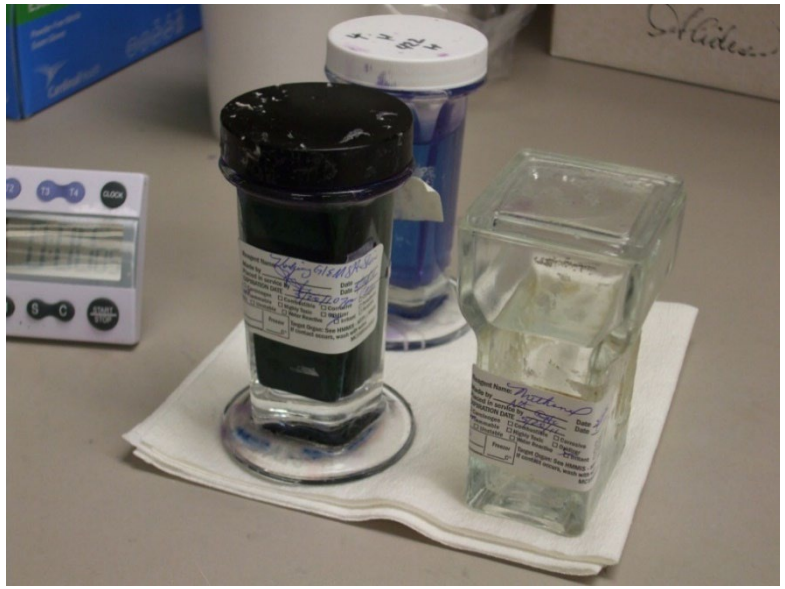


Image courtesy of Dr. Bobbi Pritt, Mayo Clinic

Quantification of Parasites

- Based on Red Blood Cells (thin film)
  - count the parasitized RBCs among 1,000-10,000 RBCs on the thin smear and express the results as % parasitemia (% parasitemia = (parasitized RBCs/total RBCs) × 100.
  - Count multiply-infected RBCs as ‘1’.
  - Do not figure gametocytes into calculations
  - Do not ‘select’ areas—pick random fields regardless of presence/absence of parasites. Pick areas with no overlapping RBCs.
- Based on White Blood Cells (thick film).
  - Tally the parasites against WBCs, until you have counted 500 parasites or 1,000 WBCs, whichever comes first. Express the results as parasites per microliter of blood, using the WBC count if known, or otherwise assuming 8,000 WBCs per microliter blood. Parasites/microliter blood=(parasites/WBCs) × WBC count per microliter<or 8,000>

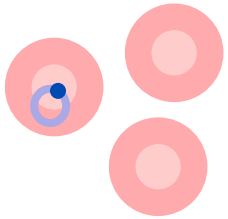
Non-microscopic Diagnostic Methods

- Molecular
  - Becoming increasingly popular, but still often not cost-effective for screening.
- Antigen Detection: Rapid Diagnostic Tests
  - BinaxNOW currently the only FDA-approved RDT for malaria in the US.
  - Used in lymphatic filariasis screening/elimination programs
- Antibody Detection
  - Not generally recommended for clinical diagnosis of malaria; better for epi investigations and trace-back investigations with transfusion-related cases.
  - Babesiosis (also helpful for traceback investigations)
  - Chronic Chagas
  - Lymphatic Filariasis

Slide courtesy of Dr. Bobbi Pritt, Mayo Clinic

Size of Infected RBCs

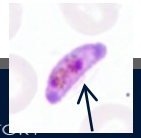
Normal or small



P. falciparum (normal RBCs) or
P. malariae (norm or small RBCs)

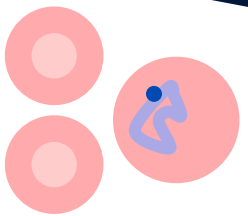
No stippling; maurer's clefts in *P. falciparum* (fewer and larger than stippling)

P. falciparum
- RINGS AND GAMETOCYTES PREDOMINATE
- Small delicate rings
- <1/3 size RBC
- Headphones forms
- Applique forms
- Multiple rings/RBC

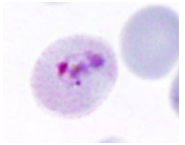


P. malariae
- ALL STAGES PRESENT
- Thick rings
- ≤1/3 size RBC
- Basket, band forms
- Rosette schizonts

Enlarged



P. vivax or
P. ovale



Supporting features:
Stippling, all stages

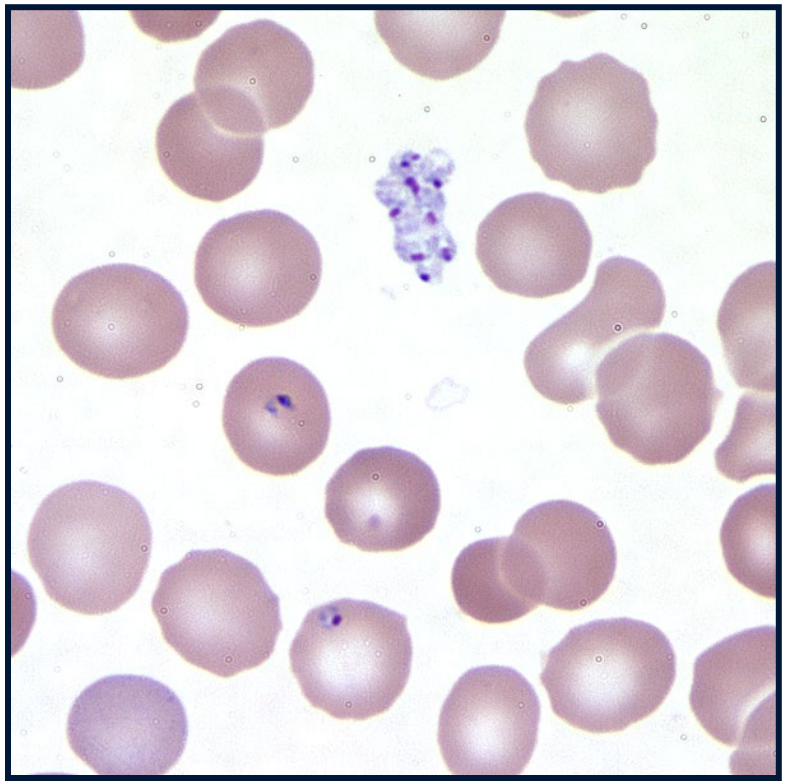
P. vivax
- Grossly ameboid trophs
- >1/3 size RBC
- Schizont with >12 merozoites
- epi

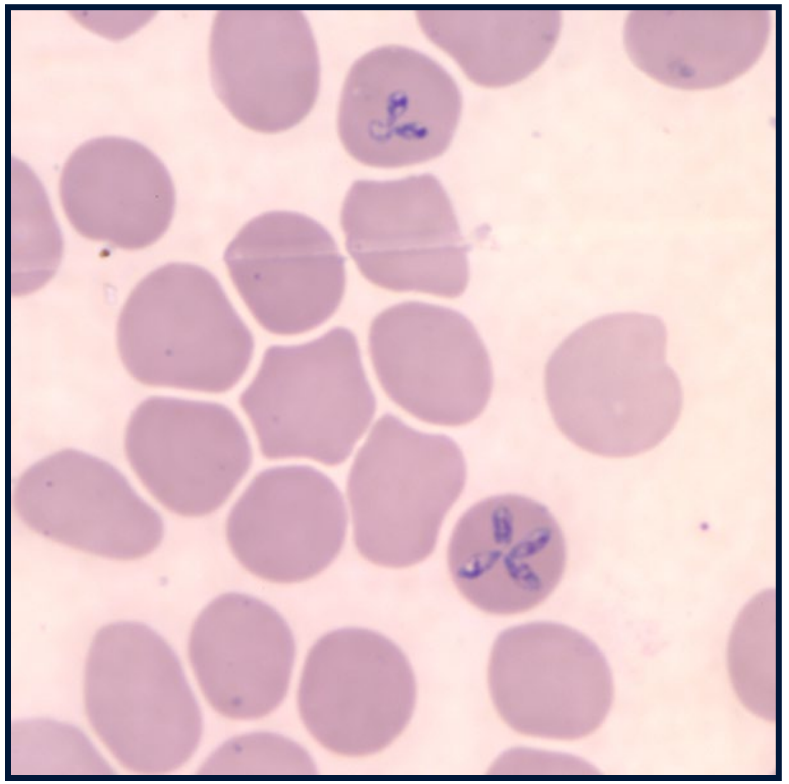
P. ovale
- Troph compact to slightly ameboid
- > 1/3 size RBC
- oval, fimbriated
- Schizont with ≤ 12 merozoites
- epi

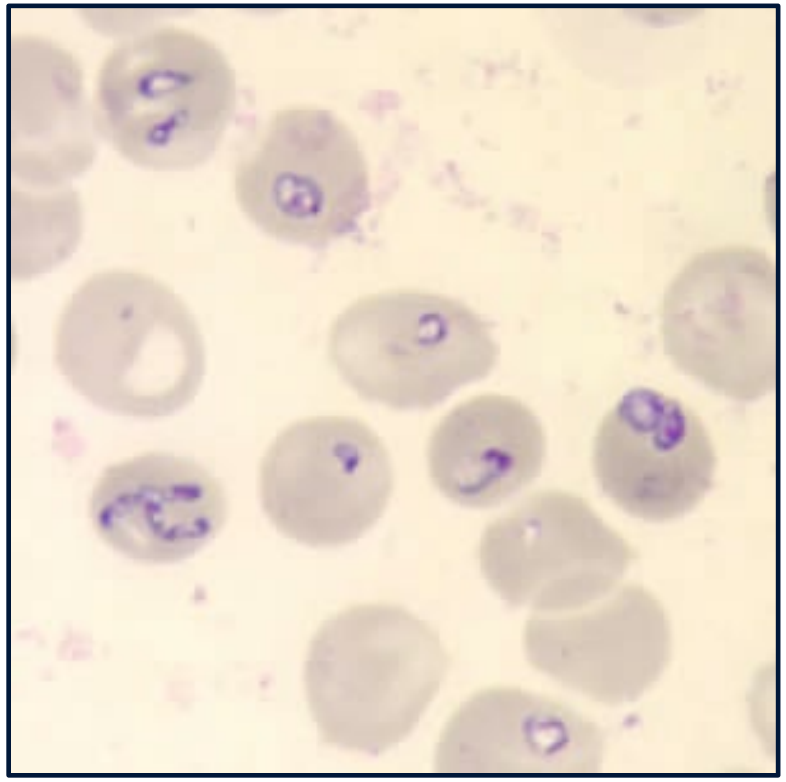
Plasmodium vs. *Babesia*

Feature	*Plasmodium*	*Babesia*
Stages seen in human blood films	Morphologically differentiated into ring-form trophozoites, mature trophozoites, gametocytes, schizonts	Most stages take on some form of a ring; 'Maltese cross'
Extracellular forms present?	No	Yes
Ring morphology	Generally consistent within a specimen	Highly pleomorphic in terms of size, shape, number
Hemozoin pigment produced?	Yes	No
Enlargement of the infected erythrocyte?	Sometimes (*P. vivax*; *P. ovale*)	Never

