

**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*)

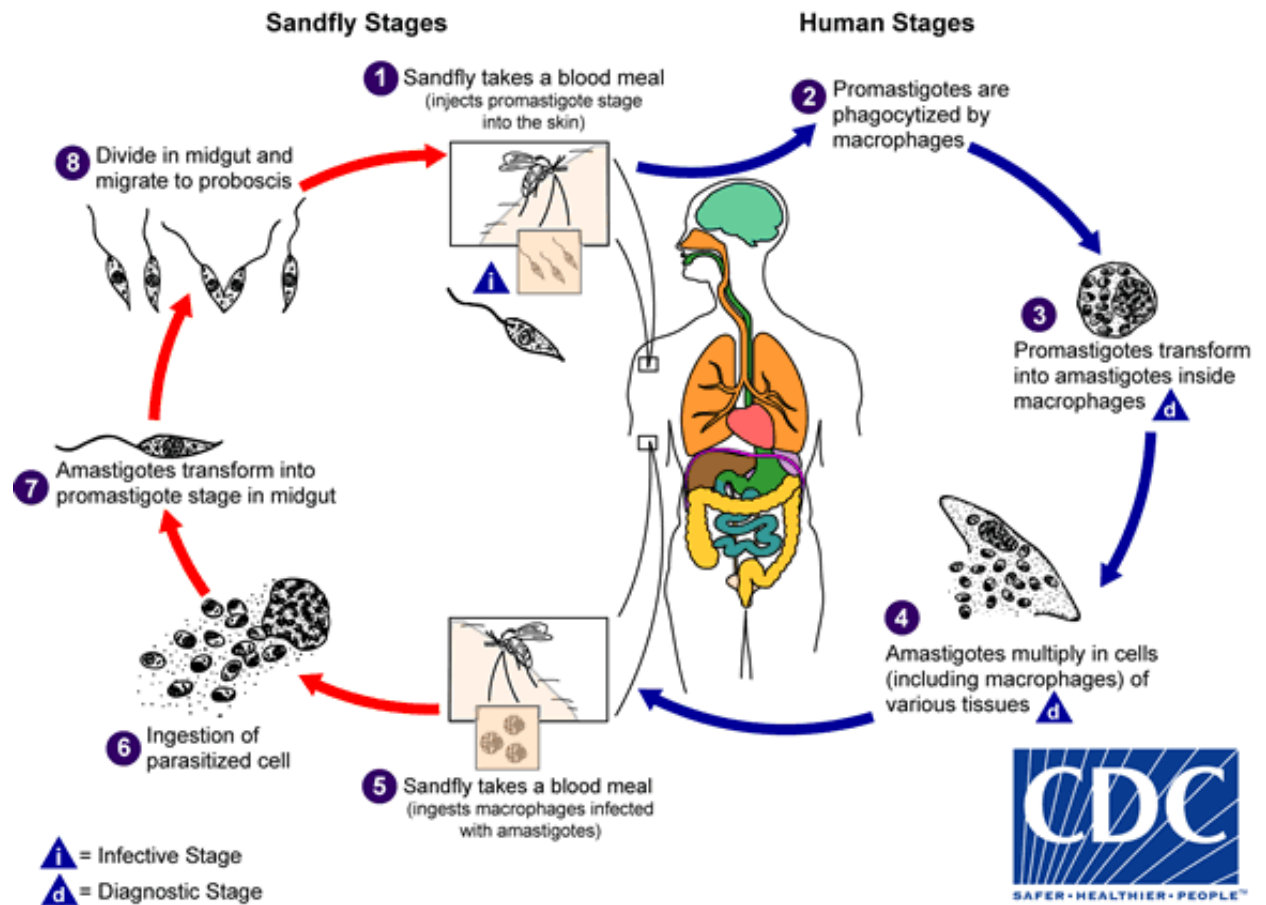
VL= visceral leishmaniasis, KALAZAR (*L.donovani*, *L. Infantum*, *L.chagasi*)

MCL=mucocutaneous leishmaniasis (*L.braziliensis*)

