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LABORATORY MANUAL ON FIELD DIAGNOSIS OF ANIMAL AND AVIAN DISEASES

First Edition

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Directorate of Research (Vety)
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LABORATORY MANUAL ON FIELD DIAGNOSIS OF ANIMAL AND AVIAN DISEASES

DEPARTMENT OF BIOTECHNOLOGY

Edited by

Dr N. N. Barman
Professor
Department of Microbiology
College of Veterinary Science
Assam Agricultural University
Khanapara, Guwahati
Assam, INDIA

FOREWORD

I have immense pleasure in writing the foreword note for the laboratory manual of disease diagnosis. The DBT Centre in NE on Advanced Animal Disease Diagnosis and Management Consortium (ADMaC) is a tripartite concept and has already completed four years of its establishment. The Project was established to strengthen Animal Disease Monitoring and Surveillance system in North Eastern region of the country. In regards to the animal disease scenario of the country, the North Eastern Region is very important because of its strategic geographical location. The North Eastern Region shares approximately 4500 km of porous international boundaries with Myanmar, Bangladesh, Bhutan, Nepal and China. Uncontrolled migration of animals from the neighbouring countries can be a great threat for incursion of emerging and transboundary diseases to this region. Rapid globalization and increasing trade has resulted free movement of humans and animals and seems to be another precipitating factor for increasing threat of infectious diseases crossing the borders. Coupled with global warming, massive deforestation resulting in increased vector population, the region becomes more vulnerable for occurrence of many exotic viral, bacterial and parasitic diseases. The region has already been experienced with the outbreak of virulent form of H5N1 in poultry, PRRS, circo virus infection in pigs, pox and PPR in goats. There are reports of occurrence of Nipah virus infection in our Country as well as in neighboring country. Therefore, we need to build up our capabilities and to strengthen ourselves in the direction of developing latest diagnostics and to undertake rigorous surveillance for the highly contagious and ravaging (selected) diseases. We must have a complete vigil on the disease situation of emerging and exotic infections and build a formidable defense to guard our territories and thereby saving the livestock wealth and livelihood of millions of human beings involved with it.

To achieve this target, one of the prime objectives of ADMaC is to strengthen the human resources of the region involved in Animal Disease Monitoring and Surveillance by training of State Veterinary personnel's on the use of advanced and molecular diagnostic techniques. Since at the base of the pyramid of animal disease monitoring and surveillance are the paravets of the region, therefore, it was felt that the paravets should be

trained first with the modern techniques of laboratory diagnosis of animal diseases. Accordingly the ADMaC organized several training programmes for the paravets and veterinary officers of all eight North Eastern states. During the course of different trainings, many trainees expressed the need of a comprehensive laboratory manual for use in the field labs of North East. I am happy to learn that the scientists and research workers of the Core Lab I of the Advanced Animal Disease Diagnosis and Management Consortium (ADMaC) have taken initiatives with active support from all other core labs and national labs in preparing a comprehensive manual entitled **"Laboratory manual on field diagnosis of animal and avian diseases"**. The manual contains much important information like laboratory safety rules, collection, preservation and dispatch of samples, preparation of laboratory items, laboratory reagents etc. and some routine diagnostic tests. The use of different illustrations/ colored photographs, flow chart has made the manual more attractive.

Since limited copies will be printed and distributed to few ADMaC attached field labs, I hope that the different State AH & Veterinary Departments may replicate some more copies or may download from the website www.neradslab.res.in with due acknowledgements to DBT and Core Lab I of the ADMaC project.

I congratulate the scientists and scientific staffs and hope that with their guidance Good Laboratory Practice (GLP) will be routinely practiced in all district level laboratories of all North Eastern Region and timely and accurate diagnosis can be given to the needy farmers.

(A.Chakraborty)

PREFACE

Herd health is a key and highly sensitive determinant of productivity within any livestock husbandry system. Deviations from the maintenance of optimal herd health can cause significant consequences for farmers and livestock as a whole. Emerging and endemic infectious diseases in animals and birds are occurring in our country and causing serious economic loss to this unique industry. Diagnosis of disease is pre-requisite to contain as well as control such outbreaks. However, trained manpower, laboratory facilities at field level are still in infancy in this North Eastern part of India. DBT-NERBPMC provided financial support for development of skilled manpower, creation of infrastructure facilities through a tripartite concept involving three NE Institutes, four National Labs and eight Directorates of Animal Husbandry and Veterinary in NER.

Diagnosis of diseases at field level is an important job as it can be a practical guidance for the farmers to take early action to contain the outbreaks. Authentic disease data generated at field level helps in depicting real-time disease map in the region. To train up field veterinarians and create diagnostic facilities in peripheral labs are challenging tasks in this region. With limited facilities our field veterinarians can make use of certain simple techniques for quickly diagnose of diseases. To overcome the difficulties commonly faced in field laboratories, this laboratory manual is compiled providing comprehensive knowledge and information on selection of suitable samples, appropriate collection procedure and proper dispatch method, diagnosis of microbial and parasitic agents, clinical pathology and histopathology from a single source. Standard disease diagnosis and reporting formats, contact details of diagnostic laboratories and companies are also included. Procedures are described briefly in simple language with the help of sketch, colour illustration so that it can be utilized by different range of laboratory workers including veterinary officers, students and technicians.

It is hoped that, this laboratory manual on diagnosis of animals and avian diseases would immensely benefit its users in the field of disease diagnosis.

N. N. Barman

ACKNOWLEDGEMENTS

On behalf of the Core lab-I of ADMaC project, Assam Agricultural University conveys our deep sense of gratitude to the Department of Biotechnology, NER-BPMC, Govt. of India for providing necessary funds under "**DBT-NER Centre for Advanced Animal Disease Diagnosis and Management Consortium**".

We also express our sincere gratitude to Dr K. M. Bujarbaruah, Vice-Chancellor, Assam Agricultural University who happens to be the architect of this project. His encouragement and support inspires the scientists and staff to achieve the desired goal.

Dr Madhan Mohan, advisor, DBT also duly acknowledged for involvement and interest of the project ADMaC since its incubation period to the present state.

Our special thanks to Prof Dr C Balachandran, Vice-Chancellor, and TANUVAS for contributing a valuable chapter in this manual.

Dr A. Chakraborty, Director of Research (Vety), Assam Agricultural University is the guiding force behind publication of this hand book and the help and co-operation received from him is duly acknowledged.

Dr R. N. Goswami, Dean, FVSc, Khanapara is also always there to encourage the activities of ADMaC project and his good gesture is also acknowledged.

The help and support extended by the Directors of co-operating ICAR institutes like ICAR-NIVEDI, ICAR-NRCE, ICAR-NIHSAD, ICAR-NRC pig is duly acknowledged. The contributions from PIs, Co-PIs of the project from the above ICAR Institutes including ICAR-VTCC is also duly acknowledged.

The Core Lab II of ICAR-NEH, Barapani and the Core Lab-III of CAU, Aizwal deserve similar acknowledgement for their active co-operation and help. We also offer our due acknowledgement to all the Directors of Animal Husbandry and Veterinary from all eight North Eastern States, their disease investigating officers and state level PIs and Co-PIs.

Special thanks are due to Dr Arun Verma, former ADG, ICAR, Sr. Consultant, Animal Biotechnology, NER-BPMC, DBT, Govt. of India for his keen interest in materializing the project proposal.

Finally, we express our thanks to Dr S. K. Das, Professor & Head, Department of Microbiology, the scientists, research scholars, project staffs of the Core Lab I and other members for their helps and co-operation in bringing out this practical hand book.

N.N. Barman

Editor

LIST OF CONTRIBUTORS

Prof Dr C. Balachandran, PhD
Vice-Chancellor, TANUVAS

Dr A.Chakraborty, PhD
Director (Vety), AAU, Khanapara

Dr. S.K.Das, PhD
Professor, CVSc, AAU, Khanapara

Dr. N.N.Barman, PhD
Professor, CVSc, AAU, Khanapara

Dr.(Ms) Rajeswari Shome, PhD
Principal Scientist, ICAR-NIVEDI, Bengaluru

Dr. Arnab Sen, PhD
Principal Scientist, ICAR-NEH, Barapani

Dr. Tapan Kumar Dutta
Professor, CVSc & AH, CAU, Selesih, Aizawl

Dr. R. K. Veid, PhD
Principal Scientist, ICAR-VTCC, Hisar

Dr. Sharmita Doley, MVSc
Jr.Scientist, ADMaC

Dr. Pallabi Pathak, PhD
JRF, ADMaC

Dr. Parikshit Kakati, MVSc
JRF, ADMaC

Dr. Arpita Bharali, MVSc
JRF, ADMaC

CONTENTS

1. LABORATORY SAFETY RULES

- **Treat all microorganisms as potential pathogens.**
- **Sterilize equipment and materials.**
- **Disinfect work areas before and after use with 10% bleach or 70% ethanol.**
- **Wash your hands with soap.**
- **Use gloves and mask as personal protective equipment for performing routine laboratory works.**
- **Never pipette by mouth and use pipette bulbs or pipetting devices.**
- **Do not eat or drink in the laboratories, nor store food in areas where microorganisms are stored.**
- **Label cultures, chemicals, and media clearly and securely with names and dates.**
- **Arrange chemicals according to their nature.**
- **Autoclave or disinfect all waste material before discarding.**
- **Incinerate or dump it in hollow pit.**
- **Clean up spills with care. Cover any spills or broken culture tubes use with10% bleach /70% ethanol.**

2. GUIDELINES FOR COLLECTION OF BIOLOGICAL SAMPLES

Accurate diagnosis is based on:

- The understanding of the host systems involved in the course of the disease.
- Localization/ site of predilection of infection in the body.
- Proper and timely collection of sample following strict aseptic measures.
- Correct packaging, proper storage and transportation of sample.

Common errors:

- Improper selection of site
- Poor accessibility of sites
- Contamination of samples by normal micro flora of animal body system

Sample collection is based on:

- Clinical signs observed
- Significant and clinical history
- Pathological changes
- Disease outbreak of a disease or
- Disease surveillance

2.1 List of items in field sample collection kit

Sl No.	Name of the Item	Quantity
1	Ice box with ice pack / vaccine carrier	1
2	Needle and syringe (2ml, 5 ml, 10 ml)	10 each
3	Needle (18, 21, 24 gauge)	10 each
4	Vacutainer (serum in yellow cap and whole blood in red cap)	20 each
5	Alcohol swab in plastic vial	2
6	Transport swab in broth / virus transport medium	10-15
7	Cotton swabs	10-15
8	Glass slide with box	10-15 pieces
9	Sterile normal saline in plastic bottle	500 ML
10	Plastic beaker 25 ml/ 50ml	1
11	Phosphate buffer glycerine in vials	10-15
12	Tissue collection vial for microbial isolation	10-15
13	Zip lock bag	20-30
14	10% formaline in sample vials	10
15	Serum storage vials (1.8, 2 ml)	20-30
16	Pasteur pipettes (disposable)	20-30
17	Rubber teats	2
18	Spatula for shearing /BP Blade	1
19	Spirit lamp & match box	2
20	Post mortem set [Knives-small, medium, large; sharpening stone; forceps; scissors-small, medium, large ; scalpel; B P blade with handle; axe; hack-saw with frame; plastic sheet; measuring tape; camera; thread ball; gloves; syringe; sterile vials; sample collection bottle; cotton; glass slide box, cover slip; marker]	1
21	Restraining rope	1
22	Adhesive tape & thread	2-3
23	Marker	2
24	Data entry sheet	
25	Hand gloves	10 pairs
26	Mask	10
27	Towel & soap	2 sets
28	First aid box	1
29	Medicine	
30	Pen & note book	2
31	Camera	1
32	Apron	1
33	Disposable bag	5

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2.2 Handling and collection of samples from pigs

In comparison to other large animals, adequate restrain of pig is necessary for any injection to be given or any sample to be collected. Reduction of stress and providing comfort to pigs during repeated handling in the experiment minimize error in generation of scientific data.

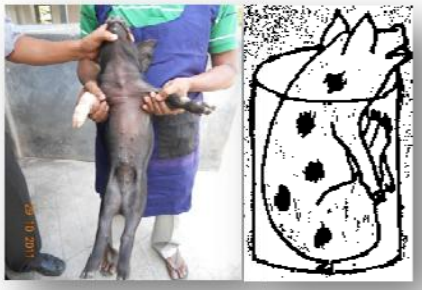
During collection of oral or nasal swabs proper restraint is necessary to prevent excessive head movement and sneezing. Pigs of 40 kg weight and larger can be restrained by use of hog snare. Animals less than 10 kg or 10-20 kg can be restrained manually. The animal should be lifted with fore legs with back towards the handler and place into the bucket, caudal end first, ensuring that the hind legs pointing up. The front legs are placed down into the bucket. With this position handler could sit on top of the bucket with pig's head and nose protruding out between the legs. Appropriate sample could be collected with minimum stress.

For collection of blood, the small pig can be picked up and secured on a lap. For large animal certain tools like hog snare, locally prepared snare string/ rope can be used. Pig should be calmed down by touching their belly/teats or scratching base of the ear. Then loop of the hog snare/pig catcher string/rope is placed around the animal's nose and the upper jaw and tighten properly. The pig reacts to this restraint device by pulling back against the snare, reaching a stalemate with the operator. Squeeze the pig against wall/post/tree and place leg of the handler behind fore legs of the animal. Alternatively, pig of 10-20 kg can be restrained in dorsal decumbency. The common sites for collection of blood are the ear vein, cephalic, saphenous veins or the cranial vena cava. The lateral, intermediate and medial ear veins are superficial and visually accessible. Marginal/lateral

ear vein is stable and it can be engorged by placing rubber band/ tourniquet around base of the ear. One must grasp the ear securely to prevent movement during collection of blood. After proper swabbing the engorged vein, a 20-22 gauge needle/ butterfly needle attached with rubber tubing can be inserted into the vein and collect 5-8 ml blood. For large volume of blood samples, venipuncture of the cranial vena cava is the method of choice. This may be done either on a standing pig or one in dorsal recumbency. To make thoracic inlet accessible on standing position, the head should be held high and on dorsal recumbency, forelegs should be drawn back along the body. The needle (20-22 gauge) of 3.8 cm is inserted on the right side in the depression between the point of the shoulder and the manubrium sterni, in order to avoid pricking vagus nerve. The needle is aimed at opposite shoulder and passed into the cranial vena cava.

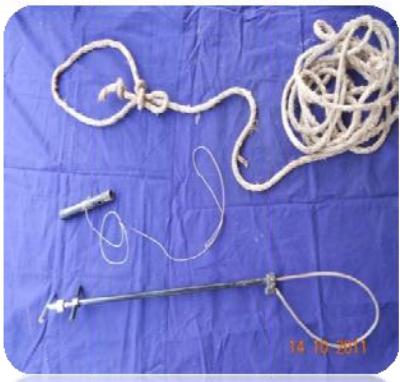
RESTRAINING TOOLS

RESTRAINING OF PIGS



Restraining of young pigs

Restraining of Adult Pig



3. COLLECTION, PRESERVATION & DISPATCH OF SAMPLES

General Considerations for collection of specimens

- The diseases most commonly encountered in animals are of bacterial, viral, parasitic, fungal and metabolic origin. Diagnosis based on symptoms and laboratory examination of the relevant materials is essential for initiating treatment at the proper time. In general the following points should be duly considered while collecting materials for laboratory diagnosis.

- All materials collected should be accompanied by detail history of disease, type of species affected, duration of disease, clinical signs, morbidity and mortality rates, disease suspected etc in standard format.

- The collected biological specimens should be transported on ice to the nearest laboratory as early as possible for diagnosis.

- Materials collected for bacteriological examination should be kept at refrigeration temperature (4^{o} C) and if a viral etiology is suspected the material can be stored at –20 to -80^{o} C in case of delay of transportation.
- For sero-diagnosis collect paired serum samples (about 2 ml sera). One serum sample should be collected at the onset of disease and second sera after recovery (2-4 weeks) from disease, preferably on 14^{th} /21^{st} day.

- If death is reported, the post-mortem examination should be conducted at the earliest as putrefied materials are unfit for laboratory examination.

- Detailed post-mortem report should be attached along with the samples collected during postmortem.

- Different virological transport media like 50% sterile phosphate buffered glycerine saline solution and phosphate buffer saline (pH 7.2-7.4) may be used. Collect samples in sterile containers if transport media is not available or transport on ice.
- For histopathology studies, tissues should be preserved in 10% formalin. The volume of formalin used should be approximately 10 times the

volume of material. Specimen bottles with wide mouth should be used for collecting tissues.

- The specimen bottles should be sealed well so as to avoid leakage and mark clearly indicating the fixative/transport media used.

- All the impression smears, should be fixed in methanol for 1-5 minutes unless otherwise specified.

- In case of outbreaks, collect materials from ailing animals (5-6 or more) and in-contact animals at the height of body temperature or flaring up of clinical signs and symptoms.

- Refer check list to arrange vials, buffer and other tools (Page No. 3).

SURVEY/OUTBREAK DATA SHEET FOR ANIMAL DISEASE DIAGNOSIS

Name of Local Vet .....Address.....
Vill.....City.....State.....PIN.....
Phone.....E-mail.....

GPS location.....
Collection Date.....
Area land mark.....

Farm Attributes

Premises/Owner' Name.....	Type Of Management & Number	Location of OB with distance (km)	Location of OB with distance (km)	**OB suspected due to**
Vill.....	.....Org farm.....	.....Weekly market	.....Major city	.....Entry of new animal
City.....	.....Small unit.....	.....Daily market	.....Slaughter house/slaughter point	.....Visitors
State.....	.....Free grazing.....	.....Livestock Transit point	.....Check point	.....Doctors/Paravet
Phone		.....Wet land/.....Dry land	.....Sanctuary/Forest	.....Contact with wild
Human density.....		.....Hilly Area.....highway	..... Water body	.....Waste feeding
Human affected.....		.....Railway station		..... Vector borne

Animal & Outbreak Attributes

Type of animal

.....Domestic
.....Wild
.....Feral

Record on outbreak

1st sign recorded.....
Last case seen.....
Duration of OB.....

Type of Breed

Pure Breed (.....)
Cross Breed (.....X.....)
Local (.....)

Type Of Breeding

.....Natural
.....A.I.

Type Of Feeding

.....Commercial feed
.....Waste Feeding
.....Grazing

Vaccination Status

.....Vaccinated.....Un-Vaccinated
.....Single Vaccination On.....
.....Repeated Vaccination On.....
Repeated Vaccination Interval:.....*6month*/.....*12month*
Name of vaccine used.....
Type of vaccine:... Live / inactivated.
Manufacturer:.....Batch No.....
Cold chain maintain/ partially maintained/ not maintained

No. affected/at risk

...../.....Young
...../.....Grower
...../..... Yearling
...../..... Adult
Total Population
Just before OB.....

No. Animal died

.....young
.....Grower
.....Yearling
..... Adult
Total Population after Subside the OB.....

Clinical Signs

.....Fever	.....Constipation
.....Hyperemia	.....Diarrhoea
.....Cyanosis	.....CNS
.....Conjunctivitis	.....Wasting
.....Respiratory Distress	.....Abortion
..... discharge	.....wound
.....skin nodule	.....Tick/mite/infestation
	.....Any other symptoms

PM Lesions

Skin :	Heart :
Brain:	Liver :
Tonsil :	Intestine :
Lymph Node :	Ileum :
Spleen :	Colon :
Kidney :	
Lung :	

Tentative diagnosis : .....Infectious ; .....Parasitic ; .....Stress ; .....Natural calamity ; .....other specific

SURVEY/OUTBREAK DATA SHEET FOR AVIAN DISEASE DIAGNOSIS

Name of Local Vet .....Address.....
Vill.....City.....State.....PIN.....
Phone.....E-mail.....

GPS location.....
Collection Date.....
Area land mark.....

Farm Attributes

Premises/Owner' Name.....	Type Of Management & Number	Location of OB with distance (km)	Location of OB with distance (km)	**OB suspected due to**
Vill.....	.....Org farm.....	.....Weekly market	.....Major city	.....Entry of new bird
City.....	.....Small unit.....	.....Daily market	.....Slaughter house/slaughter point	.....Visitors
State.....	.....scavanging.....	.....Hatchery	.....Check point	.....Doctors/Paravet
Phone	.....deep litter.....caged	.....Wet land/.....Dry land	.....other poultry farm	.....Contact with wild bird
Human density.....		.....Hilly Area.....highway	..... Water body	.....water
Human affected.....		.....Industry		.....disposed litter

Birds & Outbreak Attributes

Type of bird
.....Layer
.....Broiler
.....Other

Type of Breed
Pure Breed (.....)
Cross Breed (.....X.....)
Local (.....)

Type Of Feeding
.....Commercial feed
.....Waste Feeding
..... .Free foraging

Vaccination Status
.....Vaccinated.....Un-Vaccinated
.....Single Vaccination On.....
.....Repeated Vaccination On.....
Repeated Vaccination Interval:.....6month/.....12month
Type of vaccine:.....
Manufacturer:.....Batch No.....
Cold chain maintain/ partially maintained/ not maintained

No. affected/at risk
...../.....Chick
...../.....Grower
...../.....Pullet
...../.....Layer/adult
Total Population
Just before OB.....

Record on outbreak
1st sign recorded.....
Last case seen.....
Duration of OB.....

Type Of Breeding
.....Natural
.....A.I.

No. bird died
...../.....Chick
...../.....Grower
...../.....Pullet
...../.....Layer/adult
Total Population after Subside the OB.....

