LABORATORY MANUAL

VETERINARY PATHOLOGY

VPP- Unit-1,2&3

COURSE TITLE – GENERAL VET. PATHOLOGY, SYSTEMIC PATHOLOGY, ANIMAL ONCOLOGY, CLINICAL PATHOLOGY AND NECROPSY

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FOREWORD

I am glad to see the Laboratory Manual GENERAL PATHOLOGY, SYSTEMIC PATHOLOGY, ANIMAL ONCOLOGY, CLINICAL PATHOLOGY AND NECROPSY is prepared by ***Prof. S. D. Singh, Prof. Nem Singh, Prof .G. D. Sharma*** *Professor, Dept. of Veterinary Pathology and* ***Dr. Aditi Kumawat*** *Assistant Professor, Dept. of Veterinary Pathology. It is appreciable to note that the manual covers the practical syllabus of B.V.Sc.& A.H. course as per the standards laid down by Veterinary Council of India.*

Prof. S. D. Singh, Prof. Nem Singh, Prof .G. D. Sharma and Dr. Aditi Kumawat have devoted keenly to prepare this manual with their excellent knowledge and expertise in the field of pathology. They have covered all the aspects like objectives, outline and description, material and methods and observation to be taken care off.

Definitely this manual will be helpful for smooth and effective conduction of practicals and ensure a handbook for students for entire life in the profession.

I congratulate Prof. S. D. Singh, Prof. Nem Singh, Prof .G. D. Sharma and Dr. Aditi Kumawat for their strenuous efforts and excellent presentation of this manual.

Dean

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PREFACE

This Manual has been prepared for the undergraduate students of B.V.Sc. & A.H. in accordance with the syllabus designed by the Veterinary Council of India. The efforts have been made to make the manuscript worthy, realistic and easily understandable for the students, teachers and Veterinary Practitioners for diagnosis of different pathogens of diseases of animals. We hope this manual will serve very useful tool to the undergraduate and graduate students of Veterinary Science who are undergoing courses in veterinary Pathology.

It's our pleasure to thank Dean Sir, M.J.F College of veterinary and Animal Sciences, Chomu, Jaipur for providing necessary facilities and rendering all helps in preparing this course manual.

Computer operator and typist Mr. Ashutosh Sharma worked hard for very existence of this manual so I acknowledge his efforts.

Course Incharge

Dept. of Veterinary pathology

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EXERCISE NO. 1

DEFINITIONS

PATHOLOGY: It is the study of disease. It deals with structural, functional and compositional changes that occur in the disease along with their cause, mechanism, course, compilation and consequences.

Divisions of Pathology:

1. **General pathology:** deals with fundamental processes that are common to more than one tissue or organ.
2. **Systemic pathology:** is study of diseases peculiar to certain systems or organs.
3. **Special pathology:** is study of diseases caused by specific microbial pathogens.
4. **Clinical pathology:** is that branch of pathology used in the diagnosis of the diseases in the hospital at the patient's bedside. Pathology applied to find the solution to clinical problems especially the use of laboratory methods in clinical diagnosis.
5. **Comparative pathology:** is the study of diseases of animals and comparing them to those occurring in man.
6. **Nutritional pathology:** is the study of disease processes resulting from deficiency or excess of essential food stuffs.
7. **Experimental pathology:** means the study of disease artificially produced in animals.
8. **Chemical pathology:** deals with alterations in biochemical processes in diseases.
9. **Toxicopathology:** means the study of diseases caused by toxic substances.
10. **Oncology:** (Gk. Onco-Tumour) is study of tumours.
11. **Macroscopic or Gross Pathology:** examination of individual by systematic dissection without aid of magnifying lens.
12. **Microscopic or histopathology:** study of disease with the help of magnifying lens.

13. **Postmortem pathology:** examination of individual after death.
14. **Health:** It is the state of an individual living in complete harmonic with his environment.
15. **Etiology:** study of causation of disease.
16. **Predisposing causes:** are those which make the animal susceptible to disease.
17. **Pathogenesis:** it is the mechanism by which the cause produces the disease.
18. **Incubation period:** It is the time that elapses between the action of cause and manifestation of disease.
19. **Onset of disease: it** is the beginning of symptoms.
20. **Symptoms:** are the outword manifestations of the patient suffering from disease while alive.
21. **Lesion:** is a macroscopic or microscopic alteration in tissues.
22. **Pathognomonic lesion:** is the alteration in the tissue that indicate causes of disease.
23. **Diagnosis:** is the art of determination of the nature of the disease, its course, lesions and symptoms.
24. **Prognosis:** is the estimate by clinician of the probable severity and outcome of disease.
25. **Morbidity rate:** is the percentage of exposed animals that get affected.
26. **Mortality rate:** is the percentage of death among affected animal.
27. **Biopsy:** examination of tissue collected from living individual.
28. **Autopsy:** used for P.M. examination of human medicine.
29. **Necropsy:** term used for P.M. examination in veterinary medicine

EXERCISE NO. - 2

HISTOPATHOLOGICAL TECHNIQUES

It is the process by which gross lesions are confirmed on the basis of alterations at the cellular level. These are observed after slicing the tissue in thin sections and staining them. It includes:

1. Processing of tissue
2. Staining of sections.

1. PROCESSING OF TISSUE- It has the following steps:

(a) Collection:

(When to collect, what to collect and how to collect) Immediately after death, conduct the PM and collect the pieces of tissues representative of gross lesion with adjacent healthy tissue. Incise the tissue with sharp knife in single stroke. Size of tissue must be 2-3 cm in length, 1-2 cm in width and ½ - 1 cm in thickness should not be increased in any case.

(b) Fixation:

Rapid killing of tissue element and their preservation and hardening to keep them as near as possible the same relation they had in the living body.

Aims of Fixation:

(I) To preserve the tissue as life like manner as possible.

(II) Inhibition of autolysis and putrefaction. (Autolysis means dissolution of cells its own enzymatic action.) (Putrefaction means breakdown of tissue by bacterial action.)

(III) **Hardening-** Hardening effect of fixative allows easy manipulation of naturally soft tissue. (Like brain tissue.)

(IV) **Solidification of colloid material-** Fixation convert the normal semi fluid consistency of cell i.e. sol to gel.

(V) **Optical differentiation-** Fixation alters the refractive indices of various cells.

The most common fixative used is 10% formalin. It is used more because it is stable, inexpensive, and relatively non-toxic, penetrate rapidly, and produce no coloration and little distortion. Tissue may be stored for longer time without serious effect. It is a soft fixative in comparison, with others

Different Types of Fixatives:

(a) 10% formalin (10ml formalin + 90ml water)

37-40% formaldehyde……100ml

Tap water……900ml

It should be used 20 times more the volume of tissue.

FORMALIN-SALINE SOLUTION

37-40% formaldehyde……100ml

Sodium chloride……9 gm

Tap water……..900ml

BUFFERED NEUTRAL FORMALIN SOLUTION

37-40% formaldehyde……100ml

Distilled water…….900ml

Sodium phosphate monobasic……. 4gm

Sodium phosphate dibasic (anhydrous)……6.5 gm

(b) Zenker`s Fluid- (Mercuric chloride 5gm + potassium dichromate 2.5 gm + sodium thiosulphate 1 gm + distilled water 100ml)- Following its use tissue must be washed under running tap water overnight to remove excess dichromate otherwise with alcohol it form lower oxides which cannot be removed. Mercuric chloride pigment must be removed with iodine by oxidation into mercuric iodide which can be removed by sodium thiosulphate which converts it into mercuric tetrathionate which is water soluble.

Procedure:

(I) Place section in 0.5% iodine in 80% alcohol for 5 mins.

(II) Rinse in water.

(III) Place in 3% aqueous sodium thiosulphate.

(IV) Wash in running water.

Zenker`s fluid is good for staining nucleus and connective tissue. Fixation occurs within 12 hours.

(c) Carnoy`s Fluid- Chloroform 30ml + absolute 60ml + glacial acetic acid 10ml.- It preserves the glycogen. It is a rapid fixative nuclear staining is greatly improved. It will fix as well as dehydrate the tissue. Carnoy`s fluid is used for biopsy on which reports are required urgently. It requires half an hour for fixation.

c. Washing: Washing is done with running tap water for 6-8 hours or overnight.

d. Dehydration: It is the most essential step and must be carried out thoroughly. The dehydration is carried out in stages using various ascending grades of alcohol eg. 50%, 70%, 90% and absolute alcohol.

e. Clearing: The purpose of clearing is to remove alcohol from dehydrated tissue. It must be a solvent of wax freely mixed with alcohol. When xylene and benzene are used the tissue is rendered translucent therefore, the term clearing is used otherwise dealcoholization term will be appropriate. Other reagents are cedar wood oil, chloroform, etc.

f. Paraffin Embedding: Impregnation of tissue with molten paraffin wax and is carried out by putting wax andin different containers of metal or glass in the oven or in the vacuum oven. The temperature of the oven should be 2˚C higher than the melting point of paraffin wax. Total time given is 4-6 hours.

g. Preparation of blocks: Blocks are prepared with the help of two L-molds formed of brass. First of all adjust the mold to a suitable size on the glass piece and then fill with molten wax. Then transfer the tissue with the help of a warm forceps to the bottom of molten wax. At the same time proper number of tissue and date should be written on paper and should be placed by the side of the column. Then allow it to cool. After solidification of wax, L pieces should be separated.

h. Trimming of block: It is done with the help of warmspatula to remove the excess wax and to make the corners blunt. So that at the time of section cutting individual section can be identified due to removal of wax from the label.

I. Section of cutting

Sections are cut with the help of microtome the thickness of the section should be 4-6µ.

Flow Chart of Processing of Tissue

Collection
Length 2-3 cm
Width 1-2 cm
Thickness ½- 1 cm

→

Fixation
10% formalin
Ratio 1:20
Duration 48-72 hrs.

→

Washing
Running tap water
For 6-8 hrs or
Over night

↓

Dehydration
Ethyl alcohol

Holding point
80% Alcohol
90% Alcohol – 1 to 2 hr
95% Alcohol – 1 to 2 hr
Ab – Alcohol 1st - 1-2 hr
Ab – Alcohol 2nd - 1-2 hr
Ab – Alcohol 3nd - 1-2 hr

↓

Clearing

Xylene I – 1 hrs.
Xylene II – 1 hrs.
Xylene II – 1 hrs.

↓

Paraffin wax
P. W. I – 1 -1.30 hr

P. W. IV – 1 -1.30 hr

↓

(By L – molds)

←

Trimming of block Preparation of Blocks
By microtome
(4-6 µthickness)

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Mounting of section
Embedding
On albumenized slide

↑

P. W. II – 1 -1.30 hr
P. W. III – 1 -1.30 hr

↑

Drying of slide
(In oven for 30 min. at 60˚ C)

i. Mounting of section on aluminized slide: Smear the slide with a mixture of 50 parts of egg albumin and 50 parts of glycerin with the help of piece of nylon. Few crystals of thimol should be added to egg albumin and mix it well, as it acts as a preservative.

j. Drying of Section: Place the slide in vertical position in paraffin oven at 60˚C for 15-30 mins.

*** Meyer's Glycerine egg albumin: 1 gm sodium salisylate is dissolved in 50 ml of glycerine and 50 ml of white egg albumin and mixed and filtered. This solution is used to mount the sections.

STAINING OF SECTIONS-

Staining is mainly done by haematoxyline and eosin.

The steps of staining are as follows-

(a) Deparaffinization: It means removal of wax. This is done with xylene I and II for 5 mints in each.

(b) Hydration: Means addition of water in the tissue, because the stains are water soluble. This is done by transferring the sections in graded alcohols of descending series for 3 mints in each and then to distilled water.

(c) Staining by haematoxylin for 5-10 mints.

(d) Washing with running tap water.

(e) Differentiation is necessary where a stain is used regressively eg. When the tissue is first over stained and later the excess color is removed.

Differentiation of haematoxylin is as follows:

(1)The haematoxylin is washed with tap water

(II) The excess of haematoxylin is removed from the section by using 1% acid alcohol. (HCL 1ml, 70% alcohol- 99ml)

(f) Washing: 1% acid alcohol is washed off with running tap water.

(g) Bluing: The section is blued in tap water for 5-10 mints (we can also use 2% ammonia water, or 1% lithium carbonate). The term bluing is applied to the process when the section is transferred from and acid solution to an alkaline fluid. The fluid brings about the desired blue or black color depending upon the haematoxylin used.

(h) Washing: In running tap water for 1-2 mints.

(i) Counter staining: As done with eosin for 3-5 mints.

