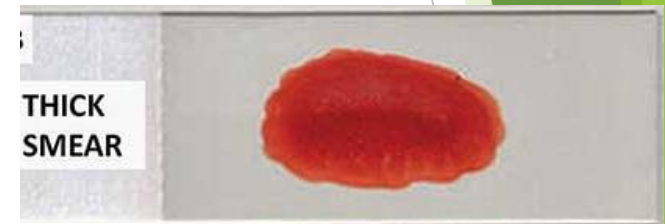


Blood Smear Preparation: Wet, Thin and Thick Smears (Practical)



Dr. AJIT KUMAR

Department of Veterinary Parasitology

Bihar Veterinary College

Bihar Animal Sciences University

Patna-800014

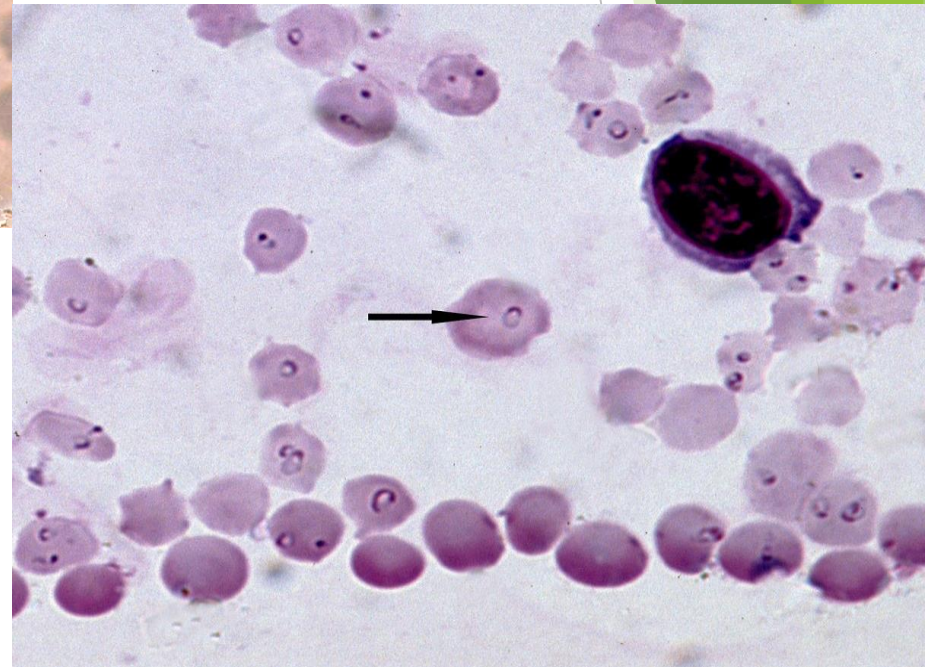
Blood Smear Preparation

Recommended for:

Diagnosis of Haemoprotozoan parasites (*Theileria*, *Babesia*, *Trypanosoma*, *Hepatozoon*, *Haemoproteus* etc.) , microfilariae of filarial worms, Rickettsial organisms (*Anaplasma* spp., *Ehrlichia* spp.)