Clinical Signs

.....Fever	.....Pasty vent
.....Hyperemia	.....Diarrhoea
.....Cyanosis	.....CNS/paralysis
.....Conjunctivitis	.....Wasting
.....Respiratory Distress	.....wound
..... Ocular discharge	.....Tick/mite/infestation
.....skin nodule	.....Bloody droppings
	.....other symptoms

PM Lesions

Skin :	Heart :
Brain:	Liver :
Spleen :	Intestine :
Kidney :	Caecum :
Lung :	
Comb & wattle:	

Tentative diagnosis : .....Infectious ; .....Parasitic ; .....Stress ; .....Natural calamity ; .....other specific

3.1 Type of materials to be collected in various disease conditions

BACTERIAL DISEASES	
Haemorrhagic Septicaemia (HS)	Blood smear, smears of fluid from swelling blood in sterile container, impression smear from heart, lungs, liver, submaxillary swellings, smears from heart blood, lymph nodes and spleen on ice
Anthrax	Blood smears from ear vein, smear of the discharges from natural orifices, smear from swelling, ear tip or a piece of muzzle in saline/ charcoal
Black Quarter (BQ)	Impression smears from the affected muscle, exudate from the swelling on ice, pieces of affected muscle on ice,LN, muscle in 10% formaline
Enterotoxaemia	Blood, smears from contents of small intestine, contents of intestine
Brucellosis	Milk, blood, serum sample (paired serum sample), vaginal mucus, uterine fluid, stomach contents of foetus, aborted foetus
Johne's Disease (JD)	Rectal pinch swab or smear, faecal sample, terminal portion of ileum with ileo-caecal valve, mesenteric lymph gland in 10% formal saline
Glander	Nasal discharge on ice, pus from skin lesions on ice, affected tissue in 10% formalin
Tuberculosis (TB)	Sputum swabs in sterile container on ice, milk in sterile vials on ice, faeces, heat fixed impression smears from lymph glands, affected tissue for histopathology in 10% formalin. lymph glands or lung lesions in sterile container in 50% glycerol phosphate buffer
Leptospirosis	Blood, blood smear, serum, urine, tissue from kidney, liver and spleen in 10% formalin, milk or urine in vials by adding 1 drop of formalin per 20 ml
Salmonellosis	Blood, faeces, intestinal content, heart blood and bile in separate sterile vials, tissues like mesenteric lymph nodes, kidney and gall bladder in 10% formalin.
Actinomycosis	Pus smear, pus in sterile container on ice, tissue in 10% formalin
Actinobacillosis	Pus smear, Pus in sterile container on ice, affected tissue in 10% formalin

Listeriosis	Blood, cerebrospinal fluid, brain, aborted foetus or placenta, all internal organs in 10% formalin or on ice
Mycoplamosis/ CCPP/CBPP/ Coryza	Swabs from sinus/trachea, nasal and vaginal swabs in PBS on ice, piece of lung preserved in 10% formalin and on ice separately, serum samples (paired serum)
Chlamydia/ Psittacosis	Nasal swab, lung pieces in sterile vials on ice, impression smears from lungs, liver, conjunctiva and foetus, serum samples (paired serum samples), internal organs in 10% formalin
Campylo-bacteriosis	Vaginal mucus, stomach content of aborted foetus
VIRAL DISEASES	
Blue tongue (BT)	Blood at the height of body temperature in heparin or EDTA for isolation. Paired sera in sterile vials on ice for serology. Spleen, lung and lymph nodes on ice for isolation. spleen, lymph nodes, intestine in 10% formol saline for histopathology
Bovine Viral Diarrhoea disease (BVD)	Nasal discharge for isolation, blood in EDTA for isolation, paired serum samples for serology, semen for isolation. Lymph nodes and spleen in sterile vials on ice for isolation, intestinal swabs for isolation
Canine Distemper (CD)	Blood smears / impression smears of conjunctiva or vaginal epithelium for viral inclusion body. Impression smears from liver for viral inclusion body, pieces of liver & spleen in sterile vials on ice for isolation, pieces of lung, urinary bladder, liver, trachea, stomach wall and brain in 10% formol saline
Foot and Mouth Disease	Vesicular epithelium in 50% phosphate buffered glycerine, oeso-pharyngeal fluid in 50% phosphate buffered glycerine
Infectious Bovine Rhinotracheitis (IBR)	Paired sera for serology, Swabs from vaginal and nasal lesions for isolation. Pieces of trachea, lungs in PBS on ice for isolation, pieces of trachea, liver, turbinate bone, lungs in 10% formol saline
Infectious Canine Hepatitis	Spleen and liver in sterile containers on ice for isolation. Liver, gall bladder and kidney in 10% formol saline for histopathology. Impression smears from liver fixed in methanol
Parvo Viral Infection of Canines	Rectal swab and faeces in PBS, paired serum samples. Pieces of intestine, heart on ice for isolation. Liver, heart, intestine in 10% formol saline

Peste des Petits Ruminants (PPR)	Eye, mouth and rectal swabs in PBS on ice for isolation. Citrated Blood for isolation. Paired sera for serology. Spleen, lymph nodes, pieces of intestine on ice for isolation. Lungs, liver, spleen, tonsil in 10% formalin
Rabies	Head / Whole carcass on ice for demonstration of viral antigen, viral inclusions and isolation of virus. Brain on ice for demonstration of viral antigen, viral inclusions and isolation of virus **Note**: It is not advisable to open the skull by persons not protected by vaccination
Swine Fever (CSF)	Heparinised blood at the height of temperature for isolation. Pieces of spleen, mesenteric lymph glands, intestine especially ileo-caecal region in 50% glycerol saline for isolation. Pieces of brain, lung, intestines, ileo-caecal region and kidney in 10% formol saline
Infectious Bursal Disease (IBD)	Ailing bird, paired serum sample. Bursa of Fabricious in 50% buffered glycerine saline. Bursa of Fabricious, kidney, spleen in 10% formol saline
Marek's disease (MD)	Ailing bird. Paired serum sample. Trachea, ovary, liver, kidney, spleen in 10% formol saline
Ranikhet disease (RD)	Serum sample from ailing bird. From dead animals –fresh carcass on ice. Liver, spleen, trachea, bronchi, lungs in 50% buffered glycerine saline. Proventriculus in 10% formol saline
BLOOD PROTOZOA, FUNGAL INFECTION	
Theileriosis	Blood smears, biopsy smears from swollen lymph nodes from early stage of disease fixed with methanol
Babesiosis	Thin blood smears fixed in methanol
Anaplasmosis	Thin blood smears fixed in methanol
Filariasis	Direct blood smear (wet film), blood smear
Trypanosomiasis	Wet film examination of blood by hanging drop, fixed blood smears, blood in anticoagulant on ice
Schistosomiasis	Dung sample (avoid contact of water which may aid in hatching of the egg). Nasal schistosomiasis –nasal discharge in normal saline, nasal granuloma in normal saline
Trichomoniasis	Vaginal or uterine discharges, prepuce scraping/ washing
Gastro-Intestinal Parasitic Diseases	Faecal sample in clean vial, Affected internal organs in 10% formalin. Intact nematode parasite in 70% warm alcohol. Trematode, cystode gently press between clean glass slides, tie with rubber band and put in 5% formol saline

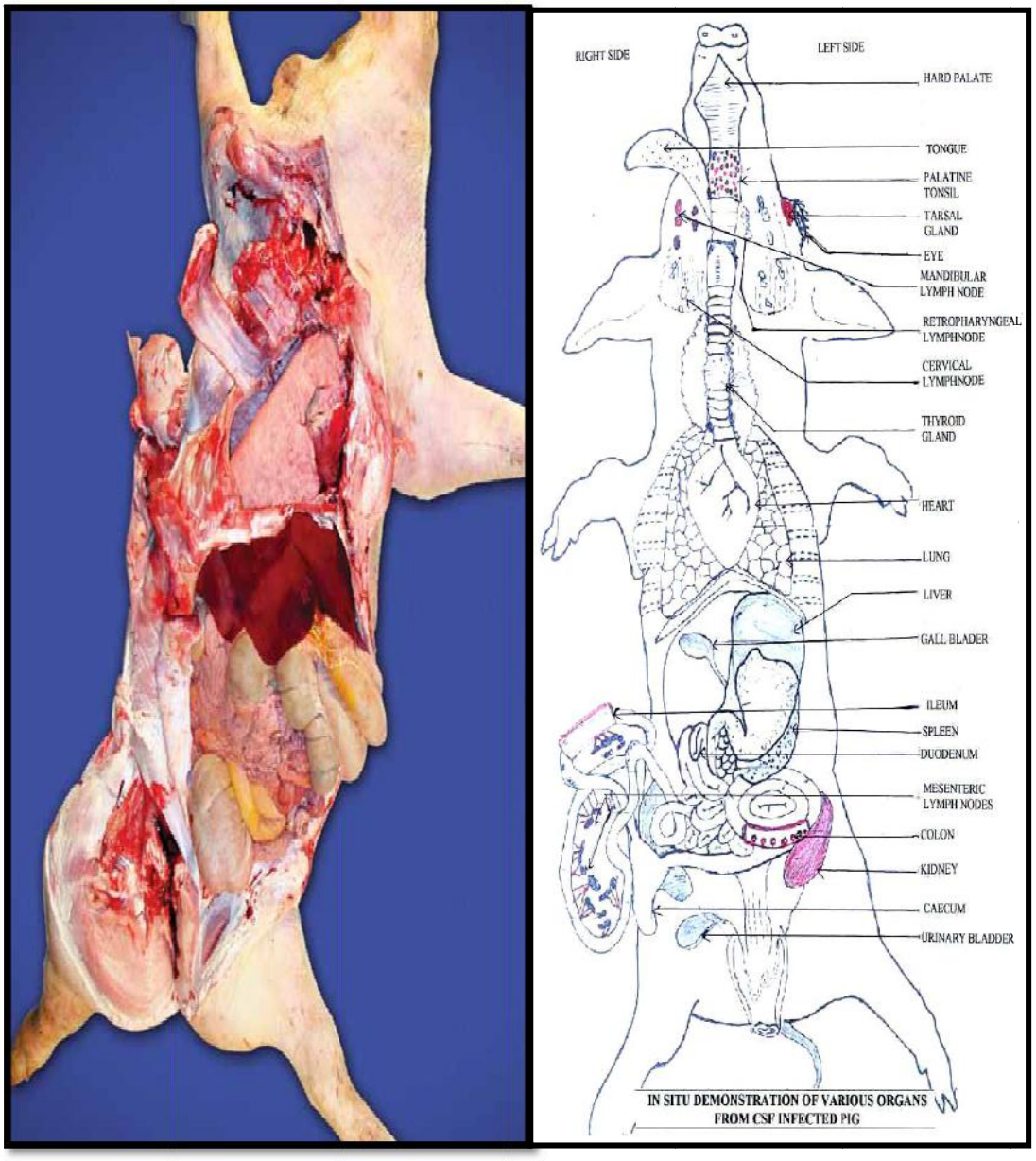
Ectoparasitic Infestations (Ringworm, Mange, Mites)	Deep skin scrapings in sterile vials, affected hair, skin, nails in dry container
Rhinosporidiosis	Nasal discharge in sterile vial, nasal swab, granulation tissue in 10% formol saline
External Fungal Infections	Skin scrapings in sterile vials
Aflatoxicosis	100 gm of suspected feed (specially groundnut cake), liver and spleen on ice, liver and spleen in 10% formol saline
OTHER DISEASE CONDITIONS	
Poisoning Cases	For chemical analysis fresh tissues(a loop of intestine, stomach) and fluids should be sent as soon as possible on ice. Avoid addition of preservatives to the samples. Stomach/ intestinal content (10 gm), liver, kidney, intestine, urinary bladder in saturated salt solution (45% sodium chloride) for **forensic analysis**. Duplicate tissue samples in 10% formol saline
Plant Poisonings	Sample of suspected grass/fodder/plants, liver on ice, stomach contents on ice
Mastitis	Milk samples (mid-stream) before onset of treatment in sterile vials on ice
Abortion	Whole foetus/ all internal organs of foetus on ice, Vaginal swab in PBS, pieces of placenta in sterile vials on ice, pieces of placenta in 10% formalin, paired serum samples
Infertility and Sterility	Semen in sterile vials, prepuce swab on ice, paired serum sample on ice
Diseases of Unknown Etiology	Feed/ fodder, blood smears, urine sample, faecal sample, blood samples collected in EDTA on ice from live animals, serum samples from live animals, stomach contents, spleen, lung, lymph node, liver, kidney, intestine in sterile vials from dead animals on ice, stomach contents, spleen, lung, lymph node, liver, kidney, intestine in 10% formol saline

3.2 Checklist for collection of samples

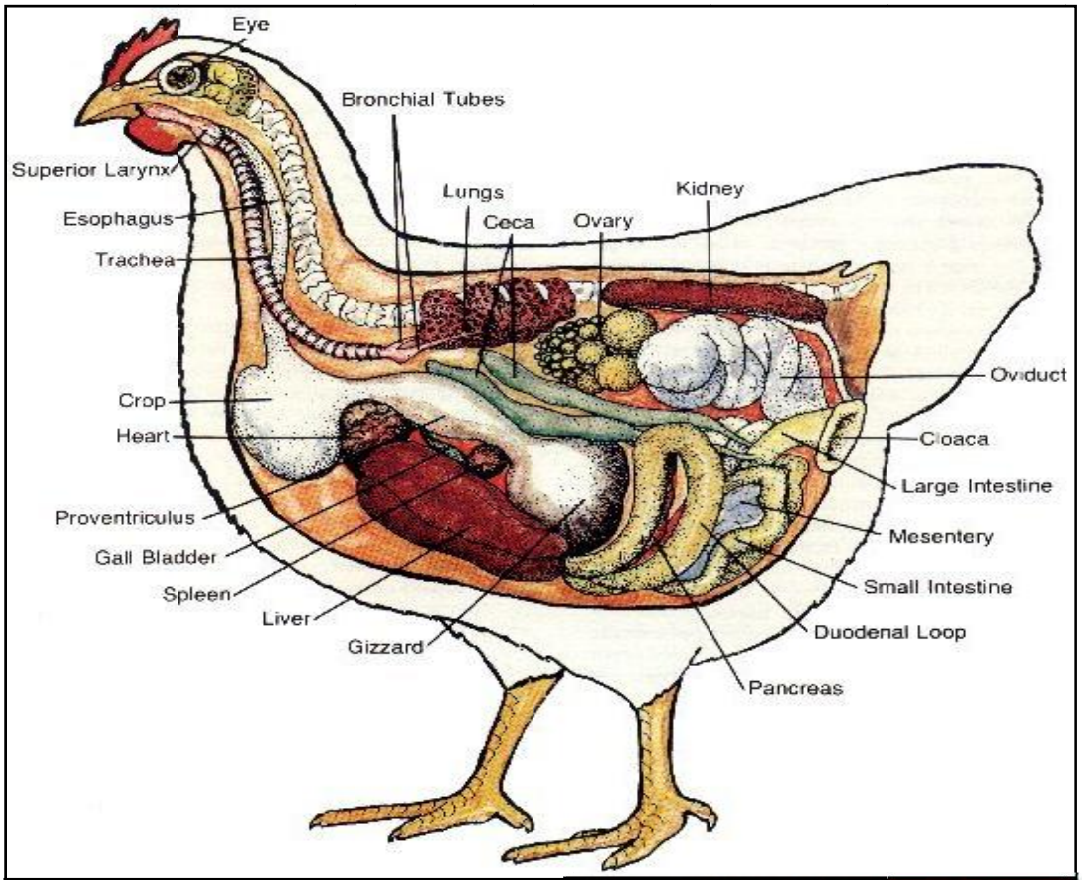
Type of Sample		Clinical samples				
		Sample on ice for			Haematology In EDTA/Heparin	Parasitology
		ELISA	PCR	Microbial Isolation		
CLINICAL	**Whole blood**					
	CSF					
	Serum					
	Nasal Swab					
	Ocular swab					
	Oral swab					
	Genital swab					
	Rectal swab					
	Faecal sample					
	Milk sample					
	Skin biopsy					
	Wound swab					
Type of Sample		**Post-Mortem Examination**				
		Sample on Ice for			*Insitu* Antigen detection, Tissue snap frozen in LN2	Histopathology Tissue in 10% Formalin
		ELISA	PCR	Microbial Isolation		
POSTMORTEM SAMPLES	**Heart blood**					
	Tonsil					
	Lymph node					
	Mesenteric LN					
	Spleen					
	Liver					
	Lung					
	Kidney					
	Small intestine					
	Large intestine/Colon					
	Urinary bladder					
	Rib bone					
	Tarsal gland					
	Nictitating membrane					
	Skin					
	Thymus					
	Brain					

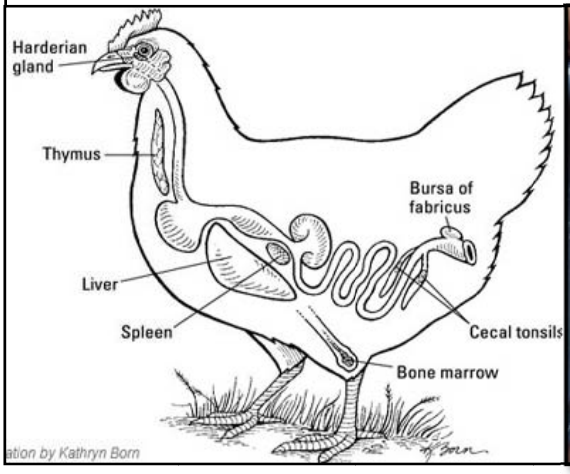
3.3 In-Situ distribution of appropriate tissue samples

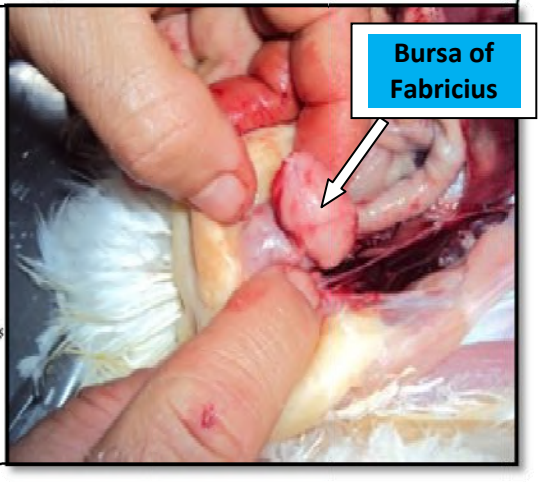
A. Location of organs showing pathological changes in large animal



B. Location of organs showing typical lesions in birds

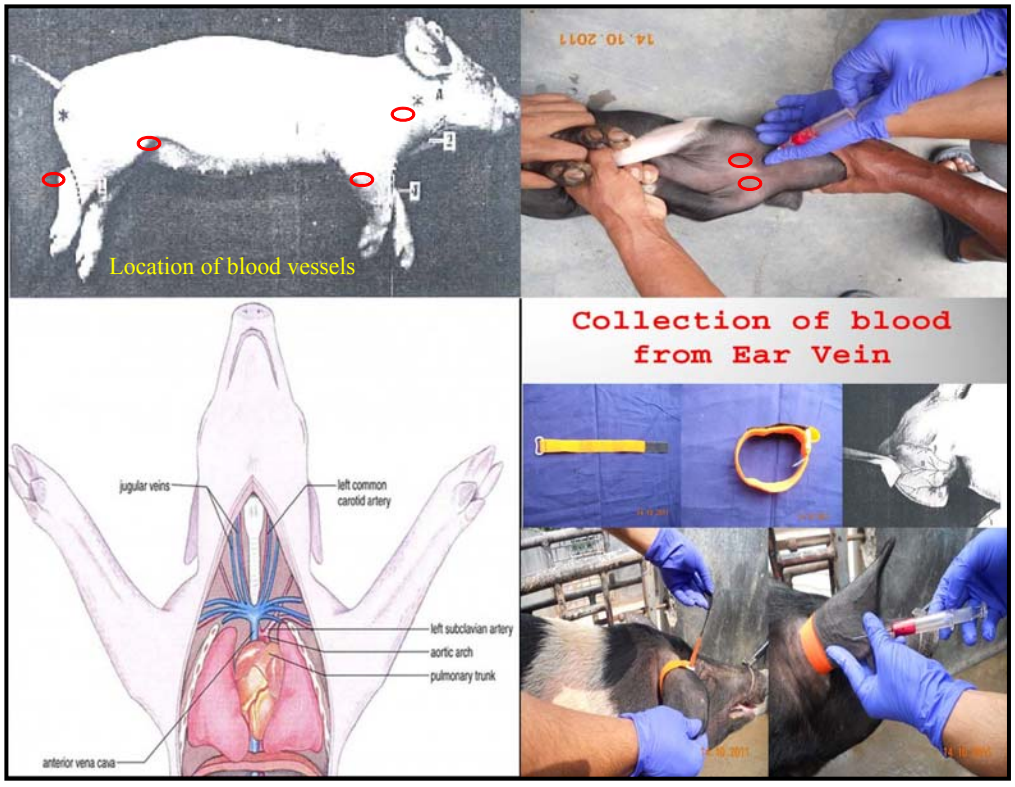






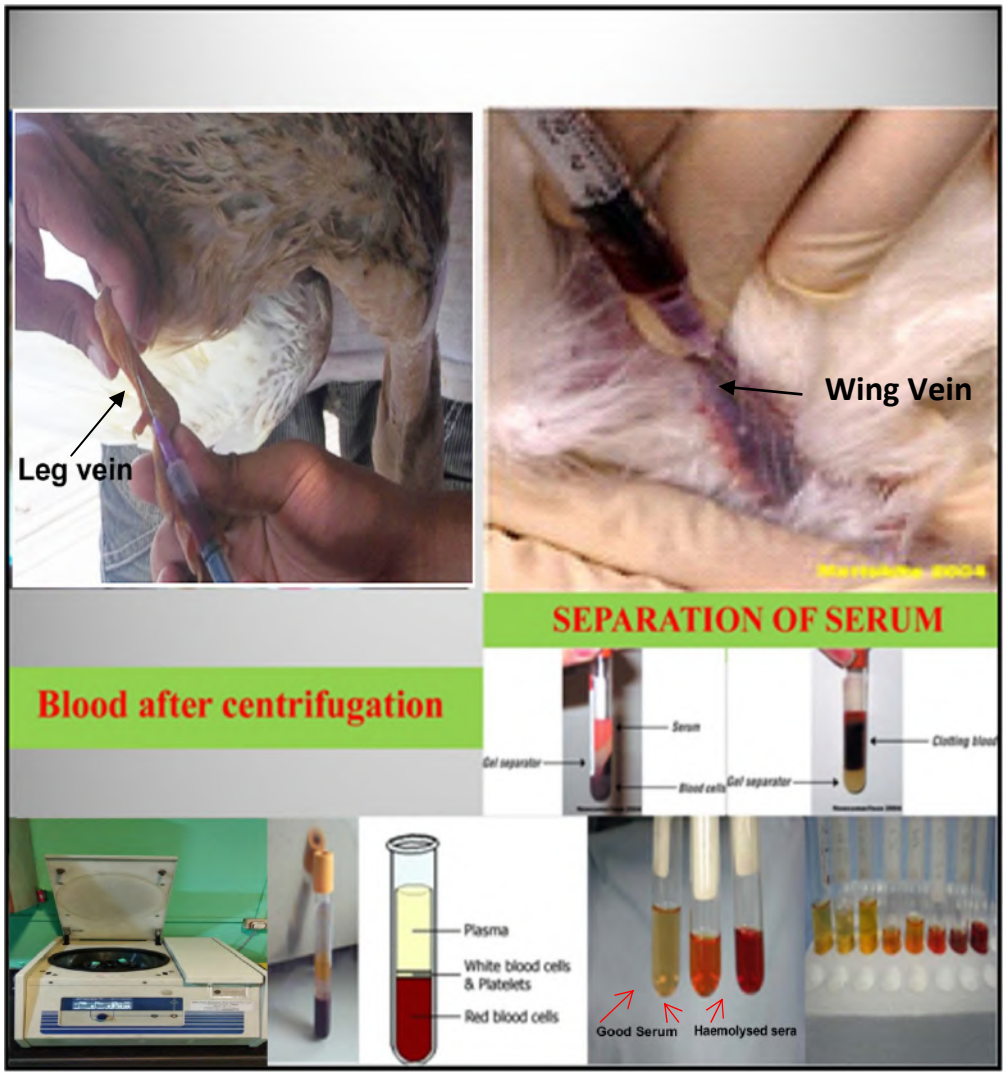
3.4 Collection of blood from animals

The common sites for collection of blood in large animals are the ear vein, cephalic, saphenous veins, abdominal vein or the cranial vena cava. The lateral, intermediate and medial ear veins are superficial and visually accessible. Marginal/lateral ear vein is stable and it can be engorged by placing rubber bang/ tourniquet around base of the ear. One must grasp the ear securely to prevent movement during collection of blood. After proper swabbing the engorged vein, a 20-22 gauge needle/ butterfly needle attached with rubber tubing can be inserted into the vein and 5-8 ml blood can be collected. For large blood samples, venipuncture of the cranial vena cava is the method of choice. In case of pigs this may be done either on a standing or one in dorsal recumbency. To make thoracic inlet accessible on standing position, the head should be held high and on dorsal recumbency, forelegs should be drawn back along the body. The needle (20-22 gauge) of 3.8 cm is inserted on the right side in the depression between the point of the shoulder and the manubrium sterni, order to avoid vegus nerve. The needle is aimed at opposite shoulder and passed into the cranial vena cava



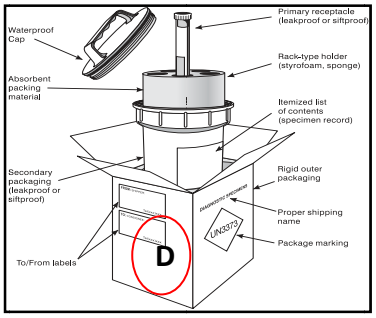
3.5 Collection of blood from birds

In poultry wing vein and additionally in duck in leg vein should be attempted to collect blood.



3.6 Packaging and transportation of samples

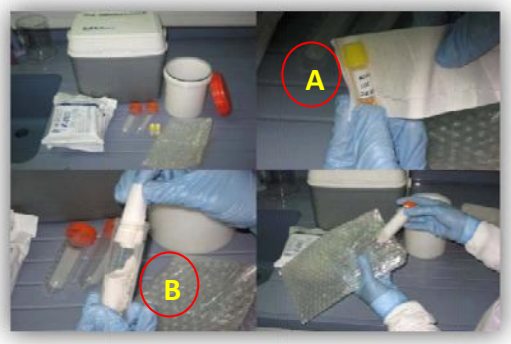
Precaution to be taken during transportation



- To prevent further contamination.
- To maintain viability of cells & there number ratio as it is.
- To avoid sample drying.

