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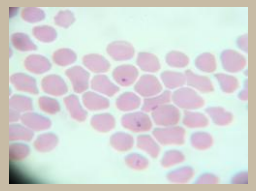
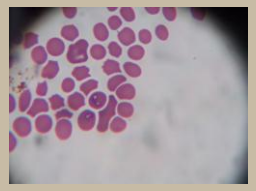
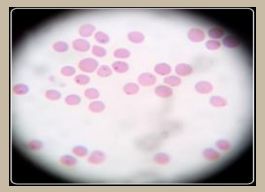
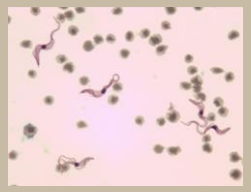
DEPARTMENT OF BIOTECHNOLOGY

Staining Kit for microscopic detection of haemoparasitic infections: Instructions and procedure.

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Introduction:

- Tick-borne infections constitute a complex of several diseases whose etiological agents are protozoal, rickettsial, bacterial or viral. Of these, protozoal and rickettsial haemoparasites namely *Babesia, Theileria, Anaplasma and Trypanosoma* are known to have negative impact on the cattle performance. Diseases produced by them are emerging and included in the OIE's list of 'B' category diseases. The resultant effects are reduced milk production, retarded growth, weight loss, reduced weight gain, delayed conception and reduced market value.

- Diagnosing haemoparasitic infection in a practical and helpful way is very important for the laboratory staff and the veterinary practitioners. There is an urgency and importance of obtaining result of blood examination in the shortest possible time for treatment of clinical cases. Microscopic examination of blood films stained with Giemsa or other blood stains is accepted laboratory practice for the diagnosis of haemoparasitic infections. This method is cost effective and test can be performed in laboratories with minimum facilities.

- The present staining kit has been assembled and standardized so as to facilitate fast and accurate detection of haemoparasites in blood smears in field laboratories.

Principle of the assay:

The staining procedure is used to differentiate nuclear and/or cytoplasmic morphology of intraerythrocytic parasites, extraerythrocytic parasites, RBCs, WBCs and Platelets.

Reagents provided with the staining kit:

SL.NO	Reagent	Quantity	Storage
1.	Fixative	100 ml × 1	Room temperature
2.	Buffer A	250 ml × 1	4 °C
3.	Buffer B	250 ml × 1	4 °C
4.	Stain	100 ml × 1	Room temperature
5.	Immersion oil	10 ml × 1	Room temperature

Consumables (Plastic/glass wares) provided:

SL. NO	Items	Quantity
1.	Glass slides	100
2.	Coplin Jar	1
3.	Marker	1
4.	15 ml Graduated conical tube.	1
5.	Pasteur Pipette	2
6.	Amber colored wide mouthed bottle for storing working buffer.	500ml × 1
7.	Reference slides *Theileria orientalis* *Anaplasma marginale* *Babesia bigemina*	1 (one) each.

Sample required & smear preparation:

- ✓ Fresh whole blood collected in EDTA containing tube for samples suspecting for *Babesia* spp., *Theileria* spp. and *Anaplasma* spp.
- ✓ For *Trypanosoma* suspected samples, blood should be collected either in heparin or sodium citrate vials.
- ✓ Blood should be preferably stained within one hour or as soon as possible to prevent changes in blood picture.
- ✓ Place a very small drop of blood (10-20μ L) on a clean grease free glass slide after properly mixing the blood.
- ✓ Prepare a thin smear and let it air dry.
- ✓ Fix the smear by briefly dipping (2 dips) in the coplin jar containing the fixative and let it air dry once again.

Working buffer and staining solution preparation:

- ✓ Add 36 ml of Buffer A and 14 ml of Buffer B to 450 ml of distilled water to prepare 500 ml of working buffer and store at 4 °C for further use.
- ✓ Staining solution should always be freshly prepared each time and 2 ml of solution is sufficient for one blood smear.
- ✓ Take 2 ml of working buffer in the 15 ml graduated tube provided and add 8 drops of the stain, which is sufficient to cover one slide.

Staining procedure:

- ➢ Gently pour the freshly prepared staining solution over the dried blood smear and let it stain for 30 mins.
- ➢ Wash the smear under slow running tap water to flush out the stain and debris and let it air dry.
- ➢ After drying, first observe the smear under low power (4X) objective of a binocular compound microscope and after clearly focusing a field, slowly turn the objective to high power (Oil immersion) and halfway through it, add a small drop of microscopic/immersion oil and fix the objective.
- ➢ Adjust the fine adjustment knob and after clearly focusing, search for presence /absence of haemoparasites(s) by slowly and carefully searching each field.
- ➢ Compare the organisms with provided reference slides or based on the observer's experience, interpretations may be drawn.

Analytical considerations:

- ✓ The buffer solution should be clear without any visible contamination.
- ✓ Macroscopically stained blood films appear purplish. If blue, then buffer solution was too alkaline and if pink, then too acidic.
- ✓ Slight variations might occur depending on the sample storage and processing but if the morphologic structures are distinct, then staining is satisfactory.

Standardized & developed by

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