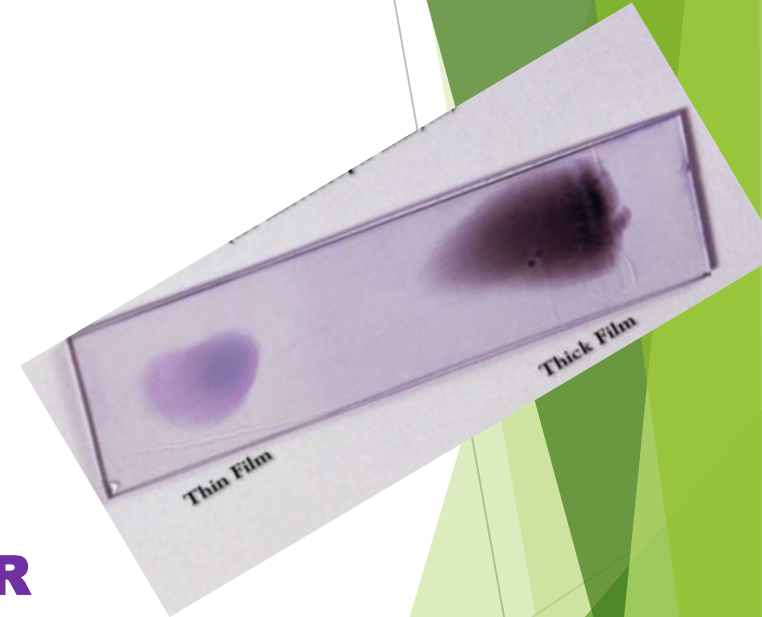
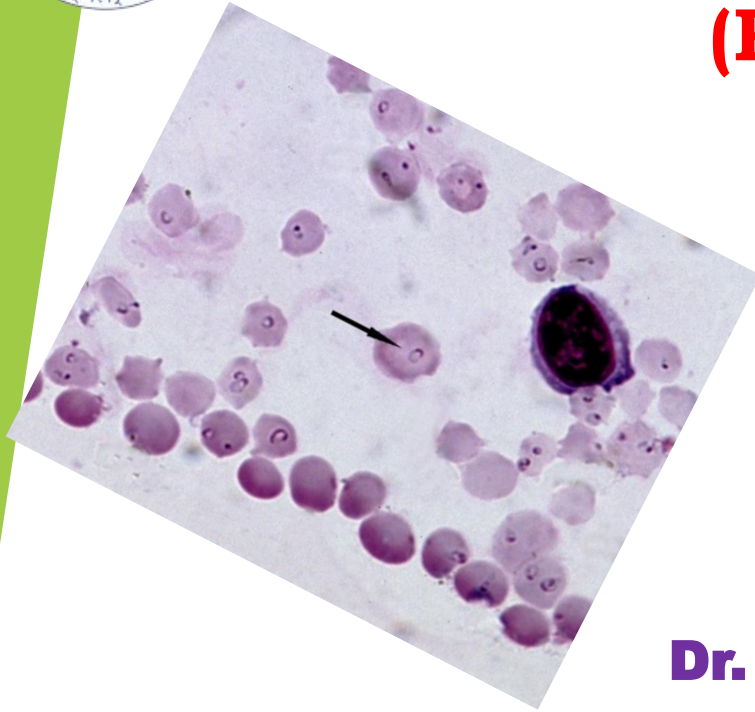


Staining of Blood Smears for Demonstration of Microfilariae and Haemoprotozoan Parasites (Practical)



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Staining of Blood Smears

Usually two types stains used in blood smears staining :-

- Giemsa stain
- Leishman stain

	Giemsa Stain	Leishman Stain
DEFINITION	Geimsa stain is a technique used primarily during differential staining of chromosomes	Leishman stain is a technique used primarily in staining of blood smears
USE	Useful in the staining of DNA regions of different chromosomes in order to investigate different aberrations such as translocations and rearrangements	Useful in blood staining in order to differentiate and identify trypanosomes, leucocytes and malaria parasites
DISCOVERY	Bacteriologist Gustav Giemsa	Pathologist William Boog Leishman
STAIN	A mixture of eosin, methylene blue and Azure B	A methanolic mixture that contains a mixture of methylene blue

Staining of Blood Smears

Leishman's stain :

This stain has a poor keeping quality in hot climate, so it is better to prepare a fresh stain from the powder every month or so.