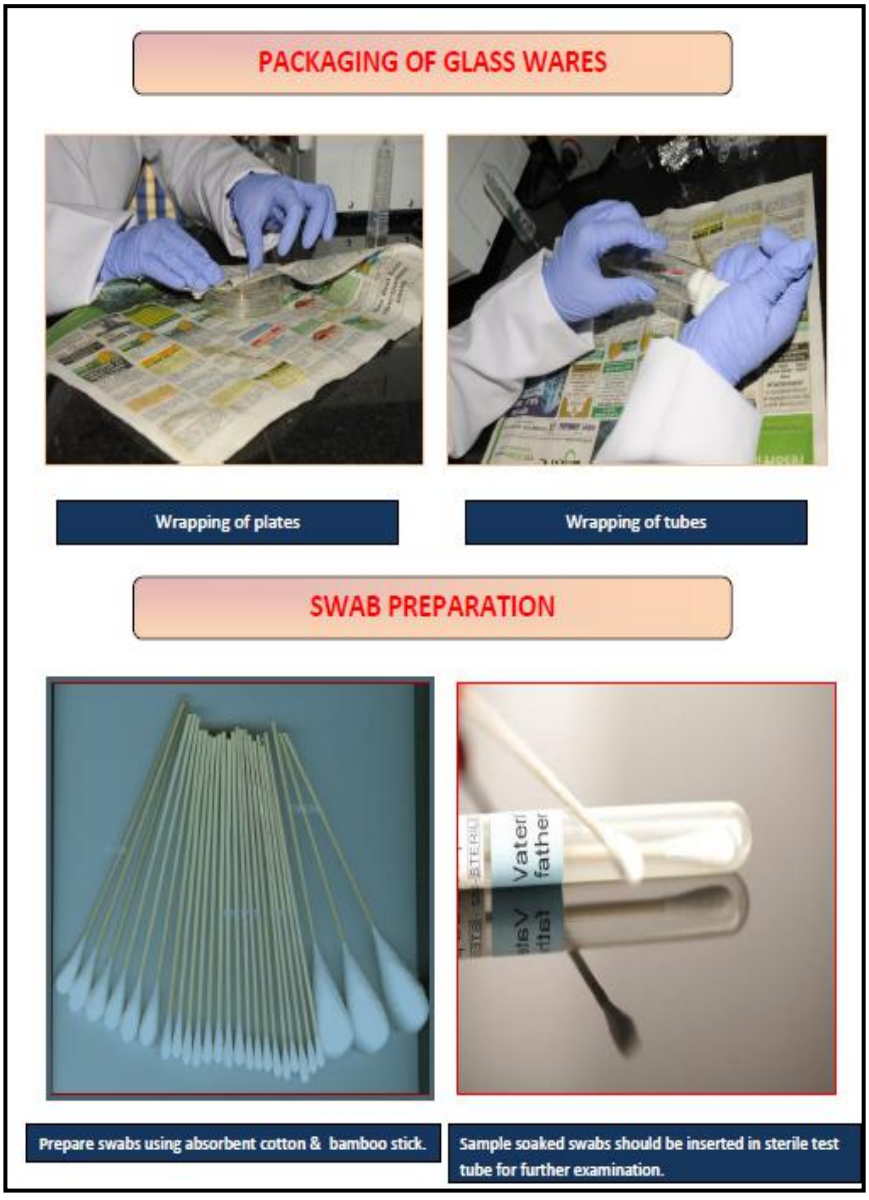
Care to be taken in Packaging

- Submit samples individually in separate leak proof containers.
- Second container around first in case of leak chances, filled with absorbent material.
- LABEL-type of animal, type of sample and date of collection, phone numbers (write using permanent marker or typed it).
- Secure/tighten the lids properly.
- If delayed, transport at 4°C on ice by ice packs.
- Avoid sending on weekends.
- Syringe can be used for fluid sample by removing needle and putting cap, expel air.
- Do not use cello tape the lids of the tubes.
- **Primary container**: containing the specimen **(A)**, should be clearly labelled, watertight and leak proof. Wrapped in sufficient absorbent material to prevent breakage and placed in a secondary container **(B).**
- **Outer shipping package**-should be a rigid box **(C):** place the secondary receptacle in an outer shipping package, with suitable cushioning that protects it and its contents from physical damage and water etc.
- Outer packing **(D)** write receiver's address and sender's address. Write in bold letter caution words - GLASS HANDLE WITH CARE, BIOLOGICAL MATERIALS, NO COMMERCIAL VALUE



4. PREPARATION OF LABORATORY ITEMS/ MATERIALS

For routine microbiological diagnostic works various glass wares, cotton swabs, inoculating loop are necessary. Glass wares should be thoroughly washed with detergent followed by plain tap water and finally air dried. Mouth of test tubes, bottles, flasks is to be plugged with non-absorbent cotton, wrapped with paper and tied securely with thread. Cotton swabs are prepared by wrapping absorbent cotton on one end of a bamboo stick and put in a test tube plugging with cotton.



Two different qualities of cotton are used in microbiological work- **absorbent** and **non-absorbent** cotton. Non-absorbent cotton will not soak water and source is seeds of tall simli tree. It is used to plug opening of test tubes, bottle, flask etc. Absorbent cotton is obtained from cotton plant and it soaks water. Absorbent cotton is used for preparing swabs.

Non- absorbent cotton used for plugging of tubes/flask

NON ABSORBENT COTTON USED FOR PLUGGING

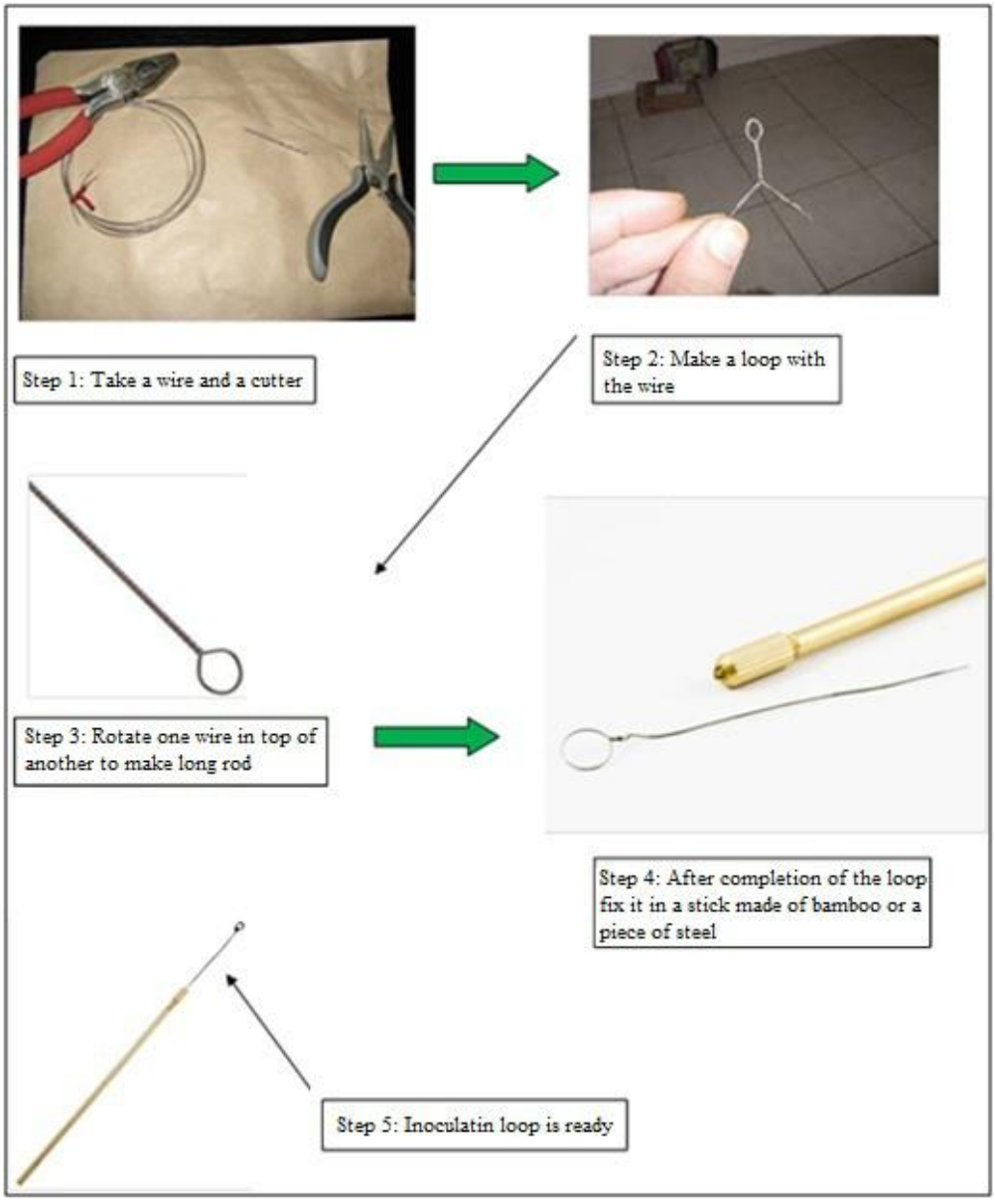
Absorbent cotton used for preparing swabs

Absorbent Cotton

Absorbent Cotton Balls

4.1 Preparation of inoculating loop

Inoculating loop is a small but essential tool used in any bacteriological laboratory for isolating bacteria from clinical as well as post-mortem samples. A 3mm diametre loop is prepared at one end of a platinum wire or suitable metal wire and fixed in a handle. Various steps are illustrated below to prepare an inoculating loop.



4.2 Sterilization

Sterilization by hot air oven : any type of microbiological works, glass wares, plastic wares, media and other reagents should be free from micro-organisms and the process is called sterilization. Commonly glass wares are sterilized by dry heat i.e by Hot air oven. Liquid media, reagents, plastic wares are sterilized by moist heat i.e. Autoclave or pressure cooker.

Sterilization of media & reagents by autoclave :

NOTE

An autoclave is used to sterilized utelsils, containers, media,soil, used infectious materials and other waste

Never autoclave toxic, flammable or radiological agents

Sterilization can also be done in pressure cooker. Cooking should be done for 20 mins at 15 psi

5. PREPARATION OF LABORATORY REAGENTS

A. Hanks' balanced salt solution

Stock solution A:

1.	NaCl	… 160g
	KCl	… 8g
	$MgSO_4.7H_2O$	… 2g
	$MgCl_2.6H_2O$	… 2g
	Distilled water	… 800ml
2.	$CaCl_2$	… 2.8g
	Distilled water	… 100ml

Mix these two solutions slowly and make up volume to 1000ml with distilled water. Add 2ml Chloroform, store at 4°C.

Stock solution B:

Na_2HPO_4	… 3.04g
KH_2PO4	… 1.2g
Glucose	… 20g
Distilled water	… 800ml

When chemicals have dissolved add 100ml of 0.4% phenol red in NaoH. Make up volume to 1000ml with distilled water. Add 2ml chloroform and store at 4°C.

For use:	Stock solution A	… 100ml
	Stock solution B	… 100ml
	Distilled water	… 800ml

Sterilize by autoclaving at 15lb for 20 minutes & store at 4°C. Add antibiotic in sterile solution

Penicillin	… 250IU/ml of solution
Streptomycin	… 100 µg/ml
Nystatin	… 25 units/ml

C. 50% Glycerine-PBS solution used for preserving tissue samples :

Glycerine pure	.. 200ml
Phosphate buffered saline solution	.. 200ml

The solution should be sterilized at 10lb pressure for 30 minutes and store at 4°C

D. Phosphate buffer saline solution (pH 7.4):

Solution A:

NaCl	… 8.00g
KCl	… 0.20g
Na_2HPO_4	… 1.15g
KH_2PO4	… 0.20g
Distilled water	… 800ml

Solution B:

$CaCl_2$	… 0.10g
$MgCl_2$	… 0.10g
Distilled water	… 200ml

Dissolve the chemicals in water in the order listed.
Sterilize the solutions A & B separately by autoclaving .Store at 4^oC till use

E. EDTA solution for blood collection: A 10% sodium salt of EDTA is dissolved in distilled water. Use one drop for each 5ml blood.

F. Dextran solution (5%) for lymphocyte separation :

Dextran sulphate	.. 5g
1.5% EDTA solution	.. at 100ml

Dissolve at 45^oC and autoclave before use

G. Alsever's solution collection of RBCs:

Glucose	… 2.05g
Sodium Chloride	… 0.42g
Sodium citrate	… 0.80g
Citric acid detergent fiber	… 100ml

Sterilize by autoclaving 10lb pressure for 15 minutes and store at 4^oC

H. Staining for gel slides:

Comassie brilliant blue staining solution (A)

Comassie brilliant blue	…	0.1g
Acetic acid	…	10ml
Ethanol	…	45ml
Distilled water	…	45ml

Destaining solution (B)

Acetic acid	…	10ml
Ethanol	…	25ml
Distilled water	…	65ml

I. Phenol saline for Brucella agglutination test :

Sodium chloride	…	0.85g
Phenol	…	0.5g
Distilled water	…	100ml

J. Phosphate buffered saline (pH-7.4):

Sodium chloride	…	8.0g
Potassium di-hydrogen phosphate	…	0.2g
Disodium hydrogen orthophosphate	…	1.16g
Potassium chloride	…	0.2g
Distilled water	…	1000ml

Adjust pH to 7.4 with 4% NaOH or 8% HCL and autoclave at 10 lb pressure for 30 minutes.

K. Lugol's Iodine Stain- is used with wet mount preparations and concentration techniques for the detection of intestinal protozoa and helminth ova and larvae.

Composition :

Iodine crystal – 5.0 gm
Potassium iodide – 10.0 gm
Dist water - 100 ml

Working dilution – 1:5 in dist water. Use for 3 weeks.

L. Lactophenol cotton blue stain (45 ml) -is used to stain fungus

Composition :

Phenol – 10 gm
Cotton blue, water soluble- 0.04 gm
Lactic acid – 10 ml
Glycerol – 20 ml
Dist water – 10 ml

M. Nutrient Agar –is used to grow bacteria

Composition:

Peptone – 0.5 gm
Beef extract/yeast extract - 0.3 gm
Agar agar- 2.5 gm
NaCl- 0.5 gm
Distilled water – 100 ml
pH is adjusted to neutral (7.4)

N. Nutrient Broth –is used to grow bacteria

(To prepare use same composition of Nutrient agar without adding agar agar)

O. Sabouraud Dextrose Agar – is used to grow fungus

Composition :

Dextrose (glucose) - 4.0 gm
Peptone - 1.0 gm
Agar - 2.5 gm
Dist water – 100 ml
Final pH 5.6

5.1 Preparation of bacteriological culture media

The survival and growth of microorganisms depend on available and a favourable growth environment. Culture media are nutrient solutions used in laboratories to grow microorganisms. For the successful cultivation of a given microorganism, it is necessary to understand its nutritional requirements and then supply the essential nutrients in the proper form and proportion in a culture medium.

Preparation of agar plates:

Materials and equipments:

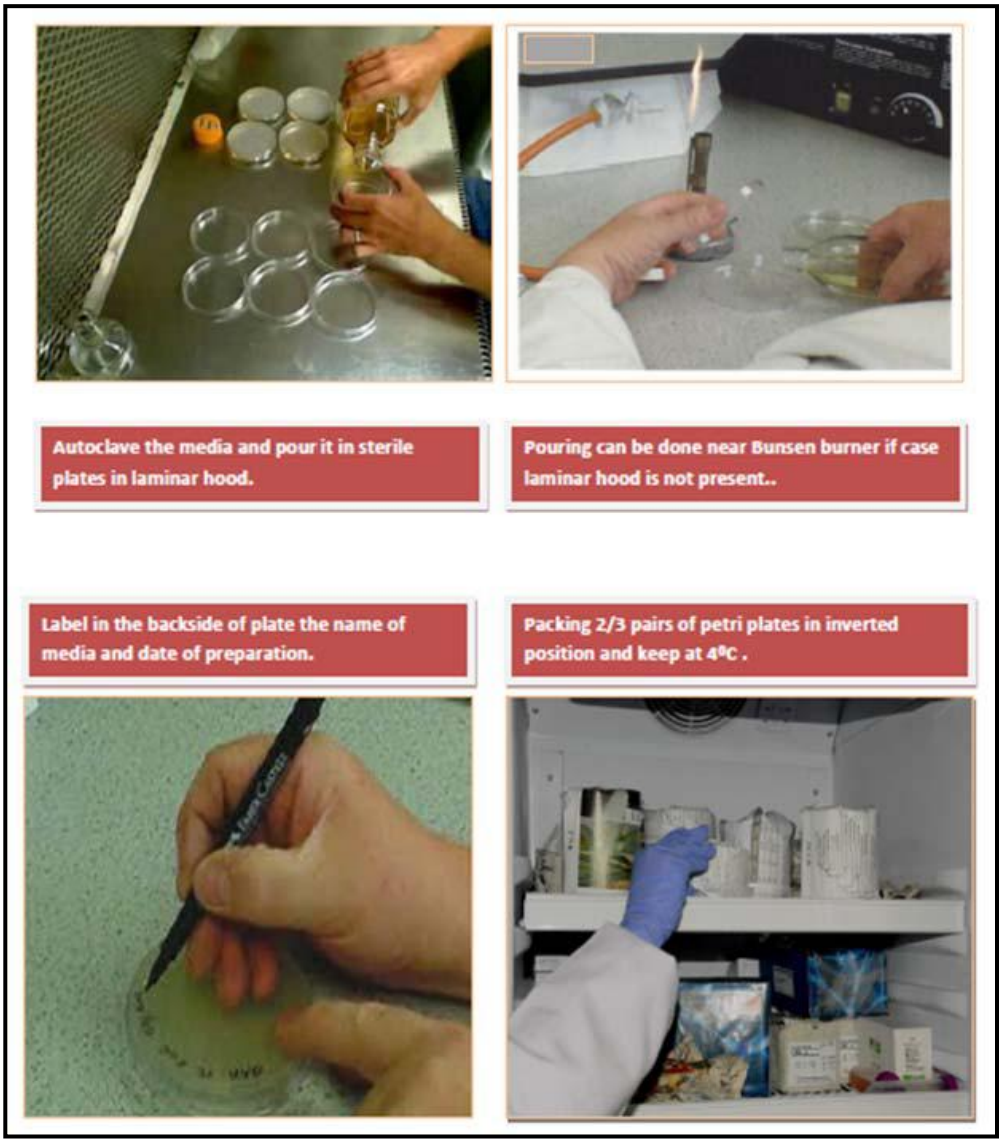
1) Distilled water
2) Measuring cylinder
3) Flask
4) Bacteriological powder media (Page No-31)
5) Spatula
6) 1N NaOH solution
7) 1N HCl solution
8) pH meter
9) Gloves
10) Sterile, empty Petri dishes
11) Bunsen burner
12) Autoclave
13) Incubator

Procedure:

- Measure the components of the medium into a flask. Use a clean spatula for every measurement. Dissolve the solid components and fill with the remaining solvent up to final volume. If the medium contains heat sensitive components (like sugars), they must be separately sterilised in solution (e.g. by filter sterilisation), and then mixed with the already sterilised and cooled agar medium. Add agar between 2.5% depending upon the climatic conditions.
- Check the pH of the medium with an indicator paper or with a pH meter and adjust to the proper value with NaOH or HCl solution.
- Close the flask with cotton plug and cover with aluminium foil, label the flask and put into the autoclave and start a sterilisation under 15 psi pressure, at 121°C for 15minutes.
- Cool the sterilized medium.
- In a laminar air flow or inoculation hood, remove the cotton plug and flame the mouth of the flask over a bunsen burner and dispense the medium into sterile Petri dishes (15-20 ml into each Petri dish) or test tube (for preparation of slant).
- Keep on the floor of the work bench until complete solidification.

- Turn the Petri dishes upside-down and stack them. The slants can be stacked separately.
- Perform a sterility test: incubate the medium at 37°C for 24 hours to check for sterility.
- In case of longer storage, Petri plates must be placed into plastic bags or boxes to avoid drying out.

Preparation of media plate



5.2 Inoculation of sample for bacterial/fungal isolation

Isolation of Bacteria -a loopful of sample is smeared on the sterile solid media at one corner and sterilized the loop by burning . Streak out three parallel lines from pooled smear after cooling down the loop, burn the loop again and draw another three parallel lines from first streak as shown in the diagram. In broth, loopful of sample shake inside tube containing sterile broth.

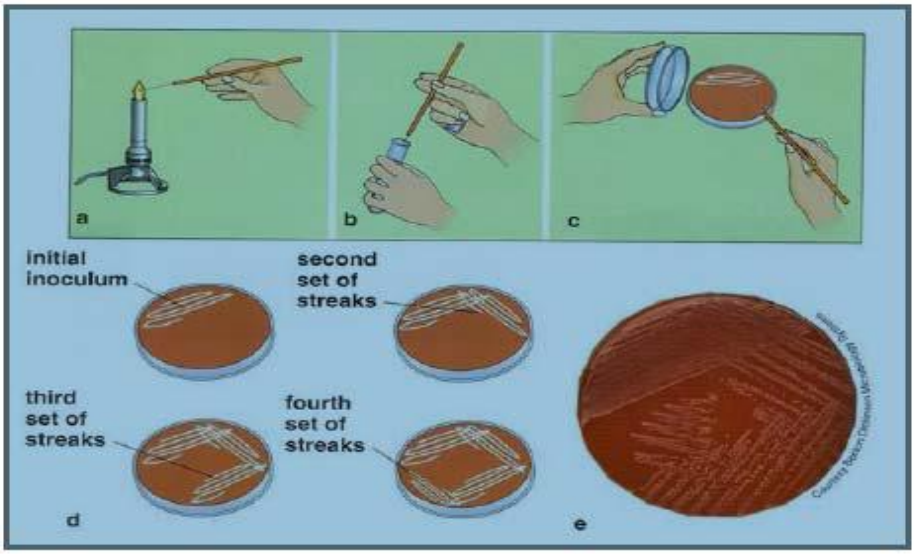
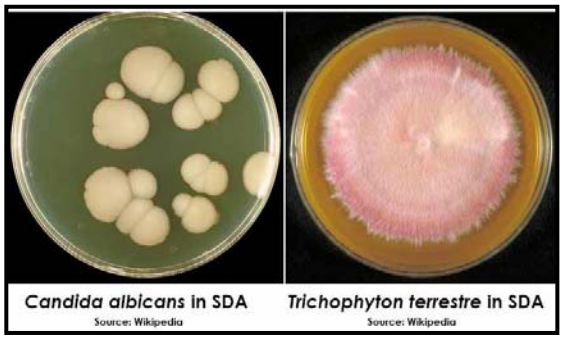


Figure indicates step wise streaking method from a **to e.**
NOTE: The procedure should be done in a laminar hood or a closed chamber.

Isolation of fungus

Sabouraud's dextrose agar media (Page No- 31) is used to isolate fungus. Suspected sample is burrowed into the media and incubate at room temperature for one to two weeks.



6. PREPARATION OF SMEAR FROM LIQUID BACTERIAL CULTURE

Materials required:

- Microscope and micro slides
- Bunsen burner/ Spirit lamp
- Inoculation loop
- Glass marking pencil
- Broth culture (Page No-31)

Procedure:

- ❖ Take a clean micro slide and pass it over the flame for 3-4 times.
- ❖ Make the surface grease free. Handle the clean slide by its edges.
- ❖ Write the identification number/ initial of organisms on the side of the slide with glass marking pencil.
- ❖ Make a circle in the centre of the slide, where the organisms are to be placed (target circle). Flame the inoculating loop until red-hot and let it cool.
- ❖ Shake the culture vigorously and transfer a loop full of organisms at the target circle. Spread the organisms over the area of the target circle. Be sure to flame the inoculating loop before placing it aside.
- ❖ Allow the slide to air dry. Do not apply heat.
- ❖ Pass the slide over the flame 3-4 times exposing the lower surface to the flame (i.e. the surface in which there is no smear) to heat kill and fix the organisms to the slide. Now the smear is ready for staining.

6.1 Preparation of smear from solid bacterial culture

Materials required:

- Microscope and micro slides
- Bunsen burner/spirit lamp
- Inoculating loop
- Glass marking pencil
- Culture plate with organisms

Procedure:

- ❖ Take a clean micro slide and pass it over the flame for 3-4 times.
- ❖ Make the surface grease free. Handle the clean slide by its edges.
- ❖ Write the identification number/ initial of organisms on the side of the slide with glass marking pencil.
- ❖ Mark a target circle in the centre of the slide with glass marking pencil.
- ❖ Flame the inoculating loop until it is red hot, let it cool and transfer a loopfull of sterilized NSS to the slide over the target circle.
- ❖ Flame the inoculating loop, let it cool and pick up a fraction of isolated colony of the target organism from the culture plate and mix it with saline on the slide. Disperse the mixture over the area of the target circle. Be certain that the organisms have been well emulsified in the liquid. Be sure to flame the inoculating loop before placing it aside.
- ❖ Allow the slide to air dry. Do not apply heat.
- ❖ Pass the slide over the flame 3-4 times exposing the lower surface to the flame (i.e. the surface in which there is no smear) to heat kill and fix the organisms to the slide. Now the smear is ready for staining.

7. STAINING OF BACTERIAL SMEAR BY SIMPLE STAIN

The use of a single stain to colour a bacterial organism is commonly referred to as simple stain.

Materials required:

- Bacterial culture
- Methylene Blue
- Wash bottle
- Blotting paper
- Micro slide, Microscope
- Glass marking pencil
- Bunsen burner
- Inoculating loop

Procedure:

- ❖ Prepare a bacterial smear
- ❖ Stain the smear with Methylene blue for 1 minute
- ❖ Wash the slide with water
- ❖ Allow the slide to dry at room temperature
- ❖ Examine under oil immersion and record the observation

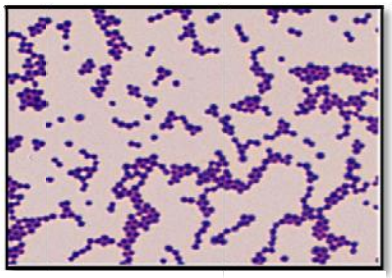
Interpretation: Organisms take blue stain. *Pasteurella* organism takes bipolar stain.

