	NATIONAL CENTRE FOR ANIMAL HEALTH **LABORATORY SERVICES UNIT**	**Second edition**
	STANDARD OPERATING PROCEDURE	**Version 2018.1**
	HAEMATOLOGY	

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TITLE: Haematogical sample collection

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: Head, LSU

DATE: 12.06.2018

1. Purpose
This document outlines the preferred methods for collecting blood from different animals based on their anatomy and morphology.

2. General information/responsibility
It is the responsibility of veterinarians, paraprofessionals/lab technicians for the collection, preservation, processing of the haematological samples.

3. Equipment/materials
3.1 There are three types of vacutainers:
- 3.1.1 Plain without EDTA (Red cap)
- 3.1.2 EDTA (Purple cap)
- 3.1.3 Heparin (Green cap).

3.2 Razor blade
3.3 Alcohol swab

4. Reagents, solution and buffer
None

5. Procedure
5.1 Site for collection of blood samples
5.1.1 Jugular Vein **-** The most commonly used site in the horse, cattle, sheep, goat, and large wild mammal, used occasionally in the dog, cat, rabbit, guinea pig and bird, when large amounts of blood is required.
5.1.2 Cephalic Vein - The most commonly used site for collection of blood in the dog, (dorsal aspect of the forelimb at the level of the elbow)
5.1.3 Ear Vein - Can be used in the cat, small dog, pig, rabbit, guinea pig, etc. A marginal ear vein on the dorsal side of the ear is selected.
5.1.4 Tail - Can be used in the pig, sheep and cattle.
5.1.5 Anterior vena cava: - Used for large Pig.
5.1.6 Wing vein: - Used for birds

5.2 Collection of blood from ear vein
5.2.1 Remove the hair with the help of scalpel blade or Scissor.

5.2.2 Clean the shaved area with cotton socked alcohol or ether.
5.2.3 Place the left index fingers under the ear at the point of applying a sharp needle with syringe.
5.2.4 Make sure that a prick is made through the skin and into vein and not through underlying cartilage.
5.2.5 When using a syringe, gentle aspiration is used to avoid collapse of the vain.

5.3 Collection from the tail

5.3.1 The tail is flexed upward and removed the hair with the help of scalpel blade or scissors.
5.3.2 Clean the shaved area with socked cotton with alcohol.
5.3.3 Prick with sterile needle, (20 to 40 drops) is enough to perform Haemoglobin estimation and packed cell volume tests.

5.4 Collection from anterior vena-cava

5.4.1 A needle is inserted just anterior and slightly lateral to the cuneiform cartilage.
5.4.2 Make sure that the needle is aimed from cartilage to the base of the ear.
5.4.3 The needle is directed upward and slightly backward until the blood start oozing

5.5 Collection from wing vein

5.5.1 Apply the socked cotton alcohol near the wing vein.
5.5.2 Then remove the feathers or pluck them with the help of hand.
5.5.3 The alar vein is seen running from beneath the pectoral muscle.
5.5.4 Prick the sterile needle below the pectoral muscle until the needle reaches in to the vain.

5.6 Plasma

It is obtained by removing all formed elements from anti-coagulated blood at 5000rpm for 3– 5 minutes. This is required for coagulation profile, fibrinogen assay etc.

5.7 Serum

It is the fluid left behind after blood has clotted. This is the most common specimen required for chemical and serological tests. A plain test tube is used for blood collection and blood is allowed to clot. The tube is then gently centrifuged to obtain clear serum.

5.8 Storage and dispatch of samples

Blood should be kept chilled, not frozen, while being held prior to dispatch and during transport to the laboratory. Blood should be submitted to the laboratory chilled in insulated containers. Avoid contact between the blood samples and ice bricks. Do not chill blood films or expose them to formalin vapour.
Blood film can be submitted in the same container provided they are securely packed and well wrapped for insulation against chilling.

6. SAFETY

6.1 When samples are taken from live animals, care should be taken to avoid injury or distress to the animal or danger to the operator and attendants.

6.2 It may be necessary to use mechanical restraint, tranquillisation or anaesthesia.
6.3 Whenever handling biological material, from either live or dead animals, the risk of zoonotic disease should be kept in mind and precautions taken to avoid human infection.

7. TROUBLESHOOT

7.1 The reasons for unable to get blood sample could be due to following touches the lower wall of the vein does not allow the blood to flow in the syringe.
7.2 Needle touches up to the upper wall of the vein does not allow the blood to flow in the syringe.
7.3 Needle partially inserted into the vein causes blood leakage into the tissue leading to swelling.
7.4 Needle inserted through both the walls of vein
7.5 Collapsed veins

Haemolysis of the blood samples can occur due to the following:

7.6 Using too small or large size needles
7.7 Pulling the plunger forcefully
7.8 Improper placing of needle in the vein
7.9 Collecting without proper drying of the antiseptics on the skin.
7.10 Transferring the blood sample to the container using needle
7.11 Vigorous shaking
7.12 Freezing of the blood sample

Therefore, take the precautions

8. REFERENCES

https://www.bd.com/.../PAS_BC_Troublehooting-hints-for-blood collection_QG_EN....Cached accessed on 16/03/2018

Preventive Veterinary Medicine Record (*RAGACOVAS*)

Manual of Histological Staining Methods of the Armed Forces Institute of Pathology Techniques. Lee G. Luna (Editor) Third Edition

USAID PREDICT. Marcela Uhart, University of California, Davis (2016) Livestock Sampling Methods: Cattle, Sheep, Goats, Camels, and Swine

Veterinary Clinical Pathology (*Ganti A. Sastry*)

TITLE: Blood Smear Preparation for Haematology

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: HEAD LSU

DATE: 12.06.2018

1. INTRODUCTION
A blood film or peripheral blood smear is a thin layer of blood smeared on a microscope slide and then stained in such a way to allow the various blood cells to be examined microscopically.
Blood films are usually examined to investigate haematological problems (disorders of the blood) and, occasionally to look for parasite within the blood such as Babesia, Theileria, Anaplasma and Trypanosomes.

2. PRINCIPLES
Small drop of blood is placed near the frosted end of a clean glass slide. A second slide is used as a spreader. The blood is streaked in a thin film over the slide. The slide is allowed to air-dry, fixed and is then stained.

3. APPLICATION
This procedure describes the method in preparation of blood smear properly which will be acceptable to the clinical laboratory

4. OBJECTIVE
A peripheral blood smear is used to complement a complete blood count (CBC) or haematology panel, to diagnose and/or monitor numerous conditions including appropriate number and morphology of white blood cells (WBCs), red blood cells (RBCs), and/or platelets (PLT), and blood parasites.