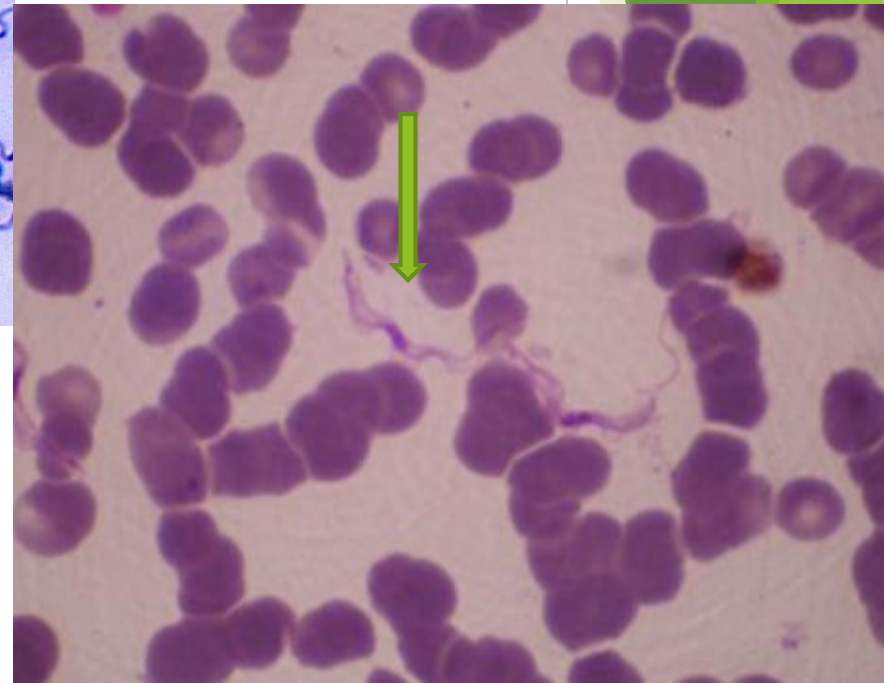
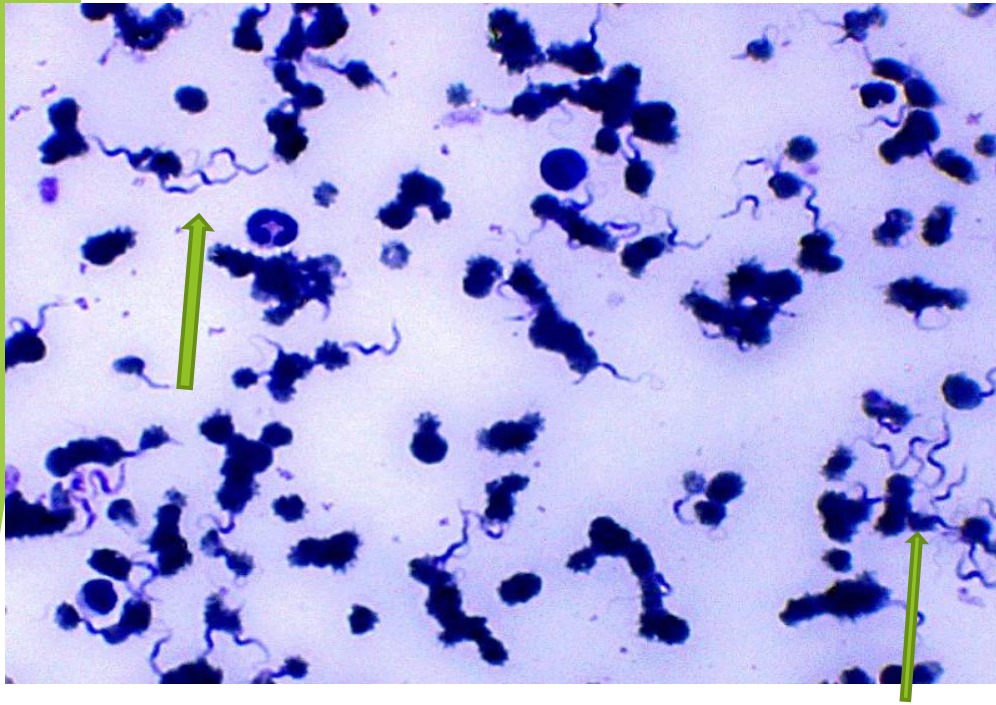
Blood Smear Preparation

Theileria spp.: Inside RBCs.



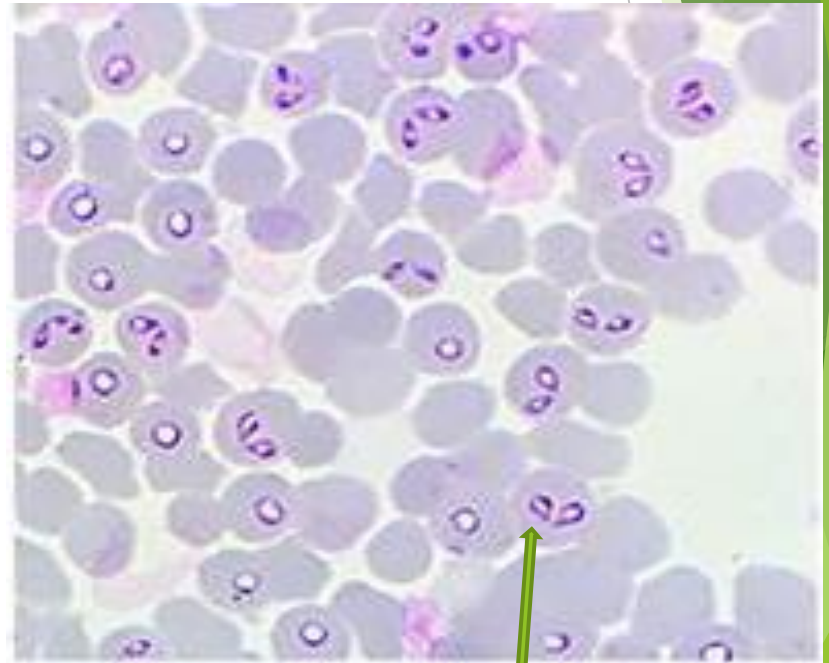
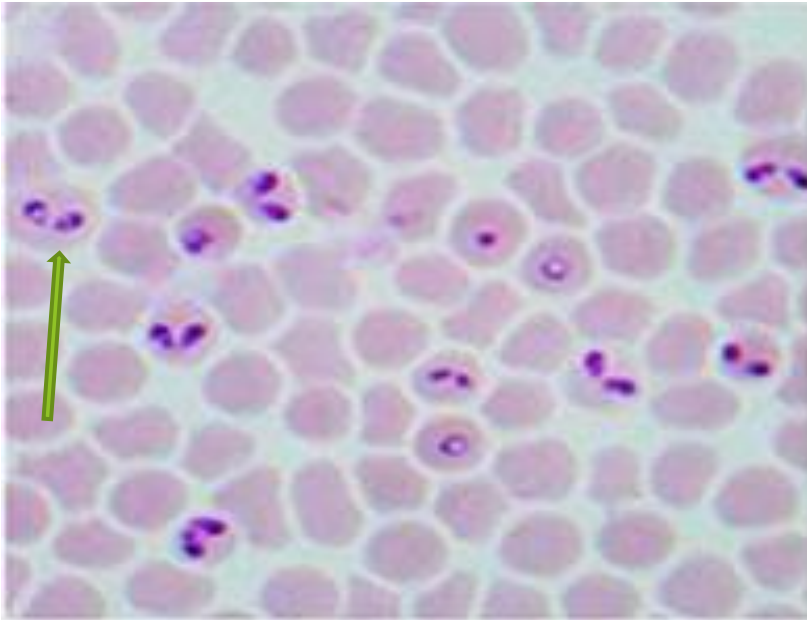
Blood Smear Preparation

Trypanosoma spp.: - in blood plasma.