Babesia spp. in thin smears







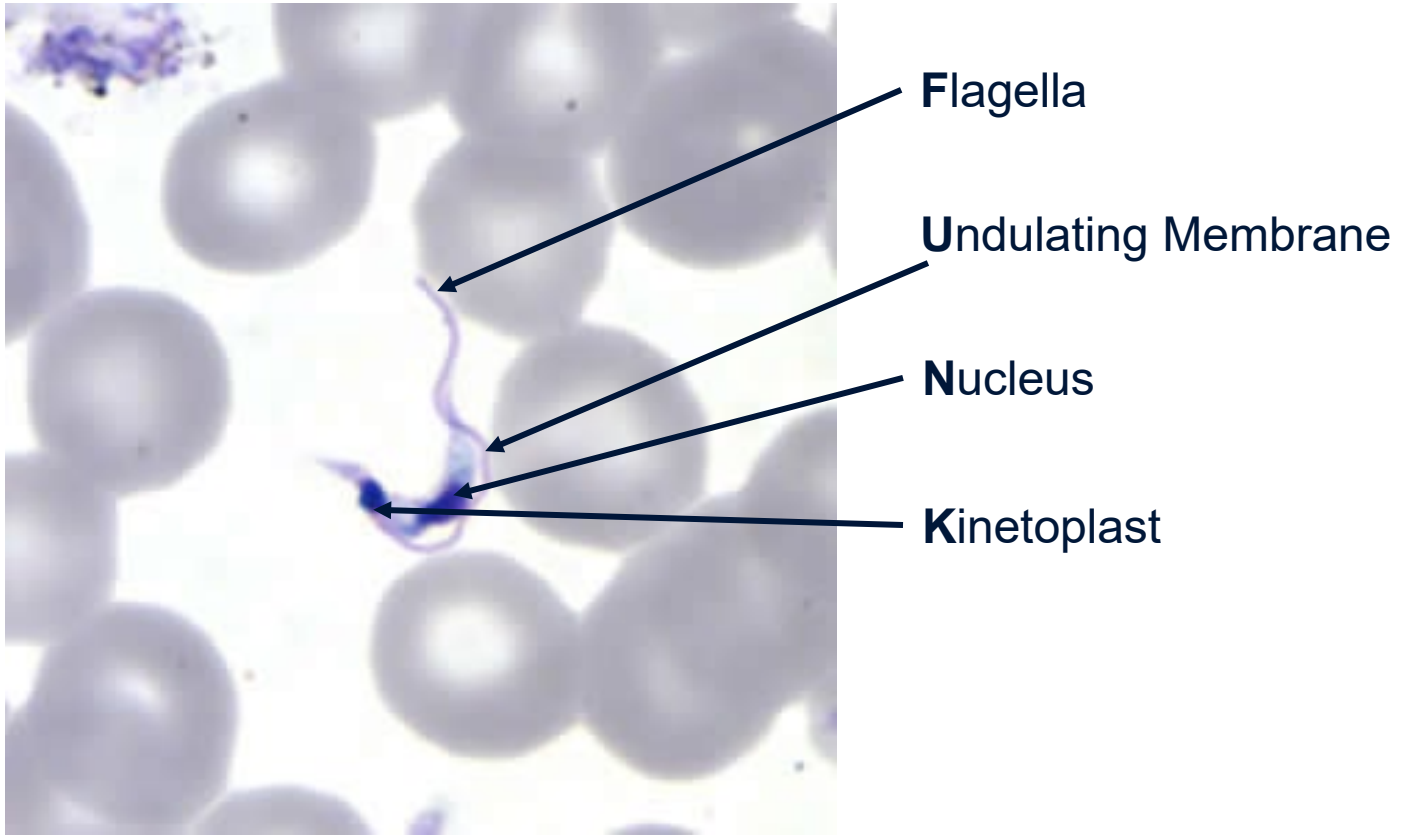
Extraerythrocytic forms

Trypanosomes

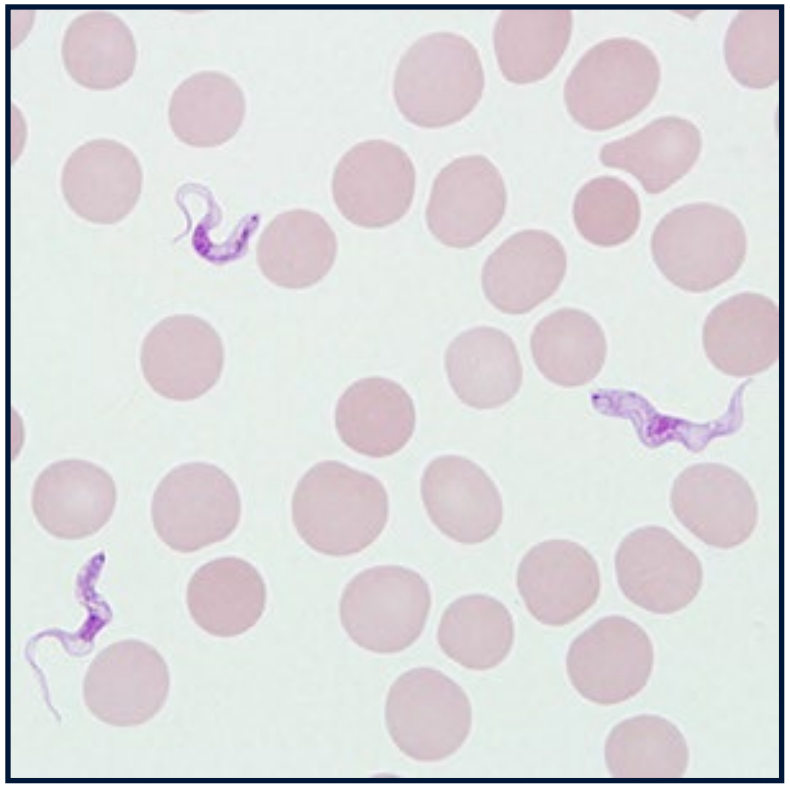
Comparison of Human Trypanosome Species

Feature	*Trypanosoma brucei*	*Trypanosoma cruzi*
Epidemiology	Central Africa	Latin America (so. USA to South America)
Vector-borne transmission	Bite of Tse-tse fly (*Glossina*)	Feces of kissing bug (triatomine bug)
Forms seen in clinical specimens	Trypomastigote (blood, CSF, bone marrow)	Trypomastigote (blood), amastigote (tissues, esp. cardiac muscle)
When to perform a blood film	Acute, chronic	Acute, reactivation
Shape of trypomastigote	Variable	Variable
Kinetoplast size	Small, often pinpoint	Large, often bulging
Dividing trypomastigotes seen in clinical specimens	Yes	No

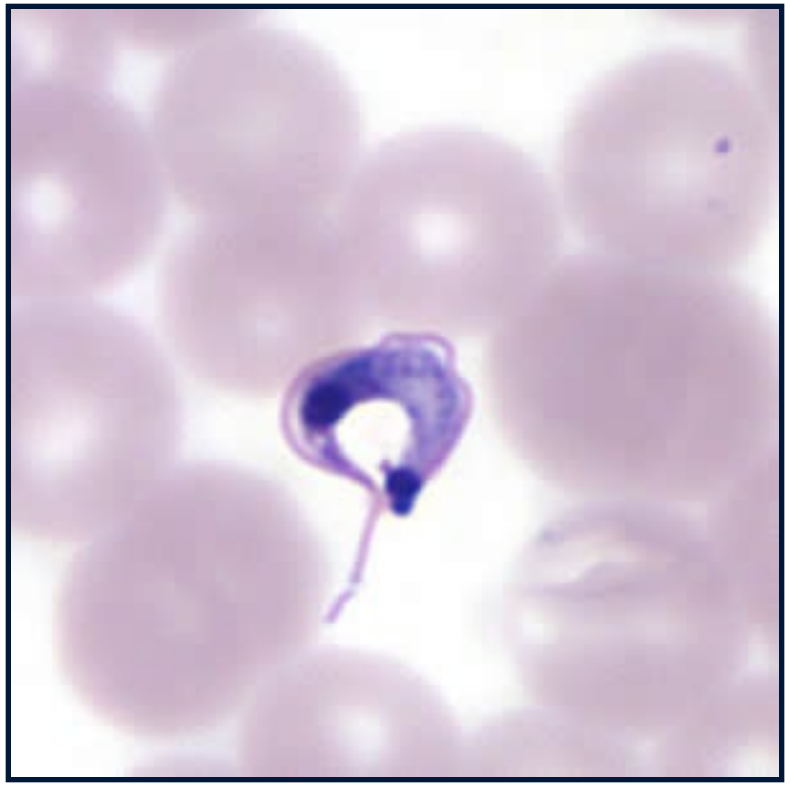
Tryps got the F.U.N.K.



Which Tryp is it?