5. APPARATUS
5.1 EDTA anticoagulated blood is preferred. Blood smears can also be made from finger stick blood directly onto a slide.
5.2 Glass slides, 3 x 1 inch with frosted edge
5.3 Stopper piercer

6. REAGENTS, SOLUTION AND BUFFER
N/A

7. PROCEDURE
7.1 Preparation of smear

7.1.1 Mix sample well, either by inversion or by mechanical rocker. Remove stopper holding tube away from face. Using two wooden applicator sticks rim the tube and check for fibrin clots.
7.1.2 Holding a 1 X 3 inch slide in your left hand by the short side, place a 2-3 mm drop of mixed whole blood about 1/4 inch from the right side of the slide, utilizing the wooden applicator sticks held in the right hand. Alternate Method: Leave slide on a flat surface.
7.1.3 Place the slide containing the drop of blood on a flat surface and hold securely.
7.1.4 Grasp a second slide (spreader slide) in the right hand between thumb and forefinger.
7.1.5 Place the spreader slide onto the lower slide in front of the blood drop and pull the slide back until it touches the drop.
7.1.6 Allow the blood to spread by capillary action almost to the edges of the lower slide.
7.1.7 Push the spreader slide forward at approximately a 30 degree angle, using a rapid, even motion. The weight of the spreader slide should be the only weight applied.
7.1.8 Do not press down. Perform this step quickly. The drop of blood must be spread within seconds or the cell distribution will be uneven.

7.2 Adjustment of the Smear Length

Increasing the angle of the spreader slide will **decrease** the length of the smear. Decreasing the angle will **increase** the smear length.

7.3 Fixing of smears

To preserve the morphology of the cells, films must be fixed as soon as possible after they have dried. It is important to prevent contact with water before fixation is complete. Methyl alcohol (methanol) is the choice, although ethyl alcohol ("absolute alcohol") can be used. To prevent the alcohol from becoming contaminated by absorbed water, it must be stored in a bottle with a tightly fitting stopper and not left exposed to the atmosphere, especially in humid climates. Methylated spirit must not be used as it contains water. To fix the films, place them in a covered staining jar or tray containing the alcohol for 2-3 minutes. In humid climates it might be necessary to replace the methanol 2-3 times a day and the old methanol can be used for cleaning slides.

7.4 Labelling of smears

A recommended method is to write the name of the patient and the date or a reference number in pencil (graphite) on the slide/film itself. A paper label should be affixed to the slide later.

8. RESULT INTERPRETATION

Good Smear should have the following characteristics-

- Thick at one end, thinning out to a smooth rounded feather edge.
- Should occupy 2/3 of the total slide area.
- Should not touch any edge of the slide.
- Should be margin free, except for point of application

9. WASTE DISPOSAL

The waste materials should be autoclave and disposed.

10. RISK ASSESSMENT

N/A

11. TROUBLESHOOTING

Common causes of a poor blood smear:

11.1 Drop of blood too large or too small.
11.2 Spreader slide pushed across the slide in a jerky manner.
11.3 Failure to keep the entire edge of the spreader slide against the slide while making the smear.
11.4 Failure to keep the spreader slide at a 30^0 angle with the slide.
11.5 Failure to push the spreader slide completely across the slide.
11.6 Rapid drying of smear
11.7 Delay in preparation (ideal within 3 hrs of collection)

Biologic causes of a poor smear

11.8 Cold agglutinin - RBCs will clump together. Warm the blood at 370 C for 5 minutes, then remake the smear.
11.9 Lipemia - holes will appear in the smear. Cannot be corrected.
11.10 Rouleaux - RBC's will form into stacks resembling coins especially in equines. Cannot be corrected.

12. REFERENCES

Maxine M. Benjamin. (2005). Outline of Veterinary Clinical Pathology. Kalyani publishers, New Delhi. p 8-9.

TITLE: Blood smear staining techniques

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: Head, LSU

DATE: 12.06.2018

1. INTRODUCTION

Any abnormalities including morphology, foreign bodies in the blood cells indicates various disease conditions in animals. Abnormalities and morphological changes in the different blood cells can be identified by various staining techniques. Romanowsky stains are most widely used staining techniques used in haematology. Romanowsky staining is a prototypical staining technique that was the forerunner of several distinct but similar methods, including Giemsa, Wright and Leishman stains, which are used to differentiate cells in pathologic specimens.

2. PRINCIPLES

When a thin film is processed, either by an automatic slide stained or by a manual method and examined microscopically, the nucleus and the cytoplasm of neutrophils, lymphocytes, monocytes, eosinophil's and basophils will show a characteristic blue or red coloration and the relative frequency of each type of leukocyte can then be established. Besides, the morphologies of the erythrocytes can also be studied.

3. APPLICATION

This technique is used for differential leucocyte count and detection of abnormal erythrocytes through thin smears. Thick smears are used to examine the blood parasites.

4. OBJECTIVE

This document outlines the procedures for staining technique of the blood smear for direct leukocyte count and study the abnormalities of erythrocytes.

5. APPARATUS

5.1 Glass slide
5.2 Cotton
5.3 Spirit

6. REAGENTS, SOLUTION AND BUFFER

- Stains – Giemsa/Leishman/Wright

7. PROCEDURE

7.1 Giemsa staining

7.1.1 Stain preparation:

7.1.1.1 Dissolve the stain with alcohol in a tightly stoppered flask using a magnetic stirrer, preferably overnight at 37^{O}C, keep stain in dark bottle without filter.

7.1.1.2 The staining quality of Giemsa improves when using a more diluted solution but prolonging the time e.g. 2% Giemsa overnight staining is preferred to 8% for 1 hour.

7.1.1.3 If a quick result is required the dilution can be increased to 10% - 20% for about 15 minutes.

7.1.1.4 Giemsa stain is probably the most popular because of its stability and reliability even, in the hands of the inexperienced worker.

7.1.2 Giemsa Staining Technique

- Fix film in methanol - 3 - 5 min.

Giemsa stain (diluted 1 in 10 (1 in 9) with buffer pH 6.4 – 6.8 7.0) -1 hour45min
Wash off with buffer solution and allow to differentiate - 30 sec.
Blot and allow drying in air.
Examine under an oil immersion objective.

7.2 Leishman staining

7.2.1 Stain preparation:

7.2.1.1 Grind 0.15gm of Leishman stain powder with small amounts of absolute methyl alcohol until an even suspension is obtained. A total of 100ml of methanol is added to produce a complete solution. Pour into a dark bottle and age for few weeks prior to use.

7.2.1.2 It is a very good stain to be used by persons accustomed to its peculiarities. Not a stable stain in the tropics.

7.2.2 Leishman staining technique:

7.2.2.1 Flood the air-dried blood film with undiluted stock Leishman stain and leave for 1-2minutes to fix.

7.2.2.2 Dilute the stain on the smear with double the volume of buffered distilled water (pH 6.8) and stain for 5-15 minutes.

