BLOOD SAMPLE

Collection of Blood Specimen

The blood provides the most common medium for recovery various stages of animal parasites. From blood specimen a diagnosis is routinely made for malaria, African trypanosmiasis, Visceral leishmanisis and most types of filariasis less frequently of chagas disease; and rarely of toxoplasmosis. Blood examination of malaria parasites needs to be collected and when ever possible reported before treatment is started. Careful attention is necessary in the collection and preparation of blood films.

The following care should be taken while collecting blood.

1. Collect sufficient quantity of blood.

a. Capillary blood from finger prick , toes, or ear lobes

b. Venous blood.

The collected blood should be enough to make wet unstained film, stained thin and/or thick films, or to be used for concentrating the parasites.

2. Time of collection

Collect the blood specimen at the appropriate time based on the clinical investigations. E.g. For microfilariae, malarial parasites, etc. Usually most malaria parasites are found in the blood towards the end of an attack of fever. Always collect blood for malaria parasite investigation before anti-malaria drugs are given to the patients. Blood should be collected in accordance with the periodicity of microfilariae of filarial worms.

3. If anti-coagulated blood specimen is to be used.

Use a suitable anticoagulant. E.g. Acid citrate dextrose (ACD) or sodium citrate for microfilariae, EDTA for malaria parasites and Trypanosomes.

4. After collection, protect specimens adequately.

The following types of blood examination can be carried out for the laboratory diagnosis of haemoparasites:

1. **Wet Blood Films**:-For microfilaria: Tube centrifugation lyzed blood techniqu10ml of venous blood is lyzed in saponin-saline. The microfilaria are concentrated by centrifugation . the addition of blue nuclear stain helps to identify the species. The number of microfilaria (mf) counted divided by 10 gives the number of mf/ml.

Microhaematocrit tube technique

Capillary blood (preferably ear lobe blood) is collected into heparinized capillary tubes or about 100µl is first collected into EDTA anticoagulant and transferred to plain capillary tubes . The blood is centrifuged in a microhaematocrit centrifuge and the buffy coat is examined for motile microfilaria.

Wet slide preparation

Collect 0.02ml of blood and mix with 2 drops of water (to lyze the RBCs) on a slide. Cover with cover glass and examine for motile using 10x objective, preferably by dark field microscopy. The technique is sometimes used as a screening test but it is not as sensitive as the above procedures.

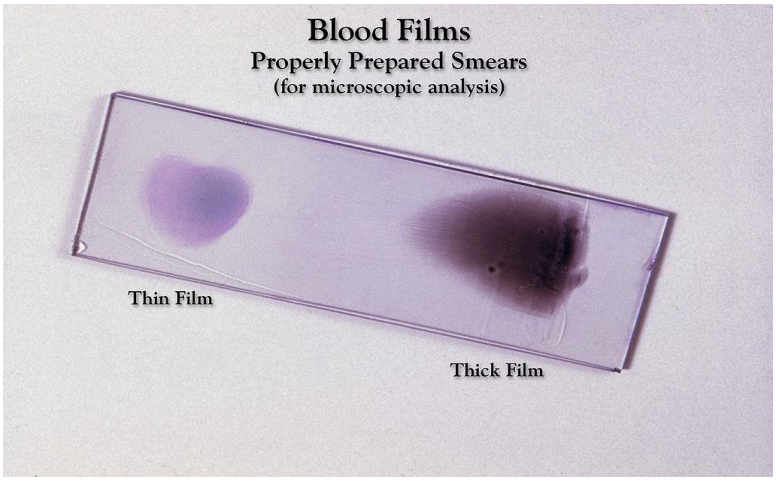
2**. Thick Blood Film**

Disinfect the tip of the finger and puncture with a needle or lancet, using and gauze sponge afterward to wipe away traces of the disinfectant and blood. Touch a clean slide to a drop of blood and using the corner of another slide, spread the blood in a rectangular pattern so that the blood slowly flows down and does not immediately form a drop at the lower edge when the slide is tilted sideways. A good thick film is the size of the postage stamp and so thick that you can just see the hands of a watch or news prints through it. The slide should be allowed to dry in a flat position such as table top, and do not use hear to dry the film. Then stain with Giemsa.