7.1 Staining of bacterial smear with differential stains

Gram staining method

Materials required:

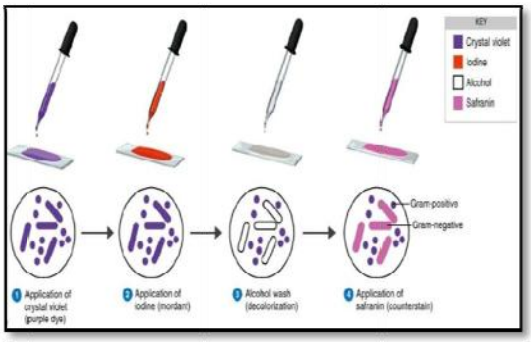
- Slides with prepared smears
- Gram staining kit
- Wash bottle
- Blotting papers
- Microscope, cedar wood oil.



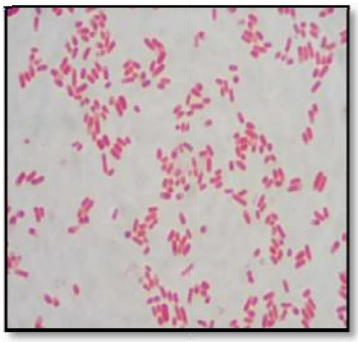
Gram +ve Bacteria stained as violet colour

Procedure:

- ❖ Prepare a smear from bacterial culture as described (Page-34)
- ❖ Flood the smear with crystal violet solution and let it stand for 45 secs
- ❖ Wash with distilled water and drain off excess water
- ❖ Flood the smear with Gram's Iodine solution and allow to act for 1 min
- ❖ Pour off the Gram's iodine and wash with distilled water
- ❖ Flood with ethyl alcohol (70 %) and gently rock the slide for 15-20 secs
- ❖ Wash with water
- ❖ Flood the smear with safranin (0.5%) or basic fuchsin for 45 secs
- ❖ Wash gently with water, blot with blotting paper and dry
- ❖ Examine under oil immersion and record the observation



Steps of Gram's stain



Gram –ve Bacteria stained as pink colour

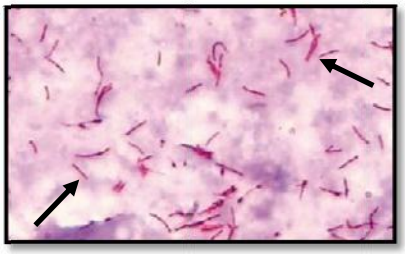
7.2 Staining of acid fast bacteria with Ziehl Neelsen stain

Materials required:

- Slides with prepared smears
- Acid fast staining kit (HiMedia)
- Wash bottle
- Blotting papers
- Microscope, cedar wood oil.

Procedure:

1. Prepare a smear from suspected bacterial culture.
2. Place the numbered slides on a staining rack with the smeared side facing up.
3. Flood the entire surface of slide with Ziehl-Neelsen 1% Carbol fuchsin solution.
4. Heat the slide slowly until steam arises. Maintain steaming for 5 minutes by using intermittent heat.
5. Rinse the slide under a gentle flow of water until all free stain washes away. Drain off excess water by tilting the slide.
6. Flood the slide with the decolorizing solution (25% Sulphuric acid) for 3 minutes. (If the slide is under decolorized after 3 minutes, further decolorize for 1 minute.)
7. Rinse the slide thoroughly with water. Drain off excess water from the slide. Wipe the back of slide with cotton soaked in decolorizer to clean the dried stains.
8. Rinse the slide again with water and drain off excess water by tilting the slide.
9. Flood the slide with 0.1% Methylene blue and counter-stain for 60 seconds.
10. Rinse the slide thoroughly with water and drain off excess stain by placing the slide under gentle stream of running water.
11. Stand the rinsed slide on the slide holding block and allow the smear to air dry.
12. Examine under oil immersion and record the observation



Acid Fast Bacilli stained as bright pink colour

7.3 Staining of fungus with lactophenol cotton blue stain

Staining of fungal cells like *Dermatophytes*, *Rhizopus, Aspergillus, Penicillium, Fusarium, Candida, Mucor* etc. Stain fungal cells by using Lactophenol cotton blue method.

Requirement

1. Young fungal culture
2. Glass slide
3. Cover slip
4. Needle
5. Lactophenol cotton blue.

Procedure

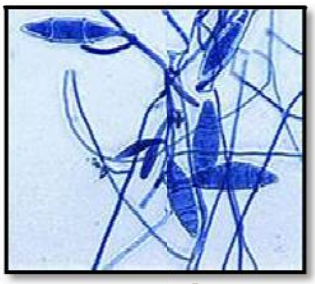
1. Take a clean grease free slide.
2. Add a drop of mounting fluid that is lactophenol cotton blue solution on a slide.
3. Sterilize the needle and cool it then transfer a 40olostr mat on fluid and press it gently so that it easily mix with the stain.
4. Take a clean cover slip and with the help of a forceps place the cover slip on 40olostr mat.
5. Take a blotting paper and wipe the excess stain .
6. Observe under low to high power objectives of microscope.

Observation

After staining fungus cell observe different parts of a cell like conidia, hyphae as well as 40olostrums40es etc.

Result

On basis of observation the type fungal organisms identified.



Dermatophyte

Penicillium

8. DIAGNOSIS OF BOVINE MASTITIS

The term mastitis refers to inflammation of the mammary gland which is characterized by physical, chemical as well bacteriological changes in the milk and pathological changes the udder tissue.

Detection of Mastitis

Physical examination of the udder: In clinical mastitis the udder may turn hard, red, and hot to the touch. Palpation of the udder may be painful to the cow. These symptoms arise from the changes in vascularity and blood flow of the gland when inflamed.

Visualization of the milk in clinical mastitis: Gross changes in the milk may be observed at the time of milking such as the presence of flakes, clots or serous milk. This is the most common means of detection of clinical mastitis. Stripping the first few squirts of milk from each quarter into a strip cup at the beginning of milking is a preferred method of detecting flakes or clots in the milk.

Visualization of the milk in sub-clinical mastitis: Sub-clinical mastitis is the most economically devastating condition in diary industry. There is no apparent gross change in the milk at the time of milking. There will be drop in milk yield, formation of curd in boiling, bitter or salty taste.

Aseptic milk sample collection:

i. Wear disposable gloves, arrange marking pen, milk sample tubes, cotton soaked in 70% alcohol, cooler with ice or freezer packs.
ii. Label the sample tube with the date, the cow ID and the quarter.
iii. Clean the udder and the teat and dry using cloth towel.
iv. Discard 3 to 4 streams of milk on the floor to minimize chances of contaminating the sample with bacteria in the teat canal.
v. Scrub teat ends using a cotton ball or gauze pad soaked in 70% alcohol.
vi. Open the sample vial immediately before the sample is taken and collect direct streams of milk into the vial without touching the teat end. Immediately place the sample vial on ice or in the refrigerator and dispatch to the lab.

8.1 California mastitis test (CMT)

Principle: The mastitis reagent reacts with genetic material of somatic cells present in mastitis milk to form a gel/precipitate.

Requirement:

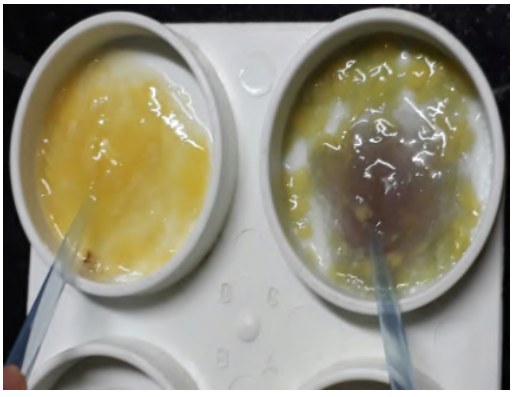
1. Mastitis reagent
2. Milk samples
3. Mastoid paddle/ Glass slide
4. Pasteur pipettes

Procedure:

A. Using mastoid paddle

- A plastic paddle having four shallow cups marked A, B, C and D for easy identification of the individual quarter is to be used.
- Add approximately 1 teaspoon (5 ml) of milk in equal amount of the reagent.
- Mix the contents thoroughly by a circular rotating motion. Formation of gel will appear within ten seconds.
- Reading should be completed within 20-40 seconds.

Interpretation of result:



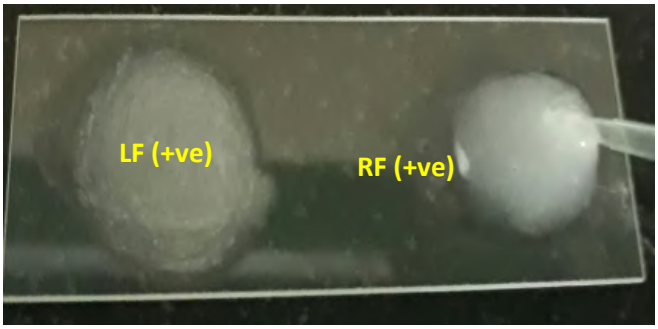
Gel formation in mastitic milk

No gel formation in normal milk

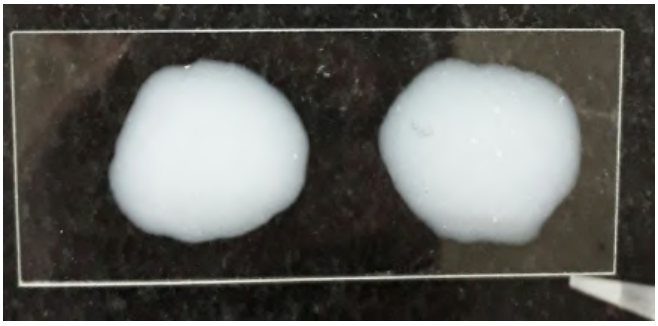
B. Using microscope slide

- Mark slide 1 as LF & RF, slide 2 as LH and RH.
- Add 3 drops of mastitis reagent to each labeled area.
- Add 3 drops of milk sample from each quarter to identified area.
- Mix thoroughly with clean individual stick for few seconds.
- Categorize milk sample based on degree of gel formation.

Interpretation of result:



Gel formation in mastitic milk



LH (-ve) RH (-ve)

No gel formation in normal milk

(LH & RH)

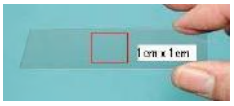
8.2 Somatic cell count (SSC) test

Due to infection in udder somatic cells, mainly epithelial cells of mammary gland and white blood cells are entered into the milk secretion. Higher the number of somatic cell count (SCC) higher the severity of inflammation of the udder.

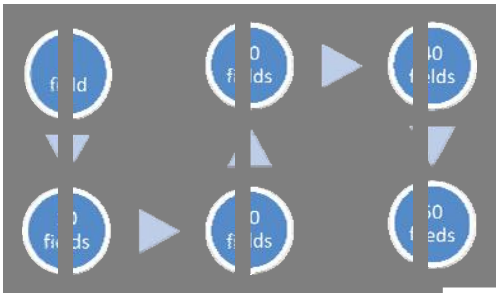
Requirements:

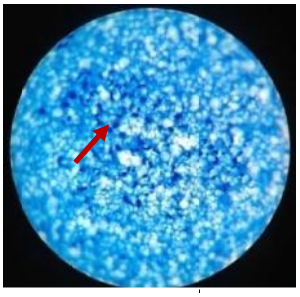
1. Milk samples.
2. Clean glass slides.
3. Staining solution.
4. Compound microscope.

Preparation of smear and staining:



- Mark a clean microscopic slide as 1 cm x 1 cm area with a marker/ diamond pencil/ glass marker.
- Transfer one loopful of properly mixed milk to the marked area with the help of wire loop .
- Make a thin smear by spreading the milk to cover the 1 cm^2 area on the glass slide and allow to air dry.
- Cover the smeared area with staining reagent (Loeffler's Methylene Blue) and allow to react for 2 – 3 minutes
- Wash the stain and allow to air dry. Examine under oil immersion microscope and count the number of Somatic cells in total 50 fields as indicated below.





Somatic Cells stained with methylene blue stain

Calculation of SCC:

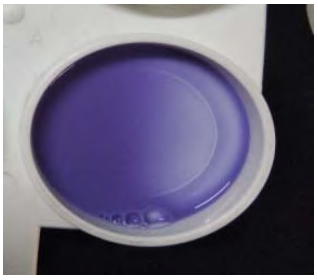
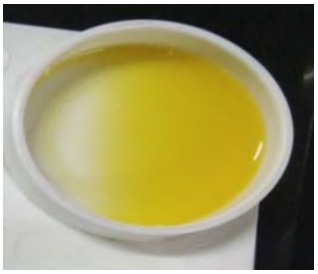
Total number of cell counts in 50 field = Y
Working factor= 10,000.
Total SCC per ml of milk = Y x 10,000

Interpretation:

- 1,00,000 cells/ml is often considered to be 'normal', reflecting a healthy mammary gland
- SCC of >2,50,000 cells/ml is suggestive of bacterial infection (Subclinical mastitis).
- Milk with an SCC of more than 4,00,000 is deemed unfit for human consumption.

Summary of test results

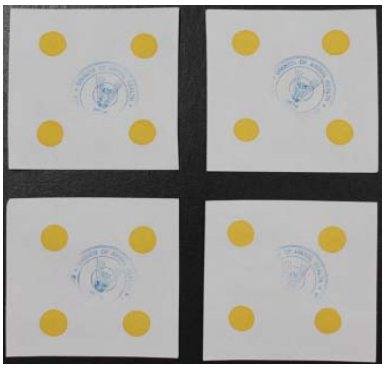
Test Score	Suggested meaning	Description of test reaction	Reaction picture in CMT	Somatic cell count with interpretation
0	Negative	The reaction mixture remains liquid		0-2,00,000 cells/ml **Normal Milk**
1	Weak positive	A distinct slime adhering towards periphery, but with no tendency towards gel formation. The reactions tend to disappear with continued movement of the fluid.		>1,50,000-5,00,000 cells/ml **Sub-clinical Mastitis**

2	Distinct positive	The reagent mixture thickens immediately with gel formation. The mixture spread out again when the motion is stopped and covers the bottom of the cup.		>5,00,000 cells/ml **Clinical Mastitis**
+	Alkaline milk, pH 7.0 or over	The reaction mixture is indicated by a contrasting deeper purple colour.		This type of milk reflects depression of secretary activity and may results either from inflammation or in drying off of the gland.
Y	Acid milk	The bromocresol purple is distinctly yellow at pH 5.2. This type of indication should be added to the score when the mixture is yellow.		Acidic milk in the udder is rare. It indicates fermentation of lactose by bacterial action within the gland.

8.3 Detection of mastitis using Mastitis Detection Card

Potential users:

- Dairy farmers
- Milk cooperative societies
- Para-veterinarians
- Veterinarians
- Byre-side test



Procedure:

1. Take out a piece of dry Mastitis Detection Card Test paper.
2. Make sure that the stamped surface is on top.
3. Put one drop of milk from each quarter of the udder on the four marked corners (already treated with indicator) of card/paper.
4. Observe the colour change, if any.
5. After brief drying, interpret the change of colour according to the colour guide provided with the kit.

Colour of paper	Mastitic condition
Colour remain as such (yellow)	No mastitis
Greenish-yellow	+
Green	++
Greenish-Blue	+++
Blue-Deep Blue	++++

PRECAUTION

1. Avoid using kit with wet hands.

9. ANTIBIOTIC SENSITIVITY TEST

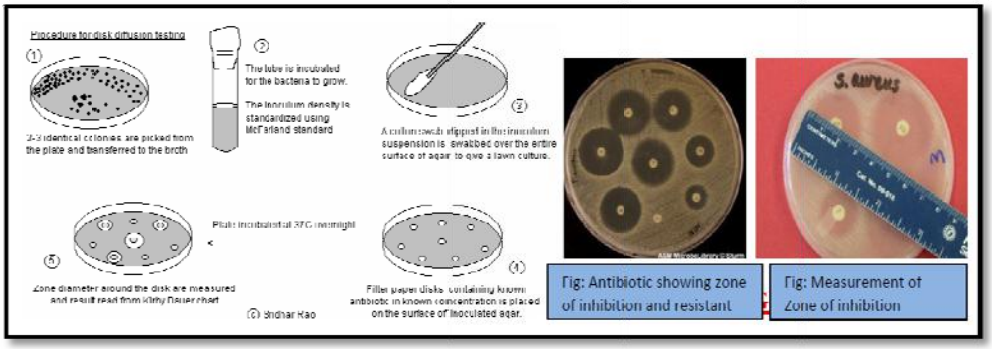
Once the causative organism of a disease is isolated and identified, it is essential for clinicians to know which antibiotic will be most effective for treatment. Antibiotic sensitivity testing by disc diffusion method (Kirby-Bauer Method) can readily provide this information.

Materials required:

1. Nutrient agar plate
2. Nutrient broth culture to be tested in tube
3. Antibiotic disc
4. Sterile swab
5. Forceps
6. Bunsen burner

Procedure:

1. Select the organism that you are going to test
2. Label the plate with isolate number and date
3. Inoculate the surface of the medium with swab after expressing excess fluid from the swab by pressing and rotating the swab against inside wall of tube above fluid level. Cover the surface of the agar evenly by swabbing in three directions. A final sweep should made on the agar rim with the swab.
4. Allow 3 to 5 minutes for the agar surface to dry before applying disc
5. Dispense discs as follow:
   Sterilize forceps first by flaming before picking discs. Keep each disc at least 15mm away from edge of plate. Apply gentle pressure to each disc on the agar with the tip of the sterile forceps.
6. Invert and incubate the plate for 16-18hrs at 37^{o}C.
7. After incubation measure the diameter of the zone of inhibition to the nearest in mm. Record the zone of inhibition and consult the code chart supplied by disc manufacturing company and accordingly determine resistant and sensitivity pattern of the test organism.



10. DIAGNOSIS OF BRUCELLOSIS

10.1 Rose Bengal Plate Test (RBPT)

Principle

RBPT is a simple spot agglutination test used in many species as a screening test prescribed for international trade. The buffered acid Rose Bengal antigen interacts with serum antibody to produce agglutination, which is used for the detection of *Brucella* specific antibodies.

Materials required

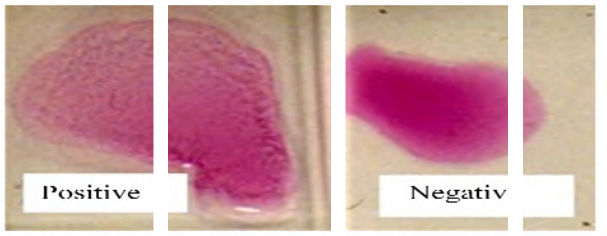
a. Rose Bengal Antigen
b. Test serum
c. Positive Control serum
d. Negative Control serum
e. Slides, tips, micropipettes, magnifying lens, etc.
f. Wooden pricks

Procedure

1. Bring the serum samples and antigen to room temperature (22 ± 4°C); only sufficient antigen for the day's tests should be removed from the refrigerator.
2. Place 25–30 µl of each serum sample on a microscopic slide, white tile, enamel or plastic plate.
3. Shake the antigen bottle well and place an equal volume of antigen near each serum spot.
4. Mix the serum and antigen thoroughly using a clean glass/ plastic rod/ micro tip for each test to produce a circular or oval zone approximately 2 cm in diameter.
5. The mixture is agitated gently for 1-3 minutes at ambient temperature on a rocker or clock and anticlock wise direction 5-10 times.
6. Read for agglutination immediately within 3-minute period. Any visible reaction is considered to be positive. A control positive and negative serum that gives a minimum positive reaction should be tested simultaneously each day to verify the sensitivity of test conditions.

Interpretation

- ✓ Any degree of agglutination varying from mild (+), moderate (++) and strong (+++) positive = recorded as positive



- ✓ No agglutination = recorded as negative.
- ✓ Include positive and negative controls while testing each time.

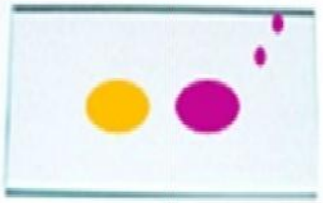
The RBPT is very sensitive. False-negative reactions occur rarely, mostly due to pro-zoning and can sometimes be detected by diluting the serum sample (1:2, 1:4, 1:8 dilution) or retesting after 3 weeks time. Nevertheless RBPT appears to be adequate as a screening test for detecting infected herds or to guarantee the absence of infection in brucellosis-free herds.

Procedure of RBPT

Bring serum & antigen to room temp (22 ± 4°C)

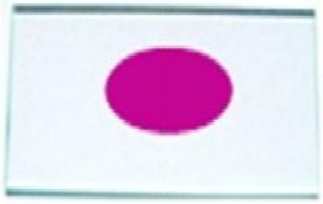
Serum

Add equal volume serum and antigen (25–30 µl) on a white tile or glass slide

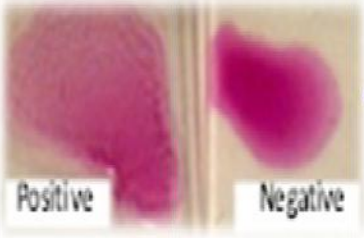


Colour antigen

Mix the serum & antigen (using a same tip) circular zone of 2 cm



Read agglutination upto 1-3 minutes



- False positive
  - Cross reactions
- False negative
  - Prozone phenomena

10.2 Milk ring test (MRT)

Principle

Milk ring test is a screening test of great value for locating infected cattle herds, especially in areas of low prevalence. An efficient means of screening dairy herds is by testing milk from the bulk/ pooled tank. The principle of milk ring test is immunoglobulins present in the milk attached to fat globules *via* the Fc portion of the molecule. If antibody to *Brucella* sp. Is present it move to the top along with the fat globules and combine with antigen to form a ring in the milk and fat interface. If no antibody is present, the fat layer will remain as a buff colour and the antigen will be distributed throughout the milk. This test may be applied to individual animals or to pooled milk samples using a larger volume of milk relative to the pool size.

Milk can be collected easily and more frequently than blood samples and is often available centrally at dairies. When a positive test result is obtained, all dairy cows contributing milk should be tested using specific test.

Materials required for test

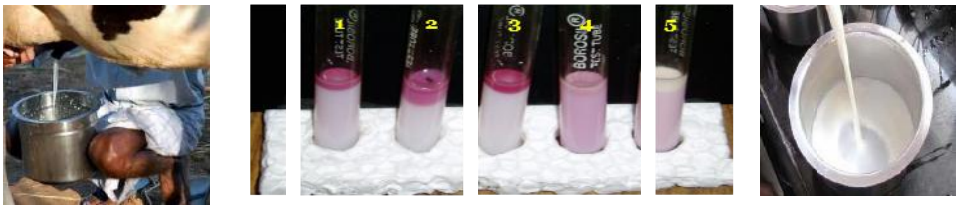
a) Brucella Milk Ring Antigen (*B. abortus* S99)
b) Pooled milk samples 5ml each
c) Known Brucella positive milk sample
d) Known Brucella negative milk sample
e) Serological tubes, tips, micropipettes, test tube racks, incubator etc.

Procedure:

1. The test is performed on pooled milk sample preferably 1 ml of sample from 100 litres of milk (one pooled milk sample from 10 cows). If necessary, samples could be pretreated with preservative (0.1% formalin) for 2–3 days at 4°C prior to use.
2. If pooled milk samples from large herds exceeding 100litres up to 300 litres are to be examined, the volume of milk should be increased to 2 or 3 ml.

3. Bring the milk samples and antigen to room temperature (20 ± 3°C); only sufficient antigen for the day's tests should be taken out from the refrigerator. Gently shake the antigen bottle well.

4. The milk samples must not get frozen, heated, subjected to violent shaking or stored for more than 72 hours. Test should be done with fresh milk (not pasteurized/ boiled/ curdled).
5. The test is performed in a serological test tube where the height of the milk column in the tube must be at least 25 mm. The volume of MRT antigen should be 2 drops / 1 ml milk to be tested from pooled milk or 50-75 µl of MRT antigen to a 2 ml volume of milk obtained from more than 100 litres and less than 300 litres of pooled milk.
6. The milk/antigen mixtures are mixed gently and incubate at 37°C for 1 hour, together with positive and negative working controls. However, further 2 hr incubation at 4°C increases the sensitivity of the test and allows for easier reading



Milk ring test: tube 1, positive control, tube 2 & 3 positive samples, tube 4, negative sample, tube 5, negative control

Interpretation

Color of cream ring	Color of milk column	MRT Reading
Definite red	white	++++
Definite red	slightly red	+++
Definite red	definite red	++
Same color as milk	same color as Cream milk	+ or – ve
White or slightly red	red milk	- ve

False positive reactions may occur in cattle vaccinated less than 4 months prior to testing, in case of colostrum and mastitic milk (OIE Manual, 2008). Therefore, it is not recommended to use this test in very small farms where these problems have a greater impact on the test results.