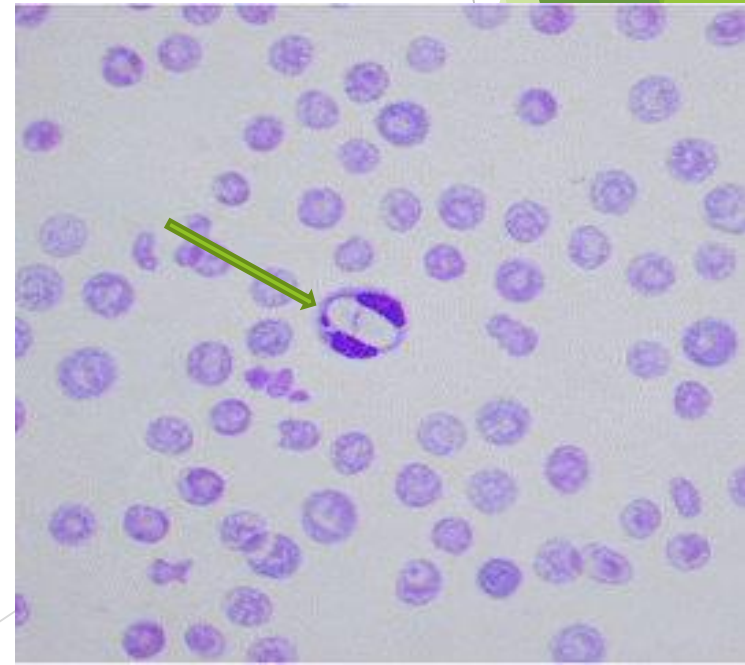
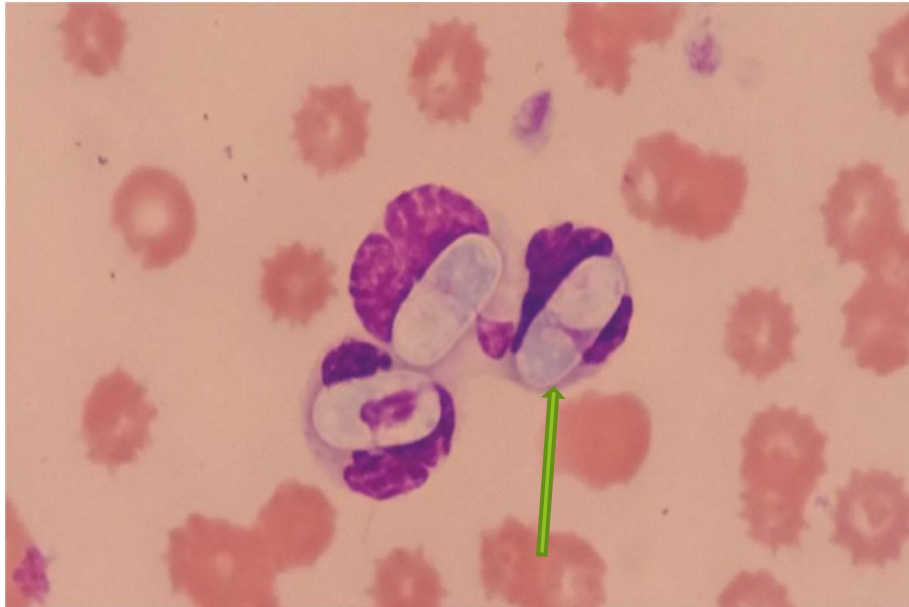
Blood Smear Preparation

Babesia spp.:- inside RBCs.