7.2.2.3 Blow the mixture or rock the slide gently to aid mixing.

7.2.2.4 Wash with distilled water until the film has a pinkish tinge (0.5-2min).

7.2.2.5 Wipe the back of the slide to remove excess stain and allow to dry in an upright position.

7.2.2.6 Examine under oil immersion microscope.

7.3 Wright's staining

7.3.1 Stain preparation:

7.3.1.1 Place 0.3gm of dry Wright's stain powder in a mortar and overlay with 3ml of glycerol. Grind thoroughly.

7.3.1.2 Rinse with 100ml of absolute methyl alcohol, added in small amounts, and place in a dark container.

7.3.1.3 Mix with magnetic stirrer about 1 week, without heat.

7.3.1.4 Filter before use.

7.3.2 Wright's staining technique:

7.3.2.1 Cover the dried blood smear completely with Wright's stain and allow to stand for 1-3minutes. The undiluted alcoholic stain will act as a fixative.

7.3.2.2 Add equal amount of buffered distilled water or neutral water (pH 6.6-6.8) distributing it over the entire smear taking care that the solution does not run over the edge.

7.3.2.3 Allow the mixture to stand for 3-5minutes. A metallic scum should appear green.

7.3.2.4 Float off the metallic scum with a stream of water from a wash bottle or the tap.

7.3.2.5 Wipe the stain from the back of the slide with a cleansing tissue while it is still wet. Otherwise, it will require alcohol to remove the dried stain.

7.3.2.6 Stand the slide on end, or wave gently in the air to dry.

7.4 Microscopic Examination of Blood Films

Every film should first be inspected at low power (x10) before general examination is undertaken with the x 40 lens. The x100 oil-immersion lens should generally be reserved for examining unusual cells and for looking for fine details of cytoplasmic granules, punctate basophilia, etc. It is essential to cover the film with a coverglass as this permits the film to be examined with the x 10 and x 40 lenses Thus, when the film is completely dry cover it by a rectangular cover-glass either permanently with a neutral mountant (DPX) or with immersion-lens oil (e.g. oil of cedarwood) as a temporary mount so that the cover-glass can be removed for re-use with successive slides. The cover-glass must be wider than the film so that cells at the edges of the film can be properly examined.

Survey the film at x10 magnification to get a general impression of its quality. Then find an area where the red cells are evenly distributed, just touching but not overlapping, and study their morphology at x 40. At the same time, scan the film to get an impression whether the leucocytes are increased or decreased, identify any unusual or abnormal cells, estimate the relative proportion of platelets and note the presence of abnormally large platelets. Use the x 100 lens for studying the fine details of the cell morphology.

8. RESULT INTERPRETATION

When viewed under microscope, the properly stained smears will reveal following structures: -

Red cells	-	Brick red
Cell nuclei	-	purple
Cytoplasm	-	blue or pink
Organisms	-	blue
Chromatin granules	-	red
Spirochetes	-	pink

9. WASTE DISPOSAL

The waste materials should be autoclave and disposed.

10. RISK ASSESSMENT

N/A

11. TROUBLESHOOTING

Characteristics of stain	***Causes***	***Rectification***
Stain too acidic (red) -RBCs are bright red-orange - WBC nuclei are pale blue -Eosin granules are brilliant orange-red	-Buffer or stain too acid - Excess buffer for stain - Insufficient staining time - Very thin films - Old stain (oxidized alcohol)	- Correct pH, remake buffer -Shorten buffer time/amount - Prolong staining time/amount - Correct film thickness - Check expiration date/stain
Stain too alkaline (blue) - RBCs are blue-green -Eosin granules are grey/blue -WBC nuclei are blue-purple -Neutrophil granules are too dark - Lymph cytoplasm is grey	-Buffer or stain too alkaline - Insufficient buffer for stain - Excessive staining time - Very thick films	- Very thick films -Increase buffer time / amount - Decrease staining time/amount - Correct film thickness
Stain too pale -Little contrast in WBCs	Weak stain solution	• Change stain • Lengthen staining interval
WBC nuclei too dark	-Stain too concentrated - Staining time incorrect	• Check dilution and adjust concentration of stain • Check timing • Reduce staining interval
Water/drying artefact	- Water contamination - Film drying too slowly	• Check humidity in air/increase drying speed if possible • Check for severe anemia
Precipitation on slides	• Unclean slides • Precipitate in stains • Insufficient rinsing	• Clean slides • Filter or replace stains • Check rinsing time • Check rinse filter (dip-type stainers)

12. REFERENCES

Maxine M. Benjamin. (2005) .Outline of Veterinary Clinical Pathology. Kalyani publishers, New Delhi. Pp 26-30

TITLE: Haemoglobin Estimation by Acid Haematin Method

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: Head, LSU

DATE: 12.06.2018

1. INTRODUCTION
Haemoglobin (Hb) refers to a protein, found in red blood cells, that is responsible for carrying oxygen from the lungs to all other tissues of the body. Changes in the level of Hb indicates various disease conditions including anemias in the animals.

2. PRINCIPLES
Anticoagulated blood is added to the 0.1 N HCl and kept for 5-7 minutes to form acid haematinThe resultant solution is then matched against a reference solution in a colorimeter or colored strip (SAHLI'S Haemoglobinometer).

3. APPLICATION
To determine the haemoglobin content in the blood of animals in the diagnosis of anemia, polycythemia.

4. OBJECTIVES
The aim of this SOP is to outline the procedure for estimation of haemoglobin content in the blood sample of animals by acid haematin method.

5. APPARATUS
5.1 Sahli's haemoglobinometer.
5.2 Sahli's pipette.
5.3 Cotton
5.4 Dropping pipette

6. REAGENTS, SOLUTION AND BUFFER
6.1 N/10 hydrochloric acid (Hcl)
6.2 Distilled water for dilution.
6.3 Blood anticoagulated with EDTA.
6.4 Rectified spirit.

7. PROCEDURE
Sahli's method
7.1 Fill graduated tube up to 20 mark with N/10 HCL.
7.2 Add 20µl blood and mix by drawing in and blowing out.
7.3 Stand for 10 minutes for reaction to complete.

7.4 Add water, little by little until blood-acid solution matches color of standard.

8. RESULT INTERPRETATION

After 10 minutes add distilled water in drops and mix the tube until it has exactly the same color as the comparison standards. Note the reading, which indicates the percentage of haemoglobin.

Read off % Hb. 100% Hb = 14.6 g Hb/100 ml.

9. WASTE DISPOSAL

- The waste materials should be autoclave and disposed.

10. RISK ASSESSMENT

NA

11. TROUBLESHOOTING

9.1 Air bubble or blood clot in the column in the pipette.
9.2 Graduations on the diluting tube should not interfere with colour matching.
9.3 The glass rod should be lifted up before colour matching and reading.
9.4 Wipe off the excess blood sticking to the sides and tip of the pipette.
9.5 Transfer the contents immediately into the diluting tube and note the time.
9.6 Take the reading without any delay because on keeping the colour will deepen

12. REFERENCES

Lewis S.M. and Lewis S.M. 2000. WHO, Guidelines on Standard Operating Procedures on Haematology. P30-32.

Maxine M. Benjamin. 2005 .Outline of Veterinary Clinical Pathology. Kalyani publishers, New Delhi. p71.