(j) Washing and dehydration: is done with ascending series of graded alcohols egs. 70%, 80%, 90% and absolute II and III.

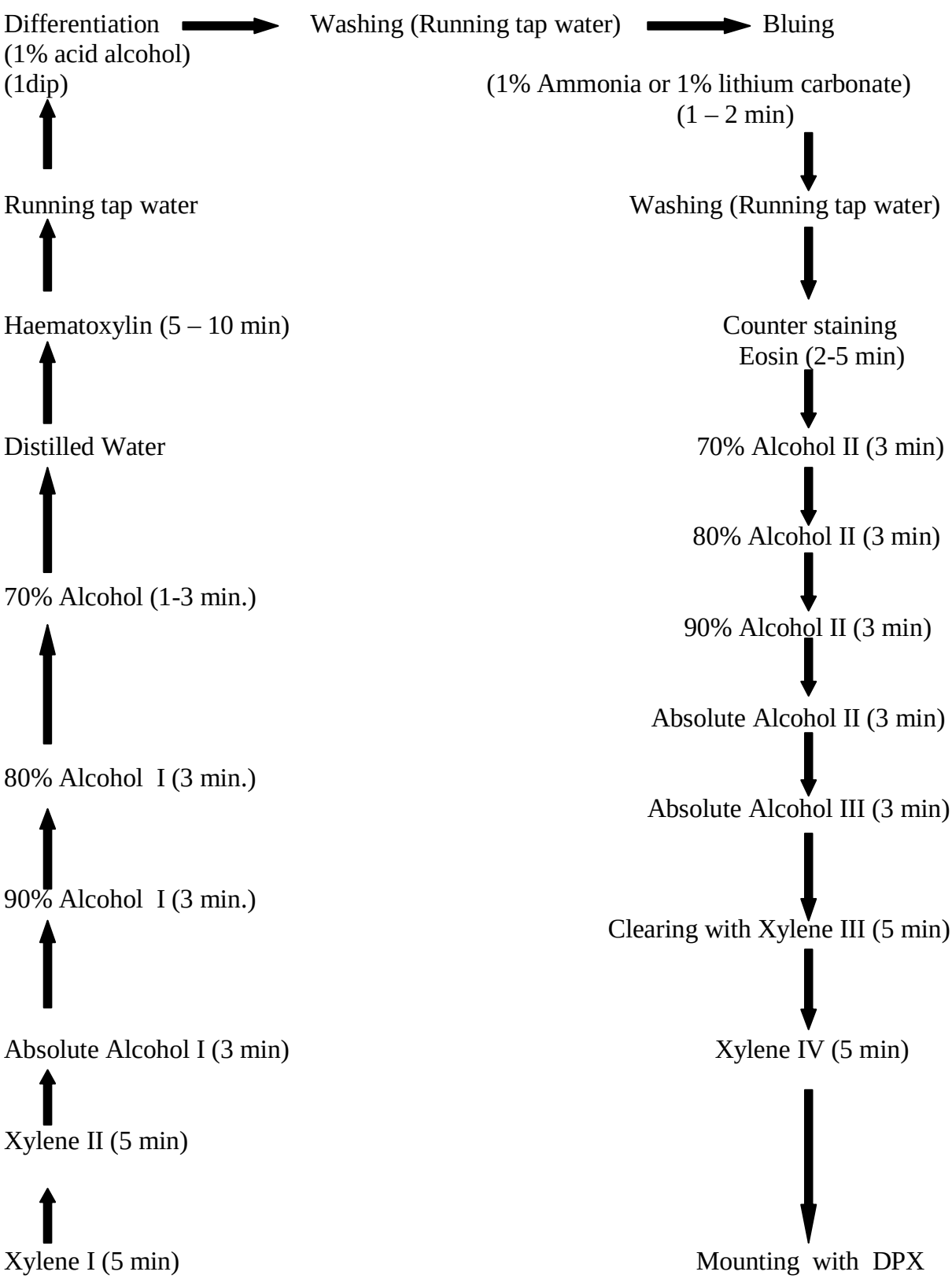
(k) Clearing: is done with xylene III and IV for 5 mints in each.

(l) Mounting of sections: to give neat finish to the stained sections. To protect it from drying, dirt, etc. It is done by putting DPX and then covering it with cover slips (DPX- Dibutyl phthalate with xylene).

The H and E stains impartscolor to the following mentioned against them.

1. Nuclei: blue-black to dark blue
2. Cytoplasm: pink
3. Erythrocytes: red
4. Muscle: red
5. Fibrous tissue: pale pink
6. Cartilage: pale to deep blue
7. Mucus: clear to pale blue
8. Calcified substance: deep blue
9. Fibrin: red
10. Protein precipitates (as in oedema fluid): pink
11. Bacterial masses: deep blue

Flow Chart of Staining of Tissue



Appearances of Tissues and Substances in special methods of staining:

- **Van Giesons's stain**
  To stain connective tissue and muscle distinctly, collagen –red; other tissues–yellow.
- **Perl's stain**
  Iron containing pigment (haemosiderin) stains blue
- **Acid fast method: (Zeihl–Neelson method)**
  To demonstrate mycobacteria; acid fast organisms stain pink.
- **Fat stain**
  Fat in tissues are dissolved out by xylol and alcohol by the conventional methods of 'passing'. To stain fat, frozen sections are prepared and stained with SudanIII, Scarletred (stains red) andOsmicacid (stains black).
- **Amyloid**
  Congo redStains-red
  Methyl violet-pink against a violet background.
- **Glycogen**
  Best's carmine–Glycogen stains bright red.
- **Fungus:**
  Grocott'sMethanaminesilvernitrate stain- stains block
  Periodic acid Schiffstain stains pink
  Gridley's stain stains deep purple
- **Gram positive and Gram negative bacteria:**
  Mac Callum Good Pasteur's stain

Questions

1. What is fixation? Why it is necessary?
2. Name the stain which is commonly used for staining tissue sections for histopathological examination.
3. What is the importance of taking different dilutions of alcohol during histopathological process?
4. What is mounting? Why Mayer's glycerine egg albumin is used before taking the section on the slides?
5. Draw flow chart of staining of tissues with H & E?

EXERCISE NO.-3

GROSS SPECIMENS

1. Organ……..

Lesions……
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10. Organ……..

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EXERCISE NO. -4

HISTO-PATHOLOGICAL CHANGES

Draw the diagram and mention the salient microscopic features of the following conditions….

CIRCULATORY DISTURBANCES

Active hyperemia:

Passive hyperemia:

Edema:

Hemorrhage:

Thrombus:

Infarction:

DISTURBANCES OF CELL METABOLISM

Cell swelling:

Hydropic degeneration:

Mucous degeneration:

Mucoid degeneration:

Hyaline degeneration:

Amyloid infiltration:

Fatty degeneration:

Gout:

ISTURBANCE IN MINERAL/PIGMENT METABOLISM

Calcification:

Anthracosis:

Silicosis:

Siderosis:

Melanosis:

Hemosiderosis:

NECROSIS AND GANGRENE

Coagulative necrosis:

Caseative necrosis:

Liquifactive necrosis:

Fat necrosis:

Gangrene:

DISTURBANCES IN GROWTH

Atrophy:

Hypertrophy:

Hyperplasia:

Metaplasia:

INFLAMMATION

Serous inflammation:

Fibrinous inflammation:

Catarrhal inflammation:

Suppurative inflammation:

Hemorrhagic inflammation:

Non-suppurative inflammation:

Granulomatous inflammation:

UNIT-2

SYSTEMIC VETERINARY PATHOLOGY

EXERCISE NUMBER-1

GROSS&MICROSCOPIC LESIONS OF CARDIOVASCULAR SYSTEM

EXERCISE NUMBER-2

GROSS&MICROSCOPIC LESIONS OF RESPIRATORY SYSTEM

EXERCISE NUMBER-3

GROSS&MICROSCOPIC LESIONS OF DIGESTIVE SYSTEM

EXERCISE NUMBER-4

GROSS&MICROSCOPIC LESIONS OF URINARY SYSTEM

EXERCISE NUMBER-5

GROSS&MICROSCOPIC LESIONS OF REPRODUCTIVE SYSTEM

EXERCISE NUMBER-6

GROSS&MICROSCOPIC LESIONS OF NERVOUS SYSTEM

EXERCISE NUMBER-7

GROSS&MICROSCOPIC LESIONS OF MUSCULO-SKELETAL SYSTEM

EXERCISE NUMBER-8

GROSS&MICROSCOPIC LESIONS ENDOCRINE SYSTEM

EXERCISE NUMBER-9

GROSS&MICROSCOPIC LESIONS OF SKIN AND APPENDAGES

EXERCISE NUMBER-10

GROSS&MICROSCOPIC LESIONS OF HEMOPOIETIC SYSTEM

EXERCISE NUMBER-11

GROSS&MICROSCOPIC LESIONS OF EYE AND EAR

UNIT-3

ANIMAL ONCOLOGY, VETERINARY CLINICAL PATHOLOGY AND NECROPSY

EXERCISE NO. -1

EXAMINATION AND INTERPRETATION OF ONCOLOGICAL TISSUE SLIDES

Neoplasm:-Neoplasm (G. neo-new; plasia- development or formation)

Definition:-The simple meaning of neoplasia is new growth.

A neoplasm is a new growth of cells which:-

. Proliferate continuously without control.

. Bearing a considerable resemblance to the healthy cells from which they arise.

. Have no orderly structural arrangement.

. Serve no useful function.

S.NO.	Histogenesis	Behaviour	
		Benign	Malignant
I	Simple tumours Epithelial cells Mesenchymal cells Others	-oma -oma -oma	-Carcinoma -Carcinoma -Sarcoma
II	Mixed tumours	Beningn mixed tumour	Malignant mixed tumour
III	Compound tumours	Mature teratoma	Immature teratoma

Histological classification of neoplasms:

	Benign	Malignant
Epithelial		
i. Epidermis	Papilloma	Squamous cell carcinoma
ii. Basal cell (Skin adnexae)	-	Basal cell carcinoma
Adnexae		
i. Hair follicle	Trichoepithelioma	Adenocarcinoma
ii. Sebaceous/Sweat/Perianal gland	Adenoma of respective gland	Adenocarcinoma
Non glandular	Papilloma	Carcinoma

epithelium		
Glandular surface	Polyp	Adenocarcinoma
Glandular epithelium	Adenoma	Adenocarcinoma
Mesenchymal		
i. Fibrocyte	Fibroma	Fibrosarcoma
ii. Mucoid connective tissue	Myxoma	Myxosarcoma
iii. Adipose connective tissue	Lipoma	Liposarcoma
iv. Cartilage	Chondroma	Chondrosarcoma
v. Bone	Osteoma	Osteosarcoma
Blood vessel	Angioma or haemangioma	Haemangiosarcoma
Lymph vessel	Lymphangioma	Lymphangiosarcoma
Smooth muscle	Leiomyoma	Leiomyosarcoma
Straited muscle	Rhabdomyoma	Rabdomyoma
Histiocyte	Histiocytoma	Malignant histiocytoma or histiocytic sarcoma
Mast cell	Mastocytoma	Malignant mast cell tumour or mast cell sarcoma
Haemopoietic tissue		
i. Lymphocyte	Lymphocytoma	Lymphosarcoma
ii. Plasma cell	-	Myeloma
iii. Monocyte	-	Monocyticleukemia
iv. Granulocyte	-	Myelogenousleukemia or granulocytic leukemia
v. Reticulum cells	-	Myelogenousleukemia or granulocytic leukemia
vi. Erythroblasts	-	Erythroidleukemia
vii. Myoloblast	-	Myeloid leukamia
Mesothelium		
i. Synovial membrane	Synovioma	Synovial carcinoma
ii. Meninges	Meningioma	Meningioma or invasive meningioma
iii. Bronchial epithelium	-	Bronchogenic carcinoma
Nervous tissue		
i. Astrocyte	Astrocytoma	Astrocytoma
ii. Oligodendroglia	Oligodendroglioma	Oligodendroglioma
iii. Ependyma	Ependyoma	Ependyoma
iv. Schwann cells	Schwannoma (neurilemmoma)	Neurilemmoma

v. Nerve cell	Neuroblastoma or Ganglioneuroma	Malignant neroblastoma or Malignant ganglioneuroma
vi. Chromaffinparaganglia (adrenal medulla)	Pheochromocytoma	Malignant pheochromocytoma
vii. Non chromaffinparaganglia (Carotid body, arotic body)	Chemodectoma or nonchromaffinparaganglioma	Malignant chemodectoma or Nonchromaffinparaganglioma or meduloblastoma
Others		
i. Neuroectoderm-Melanocyte	Melanoma	Malignant melanoma
ii. Renal epithelium	Renal tubular adenoma	Renal cell carcinoma
iii. Urinary tract epithelium (Transitional)	Transitional cell papilloma	Transitional cell carcinoma
iv. Placental epithelium (Trophoblast)	Hydatidiform mole	Choriocarcinoma
v. Spermatogenic epithelium (Testicular epithelium; germ cells)	Seminoma	Seminoma or Embryonal carcinoma
vi. Kidney	Nephroblastoma	Malignant nephroblastoma
vii. Islet cell	Insulinoma (β cell adenoma)	Malignant insulinoma
viii. Liver	Hepatoma	Hepatocellular carcinoma
ix. Sertoli cell	Sertoli cell tumour	Sertoli cell tumour