Leishman stain

- Composition
- Leishman powder (eosin-methylene blue powder) 0.5gm
- Acetone free methyl alcohol 100ml

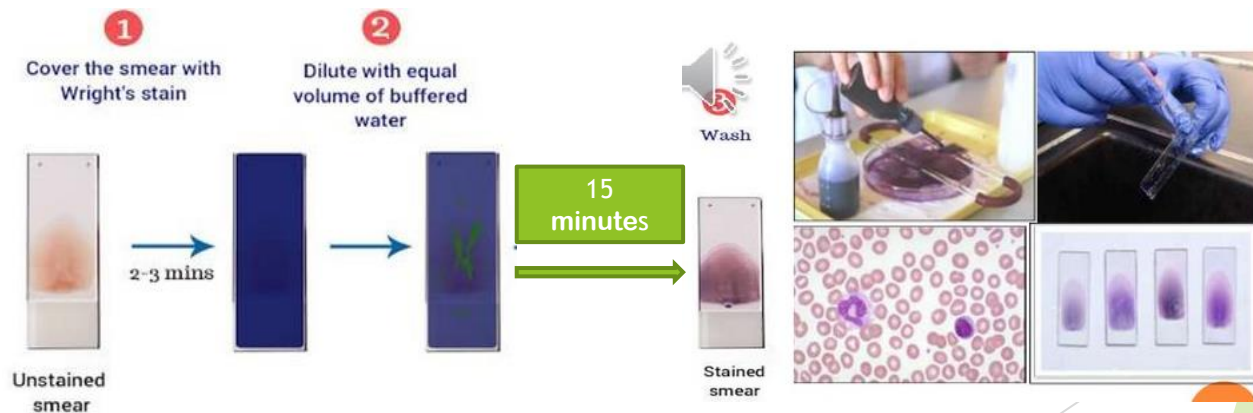


Staining of Blood Smears

Leishman stain :

Staining Procedure:

- Prepare blood smear and air dry it.
- Pour Leishman stain dropwise (counting the drops) on the blood smear and allowed to act for 1- 2 minutes.
- Add double quantity of distilled water dropwise over the blood smears (i.e. double the numbers of drops).



Staining of Blood Smears

Leishman's stain :

Staining Procedure:

- **Mixed by rocking the slide gently.**
- **15 minutes are allowed for staining**
- **Slide is washed by stream of distilled water in horizontal position until the smear looks pink.**
- **Thereafter, the slide is kept in a vertical position so that it drains and dries.**

Staining of Blood Smears

Leishman's stain :

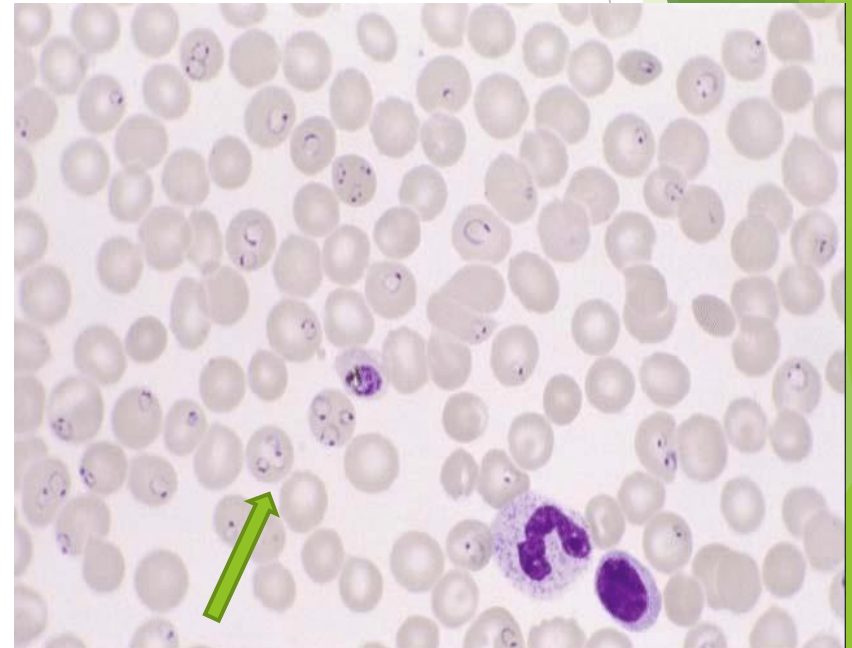
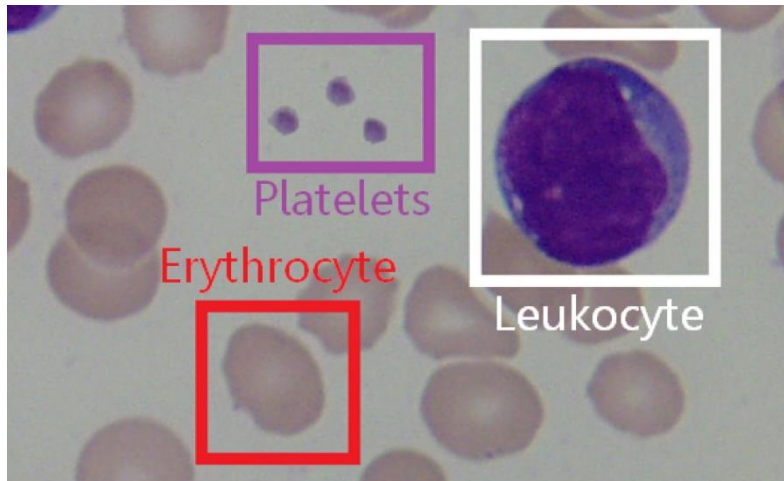
Staining Procedure:

- **Dry in air and examine under oil immersion lens of microscope.**
- **Fixation is not required in leishman's staining procedure because methyl alcohol is already mixed in leishman's stain.**

Staining of Blood Smears

Leishman's stain :

Microscopic examination.



Staining of Blood Smears

Giemsa stain :

Staining Procedure:

- Prepare blood smear and air dry it.
 - **Fixation:** Fixed in methyl alcohol for two minutes
 - Take out and dry it
 - Pour the diluted Giemsa stain (1 in 10 dilutions) over the fixed smear
- (1 part Giemsa stain + 9 parts distilled water or buffer water)

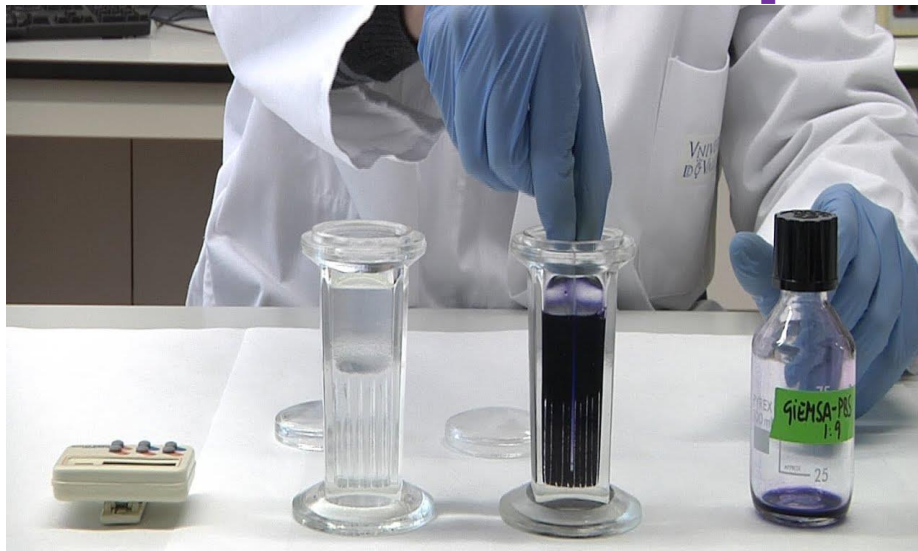


Staining of Blood Smears

Giemsa stain :

Staining Procedure:

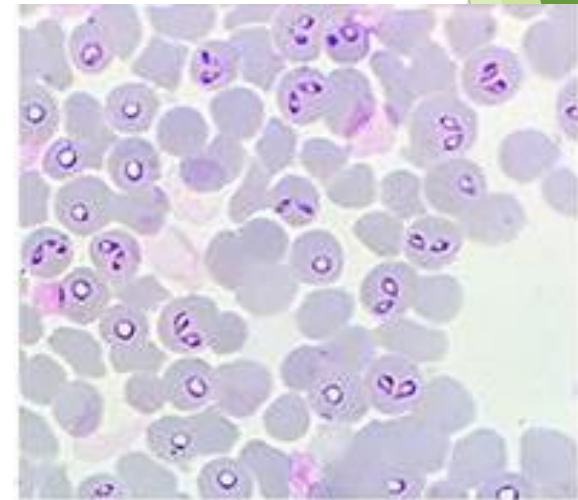
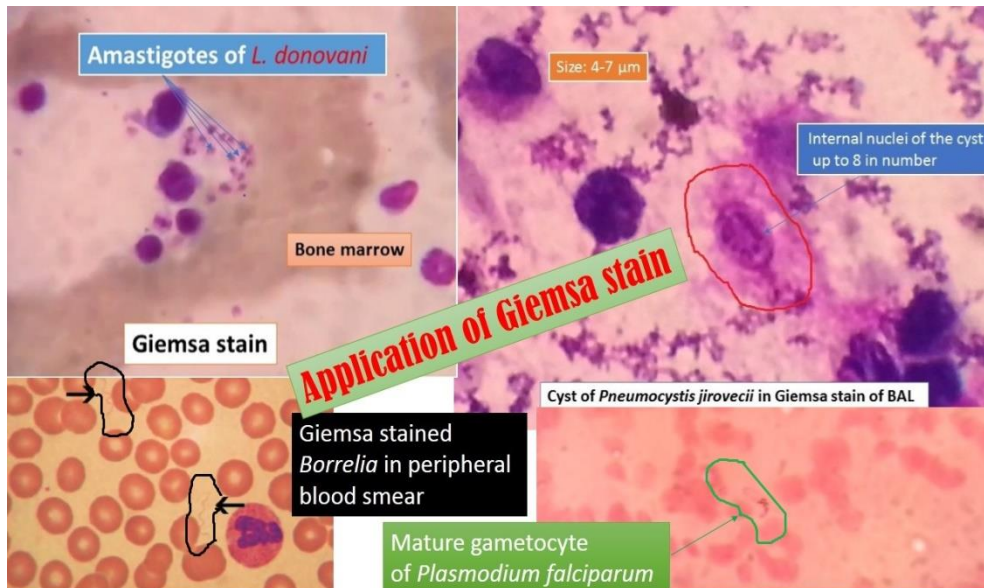
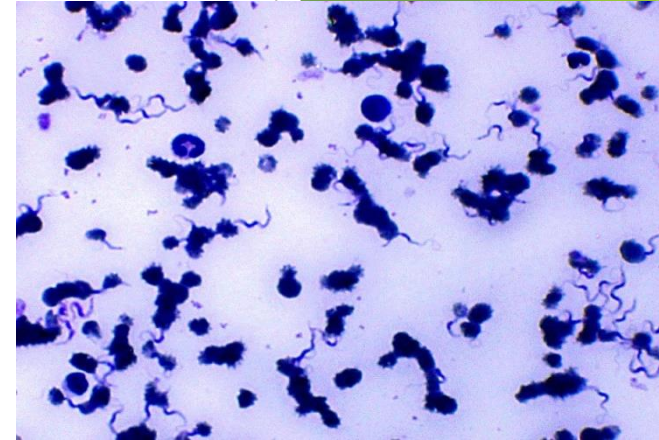
- It is kept for 45 minutes for allowing stains.
- Wash the smear under distilled water or tap water till the smear is just pink.
- Stained blood smears are dried in the air and examined under the microscope under oil immersion.



Staining of Blood Smears

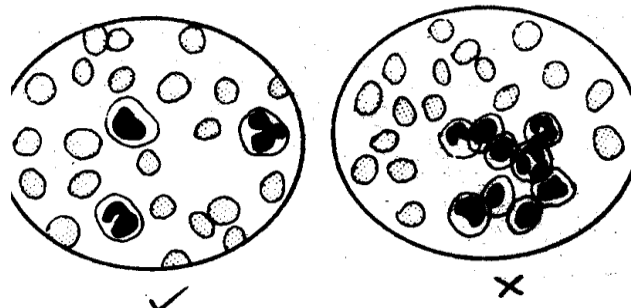
Giemsa stain :

Microscopic examination:



Staining of Blood Smears

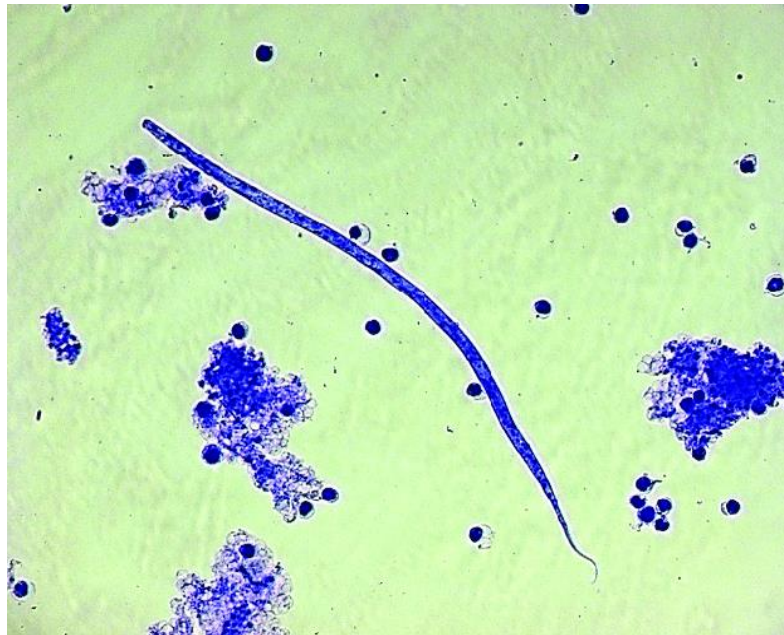
- Artifacts are liable to camouflage the precision of the microscopic findings.
- To avoid this, the slides should be placed vertically in the couplin jar having the required stain or preferably stained on a horizontal rack, diluted and washed with a buffer in the same position.
- If the stain is poured off, the scum is liable to stick to the slide and forms artifacts.



Blood smear preparation

Knott's Technique :-

- It is used for the diagnosis of microfilariae of *Dirofilaria immitis* and *Dipetalonema* spp. through lyses of the red blood cells.



Staining of Blood Smears

Knott's Technique :-

Materials:

- **Suspected heparinized blood**
- **2 % formalin**
- **Methylene blue**
- **Glass slide**
- **Coverslip**
- **Centrifuge tube**
- **Pipette**

Staining of Blood Smears

Knott's Technique :-

Procedure:

- Collect blood from the suspected dog in a heparinized vial.
- Mix 1 ml of heparinized blood and 3 ml of 2% formalin
- Centrifuge the formalin mixed blood at 1500 rpm for 5-8 minutes.
- Discard the supernatant and then sediment is stained by adding equal amount of 1:1000 methylene blue stain.

Staining of Blood Smears

Knott's Technique :-

Procedure:

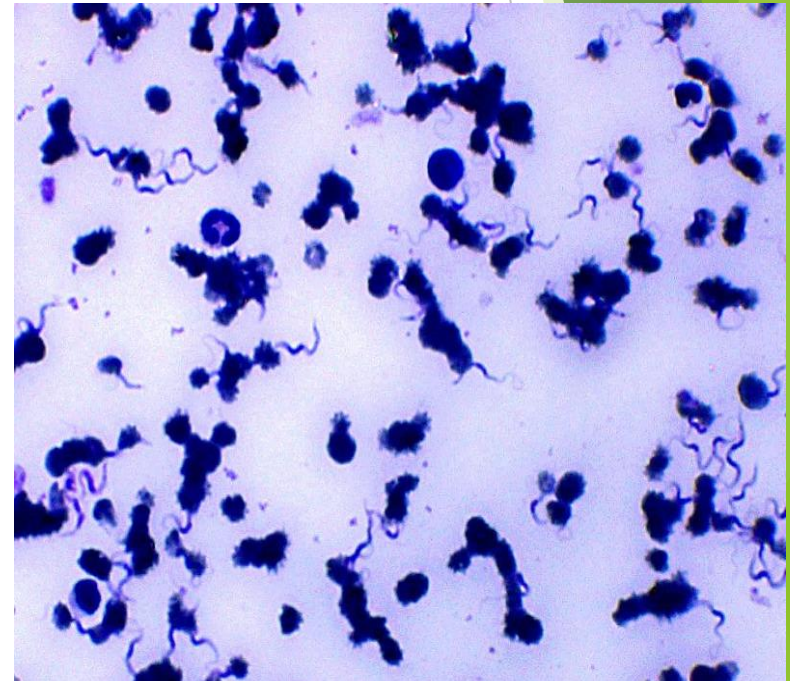
- One drop of mixture is placed on glass slide with the help of pipette and apply coverslip over it.
- Examine microfilariae under 40x or 100x



Staining of Blood Smears

Microhaematocrit centrifuge technique :-

It is used for the detection of microfilariae of filarial worms or *Trypanosoma* spp. parasite.

