

LABORATORY TESTS OF THE MOST FREQUENT TROPICAL DISEASES

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IHI

Tropical diseases

- Tropical diseases encompass all diseases that occur solely, or principally, in the tropical or subtropical regions. In practice, the term is often taken to refer to infectious diseases that thrive in hot, humid conditions, such as **malaria, leishmaniasis, schistosomiasis, onchocerciasis, lymphatic filariasis, Chagas disease, African trypanosomiasis, and dengue.**

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- **Insects** such as **mosquitoes** and **flies** are by far the most common disease carrier or "**vector**". These insects may carry a **parasite, bacterium or virus** that is infectious to humans and animals. Most often disease is transmitted by an insect "bite", which causes transmission of the infectious agent through subcutaneous blood exchange.

Additional Health Problems

- Other diseases for which treatment is available but which are still common in developing countries include **tuberculosis, cholera, yellow fever, yaws, and amoebic dysentery** and **trachoma**, and virus-caused fevers that are found predominantly in tropical regions i.e. **Lassa, Ebola, Marburg, Bunya**, and **Chikungunya** fevers (these are rare).

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- **Prompt** and **accurate** diagnosis of the diseases is part of effective disease management and will, if implemented **effectively**, help reduce unnecessary use of the medicines.

The two diagnostic approaches currently used are based on:

- the symptoms and signs of the disease - i.e. a clinical diagnosis and
- **detection of the causative agent or its product(s).**

Malaria

- **A** vector-borne infectious disease caused by protozoan parasites.
 - Species responsible: *P. falciparum*, *P. vivax*, *P. ovale*, and/or *P. malariae*
- **Microscopic diagnosis**
 - Conventional light microscopy is the established method for the laboratory confirmation of malaria. Examination by an expert microscopist of a well prepared and well stained blood film remains currently the "**gold standard**" for detecting and identifying malaria parasites.

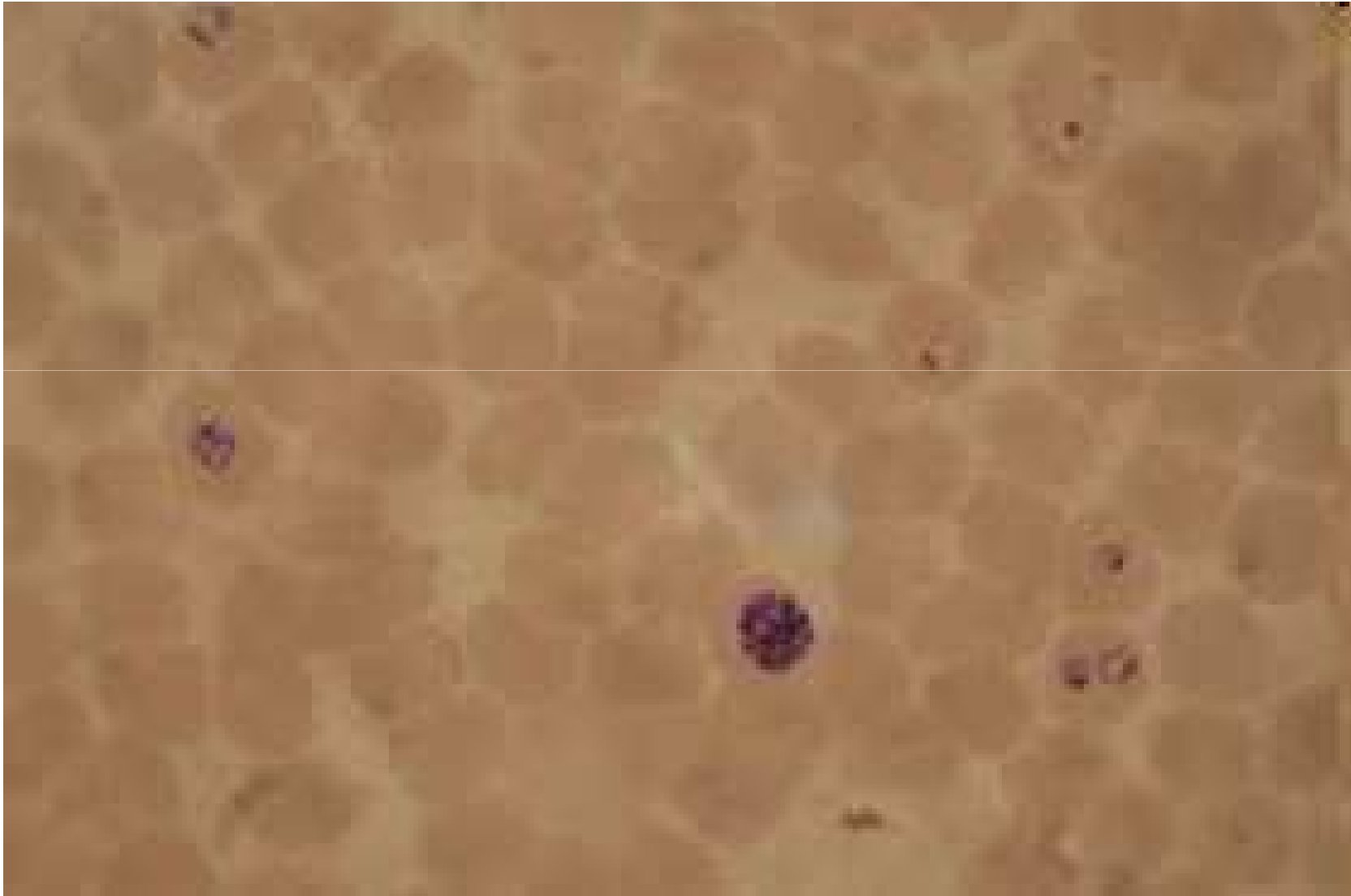
It involves:

- Collecting a finger-prick blood sample;
- Preparing a thick/ thin blood smear; staining the smear (most frequently with Giemsa); and
- Examining the smear through a microscope (preferably with a 100X oil-immersion objective) for the presence of malaria parasites.