3. **Thin Blood Film**

Collect a drop of blood on a clean slide usually from the finger tip. Touch a clean slide to a small drop of blood so the blood is near one end of the slide. Place the slide blood side up on the table. Quickly take a second new clean slid. And holding it at an angle of 30 degrees to the first slide: draw it back until lt touches the drop of blood and then push forward so that blood spreads out behind. The amount of blood should be small enough so that it is used before the spreader slide reaches the end of the first slid. In this manner a smooth film. One-cell in thickness can be prepared. Such a film

is best for the study of blood cells as well as parasites. Then air dry and stain either with Geimsa or wright stain.



Significance of Thick and thin Blood film

- About twenty times more blood can be examined in a thick film than in a thin film in the same period of time. A thick film is therefore the most suitable for the rapid detection of malaria parasites. In area where P.malariae exists, unless a thick film is examined, infection is likely to be missed because parasitaemia is normally low with this species .

- More blood can be examined in a thick film because the film is not fixed and therefore the red cells are lyzed during staining. The parasites are not destroyed and after being stained they can be detected among the white cells, against a background of lightly stained hemoglobin.

- A thin blood film is required to confirm the Plasmodium species if this is not clear from the thick film. A thin film is fixed and therefore the parasites can be seen in the red cells. Depending on the species, parasitized red cells may become enlarged,ovalin shape and show stippling. These features, together with the parasitic forms present can greatly assist in confirming a mixed infection and in identifying P.ovale and P.malariae which are more difficult to differentiate in thick film.

Making of Thick and Thin Blood film:

Blood from a finger prick is usually used for malaria smears. The thin and thick smear may be stained with wrights or Giemsa stain. A thin smear is best stained with wrights and thick best stained with Giemsa.

A. Counting the Percentage of Parasitized Red Cells in a thin film

To estimate the parasitized cells, count a total of at least 500 red cells making a note of the number that contain parasites excluding gametocytes and at the end report in percentage. For this procedure the best method is to insert in the eye piece of the microscope a disc with a central square to reduce the size of the field. This will make counting easier by reducing the number of red cells soon in the field.

Membrane filtration method for microfilaria detection

5 mL of venous blood collected in EDTA anticoagulant tube at night (between 11 pm and 1 am) in order to test the presence of circulating microfilaria and the collected blood filtered through millepore membrane filters (pore size 0.22). It enables an easy detection of microfilaria and quantifies the load of infection. They are usually observed in the early stages of the disease before clinical manifestations develop. Once the lyphoedema develops, microfilaria disappear from peripheral-blood.