Difference between Benign and Malignant tumour:

S.No.	Features	Benign	Malignant
1.	Occurrence of nodule or mass	Single	Single or multiple
2.	Shape of nodule	Round, elliptical or wart-like and pedunculated	Irregular
3.	Encapsulation	Present	Absent
4.	Rate of growth	Slow	Rapid
5.	Growth	Limited	Unceasing
6.	Spontaneous regression	Occurs	Do not occur
7.	Invasion	Absent	Present
8.	Metastasis	Absent	Present

9.	Basement membrane	Intact	Broken
10.	Blood vessel formation	Moderate	Numerous
11.	Degenerative and necrotic changes	Absent as the blood supply is adequate	Present because of inadequate blood supply
12.	Recurrence	Do not recur	Recurs after apparent removal
13.	Destruction of adjacent tissues	Little	Extensive
14.	Cell structure	Typical to adult tissue	Not typical to that of adult tissue
15.	Anaplasia	Absent, resembles cells from which they originate	Present
16.	Polarity	Maintained	Lost
17.	Cellular pleomorphism	Absent	Present
18.	Anisokaryosis	Absent	Present
19.	Number of nucleus	Not altered	Multiple (Tumour giant cell)
20.	Nucleolus	No change	Enlarged, prominent and multiple
21.	Nucleolar to nucleus ratio	Not altered	Increased
22.	Cytoplasm to nuclear ratio	Not altered	Decreased
23.	Mitosis	A few in number; Typical	Abundant, some are atypical
24.	Death	Do not occur except if the tumour involves vital organs like heart, brain	Usually occurs depending on the invasion, metastasis and tissue destruction

Grading and staging of carcass:

Based on the extent of malignant features like cellular characters (differentiation and anaplasia), invasion, metastasis and number of mitoses, tumours can be classified as grade I. II. III, IV (grade I for the least and grade IV for the most anaplastic).

Clinical staging of cancer (TNM classification)

This is based on

. Size of the primary tumour – T

. Extent of spread to regional lymph node – N

. Presence or absence of metastasis – M

Clinical staging should be combined with histological analysis such as grading of tumours which is helpful in prediction of survival of cancer patients.

Primary tumour

. To - no evidence of tumour

. T1 - tumour confined to primary site

. T2 – tumour invades adjacent tissues

Lymph nodes

. No- no evidence of tumour

. N1- Regional lymph node involvement

. N2- distant lymph node involvement

Metastases

. Mo - no evidence of tumour

. M1 - tumour in same organ or cavity as primary

. M2 - distant metastases

GROSS SPECIMENS

1. Organ……..

Lesions……
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MICROSCOPIC SPECIMENS

EXERCISE NO. 2

POSTMORTEM EXAMINATION OF LARGE AND SMALL ANIMALS

POSTMORTEM is the procedure carried out after death to determine the cause of death. It is specialized procedure, requiring a good knowledge of animal anatomy.

"Systemic exposure and critical examination of carcass for the study of gross pathological lesions with an objective to ascertain the cause of disease is called post-mortem, necropsy".

***NOTE:** Post-mortem (PM) should be conducted only when written request is received from the owner having details of animals, history of disease, treatment given etc. other wise not.

PRECAUTIONS:

1. Obtain permission of the owner in writing before post-mortem examination.
2. Request from the local police is must in vetro-legal case.
3. Conduct post-mortem examination as early as possible to avoid putrefaction.
4. Examine the smear from peripheral blood to rule out the Anthrax. Beside bacilli examination, blood examination may be carried out for parasites, other bacteria and post-mortem invaders.
5. P.M examination should be done in day time to appreciate the accurate changes in the color of the tissues. This is not possible with artificial light.
6. Conduct P.M far away from animal houses and farm premises and preferably in a government land to avoid litigation.
7. Obtain history, symptoms and treatment done etc.
8. Wear gloves, masks, apron and gumboots to avoid contact with zoonotic agents.
9. Record the P.M finding immediately.
10. Burry the carcass in deep ditch layered with lime. Carcass can be burn to ashes if incinerator is available.

SYNONYMUS: Necropsy (Veterinary Science)

Autopsy (Medical Science)

Types of necropsy:

1. **Partial Necropsy:** In case of rabies, only the brain of the carcass is examined for diagnosis. Here only a part of the body that is opened for the purpose other parts of the body are not opened.
2. **Complete Necropsy:** It should be done as a matter of routine when body of carcass is available. All parts of the body are thoroughly examined to arrive etiological diagnosis.

   In Veteroligal cases it is necessary that complete PM examination should be done.
3. **Cosmetic Necropsy:** Examination of carcass is done with very less mutilation i.e. injury of cutting and incision are sewed together and the body is washed to appear as nearly intact as possible. It is done in case of pet and wild animals

The five main steps for conduction of necropsy are

1. Perform gross examination
2. Open the body cavity (examine organs *in situ*)
3. Removal of organs
4. Examination of remaining carcass
5. Systemic examination of organs

OBJECTIVES:

1. To find the cause of death.
2. Experimental studies of lesions.
3. Studies of clinical manifestation correlating with severity of lesions of disease.
4. For the advance knowledge in the field of anatomy, surgery and pathology.

PREPARATIONS:

Before conducting any PM the following preparations are to be done-

- Wear long full sleeve apron, gum boots or shoes, latex gloves, face shields.
- While doing PM don't use finger rings, wrist watches, bangles, necklace etc.
- A post mortem kit should be kept ready having
    i. Long scissors and small scissors
    ii. Long autopsy knife/special knife (butcher's knives)
    iii. Dissecting forceps
    iv. Chisel and hammer
    v. Cutting saw
    vi. Magnifying glass
    vii. Gloves, slides
    viii. Antiseptic lotion, powder or cream
    ix. Scalpel

Students are advised to keep mini kit of postmortem containing

b. Large and small scissors
c. Scalpels
d. Forceps
e. Hand gloves
f. Antiseptic lotion

a) EXTERNAL EXAMINATION OF CARCASS:

Following steps to be followed for external examination

- Note the identification number/tag number, species, breed, age and sex of the animal.
- Observe general condition and examine the body surface (fair/poor/emaciated/cachectic) for any kind of injuries, burns, swelling, abscess etc.
- Record whether rigormortis is present or not.
- Natural orifices: Look for any discharges from the natural orifices. In Anthrax tarry coloured blood oozes from natural orifices because the blood fails to clot.
- Visible mucus membrane: Examine the visible mucus membrane (color- pink or pale, icteric (Yellow), congested, haemorrhagic and cynotic) and also the external genitillia.
- Skin and coat: Hair loss (Patchy or complete), look for presence of wounds, abraisons, lacerations, perforations, abcess and tumor.
- Snake bite: Fang marks with swelling and haemorrhage

- Canine distemper: Pustules on the ventral side of the abdomen
- Examine the skin for ring worm and scabies or mange, ticks, lice, flea etc.
- Examine the superficial lymphnodes: Prescapularlymphnodes swollen in Thileriasis.
- Examine all the bones of the body for presence of fractures and also the joints for any dislocation.

b) INTERNAL EXAMINATION:

- For examining the internal organs of the carcass following procedures are to be adopted in different species of animals.

SUBCUTANEOUS TISSUE:

- Normally fair and mosit, however, it may be dry, congested and haemorrhagic (Electrocution).

ABDOMINAL AND THORACIC CAVITIES:

- Examine the nature of the exudate, if present and record whether there is any abomasal displacement, intussusception, hernia etc.

HEART:

- Look for epicardial haemorrhages.
- Record the nature of the pericardial fluid, fibrinopurulent (Traumatic pericarditis) exudates and foreign body may be seen.
- Open the heart and examine the endocardium and heart valves (Bicuspid, tricuspid and semilunar valves) for vulvular growth (eg. vegetative growth in TRP), thrombus etc.

RESPIRATORY SYSTEM:

- Mucosa of larynx, trachea and bronchi may be congested. They may contain frothy exudates in pulmonary edema, aspirated substances etc.
- Examine the lungs and associated lymphnodes (mediastinal and bronchial).

- Make incisions of lungs at different places and examine for exudates and parasites. Put a piece of lung in water to ascertain consolidation.
- Look for granulomatous nodules, black spots (Anthracosis), abcess, brown indurations etc.

BRAIN:

- Disarticulate the head at the atlanto-occipital joint and strip off the skin and muscles.
- Make a transverse cut behind the posterior margins of the orbits.
- Make lateral cuts at angle of 34-40^{O}C.
- From a sagital plane along the dorsal aspect of cranium and they should meet interiorly and posteriorly.
- Tie up the calverium and remove with a chisel.
- Severe the optic and olfactory nerves.
- Lift the brain from the front and remove.
- Examine the various parts of the brain for presence of parasites, cysts, tumors, congestion and hemorrhage.

DIGESTIVE TRACT:

- Open the digestive tract by enterotome at various portions and examine its contents and mucus membrane.
- Examine the mesenteric lymph nodes and blood vessels.
- Open the rumen, reticulum and omasum in case of ruminants.
- Remove the content and examine for any lesion or parasite.
- Examine the reticulum for the presence of any foreign body like needle or any sharp object.
- Examine the omasum for congestion, ulcers, hemorrhage, swelling of folds and edema.

URINARY TRACT:

- Examine the subcapsular haemorrhages.
- Examine kidneys, urethra and urinary bladder for any haemorrahages, stones.

PARENCHYMATOUS ORGANS:

- Examine the liver for congestion, hemorrhage, nodules, enlargement and also the gall bladder.
- Examine spleen for size, shape, consistency, congestion hemorrhage, necrotic foci etc.
- Examine the sub scapular, mediastinal and popliteal lymph nodes.
- Examine the ureters for enlargement or obstructions.

GENITAL TRACT:

A. Female:

- Separate the female genital tract from the rectum and take it out. Open the vulva to the tips of cornua.
- Examine the vagina for congestion, hemorrhage, trauma, granular structure and any foreign body.
- Open the fallopian tubes and uterus and examine for edema, congestion and hemorrhage etc.
- Examine the ovaries by incising them.

B. Male

- Make an incision from the neck of the bladder to the urethra.
- Open the urethra and examine for any lesion or ulcer.
- Examine the testis for atrophy, enlargement, edema, congestion and adhesion with skin and epididymis.

POST MORTEM CHANGES

1. Autolysis:

Autolysis is the digestion of tissue brought about by the tissue's own enzymes.

2. Algor mortis:

It is the cooling of the body at or before the stoppage of blood flow. The faster is the algor mortis the greater is the preservation of the carcass.

3. Putrification:

It is digestion of the tissues brought about by the bacterial enzymes. After death the body defences are no larger functioning. Hence, saprophytic bacteria from the intestine and from the surface of the body invade the tissue, grow and multiply there and bring about the digestion of the tissue by their enzymes.

4. Rigor mortis:

It is shortening and contracting of the muscle after death so that the body becomes rigid and immobilize. Evidently, rigor mortis is associated with cell metabolism. Many theories have been advanced to explain rigor mortis but none of them is found to be satisfactory. It is believed that the contraction is due to the accumulation of catabolic substances with the cell but it is not explained that why the cell remains in a state of contraction even after death. Rigor mortis appears in about one to eight hours after death and order of its appearance is head, then neck then trunk and finally the limbs. Higher external temperature augments rigor mortis. Other factors which facilitate the rigor mortis are violent exercise (Fighting, racing, struggling etc.) and violent muscular contraction as in tetanus or strychnine poisoning.

Lower external temperature delays the appearance of rigor mortis. In weak and debilitated animals the rigor mortis is delayed and is never very pronounced or it may not appear at all. Rigor mortis disappears after about 20 to 30 hours. The disappearance of rigor mortis is in the same order as it had appeared i.e. head then neck then trunk and finally limbs.