11. DIAGNOSIS OF BOVINE TUBERCULOSIS

Single intra dermal test:

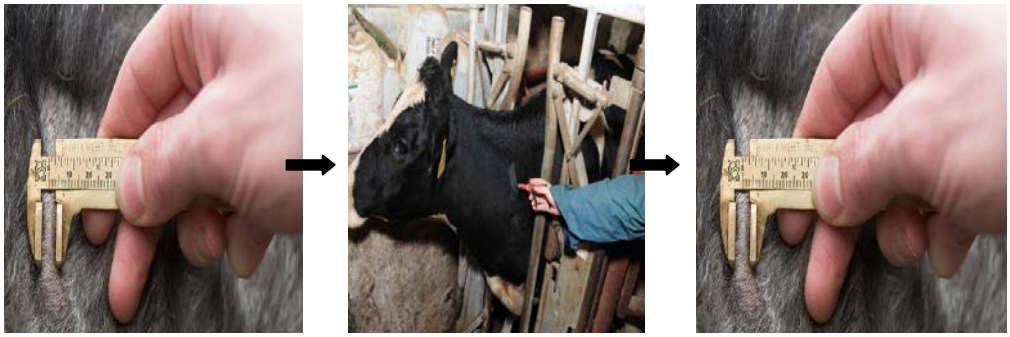
Requirements:

i. Tuberculin (Mammalian Tuberculin)/PPD
ii. Vernier calipers
iii. Intra dermal needle

Procedure:

- An area on the side of the neck of the suspected animal is clearly marked.
- Shave the area aseptically.
- Measure the thickness of the skin with the aid of a vernier caliper and record the thickness.
- Inject 0.1 ml of the tuberculin intra-dermally using a intra dermal needle.
- Observe for any diffuse swelling or redness in the area after 48- 72 hours and also record the thickness of the skin with a vernier caliper.

Observation:
Increase in the thickness of the skin by **> 4 mm** & **diffuse swelling**: **Positive result**.
Increase by **3mm: Doubtful reaction**.



Step -1 Step-2 Step-3(After 48-72 hrs)

12. AGAR GEL PRECIPITATION TEST (AGPT) FOR DETECTION OF IBD IN POULTRY

Principle: Antigen and antibody placed in the wells of the agar or agarose gel diffuse towards each other and form a visible opaque band of precipitation in the optimal proportion.

Materials required:

1. Microslides
2. 1% agarose in PBS (pH-7.2-7.4) 0.02% sodium azide
3. Antigen (Bursa of Fabricius suspension)
4. Serum sample (Known positive IBDV sera)
5. Gel puncher
6. Template
7. Needle
8. Micropipette

Precoating of slides:

1. Dissolve the agarose by boiling and add sodium azide@0.02%.
2. Place the slide on a horizontal surface and slowly pour the agarose on the slide.
3. Allow the agarose to solidify.

Procedure for AGPT:

1. Prepare the slide as described above.
2. Place the slide over a template and punch wells of 2 to 3 mm in diameter and 4 to 5mm apart with gel punch.
3. Remove the cut gel from the wells by scooping with needle.
4. Fill the central well to brim with 20µl neat serum and peripheral wells with 20 µl antigen. Alternatively fill one peripheral well NSS or PBS as control.
5. Place the slides in humid chamber and incubate overnight at room temperature.
6. Examine the slides for precipitin lines.

Interpretation: Precipitin lines appear between antigen and antibody wells in positive reaction and interpreted as bird infected with IBDV.

13. VIRAL HAEMAGGLUTINATION TEST

Principle: The test is based on the binding of certain viruses to the erythrocytes receptors of certain avian and mammalian species and thereby causing agglutination of the erythrocytes. Unlike other serological test, viral haemagglutination test does not involve any antibody. It is used for identification and quantification of certain viruses like Newcastle disease virus in infected tissues of poultry.

Materials required:

1) U/V bottomed microtitre plate
2) Pipette (20-200 µl)
3) Disposable syringe-2ml
4) Tips-20-200 µl
5) Centrifuge tube-15ml
6) Ice Box
7) Centrifuge machine

Chemicals required:

Sl No	HAEMAGGLUTINATION TEST		HAEMAGGLUTINATION INHIBITION TEST	
	Reagents	**Storage**	**Reagents**	**Storage**
1	Phosphate buffer saline (pH-7.5)	4^0C	Chicken sera (Hyper immune sera)	-20 ^{0}C
2	1% Chicken red blood cells (RBCs) in Alsever's solution (Page No. 29)	4^0C	Antigen 4HAU	-20 ^{0}C
3	Antigen	-20 ^{0}C	Chicken red blood cells (RBCs) in Alsever's solution	4^0C

Preparation of antigen:

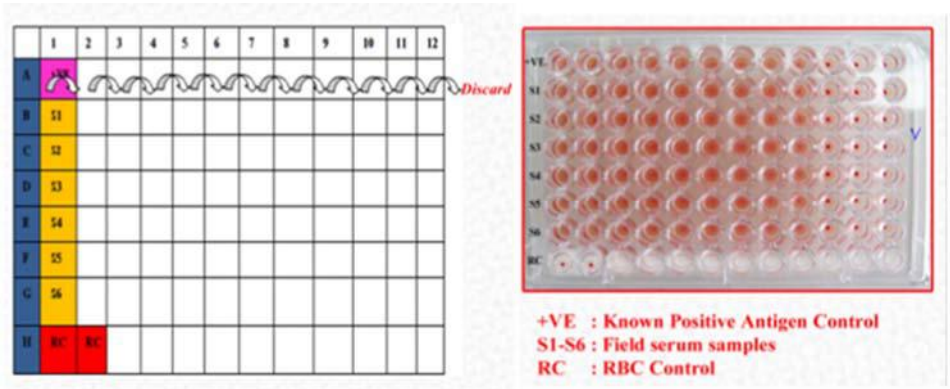
A 20% suspension of spleen and lung tissue of dead bird is prepared in PBS.

Procedure:

- 25µl of PBS is dispensed into each well of a plastic V-bottomed microtitre plate.
- 25µl of the viral antigen is placed in the first well of A1.
- 25µl of respective test samples are distributed in the first well of B1,C1…..G1.
- Two fold serial dilutions of 25µl volume of the virus antigen is prepared separately across the plate (from well-1 to well-12 of each row) using individual tip and 25µl volume of virus suspension from the last well is discarded.
- 25µl of 1% (v/v) chicken RBCs is dispensed to each well along with control RBCs well in H1 & H2.
- The solution is mixed by tapping the plate gently. The RBCs are allowed to settle for about 30 minutes at room temperature.

Observation and interpretation:

In positive cases, a layer of agglutinated RBCs in the form of a matt on the bottom of the well is formed, whereas no agglutination of RBCs, it appears as sharp button of RBCs. The highest virus causing complete agglutination of RBC is termed as one haemagglutination unit-HAU. HA is determined by tilting the plate and observing the presence or absence of tear shaped streaming of the RBCs.



13.1 Viral haemagglutination inhibition test

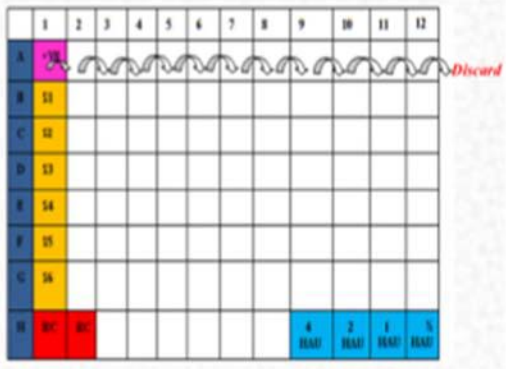
Principle: When certain viruses like ND virus mixed with serum containing specific antibodies, an Ag-Ab reaction occurs which subsequently inhibits the haemagglutinating property of the virus. It is used for detection and estimation of antibody in the serum of birds infected with ND virus.

Procedure:

- 25µl of PBS is dispensed into each well of a plastic V-bottomed microtitre plate.
- 25µl of serum is placed into the first well of the plate (A1) and test serum samples are in B1 to G1 of the plate.
- Two fold serial dilutions of 25µl volumes of the serum are made across the plate and discard from the last well.
- 4 HAU virus/antigen in 25µl is added to each well and the plate is left for a minimum of 30 minutes at room temperature.
- HAU control is kept at wells H9, dilute serially up to H12 yielding 4 HAU, 2 HAU, 1 HAU and ½ HAU, respectively.
- 25µl of 1% (v/v) chicken RBCs is added to each well including HAU control (H9 to H12) and RBC control (H1 and H2). After gentle mixing, the RBCs are allowed to settle for about 30 minutes at room temperature.

Observation and interpretation:

The HI titre is the highest dilution of serum (i.e. prior to agglutination well) causing complete inhibition of 4HAU of antigen. The agglutination is assessed by tilting the plates where the RBCs stream at the same rate as the HAU control wells.



+VE : Known Positive serum
S1-S6 : Field serum samples
RC : RBC Control
HAUC: Haenagglutination Unit Control

14. In-House Indirect ELISA for detection of CSFV antibodies in serum and plasma from pigs

Principle of the kit

The CSFV antigen used is a RK-13 adapted cell culture derived viral antigen stabilized using the Lactalbumin hydrolysate (LAH) stabilizer. This assay is an indirect ELISA which utilizes microplate coated with CSFV antigen. The antigen-antibody (specific to CSFV) forms a complex and an anti-porcine horseradish peroxidase conjugate is added which binds to the antibody indicating color

KIT COMPONENTS and STORAGE				
Sl. No	Components	Qty	Vol.	Storage
1.	Test plate	4	-	4°C
2.	Antigen (freeze dried)	1	-	-20°C
3.	Coating Buffer (Ready to use)	1	50 ml	4°C
4.	Wash concentrate (10X)	1	200 ml	4°C
5.	Blocking 1	1	4 g	4°C
6.	Blocking 2	1	10 g	4°C
7.	Diluent (Ready-to-use)	1	70 ml	4°C
8.	Positive control	1	10 µl	-20°C
9.	Negative control	1	10 µl	-20°C
10.	Conjugate	1	7 µl	-20°C
11.	OPD Tablets	4	-	4°C
12.	H_2O_2	1	500 µl	4°C
13.	Stop Solution(Ready-to-use)	1	50 ml	4°C

Preparation of reagents (one plate)

Antigen preparation

- Stock Antigen- dilute component 2 with 1ml of component 7 (Mix properly)
- Working Antigen (10ml)- take 500µl of the stock antigen and dilute with 9.5 ml of component 3 (Mix properly)

Wash solution

- The wash concentrate (10X) (Component 4) must be diluted 1:10 in distilled water. Example: for 500 ml wash solution, dilute 50 ml Wash Concentrate (10X) with 450 ml distilled water and mix well to give 1X Wash solution

Blocking solution

- 0.6 g of component 5 and 1.5 g of component 6 to be dissolved in 30ml of the Wash solution (1X)
- **Test sera (To be prepare in a deep well plate)**
- Add 100 µl of component 7 to the entire well. Add 0.5 µl of component 8 to wells A1 & B1 and component 9 to wells C1 & D1. Add 1 µl of the test sera into the remaining wells

Conjugate (Prepare before use)

- 1 µl of the conjugate (Component 10) added to 15 ml of 1X Wash Solution and mixed well

Substrate (Prepare before use)

- Take 10 ml of distilled water and add 1 tablet (light sensitive) (Component 11). Dissolve the tablet; add 40µl of component 12. (To be prepared away from direct light)

TEST PROCEDURE

1. Add 100µl of the diluted Antigen (working Antigen) to all the wells
2. Seal the plate and incubate at 37^{o}C for 1 hour
3. Wash each well with approximately 300µl of Wash solution 5 times. Aspirate liquid contents of all wells after each wash. Following the final aspiration, firmly tap residual wash fluid from each plate onto absorbent material. Avoid plate drying between washes and prior to next step
4. Add 300µl of Blocking solution to all the wells
5. Seal the plate and incubate at 37^{o}C for 1 hour
6. Repeat Step 3
7. Add 100µl of the Control sera and the Test sera from the deep well plate into the corresponding wells of the Test plate. (Contents should be mix by pippetting before adding to the test plate). Use a separate pipette tip for different Controls and Test sera
8. Seal the plate and incubate at 37^{o}C for 1 hour
9. Repeat Step 3
10. Add 100µl of the diluted Conjugate to all the wells
11. Seal the plate and incubate at 37^{o}C for 30mins
12. Repeat Step 3
13. Add 100µl Substrate (light sensitive) to all wells and incubate for 15mins in dark
14. Stop the reaction by adding 100µl Stop Solution (Component 13). Add the Stop Solution in the same order as the Substrate Solution was added
15. Measure the absorbance at 490 nm on a microplate reader.

N.B.: All reagents must be allowed to come to room temperature before use and mixed by gentle swirling or vortexing. Use separate tip for each test sample

CUT-OFF CALCULATION

Positive cut-off= Negative control x 2.5

INTERPRETATION

Equal or more than positive cut-off = Positive
Less than positive cut-off = Negative

	1	2	3	4	5	6	7	8	9	10	11	12
A	+ve C	TS 1	TS 9	TS 17	TS 25	TS 33	TS 41	TS 49	TS 57	TS 65	TS 73	TS 81
B	+ve C	TS 2	TS 10	TS 18	TS 26	TS 34	TS 42	TS 50	TS 58	TS 66	TS 74	TS 82
C	-ve C	TS 3	TS 11	TS 19	TS 27	TS 35	TS 43	TS 51	TS 59	TS 67	TS 75	TS 83
D	-ve C	TS 4	TS 12	TS 20	TS 28	TS 36	TS 44	TS 52	TS 60	TS 68	TS 76	TS 84
E	Ag blank	TS 5	TS 13	TS 21	TS 29	TS 37	TS 45	TS 53	TS 61	TS 69	TS 77	TS 85
F	Ag blank	TS 6	TS 14	TS 22	TS 30	TS 38	TS 46	TS 54	TS 62	TS 70	TS 78	TS 86
G	CC	TS 7	TS 15	TS 23	TS 31	TS 39	TS 47	TS 55	TS 63	TS 71	TS 79	TS 87
H	CC	TS 8	TS 16	TS 24	TS 32	TS 40	TS 48	TS 56	TS 64	TS 72	TS 80	TS 88

+ve C	*Known positive serum control*
-ve C	*Known negative serum control*
Ag blank	*Antigen Blank*
CC	*Conjugate Control*
TS1-TS88	*Test samples (At the ratio 1:100)*

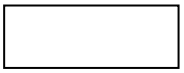
15. MICROSCOPIC EXAMINATION OF HELMINTH OVA (QUALITATIVE METHODS)

A) Direct method:

Requirements:

- Faecal Sample
- Microscopic slide & cover slip
- Match stick or bamboo stick
- Forceps
- Compound microscope

Procedure:

Take clean microscopic slide 

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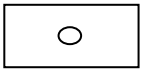
Place a drop of water at the centre of the slide 

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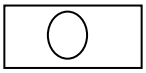
With a match stick or bamboo stick place a mass of the faecal sample over the drop of water 

⇩

Mix the sample with water 

⇩

Remove coarse materials with a stick or forceps 

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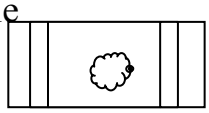
Put a cover slip over the sample 

⇩

First examine under low power and then under high power of a compound microscope for confirmation

C. Salt floatation method of faecal examination:

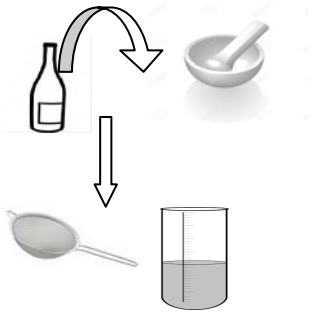
Take 2-3 g of faecal sample in a clean mortar 

⇩

Add a little amount of tap water and gently triturate the mixture to make it uniform using a pestle

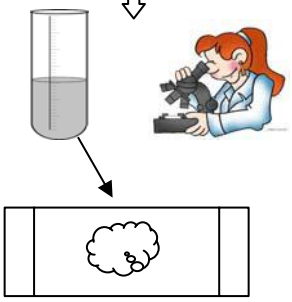
⇩

Add 10-15 ml of floatation fluid & mix thoroughly with the faecal sample



Strain the suspension through a sieve in a clean floatation tube and Allow to stand undisturbed for 10-15 minutes

⇩



With the help of a clean wire loop, transfer a drop of suspension from the surface on to a clean microscopic slide, put a cover slip over the drop and examine under low power and then under high power using a compound microscope

Alternative method:

- ✦ Make a suspension of the faecal sample with saline solution in a mortar using a pestle
- ✦ Strain the solution to a centrifuge tube and centrifuge for 1500 rpm for 5 minutes
- ✦ Discard the supernatant and add floatation fluid up to the brim
- ✦ Put a cover slip over the tube and centrifuge again for 5 minutes at 1500 rpm
- ✦ Carefully remove the cover slip and place it over a microscopic slide and examine under a compound microscope.

C) Sedimentation method:

Requirements:

- Pestle & mortar
- Water
- Strainer/sieve
- Microscopic slide
- Centrifuge tube
- Centrifuge machine
- Compound microscope

Procedure:

- ➢ Place about 1 g of faeces in a mortar and add a little quantity of water, triturate it using pestle
- ➢ Mix it thoroughly and strain to a centrifuge tube
- ➢ Fill the tube with water and centrifuge at 1500 rpm for 5- 10 minutes
- ➢ Discard the supernatant and repeat the above step by adding and discarding the water until the supernatant becomes clear
- ➢ Using a pipette, take a drop of the sediment onto a clean microscopic slide and add a drop of 1% iodine
- ➢ Place a cover slip and examine under the low and high power of a compound microscope

Alternative method:

- Place about 1 g of faeces in a mortar and add a little quantity of water, triturate it using pestle
- Mix it thoroughly and strain it to a petridish
- Allow to stand undisturbed for 10-15 minutes
- Discard the supernatant and examine the sediment directly under a compound microscope

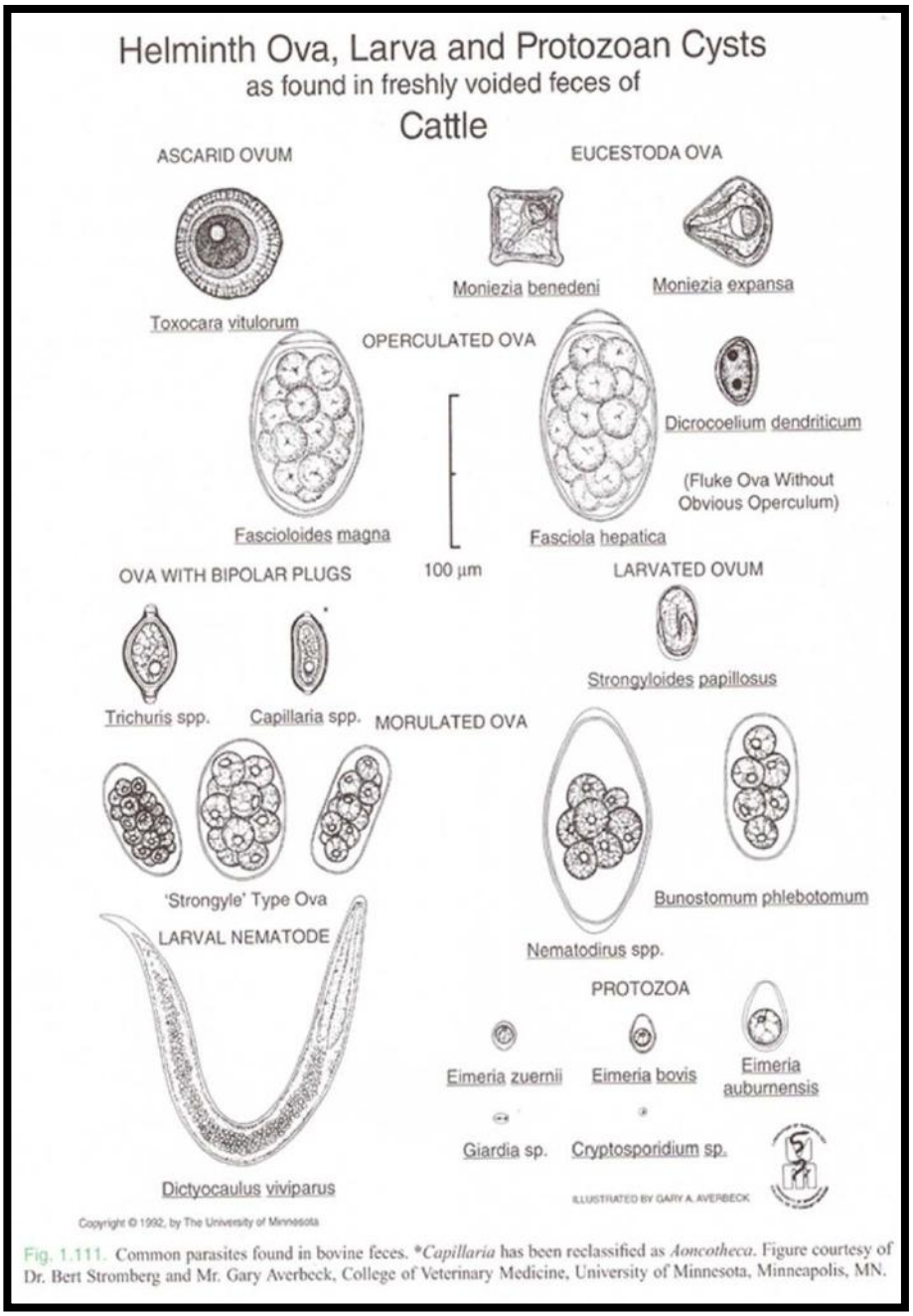


Fig. 1.111. Common parasites found in bovine feces. **Capillaria* has been reclassified as *Aoncotheca*. Figure courtesy of Dr. Bert Stromberg and Mr. Gary Averbeck, College of Veterinary Medicine, University of Minnesota, Minneapolis, MN.

Courtesy: Veterinary Clinical Parasitology, 8th Edition by Anne M. Zajac and Gary A. Conboy

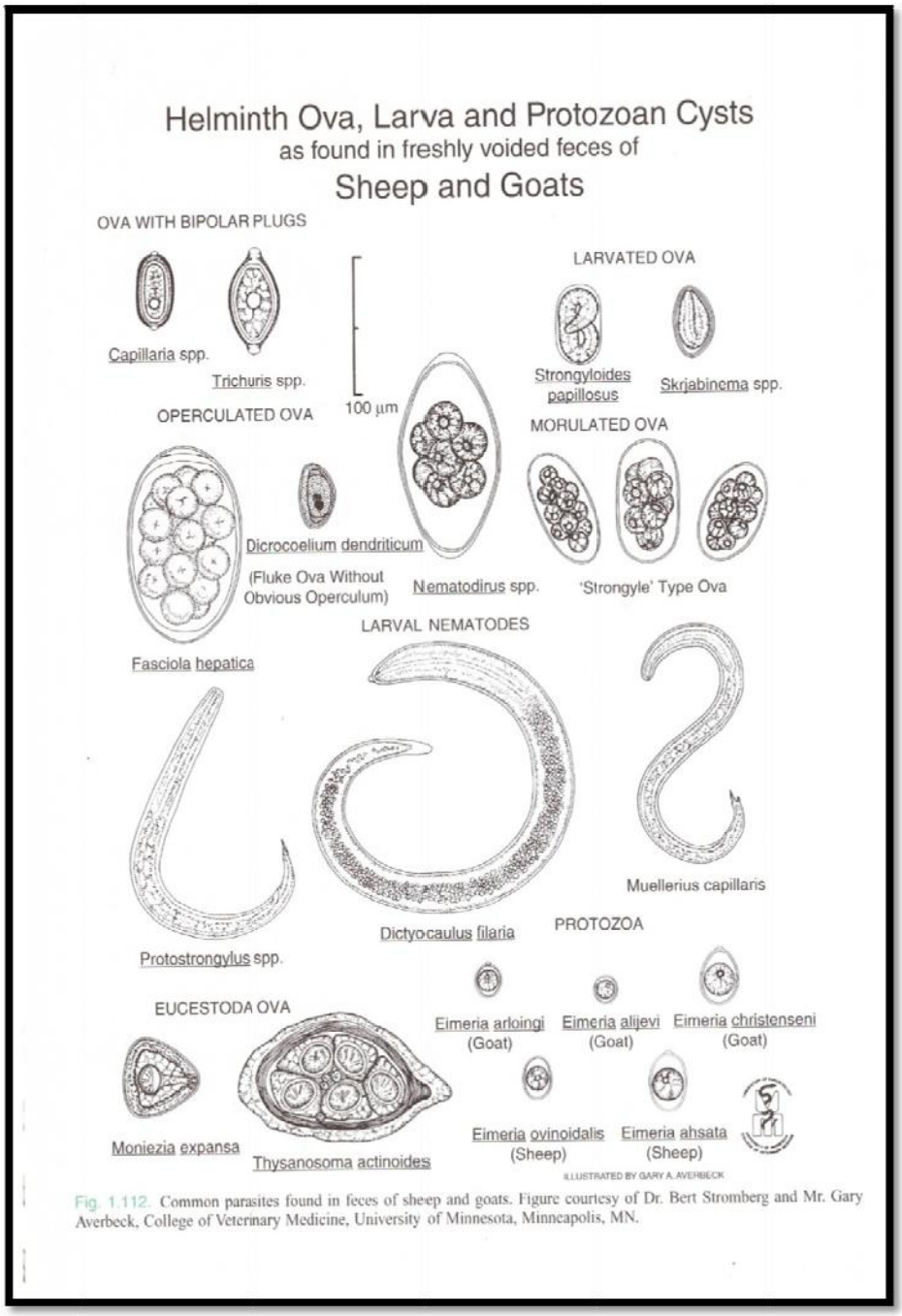


Fig. 1.112. Common parasites found in feces of sheep and goats. Figure courtesy of Dr. Bert Stromberg and Mr. Gary Averbeck, College of Veterinary Medicine, University of Minnesota, Minneapolis, MN.