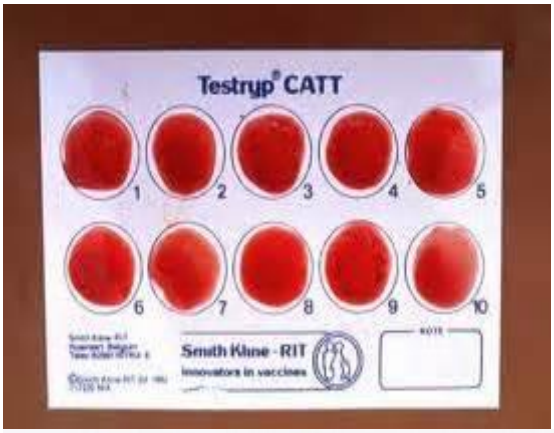
Serology-Based Assays

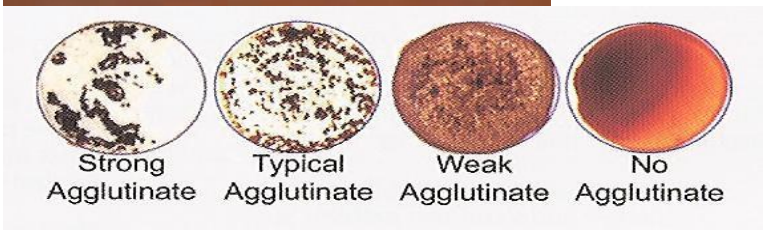
In situations where biologic samples or tissue specimens are unavailable, serology alone is the gold standard for diagnosis. Serology-based diagnosis tools can be divided into two categories: antigen-detection assays and antibody-detection assays. These include the enzyme-linked immunosorbent assay (ELISA), also called enzyme immunoassay (EIA), and all its derived tests such as the Falcon assay screening test ELISA (FAST-ELISA) and the dot-ELISA. Other assays include the hemagglutination (HA) test, indirect or direct immunofluorescent antibody (IFA or DFA) tests, complement fixation (CF) test, and immunoblotting and rapid diagnostic tests (RDTs).

Although the ease of use and turn around times for serologic assays are similar to microscopy, serology-based assays are more sensitive and specific. It becomes important for individuals whose blood smears do not permit identification of the parasite (e.g., differentiating between Babesia and Plasmodium) or for patients exhibiting low-parasitemia and/or who are asymptomatic (e.g., Chagasic patients). Classifying an infected asymptomatic patient as negative could lead to transmission of the parasite during blood transfusions or organ transplants.

Card agglutination test for trypanosomiasis (**CATT)** is the preferred first-line serological detection method for *T. b. gambiense*, but must be followed by parasitological confirmation and stage determination.

- Pricking the patients finger
- Collecting the blood sample
- Mixing the blood with heparin
- Adding the CATT antigen
- Adding the sample
- Agglutination reaction





4.1. Nucleic Acid-Based Approaches

The many limitations of microscopy and serology-based assays have influenced parasitologists towards the use of gene amplification methods made possible with the advent of the polymerase chain reaction (PCR). Besides the traditional PCR, including nested and multiplexed PCR, we have seen the implementation of the real-time PCR (RT-PCR) for the detection of several parasitic infections. Newer technologies such as loop-mediated isothermal amplification and Luminex-based assays have also emerged as possible new approaches for the diagnosis of parasitic diseases.

Molecular-based approaches based on nucleic acids offer greater sensitivity and specificity over the existing diagnostic tests. They permit the detection of infections from very low parasitized samples including those from asymptomatic patient's samples. Moreover, multiplexed PCR allows for the detection of multiple sequences in the same reaction tube proving useful in the diagnosis of several parasitic infections simultaneously.

Loop-mediated isothermal amplification (LAMP) is a unique amplification method with extremely high specificity and sensitivity able to discriminate between a single nucleotide difference. It is characterised by the use of six different primers specifically designed to recognise eight distinct regions on a target gene, with amplification only occurring if all primers bind and form a product

Unlike a regular PCR reaction, LAMP is carried out at a constant temperature (usually in the range of 60–65°C). This unique feature not only results in higher yields, but also eliminates the need to buy a thermal cycler and shortens the reaction time by eliminating time lost during thermal changes. In addition, the reaction can be carried out without extracting the DNA from the collected samples. In 35 minutes, using a simple water bath, LAMP was able to detect both T. b. gambiense and T. b. rhodesiensedirectly from blood, serum, and CSF samples.

Luminex xMAP Technology

Luminex technology is a bead-based flow-cytometric assay that allows the detection of various targets simultaneously. The microsphere beads can be covalently bound to antigens, antibodies, or oligonucleotides that will serve as probes in the assay. Up to 100 microspheres are available each emitting unique fluorescent signals when excited by laser therefore allowing the identification of different targets. Adapted to the study of parasites, the Luminex assay could identify multiple organisms or different genotypes of one particular organism during the same reaction utilizing very low volume. The approach could prove useful in the study of antigenic diversity and drug-resistance alleles and for the diagnosis of parasitic diseases.