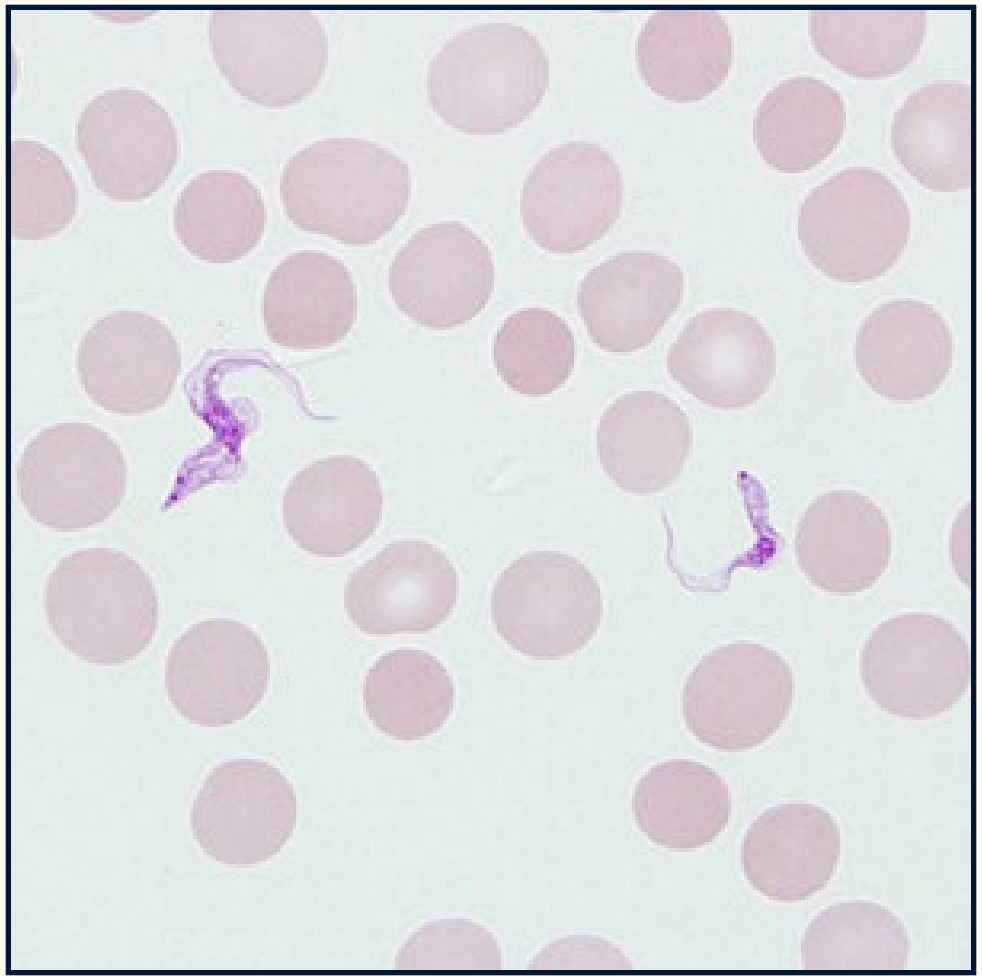


Trypanosoma brucei

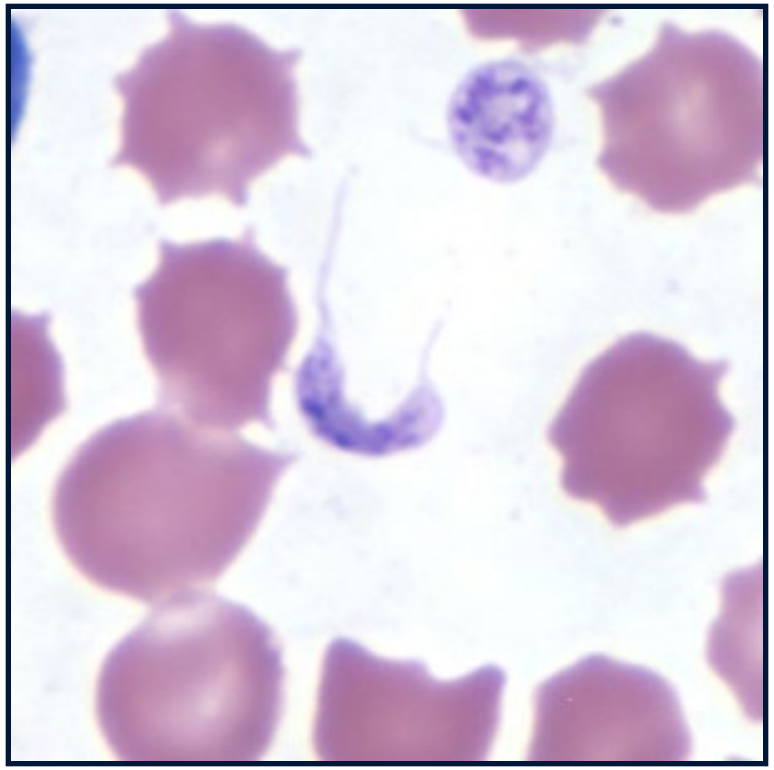


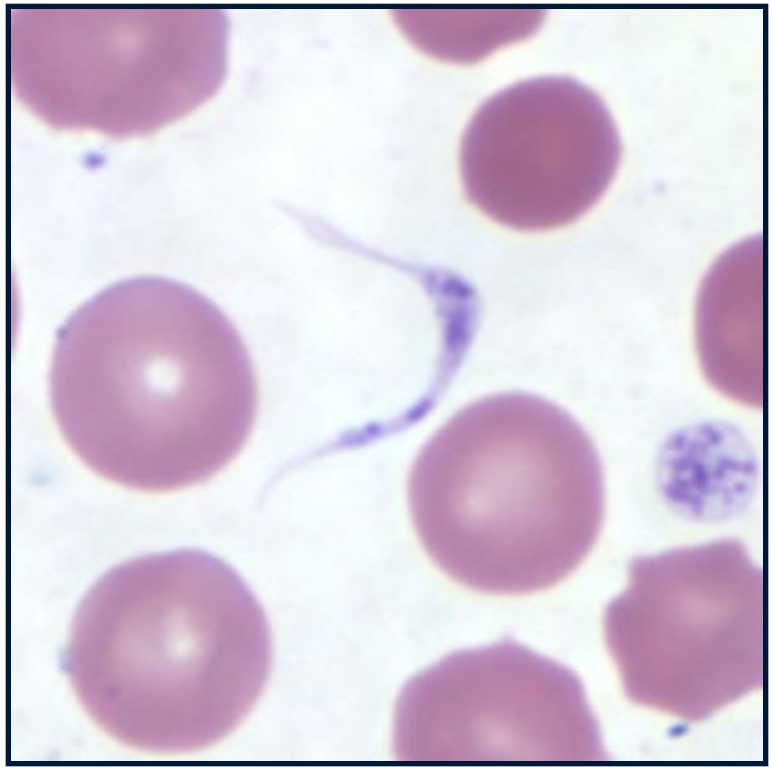
Trypanosoma cruzi

Dividing forms of *Trypanosoma brucei*



Elongated and Degenerating Platelets



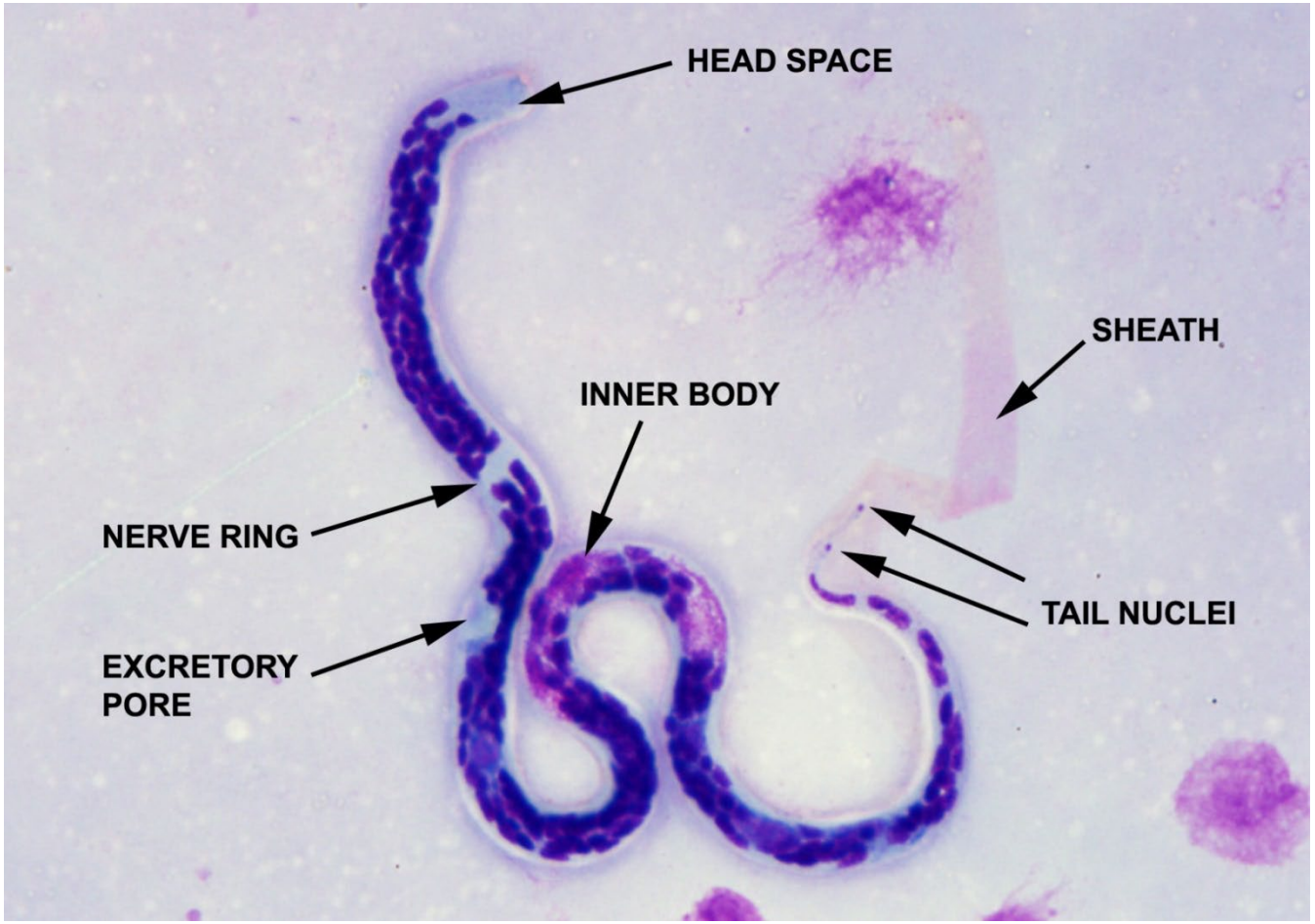


Microfilariae

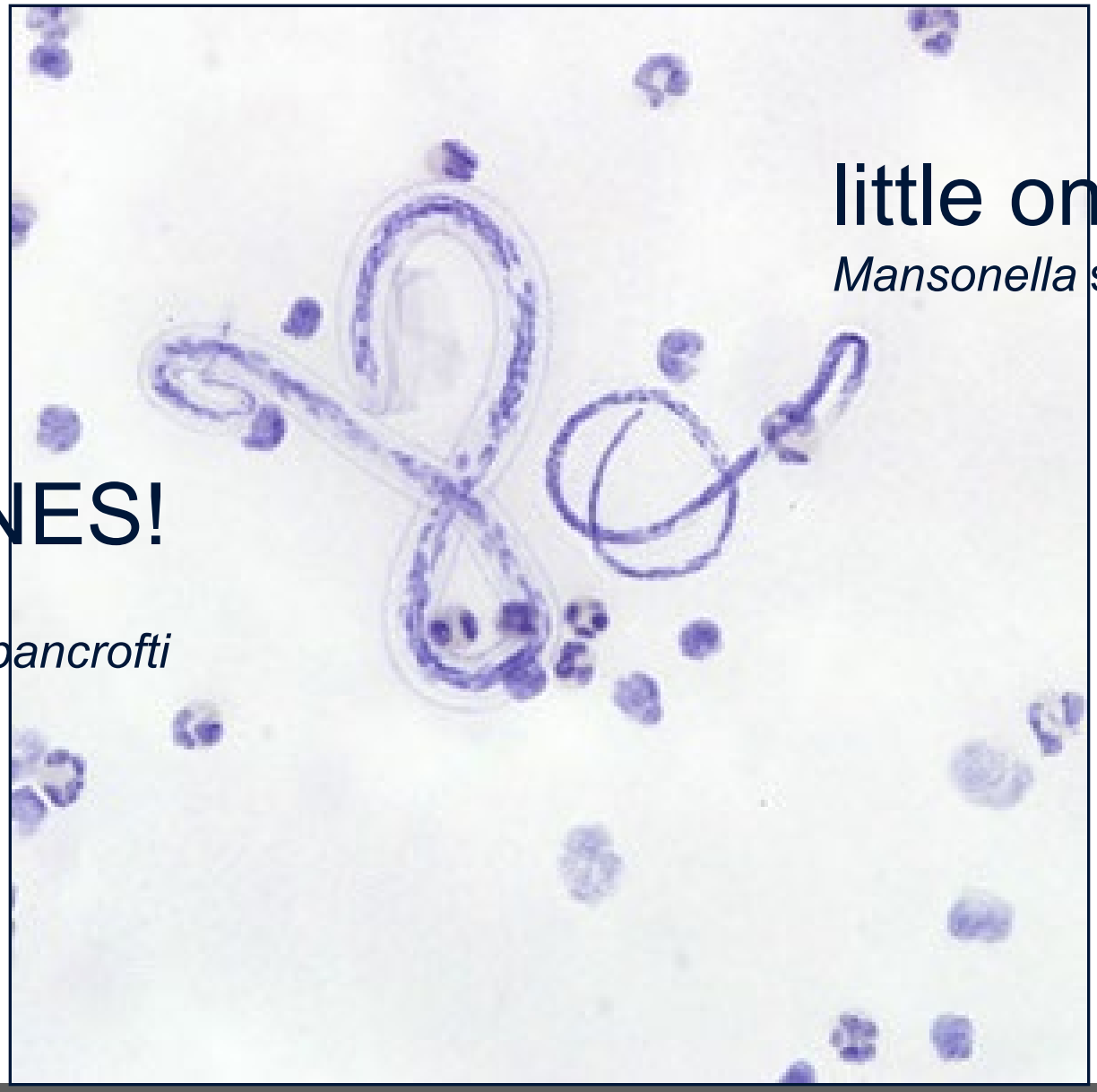
Filariasis – Important Diagnostic Criteria

- Specimen type – blood or skin snips
- Size (approximate or measured lengths).
- Geographic Distribution
- Periodicity
- Sheath – its presence or absence and, when present, color when stained with Giemsa.
- Arrangement of nuclear column, in particular the arrangement of tail nuclei