Courtesy: Veterinary Clinical Parasitology, 8th Edition by Anne M. Zajac and Gary A. Conboy

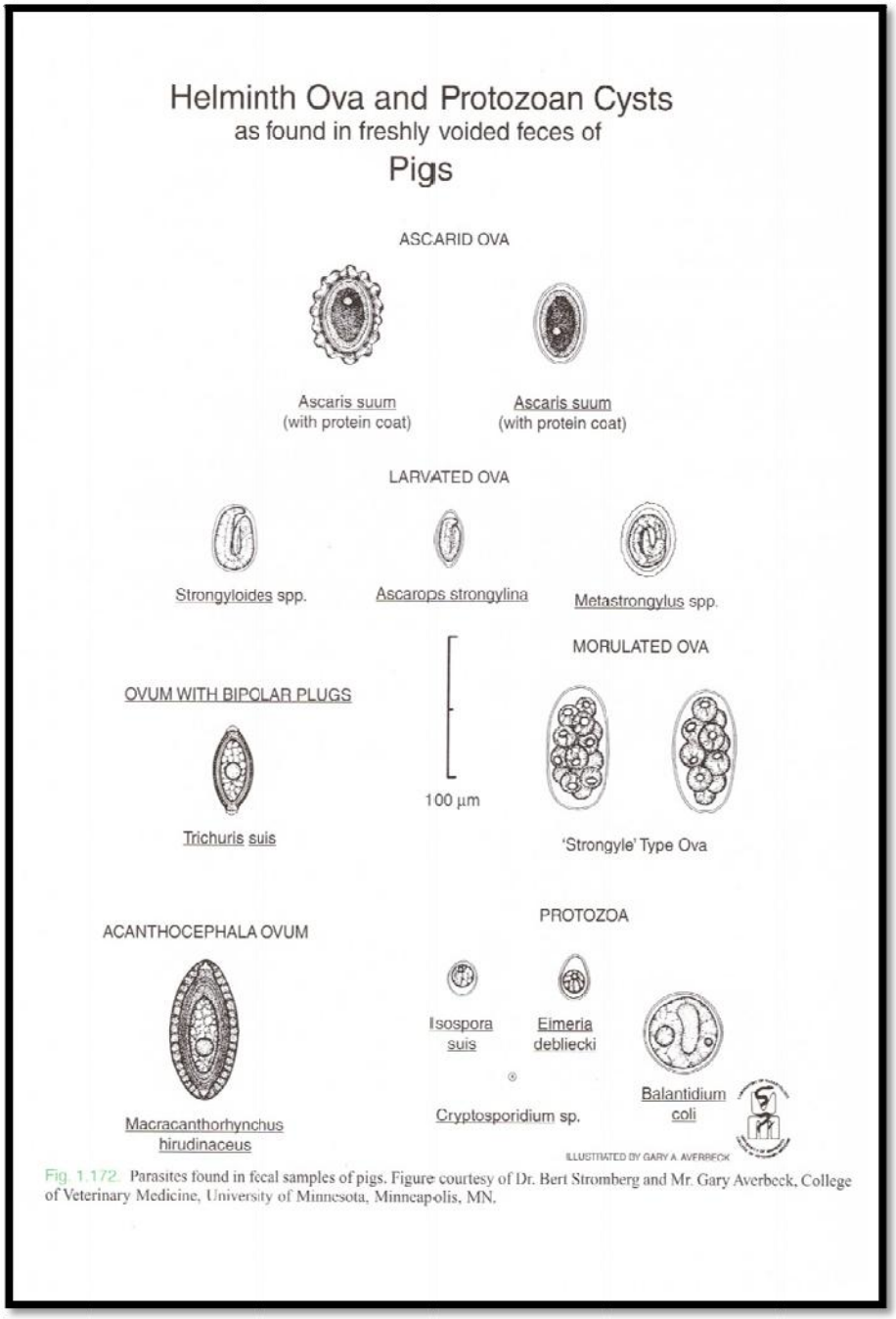


Fig. 1.172. Parasites found in fecal samples of pigs. Figure courtesy of Dr. Bert Stromberg and Mr. Gary Averbeck, College of Veterinary Medicine, University of Minnesota, Minneapolis, MN.

Courtesy: Veterinary Clinical Parasitology, 8th Edition by Anne M. Zajac and Gary A. Conboy

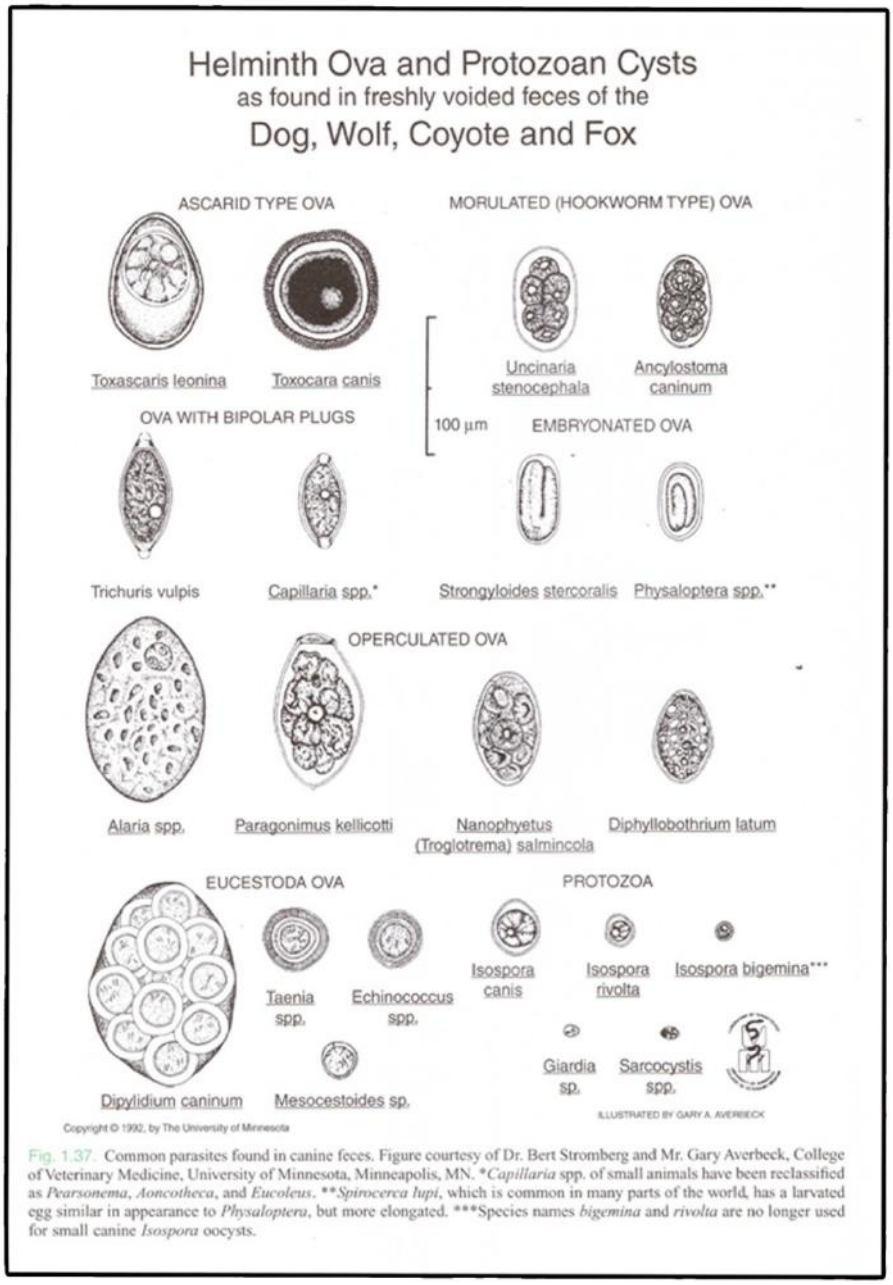
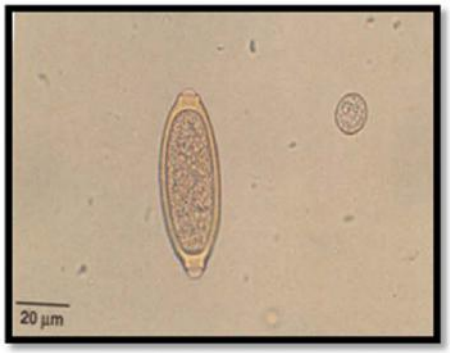


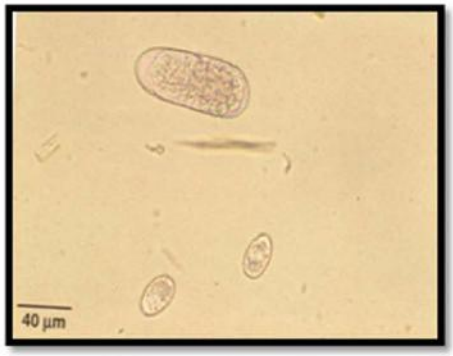
Fig. 1.37. Common parasites found in canine feces. Figure courtesy of Dr. Bert Stromberg and Mr. Gary Averbeck, College of Veterinary Medicine, University of Minnesota, Minneapolis, MN. **Capillaria* spp. of small animals have been reclassified as *Pearsonema*, *Aoncotheca*, and *Eucoleus*. ***Spirocerca lupi*, which is common in many parts of the world, has a larvated egg similar in appearance to *Physaloptera*, but more elongated. ***Species names *bigemina* and *rivolta* are no longer used for small canine *Isospora* oocysts.

Courtesy: Veterinary Clinical Parasitology, 8th Edition by Anne M. Zajac and Gary A. Conboy

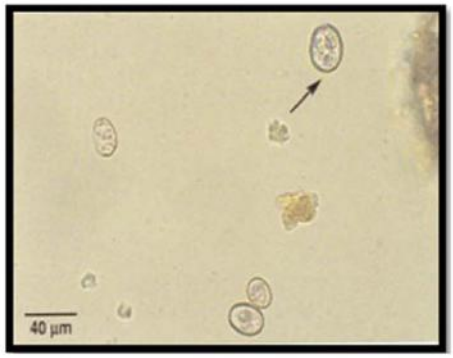


Eggs of *Capillaria Spp.* in avian faecal sample

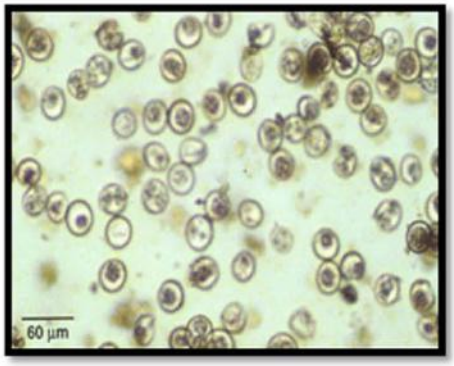
Eggs of *Capillaria Spp.* in avian faecal sample



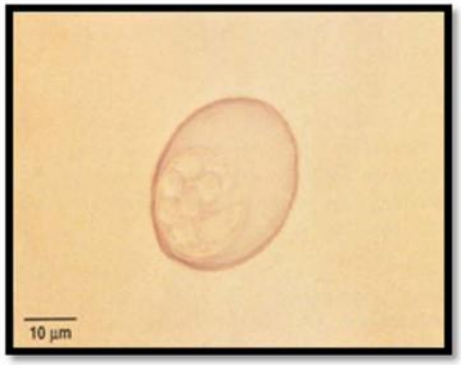
Strongylied eggs and ***coccidia*** oocysts in avian faecal sample



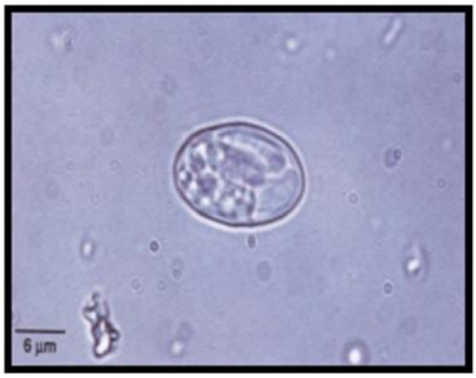
Sporulated oocyst of ***Eimeria*** Spp.in avian faecal sample



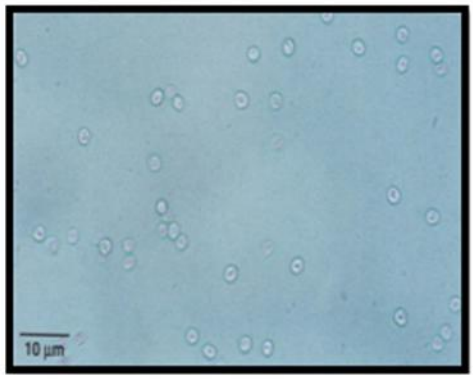
Oocyst of ***Isospora*** Spp. in avian faecal sample



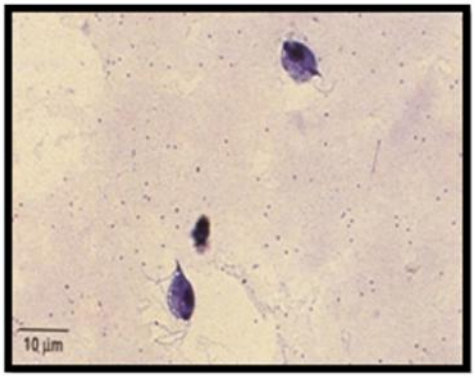
Oocyst of ***Caryspora*** Spp. in avian faecal sample



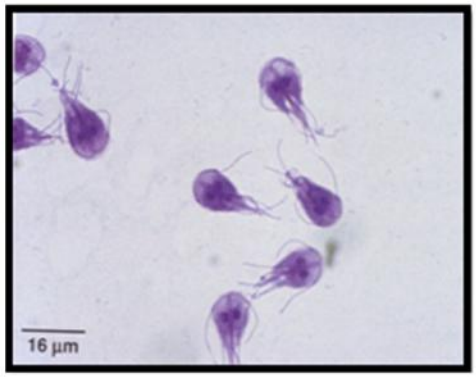
Oocyst of ***Sarcocystis*** Spp.
in carnivorus birds faecal sample



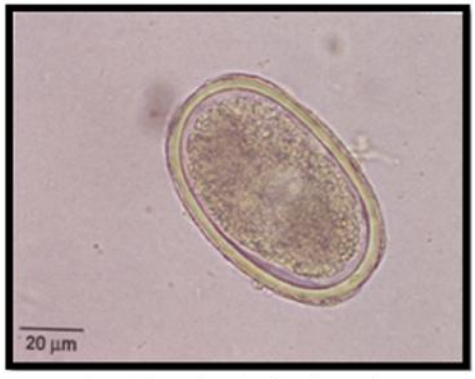
Oocyst of ***Cryptosporidium*** Spp.
in avian faecal sample



Oocyst of ***Trichomonas*** Spp.
in avian faecal sample



Giardia trophozoites
in avian faecal sample



Ascarid eggs in avian faecal sample

15.1 Microscopic examination of protozoa in faecal sample

A) Direct examination of fresh faecal smear:

Requirement:

- Fresh faecal sample
- Microscopic slide
- Cover slip
- Tooth pick/ wooden prick
- Saline solution
- 1:1000 aqueous solution of eosin
- Lugol's iodine (Page no-31)

Procedure: (Without staining)

- Place a drop of saline solution on a clean glass slide
- Take a small amount of freshly collected faecal sample with a tooth pick on a glass slide and mix thoroughly with the salt solution
- Remove the coarse particles aside using the tooth prick
- Place a cover slip over the drop of wet smear
- Examine over low and high power of a microscope

Procedure: (With stain):

- Place a drop of saline solution on a clean glass slide
- Take a small amount of freshly collected faecal sample with a tooth pick on a glass slide and mix thoroughly with the salt solution
- Remove the coarse particles aside using the tooth prick
- Add a drop of 1:1000 eosin and mix thoroughly to stain the debris pink leaving the trophozoites and cysts unstained
- Add a drop of iodine to stain the nucleus and the chromatoid bodies
- Place a cover slip and examine under low and high power of a compound microscope

B) Floatation method:

Requirements:

- Faecal sample
- Pestle & mortar
- Strainer/sieve
- Narrow cylindrical container
- Coverslip
- Micro slide
- Wire loop
- Compound microscope
- Floatation solution
- Centrifuge tube
- Centrifuge machine

Procedure:

- Take 1 gm of faecal sample in a clean mortar
- Add a little quantity of tap water and homogenize the mixture using a pestle
- Add 10-15 ml of floatation fluid and mix well
- Strain the suspension through a strainer to a narrow cylindrical tube
- Allow to stand in the tube undisturbed for 10-15 minutes with or without putting a cover slip

Or

- Strain the suspension to a centrifuge tube and centrifuge at 1500 rpm for 5 minutes
- Transfer a loop of the suspension from the surface of the tube onto a clean microscopic slide and place a cover slip
- Examine under low and high power of a compound microscope

B) **Sedimentation method**:

Requirements:

- Pestle & mortar
- Strainer/sieve
- Petridish
- Tap water
- Microscope
- Microscopic slide
- Pipette
- Centrifuge tube
- Centrifuge machine

Procedure:

- ✓ Place 1 gm of faeces in a mortar and mix thoroughly with a little quantity of water with a pestle
- ✓ Strain in to a Petridish or a centrifuge tube
- ✓ Allow the petridish to stand undisturbed for 10-15 minutes or centrifuge the tube at 1500 rpm for 5 minutes
- ✓ Discard the supernatant gently from the petridish or centrifuge tube
- ✓ Examine the petridish directly under the microscope or place a drop of the sediment from the centrifuge tube on a clean micro slide and examine under the microscope.

C) Formol ether technique for detection of *Giardia* & *Entamoeba* cysts:

Requirements:

- Pestle & mortar
- Strainer
- Petridish
- Tap water
- Microscope
- Microscopic slide
- Pipette
- Centrifuge tube
- Centrifuge machine
- Formol saline
- Ether
- Iodine (1%)

Procedure:

- Take about 1 g of faeces in a mortar and emulsify with 5 ml of 10% formol saline
- Strain through a strainer in to a clean centrifuge tube
- Add equal volume of ether and shake vigorously after inserting a rubber stopper
- Remove the stopper and let it stand for 2 minutes
- Centrifuge at 2000 rpm for 2 minutes and a ring of faecal debris will appear between the ether and formalin layer, leaving the sediment at the bottom
- Loosen the debris ring with an applicator
- Pour off the supernatant and debris ring carefully without disturbing the sediment
- Add a drop of saline solution to the sediment and mix well
- Take a drop of sediment with a pipette on a clean glass slide and add a drop of iodine solution
- Place a cover slip over the drop and examine under the low and high power of a compound microscope.

D) **Ziehl Neelsen staining technique for detection of cryptosporidium oocysts**:

Requirements:

Faecal sample	Immersion oil
Acid alcohol	Microscopic slide
Carbol fuchsin	Cover slip

Procedure:

- ✦ Prepare a thin faecal smear, air dry or pass over a flame
- ✦ Stain with carbol fuchsin for 2 minutes
- ✦ Rinse with tap water
- ✦ Rinse with acid alcohol for a 3-5 seconds
- ✦ Rinse again with tap water
- ✦ Counter stain with brilliant green or 1 % aqueous methylene blue for 1-2 minutes
- ✦ Rinse again with tap water
- ✦ Air dry
- ✦ Apply cover slip and examine under 100 X using oil immersion.

Commonly used floatation fluids:

1. Sodium chloride: sp. Gravity-1.2
2. Zinc sulphate: Sp. Gravity-1.18 (386 gm in 1000 ml dist. Water)
3. Sheather's sugar solution: Sp. Gravity-1.12-1.30 (Sucrose-500 gm, Phenol -6.5 gm melted in hot water bath and dist. Water-320 ml)

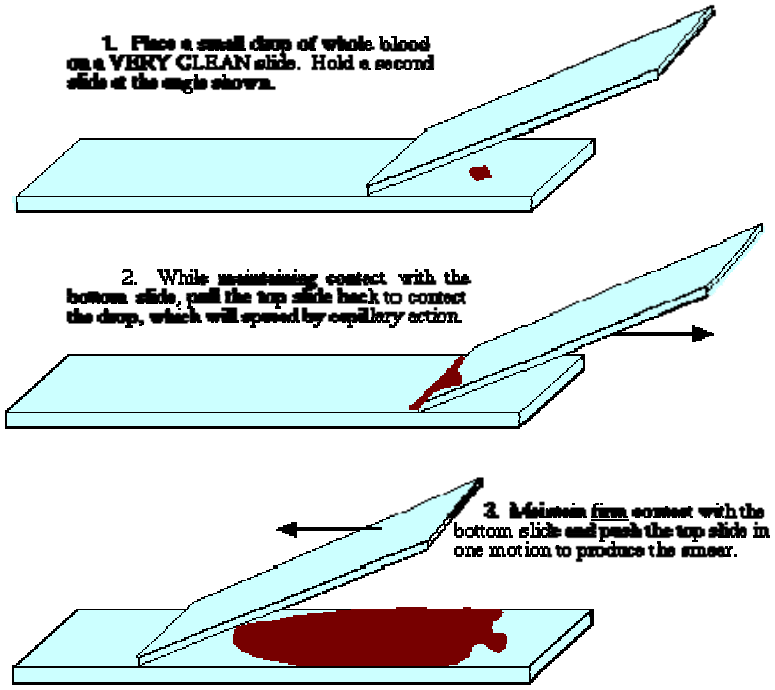
15.2 Microscopic examination of blood smears for diagnosis of haemoprotozoan parasites

A) Preparation of thin blood smear :

Requirements:

- Anti coagulated blood sample in EDTA/ HEPARIN
- Microscopic slide
- Methanol

Procedure:



Air dry the smear and dip in methanol in a coupling jar for fixation

⇩

Air dry the methanol fixed smear before storing for further use.

B) Giemsa staining of blood smear:

Requirement:

- Methanol fixed blood smear
- Sorensen Buffer (pH:7.2)
- Giemsa stain (Stock solution)
- Staining rack

Procedure:

- Prepare a fresh working solution of Giemsa stain by mixing with Sorensen buffer @1:10
- Place the methanol fixed blood smear on a staining rack
- Pour the stain solution over the smear to cover the whole slide
- Allow it to stand for 30- 45 minutes
- Wash under running tap water directly without pouring off the stain
- Air dry the slide
- Observe under low, high and finally under oil immersion of a compound microscope

Sorensen buffer: (working solution)

DiSodium hydrogen phosphate solution: 72 ml
Potasium dihydrogen phosphate solution: 28 ml
Distilled water: 900 ml.