Sastry G.A. 2009, Veterinary Clinical Pathology, CBS Publishers & Distributors. P1-103.

TITLE: Packed Cell Volume (PCV)

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: Head, LSU

DATE: 12.06.2018

1. INTRODUCTION
When red blood cells in blood are separated by centrifugation in a tube, the volume of the red cells per unit volume of blood is termed as packed cell volume or the percentage volume of red cells in a volume of whole blood. It is expressed in liters of RBC per liter of blood. It may also be expressed in percentage when it is called haematocrit**.**

2. PRINCIPLES
Haematocrit is defined as the volume occupied by erythrocytes (RBCs) in a given volume of blood and is usually expressed as a percentage of the volume of the whole blood sample. The hematocrit is usually determined by spinning method using a blood filled capillary tube in a centrifuge machine. To RBCs are packed using the centrifugal force, forcing all red cells below and plasma above.

3. APPLICATION
To diagnose anaemia in animals

4. OBJECTIVE
To outline the method of estimating the packed cell volume in the blood of animals.

5. APPARATUS
5.1 Capillary tubes, plain (75 mm)
5.2 Micro hematocrit centrifuge
5.3 Micro hematocrit reader
5.4 Hematocrit tube sealant
5.5 Capillary and venous blood in an anticoagulated EDTA tube

6. REAGENTS, SOLUTION AND BUFFER
N/A

7. PROCEDURE
Microhaematocrit method

7.1 Mixed the anti-coagulated venous or capillary blood gently 5 times.
7.2 Fill the capillary tube with blood. Preferably two tubes should be used for each sample as breakage or leakage is common.

7.3 Seal one end of the tube with plasticine (sealing wax), and place these tubes in the micro hematocrit centrifuge.
7.4 Centrifuge for 5 minutes at a speed of 10,000 r.p.m. This will separate RBCs from plasma and leave a band of buffy coat at the interface consisting of WBCs and Platelets.
7.5 Take out the tube and place it in the holder of micro-hematocrit scale in such a way that the bottom margin of red cells layer is against the 0 mark of the scale.
7.6 Similarly adjust the capillary tube so that the top margin of plasma layer coincides with the slanting line of 100 mark.
7.7 Now adjust the sliding line so that it cuts between the red cell layer and the buffy coat.
7.8 Note the reading. This is the packed cell volume.
7.9 Different layers of blood in micro-haematocrit tube after centrifugation:
- Plasma
- Buffy coat
- Red Blood Cells

8. RESULT INTERPRETATION
- The result as per the reader is expressed as percentage (%).

9. WASTE DISPOSAL
- The waste materials should be autoclave and disposed.

10. RISK ASSESSMENT
N/A

11. TROUBLESHOOTING
11.1 Incomplete sealing of the capillary tubes will give falsely low results because in the process of spinning, RBCs and a small amount of plasma will be forced out from the tubes.
11.2 Shortened spin time or slowed centrifugation speed may yield falsely elevated results.
11.3 Micro-haematocrit centrifuge should never be forced to stop by applying pressure to the metal cover plate. This will cause the RBC layer to sling forward and results in a falsely elevated value. The hematocrit is usually three times the haemoglobin.
11.4 The EDTA (dipotassium salt for choice) should be in a concentration of 1.5 mg/ml. This concentration will be exceeded if a reduced amount of blood is added to a standard specimen container or if the blood is taken up in a capillary which contains anticoagulant. This will cause shrinkage of the red cells with falsely low PCV. Storing blood beyond 6-8 hours results in an artefactual increase in PCV, especially in hot climates
11.5 Inadequate mixing of blood before sampling.
11.6 A clot in the specimen.
11.7 Inadequate packing in prescribed time (see 'Maintenance' above)
11.8 Including the buffy-coat layer in the reading of the red cell level.
11.9 Continuous use of centrifuge for several hours, especially in hot climates, will result in its becoming over-heated, causing the samples to lyse (haemolysis).

11.10 Evaporation of plasma during centrifugation, especially if the centrifuge over-heats, or if the spun sample is left for a time before being read.
11.11 Storage for 6-8 hours

12. REFERENCES

Lewis S.M. and Lewis S.M. 2000. WHO, Guidelines on Standard Operating Procedures on Haematology. p 30-32.

Maxine M. Benjamin. 2005 .Outline of Veterinary Clinical Pathology. Kalyani publishers, New Delhi. p71.

Sastry G.A. 2009. Veterinary Clinical Pathology. CBS Publishers & Distributors. p1-103

TITLE: Total Leukocyte Count (TLC)

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: HEAD LSU

DATE: 12.06.2018

1. INTRODUCTION
White blood cells (WBCs), or leukocytes, are a part of the immune system and help our bodies fight infection. They circulate in the blood so that they can be transported to an area where an infection has developed. Increase or decrease in count indicates some kinds of ailments in the animals.

2. PRINCIPLES
A sample of whole blood is mixed with a weak acid solution that lyses non-nucleated red blood cells. Following adequate mixing, the specimen is introduced into a counting chamber where the white blood cells (leukocytes) in a diluted volume are counted.

3. APPLICATION
To estimate the total leukocyte count in the blood sample.

4. OBJECTIVES
To outline the procedure for counting the total leukocytes in the blood.

5. APPARATUS
5.1 Neubaur counting chamber
5.2 Thomas diluting pipette
5.3 Cover slip
5.4 Compound microscope

6. REAGENTS, SOLUTION AND BUFFER
Diluting fluid - Either of the following diluting fluids may be used:

6.1 Two percent acetic acid - Add 2 ml glacial acetic acid to a 100 ml volumetric flask. Dilute to the mark with distilled water.

6.2 One percent hydrochloric acid - Add 1 ml hydrochloric acid to a 100 ml volumetric flask. Dilute to the mark with distilled water.

7. PROCEDURE
7.1 Draw well-mixed capillary or venous blood exactly to the 0.5 mark in a Thomas diluting pipette. This blood column must be free of air bubbles.

7.2 Wipe the excess blood from the outside of the pipette to avoid transfer of cells to the diluting fluid. Take care not to touch the tip of the pipette with the gauze.

7.3 Immediately draw diluting fluid to the "11" mark while rotating the pipette between the thumb and forefinger to mix the specimen and diluent. Hold the pipette upright to prevent air bubbles in the bulb.

7.4 Mix the contents of the pipette for 3-5 minutes to ensure even distribution of cells. Expel unmixed and relatively cell-free fluid from the capillary portion of the pipette (usually 4 drops).

7.5 Place the forefinger over the top (short end) of the pipette, hold the pipette at a 450 angle, and touch the pipette tip to the junction of the cover glass and the counting chamber.