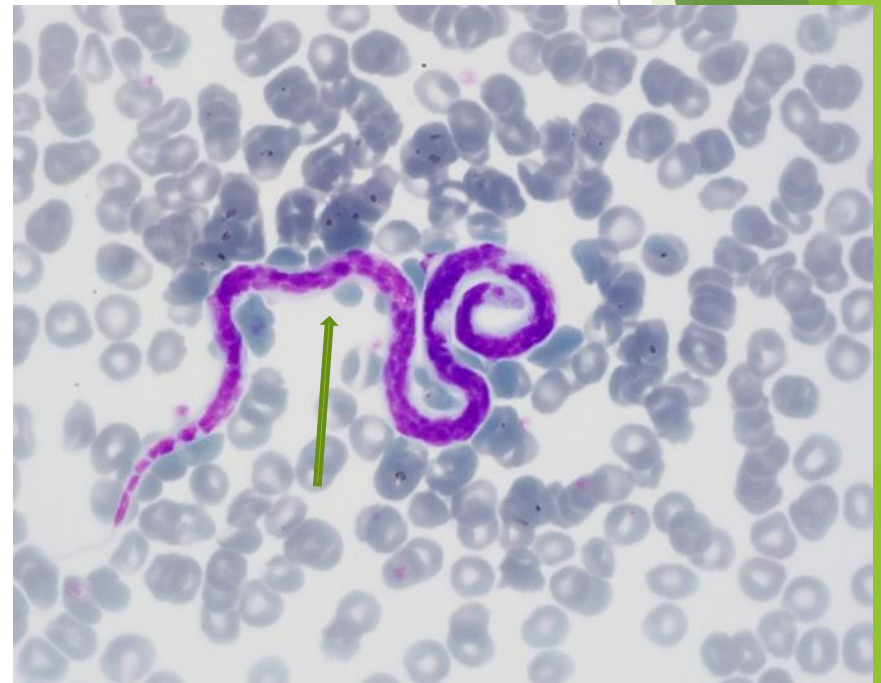
Blood Smear Preparation

Hepatozoon canis:- inside neutrophil



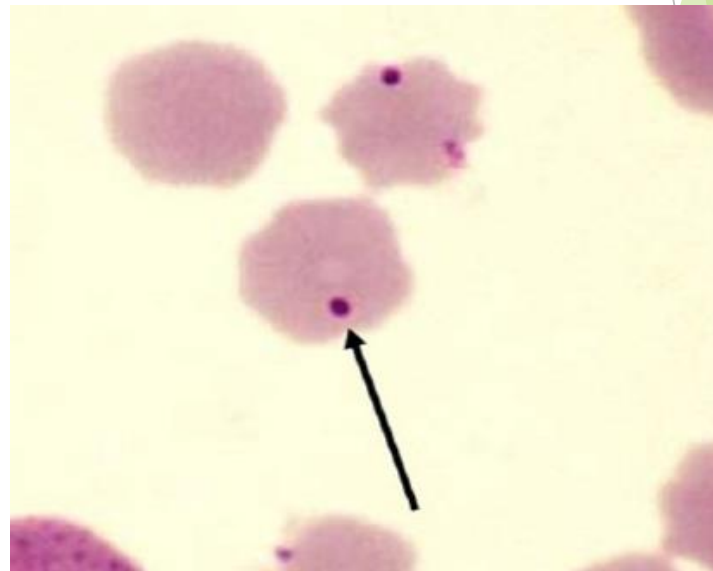
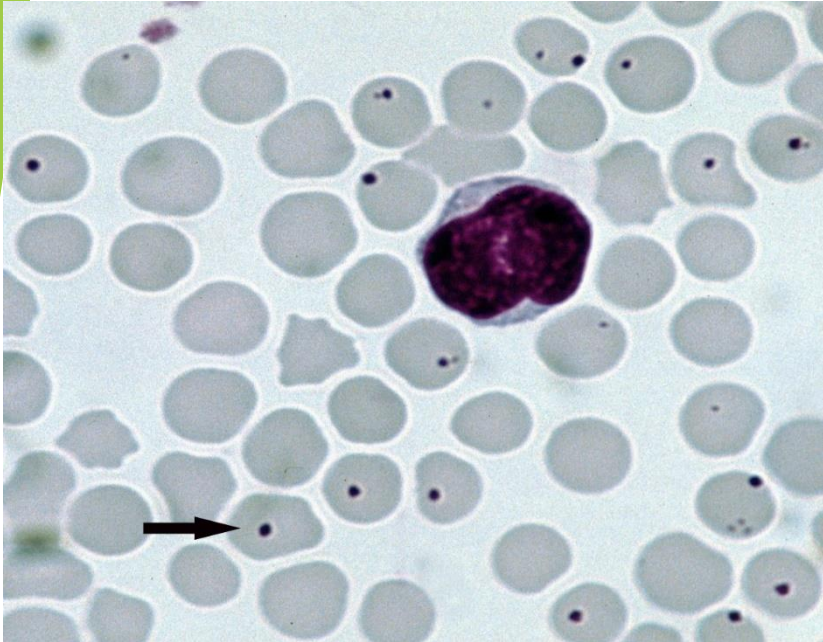
Blood Smear Preparation

Microfilaria of filarial worms: in blood plasma.



