Laboratory diagnosis of Blood and tissue flagellates

(Leishmania and trypanosma)

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**Leishmania and trypanosma:**
- They are transmitted by insect vector
- They have different life cycle stages for diagnosis, the trypomastigote and amastigote are the diagnostic stages found in humans.
- The **amastigote** is an obligate intracellular organism, found within macrophages, liver, or spleen cells, or in bone marrow cells in disease caused by *Leishmania spp*.
- The **trypomastigote**, a flagellated form is found in the blood, CSF, lymphatic fluid of patients infected with *Trypanosoma spp*.
- The epimastigote and promastigote stages are seen in the insect vectors.

**Thick and thin smears:**
- Thin smears must first be fixed in methanol before staining with giemsa stain.
- RBCs in the unfixed thick smear will lyse during the staining procedure
- A thick film is best for detection of parasites (high sensitivity), because of the larger volume of the blood and the fact that orgs are concentrated in a small area.
- In the thick film, the RBCs are destroyed so that only white cells, platelets and parasites are visible.
- In a thick film the organisms may be difficult to identify and there is no way to compare size of infected and noninfected erythrocytes. Therefore, species identification should be made from a thin film because the characteristics of the parasite and RBC can be seen.

**Leishmaniasis**
- Transmitted by sandflies. Approximately 30-50 phlebotomus spp and lutzomyia spp transmit leishmania.
- Human infection is caused by about 21 of 30 species that infect mammals. These include the *L. donovani* complex with 3 species (*L. donovani, L. infantum*, and *L. chagasi*); the *L. mexicana* complex with 3 main species (*L. mexicana, L. amazonensis*, and *L. venezuelensis*); *L. tropica; L. major; L. aethiopica*; and the subgenus *Viannia* with 4 main species (*L. (V.) braziliensis, L. (V.) guyanensis, L. (V.) panamensis*, and *L. (V.) peruviana*).

- **Leishmaniasis types of disease:**
  - LCL= localised cutaneous leishmaniasis *(L. Tropica, L.major, L.aethiopica)*
  - VL= visceral leishmaniasis,KALAZAR *(L.donovani,L. Infantum, L.chagasi)*
  - MCL=mucocutanous leishmaniasis *(L.braziliensis)*
  - PKDL= post Kala-azar dermal leishmaniasis
  - DCL = Diffuse cutaneous leishmaniasis
- Life cycle of leishmania:
**Lab diagnosis:**
- The amastigote is the diagnostic stage in humans.
- The diagnosis of leishmaniasis is commonly made by microscopic identification of the nonmotile, intracellular form (amastigote) in stained sections of lesions, and of the motile, extracellular form (promastigote) grown in a suitable media or in experimental animals.
- Amastigote is a small intracellular stage found in macrophages or histocytes around the periphery of the skin lesion (L.tropica or L.brazilianis) or within cells of a bone marrow aspirate or liver or spleen biopsy specimens (L.donovani)

- Amastigotes of *Leishmania* are spherical to ovoid and measure 1-5 µm long by 1-2 µm wide. They possess a large nucleus, a prominent kinetoplast, and a short axoneme, the last of which is rarely visible by light microscopy. The organisms reside in macrophages of the host and can be found throughout the body.

A, B: *Leishmania* spp. amastigotes; touch-prep stained with Giemsa.
Trypanosmiasis:

- The tsetse fly (genus Glossina) is the vector for transmission of trypanosomes.
- Trypanosoma brucei rhodesiense and T. B. gambiense are the causative agents of sleeping sickness, which is seen primarily in central Africa.
- T. cruzi is the gent of American trypanosmiasis or chagas disease.
Lab diagnosis:

- The diagnostic stage in humans is the trypomastigote, which is usually seen in a wright stained blood smear. Wet films may be used to detect the motile trypomastigote.
- Trypomastigote is an elongated structure that often appears in a C or U shape.
- It shows a single large nucleus midbody, a single anterior flagellum, and a posterior kinetoplast to which is attached the undulating membrane.
- In a cardiac or other tissue biopsy specimen, the organism can be seen in the amastigote stage.
- The organism can also be detected in lymphatic fluids and CSF.
- The CSF in infected individuals will often show increased lymphocytes and elevated protein levels.

- **Diagnosis of *T.b* gambiense (HAT):**
  1) find trypomastigote in: gland aspirate/thin or thick stained blood films.
  2) sensitivity of blood films is low: increase it by concentrating organisms using:
     - Micro-haematocrit
     - quantitative buffy coat
     - mini anion exchange column
  3) for confirmation of CNS involvement examine CSF For:
     - parasites (diagnostic)
     - high IgM
     - morula cells
     - high lymphocytes
     - High CSF total protein (no longer recommended)
  4) serology: antibody detection possible (IFAT,ELISA,CATT” card agglutination trypanosome test”)

- **Diagnosis of *T.b* rhodesiense infections:**
  diagnosis by stained blood films is easier as paracitaemia is high.
Life cycle of *Trypanosoma cruzi*

**Diagnosis of T. cruzi:**

- During acute phase, detect parasites in blood in thin and/or thick stained blood films.

- During the chronic phase very low numbers of parasites in blood; therefore: (symptoms, X-ray, PCR, Xenodiagnosis)

- Serology (IFAT, ELISA) during both phases.