Trophozoite and Gametocytes



Trophozoite and schizont



Advantages

- It is **sensitive**. When used by skilled technicians, microscopy can detect densities as low as 5–10 parasites per μl of blood.
- It is **informative**. Parasites can be characterized in terms of their **species** (*P. falciparum*, *P. vivax*, *P. ovale*, and/or *P. malariae*) and of the **circulating stage** (e.g. trophozoites, schizonts, gametocytes). In addition, the parasite **densities** can be quantified (from ratio of parasites per number of leukocytes or erythrocytes). Such quantifications are needed to demonstrate hyperparasitaemia = severe malaria or to assess parasitological response to chemotherapy.

Advantages cont..

- It is relatively **inexpensive**. Cost estimates for endemic countries range from about US\$ 0.12 to US\$ 0.40 per slide examined.
- It is a general diagnostic technique that **can be shared with other disease control programmes**, such as those against tuberculosis or sexually transmitted diseases.
- It can provide a **permanent record** (the smears) of the diagnostic findings and be subject to quality control.

Disadvantages

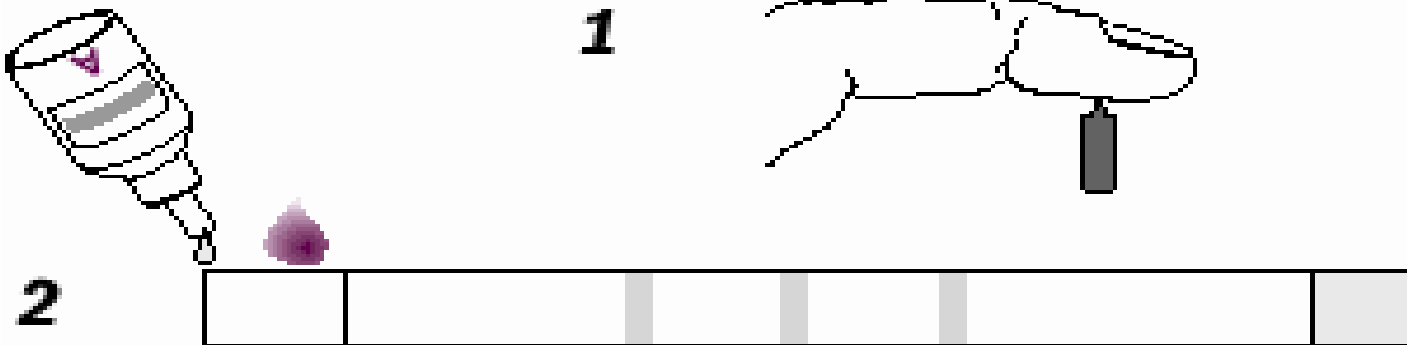
- **Labour-intensive** and **time-consuming**, normally requiring at least 60 minutes from specimen collection to result.
- Depends absolutely on **well trained and well supervised technicians, good techniques, reagents, microscopes**. Unfortunately these conditions are often not met, particularly at the more peripheral levels of the health care system.
- There are often long **delays** in providing the microscopy results to the clinician, so that decisions on treatment are often taken without the benefit of the results.

Rapid diagnostic tests (RDTs)

- Also called **Malaria Rapid Diagnostic Tests, Antigen-Capture Assay** or "**Dipsticks**".
- They are based on the detection of **antigens** derived from malaria parasites in lysed blood, using **immunochromatographic** methods.
- Employs a dipstick or test strip bearing **monoclonal antibodies** directed against the target **parasite antigens**

PROCEDURAL STEPS

Collect blood



2 Mix blood with lysis buffer and detection antibodies on strip (or in well or test tube)

Lysed blood and reagents migrate up strip



Antigen-antibody complexes bind to capture antibodies in detection lines



Add washing buffer

Buffer migrates up strip, making detection lines visible

Antigens targeted by currently available RDTs

- **Histidine-rich protein II (HRP-II)** - a water-soluble protein produced by trophozoites and young (but not mature) gametocytes of *P. falciparum*. Commercial kits currently available detect HRP-II from *P. falciparum* only.
- **Parasite lactate dehydrogenase (pLDH)** - produced by asexual and sexual stages (gametocytes) of malaria parasites. Test kits currently available detect pLDH from all four Plasmodium species that infect humans. Can distinguish *P. falciparum* from the non-falciparum species only, but not between *P. vivax*, *P. ovale* and *P. malariae*.

Comparison of the requirements, performance and direct cost of microscopy and RDTs

	MICROSCOPY	RDTs
REQUIREMENTS		
Equipment	Microscope	None
Electricity	Preferred, not necessary	None
Supplies	Blood collection, staining reagents and supplies, water	Blood collection (supplied in some kits)
Training	Trained microscopist	Only minimal training required
PERFORMANCE		
Test duration	Usual minimum 60 minutes	15-20 minutes
Labour-Intensiveness	High	Low
Subjectivity	High	Low
Robustness	Average	High
DIRECT COSTS		
Cost per test	US\$ 0.12-0.40	US\$ 0.60-2.50

Comparison of the technical specifications of microscopy and RDTs

TECHNICAL SPECIFICATIONS		
Detection threshold	5-10 parasites/ μ l blood	40-100 parasites/ μ l blood
Detection of all four species	Yes	Some RDTs
Quantification	Possible	Not possible
Differentiation between <i>P. vivax</i> , <i>P. ovale</i> and <i>P. malariae</i>	Possible	Not possible
Differentiation between sexual and asexual stages	Possible	Not possible
Detection of (<i>P. falciparum</i>) sequestered parasites	No	Yes
Antigen persistence	Not applicable	Some RDTs

Other techniques

- Other diagnostic methods are available, but they are not as suitable for wide field application as microscopy or RDTs and are unsuitable for use in routine disease management.