15.3 Examination of skin scrapping for ectoparasites

A. Direct method:

Requirements:

- Scalpel
- Lubricating oil
- Clipping scissors
- Microscopic slide
- Cover slip
- Microscope

Procedure:

- Clip off the hairs of the affected area
- Smear the area with liquid paraffin or lubricating oil
- Scrap the lesion with the help of an oily scalpel till blood oozes out
- Transfer the scrapping material from the scalpel onto a clean microscopic slide containing a drop of oil
- Examine the preparation under low and high power of a compound microscope

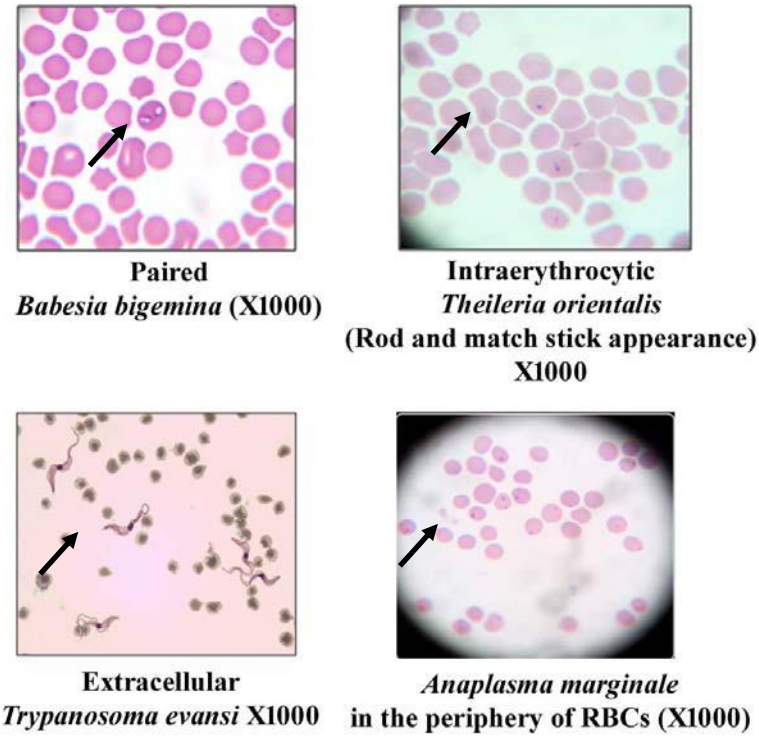
B) Indirect method:

Requirements:

Scalpel	Test tube holder
Lubricating oil	Spirit lamp
Clipping scissors	Centrifuge tube
Micro slide	Centrifuge machine
Cover Glass	Sodium or
Test tube	potassiumhydroxide (10%)

Procedure:

- The materials should be collected as described earlier in the direct method
- Transfer the scrapping materials to a clean test tube/ centrifuge tube
- Add required quantity of Sodium/ potassium hydroxide and boil the contents of the test tube over a spirit lamp till the hairs and tissue debris are dissolved
- Discard the supernatant and take a small amount of sediment on a clean micro slide
- Cover the material with a cover slip and examine under low and high powers of a compound microscope



Paired
***Babesia bigemina* (X1000)**

Intraerythrocytic
Theileria orientalis
(Rod and match stick appearance)
X1000

Extracellular
***Trypanosoma evansi* X1000**

Anaplasma marginale
in the periphery of RBCs (X1000)

Fig: Common haemoparasites encountered in cattle

16. CYTOPATHOLOGICAL DIAGNOSIS OF DISEASES IN LIVESTOCK AND POULTRY IN CLINICAL AND NECROPSY SAMPLES

Diagnosis of diseases in animals is an interesting job that too under field conditions. Field veterinarians can make use of certain simple techniques which will aid in quick diagnosis of diseases. One among them is the cytological technique which does not require elaborate procedure and equipment. All you need are suitable syringes and needles, glass slides, staining solutions and a good microscope. Usually lower and higher magnification examinations are sufficient and in certain situations, oil immersion objective is required e.g. identification of microorganisms like bipolars and blood parasites.

Cytology is defined as the examination of cells without regard to the tissue's architectural details. This diagnostic process is suggested, when clinical examination, radiographic evaluation and laboratory data could not confirm the diagnosis. The **advantages** of cytological sampling technique are quick, easy, inexpensive, less invasive than surgical biopsy, no need of general anaesthesia, if required light sedation, short processing time, results available in short time (5 to 30 in minutes) and a fewer complications. Be **cautious** about haemorrhagic tendencies and gain expertise under suitable guidance.

The limitations of cytological technique are no tissue architecture, tissue of origin, biologic behavior, grading, degree of tissue infiltration, presence and absence of necrosis and not a representative of the lesion. Small mobile or very firm lesions don't yield sufficient materials. In obesity, perilesional fat may come instead of the tissue of interest. In large lymphoma, lymph node may be necrotic and is non–diagnostic. While examining the cytological preparations, note that the cellular cytoplasm and nucleus are clearly visible. In poor smear preparation, do not attempt diagnosis. The bad smears will show smudged cells, "bare" nuclei without cytoplasmic details and nuclear and cytoplasmic vacuoles. Artifacts will be a problem with dirty slides, water, starch granules from gloves, ultrasound gel and stain precipitates.

Sample collection techniques: various approaches are adopted to collect cytopathological samples.

i). Fine needle aspiration biopsy (FNAB): It is the most used method. In short, it is known as FNAB and is employed to identify cells and differentiate

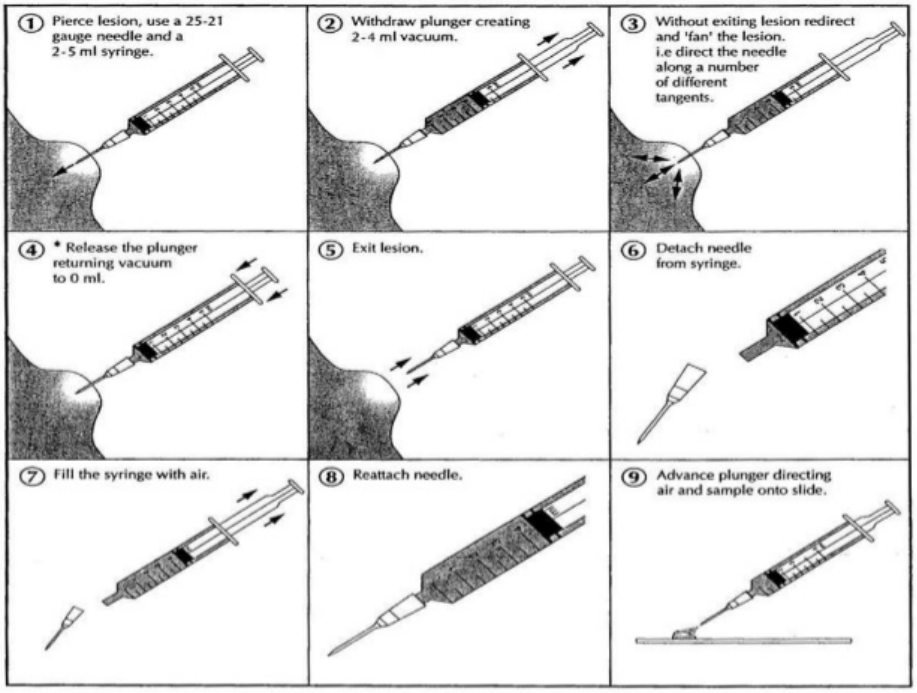
inflammation and neoplasia. The advantages are less time consuming, multiple site sampling in single procedure, consecutive sampling at intervals are possible. Diagnosis may eliminate surgery and specimens can also be used for histopathology.

"***Stop collecting sample if blood appears"" Metastasis is remote with FNAB sampling; Hence, do not hesitate to get sample"***

Procedure:

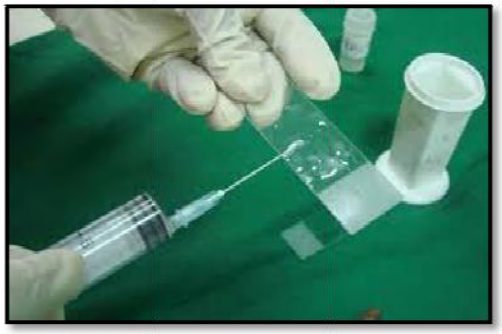
- ✦ Clean the area aseptically
- ✦ Anaesthetize the area
- ✦ Hold the mass firmly in hand
- ✦ Use 3 to 20 mL syringe with 21 to 25 gauge needle
- ✦ Insert needle into the mass, apply negative pressure and move the needle in various directions while aspirating
- ✦ Release negative pressure, withdraw needle out
- ✦ Dislodge needle and air drawn into the barrel
- ✦ Push the contents of the needle on clean glass slides
- ✦ Prepare smear

This technique is referred to as "continuous suction" type.



Preparation of slides

- ✓ Fluid – Smears
- ✓ Small fragments – Squash
- ✓ Large tissue fragment – Imprints/ histopathology
- ✓ Culture – Swirl the needle in medium before fixation for histopathology /slide preparation



The next procedure known as **"intermittent suction"** is more suitable for smaller lesions, where it is not possible to advance or redirect the needle without exiting the mass. Use 23G needle and 5 ml syringe. Withdraw and release the plunger several times with the needle inserted in the mass, release the suction, remove the needle from the mass, disconnect the needle from the syringe and obtain smears as described earlier.

Another procedure called as "**non-suction"** type causes less damage to fragile cells e.g. lymphoid cells. This procedure also minimizes blood contamination, useful for aspirating highly vascular masses and lymph nodes and also obtaining ultrasound guided samples of lesions in body cavities.

ii). Impression smears with microscope slide

- Ulcerated surface
- Cut surface of excised tissues or surfaces of tissues in biopsy or PM specimens
- Carefully blot the cut surface of the tissue with absorbent material/paper towel to remove the blood and other body fluid
- Make impression smears by gently touching flat cut surface
- Make several imprints on the same slide
- Air dry or wet fix
- Remaining portions sliced into thin sections and fixed for histopathology

Impression - Disadvantages

It collects relatively a few cells. Cells collected may not necessarily be representative of the lesion under investigation e.g. Ulcerated lesions are often secondarily inflammed or infected which may result in only inflammatory cells of interest or may induce in the cells of interest dysplastic changes that mimic those associated with neoplasia. It has limited use in superficial neoplasia but, useful for intraoperative confirmation of neoplasia in biopsy samples or excised tissue.

iii). Impression with cello tape– "No swelling but surface lesions are present"

- Useful technique in diagnosis of malasseziasis in dogs
- Take a regular cellophane tape and stick on to the affected area (Erythematous to hyperpigmented skin)
- Leave it for a minute
- Remove the tape, reverse and stick on to a glass slide with the impression smear facing out
- Stain with **Leishman-Giemsa stain** as described below
- Examine under microscope

C. Impression smear taken from lesion paste on slide keeping impressed smear upside

B. Stain the impressed cello tape

iv). Scrapings

- Touch imprints from solid tissues acellular
- Harvesting cells from lesions unlikely to yield large no. cells on FNAB (Hard tissues - Mesenchymal tissue tumours – fibroma)
- So scrape smears are prepared
- Scrape cut surface carefully with a scalpel blade
- Discard the first scraping contaminated with the blood and again scrape carefully
- Spread materials in a thin layer/squash on glass slides
- Air dry or wet fix

v). Incisional Biopsy

Small portion of a lesion surgically removed is utilized. Impression or scraping smear can be prepared and used.

Fixation of smears

i. **Dry fixation** is generally adopted and is done by rapidly agitating the slide in air (As in the case of blood smear preparation).

ii. **Wet fixation** is advised if staining is to be delayed for a few days to enhance cell preservation.
   - Fix slide within 30-60 seconds / Spray
   - 95% ethanol/absolute isopropanol /Methyl alcohol/ for a few minutes to 30 min
   - RBC lysis improves examination of clumps of cells

Cytological stains

For routine staining, use Leishman-Giemsa **(LG)** staining. On occasions, haematoxylin-eosin staining procedure can be adopted.

Leishman-Giemsa Staining

Preparation of LG stain

Leishman powder	120 mg
Giemsa powder	30 mg
Absolute methanol	100 mL

- Cover the smear with LG stain for a minute
- Dilute with tap water/Distilled Water /Phosphate Buffer Saline pH 6.8-7.2 in that order if there is a problem in staining character of cells i.e. cytoplasm and nucleus
- Allow for about 20 min
- Wash in tap water/Distilled Water /Phosphate Buffer Saline
- Air dry
- Examine under microscope first under low power and then at high power field (Place a cover slip over the smear while examination)
- If oil immersion objective is to be used, take out the coverslip

Haematoxylin and Eosin Staining

Preparation of Harris' haematoxylin

Hematoxylin crystals	5.0 g
Alcohol, 100 %	50.0 mL
Ammonium or potassium alum	100.0 g
Distilled water	1000.0 mL
Mercuric oxide (red)	2.5 g

Dissolve the hematoxylin in the alcohol, the alum in the water by the aid of heat. Remove from heat and mix the two solutions. Bring to a boil as rapidly as possible (Limit this heat to less than 1 minute and stir often). Remove from heat and add the mercuric oxide slowly. Reheat to a simmer until it becomes dark purple, remove from heat immediately and plunge the vessel into a basin of cold water until cool. The stain is ready for use as soon as it cools. Addition of 2 – 4 mL of glacial acetic acid per 100 ml of solution increases the precision of the nuclear stain. Filter before use.

- Wet fixed smears kept in Harri's haematoxylin for 20 min
- Wash in running water
- Dip in 1% acid alcohol, wash immediately
- Keep in running water for 5-10 min for "bluing"
- Stain with 1% aqueous solution of eosin for 1-2 min
- Wash, dry and dip in xylol
- Mount on DPX mountant with a clean cover slip

If **Diffquik stains** are available, they can be used much easily following manufacturers instruction. May take only a few minutes.

Special stains like Gram (bacteria), acid fast (TB, JD), toluidine blue (Mast cells) and Periodic Acid Schiff (PAS) staining and Grocott-Gomori silver staining (Fungi) as it demands.

Staining characters

Romanowsky stains and combinations with Giemsa stain nucleus purplish and cytoplasm bluish. Haematoxylin & eosin stain the nucleus bluish and cytoplasm eosinophilic.

Mast Cells – Toluidine Blue Staining

Toluidine blue stock solution

Toluidine Blue O	1.0 g
70% alcohol	100.0 mL

Mix, solution is stable for 6 months

1% Sodium chloride:	0.5 g
Sodium chloride	
Distilled water	50.0 mL

Make fresh

Working solution

Toluidine blue, Stock	5.0 mL
1% Sodium chloride	45.0 mL

Make fresh, discard after use

Procedure

- To the smear add working toluidine blue, 1 – 2 minutes
- Rinse in distilled water, 3 changes
- Dehydrate quickly through the 95% and absolute alcohols
- Clear in xylene and coverslip

Result

Mast cells will take violet stain of the cytoplasmic granules with a background of shades of blue.

Diagnosis and interpretation of results

Any swelling and mass may be an

Abscess: Pus may come into syringe depending on the consistency

Haematoma: Blood tinged fluid will flow into the syringe

Cyst : Clear fluid will flow into syringe

Granuloma: TB, parasitic, pyogranuloma

Or A tumour :

Any surface swellings which are commonly observed and need proper diagnosis. Hence, the condition has to be differentiated whether it is inflammatory / infection or neoplastic.

Infections

Infectious agents like bacteria, virus, parasite and fungi may be identified. In **malasseziasis** affected dogs **(Fig. 1),** basophilic, peanut or double bottle or foot print shaped organisms are found. In abscess, **bacteria will be detected** – cocci, bacilli; Gram +ve or Gram –ve (**Fig. 2,3,4).**

In pox virus infection, epithelial cells may show intracytoplasmic inclusions. In granulomas, bacteria (TB / JD: Acid fast bacilli – Necropsy: TB-Lungs, lymph nodes-{Caseous / calcified nodules}, intestine {JD – Corrugated appearance}; Actinomycosis: Chinese letter appearance of bacilli or fungi {Aspergillosis: Branching hyphae, Blastomycosis: Spherical bodies, branching elements}). Necropsy: Suspected canine distemper cases, urinary bladder / stomach / lung impressions, intranuclear / intracytoplasmic inclusions see **(Fig. 5);** ICH – Intranuclear basophilic inclusions; PPR: Lung lesions, you may find intracytoplasmic inclusions.

Inflammations

In acute inflammation, the neutrophils predominate in non-septic condition. In septic condition neutrophil associates with bacteria. Neutrophils may be intact or degenerate (swollen nucleus). **Macrophages** predominate in **chronic inflammation** and accompanied by epithelioid cells and giant cells in granulomatous inflammation. **Subacute inflammation** is characterized by the presence of lymphocytes and plasma cells. In **allergic conditions**, eosinophils predominate.

Diagnosis of lymphadenopathies – "Do not sample when lymph node is not swollen"

Lymph node enlargement is commonly encountered condition in animals. FNAB smears are handy in making the diagnosis. Five different observations can be made

i. **Specific diseases:** Cattle: In theileriosis, the Koch's blue bodies can be detected in the cytoplasm of lymphocytes **(Fig. 6)**. Bacterial infections (Abscesses) and blastomycosis may be seen.

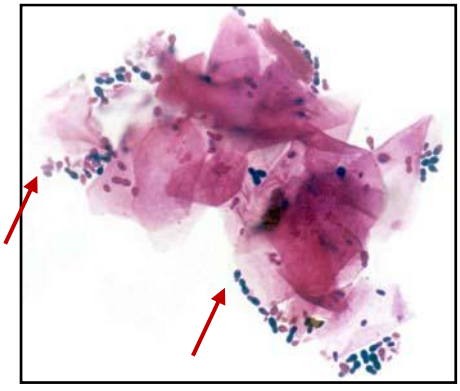


Fig. 1. Dog-Malassezisasis-Skin impression-Bluish peanut / double bottle / foot print shaped organisms (arrow). Red keratinized cells

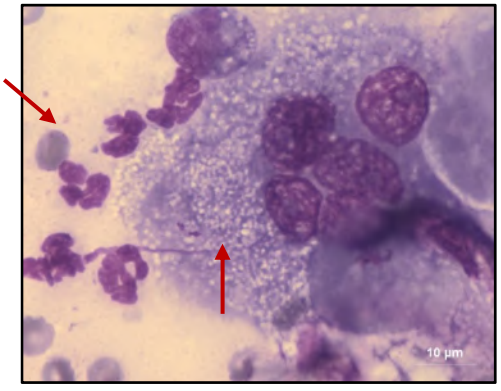


Fig.2. Dog-Skin-Chronic (active) inflammation-Granuloma-Multinucleate cell and neutrophils (arrow)

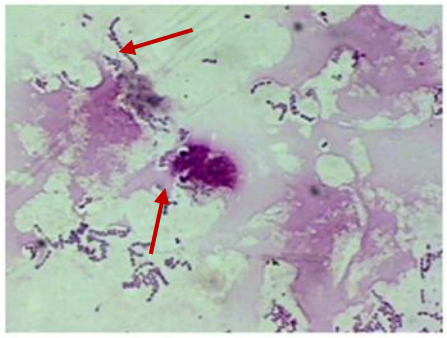


Fig. 3. Acute septic inflammation-Calf-Streptococci organism free and in the cytoplasm of neutrophil (Phagocytosed)

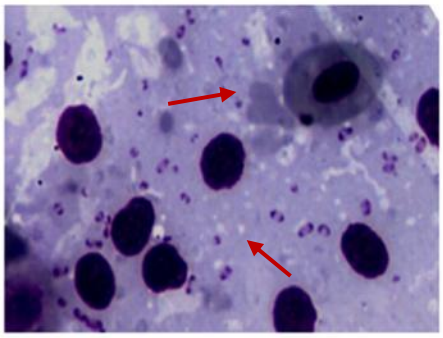


Fig. 4. Fowl cholera-Necropsy-Liver impression smear-Bipolar organisms (arrow). Note a nucleated avian RBC

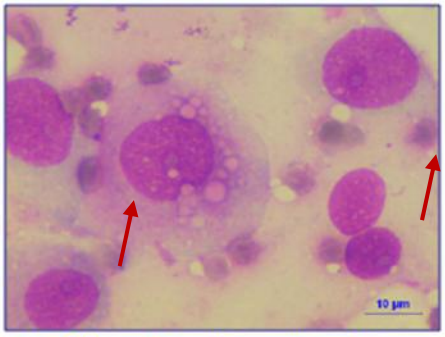


Fig. 5. Canine distemper-Necropsy-Urinary bladder smear-Intracytoplasmic & intranuclear inclusions (arrow) in epithelial cell

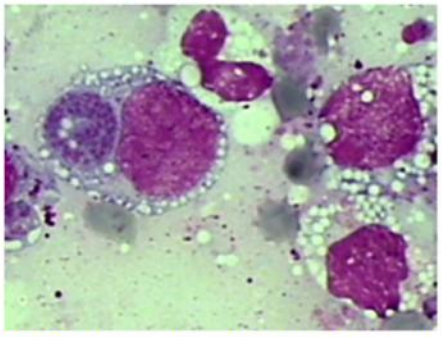


Fig 6. Cattle-Lymph node smear-Koch's blue bodies (arrow) in the cytoplasm of lymphocyte of Theileria infected cattle

i. **Reactive hyperplasia**: Wherein plasma cells are found. These are round to oval cells having eccentric nuclei and hyper-basophilic cytoplasm.

ii. **Inflammations-lymph adenitis**: As described earlier, it may be acute or chronic conditions.

iii. **Primary tumours:** In lymphomas especially dogs, mixed lymphocytic cell population is seen (Intermediate lymphoma). Sometimes, large neoplastic lymphoid cells with multiple nuclei will be present (Poorly differentiated lymphoma). At times, large undifferentiated cells may be seen (Undifferentiated lymphoma).

iv. **Secondary tumours**: Tumours of skin may spread to local lymph nodes. Look for characteristic of epithelial or mesenchymal cells and presence of tumours nearby the lymph nodes.

Neoplastic conditions

Evaluation criteria

Nuclear criteria - These are anisokaryosis (Variation in size of nucleus), multinucleate cells, giant cells (Large sized cells), mitotic figures, abnormal shape, N : C ratio decreased due to enlargement of nucleus, vacuolation.

Chromatin criteria- coarse, reticulate or sometimes fine.
Nucleolar criteria- size, shape and number variation.

More than three characters indicate malignancy.

Classification

Based on cytology, neoplastic conditions are classified into three categories viz.
i. Discrete cell tumours ii. Epithelial cell tumours and iii Mesenchymal cell tumours.

Discrete cell tumours (cells are discrete) -useful in dogs – easy to diagnose

These tumours can be easily diagnosed and yield high cellularity. Cells are discrete and mostly round in shape. Cells of mast cell tumours show cytoplasmic granules (Leishman-Giemsa), seen in a pole or either pole or distributed throughout the cytoplasm. Histiocytomas **(Fig. 8.)** will show indented nucleus and multinucleate cells (Binucleate / trinucleate or more number). TVT cells show cytoplasmic vacuoles **(Fig 7).** Cells of plasma cell tumours are round to oval and cytoplasm is deeply basophilic and show pale Golgi zone near nucleus. Melanoma cells contain brownish to black cytoplasmic pigments **(Fig. 9).** Lipomas will show variable sized cells with cytoplasmic vacuoles seen almost occupying the entire cytoplasm with eccentrically placed nucleus **(Fig.10).**

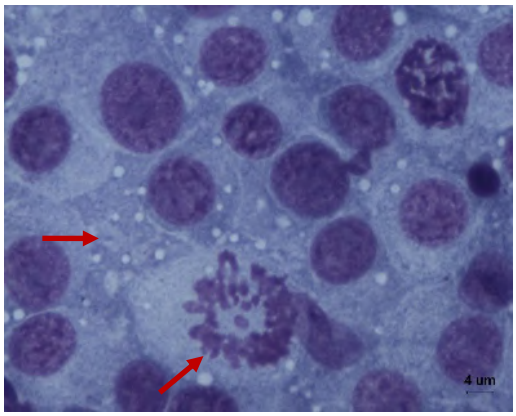


Fig. 7. Transmissible veneral tumour (TVT)-Cells showing punctate vacuoles in the cytoplasm; Mitotic figure (arrow)

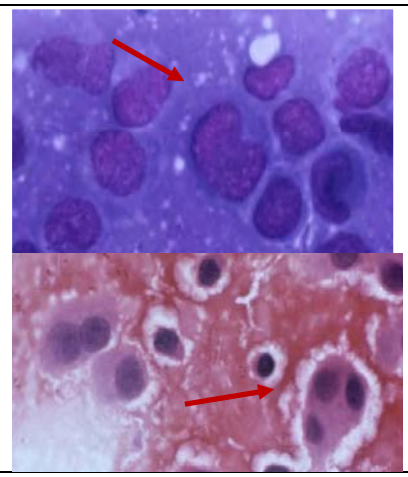


Fig.8. Dog-Skin-Histiocytoma-Large cells, abundtan cytoplasm and indented nuclues(arrow) LG stain. Right-Multinuclate cells H&E stain

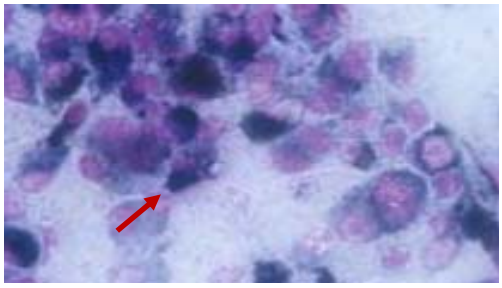


Fig 9: Dog-Skin-Malignant melanoma-Variable sized cells showing blue-black granules in the cytoplasm-Anisokaryosis and nucleoli present.