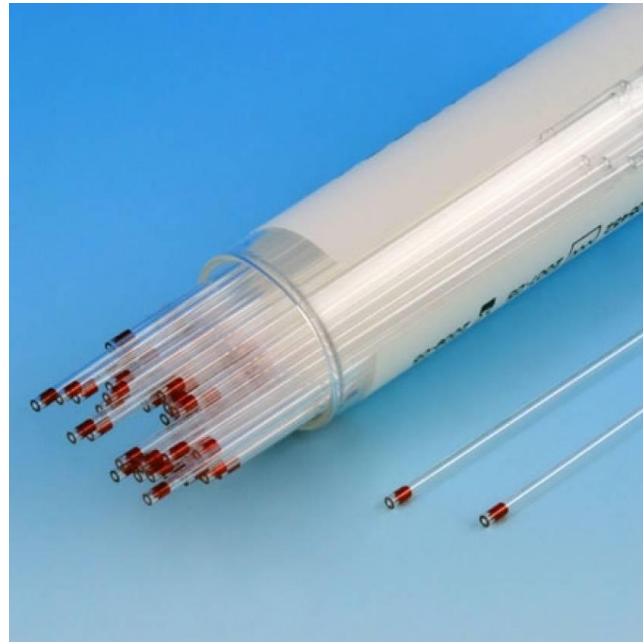


Staining of Blood Smears

Microhaematocrit centrifuge technique :

Materials:

- ❖ Suspected blood samples
- ❖ Microhaematocrit tube
- ❖ Sealing materials or wax
- ❖ Microscope

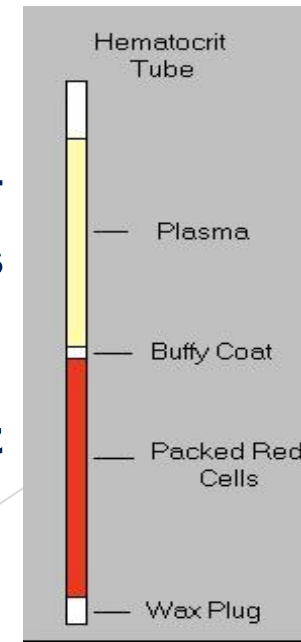
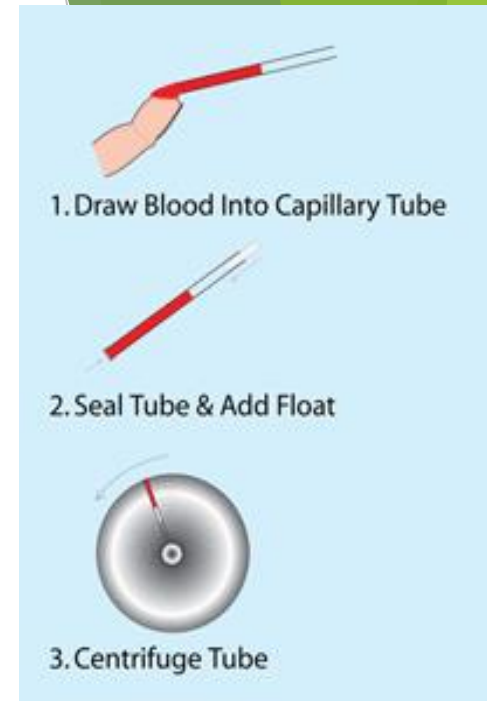


Blood smear preparation

Microhaematocrit centrifuge technique :

Procedure-

- After filling the microhaematocrit tube with blood, centrifuge it for one minute.
- When suspected blood contained in microhaematocrit tube is centrifuged, three layers i.e. plasma layer, WBC or buffy coat layer and RBC layer.