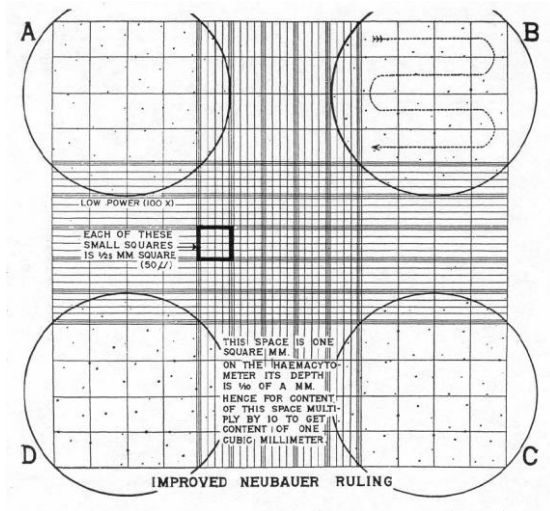
7.6 Allow the mixture to flow under the cover glass until the chamber is completely charged. Similarly, fill the opposite chamber of the haemocytometer.

7.7 If the mixture overflows into the moat or air bubbles occur, clean and dry the chambers, remix the contents of the pipette and refill both chambers.

7.8 Allow the cells to settle for about 3 minutes. Under low-power magnification and reduced light, focus on the ruled area and observe for even distribution of cells.

7.9 Count the white cells in the four 1 sq. mm corner areas corresponding to those marked A, B, C, and D of the following figure -1 in each of two chambers.

7.10 Count all the white cells lying within the square and those touching the upper and right-hand center lines. The white cells that touch the left-hand and bottom lines are not to be counted. In each of the four areas, conduct the count as indicated by the "snake-like" line in figure 5-1. A variation of more than 10 cells between any of the four areas counted or a variation of more than 20 cells between sides of the haemocytometer indicates uneven distribution and requires that the procedure be repeated.



a.

Figure - Haemocytometer counting chamber (WBCs). Areas marked A, B, C, and D are used to count white blood cells.

a) Calculation

i. Routinely, blood is drawn to the 0.5 mark and diluted to the 11 mark with WBC diluting fluid. All the blood is washed into the bulb of the pipet (which has a volume of 10). Therefore, 0.5 volumes of blood are contained in 10 volumes of diluting fluid. The resulting dilution is 1:20.

(These figures are arbitrary and refer strictly to dilution and not to specific volume).

ii. The depth of the counting chamber is 0.1 mm and the area counted is 4 sq. mm (4 squares are counted, each with an area of 1.0 sq. mm therefore, 4 x 1.0 sq. mm = a total of 4 sq. mm). The volume counted is: area x depth = volume. Four sq. mm x 0.1 mm = 0.4 cu mm.

iii. The formula is as follows:

$$\text{WBCs per cu mm} = \frac{\text{Average number of chambers (2) WBCs counted x dilution (20)}}{\text{Volume (0.4)}}$$

For example:

First Chamber Cells counted in each square	Second Chamber Cells counted in each square
35	45
40	37
44	36
39	44
158 WBCs counted	162 WBCs counted

Calculate the average number of WBCs per chamber:

$$\text{Ave num WBCs} = \frac{158+162}{2} = 160$$

Calculate the number of WBCs per cubic mm:

$$\text{WBCs per cu mm} = \frac{160 \times 20}{.4} = 8000$$

8. RESULT INTERPRETATION
Calculation

Routinely, blood is drawn to the 0.5 mark and diluted to the 11 mark with WBC diluting fluid. All the blood is washed into the bulb of the pipet (which has a volume of 10). Therefore, 0.5 volumes of blood are contained in 10 volumes of diluting fluid. The resulting dilution is 1:20. (These figures are arbitrary and refer strictly to dilution and not to specific volume).

iv. The depth of the counting chamber is 0.1 mm and the area counted is 4 sq. mm (4 squares are counted, each with an area of 1.0 sq. mm therefore, 4 x 1.0 sq. mm = a total of 4 sq. mm). The volume counted is: area x depth = volume. Four sq. mm x 0.1 mm = 0.4 cu mm.

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Calculate the number of WBCs per cubic mm:

$$\text{WBCs per cu mm} = \frac{160 \times 20}{.4} = 8000$$

9. WASTE DISPOSAL

The waste materials should be autoclave and disposed.

RISK ASSESSMENT

N/A

11. TROUBLESHOOTING

11.1 Poor pipetting technique causes high or low counts. Poor pipetting technique includes:

- Undershooting desired line with blood or diluting fluid.
- Overshooting desired line with blood or diluting fluid.

- Air bubbles in the column on bulb.
- Failure to wipe the tip free of blood.
- Too slow manipulation following the withdrawal of the specimen thus allowing some of the blood specimen to coagulate.
- Failure to mix the blood and diluent properly.

11.2 Failure to expel 2 or 3 drops in the pipette tips before charging the haemocytometer.
11.3 Overfilling the chamber of the haemocytometer, which causes erroneously high counts.
11.4 Wet or dirty cover glasses and hemacytometers.
11.5 Uneven distribution of cells in the counting chamber causes erroneous results.
11.6 Inaccuracy or carelessness in marking counts.
11.7 Diluent that is cloudy or contains debris.
11.8 Failure to mix anticoagulated blood thoroughly before use.
11.9 The available error when four large squares are counted is +20 percent. Counting eight large squares decreases the error to +15 percent.
11.10 The importance of clean, dry diluting pipettes cannot be stressed too much as the greatest source of error in the counting of WBC is the use of wet and/or dirty pipettes.
11.11 The counting chamber must be scrupulously clean and free of debris that might be mistaken for cells.
11.12 The minimum blood sample recommended for performing routine white blood cell counts is that obtained using one pipette and counting two chambers as previously outlined.
11.13 In cases where the WBC count is exceptionally high, as in leukemia, the dilution should be made in the red blood cell diluting pipet. The blood is drawn to the 1.0 mark and the diluting fluid is drawn to the 101 mark. The resulting dilution is 1:100.
11.14 In cases of leukopenia, the white pipette should be filled to the 1.0 mark and diluted to the 101 mark with 2 percent acetic acid. The resulting dilution is 1:10.
11.15 If nucleated erythrocytes are present, the count is corrected by the following formula:

$$\text{correct count} = \frac{\text{observed count} \times 100}{100 + \text{percent nucleated erythrocytes}}$$

12. REFERENCES

Lewis S.M. and Lewis S.M. 2000. WHO, Guidelines on Standard Operating Procedures on Haematology. p 30-32.

Maxine M. Benjamin. 2005 .Outline of Veterinary Clinical Pathology. Kalyani publishers, New Delhi. p71.

Sastry G.A. 2009. Veterinary Clinical Pathology. CBS Publishers & Distributors. p1-103

TITLE: Total Erythrocyte Count (TRBC)

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: Head, LSU

DATE: 12.06.2018

1. INTRODUCTION
Red blood cells or erythrocytes are the most common type of blood cell in the animals which are principal means of delivering oxygen (O2) to the body tissues. Its total count alters (increase or decrease) in different kinds of ailments in the animals. Hence, the count of total RBCs will provide clue in diagnosing certain conditions in the animals.