Anatomy of a Microfilaria



Size



little ones

Mansonella spp.

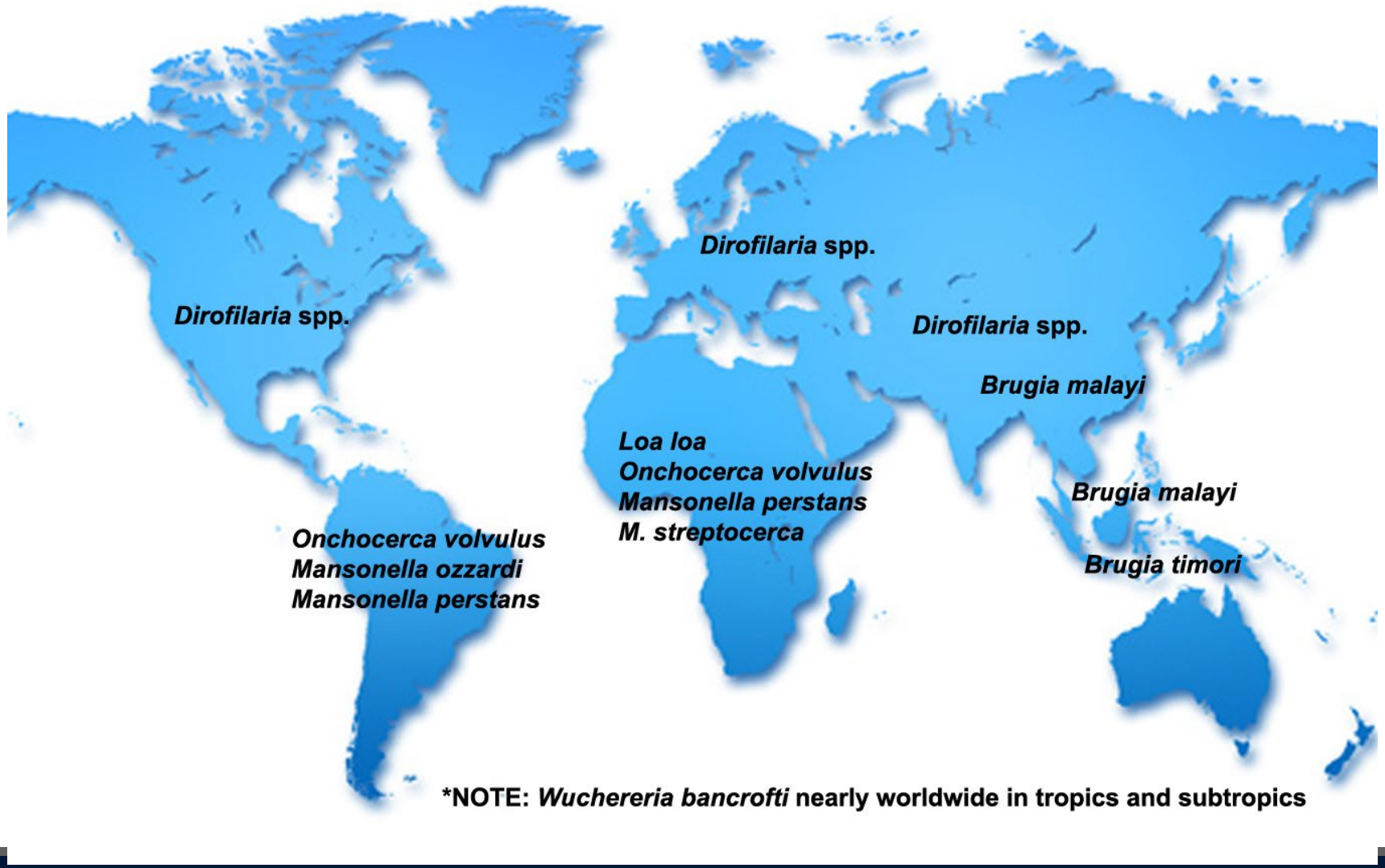
BIG ONES!

Loa loa

Wuchereria bancrofti

Brugia spp.

Geographic Distribution



Periodicity

Periodicity is reflected in the habit of the microfilariae of certain species to be in peripheral blood to match the optimal feeding times of the vector.

- Diurnal periodicity: microfilariae circulate during the day (*Loa*)
- Nocturnal periodicity: microfilariae circulate at night (*Wuchereria**, *Brugia*)
- Aperiodic: no periodicity (*Mansonella*)

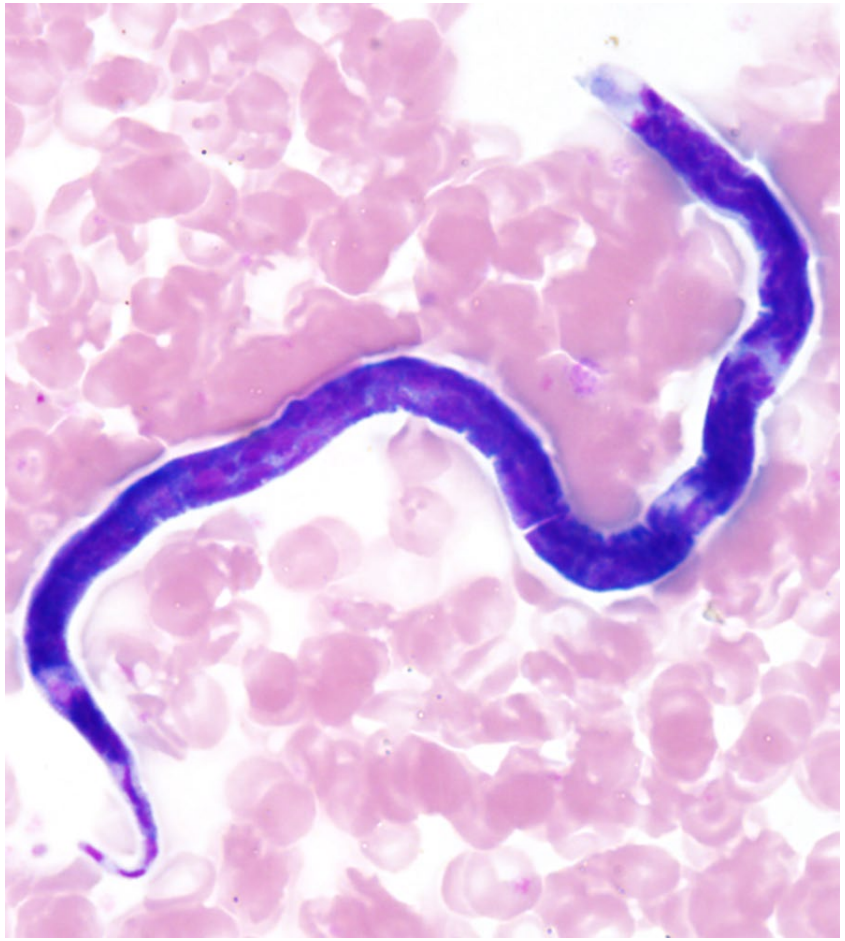
*note: populations of *W. bancrofti* in South Pacific circulate primarily between noon and 6PM.