They include:

- **Microscopy using fluorochromes** such as acridine orange, either on blood smears or on centrifuged blood specimens (QBC ® technique) is expensive and requires special equipment and supplies (centrifuge and centrifuge tubes, special light sources and filters).

OTHER TECHNIQUES cont..

- Polymerase chain reaction (PCR) based tests: Parasite nucleic acids are detected using PCR. PCR is used to amplify specific regions of a DNA strand (the DNA target). This technique is **more accurate than microscopy**. However, it is **expensive**, and requires a **specialized and costly equipment** and **laboratory conditions** that are often not available in the field.

OTHER TECHNIQUES cont..

- Some molecular methods are available in some clinical laboratories and rapid real-time assays (for example, QT-NASBA based on the polymerase chain reaction) are being developed with the hope of being able to deploy them in endemic areas.
- **Antibody detection by serology** only measures prior exposure and not specifically current infection.

Leishmaniasis

- A vector-borne disease caused by obligate intra-macrophage protozoa belonging to the genus *Leishmania*.
- Transmitted by the bite of species of **sand fly** of the genus *Lutzomyia* and *Phlebotomus*.
- Also known as **Leishmaniosis, Leishmaniose, Orient Boils, Baghdad Boil, kala azar, black fever, sandfly disease, Dum-Dum fever or espundia.**

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- Confirmatory tests are required to decide which patients should be treated. A test should be able to make the distinction between **acute disease and asymptomatic infection**, because none of the drugs currently available is **safe** enough to treat asymptomatic infections.

Non-leishmanial tests

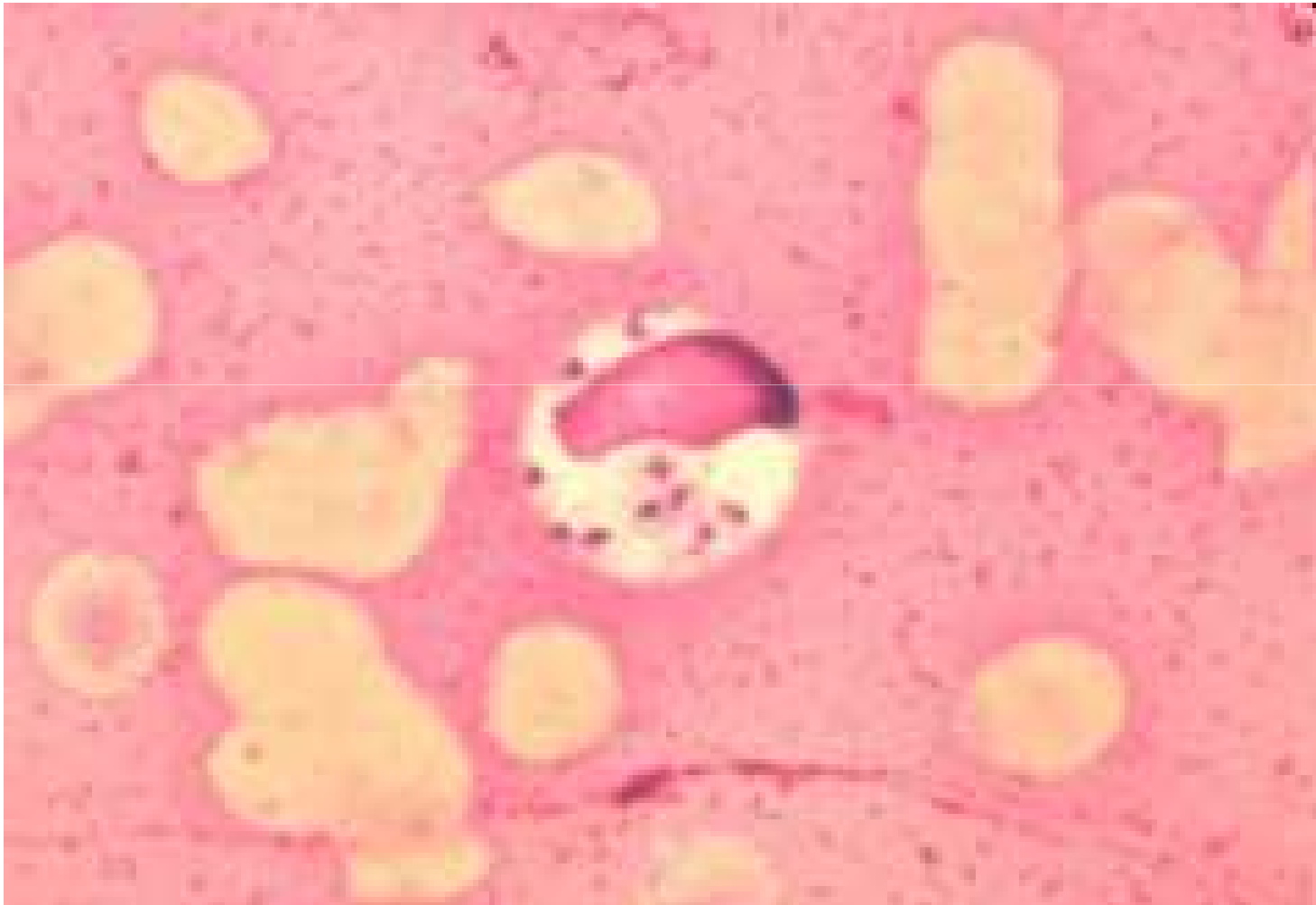
- A reduction in the number of red and white blood cells and platelets (**pancytopenia**), highly specific (98%) for Visceral Leishmaniasis (VL) in suspected clinical patients in Nepal but the sensitivity was low (16%).
- Marked **polyclonal hypergammaglobulinemia** (the production of high titres of non-specific antibody), a common finding in VL, can be detected by a **formol gel test** (FGT; also called the aldehyde test), which is still used in East Africa and Asia because of its simplicity and low cost. However, it has low sensitivity (34%), some experts have recommended its use be discontinued.