2. PRINCIPLES
The fluid will lyse the WBCs and platelets keeping RBCs intact which can be counted.

3. APPLICATION
To diagnose erythropoietic conditions through the counting of total red blood cells.

4. OBJECTIVE
To describe the procedure for counting of Total erythrocytes in the blood sample of animals.

5. APPARATUS
5.1 *Thomas (Diluting) Pipette* - This diluting pipette consists of a calibrated stem portion and a bulb chamber containing a red bead to facilitate uniform mixing, giving the blood a 1: 200 dilution and 1: 100 dilution.

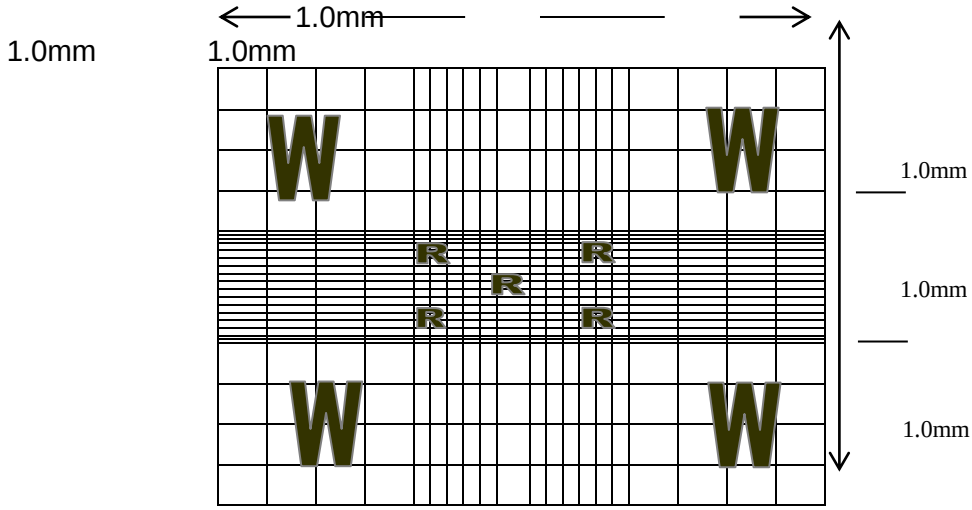
5.2 *Haemocytometer (Counting chamber) -This consists of a heavy glass slide with two* counting areas situated on the central platform, which is 0.1 mm lower than the two adjacent platforms giving the chamber a depth of 0.1 mm. The Improved Neubauer Haemocytometer is generally used for the counting of RBC because of the clearer demarcation of the ruled area as shown below in the diagram.

5.3 *Cover glass or cover-slip* - The cover glass or cover-slip used on the Haemocytometer should be: Optically flat- BSS 0.45 mm thick, 22 x 23 mm (for double side), 22 x 16 mm (for single side)

The figure of an improved Neubauer Hemocytometer is shown below with the following zones:

R – zones to be counted under high power for erythrocytes

W – zones to be counted under low power for leukocytes



6. REAGENTS, SOLUTION AND BUFFER

6.1 Dacie's fluid (modified) for counting RBC

7. PROCEDURE

7.1 Draw blood using Thomas diluting pipette to 0.5 mark.
7.2 Wipe outside of pipette clean with a piece of tissue.
7.3 Dilute blood with Dacie's Fluid to 101 mark.
7.4 Wipe outside of pipette clean; mix blood well by hand or an automatic shaker.
7.5 Prepare Haemocytometer by sliding the cover-slip in to obtain Newton rings.
7.6 Mix blood again and discard at least 1/3 of mixture.
7.7 Carefully fill up chambers and allow cells to settle down before counting.
7.8 Using low m7agnification to scan the distribution of cells before using x 25 or x 40 objectives for counting.
7.9 Count the four secondary squares together with the centre secondary square of the Central Primary Square.
7.10 Record the count of each secondary square individually to ensure even distribution of cells.

8. RESULT INTERPRETATION

Calculation:

Let R = No. of cells counted.
Volume of 1 secondary square = 0.2 x 0.2 x 0.1 cu. mm.
Volume of 5 secondary squares = 0.2 x 0.2 x 0.1 x 5 cu. mm = 0.02 cu. mm
In 0.02 cu. mm there R cells
In 1 cu mm or in 1µl there are R /0.02
But the dilution of blood is 1:200
In 1 µl there are R x 200 /0.02 cells
i. i.e. R x 10,000 cells
ii. i.e. R x 10^4 cells

9. WASTE DISPOSAL

The waste materials should be autoclave and disposed.

10. RISK ASSESSMENT

N/A

11. TROUBLESHOOTING

11.1 Greasy chamber or chamber containing dust particles.

11.2 Fluid runs into moats--Flooding the chamber so that the Presence of yeast cells-contamination of the diluting fluid with yeast cells.

11.3 Drying of the fluid in the chamber before and during counting.

12. REFERENCES

Lewis S.M. and Lewis S.M. 2000. WHO, Guidelines on Standard Operating Procedures on Haematology. p 30-32.

Maxine M. Benjamin. 2005 .Outline of Veterinary Clinical Pathology. Kalyani publishers, New Delhi. p71.

Sastry G.A. 2009. Veterinary Clinical Pathology. CBS Publishers & Distributors. p1-103

TITLE: Differential leucocytes count (DLC)

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: Head, LSU

DATE: 12.06.2018

1. INTRODUCTION

Differential leucocytes count is method to enumerate different leucocytes levels in the blood. Variations found in the number of different leucocytes from the normal count gives fair idea on the types of infection or conditions that animal are stressed upon by.

2. PRINCIPLES

A stained smear is examined in order to determine the percentage of each type of leukocyte present and assess the erythrocyte and platelet morphology.

3. APPLICATION

This count is normally performed when an animal is suspected to be suffering from an infection or when the total WBC counts is abnormal. It is important to perform the total WBC count before attempting to do the differential WBC. A good blood film has to be made and stained by a Romanowsky stain.

4. OBJECTIVE

To outline the procedures for estimating each type of WBC in whole blood.

5. APPARATUS

5.1 Microscope
5.2 Cedar oil
5.3 Cotton
5.4 Spirit
5.5 Glass slide

6. REAGENTS, SOLUTION AND BUFFER

6.1 Giemsa stain

7. PROCEDURE

7.1 Stain preparation:

7.1.1 Dissolve the stain with alcohol in a tightly stoppered flask using a magnetic stirrer, preferably overnight at $37^{O}C$, keep stain in dark bottle without filter.

7.1.2 The staining quality of Giemsa improves when using a more diluted solution but prolonging the time e.g. 2% Giemsa overnight staining is preferred to 8% for 1 hour.

7.1.3 If a quick result is required the dilution can be increased to 10% - 20% for about 15 minutes.

7.1.4 Giemsa stain is probably the most popular because of its stability and reliability even, in the hands of the inexperienced worker.