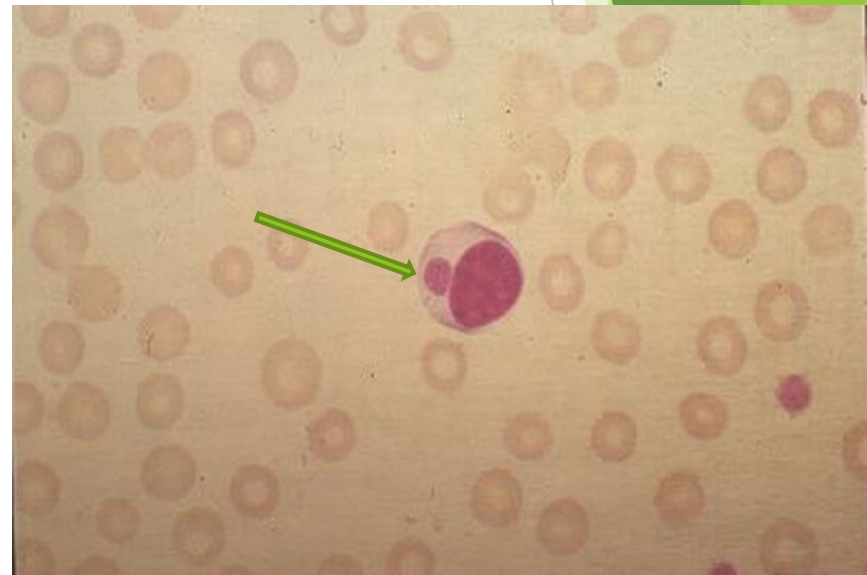
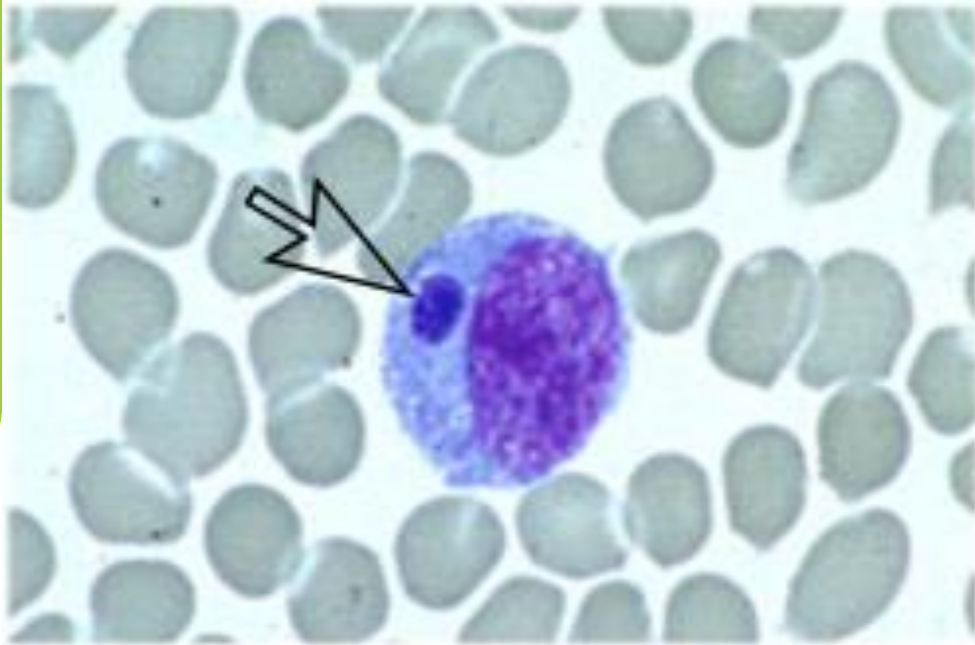
Blood Smear Preparation

Anaplasma marginale:- near margin of RBCs.



Blood Smear Preparation

Ehrlichia spp.:- inside leucocytes.



Blood Smear Preparation

Cleaning new slides:

- Slides should be absolutely grease free and clean.

Procedure:

- To make it absolutely grease-free the slides are soaked overnight in 2% glacial acetic acid in ethyl alcohol, washed in distilled water, dried and cleaned with dry muslin cloth before use



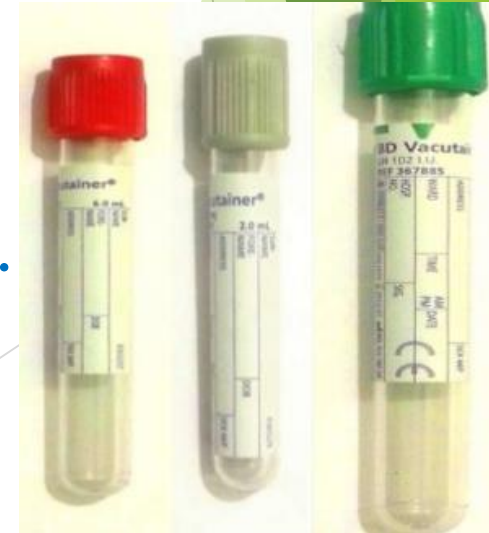
Blood Smear Preparation

Blood collection vials :

- EDTA containing blood collecting vial



- Simple vial (without anticoagulant)
- to be add anticoagulant before blood collection.



Blood Smear Preparation

Anticoagulants :

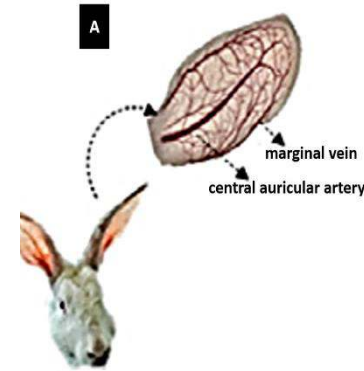
- Ethylene diamine tetra acetic acid (EDTA) : 1 mg of powder to 5 ml of blood.
- Heparin : 75 units for 10 ml. of blood.
- Sodium oxalate 20% : use @ 0.01 ml/ml of blood.
- Sodium citrate 25% : use @ 0.01 ml/ml of blood.