Microscopy

- Parasite detection microscopy: The visualization of the **amastigote** form of the parasite by microscopic examination of aspirates from lymph nodes, bone marrow, spleen or buffy-coat preparations of peripheral blood is the classical confirmatory test.
- The accuracy of microscopic examination is influenced by the ability of the laboratory technician and the quality of the reagents used.

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- The sample should be spread on a slide to make a thin smear, and stained with leishman`s or Giemsa`s stain (pH 7.2) for 20 minutes.
 - **Amastigotes** are seen with monocytes or, less commonly in neutrophil in peripheral blood and in macrophages in aspirates. They are small, round bodies 2-4um in diameter with indistinct cytoplasm, a nucleus and a small rod shaped **kinetoplast**. Occasionally **amastigotes** may be seen lying free between cells.

***Leishmania donovani* in bone marrow cell**



Antibody-detection tests

- Indirect fluorescence antibody (IFA),
- Enzyme-linked immunosorbent assay (ELISA)
- western blot

Have shown high diagnostic accuracy in most studies but are poorly adapted to field settings.

- Two serological tests have been specifically developed for field use and have been sufficiently validated — the **direct agglutination test** (DAT) and the **rK39**-based **immunochromatographic test** (ICT).

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- The DAT is a semi-quantitative test that uses microtitre plates in which increasing dilutions of patient's serum or blood are mixed with stained killed *L. donovani* promastigotes. If specific antibodies are present, agglutination is visible after 18 hours with the naked eye. The DAT is simpler than many other tests but it requires equipment such as microtitre plates and micropipettes, well-trained laboratory technicians and regular quality control.

- rK39 is a 39-amino acid repeat that is part of a **kinesin-related protein** in *Leishmania chagasi* and which is conserved within the *L. donovani* complex. An rK39-based ELISA showed excellent sensitivity (93–100%) and specificity (97–98%) in many VL-endemic countries. The test has been developed into an ICT. This test has been shown to be less accurate in East Africa for reasons that remain unclear.
- rK39 ICTs are easy to perform, rapid (10–20 minutes), cheap (around US\$1 per test) and give reproducible results. They are currently the best available diagnostic tool for VL for use in remote areas.

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- Tests that detect specific anti-leishmanial antibodies suffer two major limitations:
 - First, serum antibody levels remain detectable up to several years after cure. Therefore, relapse cannot be diagnosed by serology.
 - Second, a significant proportion of healthy individuals with no history of Leishmaniasis are positive for anti-leishmanial antibodies owing to asymptomatic infections. The seroprevalence in healthy populations varies from <10% in low to moderate endemic areas, to >30% in high-transmission foci or in household contacts.
 - Antibody-based tests must therefore always be used in combination with a standardized clinical case definition for VL diagnosis.

Antigen-detection tests

- In theory, antigen-detection tests should be **more specific** than antibody-detection tests as they **avoid cross-reactivity** and can **distinguish active from past infections**.
- A latex agglutination test detecting a heat-stable, low-molecular-weight carbohydrate antigen in the urine. It has a good specificity but only low to moderate (48–87%) sensitivity.
- There are two practical limitations: the urine must be boiled to avoid false-positive reactions and it is difficult to distinguish weakly positive from negative.

Other methods

- The detection of parasites in the blood or organs by culture or by using molecular techniques such as **PCR** is more sensitive than microscopic examination but these techniques remain restricted to referral hospitals and research centres, despite efforts to simplify them.

Schistosomiasis

- Also called **bilharzia**, **bilharziosis** or **snail fever** is a parasitic disease caused by five species of water-borne flatworm, or blood flukes, of the genus *Schistosoma*:
 - **Intestinal schistosomiasis** caused by *Schistosoma mansoni* occurs in Africa, the Eastern Mediterranean, the Caribbean and South America.
 - **Oriental or Asiatic intestinal schistosomiasis**, caused by the *S. japonicum* group of parasites (including *S. mekongi* in the Mekong river basin), is endemic in South-East Asia and in the Western Pacific region.
 - Another form of **intestinal schistosomiasis** caused by *S. intercalatum* reported from central African countries.
 - **Urinary schistosomiasis**, caused by *S. haematobium*, is endemic in Africa and the Eastern Mediterranean.

