Diagnostic Parasitology: Blood and Other Body Fluids

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• Major (related to this talk)
  – None

• Other
  – American Society for Microbiology (speaker/honoraria; Editorial Board, *Journal of Clinical Microbiology*)
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  – 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)
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 traceback investigations with transfusion-related cases.
  - babesiosis (also helpful for traceback investigations)
  - Chronic Chagas
  - Lymphatic Filariasis
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

Slide courtesy of Dr. Bobbi Pritt, Mayo Clinic
# Plasmodium vs. Babesia

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

Extraerythrocytic forms
Trypanosomes
## Comparison of Human Trypanosome Species

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

BIG ONES!
Loa loa
Wuchereria bancrofti
Brugia spp.

little ones
Mansonella spp.
Geographic Distribution

*NOTE: Wuchereria bancrofti nearly worldwide in tropics and subtropics*
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.
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
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Size and Sheath

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

Size large (>200µm)
Sheath may be present
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*

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Review: Key to the microfilariae in blood

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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
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  - Sheath may be present
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Size large (>200µm)
Sheath may be present

- Tail anucleate
  *Wuchereria bancrofti*

- Tail with nuclei at or to the tip
  *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
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