Blood Smear Preparation

Collection of blood :

- Small amount of blood for making smears may be taken from the ear vein of horse, cattle, sheep, goat, pig, rabbit and dog. When a blood sample from a bird is required, the vein near the elbow joint under the wing is punctured.
- The hairs from the area should be clipped and the part cleaned and disinfected with 70% alcohol or methylated spirit.



Blood Smear Preparation

Blood collecting Syringe and Needle :

- The gauge of the needle should be 16-18 for horse, camel, cattle, sheep and goat ; 16-20 for pigs; 18-20 for rabbits; 20-22 for dogs; and 20-24 for cat, poultry and man.



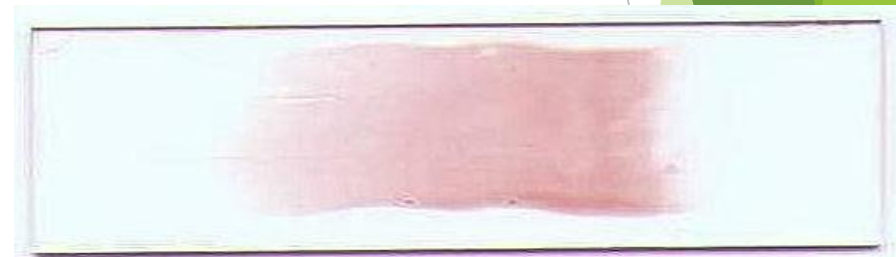
Blood Smear Preparation

Types of blood smear:

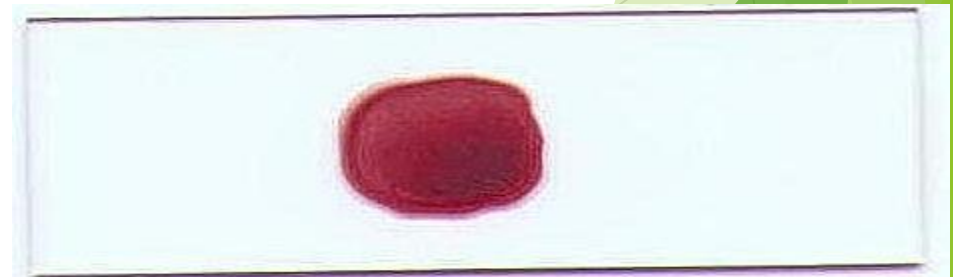
- Wet smear (Wet blood film)



- Thin



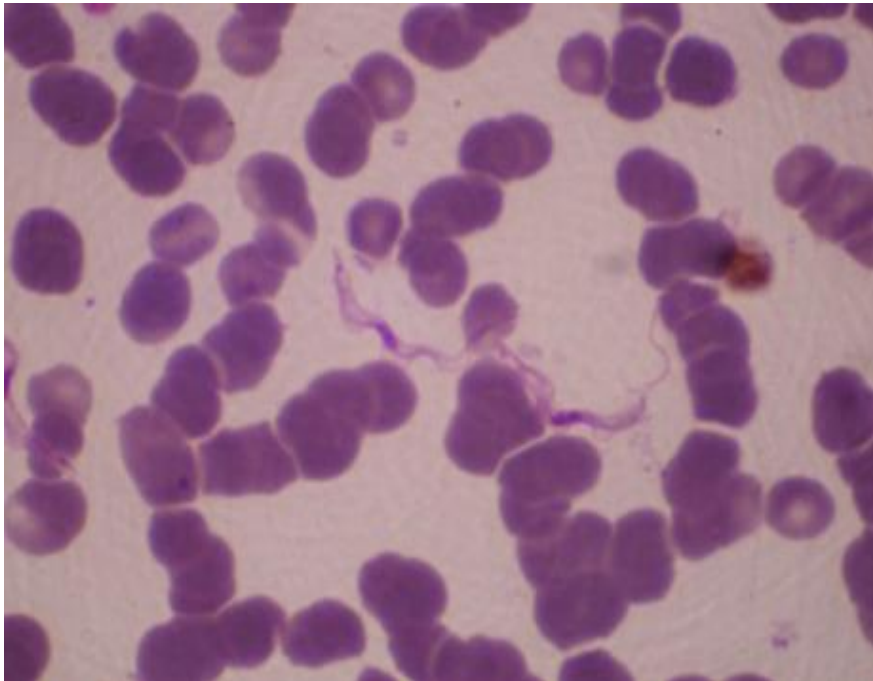
- Thick smears



Blood Smear Preparation

Wet smear:

- A wet blood film is used for the detection of living trypanosomes, microfilariae of filarial worms etc.

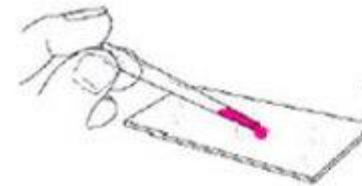
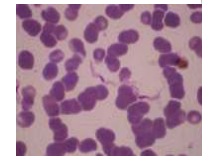


Blood Smear Preparation

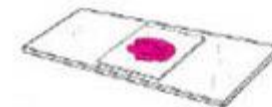
Wet smear (Wet blood film):

Preparation Method :

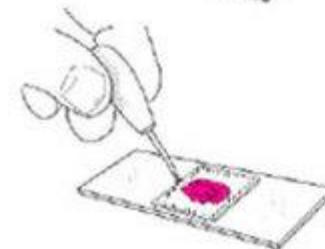
- Place a small drop of blood on to a clean grease free slide and put a cover slip on it and examine under low power (10x or 40x) of the microscope.
- This is an easy and rapid method of detection of live *Trypanosoma* spp. and microfilaria or first larval stage of blood filarial worms in acute infection.