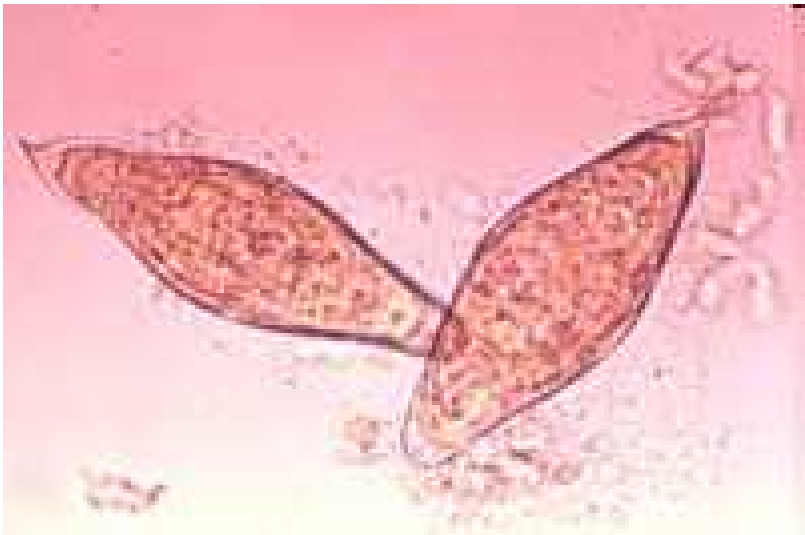
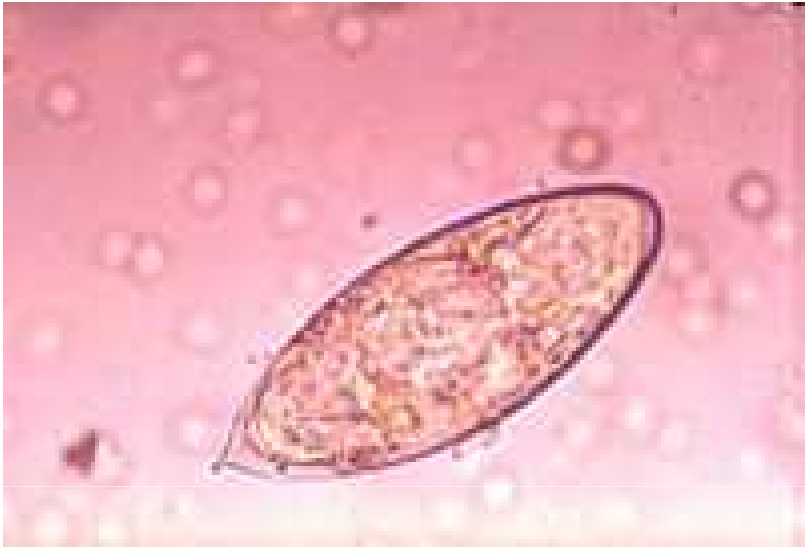
laboratory tests

- **Microscopic** identification of eggs in stool or urine is the most practical method for diagnosis.
- Eggs may be passed intermittently or in small amounts - repeated examinations or use of **concentration** methods – formal-ether, MIF, SAF.
- The Kato-Katz technique is now most commonly used for detecting schistosome eggs in the field. Not good for hookworm eggs because they collapse within 30 to 60 minutes of preparation using this method.

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- It involves examining under a microscope a sieved fecal sample covered with cellophane stained with malachite green. The total number of stained eggs are counted and used to calculate the number of eggs per gram.
 - For *S. haematobium* urine centrifugation for eggs in sediment is routinely done.

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- Another field method is urine filtration using a standard volume through a nucleopore membrane followed by egg counts on the membrane.
 - Tissue biopsy (rectal biopsy for all species and biopsy of the bladder for *S. haematobium*) may demonstrate eggs when stool or urine examinations are negative.
 - The eggs of *S. haematobium* are ellipsoidal with a terminal spine, *S. mansoni* eggs are also ellipsoidal but with a lateral spine, *S. japonicum* eggs are spheroidal with a small knob.