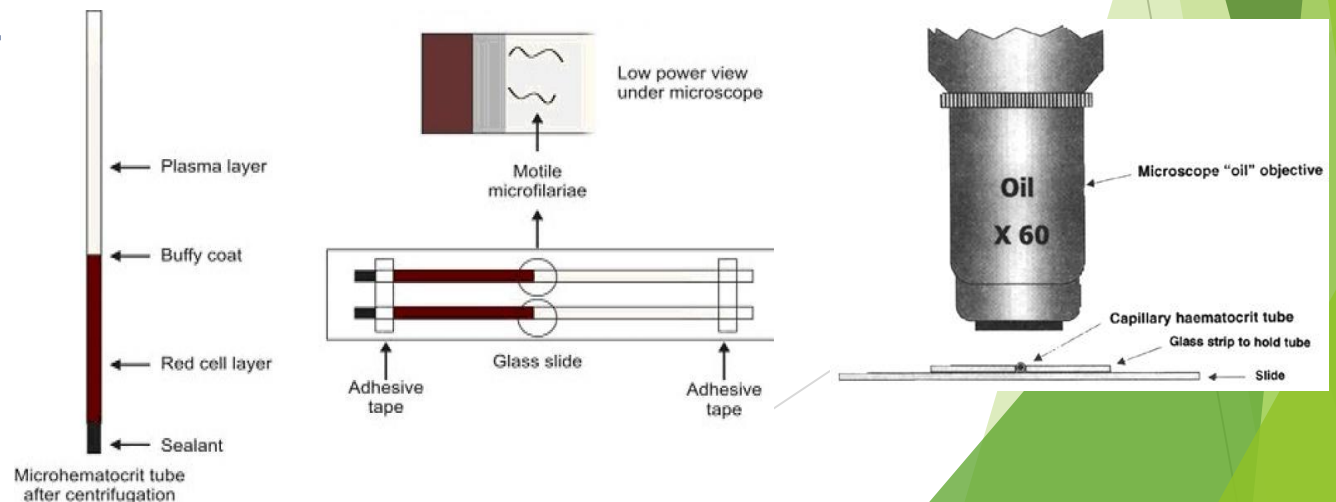


Blood smear preparation

Microhaematocrit centrifuge technique :-

Procedure-

- Under a dissecting microscope the tube is placed. Then examine at the interface of the plasma and buffy coat for mortality. In the plasma microfilariae will be observed in active swimming condition

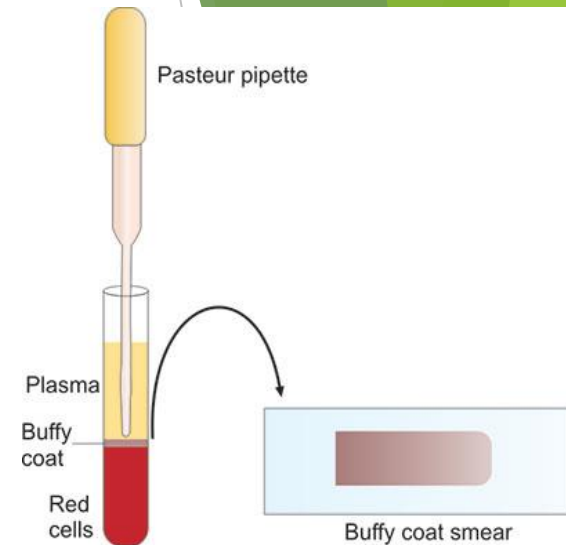


Blood smear preparation

Microhaematocrit centrifuge technique :-

Procedure-

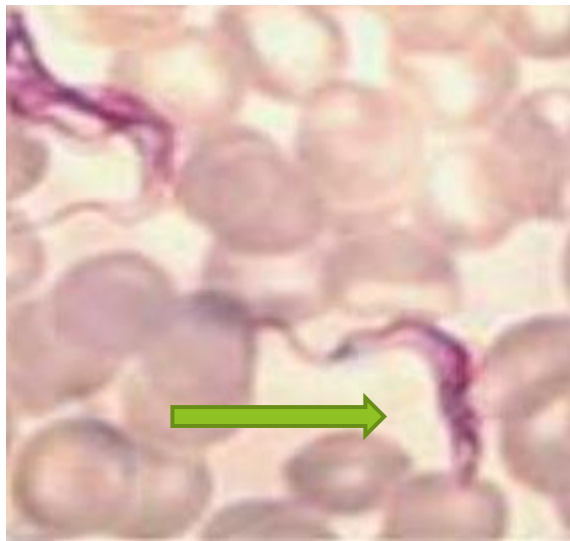
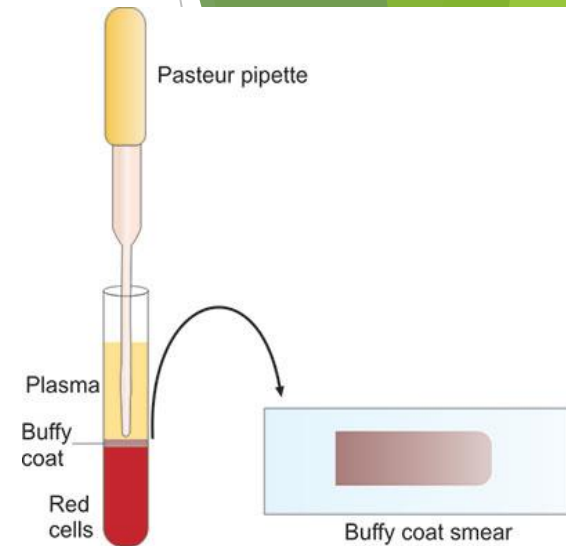
- At the buffy coat interface break the tube and tab the buffy coat and a small amount of plasma on a slide; after placing a cover glass live microfilariae/ *Trypanosoma* spp. can be examined or allow it to air dry, fix for one minute with methyl alcohol and stain with Giemsa for 30 minutes for identification .