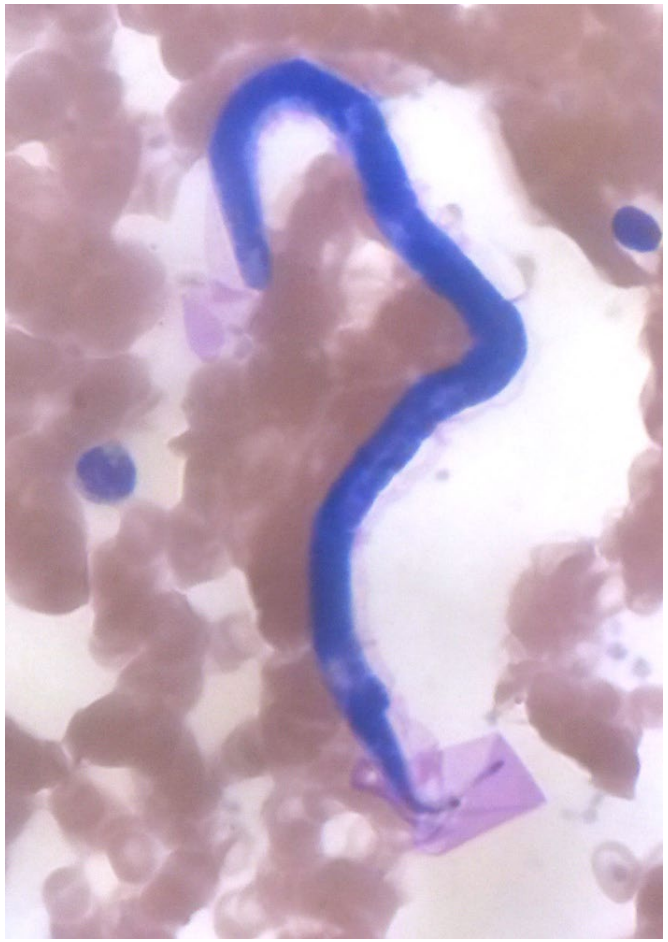
That Pesky Sheath!

The sheath is a membrane surrounding the microfilariae of some filarial nematodes.

- May be present (*Loa*, *Wuchereria*, *Brugia*) or absent (*Mansonella*, *Onchocerca*)
- Absence of sheath in clinical specimen does not mean it is not an unsheathed species!!!!
- May be colorless to hot pink; color is pH-dependent and not strictly a biological phenomenon.

image courtesy of the South Carolina PHL





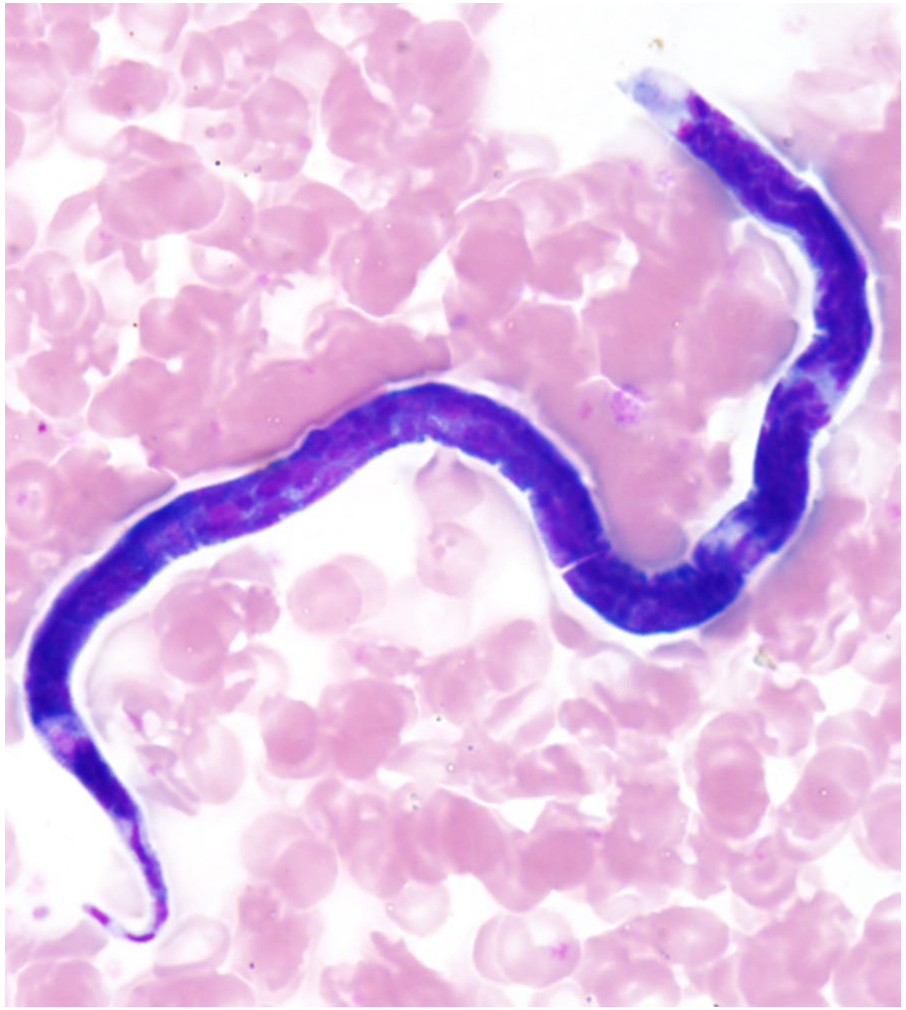
Case 1:

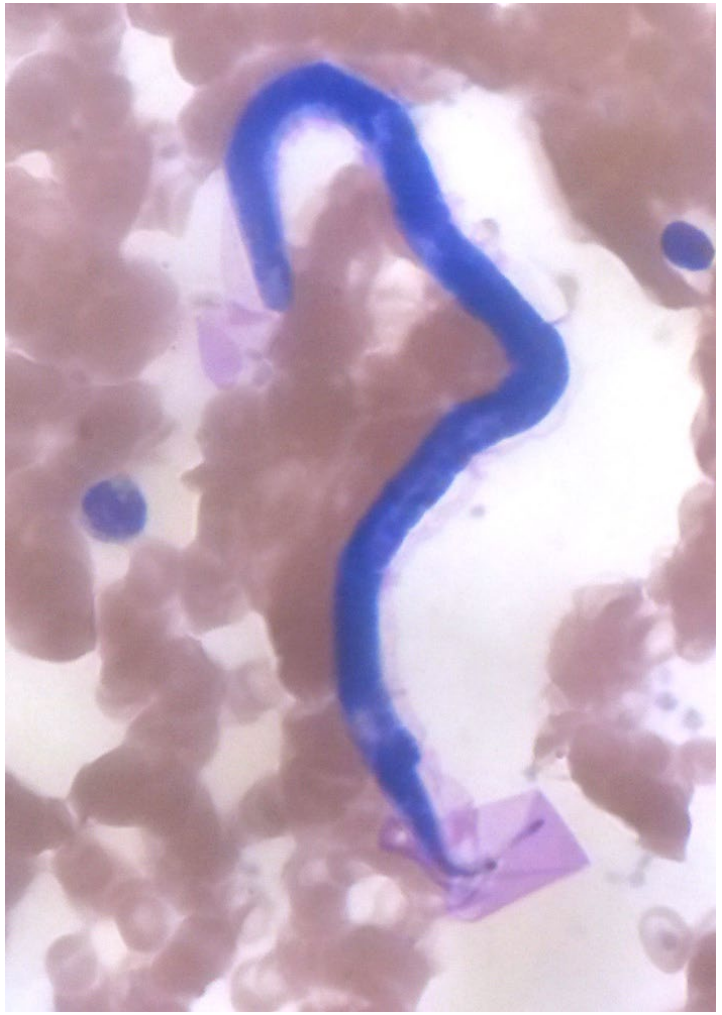
- Size 245 µm
- Travel: Cameroon
- No sheath

Case 2:

- Size 230 µm
- Travel: Gabon, Nigeria, Mozambique
- Pink sheath

image courtesy of the South Carolina PHL

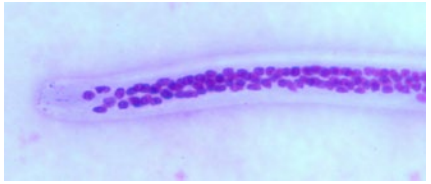




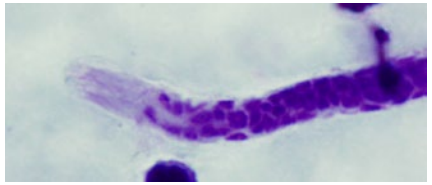
BOTH are *Loa loa*!