How rapidly rigor mortis disappear depends upon the rate at which autolysis and putrefaction takes place. The faster is the autolysis and putrefaction quicker is the disappearance of rigor mortis. The rapidity of appearance and disappearance of rigor mortis is utilized to indicate the length of time, the animal has been dead.

5. Post mortem clotting of blood:

After death, the endothelial cells, the leucocytes and the thrombocytes release an enzyme thrombokinase or thromboplastin which initiates the cloting process. The clot is found in heart, the veins and smaller arteries. The arteries are mostly found to be empty because of their contraction in rigor mortis, emptying out most of the blood. If the animal has been dead for quite some time then the blood clot is digested and liquefied by the action of enzymes. During the post

mortem examination it is important to remember the various factors which may cause failure of clotting.

6. Post mortem emphysema:

It is the accumulation of gases in the tissues during the putrefaction produced by bacterial fermentation. The nature and quantity of gases produced is depending upon the nature of invading bacteria. However, the predominant gas produced is H_2S responsible for offensive odour.

7. Hypostatic congestion:

It is the accumulation of blood in the ventral portion of the organs or in general in the influence of gravity.

8. Pseudomelanosis:

After death, the carcass is found discoloured to various shapes of green, grey or black colour. During putrefaction H_2S is produced which combines with the iron of hemoglobin to from iron sulphide which is black in colour. Depending upon the concentration of this pigment and its combination with other tissue pigments we get various shades of green, gray and black colour.

9. Imbibition with haemoglobin:

Following haemolysis the haemoglobin pigment is released which is soluble in body fluid and diffuses out in the surrounding tissue around a blood vessel staining the tissue to a pink colour. If there is too much of the haemoglobin pigment, then the tissue may assume dark red colour.

10. Imbibition with bile:

The tissue surrounding the gall bladder is staining to the yellow colour due to diffusion of chole bilirubin from the gall bladder.

11. Rupture of organ or tissue:

During putrefaction gases are produced and accumulation in the organ and tissues causing distension, until a stage is reached when due to pressure of accumulated gas they can hold no longer and rupture releasing gas. Stomach, intestine, diaphragm and ventral abdominal wall are found to be commonly involved.

12. Displacement or organ:

The segment of containing the gases being lighter, rise above while those containing the feed being heavier lie ventrally in the abdomen. When the carcass is rolled then these become displaced or rotated. This must be distinguished from autemortem volvulus or torsion in which there will be which there will be acute local passive hyperemia.

NECROPSY PROCEDURES FOR HORSES

- Place the carcass of the horse on the right side down
- Make an incision through the skin in the ventral median line from chin to anus dorsolaterally around the mammary glands or penis
- Reflect back the skin of neck, thorax and abdomen of left side as much as possible without making further incision into the hide
- The left fore and hind limbs are severed by cutting all muscles and attachments
- Finish skinning the thorax and abdomen to the tops of the spinous processes of the vertebrae
- Incise through the midline from the Xiphoid to the pubis and from pubis to the lumbar region through the abdominal wall
- Pull upword and forward the large flap of abdominal wall and cut the diaphragm dorsoventrally close to its attachments
- The abdominal viscera are removed in the following order
  Great colon and caecum
    - Left kidney
    - Left adrenal
    - Small intestine
    - Liver
    - Right kidney
    - Right adrenal
    - Stomach
- Open the thoracic cavity by cutting the ribs along their ventral ends from the thoracic inlet to the last rib.
- Remove thoracic organs by severing the esophagus, trachea and other attachments, then lifting the heart and lungs out while cutting the remaining attachments.
- Cut the symphysis of pubis. Saw through the shaft of the ileum above the coxifemoral joint. Examination the internal genital organs, rectum and urinary bladder.

EXERCISE-3

NECROPSY PROCEDURE FOR CATTLE, BUFFALO, SHEEP AND GOAT

- Place the carcass left side down in order to keep the rumen out of the way.
- Sever the right fore and hind limb by cutting all the muscles and attachments (better disarticulate the right hind limb).
- Make an incision through the skin from the space between the jaws into the perineal region. Incise around the mammary glands or penis.
- Reflect back the skin of right side as much as possible.
- Remove the abdominal wall by making incision through its periphery
- Open rumen, reticulum, omasum and abomasum in situ and remove all the contents and examine thoroughly.
- Expose the thoracic cavity in the same manner as in case of horse.
- Remove the lungs and heart along with trachea and esophagus.

NECROPSY PROCEDURE FOR SWINE

- Place the carcass on its back.
- Make an incision so as top drop the limbs to horizontal position and thereby expose the trachea, esophagus, thoracic and abdominal cavities.
- Make an incision at the sternum passing through the costal cartilage (raise the anterior end of the sternum with left hand so that the heart is not cut).
- Incise the ventral abdominal wall towards the pubis to expose the abdominal organs.
- The ventral abdominal wall left attached to the pubis to cover the abdominal cavity after necropsy.
- Open the thoracic cavity by breaking the ribs
- Remove the thoracic organs attached to trachea and esophagus
- First remove the spleen and omentum
- Push the small intestine to the left side to examine the rectum
- Cut the esophagus posterior to the diaphragm.
- Remove the liver, stomach and intestine together
- Remove the adrenal glands and then remove the kidneys
- Sever the uterus posteriorly as possible in case of female
- Examine the seminal vesicle and other accessory organs

Necropsy procedure for dogs and cats

- Place the animal with its dorsal surface on the table.
- Extend the head and straighten the neck.
- Make an incision from the point of mandibular symphysis to the ischial arch.
- Separate the skin from the underlying surface.
- Pull the legs to the side.
- Cut the coxifemoral joint and separate the hind limbs.
- Make an incision from the xyphoid cartilage to the pubis.
- Make the transverse incision from the midline to the dorsal extent of the body cavity.
- Expose the abdominal cavity by removing the flaps.
- Cut the sternal cartilage or ribs and expose the thoracic cavity.
- Examine all organs in-situ with out severing the attachment.
- Lift the organs up and incise for closer examination and return them to the body cavity at their positions.
- Press the knife under the tricuspid valve into the pulmonary artery and open it.
- Do it similarly at the left side also.
- Open the pulmonary vein by passing knife through the bicuspid valve.
- Examine the vessels of the heart, endocardium and myocardium.

Necropsy procedure of Poultry

- Dip the dead bird in antiseptic solution or in water to avoid feather contamination.
- Keep the bird on post-mortem table at vertebral column and look for any lesion or parasites on skin.
- Examine the eyes, face and vent.
- Remove skin through a cut with knife and with the help of fingers. Expose thymus, trachea oesophagus in neck.
- Break the cox femoral joint by lifting the legs. Examine the chest and thigh muscle.
- Cut on lateral side of chest muscles. Lift the chest muscle dorsally and break bones at joints with thorax. Cut bones at both sides and remove muscles, bones to expose thorax, abdomen.
- Examine different organs.

- Cut proventriculus and pull the organs of digestive tract out. Separate liver, spleen, intestine, caecum, proventriculus, gizzard etc.
- Expose bursa just beneath the cloaca.
- Cut beak at joint, examine mouth cavity and expose oesophagus and trachea.
- Remove skin of head and make a square cut on skull to expose brain.
- Take a forceps and place in between thigh muscles, remove fascia and expose the sciatic nerve.
- Separate each organ; examine them for the presence of liver.

QUESTIONS

1. Give one difference between necropsy and biopsy.
2. Mention the importance of P.M examination in Veterinary Science.
3. What do you understand by the term Rigor-mortis?
4. Difference between Autolysis and putrefaction.
5. Define Adipocere, Mummification, Anthropophagy, Algormortis etc.

EXERCISE-4

WRITING POSTMORTEM REPORT

POSTMORTEM REPORT No./

Notes on the postmortem examination on the body of a……....

Belonging to …….

Result of examination of blood smear taken after death:

A. Precise of the case

1. Date of admission, ward and case No.
2. Date and time of death reported
3. Date and time of making P.M.
4. History
5. Clinical diagnosis

B. External Examination

1. Class of animal, sex, age, breed
2. Description marks
3. Condition of the body
4. Rigor mortis
5. Natural orifices
6. Visible mucous membranes
7. Presence of wounds, if any
8. Superficial lymph nodes
9. Any other abnormalities

C. Internal Examination

1. Subcutaneous tissue
2. Abdominal cavity
    1. Peritoneal cavity and peritoneum
    2. Position of organs (organs inspected *in situ*)
3. Thoracic cavity
    1. Pleural cavity of pleura
    2. Position of organs
4. Pericardial sac

5. Heart
    1. Gross appearance, colour, size etc.
    2. Chambers
    3. Valves/Endocardium
    4. Myocardium
    5. Blood vessels
6. Larynx, Trachea and Bronchi
    1. Abnormalities
    2. Parasites
    3. Bronchial lymph nodes
7. Lungs
    1. Gross appearance, colour, size etc.
    2. Palpable abnormality
    3. Section
    4. Parasites
    5. L. nodes
8. Diaphragm
9. Liver
    1. Gross appearance, colour, size etc.
    2. Surface
    3. Borders
    4. Parenchyma
    5. Portal lymph nodes
    6. Gall bladder (wall and contents)
    7. Parasites
10. Spleen
11. Kidneys
    1. Gross appearance, colour, size etc.
    2. Capsule
    3. Cortical surface
    4. Section
    5. Renal pelvis
    6. Parasites and caliculi
12. Adrenal glands
13. Head

1. Mouth (lips, teeth, gums, tongue, palate, salivary gland etc. and associated lymph nodes)
   2. Eyes
   3. Ears
14. Nasal cavity
   1. Mucous membrane
   2. Sinuses
   3. Pharyngeal mucous membrane
   4. Tonsils
   5. Guttural pouches
   6. Retropharyngeal lymph nodes
15. Neck
   1. Oesophagus
   2. Thyroids
   3. Parathyroids
   4. Thymus

16. Stomach (s)-Forestomach in ruminants
   1. Serous surface
   2. Mucosa and contents
   3. Parasites
17. Intestines
   1. Mesentery, mesenteric blood vessels, lymph nodes and parasites
   2. Surface (serous and mucous)
   3. Ileocaecal valve
   4. Contents (Parasites)
18. Pancreas
19. Pelvic cavity
   1. Urinary bladder
   2. Generative organs
   3. Accessory sexual glands
   4. L.nodes
20. Brain and spinal cord
21. Skeleton and musculature
22. Clinical Laboratory Examination

D. Appearance found

Diagnosis:

Materials collected:
Result of examination:

POULTRY POSTMORTEM REPORT

. Name of the institution:……Poultry necropsy No.:……

. Date & Time of making necropsy:…….. . Reference:…….

. Owner Name and Address:…… . Date, time and place of death:…….

. Particulars of the bird:……Species: Breed: Type of bird: Commercial/ Breeder/

Fancy/ Wild Broiler/……Age:……Sex: M/F……..Identification/ Wing/ Leg band No……..

. Colour:……History:…….Total stock:……..

. Mortality:……..Pattern for last 7 days:…….

. Percentage:…….Signs:…….

NECROPSY FINDINGS

EXTERNAL EXAMINATION FINDINGS

. Condition of the carcass:
. Eyes:
. Beak:
. Feathers:
. Skin:
. Presence of parasites, if any:
. Rigor mortis:
. Visible mucous membrane:
. Natural orifices:
. Presence of wounds, if any:
. Any other abnormality, if any:

INTERNAL EXAMINATION FINDINGS

. Digestive system:
. Circulatory system:
. Respiratory system:
. Uro-genital system:
. Nervous system:
. Musculo-skeletal system:
. Lymphoid organs:

DIAGNOSIS

. Museum specimens collected:
. Tissues for histopathology:
. Other laboratory investigation:
. Necropsies by:

EXERCISE NO. – 5

COLLECTION AND PRESERVATION OF BLOOD AND USE OF ANTICOAGULANTS

Blood is a mobile fluid tissue .It is composed of formed elements and plasma formed elements are: Erythrocytes, leucocytes and thrombocytes

Plasma:it is liquid which suspend cells in it

The quantitative and qualitative studies in blood help us in assessment of health status of the animal body .As the blood circulates through whole body and performs vital functions and mal functions of different organs or systems.Apart from diagnosis of disease ,haematology also help in tratment of disease

REQUIREMENTS

Hypodermic needles,syringe,scissors ,anticoagulents

PROCEDURE

1)Take appropriate size sterile needle and mount it on the syringe.

2) Clip the hairs and wash the area with soap and water should be done .

3) Locate the vein under the skin,apply thumb pressure or tourniquet towards heart to make the syringe.

4) Insert the needle obliquely through skin into vein and draw the required quantity of blood in to the syringe.