7.2 Giemsa Staining Technique

7.2.1	Fix film in methyl alcohol	-	3 min.
7.2.2	Giemsa stain (diluted 1 in 10 with buffer pH 7.0)	-	1 hour
7.2.3	Wash off with buffer solution and allow to differentiate	-	30 sec.
7.2.4	Blot and allow drying in air		

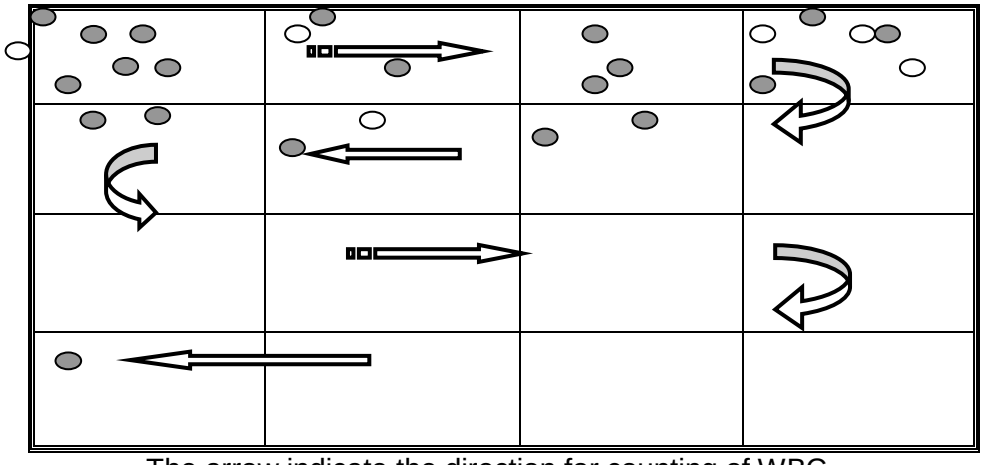
7.3 Methods of counting

Triple ruling:

- Cells touching top and left center lines are counted.
- Cells touching bottom and right center lines are not counted.

Double ruling:

- Cells touching top and left outer lines are counted.
- Cells touching bottom and right inner lines are not counted.



The arrow indicate the direction for counting of WBC

The cells can be counted in a counting chamber.

8. RESULT INTERPRETATION

In total of 100 cells are counted and each type of cells are calculated as percentages.

9. WASTE DISPOSAL

- The waste materials should be autoclave and disposed.

10. RISK ASSESSMENT

- N/A

11. TROUBLESHOOTING

- NA

12. REFERENCES

Lewis S.M. and Lewis S.M. 2000. WHO, Guidelines on Standard Operating Procedures on Haematology. p 30-32.

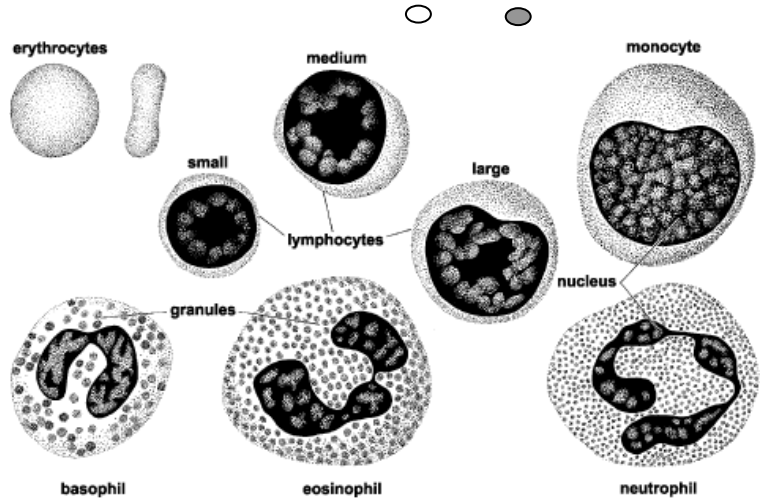
Maxine M. Benjamin. 2005 .Outline of Veterinary Clinical Pathology. Kalyani publishers, New Delhi. p71.

Sastry G.A. 2009. Veterinary Clinical Pathology. CBS Publishers & Distributors. p1-103

13. APPENDIX

Cells under microscope - It is important to count and differentiate at least 200 WBCs into these categories of cells:

Cell Type	Appearance	Relative Diameter
RBCs	Pink-orange colour No nucleus	1
Lymphocytes	Nucleus : large, rounded, deep purple Cytoplasm : narrow rim around nucleus, pale blue	1 – 1.3
Monocytes	Nucleus : indented (Kidney shaped), purple Cytoplasm : pale blue	2.0 – 2.5
Neutrophils	Nucleus : multi-lobed, purple Cytoplasm : pale pink, small pink or purple granules	1.3 – 1.8
Eosinophils (rare)	Nucleus : two lobes ('spectacles'), purple Cytoplasm : pale blue, many large dark blue granules	1.3 – 1.8
Basophils (very rare)	Nucleus : indented (Kidney shaped), purple Cytoplasm : pale blue, many dark blue granules	1.3 – 1.8
Platelets	Blue with pink granules No nucleus	0.2 – 0.3



Basic structures of leukocytes

TITLE: Erythrocyte Indices

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: Head, LSU

DATE: 12.06.2018

1. INTRODUCTION
The erythrocytes indices define the size and Hb content of erythrocytes from values obtained from erythrocytes count, Hb concentration and the PCV. It includes MCV, MCH and MCHC.

2. PRINCIPLES
The Hb estimation, PCV and TRBCC are obtained from the complete blood count parameters and the erythrocyte indices are calculated by the given formula.

3. APPLICATION
These indices are useful in morphological classification of anaemia and to evaluate erythropoietic responses.

4. OBJECTIVE
To outline the methods to calculate erythrocyte indices like MCV, MCH and MCHC from Hb, PCV and TRBC parameter values obtained from respective independent tests.

5. APPARATUS
N/A

6. REAGENTS, SOLUTION AND BUFFER
None

7. PROCEDURE
7.1 Mean corpuscular volume (MCV)
MCV is the average volume of the individual erythrocytes and is calculated from the formula-

$$MCV = \frac{PCV\ (\%) \times 10\ (\text{femtolitre})}{TRC\ (\text{million/microlitre})}$$

7.2 Mean corpuscular Haemoglobin concentration(MCHC)
MCHC is the concentration of haemoglobin in the average erythrocytes, or ratio of the weight of haemoglobin to the volume in which it is contained, and is calculated from the formula:

$$MCHC = \frac{Hb\ (g/dl) \times 100}{PCV\ (\%)}$$

7.3 Mean corpuscular haemoglobin (MCH)

MCH is the amount of Hb, in the average erythrocytes and is calculated from the formula:

$$MCH = \frac{Hb\ (g/dl) \times 10}{TRC\ (million/microlitre)}\ (picogram)$$

8. RESULT INTERPRETATION

Record the above calculated values in unitsMean corpuscular volume (MCV) =fL
Mean corpuscular Haemoglobin concentration (MCHC) =Pg
Mean corpuscular haemoglobin (MCH) =g/dL

9. WASTE DISPOSAL

- The waste materials should be autoclave and disposed.