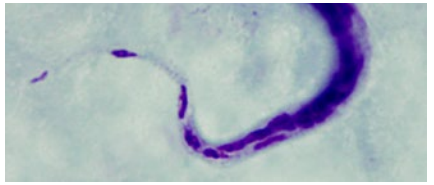
Head and Tail Nuclei of the Microfilariae

Wuchereria bancrofti

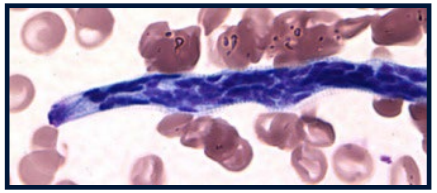


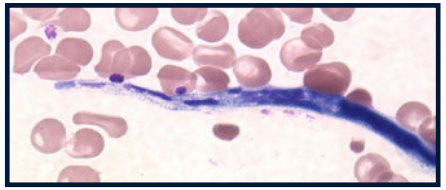
Brugia malayi
B. timori



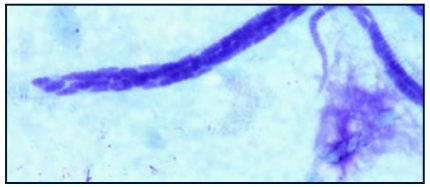


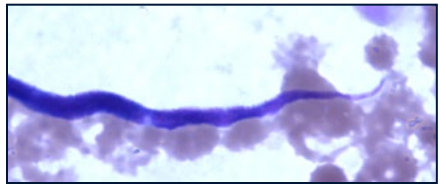
Loa loa



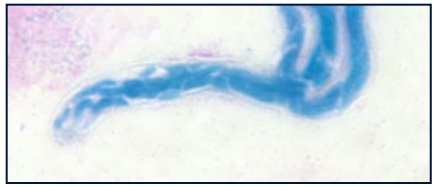


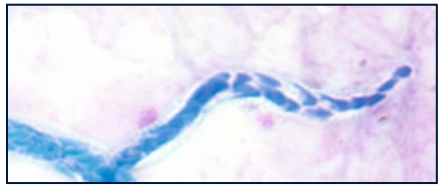
Mansonella ozzardi





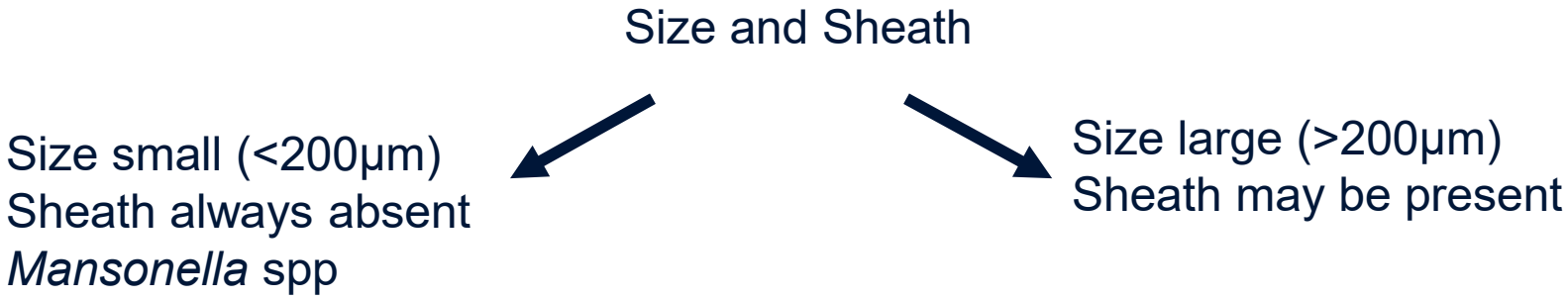
Mansonella perstans





Review: Key to the microfilariae in blood

Review: Key to the microfilariae in blood



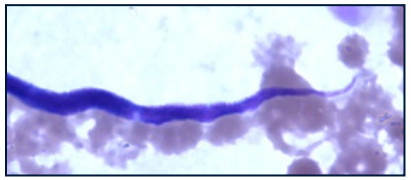
Review: Key to the microfilariae in blood

Size and Sheath

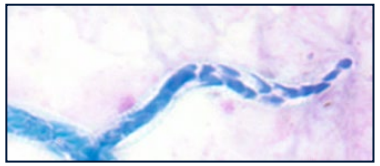
Size small (<200µm)
Sheath always absent
Mansonella spp.

Size large (>200µm)
Sheath may be present

Tail tapered, pointed; anucleate
M. ozzardi



Tail blunt; nuclei to the tip
M. perstans

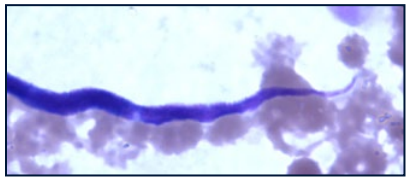


Review: Key to the microfilariae in blood

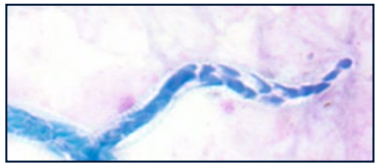
Size and Sheath

Size small (<200µm)
Sheath always absent
Mansonella spp.

Tail tapered, pointed; anucleate
M. ozzardi



Tail blunt; nuclei to the tip
M. perstans



Size large (>200µm)
Sheath may be present

Tail anucleate
Wuchereria bancrofti

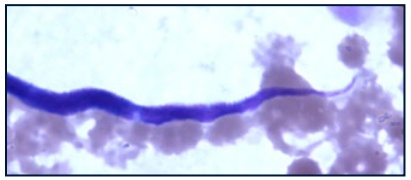
Tail with nuclei at or to the tip

Review: Key to the microfilariae in blood

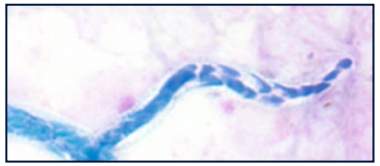
Size and Sheath

Size small (<200µm)
Sheath always absent
Mansonella spp.

Tail tapered, pointed; anucleate
M. ozzardi



Tail blunt; nuclei to the tip
M. perstans

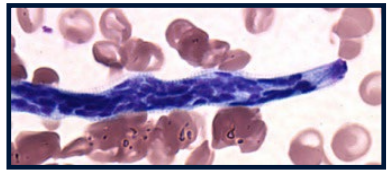


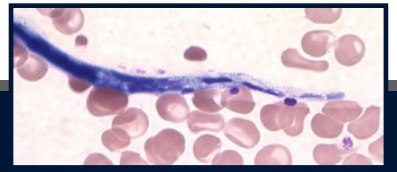
Size large (>200µm)
Sheath may be present

Tail anucleate
Wuchereria bancrofti

Tail with nuclei at or to the tip

Tail with irregular nuclei to tip; short head space
Loa loa



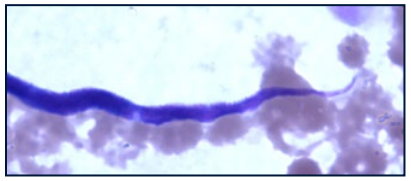


Review: Key to the microfilariae in blood

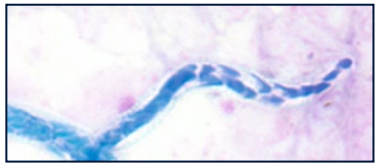
Size and Sheath

Size small (<200µm)
Sheath always absent
Mansonella spp.

Tail tapered, pointed; anucleate
M. ozzardi



Tail blunt; nuclei to the tip
M. perstans

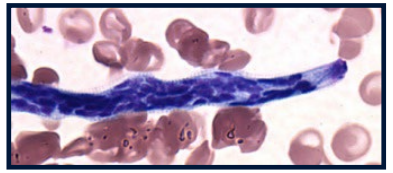


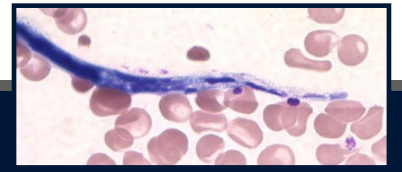
Size large (>200µm) Sheath may be present

Tail anucleate
Wuchereria bancrofti

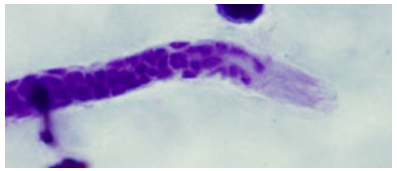
Tail with nuclei at or to the tip

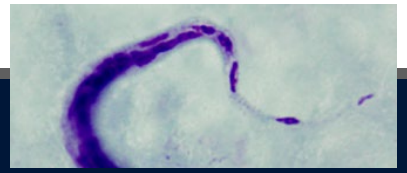
Tail with irregular nuclei to tip; short head space
Loa loa





Tail with terminal and subterminal nuclei separated by gap; long head space
Brugia spp.