5) Remove the needle from vein and apply fingure pressure for 1-2 min on punctured site followed by spirit swab application.

6) Remove the needle from syringe and gentle pour blood into the vial containing suitable anticoagulant

7) Stopper the vial and rotate it gently inorder to mix the anticoagulant with blood without frothing.

PRECAUTIONS TO PREVENT HAEMOLYSIS

1) Needle of small gauge should be used as have decreased flow rate which decreases velocity turbulence .Haemolysis is directly related to flow velocity and conduit radius.

2) Needle and syringes must be dry .presence of moisture may cause haemolysis.

3) Suction or pumping action should be used minimum the syringe the blood should flow freely.

4) Needles should be removed before transferring the blood to the vial blood from syringe because forcing the blood through the needle under the pressure may rupture red blood cells

5) After obtaining the blood the syringe should be taken apart and rinsed immediately to prevent sticking and plugging of barrel.

6) Mixing of the blood should always be done with gentleness to dissolve the anticoagulant .rough handling may lead to rupture of the blood cells.

SPECICES WISE COLLECTION SITE AND NEEDLE DIMENSIONS

S.NO	SPECIES	SITE/VEIN	GUAGUE NO	LENGTH
1.	Cattle ,buffalojugularvein	16-18	1.5-2.0	
horse,camel				
2.	sheep,goat	jugular vein	18-20	1.5-2.0
3.	pig	anterior venacava	20	1.5-2.0
4.	dog	cephalic /saphenous	20-22	1.5-2.0
5.	cat	ear/cephaliv vein/jugular	22-25	1.0
6.	rabbit	ear/cardiacpunctures	18-22	3.0
7.	poultry	wing/cardiac puncture	22-24	1.5

ANTICOAGULANTS:

Anticoagulants are subsances ,that prevent the formation of clot .many coagulants can be used to obtain blood sample free from clot for blood transfusion and analytical work .there are four common coagulants in use:Oxalates,Trisodiumcitrate,Diphotasium or disodium or ethylediminetetra-acetic acid.(EDTA) and heparin .The yse of dry anticoagulant is preffered to avoid dilution of blood .good anticoagulants should have the following qualities:

1. It should not alter the size and shape of red cells.

2. It should not cause haemolysis

3. Leukocytes destruction should be minimum.

4.Platlets aggregation should be minimum

(A) EDTA (Ethylene diamine tetra-acetic acid)

1.The sodium or pottasium salts of EDTA are powerful anticoagulant.It act by chelating effect on calcium molecules in blood and it renders ionic calcium unavailable for clotting .This require concentration of 1.2 mg of the anhydrous salt per ml of blood .

2. The anticoagulant of choice is the dipottasium salt as it is very soluble .It is preffered over disodium salt which is less soluble.

3.Excess EDTA(>2mg/ml of blood)would affects both RBCS and WBCS causing shrinkage and degenerative changes,decreases PCV & MCH, and causes platelets to swell and then to disintegrate ,resulting in high platelets count.

4.EDTA not soluble for use in the investigation of coagulation studies such as in estimation of prothrombin time.

5. It is the anticoagulant used in ESR estimation.

(B)TRISODIUM CITRATE

1.It is the anticoagulant of choice of coagulation studies ,for this nine volumes of blood added to one volume of sodium citrate solution(10%dilution)

2.It acts by its chelating effect that is by forming insoluble salt with calcium molecules

3.It most widely used in determination of ESR

(C)HEPARIN

1. It is an effective anticoagulant at a concentration on 10-20 IU/ML of blood (I.U=0.01MG).It doesnot alter the size of rbc.

2.It should not be used for making blood films as it gives a faint blue coloration to the background when the films are strained

3.It is the best anticoagulant to use for osmotic fragility

4.It should not be used for WBC count as it tends to cause the webs to clump

5.It acts by facilitating action of antithrombin IIItherby inhibiting the active form of clotting factors Ix,x,xI,andxII.

(D)OXALATES

1.Acombination of 0.8 pottasium oxalate and 1.2 gram ammonium oxalate dissolve in 100ml distilled water

2. It is used at the rate of the 0.1 ml of blood .

3. Oxalate combines with calcium to form insoluble calcium oxalate.

4.Pottasium oxalate cause shrinkage while ammonium oxalates swelling of erythrocytes.

PREPARATION OF SERUM

Blood is collected without any anticoagulant and allowed to clot in slant position for 10 minutes till blood is solidified .Incubate the tube at 37degree centigrade for 1 hour and then leave overnight in refrigerator and drain out clear serum in centrifuge tube .centrifuge at 1500 rpm for 5 minutes .and collect serum

PRESERVATION OF SERUM: for saturation for long time and preservatives, sterilization by filtration or freeze under vacuum (lyophilisation)

DEFIBRINATED BLOOD:

Transfer 10 ml freshely collected blood in to a clean dry conical (50 ml capacity)withoutanticoagualnt .place a thin glass rod or bamboo stick and gently rotate the blood for about 10 min .In this way all the fibrinogen becomes converted

in to fibrin in a short time and is quickely removed from the blood sample and remaining blood is known as defibrintedblood.such blood may be required for

1. Quick seperation of serum

2. Isolation of leukocytes

3. Collection of erythrocytes for osmotic fragility test .

QUESTIONS;

1). How serum does differ from plasma?

2.Why container is kept in slanting position for harvesting serum ?

3.What is the role of heparin in the body?

4.What will happen if water comes in contact with blood?

EXERCISE NO. – 6

ESTIMATION OF HEMOGLOBIN

Hemoglobin: Hemoglobin (Hb) is the main component of the erythrocyte which is a conjugated protein and serves to transport of CO2 and O2 in the body.

Hb is estimated by following methods:

1. Sahil's Hemoglobinometer
2. Spencer Hemoglobinometer
3. CyanmetHemoglobin Method

1. Shali's hemoglobinometer method

Principle

A measured volume of blood is converted to acid hematin with dilute hydrochloric acid and the hematin solution is diluted drop-wise until it matches with yellow- glass standards of the hemoglobinometer and the reading is noted.

Materials required

Sahli's hemoglobinometer, hemoglobin pipette and stirrer.

Reagents required

0.1N HCI, distilled water and blood sample.

Procedure

Take 4-5 drops of 0.1N HCL in the calibrated tube of sahli's hemoglobinometer and then add exactly 20µl (0.02ml) of blood sample to the hemoglobinometer tube with the help of hemoglobin pipette and mix properly with the help of stirrer. Allow the tube to stand for 10 minutes for formation of acid hematin. Then dilute the hematin solution with

distilled water till the color matches with glass standards and read the lower meniscus while taking the readings ,this will give Hb concentration as gm per 100 ml of blood or gm% or gm per dl of blood

Advantages of Sahil's method

- Low cost
- Being portable can be taken in field condition.
- Give immediate results.

Precautions

1. Take exactly 0.02 ml (20µl) of blood and wipe excess of blood adhering to outside of pipette,
2. Concentration of HCI should be exactly 0.1N.
3. There should be proper mixing of the acid and blood for proper formation of acid hematin.
4. Properly cell mixed blood should be taken.
5. There should not be any air bubble in the pipette.
6. Read lower meniscus while taking the reading in the hemoglobinometer tube.

Hemoglobin estimation in case of birds

10 ml of 0.4% ammonium hydroxide solution is mixed with 20µl of blood sample. And then add 0.36% ml of concentrated HCI and mix. The precipitates of nucleic acids will settle at the bottom or float at the top and precipitates do not interfere with the color development.

Then density of the color is calorimetrically determined at 410 mµ wavelength and values are compared with standard curve as in case of cyanmet Hb method

EXERCISE NUMBER- 7

TOTAL LEUCOCYTE COUNT (TLC)

Principle

Blood is diluted in a special pipette with WBC diluting fluid which will hemolyzed the erythrocytes and not leucocytes. These diluted cells are placed on hemocytometer and then counted under low power of microscope.

Materials required

Neubauer hemocytometer, microscope, tissue paper, WBC counting Thomas pipette which is marked as 0.5,1.0 and 11 and has a white bead in its bulb. Fill the blood up to 0.5 marks and diluting fluid up-to 11 marks, making the dilution of 1:20.

Reagents required

1. WBC diluting fluid, blood sample

   Different WBC diluting fluids are

(i) Turks WBC diluting fluid

Glacial acetic acid	2ml
Gention violet (1% aq.solution)	1ml
Methyl violet	1 drop
Distilled water to make	100ml

(ii) Rees and Eaker solution

Sodium citrate	3.8 gm
Formalin	0.2ml
Brilliant cresyl blue	0.5gm
Distilled water to make	100 ml

This fluid is good for WBC counting in birds.

WBC diluting fluid should have the following properties:

(a) Should be hypotonic to blood i.e it should cause lysis of RBC and WBC loose their cytoplasm and their neuclei become prominent.
(b) Should have fixative to fix WBC.
(c) Should have stain to stain WBC.

Best results are obtained when these solutions are used within three months of their preparation.

Procedure

Fill the blood up to 0.5 mark in WBC diluting pipette and wipe off the blood adhering to the outside of the pipette and draw the WBC diluting fluid to the 11 mark and mix the diluting fluid with blood by rotating the pipette for two minutes. Then discard the first few from the stem of the pipette and then put the cover slip on the hemocytometer. And then load the chamber of hemocytometer by touching the tip of pipette at the junction of edge of hemocytometer and cover slip till the hemocytometer chamber is filled with the diluted blood without any air bubble. Then allow the cells to settle down in the hemocytometer chamber for five minutes. Then count the leucocytes under low power (10x) of the microscope in the four corner large squares. Count the cells which are on left and lower margin of the square and discard the cells which are on right upper margin

Then calculate the total leucocytes as follows.

Let number of leucocytes in four large squares =	x
Volume of one large square = 1x1x0.1=	0.1cmm
Volume of four large square = 4x0.1 =	0.4 cmm
Thus 0.4 cmm of diluted blood contains cells =	X
1 cmm of diluted blood contain cells =	Xx10/4

Dilution factor 1:20

So, 1 cmm of undiluted blood contain = Xx 10/4x20=X x50 = leucocytes per cmm

Precautions

- Pipette and hemocytometer should be clean and dry.
- Exact measuring of 20µl (up to mark 0.5) blood in the pipette and proper dilution of sample should be there.
- Wipe off the excess blood from the tip of the pipette.
- There should be proper mixing of blood and diluting fluid.
- Proper filling of the counting chamber without any air bubble should be done.
- Discard 2-3 drops of solution from stem of the pipette while charging the hemocytometer.

EXERCISE NUMBER -8

DETERMINATION OF TOTAL ERYTHROCYTE COUNT (RED BLOOD CELL)

Principle:

Since the RBC count runs in millions, the count is made possible by diluting the sample of the blood before counting and subsequently multiplying the count by the dilution factor.

Apparatus :

1. Improved Neubeur's counting chamber

2. RBC pipette

3. RBC diluting fluid (Haem's fluid)

4. Compound microscope, coverslips

RBC Diluting Fluid (Haem' s fluid)

Composition: Each 100 ml of fluid contain:

1. Sodium sulphate (Na_2So_4) :2.5 gm (to prevent aggregation of RBCs i.e Rolex formation)
2. Sodium chloride (NaCl) :0.5 gm (to maintain isotonicity of the fluid ,RBCs remain suspended in the solution).
3. Mercuric Chloride ($HgCl_2$) :0.25 gm (preservative : antibacterial and antifungal)
4. Distilled Water :100 ml (solvent)

Qualities of an ideal diluting fluid:

1.It should be isotonic.

2.It should prevent agglutination.

3. It should not cause hemolysis

4. It should not contain fixative to preserve the red cell.

5. It should be cheap and easily available.

Haemoctometer :

A haemocytometer is an apparatus used for counting of blood cells. It consists of two pipettes (RBC and WBC) and a ruled slide called the **Improved Neubauer's Counting Chamber**

A. **Pipette**

The pipette is used to dilute the blood with a suitable fluid in an accurately known proportion Since RBCs are more numerous than WBCs, different dilutions have to be made for the RBC and WBC count .Hence two pipettes are provided.

1. **RBC pipette:**

   (i)This consists of a capillary tube ,the stem that widens into a bulb containing a small red glass bead .

   (ii) The pipette is calibrated in to 0.5 and I mark on the stem and 101 above the bulb. These markings refer to relative rather than absolute volumes.

   (iii) The pipette is supplied with a rubber tubeand mouth piece to facilitate filling by suction .This rubber tubing is sufficiently long to enable the observer to read the pipette calibrations comfortably during the process of filling.