PKDL= post Kala-azar dermal leishmaniasis

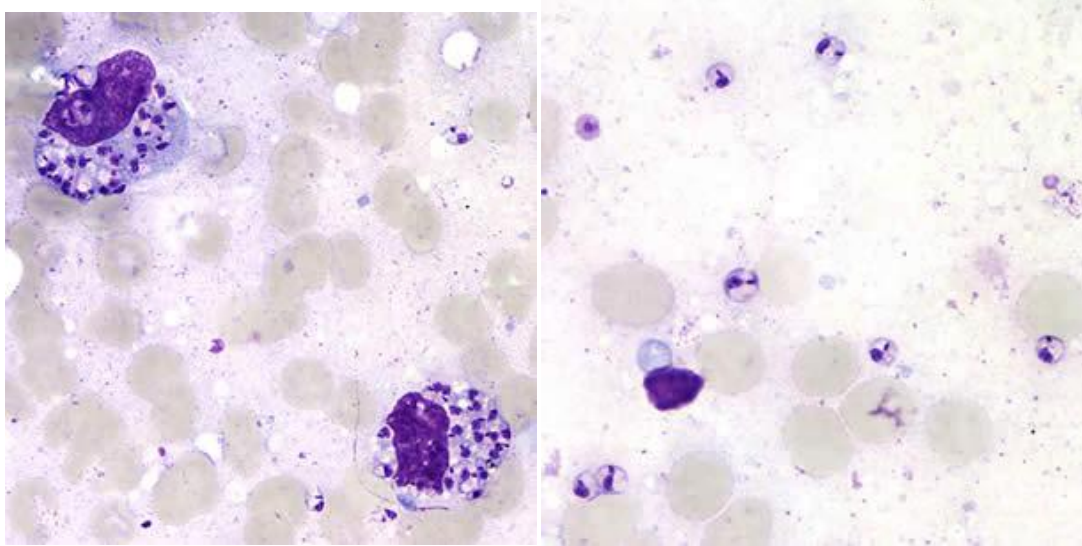
DCL = Diffuse cutaneous leishmaniasis

- 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. braziliensis*) 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  $\mu\text{m}$  long by 1-2  $\mu\text{m}$  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.