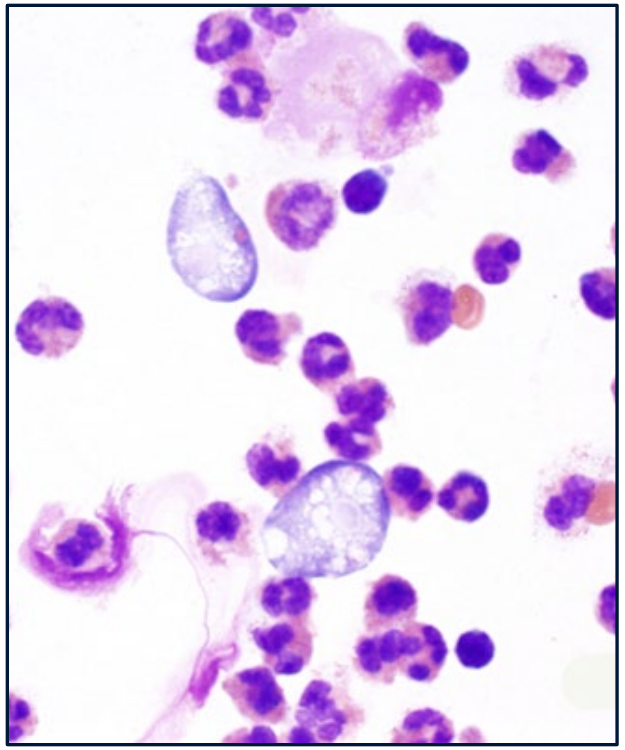


Other Body Fluids

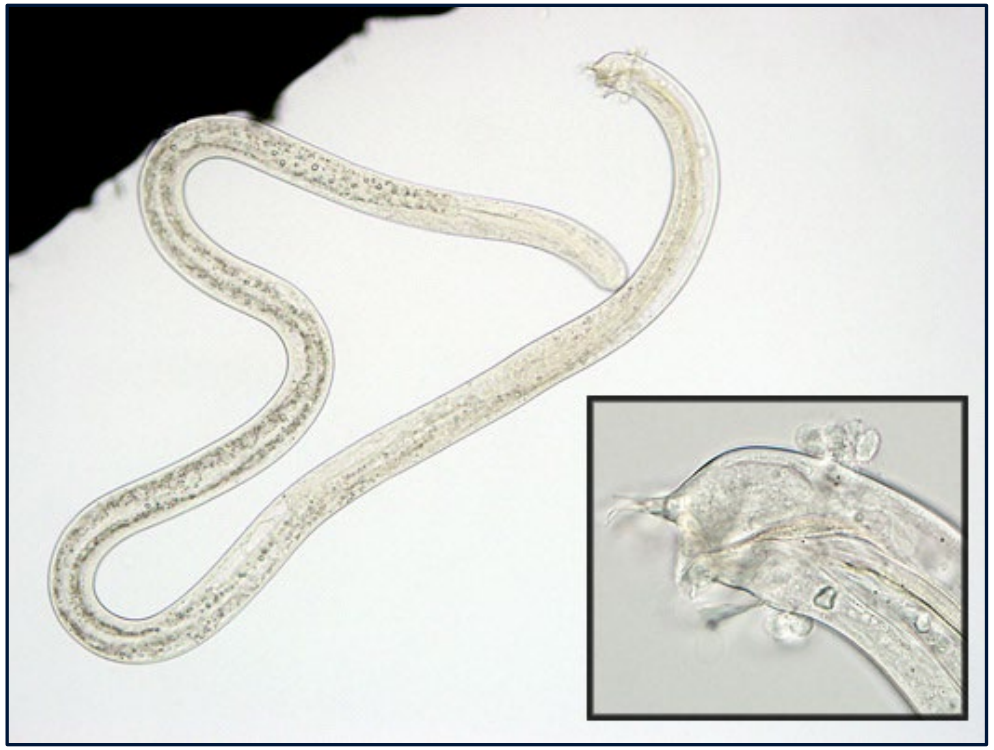
Cerebral Spinal Fluid

- *Naegleria fowleri* (trophozoites)
- *Trypanosoma brucei* (trypomastigotes)
- *Strongyloides stercoralis* (L3 larvae; hyperinfection; rare)
- *Angiostrongylus cantonensis* (L4 larvae, sub-adults; rare)
- *Trichomonas vaginalis* (trophozoites; rare)
- CSF can be used for some antibody detection assays and PCR
  - Free-living amebic infections
  - Neurocysticercosis
  - angiostrongyliasis

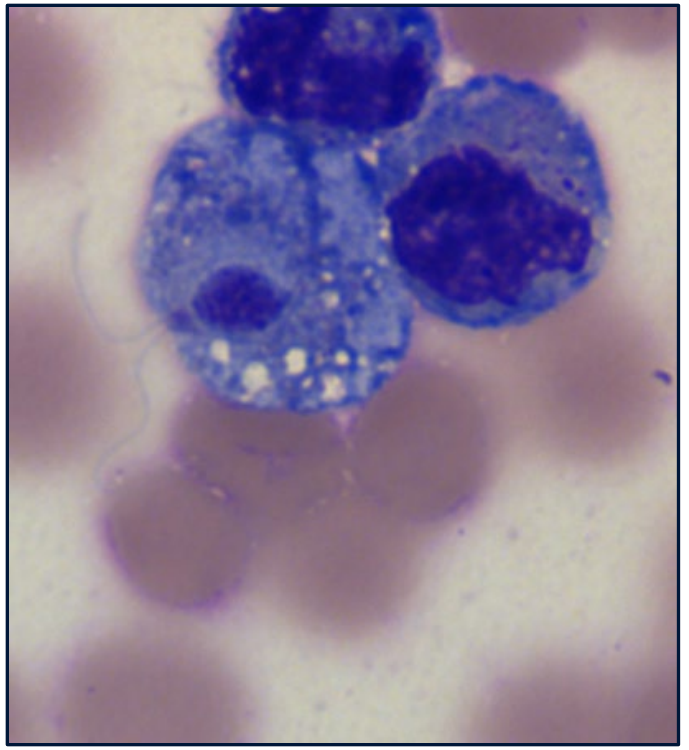
Parasites in CSF



Naegleria fowleri



Angiostrongylus cantonensis



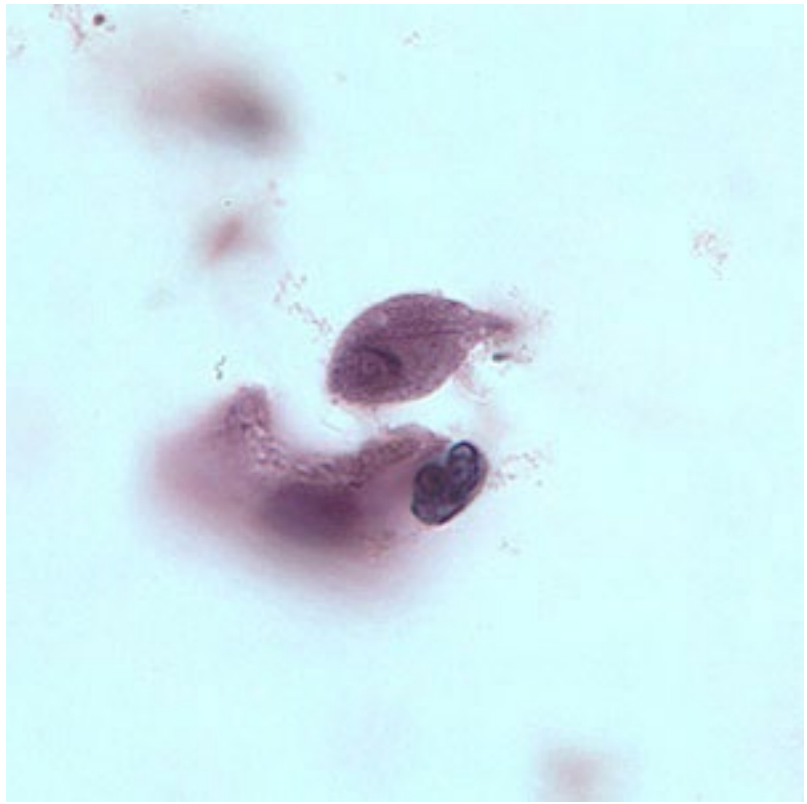
Trichomonas vaginalis

Urine

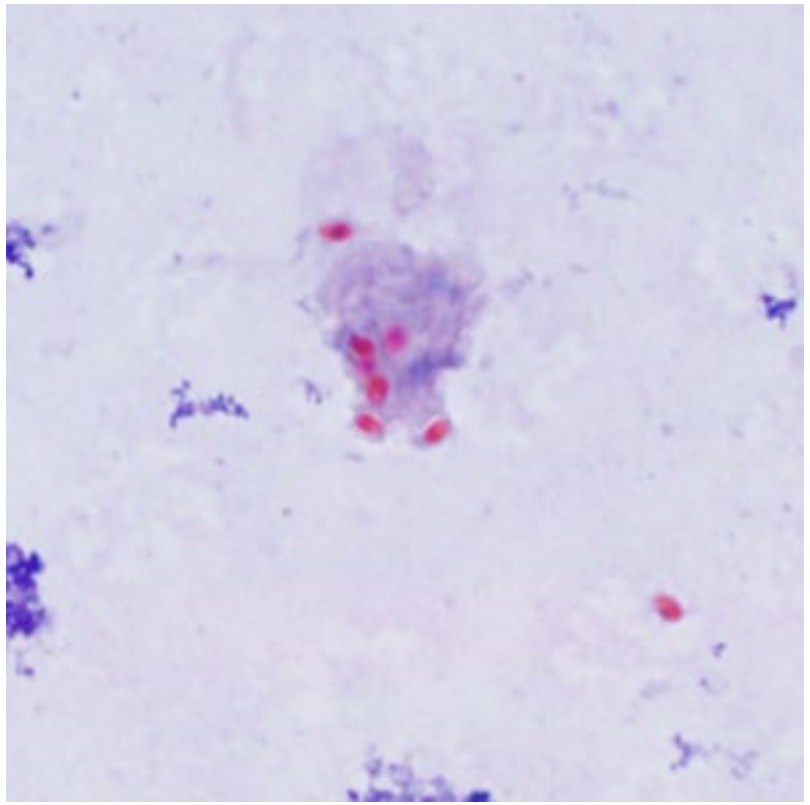
- *Schistosoma haematobium* (eggs)
- *Enterobius vermicularis* (eggs; female patients; could be contaminant)
- *Trichomonas vaginalis* (trophozoites)
- microsporidia (spores)

Parasites In Urine

Schistosoma haematobium



Trichomonas vaginalis

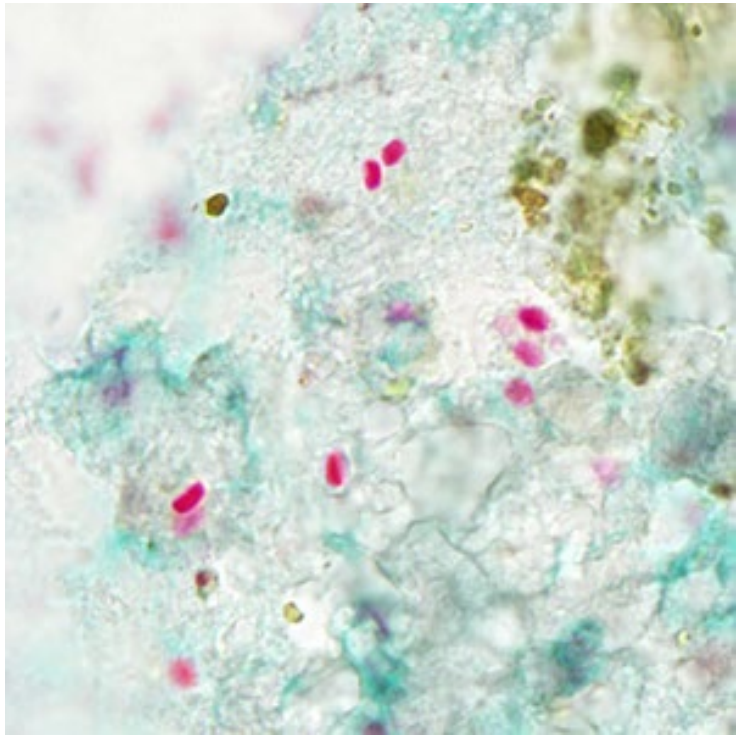


microsporidia

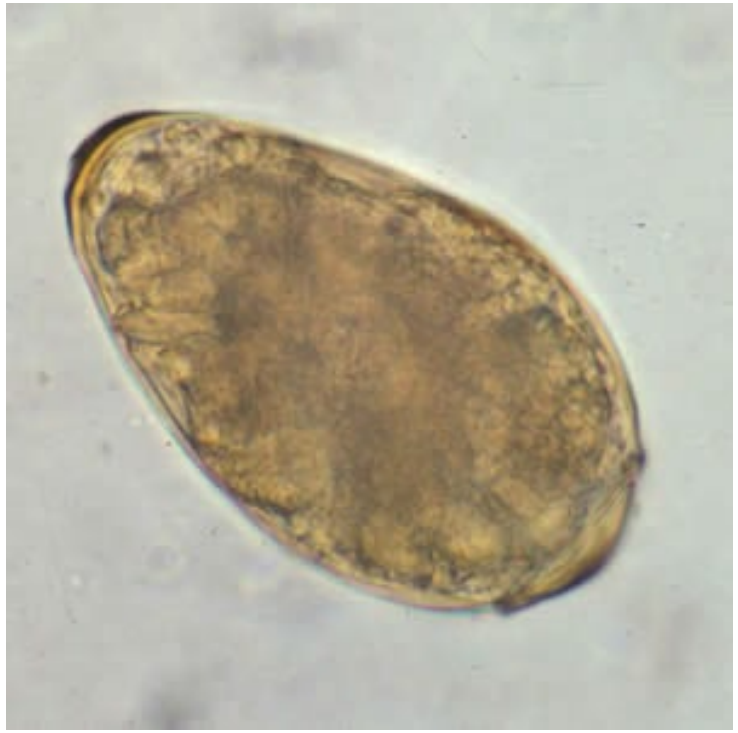
Respiratory Specimens (BAL, sputum)