2. **WBCpipette:**

   It is similar to the RBC pipette but has a similar bulb with a white glass bead .the graduation are 0.5 and 1 on the stem and 11 above the bulb.

B. **Improved Neubaur's Counting Chamber:**

1. It is used to obtain a very thin film of fluid of known volume for cellular counts .It consists of a thick glass slide with a polished central platform divided by a short transverse gutter in to two portions: each portion of which is ruled with a counting grid.

2. on either side, the central platform is bounded by a groove called as **a Moat**. Each moat,inturn,is bounded on its outer side by another platform ,which is slightly higher than the central platform.

3. A perfectly ground coverslip rests upon the two lateral platforms ,thus the bridging the moats and covering the central platforms .The chamber is so constructed that there is a space of 0.1 mm between the ruled platform and the cover slip .

4. The counting grid is made up of a ruled area measuring 3mm x 3mm .This area is divided by triple lines into 9 large equal shares each having an area of 1sq.mm.(1mmx1mm).of these the four large squares at the

corners are used for the WBC counting while the larger square in the center is used for RBC counting.

5. Each of four large squares at the corners of the ruled area are used for WBC counting and are called **WBC squares.**each squares is further divided in to 16 smaller squares each having an area of 1/16 sq.mm(1/4mmx1/4mm).

6. The central large square is for RBC counting and is called **RBC square .It has** an area of 1 sq.mm. (1mmx1mm).It is further divided by means of triple lines(width 0.01 mm)into 25 medium sized squares ,each with an area of 1/25 sq.mm(1/5 mm x 1/5 mm).Each medium sized square is further divided in to 16 small squares , each with an area of 1/400 square mm (1/20 mmx1/20mm).

Important note: With a x10 eyepiece lens and 10 objective, one large square (area 1 sq .mm) usually occupies the microscope field.

7. For the total RBC count, the central squares containing 400 smallest squares are used .Each smallest square as an area of 1/400 sq.mm. If a coverslip is placed the depth of the chamber is 1/10 mm making the total volume over each of smallest square as 1/4000cu.mm

8. For total leucocyte count (TLC)four corner squares are used .Each of these corner square has a slide of 1mm and depth of 1/10 mm, so that the volume of fluid in one square is 1/10 cu.mm

Procedure:

1. Ensure that the improved Neubauer's chamber, cover slips and the lenses of the microscope are absolutely clean and the RBC pipette is dry and patent.

2. Take adequate RBC diluting fluid in a watch glass.

3. Prick the figure or the tip of ear taking necessary precautions or suck the blood from the vial

4. Wipe off the first drop of the blood .suck the next drop of the blood in RBC pipette exactly up to 0.5 mark,taking care that there should be no air bubbles (any excess of blood drawn in can be removed by tapping the tip of the pipette against the palm of your hand)

5. Wipe of the blood sticking to the tip of the pipette.

6. Immediately, thereafter, holding the pipette in a nearly horizontal position, Hayem's fluid sucked in to the pipette exactly up to 101 mark ,just above the bulb .

7. The pipette is kept horizontally between the palms and rolled gently for about a minute to ensure thorough mixing of the blood and the fluid. This will give a suspension of RBCs in the bulb of pipette (dilution: 1/200)

8. A clean cover slip is placed in the Neubauer's chamber so as to cover the ruled area on both slides.

9. Focus the RBC squares of Neubauer's ruling under the low power objective (x10) of the microscope .The chamber is then removed from the microscope and placed on the table .The focus of the microscope is not disturbed.

10. Discard the first few drops of the fluid from the RBC pipette. They constitute the unmixed Haem's fluid present in the stem of the pipette.

11. Charging the Neubauer's chamber

(i)A small drop of the fluid is allowed to form at the tip of the pipette and this drop is gently brought in contact with the edge of the cover slip. The fluid is drawn in to the chamber by capillary action .Both the sided of the Neubaur's chamber are changed.

(ii)An ideally charged chamber is one which has been charged with a singly adequate sized drop which just fills the chamber without leaving any air bubbles.

(iii) if the fluid overflows in to the gutters, it is called Overcharging. If it is insufficient to fill the chamber or having air bubbles, it is said to be undercharged.

12. Allow some time (about 1 minute) for cells to settle down in the counting chamber so that all the cells present wil be in the same plane.

13. The charged Neubauer's chamber is now kept under the low pressure of the microscope (already in focus).Using only the fine adjustments, the cell along the ruled area brought in to the focus.

14. Check whether the cells are uniformly distributed or not, if not clean the Neubauer's chamber and recharge it.

(Note: if the difference in the total count of RBCs in different medium sized squares is more than 20 .It indicates an uneven distribution of the cells.)

15.focus the RBCs squares (central squares of Neubauer's chamber)under high power objective (x40)and count the RBCs in 4 medium size corner squares and one medium small squares (1/20 mm side)

16. Rules for counting cells in order to avoid counting the same cell twice, following rules needs to be observed.

(i)any cell which is lying on the upper or left border of the square is counted in that particular square ;and

(ii)omit the cells lying on the lower or right border of a square .Vice-versa ,if any one may desire :the cells lying on the right or upper border of a square can be counted in that particular square ,an omit the cells lying on the left or lower border of the square .

(iii) Count the RBCs in four medium size corner squares and one medium central square.

Enter your observations in the corresponding squares.

Calculations:

a. calculation of diluting factor:

0.5 part of blood and 100.5 parts of RBC diluting fluid are present in the pipette .As blood gets mixed up with the diluting fluid only in the bulb of the pipette :therefore ,1part of diluting fluid which remains in the stem of the pipette ,does not mix with the blood .thus 0.5 part of the blood mixes with 99.5 parts of the diluting fluid in the bulb of the pipette to form 100 (0.5 +99.5) parts of the solution .Hence ,dilution factor is :

Final volume achieved (100parts)

----------=200

Original volume taken (0.5 parts)

b. **Calculation of volume of fluid examined.**

Area of five medium sized RBC squares = 5x1/5 mm x1/5mm = 1/5 sq.mm

Depth of the chamber =1/10 mm.

Hence, volume of fluid in 5 RBC squares = 1/5 sq.mm x 1/10 mm= 1/50 cumm

c. Calculation of the total RBC Count

Let 'N' be the total no. of RBC counted in 5 medium size squares i.e

1/50 cumm of the diluted blood.

Therefore, no. of RBCs in 1 cumm of undiluted blood = N x 50 xdiluted factor (200) = N x 10000

Important note: Inspite of the utmost precautions the error is count is ± 20%

Because of the following factors:

1. Pipette error – the marks on the pipette may not be accurate.
2. Chamber error – the depth of the chamber may not be accurate.
3. Statistical error- as the multiplication factor is 10000 therefore; error in counting of even one RBC will cause variation of total RBC count by 10,000/ cumm of blood. This can be reduced by counting more no. of cells.
4. Field error – after charging the cells may not distribute uniformly throughout the chamber. The error in counting RBC can be reduced to 5% if doubled the no.s of cell are counted.
5. Normal values of domestic animals (millions/cumm or RBC $x10^6$µl)

Horse	6.5-13	Dog	6.4-8
Cattle	5.4-9	Cat	6.2-10
Sheep	8.5-13.9	Buffalo	5.4-7.4
Goat	8-17	Swine	2.8-4.5
Camel	7-13		

Precautions:

1. Both haemocytometer and coverslip must be dry and free from grrese
2. Use only dry pipette
3. .never use a broken coverslip
4. Before charging the chamber ,the fluid in the stem of the pipette has to be discarded
5. The coverslip should be placed symmetrically so as to cover the ruled area completely
6. There should be no under or over charging of the chamber (count will be low in the bith cases

Discussion:

High red blood cell count

a) Physiological- lowered oxygen tension, new born animals, stress, dehydration

b) pathological-acidiosis, vomiting,bacterialpoisions, diarrhea ,bronchitis

2. Low red blood cell (Oligocythemia)

a)Physiological-temporarly following injections of fluids

b) Pathological- anaemia, leukemia, haemorrhage

Questions:

1 .why there is need to dilute the blood before counting RBCs

1. **Why any small excess of blood is drawn in to pipette should be removed by piece of cotton**
2. **How is RBC count affected when the chamber is under or over charged**
3. **Why you should charge both sides of chambers**
4. **Why is it important to observe the rule of counting**
5. **What are the functions of bead in the pipette**
6. **What is the unit of Total Erythrocyte Count**
7. **List uses of RBC pipette other than counting the RBCs**

Observations:

Results:

Interpretations:

EXERCISE NUMBER-9

DETERMINATION OF DEFFERENTIAL LEUKOCYTIC COUNT (DLC)

APPARATUS:

4-5 glass slide, compound microscope, cedar wood oil and leshmain stain.

it belongs to romanowsky group of stains containing an acidic and a basic dye .The remarkable property of this group of stain is:

1)of making clear distinction in shade of staining and

2)of staining granules differently

1.EOSIN- It is in acidic dye (negetivelycharged)andstainespostively charged (basic)particals like RBC S granules of eosinophils

2.METHYELENE BLUE-Itis a basic dye (positively charged)and stains negetively charged (acidic)particals like cytoplasm,nuclei of WBCs and granules of basophils

3.ACETONE FREE METHYL ALCOHOL-It is a fixative .it preserves the cells in whatever chemicals and metabolic state they are at the time of staining .The blood smears also get fixed to the slide so that it cannot be washed off.

NOTE:the methyl alcohol should be acetone free since acetone causes shrinkages of the cells and may even produce crenation and lysis of the cells .It should be completely water free since water may affect apppearence of the film by causing rouleauxformation)

PROCEDURE:

A.PREPARATION OF BLOOD SMEAR

1.Take four or five clean grease -free glass slides with smooth edges ,3-4 to be covered with the blood film and one to be used as a spreader .

2.Lay 3-4 glass slides on the table ,prick the fingure,touch the bleeding point lightly in the center line about 1-2 cm from one end of the slides to obtain a small drops of blood on each.

3.place the narrow edge of the spreaders on the surfaces of slide just in the front of the drop of blood at an angle of 45degree centigrate.draw the spreader backward so as to touch the drop and hold there till the blood run along the full width of the of the spreader at the line of the junction .

4. The spreader is then moved slowly and smoothly to the other end of the slide maintaining the angle of 45 degree centigrade .

NOTE :A properly made blood film wil be fairly uniform without uneven streaks of spot/ vacuoles in it and neither too thick nor too thin)5.allow the film to draw in air .repeat the procedure with other slides

(Notes :ROULEAUX formation is usually seen in varying degrees in wet preparations of whole blood)

criteria for a good blood smear-

1.It should cover almost the entire width about 3-4 cm length of the slide.

2.it is tongue shaped with no tails at the ends

3. it is of uniform thickness that is neither too thin nor too thick .(a thick smear ,when placed on a white background appears red.

4.there are no longitudinal /cross stritations or air gaps in the smear .

B.FIXING AND STAINING OF THE BLOOD SMEAR

1.FIXING THE BLOOD SMEAR

I)place the blood smears across the two of the parellel supports on the glass rods ,so that the slides are horizantal

ii)pour 8-12 drops of leishman s stain on each side ,just enough to cover the smear ,note the time .leave it for about two minutes.

(IMPORTANT NOTE:Note the number of leishmans strain drops poured In hot weather , the strain should be watched to see that it doesnot become thick from the evoparation of the alcohol.if this occurs,the stain will precipitate on the film .This can be avoided by adding more stain to the one already on the slide)

2)STAINING THE SMEAR :

i)At the end of the 2minutes ,add an equal number of drops of buffered water (ph-6.8)over the stain,taking care that the water is not spilled over

(NOTE:Double the number of drops of the distilled water may be used in case of non availability of buffered water .)

ii)Mix the stain and water evenly by gently blowing air intermittently with the help of dropper .

iii)If the dilution of the stain is correct ,the fluid will be covered by a thin greenish scum,leave the stain to act for 10 min (staining time)

(IMPORTANT NOTE:depending on the strength of the leishman stain the fixation time and the staining time or variable)

3.At the end of 10 minutes ,pour off the strain ,hold the strand in the slanting position below the tap and the water is allowed to flow over the smear .

4.Wash the slide (up to 2 minutes)with tap water gently and thorughly till the film gets a pinkish tinge.make sure that :

i) None of the greenish scum settles on the surface of the blood film and

ii) Water stream doesnot strike the blood smear directly or else it may be washed off .

5.Wipe clean the back of the slide and set it upright to dry

C.ASSESSMENT OF THE QUALITY OF THE STAINED SMEAR:

Check the staining and thickness of the blood smear by examining it first under the low power objective and then high power objective.Awell stained blood smear has the following characteristicCs:

1.The smear is single cell thick ,with not much overlapping of the cells and the cells are uniformly distributed .