(S) haematobium, mansoni, intercalatum, and japonicum



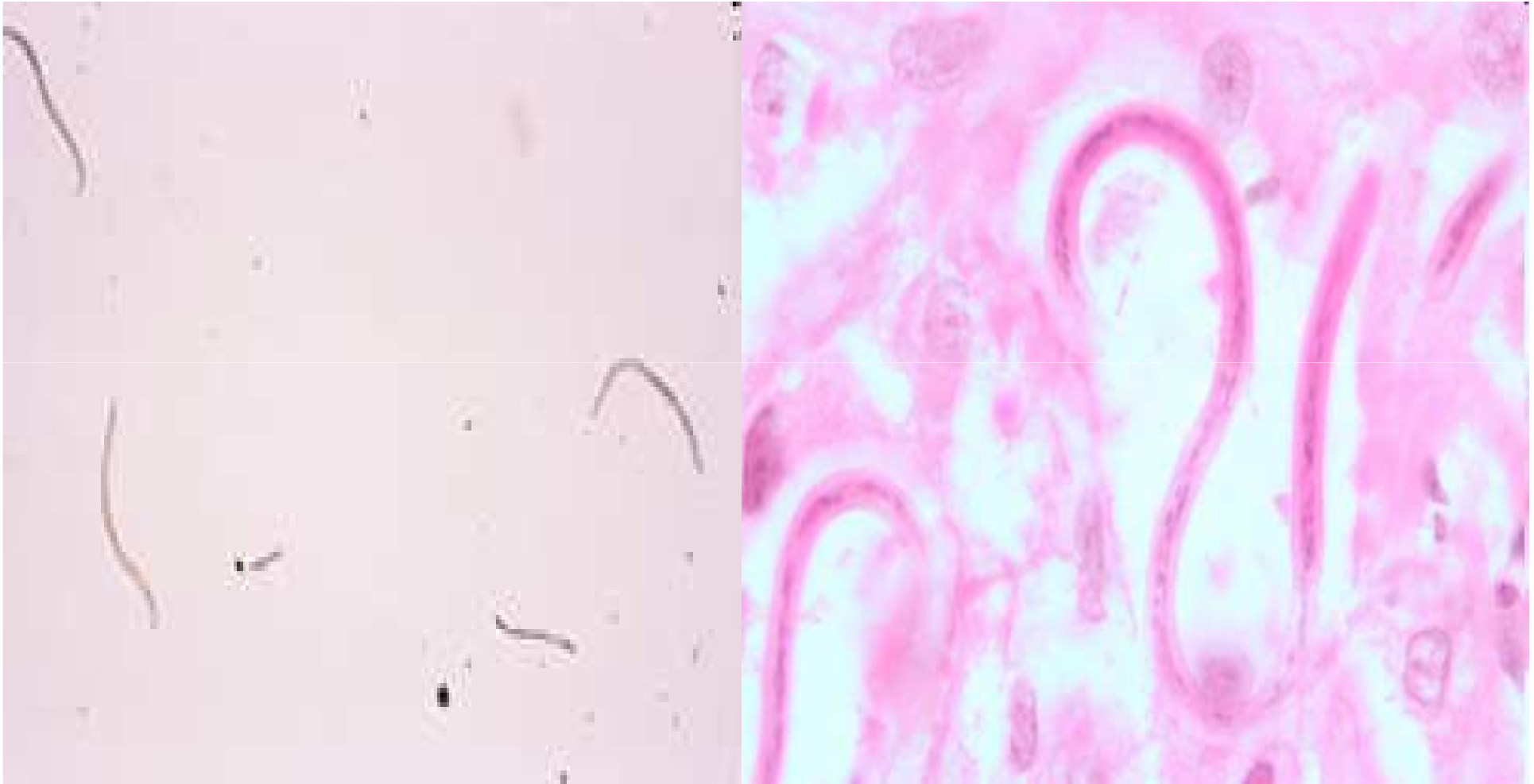
Onchocerciasis

- **Also called river blindness** is the world's second leading infectious cause of blindness. It is caused by *Onchocerca volvulus*, a nematode that can live for up to fifteen years in the human body.
- It is transmitted to people through the bite of a **black fly**.

Diagnosis

- Examination of **skin snips** will identify microfilariae of *Onchocerca volvulus* and *Mansonella streptocerca*.
- Skin snips can be obtained using a corneal-scleral punch, or more simply a scalpel and needle. The sample must be allowed to incubate for 30 minutes to 2 hours in saline or culture medium, and then examined for microfilariae that would have migrated from the tissue to the liquid phase of the specimen.
- Smears can also be prepared and **stained** with Giemsa or hematoxylin and eosin.

Microfilariae of O. volvulus from a skin nodule



Lymphatic Filariasis

- More commonly known as *elephantiasis* is a parasitic and infectious tropical disease, that is caused by thread-like parasitic worms, of the type filarial nematode. Examples are *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*.
- The parasites are transmitted by **insect bites**, usually **mosquitoes**. Lymphatic filariasis is extremely rare in Western countries.

Diagnosis/Lab tests

- Until very recently, diagnosing lymphatic filariasis had been extremely difficult, since parasites had to be detected **microscopically** in the blood, and the parasites have a "**nocturnal periodicity**" that restricts their appearance in the blood to only the hours around midnight. ?when their vector, the mosquito, is most likely to bite.
- It involves Identifying microfilariae on a Giemsa stained thick blood film.

Microfilaria of *W. bancrofti* and *B. malayi* in a thick blood smear, stained with Giemsa



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- The new development of a very sensitive, very specific simple "**card test**" to detect circulating parasite antigens without the need for laboratory facilities and using only finger-prick blood droplets taken anytime of the day has completely transformed the approach to diagnosis.
 - The ICT Filariasis, a rapid card test format, which is based on qualitative detection by monoclonal antibody of the circulating antigen of *Wuchereria bancrofti* adult worm, is a new diagnostic test of choice for determining the infections under field conditions. Circulating filarial antigens (CFA) assays and Og4C3 ELISA are also applied.

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- **Concentration methods:** Knotts technique and filtration using membrane filters
 - There are also **PCR assays** available for making the diagnosis. It is rapid and specific method to detect filarial parasite DNAs in blood and mosquito samples using the PCR technique.
 - This PCR-RFLP technique can be apply to use in diagnosis and to differentiate between species of filaria in humans the reservoir host and the mosquito vector in endemic areas.