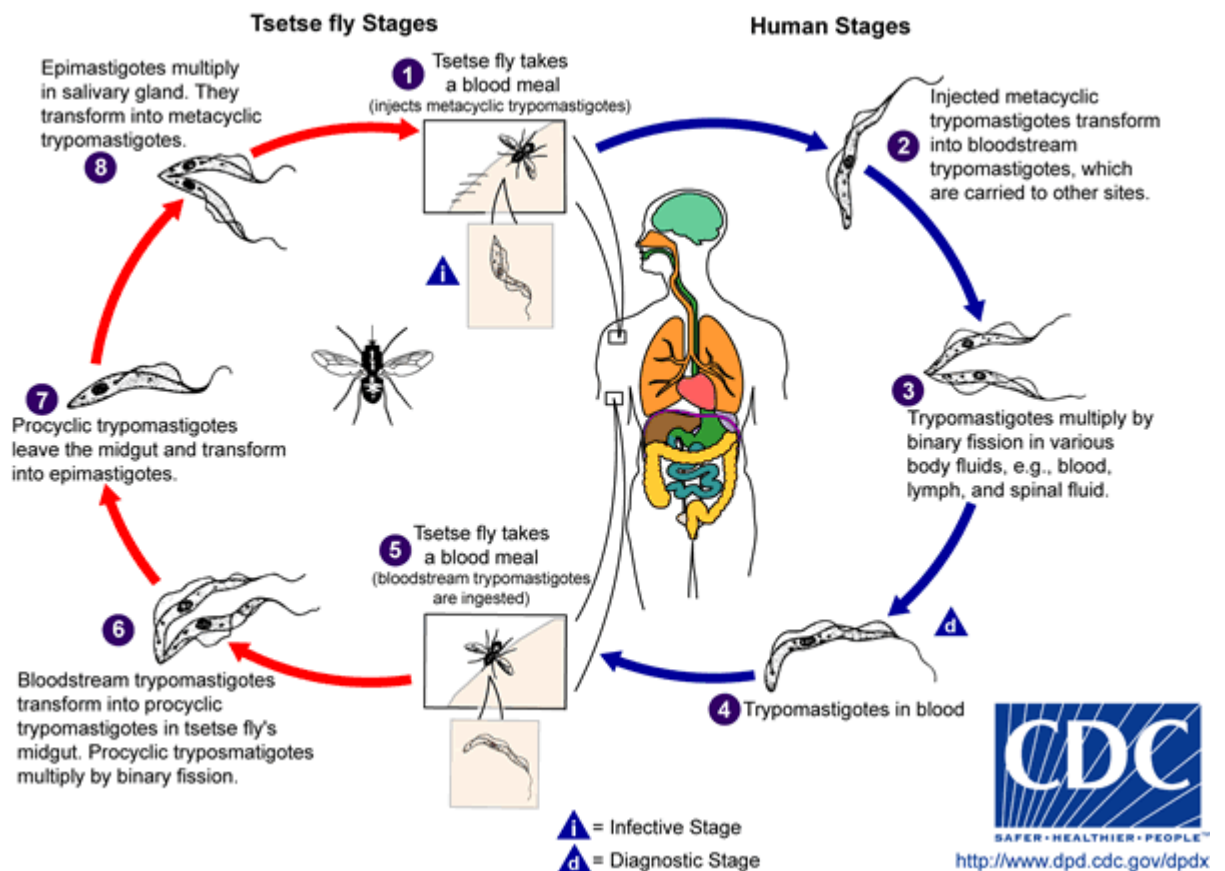


**A, B:** *Leishmania* spp. amastigotes; touch-prep stained with Giemsa.

## 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 agent of American trypanosomiasis or Chagas disease.

### Life cycle of African Trypanosomes:



### **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:

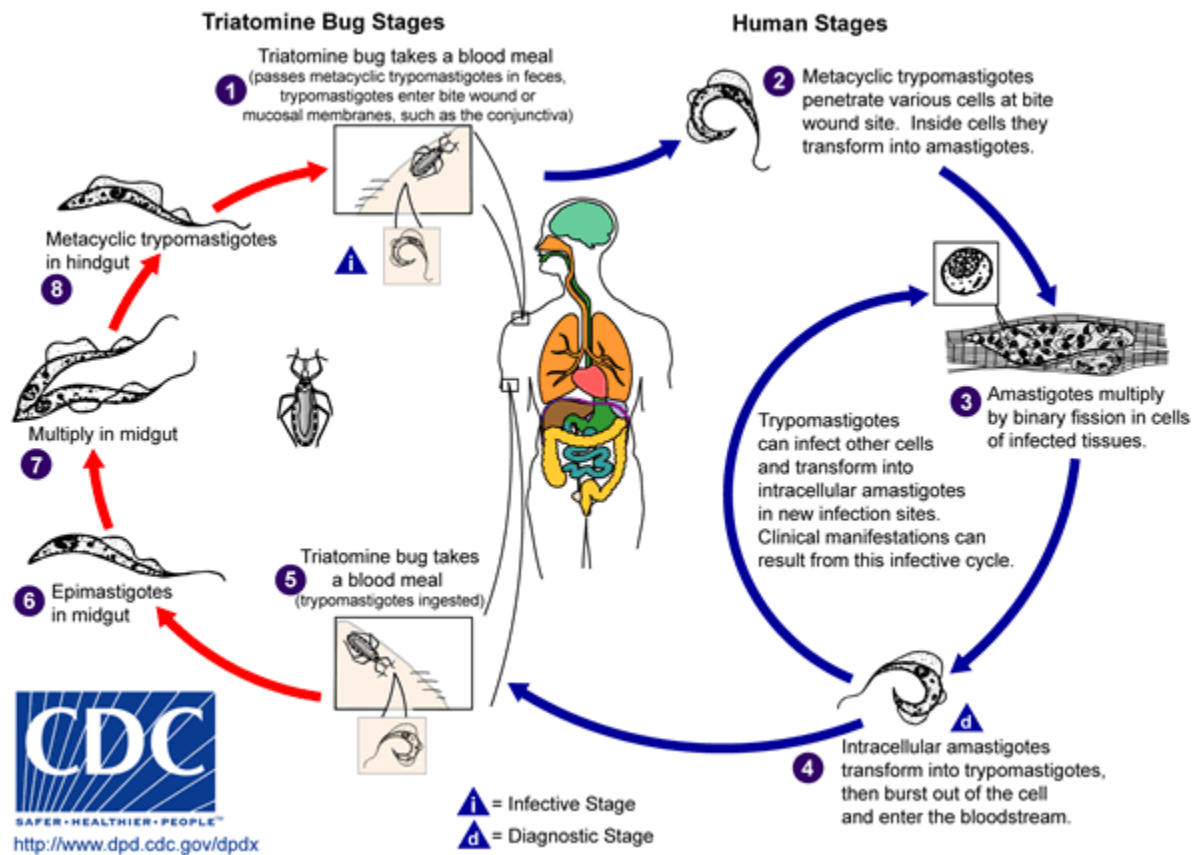
- parasites(diagnostic)
- high IgM
- morula cells
- high lymphocytes
- High CSF total protein (no longer recommended)

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.