Blood smear preparation

Microhaematocrit centrifuge technique:-

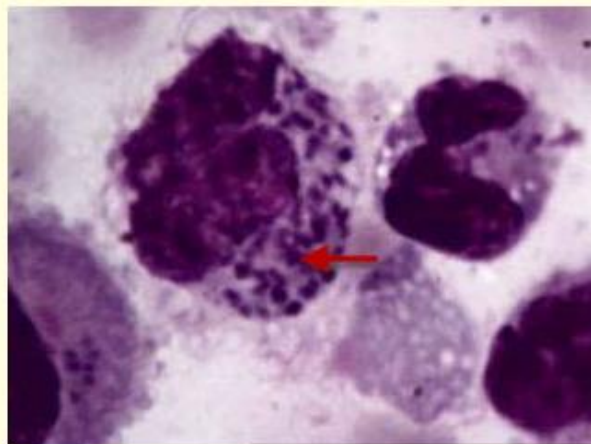
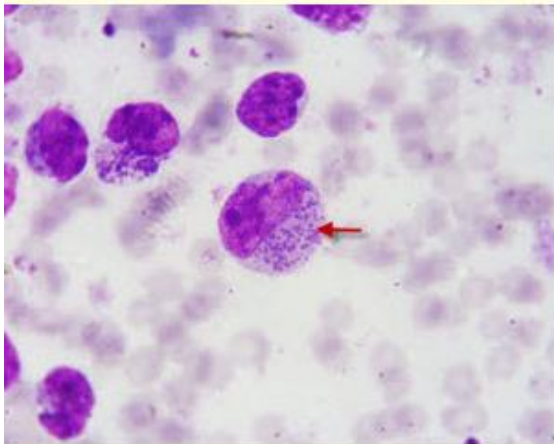
Results: -*Trypanosoma* spp. and microfilaria may be observed in buffy coat smear after microscopic examination.



Staining of Blood Smears

Lymph Gland Biopsy:

- A lymph node smear is sometimes used in preference to a blood smear, for example during an investigation of theileriosis or even trypanosomosis.
- Common lymph node used for biopsy is prescapular.



Kochs blue bodies



Staining of Blood Smears

Lymph Gland Biopsy

Materials:

- ❖ Glass slide
- ❖ Shaving blade
- ❖ Methylated spirit
- ❖ Needle (16-18 gauze for cattle)

Enlargement of prescapular lymph nodes



Staining of Blood Smears

Lymph Gland Biopsy: -

Procedure:

- A suitable superficial lymph node is selected and palpated so that the site is fully known
- Selected site is shaved with shaving blade and make antiseptic with the help of spirit
- Punctured the palpated lymph node by a sterile hypodermic needle and lymph is gradually drawn into a clean syringe.
- Collected lymph fluids are ejected on a clean glass slide and make lymph smear with the help of a spreader slide
- Then stain the lymph smear either with Giemsa or Leishman stain and examine under oil immersion lens of microscope.



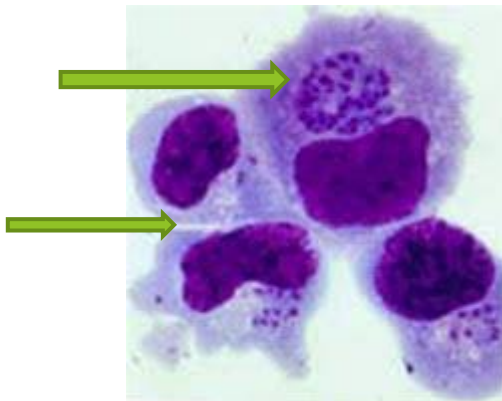
Staining of Blood Smears

Lymph Gland Biopsy:-

Result:

Schizont stages (Koch's blue bodies) of *Theileria* species found inside the cytoplasm of lymphocyte or Leaf like *Trypanosoma* sp. may be observed in lymph fluids.

Lymphocyte
containing
Schizonts



THANK U