- *Strongyloides stercoralis* (L3 larvae)
- *Paragonimus* spp. (eggs)
- *Echinococcus* (protoscoleces, hydatid sand; very rare)
- microsporidia and *Pneumocystis* (fungi historically considered protozoans)

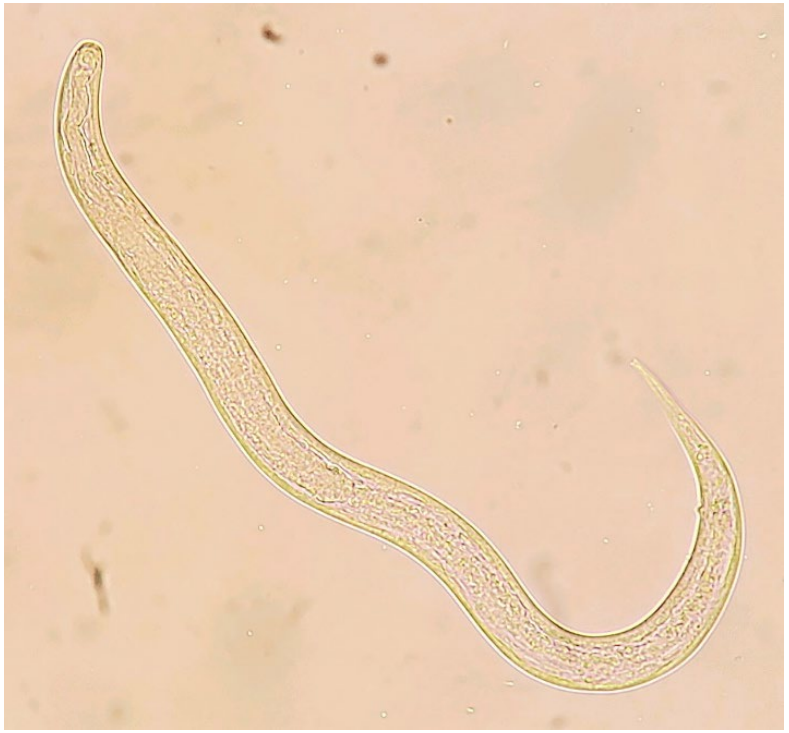
Parasites in Respiratory Specimens



microsporidia



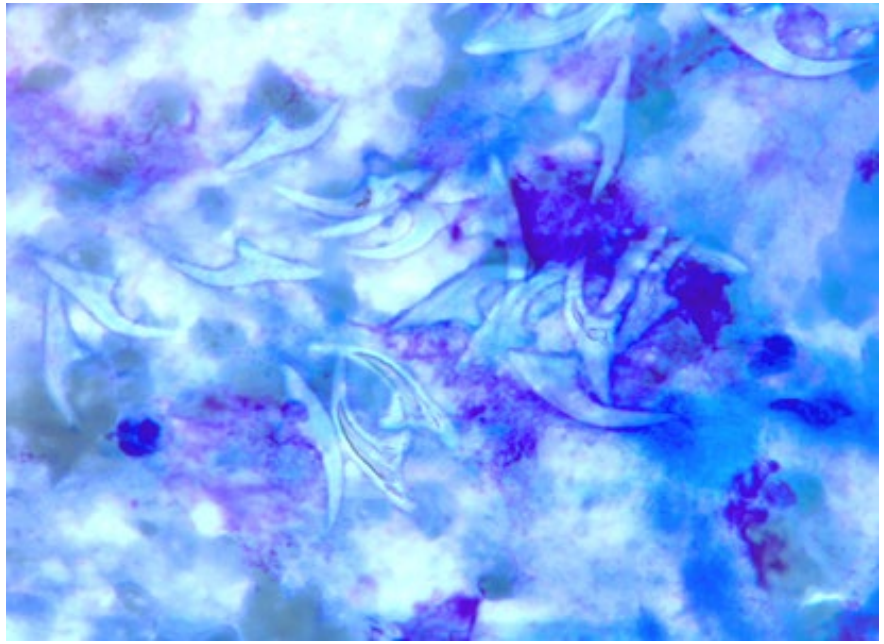
Paragonimus spp.

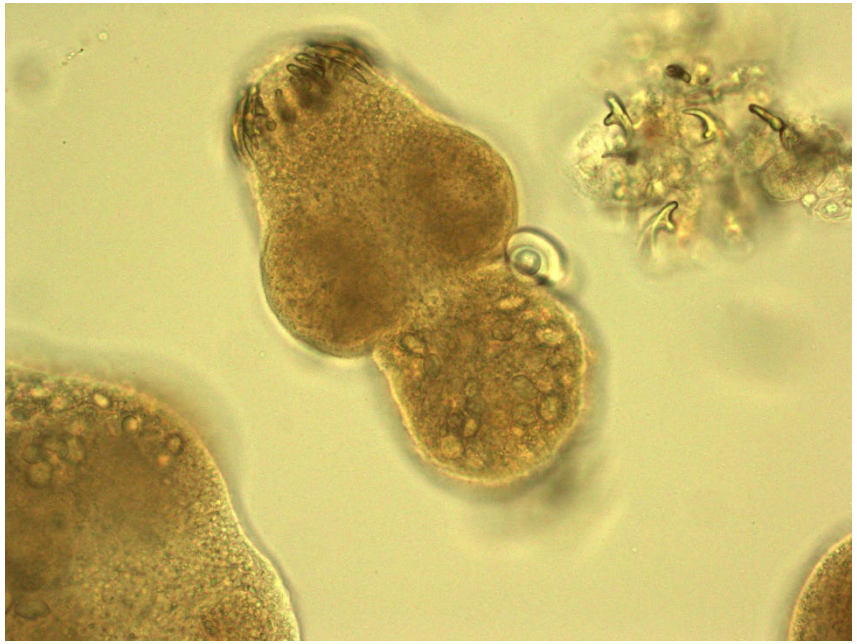


Strongyloides stercoralis (L3)

Liver Aspirates

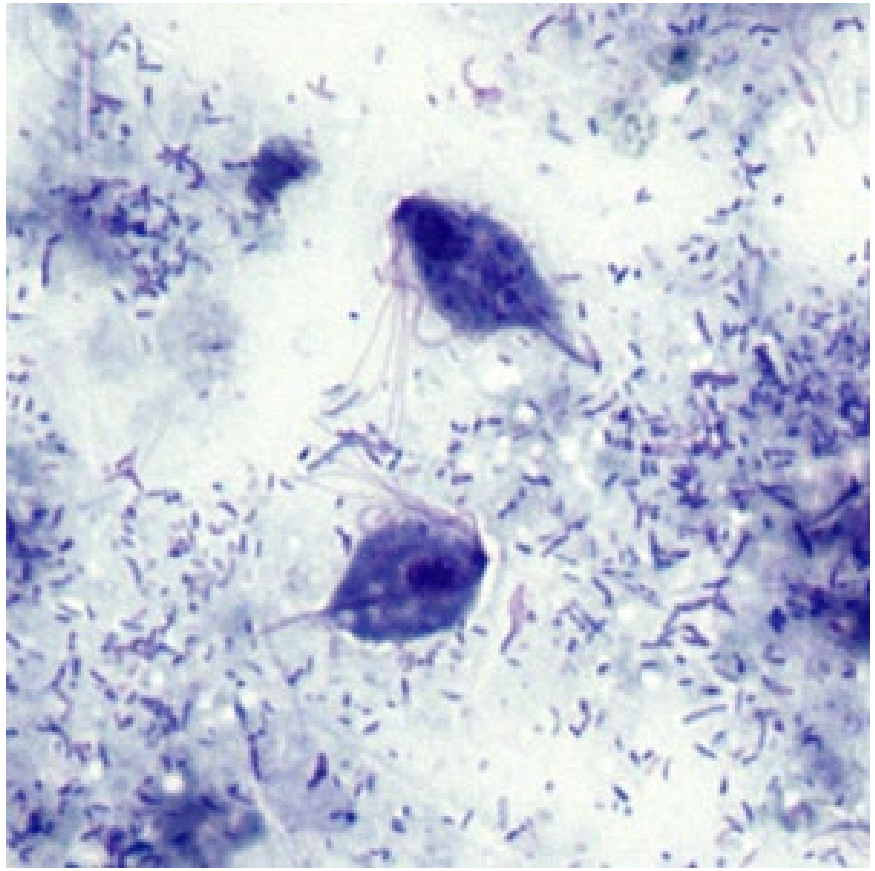
- *Echinococcus* spp. (protoscoleces, hydatid sand)
- *Entamoeba histolytica* (rare; very difficult to separate from PMNs)





Pleural Fluid

- *Trichomonas tenax* (trophozoites)
- *Echinococcus* spp. (protoscoleces, hydatid sand)
- *Strongyloides stercoralis*
- microsporidia



Acknowledgements

- Marc Roger Couturier and the folks at APHL for the invite
- Co-presenters Marc R. Couturier and Henry S. Bishop
- CDC-DPDx (images)
- Dr. Bobbi Pritt (Mayo Clinic, Rochester, MN), images and algorithms