10. RISK ASSESSMENT

- N/A

11. TROUBLESHOOTING

- N/A

12. REFERENCES

Lewis S.M. and Lewis S.M. 2000. WHO, Guidelines on Standard Operating Procedures on Haematology. p 30-32.

Maxine M. Benjamin. 2005 .Outline of Veterinary Clinical Pathology. Kalyani publishers, New Delhi. p71.

Sastry G.A. 2009. Veterinary Clinical Pathology. CBS Publishers & Distributors. p1-103

TITLE: Erythrocyte Sedimentation Rate (ESR)

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: HEAD LSU

DATE: 12.06.2018

1. INTRODUCTION

In certain destructive and inflammatory conditions, ESR may be accelerated ,this may be due to some alteration in the physio-chemical properties of blood, in which the surface charges of the erythrocytes is altered, rendering them to aggregate together and form rouleaux. Rouleaux formation is accompanied by accelerated ESR. The larger the rouleaux formation, speeder the sedimentation occurs. There is also a rise in the fibrinogen and globulin content of blood.

2. PRINCIPLES

The erythrocytes gravitate towards the bottom due to its weight when the anticoagulated blood is kept vertical in a hematocrit tube. The speed of the fall in erythrocytes level in tube differs in different animals and this speed of the fall is measured to get the ESR**.**

3. APPLICATION

To diagnose the destructive and inflammatory conditions in animals through the estimate of the erythrocyte sedimentation rates.

4. OBJECTIVE

This SOP outlines the procedures for estimation of sedimentation rate of the erythrocytes.

5. APPARATUS

5.1 Win Trobe tube
5.2 Pasteur pipette
5.3 Tube stand
5.4 clock

6. REAGENTS, SOLUTION AND BUFFER

- Blood samples in EDTA as anticoagulant

7. PROCEDURE

7.1 Use Heller's and Paul's oxalate mixture or EDTA as anticoagulant.
7.2 Fill Win Trobe tube to mark 0 with a long Pasteur pipette from bottom upwards as explained earlier, taking care to see that no air bubbles are present.
7.3 Keep the tube in a special stand, taking care to see that it is perfectly vertical. Even a slight enhance sedimentation

7.4 Observe and measure fall in the erythrocytes column in millimeters from above (left side calibration) every 10 minutes.

8. RESULT INTERPRETATION

Record the fall of erythrocyte column in mm

9. WASTE DISPOSAL

Should be disposed after autoclaving.

10. RISK ASSESSMENT

Blood samples should be handled with precaution as there are chances of getting zoonotic infection if the animals are infected.

11. TROUBLESHOOTING

11.1 The concentration of oxalate must be exactly 2mg/ml of blood. If it is higher, ESR may be slower.
11.2 Keep the hematocrit free from dirt. If it has been cleaned with ether or alchohol, dry it thoroughly because dirt, alcohol and ether influence the rate of fall.
11.3 Keep the tube absolutely vertical.
11.4 See that no air bubbles are trapped while filling the tube since these influence the rate.
11.5 Conduct the test immediately after the collection of blood, since too old a samples gives erroneous result.
11.6 See that hemolysis of blood does not occur, since it may modify the ESR.
11.7 See that the blood samples have normal temperature. Also see that the temperature of the place where the test is conducted is cool and between 22º C and 27ºC. Higher temperature increases ESR.
11.8 See that the tube is kept in a place free from any disturbances or vibration since these influence the ESR. Do not keep the tube near a running centrifuge.

12. REFERENCES

Lewis S.M. and Lewis S.M. 2000. WHO, Guidelines on Standard Operating Procedures on Haematology. P 30-32.

Maxine M. Benjamin. 2005 .Outline of Veterinary Clinical Pathology. Kalyani publishers, New Delhi. p71.

Sastry G.A. 2009. Veterinary Clinical Pathology. CBS Publishers & Distributors. p1-103

TITLE: Wet Film Examination

PREPARED BY: Haematology section

REVISED BY: Haematology section

APPROVED BY: Head, LSU

DATE: 12.06.2018

1. INTRODUCTION
The confirmatory test for diagnosis of blood parasites is made through the examination of fresh blood and preparation of blood smears which are then examined for the presence of the parasites. Examinations of blood smears are very time consuming. The parasite may be present in the peripheral blood in very small numbers. Careful examination of the blood smear is required before reporting the smear out as "no parasites seen."
It takes experience to accurately examine blood smears for parasites. Platelets sitting on top of red blood cells are commonly mistaken for blood parasites.

2. PRINCIPLES
The examination of blood parasites is done through freshly prepared blood smear and examination with the help of microscope. The presence of blood parasites in the sample is indicated by movements of the parasites.

3. APPLICATION
Used to detect important parasites/protozoa like microfilaria and trypanosomes in blood through wet film examination technique.

4. OBJECTIVE
To outline the procedure to detect and identify blood parasites in wet films by light microscopy.

5. APPARATUS
- 5.1 Clean, grease free slide
- 5.2 Microscope
- 5.3 Cotton

6. REAGENTS, SOLUTION AND BUFFER
- 6.1 Whole blood in EDTA
- 6.2 Spirit

7. PROCEDURE
- 7.1 Place a small drop of blood on the clean, grease free slide with the help of a dropper.
- 7.2 Cover the blood with cover glass to prevent clotting.

7.3 The blood is examined under low power microscope

8. RESULT INTERPRETATION

8.1 Microfilariae is identified by whip-like motions.
8.2 Trypanosomes are identified by the rapid undulating and twisting movements.

9. WASTE DISPOSAL

The waste materials should be autoclave and disposed.

10. RISK ASSESSMENT

N/A

11. TROUBLESHOOTING

None

12. REFERENCES

Lewis S.M. and Lewis S.M. 2000. WHO, Guidelines on Standard Operating Procedures on Haematology. p30-32.

Maxine M. Benjamin. 2005 .Outline of Veterinary Clinical Pathology. Kalyani publishers, New Delhi. p71.

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HAEMATOLOGY TEST CATEGORIZATION

Sl. No.	Procedure / SOP	DVL	SVL/TVH	RLDC/ NVH	NCAH
A	**Complete Blood Picture (CBC)**				
1	Hematocrit or PCV	X	X	X	X
2	Haemoglobin estimation	X	X	X	X
3	Erythrocyte indices - MCV/MCH/MCHC	X	X	X	X
4	TRBC count	X	X	X	X
5	TWBC count	X	X	X	X
6	Erythrocyte sedimentation rate (ESR)	X	X	X	X
B	**Differential Leukocyte Count (DLC)**	X	X	X	X
C	**Staining**				
1	Giemsa staining	X	X	X	X
2	Leishman staining	X	X	X	X
3	Wright's staining	X	X	X	X
D	**Wet film examination for microfilaria, trypanosome, etc**	X	X	X	X