2.Atleast one WBC is seen per high power field (x100)

3.The RBCS are stainined light pink

4.In an overstained smear ,RBCs look bluish black and WBCS will take up more methylene blue ,hence look totally purple

5.In an understainedsmear ,RBCs appear very pale and WBCs look almost colourless.

D.EXAMINATION OF THE STAINED SMEAR UNDER OIL IMMERSION

1.Place a drop of cedar wood oil on the blood film .swing the oil-immersion objective into the position .carefully lower the objective untill it just touches the drop oil.Now raise the objective and focus the cells using fine adjustment

2. Study the distribution and characteristics of different types of the cells in different parts of the smear.

i).The most abundunt cell type found anywhere in the smear is of course the RBCs.These are seen as light pink, non-nucleated discs of uniform shape and size.

ii)At the head end of the smear the RBCs are crowded and superimposed ,and the WBCs are poorly stained .

iii) At the extreme tail end ,the cells are wide apart and the WBCs are distorted.

iv) At the upper and lower edges of the smear the WBCs are found in plenty but are poorly stained and abnormally rich in granulocytes.

E.IDENTIFICATION OF DIFFERENT TYPES OF LEUKOCYTES:

Identification and counting of different types of leucocytes is done under oil-immersion objective.The WBCs can be differentiated from RBCs by the presence of the nucleus and large size.

while identifying the WBCs keep 4 factors in mind:

1.size of the cells(compare with the surrounding RBCs which are of uniform size ,7.2)

2. Features of the nucleus(colour ,number of the lobes)

3. Features of the cytoplasmic granules (pink or blue,fine or course)

4. Nuclear/cytoplasmic ratio

F.COUNTING OF THE DIFFERENT TYPES OF THE LEUCOCYTES:

A minimum of 100 WBCs are identified in asystemic matter and counting is made using the tally-bar methos.ideally all the cells should be counted in a single strip running the length of the smear ,proceeding from base to apex ,use the mechanical stage to traverse the full length of the film .move the slide along vertically by a distance equivalent to 2mm and again traverse the full length of the film ,this time in the opposite direction .This method ensures that cells are counted and no more than once.

leucocytes	DOG	CAT	COW	CAMEL	HORSE	SHEEP	GOAT	PIG	CHICK
neutrophils	65-70	55-60	25-30	65-75	50-60	25-30	35-40	30-35	
lymphocytes	20-25								
eosinophils	2-5								
basophils	<1								
monocytes	5								

CHARACTERISTICS FEATURES OF THE DIFFERENT TYPES OF LEUKOCYTES

CELL TYPE	CELL SIZE(diameter)	NUCLEUS	CYTOPLASAM	GRANULES
Neutrophils (60-70%)	10-14 (1.5 to 2times the size of surrounding RBCs)	Multilobed (1-5 lobes),purple in colour	Slight bluish	Very fine like particals ,numerous red brown or purplish in colour
Eosinophils (1-4%)	- do-	Usually bilobed (spectacle shaped) purple in colour	Eosinophilic (acidic),light,pink granular	Large,coarsebrick red in colour donot cover the nucleus
Basophils (<1%)	-do-	Usually bilobed purple in colour	Basophilic(basic) Appears ,blue,granular.	Large,coarse,purple Or blue,overlying the nucleus

Lymphocytes (20-40%)	Small:7-10 (Size of an RBC)Large: 10-14(1.5-2 times the surrounding RBCs) Irregular outline	Single,verybig Occupying whole of the Cell,purple in colour,oval or rounded,central in position	Scanty,skybluecolour,amount less than amount of nucleus	Absent
Monocytes (2-8%)	10-18 (2 to 2.5 times the size ofaRBC)irregular outline	Single,usually intended kidney shape,pale in colour,may be oval or circular ,peripheral in position	Abundant (amount more than thenucleus amount),paleblue in colour.	Absent

PRECAUTIONS:

1.The slides should be clean and grease free.

2.Stored blood should not be used for for making smear.

3.Dont not heat dry or blot dry the smear .

4.The smear should stained not later than two hours after it has been prepared.

QUESTIONS:

1)Why is it necesseary to dry the film before putting the stain on it?

2)Why a blood film must not be too thin?

3)What are the functions of various constituents of leishmans stain.

4) Do you notice any other cells besides WBCs and RBCs in the smears. How do you distinguish then.

5)Why the cells donot get stained in the first two minutes of staining procedure.

6)Why should the leishmans stain be acetone free.

7)Is there any other way to fix the blood smear ,ifleishmans stain is not available .

8)What is the buffer water,what is its function

9)Can tap water ever be used for dilution purpose ,ifnot,why?

10)Which part of the smear should be avoided for counting the cells and why?

11) Is it necessary to study the whole length of the smear for cell counting purpouses and why?

12) What all informations one can get gather from a pheripheralsmear .

13) How can we estimate approximate size of the WBCs under the microscope.

OBSERVATIONS:

RESULTS:

INTERPRETATION:

SIGNATURE.....

EXCERCISE NUMBER -10

DETERMINATION OF BLOOD GROUPS

APPARATUS:

Antiserum-a,antiserum-b,antiserum-rh,isotonic saline (0.9%NACL),glass slides ,applicator sticks,compoundmicroscope,glass marking pencil,and capillary dropper.

PRINCIPLE:

More tham 30 blood group specific antigen(agglutinogens)can be recognized on the membrane of human RBCs .These antigens enable the blood groups of different individuals to be differentiated

The cheif blood groups are:

1.Classical ABO blood groups

2.Rhesus (RH)blood group,and

3. Mand N blood group

1.The ABO blood group system was first discovered by Landsteiner in 1900 .it is based on the presence or absence of group specific substances A,B and O in the RBCs .Accordingly human beings can be divided in to 4 main groups Viz,A,B,ABandOblood groups

2.The Rh blood group system was discovered by landsteiner and weiner in 1940.RBCs of Rhesus monkeys when injected in to the rabbits,the rabbits responded to the presence of an antigns in these cells by forming an antibody which agglutinated Rhesus RBCs.If the immunized rabbits serum is tested against human RBCs ,agglutination occur in 85%in men ,these people are called RH+(positive) and their serum contains no rh antibody.No agglutination occur in 15%these are called rh-(negative)and their serum also contain no RH antibody.

3.The procedure is based on the principle of agglutination i.e.clumping of RBCs .The phenomenon of agglutination is due to the interaction between the factors - agglutinogen (antigen)present on the RBCs membrane and agglutinin(antibody)present in the plasma.

4.Normal human RBCs possessing a particular antigen will show the agglutination in the presence of the corresponding antibody.This may occur quite soon but may not develop for several minutes .if the agglutnation titre of the serum is low.

PROCEDURE:

A.Preparation of red cell suspension:

Take 3ml of isotonic saline(0.9%NACL)in a clean test tube.Fingure or tip of the ear is pricked under aseptic precautions so as to assure free flow of blood ,the first drop of the blood is ,wiped away with clean piece of clothand a drop of blood is added to test tube.Alternately ,red cell suspension may be prepared simple by inverting the test tube over the fingure pricked.

B.Determination of blood groups:

1.Take an ordinary grease free,clean glass slide and one drop each of antiserum A(i.e.serum containing antibodies),anti-serum b(i.e serum containing antibodies)and anti-serum Rh (i.e.serum containing antibodies ,anti-D)is placed there on with the help of a dropper .In addition one drop of isotonic saline (used as control)is also place on the slide.The slide is accordingly labeled as anti-A,anti-B,anti-Dand control.

2.The red cell suspension is drawn from the bottom of the test tube into the capillary dropper and a dropof itis added to each of the drops in the slide .The two are mixed with the help of seperate application sticks.

3.wait for 10 minutes,the slide is then gently rocked back and forth and examined for the presence of agglutination (clumping of RBCs).confirm the findings under the low power of the microscope .

i)If there is no agglutination ,the RBCs remain seperated and evenly distributed ;and

ii)If agglutination occur ,the RBCs are messed together in clumps and lose their outline.

3. The blood group is determined as indicated in the table below

+':Agglutinationi.e RBCs are messed together in clumps and lose their outline .

_':No Agglutination i.e.RBCs remain seperate and evenly distributed

IMPORTANT NOTE:

i)False positive reaction:can occur due to use of antisera contaminated with bacterial growth or infection of RBCs suspension by bacteria.

ii)False negative reaction :Due to loss of potency of this antisera because of improper storage**.**

PRECAUTIONS:

1.The slides should be throughly cleaned with soap and water

2.The slide should be labeledbefore hand after placing the antisera A,B and Rh and isotonic saline .

3.There should not be any intermixing of the four drops placed on the slide .

4.The slide should be examined for agglutination before the solution dries up.confirm the findings under the low power of the microscope.

QUESTIONS:

1.What is the use of control.

2.Why we should wait for 10 minutes before looking for agglutination

3.Why the prepration should be examined before its starts getting dried up.

4.What are the use of blood grouping tests.

5.What basic rules need to be observed before blood transfusion.

OBSERVATIONS:

RESULTS:

Interpretations:

Signature.....

EXERCISE NUMBER -11

DETERMINATION OF THE RBC INDICES (BLOOD STANDEREDS)

APPARATUS:

As for the estimation of haemoglobin,RBC count and packed cell volume -PCV

PRINCIPLE:

The values for RBC count, haemoglobin content and packed cell volume (PCV)can be used to obtain certain RBC indices (also called absolute value of blood indices).These indices indicats the size and the haemoglobin concentration within the RBC and thus help in diagnoising the type of anaemia.The various blood indices are given as under:

a) **MEAN CORPUSCULAR VOLUME (MCV)-**Volume of a single RBC in cubic microns ( ).It can be computed as :

$$\text{MCV ( )} = \frac{\text{PCV x 10}}{\text{RBC count in million/cmm}}$$

$$= 45/5 \times 10$$

$$= 90 \text{(average)}$$

1.RBCs with normal MCV called Normocytes

2.RBCs whose MCV exceed normal range are macrocytes .

3.RBCs with MCV below normal range are microcytes .

b.MEAN CORPUSCLES HAEMOGLOBIN (MCH)-Average amount of haemoglobin in a single RBC in picogram (10^{-12})

It can be computed as :

$$\text{MCH (PG)} = \frac{\text{Haemoglobin in gms\% x 10}}{\text{RBC count in million /cumm}}$$

=15/5x10

=30(average)

C.MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC):

Amount of haemoglobin expressed as percentage of the volume of the RBC or it is the haemoglobin cocentration in a single RBC

MCHC=Haemoglobin in gm% X100

PCVin percent

==15/5x100

=33(average)

1.If the MCHC is within the normal range ,the RBC is normochromatic.

2.If the MCHC value is below normal range ,therbc is Hypocromatic This usually indicates that an individual is suffering from iron defeciency.

3.MCHC value never exceed more because RBCs cannot hold haemoglobin beyond its saturation point i.e. metabolic limit of the cells haemoglobin forming mechanism ,therefore,anaemic can never be hyperchromic.

d.COLOUR INDEX (CI)-It denotes the ratio of haemoglobin to RBC

CI= Haemoglobin percentage*

RBC percentage**

=100/100

=1(average)

Normal range =0.85-1.15(1.015)

*14.8gm%haemoglobin=100%

**5 million /cumm RBC count =100%

NORMAL VALUES:

ANIMALS	MCV(f1)	MCH (pg)	MCHC(g/dl)
Cattle	40-60	15-20	32-39
Sheep	25-50	10-14	27-36
Goat	15-25	8-10	32-34
Pig	50-70	16-20	28-35
Horse	*35-60*	13-19	33-35
Dog	60-78	20-24	30-35
Cat	40-55	13-17	32-34
Camel	25-50	13-18	40-45

OBSERVATIONS:

RESULTS:

INTERPRETATIONS:

SIGNATURE.....

EXERCISE NUMBER -12

DETERMINATION OF PLATELET COUNT

PRINCIPLE:

The number of platlets in a sample of diluted blood is counted in a haemocytometer of known dimension.From the number of cells seen the total platelet count ofthe undiluted sample is calculated.

APPARATUS:

Improved Neaubaure' counting chamber ,RBC,pipette platlets,diluting fluid (1%ammonium oxalate)

Compound microscope ,coverslip,pricking apparatus

PLATLETS DILUTING FLUID:

1%ammonium oxalates its acsas :

1). Anticoagulant

ii) Preserves the platelets

iii) Destroys the RBCs

PROCEDURE:

1.Draw the blood upto 0.5 %mark in the RBC pipette and then suck the platlet diluting fluid upto 101 mark (dilution factor is 1:200)

2. Mix the solution and blood ,wait for 10-15 minutes

This is important for complete haemolysisof RBCs, thus to get clear background at the time of counting the platlets.