1. Using a capillary pipette, place a small drop of culture broth on the center of the slide.



2. Carefully place a clean coverslip over the drop, avoiding bubbles.



3. If no fluid has escaped from under the edges of the coverslip, seal the preparation with clear nail polish. Allow the polish to dry thoroughly before examining preparation under the microscope.

Blood Smear Preparation

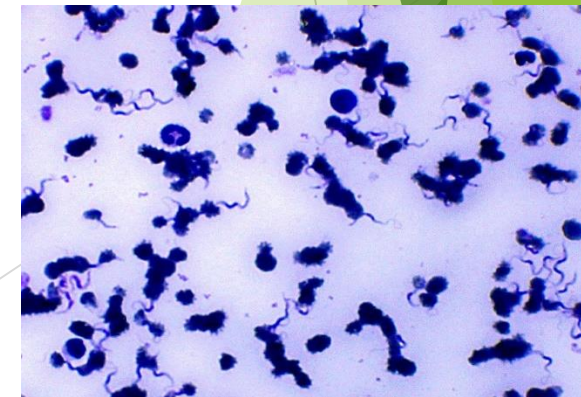
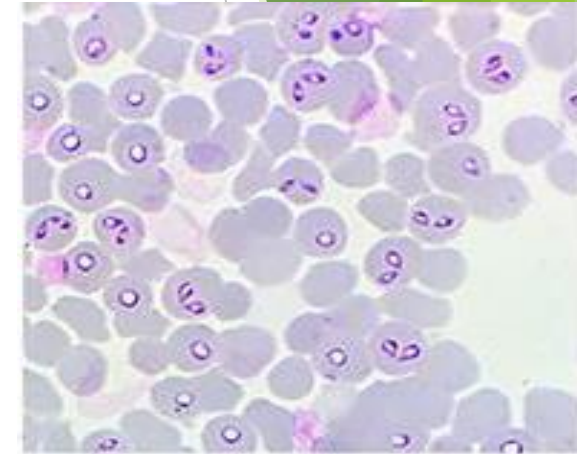
Wet smear:

- For delay preparation of wet film, the blood should be collected in Alsever solution.
- Alsever's solution contains Dextrose (5.125 gm), Sodium citrate (2 gm) and Sodium chloride (1.05 gm), Citric acid (0.137 gm) and distilled water (250 ml).

Blood Smear Preparation

Thin Smear method :

- Thin blood smears are used when the parasites are plenty.
- In pyrexial phase(at the time of high temperature), It should be prepared, when blood parasites are present in large numbers (in acute infection) in the blood.
- Useful in diagnosis of Haemoprotozoan parasites (*Theileria*, *Babesia*, *Trypanosoma*, *Haemoproteus* etc.), Rickettsial organism (*Anaplasma* spp. etc.)



Blood Smear Preparation

Thin Smear method :

Preparation Method :

- The site of the vein is cleared with non-fluffy cotton and ethyl alcohol to remove the contaminants and the slide is dried.
- The vein is punctured using a clean needle.
- A small drop of blood, less than a pin's head is placed in the middle, near one end of the slide.