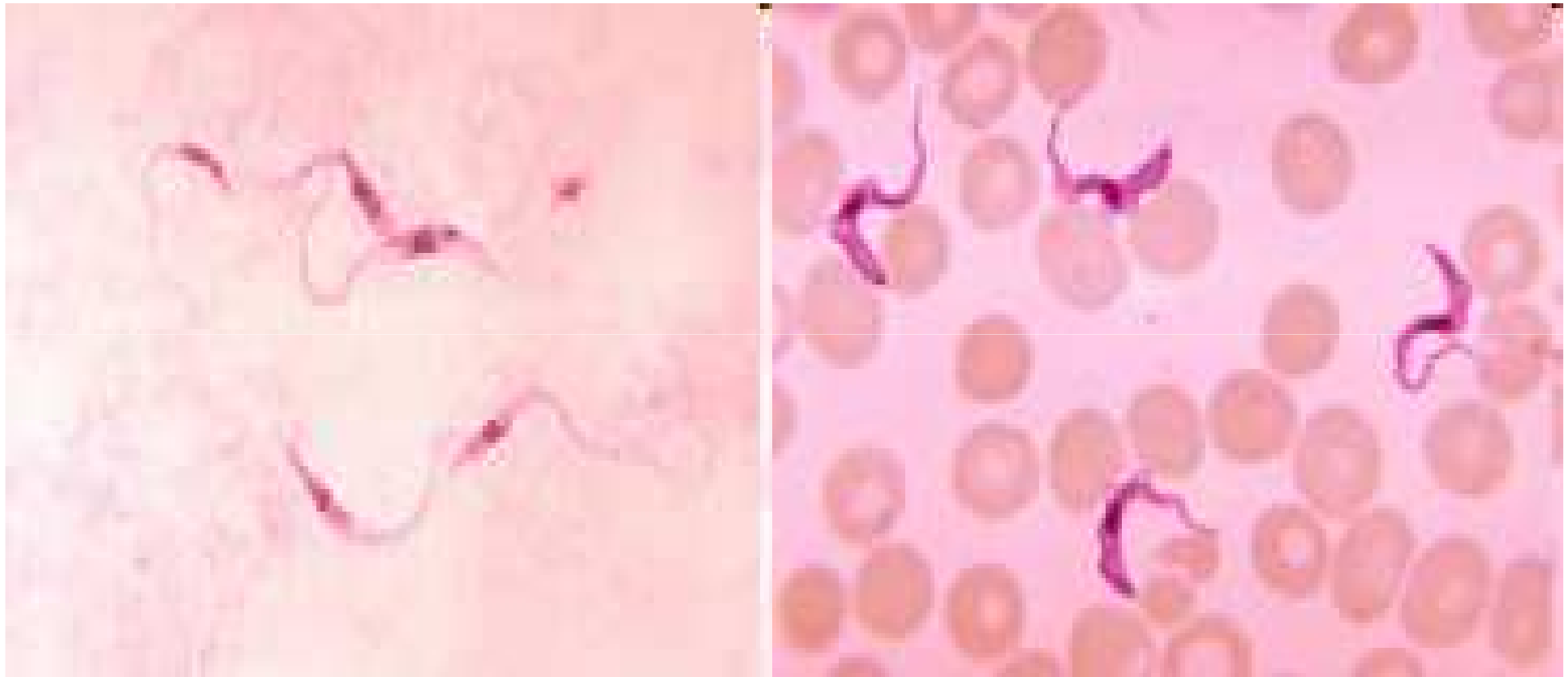
American trypanosomiasis

- Also known as Chagas disease
- It is a tropical parasitic disease caused by the flagellate protozoan *Trypanosoma cruzi*.
- Commonly transmitted to humans and other mammals by an insect vector, the blood-sucking **assassin bugs** of the subfamily Triatominae (family Reduviidae).
- Blood transfusion, contaminated food, mother to her fetus.

Laboratory tests

- **Microscopic examination** of fresh anti-coagulated blood (5ml), or its buffy coat (HCT), for allowing the observation of the rapid movements of live *T cruzi* trypomastigotes (at 400X), QBC method
- Examination of at least 100 microscopic fields.
- Use of thin and thick blood smears stained with Giemsa, for direct visualization of parasites.
- Stained thin and thick blood smears are less sensitive methods. However, a thin blood smear allows visualization of the nucleus, the kinetoplast, and the flagella, making easier differentiation of species.

Giemsa-stained *Trypanosoma* forms



Indirect methods

- Isolation of *T. cruzi* can occur by inoculation into mice, by **culture** in specialized media (e.g., NNN, LIT);
- **Xenodiagnosis**, where uninfected Reduviidae bugs are fed on the patient's blood, and their gut contents examined for parasites.

Other tests

- **Immunoassays**, using the reaction of an antibody or antibodies to its antigen and can be used to distinguish among strains.
- Include: detecting **complement fixation, indirect hemagglutination, indirect fluorescence assays, radioimmunoassays, and ELISA.**
- Alternatively various polymerase chain reaction (**PCR**) procedures have been described that use specific primers to detect *T cruzi* kinetoplastic or nuclear DNA but has low sensitivity