3. Discard first few drops and charge the number .place the charged chamber in a moist petridish (wet filter paper at the bottom)wait for another 20-30 minutes to allow the platletsto settle down

4. Under high power objective (x40)of microscope ,count the platlets in all the 25 RBC squares i.e.central squares (1mmx1mm).Count the platelets on both sides of the chamber.

NOTE: The platelets can been seen as tiny (2-4)diameter well seperated highly refractile rounded bodies with silvery appearence .They can be recognised by carefully altering the focus of the microscope with fine adjustment and by reducing the illumination by closing iris diaphragm

4.Count the platlets in all the 25 RBCs central squares under high power

CALCULATIONS:

A.dilution factor :200

B.volume of fluid examined in 25 RBCs squares

=1mmx1mmx0.1mm

=0.1cumm

C.Total number of platlets per cumm of blood.

Let "N'be the total number of platlets in 1 cumm of undiluted blood

=

=nx10x200

=nx2000

NORMAL VALUES:

The normal platlets count in domestic animals ranges from 175000/ to 500000 with an average of 4,50,00/ 10^{-6}

Camel-

horse-

pig-

DISCUSSION:

Decreases in the count may occur in the following pathological conditions (thrombocytopenia)

1.Any disease condition of bone marrow

2.Severe haemorrhage

3.Uremia

4.Idiopathic

Increase in the counts may occur in the following pathological conditions:

1. Chronic haemolytic icterus

2. Haemolytic anaemia

3. Sickle cell anaemia

QUESTIONS:

1. Name the other diluting fluids which may be used for platelet count

2.Give the normal range of platlet count

3. Enumerate the functions of platlets

4.Give the live span and site of destruction of platlets

5. Define thrombocytosis and thrombocytopenia .Give their common causes

6.What do the platlets look like in a pheripheral blood smear,stained with leishman'sstain.Is it possible to make platlet count in stained manner?

OBSERVATIONS:

RESULT:

Interpretation:

Signature.....

EXERCISE NUMBER -13

DETERMINATION OF ERYTHROCYTE SEDIMENTATION RATE (ESR)

ESR: It is defined as the speed at which erythrocytes fall down in a vertical column of blood samples.

Principle

Blood cells being heavier than plasma fall down when they are allowed to stand in a vertical column.

Materials required

Wintrobehematocrit tube or Westergren ESR tube with stand, with long needle.

Procedure

It can be estimated by two methods:

1. Wintrobehematocrit tube method
2. Westergren's ESR tube method.

1. Wintrobehematocrit tube method

Wintrobehematocrit tube is 11 cm long tube having an uniform bore of 3 mm . it is calibrated by a double 10cm scale, on the left side calibration is from top to bottom and on right side calibration is from bottom to top. Fill the Wintrobehematocrit tube to zero mark at the top with the help of a syringe with long needle and keep this tube in Wintrobe stand in vertical position at a constant temperature and note the initial reading at zero and then take final reading after half an hour or one hour and record ESR in mm per hour.

2. Westergren's method

In this method there is a Westergren's tube which is 33 cm long and its diameter is 2.5 mm with calibrations from 0-200 mm from top to bottom. In this method the bottom of Westergren tube is dipped into the blood and the blood is drawn in the tube by sucking up to 0 mark and tube is placed in upright vertical position in a special rack having a soft rubber cushion at the bottom so that the tube is sealed when inserted into the rack. Fall in erythrocytes in the given period of time represent ESR in mm.

ESR:-Is affected by aggregation of RBCs to form rouleaux. More rouleaux formation causes increased ESR. Equines show more rouleaux formation so they have more ESR value. Young erythrocytes of reticulocytes do not form rouleaux so there in no ESR.

EXERCISE NUMBER -14

COLLECTION EXAMINATION OF URINE FOR DIAGNOSTIC OF SYSTEMATIC AFFECTION

Principle

Urine is formed after filtration of plasma followed by its selective re-absorption and secretion in the renal tubule. Physiological analysis of urine provides valuable information regarding overall health status of the body.

Detailed systematic analysis of a urine sample requires following types of tests:

(a) Physical Examination:

Volume, colour, appearance, specific gravity, pH and Titrable acidity.

(b) Chemical Examination:

For qualitative and quantitative detection of normal (urea, creatinine, uric acid,electrolytes etc) and abnormal (reducing sugar, proteins bile salts bile pigments blood or hemoglobin indicant bodies etc.)

(c) Microscopic Examination:

For detection of blood cells epithelial casts crystals micro-organisms etc.

Requirements

Urinometer, P^H indicator paper

Procedure (physical examination of urine):

1. **Collection of sample:**

Early morning sample is useful for most routine analysis; however a 24 hours sample become necessary for recording daily urinary volume output and for quantitative estimations. First few ml of sample is usually discarded as it may contain dust and contents from urethra, prepuce or genital tract. The container (glass vial, plastic bottle/ cylinder, or dark glass bottle) should be clean and dry. The urine sample must be analyzed immediately because the sample is quickly spoiled upon exposure o air and light.

(a) Direct collection: Urine can be collected from female animals by tapping the vula with fingers camel, Bullocks and other draft animals generally urinate when brought to rest after physical work in males, a canvass or rubber urine collecting may be strapped under the belly and urine is collected.

(b) Collection with urinary catheter: Appropriate sized (length & diameter) urinary catheter is inserted into the urinary bladder through urethra and clean, uncontaminated urine is collected. Catheterization is easy in females due to shorter, wider and simple urethral anatomy; Catheters should be properly sterilized and lubricated to avoid any injury or infection to the urinary tract.

2. **Urine volume:**

Volume of urine discharged per day varies with age, size species of animal and its water/dietary intake, body temperature, climate physical exercise and physiological state.

Increased volume (polyuria) is recorded after excess water intake, during winter months, low protein diet, pregnancy, diabetes and many other diseases.

Decreased volume (oligouria) can be recorded after low water intake, during summer season, after exerices, profuse sweating, high fever, high protein diet, dehydration and diseases.

3. **Colour:**

For recording colour, a sample of urine is taken in a transparent glass tube and colour is noted down. Normal colour of urine is pale yellow due to presence of urobilin pigment. The normal colour tends to be lighter after polyuria and becomes deepened/ intense due to oligouria. Urine colur can change due to different diets and drugs administration also. Abnormal colours could be as under:

Reddish	-	due to intact erythrocytes
Coffee	-	due to hemoglobin
Greenish yellow	-	due to jaundice (bile pigments)

4. Appearance (Transparency):

Normal fresh urine tends to remain clear and transparent in most species. However, equine urine appears thick and cloudy or opaque due to presence of calcium carbonate and mucus. Sample is kept undisturbed for turbidity to settle down and supernatant is used for analysis.

Turbidity can also appear after prolong standing (because of precipitation of calcium salts) and due to presence of cells mucus, epithelial casts, bacteria and crystals.

5. Specific gravity:

Pour the urine sample in the urinometer cylinder up to three fourth capacities. Dip the urinometer gently into urine and give a little rotation so that urinometer does not touch the walls of cylinder. Note down the reading on the stem of urinometer at the interface of urine and air. Record the urine specific gravity by placing the urinometer reading at 2^{nd} and 3 rd decimal position of 1000 (e.g. if urinometer reading is 18, then specific gravity is noted as 1018 and so on)

Correction of urinometer reading of temperature:

Note the temperature of urine in c and the calibration temperature of urinometer and find out the difference. For 3ºC deviation in temperature, add or subtract one unit in specific gravity at third decimal position. For example it urinometer is calibrated at 20ºC and observed specific of urine is 1.032 and urine temperature is 14ºC then corrected specific gravity will be: - 1.032-0.001 (20-14/3)=1.030

Normal specific gravity of urine ranges between 1.001 to 1.060. Urine specific gravity varies due to water intake, nature of diet, season of the year, physical exercise and physiological state of the body.

Under most physiological conditions specific gravity and volume of urine discharged bears a reciprocal relationship (exception diabetes mellitus). Urine specific gravity reflects concentrating and diluting ability of the kidneys.

6 Odour:

Freshly voided urine bears a typical ammonical smell due to decomposition of urea. Abnormal sweetish/fruitish odour is observed due to presence of ketone bodies in urine.

7 Urine pH:

Urine pH can be measured using pH indicator paper or digital pH meters. Dip a piece of pH paper strip in the urine sample and match the colour of strip with standard colours while strip is wet. Not the pH directly.

Urine pH is highly variable (4.5to 8.5) due to nature of diet, body metabolism and species variation.

Acid urine is characteristic of carnivores, young herbivores high protein diet, starvation and heavy exercise etc.

Alkaline urine is characteristic of herbivores, high vegetable diet, excess vomition, urine retention etc.

If urine sample is stored at room temperature for sometime, its pH drifts toward alkalinity due to liberation of NH_3 from urea degradation by bacteria.

2. CHEMICAL EXAMINATION OF URINE

BENEDICT'S TEST:-

Test used for glucose in urine

Procedure:-

Add 8 drops of urine in 5.0 ml of benedict's solution

⇩

Boil for 2 minutes

⇩

Results:---------

(a) No change in colour- Negative

(b) GREEN colour - [+] [0 - 0.5% sugar]

(c) YELLOW colour - [++] [0.5- 1% sugar]

(d) ORANGE colour - [+++] [1-2% sugar]

(e) RED colour - [++++] [2< % sugar]

RESULTS------------------------------

HAY'S TEST:-

Test use for bile salt in urine

Procedure:-

Take 5 ml urine in test tube and sprinkle sulphur powder on the urine surface

⇩

Powder sinks down to the test tube

⇩

Bile salt present in urine

Results----------

HELLER'S TEST (per form to detect albumin protein in urine)

Used for Estimation of protein in urine

Procedure:-

Take 2 ml concentrate nitric acid in a test tube

⇩

Add urine drop by drop to the test tube

⇩

Appearance of white ring at the junction indicates presence of protein

Results----------

ROTHERA'S TEST

Used for test for ACETONE/KETONE in urine

Procedure:-

Saturated 5ml of urine with crystalline ammonium sulphate in a test tube

⇩

Add 2-3 drops of 5% sodium nitropruside solution and then add strong ammonium hydroxide

⇩

Appearance of violet colour

(Positive test)

GMELIN'S TEST:-Test used for bile pigments in urine

Procedure:-

Add 5 ml Nitric acid (HNO_3) in 5 ml urine

⇩

Various colour ring at the junction of two liquids

(green, blue, red, violet)

⇩

Bile pigment present

Normal Hematological values of Domestic Animals

Parameter	Cow	Buffalo	Horse	Sheep	Goat	Pig	Chicken	Dog	rabbit
Bleeding time (min)	5.5	4.5	10	2-4	2-4	3-4	2-4	3-4	
Clotting time (min)	6.5	4-6	11.5	2-3	2-3	3-4	4-5	3.0	
ESR (mm)	2-4 /7h	30-40/h	15-40/20min	15/20 min	0-1/h	1-10/h	1-3/h	5-10/h	3-4/h
PCV (%)	35	32	34	32	34	42	30-35	45	41
Hb.conc (g/dl)	14	12	12	12	11	12	12	13	10
TEC (10^6/µl)	6-8	6-8	8-12	10-13	13-18	6-8	2.5-3.2	6-8	5-7
MCV (fl)	54		47	37	17.5	58	120	64	65
MCH (pg)	17		15	12	7.5	19	26	22	21
MCHC (%)	33		33	32	34	33	22	32	33
TLC (103 µl)	7-10	9-15	8-11	7-10	8-12	7-20	20-30	9-13	7-9
Neutrophil (%)	27	27	55	27	37	38	27	67	43
Lymphocyte (%)	63	52	35	63	53	50	55	23	42
Eosinophil (%)	2-5	15	2-5	2-5	2-5	6	5-6	2-5	2
Monocyte (%)	5-6	9	5-6	5-6	5-6	6	10-11	5-6	9
Basophil (%)	<1	<1	<1	<1	<1	<1	2-4	<1	4
Platelet no (10^3/µl)	350		176	441	2500	403	254	155	536

Appendix (ii)

Normal Physiological Values of Domestic Animals

Species	Heart Rate (per min)	Arterial Blood Pressure (mmHg)
Cow	40-80	140:95
Buffalo	40-60	
Horse	28-40	110:60
Sheep	70-80	120:70
Goat	70-80	
Pig	65-110	150:100
Dog	70-120	130:85
Chicken		175:145
Rabbit	200-250	110:80