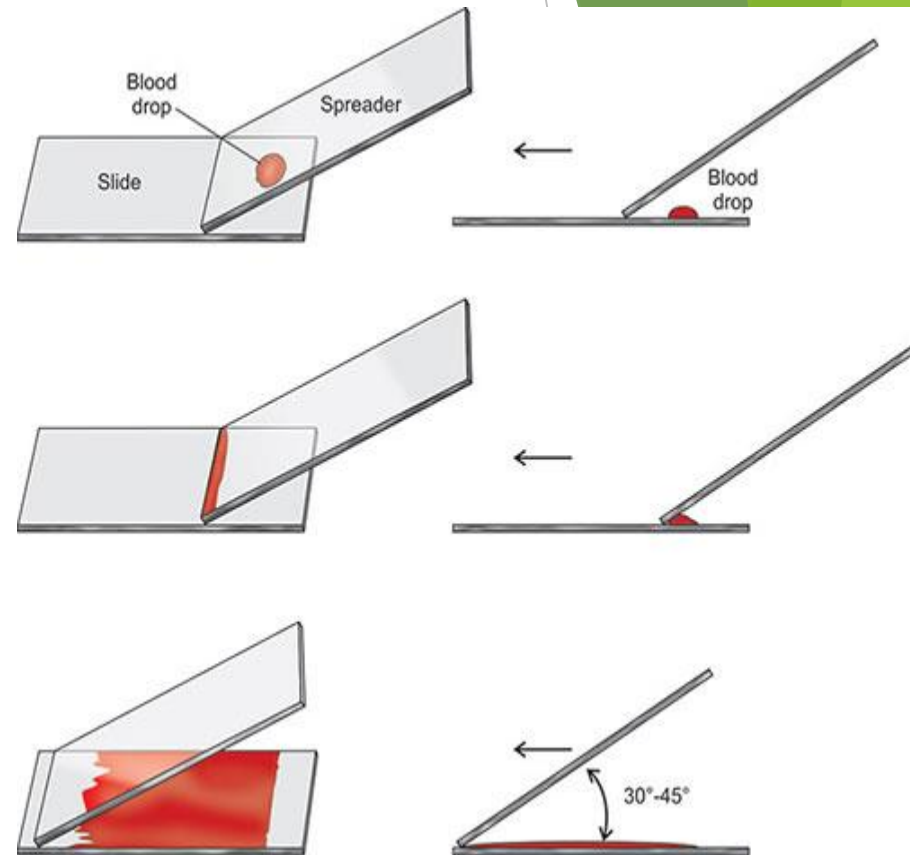


Blood smear preparation

Thin Smear method :

Preparation Method :

- Slide is held firmly between the middle finger and thumb of the left hand and another clean slide with straight and smooth edges (spreader slide), is placed on the center of the examination slide.
- Lower edge of the spreader slide is held at an angle of 30 to 45 degrees and is drawn up to make contact with the drop of blood and wait until drop of blood flows both end of the spreader slide.
- Draw the spreader slide away from the blood drop with a smooth rapid movement. This action results in thin and even blood smear.

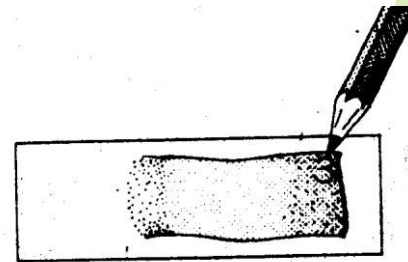
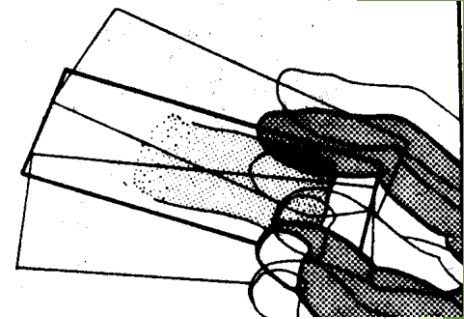


Blood Smear Preparation

Thin Smear method :

Preparation Method :

- Blood film is dried by waiving it in the air.
- Identity of blood smear with respect to its host etc. is recorded on the slide by making.



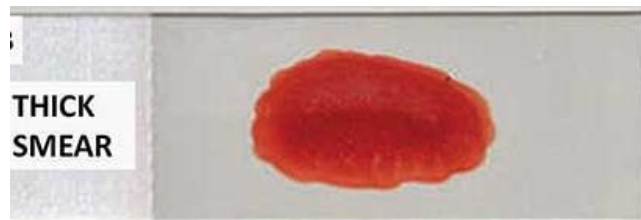
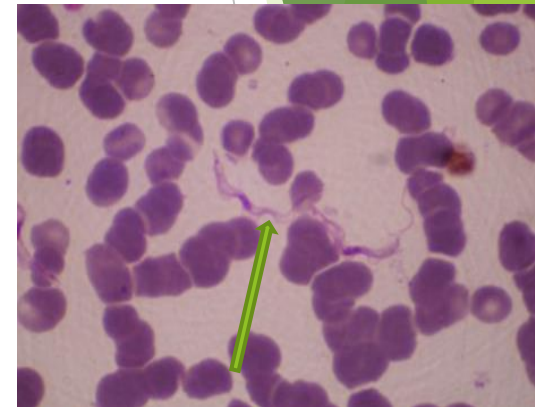
Blood Smear Preparation

Thick Blood Smear:-

The diagnosis of milder infection or chronic infections when only a few *Trypanosoma* spp. are present in the entire blood smear.

Procedure:

- In such cases prepare a thick blood smear in which large quantity of blood can be examined. A large drop of fresh blood is placed on a glass slide.
- Thick smear must be dehaemoglobinised (removal of hemoglobin) before it is stained. This is done by placing the slides in water until the colour disappear.
- Thick blood smears cannot be used with fowl or camel blood because of the nucleated R.B.C.

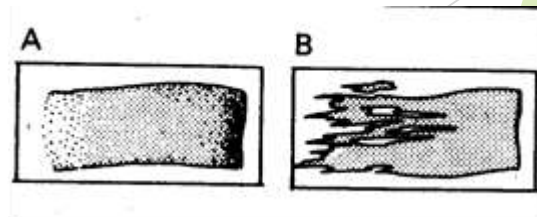


Blood Smear Preparation

Points of a good blood film or smear :

- The film should occupy about 1/3 of the length of the slide.
- The greater part of it should consist of a uniform single layer of blood cells.
- The edges of the film should be as unbroken as possible.
- The film should not be so thin to break the continuity of the film.

A. Good smear B. Bad smear



Blood Smear Preparation

Fixation of blood smears:

- Fixation is required to prevent any type of autolytic changes.
- Otherwise the smears would deteriorate, the cells may shrink or stretch due to osmosis or be digested by their own cellular enzymes or the smears may also be affected by bacteria or fungi such as moulds.
- Chemical fixatives like methyl alcohol is used to fix the smear.

THANK U