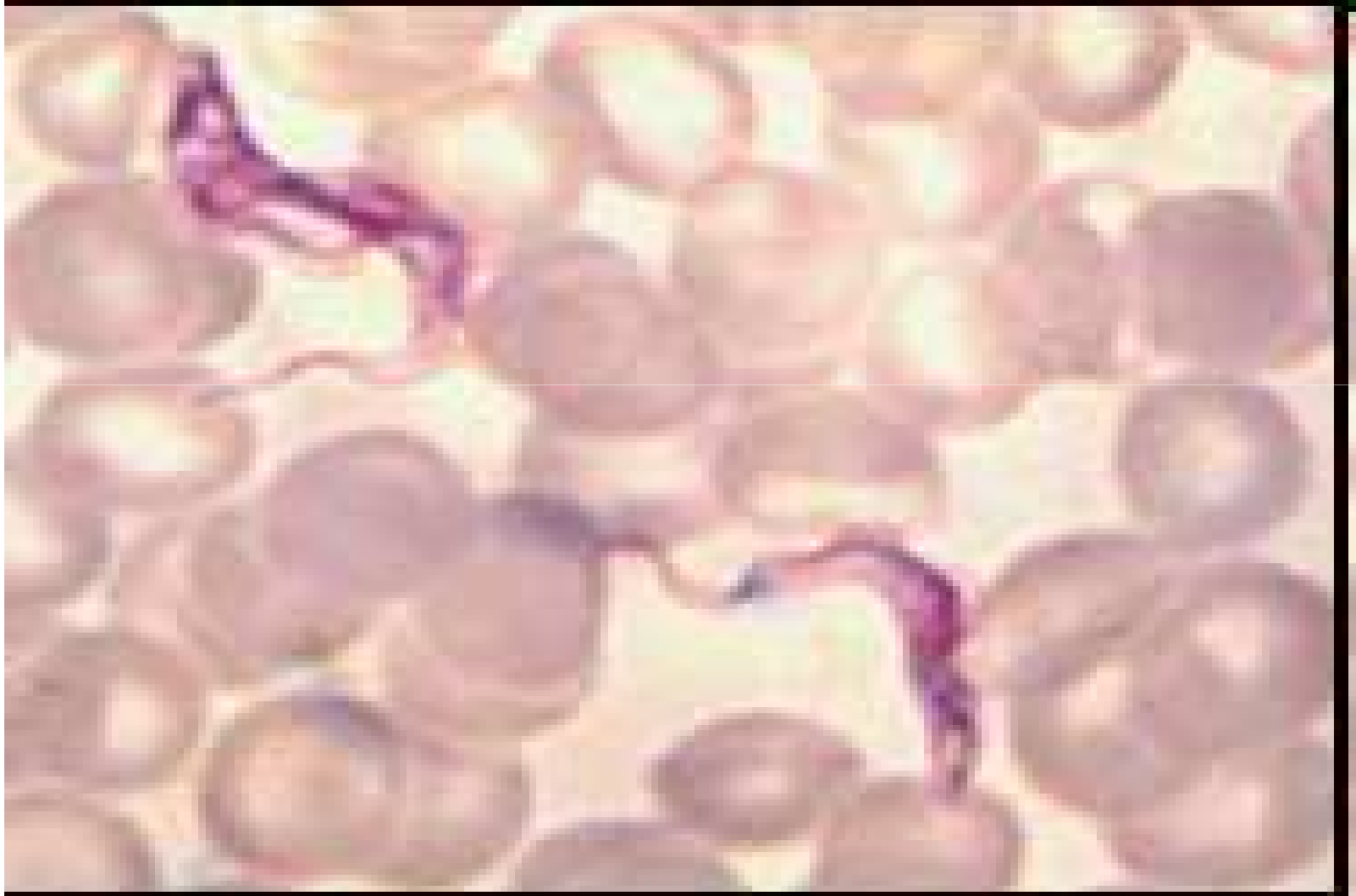
Human African trypanosomiasis

- Also called “**sleeping sickness**”
- It is a parasitic disease of people and animals, caused by protozoa of species ***Trypanosoma brucei*** and transmitted by the **tsetse fly**.
- The disease is endemic in certain regions of Sub-Saharan Africa

Laboratory tests

- Diagnosis of infection requires **actual detection of trypanosomes** in blood, lymph nodes, CSF, skin chancre aspirates, or bone marrow.
- Lymph node aspirate is commonly used as a rapid test for trypanosomes at a high dry magnification (X 400).
- It requires immediate search for parasites because they are mobile for only 15-20 minutes

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- Blood smear
 - A wet smear of unstained blood or Giemsa-stained thick smear (more sensitive) is used to observe the mobile trypanosomes, again for 15-20 minutes. Wright and Leishman stains are inadequate.
 - Other assays includes the hematocrit centrifugation technique for buffy coat examination, and the miniature anion-exchange centrifugation technique, which filters out the red cells but not the trypanosomes



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- Serologic antibody detection
 - The standard serologic assay to diagnose West African trypanosomiasis is the card agglutination test for trypanosomiasis (CATT).
 - The CATT can be conducted in the field without electricity, and results are available in only 10 minutes. It is highly sensitive (96%) but less specific because of cross-reactivity with animal trypanosomes.
 - Commercial antibody tests for Eastern African trypanosomiasis are not available.

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- Other tests developed but not frequently used clinically include antibody detection in the CSF and intrathecal space, enzyme-linked immunoassay (ELISA), polymerase chain reaction (PCR), and serum proteomic tests.

Dengue fever

- **Dengue fever (DF)** and **dengue hemorrhagic fever (DHF)** are acute febrile diseases, found in the tropics and subtropics, and caused by four closely related but antigenically distinct, virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4), of the genus *Flavivirus*, family Flaviviridae.
- The geographical spread is similar to malaria, but unlike malaria, dengue is often found in urban areas of tropical nations.
- Dengue is transmitted to humans by the *Aedes aegypti* (rarely *Aedes albopictus*) mosquito, which feeds during the day.

Laboratory tests

- The diagnosis of dengue is usually made **clinically**.
- Indirect – Thrombocytopenia ($<100,000$ platelets per mm^3 or estimated as less than 3 platelets per high power field)
- Evidence of plasma leakage (hematocrit more than 20% higher than expected-haemoconcentration)
- leukopenia (neutropenic)

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- Several diagnostic techniques are available to document an infection by the dengue virus at reference labs.
 - Virus isolation and
 - immunoglobulin M enzyme-linked immunoassay, or IgM ELISA.
 - Virus isolation is attempted to determine the serotype of the infecting virus. This can be performed either by using mosquito cell cultures or by mosquito inoculation.
 - The anti-dengue IgM ELISA is the basic test for serologic diagnosis.

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- Plaque reduction neutralization test/DENV neutralizing antibody assay (ELISA based micronutrialization)
 - Polymerase chain reaction (PCR) can be done to confirm the diagnosis of dengue if clinically indicated.

End

Thank you