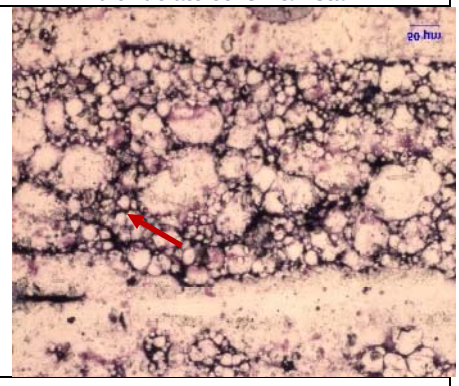


Fig.10. Dog-Skin-Lipoma-Variable sized cells with vaculated cytoplasm

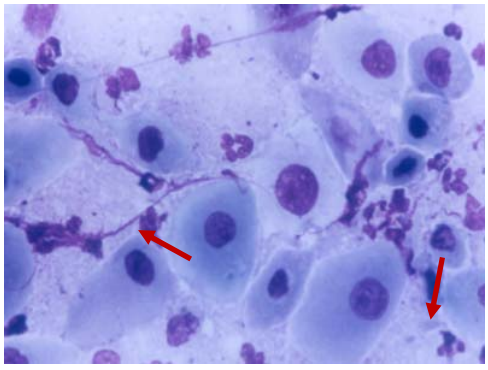


Fig. 11. Dog-Skin-Squamous cell carcinoma-Polyhedral cells and a few intermediate cells (arrow)

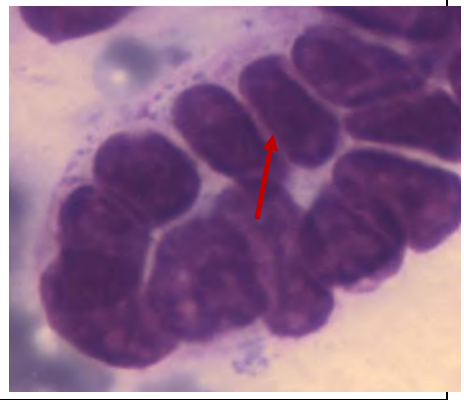


Fig.12. Dog-Skin-Basal cell carcinoma-Cluster of cylindrical cells

Epithelial cell tumours – moderately easy to diagnose

Squamous cell carcinoma will show cluster of squamous cells and intermediate cells **(Fig.11.).** Look for tadpole cells. Basal cell carcinomas will reveal cluster of cylindrical cells **(Fig.12).** In benign tumours, cells and their will show slight variation in size and carcinomas will show cluster of cells (and nuceli) varying in size and shape. Perianal adenomas will reveal hepatoid cells **(Fig. 13).**

Mesenchymal tumours – needs expertise gained over a period of time

These tumours yield low cellularity. Fibromas will show spindle cells and fibrosarcomas spindle to plump cells **(Fig.14.).** However, osteosarcomas **(Fig.15)** can be diagnosed better and the cells will be discrete, round to oval and show eccentric nuclei (plasmacytoid appearance).

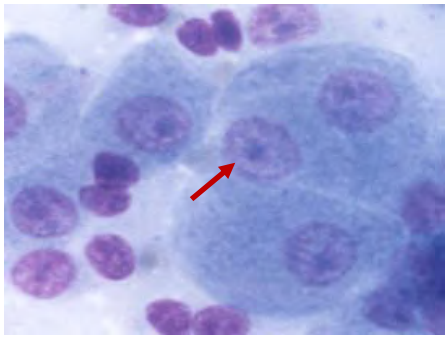


Fig.13. Dog-Perianal adenocarcinoma-Variable sized hepatoid cells and nucleoli (arrow)

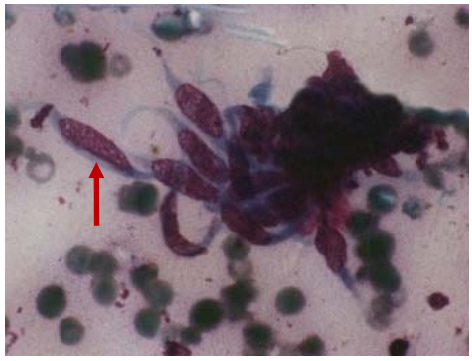


Fig.14. Dog-Skin-Fibroma-Note spindle shaped cells

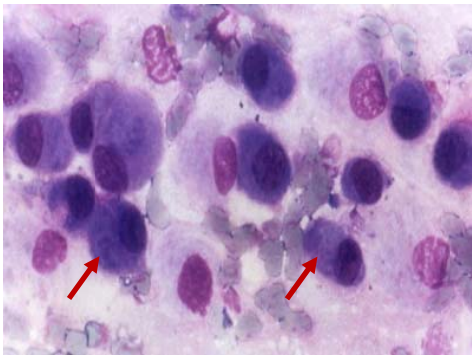


Fig.15. Dog-Bone-Osteosarcoma-Variable sized oval cells, eccentric nuclei, highly basophilic cytoplasm (arrow)

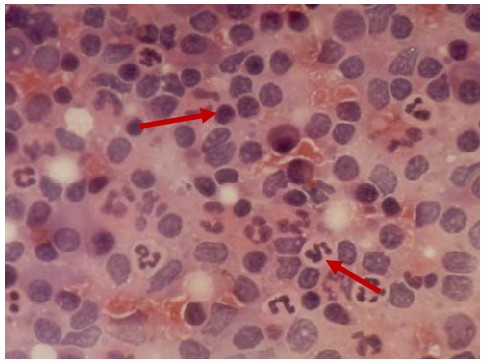


Fig. 16. Dog-Lymph node-Acute lymph adenitis-Note many neutrophils (arrow) are present. H&E stain

Poultry disease diagnosis

Impression smears from liver of suspected cases of fowl cholera (Multifocal necrotic hepatitis) will reveal bipolars (LG stain). Similarly, impression smears from nodular or diffuse (greyish-white) lesions will show heterogeneous population of neoplastic lymphoid cells in Marek's diseases (MD) and homogeneous neoplastic lymphoblastic cells in lymphoid leucosis (LL). One will find septate hyphae crushed preparations from lesion of lungs and air sacs in aspergillosis. In granulomatous lesions, FNAB smears will show macrophages and multinucleated giant cells (Look for any bacterial cocci). In duck plague, attempts can be made to find out inclusions in epithelial cells of intestinal impressions.

Conclusion

A judicious use of cytology will be beneficial to the practicing veterinarians as diagnosis is made available within a short period of time and can decide on proper treatment, prognosis and further investigative process e.g. TVT can be successfully treated medically without surgical intervention.

Thanks are due to Dr. K.Krithiga, Dr.M.Thangapandiyan, Dr.S.Vairamuthu, Dr.N.Jayanthi and Dr.B.Murali Manohar, TANUVAS for their help.

17. PROCESSING OF TISSUE FOR HISTOPATHOLOGICAL EXAMINATION

Collection:

Tissue for histopathological examination should be collected immediately upon removal from the carcass as soon as after death as possible. The portion representing the characteristics lesion should be selected together with a healthy portion and that should not be more than 5-10 mm in thickness. After removal it should be kept in fixative, which should be at least 10 times more than volume of the tissue. The tissue specimen should be properly labelled and stored.

Fixation:

The foundation of all good histological preparation depends on complete fixation. Fixation is required to-

1. Prevent post mortem changes such as putrefaction and autolysis.
2. Preserved various cell constituents in as life like manner as possible.
3. Protect by hardening the naturally soft tissue, thereby allowing easy manipulation during subsequent processing.
4. Convert normal semi-fluid consistency of cells to an irreversible semi solid consistency.
5. Aid visual differentiation of structure by application of biological dyes and stains.

Choice of fixatives:

Selection of fixative depends on purpose for which the tissue is preserved. Following are few commonly used fixatives:

1. ***10% formalin: Commonest fixative widely used in the laboratories***.

Concentrated formalin (37-40%)	…	100ml
Water	…	900ml

2. ***Formalin saline solution:***

Concentrated formalin (37-40%)	…	100ml
Sodium chloride	…	9g
Water	…	900ml

3. **Buffered neutral formalin solution:**

Concentrated formalin (37-40%)	…	100ml
Distilled Water	…	900ml
Sodium phosphate monobasic	…	4.0g
Sodium phosphate dibasic	…	6.5g

4. **Ethyl alcohol 70-100%**

It is used for preservation of glycogen. It is slow in penetration, hardens and shrinks the tissue

5. Zenker's solution:

Distilled Water	…	1000ml
Mercuric chloride	…	50.0g
Potassium dichromate	…	25.0g
Sodium sulphate	…	10.0g

Add 5 ml glacial acetic acid to 95ml of Zenker's solution before use (The solution does not keep well after the adition of acetic acid).

Tissue preserved by this method stain well with many techniques, but it is suggested that they be post fixed in 2.5% aqueous solution of potassium dichromate for 2 hours following Zenker's fixation.

6. Bouin's solution:

Picric acid, saturated aqueous solution	…	750.0ml
37-40% formalin	…	250.0ml
Glacial acetic acid	…	500.0ml

Fix blocks for 4-12 hours. Wash the tissue thoroughly in 50% alcohol for 4-6hrs, agitating constantly for proper removal of the picric acid. Removal of picric acid is essential for proper staining of the tissue section.

7. **Carnoy's solution:**

Absolute alcohol	…	60.0ml
Chloroform	…	30.0ml
Glacial acetic acid	…	10.0ml

One of the best penetrating and quickly acting fixatives. Generally 3 hours is adequate for normal size tissue. No washing is necessary and tissue may be transferred to absolute alcohol + haemolyzes RBC.

8. **Glutaraldehyde:** 2.5 percent glutaraldehyde in 0.1 M Sodium cacodylate buffer solution is used as fixatives for electron microscopy.

Decalcification:

Calcium salt provides hardness and rigidity to the bone and other tissue and that must be removed to ensure the specimen is soft for microtome section.

Bone and other calcified tissue should be cut into small pieces before fixation. After adequate fixation, place the tissue in a large quantity of decalcifying solution. The tissue should be checked every day by pricking with a pin and if decalcification is not proper the solution should be changed. After decalcification is over the tissue should be washed in running water for several hours to remove the traces of the decalcifying solution. Since the decalcifying acids continue to act on tissue during subsequent processing. One of the decalcifying solutions is

Perenyi's fluid:

10% nitric acid, aqueous	…	40.0ml
Absolute alcohol	…	30.0ml
0.5% chromic acid, aqueous	…	30.0ml

Processing of tissue:

A specimen brought to the laboratory is usually marked with identifying number and name. Keep this identification with the specimen thorough out the processing. All identifying marks should be made with a soft lead pencil. For processing of tissue, tissue capsules are used. Tissue

from different animal can be processed together by keeping them separately in different capsule with individual identification numbers.

a) Washing:

The first step of tissue processing is washing. The fixed tissue should be washed thoroughly in running tape water at least for 6-8hrs preferably overnight to remove the traces of fixatives. It is important, to obtain a desired tissue section.

b) Dehydration:

It is the process of removal of all extractable water from the tissue. It is usually accomplished by treating the tissue with ascending grades of alcohol i.e.

50% Alcohol	---	1 hour
70% Alcohol	…	1 hour
80% Alcohol	…	1 hour
90% Alcohol	…	1 hour
Absolute alcohol I	…	1 hour
Absolute alcohol II	…	1 hour
Absolute alcohol III	…	1 hour

c) Clearing*:*

The clearing reagents miscible with dehydrant. As the dehydrant is removed, the tissue clear, becoming translucent signifying the completion of the process. Xylene is most wide used clearing reagent. Clearing with xylene is done by

Absolute alcohol xylene mixture (50 parts each)	…	1 hour
Xylene I	…	1 hour
Xylene II	…	1 hour
Xylene III	…	30 minutes or until clear

If clearing time is not properly maintained the tissue become hard and leading to difficulty in sectioning of it.

d) Impregnation:

It is complete removal of the clearing reagents by substitution with melted paraffin. A paraffin bath at 60^{o}C is used for this purpose with at least three paraffin cups. Paraffin (58-60^{o}C melting point) are allowed to melt in the cups. The cleared tissues are immediately transferred to the paraffin cup and impregnation in accomplished by three changes of melted paraffin of one to two hours each.

e) Embedding:

It is the orientation of the tissue in melted paraffin, which after solidification provides a firm medium for keeping intact all parts of the tissue when section are cut. For this purpose, a pair of "L" mould is used. A rectangle is made over a plain surface with the pair of "L" mould. Freshly melted paraffin (60^{o}C) is poured in the rectangle and the tissues are placed immediately after removing from the paraffin bath. While placing the tissue care must be taken so that the surface of the tissue from which sections are required facing downward. Then it is allowed to cool down for solidification of the paraffin. After solidification tissue blocks are ready for sectioning.

f) Sectioning:

Tissue blocks are sectioned at 4-5 micron thickness with the help of a microtome. The blade of which should be very sharp with even edge.

The tissue blocks are fitted to the block holder at opposite side of the surface of the tissue to be cut. Then the block holder is fixed in the microtome and sections are made gently rotating the microtome. This tissue sections are the put in to 50% alcohol for initial stretching. Thereafter they are allowed to spread in tissue floatation bath, temperature of which is maintained at 56-58^{o}C. After proper spreading the tissue sections are mounted in clean microslide and allowed to dry either at room temperature or in hot air oven. Before mounting, the microslide should be smeared with a small drop of Mayer's egg albumin as an adhesive.

Composition of Mayer's egg albumin

Egg white	…	50ml
Glycerine	…	50 ml

Mix well and filter through a coarse filter paper and add crystal of thymol as preservative.

g) Staining:

A) Routine staining:

The tissue sections are stained routinely with Haematoxyline and Eosine (H&E). Haematoxyline stains the acidic part of the cell i.e. Nucleus (contains nucleic acid) while the eosine imparts colour to the basic part of the cell i.e. cytoplasm. There are different types of haematoxyline e.g. Mayer's, Delafield's, Harri's, Ehrlich's etc. Delafield's haematoxylin is most commonly used in the laboratory.

Preparation of Delafield's haematoxylin:

Haematoxyline crystals	…	8.0g
Alcohol 95%	…	50 ml
Ammonium or potassium alum, saturated aqueous solution (approx. 15g/100ml)	…	800ml

Add the haematoxyline dissolved in alcohol to the alum solution and expose to light and air in an unstopped bottle for 3-5 days. Filter and add

Glycerine	…	200ml
Alcohol 95%	…	200ml

Allow the solution to stand in the light approx. 3 days, filter and keep in tightly stoppered bottle.

Preparation of Eosin Solution:

Stock Eosin Solution:

Eosin Y, water soluble	…	1.0g
Distilled water	…	20.0ml
Dissolve and add alcohol 95%	…	80.0ml

Working Eosin Solution:

Eosin stock solution	…	1part
Alcohol 80%	…	3 parts

Just before use add 0.5ml of glacial acetic acid to each 100ml stain

Routine staining procedure:

The dried tissue sections are taken in a rectangular staining jar and staining is proceeded as follows:

a) Deparaffinization: The first step is the removal of the paraffin from the sections. This is done by immerging in xylene, 3 changes of 10 minutes each.

b) Rehydration: This is the addition of water, done with descending grades of alcohol, as follows

Absolute alcohol I	… 2min
Absolute alcohol II	… 2min
Alcohol 90%	… 2min
Alcohol 80%	… 2min
Alcohol 70%	… 2min
Tape water	… 6 minutes

c) Staining with haematoxyline: After hydration the sections are immersed in Delafield's haematoxyline for 15-20 minutes.

Rinse in tape water.

↓

Differentiate in acid alcohol, 3-5 quick dips.
(Conc.HCL-1ml70% alcohol-99ml).

↓

Wash in tape water.

↓

Dip in ammonia water until sections are bright blue in colour(4-6 dips)

↓

Wash in running tape water for 10-20 minutes. If washing is inadequate eosin will not stain evenly.

d) Staining with eosin: Stain with eosin for 15 second to 2 minutes depending on the age of the eosin and the intensity of the stain required.

e) Dehydration: It is done with 2 changes of 90% alcohol of 2 minutes each and then 2-3 changes of absolute alcohol for 2 minutes each.

f) Clearing: Is done with 3 changes of xylene of 2 minutes each.(Slides may remain in xylol until cover slipped.)

g) Mounting: The stained sections are then cleaned with a soft and clean cloth and mount a cover slip with DPX.

h) Mounted slides are then allowed to dry and after drying it is ready for examination.

Result: Nuclei: Purple
Cytoplasm: Pink
RBC: Red

B) Special Staining

In histopathological techniques, i.e. in tissue sections one can demonstrate bacteria, fungus, chlamydia, rickettsia or viral inclusions by using special staining procedure. Though it requires specific expertise it can be done in diagnostic laboratories as a routine procedure.

i) Brown and Brenn stain for bacteria in tissue (Fig 1,2,3).
Result – gram positive - bacteria blue coloration
gram negative bacteria – red coloration
nuclei – red
other tissue element – yellows.

ii) MacCullum – Goodposture stain for bacteria in tissue.
Result – gram positive organism – blue
gram negative organism – red
other elements – various shade of red to purple.

iii) Ziehl-Nelsen stain for acid fast bacteria.
Result – acid fast bacilli – bright red
erythrocytes – yellowish orange
other elements – pale blue.

iv) Levaditis method for staining spirochetes in blocks
Result – spirochetes – intensely black
background – yellow to light brown

v) Silver method for spirochetes in sections
Result – spirochetes – black
background – yellow to brown

vi) Gridley's stain for fungi.
Result – mycelia – deep blue
conidia – deep red to purple
background – yellow

vii) Grocott's method for fungi (Fig. 5)
Result – Fungi – sharply delineated in black
mucin – deep gray
inner part of mycelia & hyphae – old rose.
background – pale green

viii) Phloxine Tolidine Blue stain for Malaria parasite.
Result – malaria parasite – pale blue cytoplasmic structures within erythrocytes

ix) Giemsa stain for Rickettsia
Result – Rickettsia – violet
nuclei – blue

x) Schleifstein stain for Negri bodies
Result – Negri bodies – deep magenta
cytoplasm – bluish violet

xi) Haematoxylin – Shorr S3 stain for inclusion bodies (Fig.4)
Result – inclusion bodies – brilliant red.

The detail procedure for these special stains one can refer any book on manual of histologic and special staining techniques.

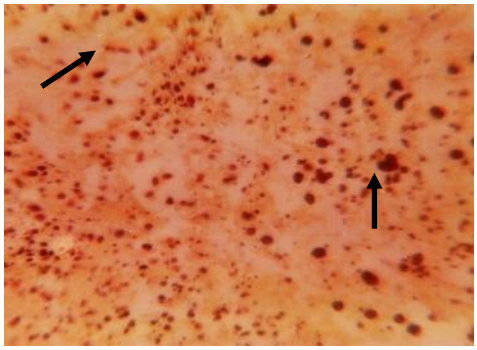


Fig 1: Mixed infection of Candida & E. coli. Brown & Brenn X400

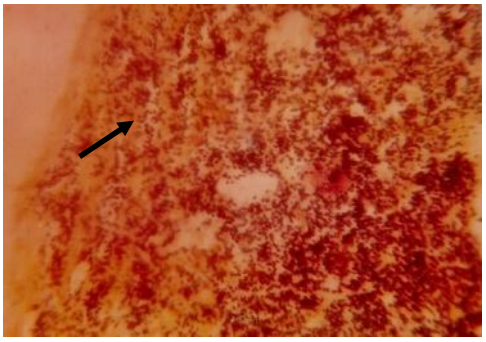


Fig 2: Streptococcal colonies. Brown & Brenn X400

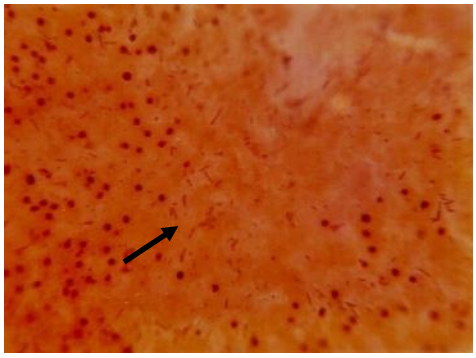


Fig 3: Bacilli in Necrobacillosis. Liver .Brown & Brenn X400

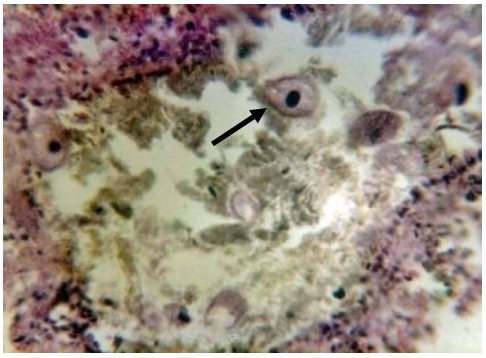


Fig 4:Plant material and Blantadium coli in bronchi H &E X400

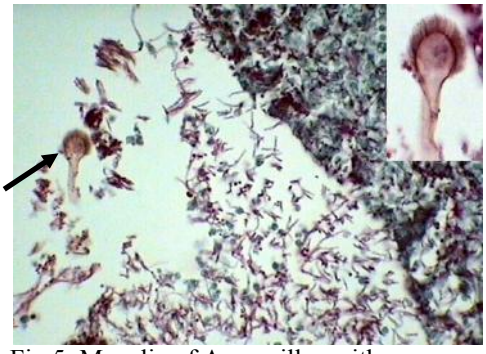


Fig 5: Mycelia of Aspergillus with conidiopore with in bronchi. Grocotts X100.Coniodiospore in inset. Grocotts X1000

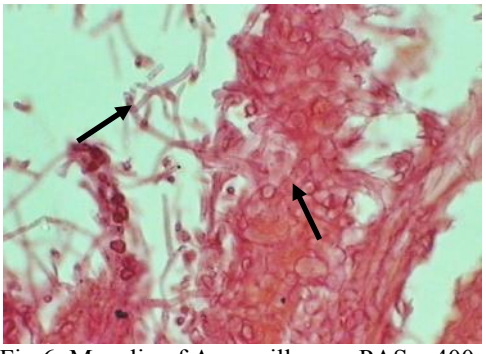
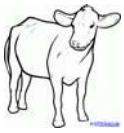


Fig 6: Mycelia of Aspergillus sp. PAS x400

18. STANDARD SURVEY FORMATS



Survey on Cattle herd & Health Management

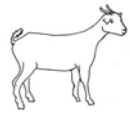
Sl No. ___ Name of Village _____ _____Tehsil_____
Dist._____State_____ GPS location : Longitude _____
GPS location : Longitude _____ and Latitude _____
Name of Veterinarian / AHW_____ Doc Ph number_____
Date form completed: / /

Variables	Number herd/ Farm/Unit (Frequency %)		
	Semi-commercial Farms (n=)	***Backyard Farms (n=)***	***Free grazing Farms (n=)***
Farm Attributes			
Dry Plane			
Wet land			
Hills			
Forest			
Production system			
Milk purpose			
Draught purpose			
Breeding Farm			
Animal raising			
Indoor			
Partially Outdoor			
Scavenging			
Tethering			
Pen type			
Breeds of cattle			
Local			
Exotic			
Cross-breed			
Origin of stock			
Own Farm bred			
Live market			
Breeder - other farm			
Breeding			
Natural service			
Artificial insemination			
Total Herd size / Categories			
0—6 months			
6 Month—2 Years			
2 Years—6 Years			
Above 6 Years			
Bull			
Feeds			
Commercial			
Vegetable /Grass/leaves			
Market waste			

Cattle Health & Farm Hygiene

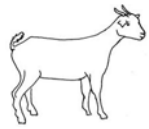
Variables	Number herd/ Farm/Unit (Frequency %)		
	Semi-commercial Farms (n=)	***Backyard Farms (n=)***	***Free grazing Farms (n=)***
According to you which are the common vaccines used			
B Q			
FMD			
HS			
Anthrax			
Brucellosis			
Regular deworming			
According to you which are the top ten infectious diseases in your locality			
CBPP			
FMD			
B Q			
Pneumonia			
Diarrhoea			
Abortion/still birth			
Nervine symptoms			
Impaction			
Mastitis			
Hump sore/ skin disease			
Babesiosis			
Plant toxicity			
Ectoparasite, vector prevalent			
Any other diseases			
According to you, which of these actions farmers in your area do when they notice a contagious disease			
Quarantine of new cattle introduced			
Isolation Sick animals			
Treatment even healthy to sick animals			
Sale of animals without signs			
Sale of diseased animals only			
Slaughtering diseased animals			
Slaughtering all animals			
Share bullock in agriculture			
Burying of carcass in the farm			
Cleansing and disinfection of shed			
Continue selling milk			

Goat Health & Farm Hygiene



Variables	Number herd/ Farm/Unit (Frequency %)		
	Semi-commercial Farms (n=)	***Backyard Farms (n=)***	***Free ghrazing Farms (n=)***
Farm Attributes			
Dry land/ Plain			
Wet land			
Hills			
Forest			
Production system			
Meat purpose			
Dual purpose			
Breeding Farm			
Animal raising			
Indoor			
Partially Outdoor			
Scavenging			
Tethering			
Pen type			
Breeds of Goats			
Local			
Exotic			
Cross-breed			
Origin of stock			
Own Farm bred			
Live market			
Breeder - other farm			
Breeding			
Natural service			
Artificial insemination			
Total Herd size / Categories			
0—3 months			
3 Month—1 Year			
1 Year—2 Years			
Above 2 Years			
Buck			
Feeds			
Commercial			
Vegetable /Grass/leaves			
Market waste			

Goat Health & Farm Hygiene

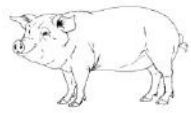


Variables	Number herd/ Farm/Unit (Frequency %)		
	Semi-commercial Farms (n=)	***Backyard Farms (n=)***	***Free grazing Farms (n=)***
According to you which are the common vaccines used			
PPR			
FMD			
HS			
Anthrax			
Enterotoxaemia			
Regular deworming			
According to you which are the top ten infectious diseases in your locality			
PPR			
FMD			
Enterotoxaemia			
Pneumonia			
Diarrhoea			
Abortion/still birth			
Nervine symptoms			
Coccidiosis			
Enteric Parasites			
Mange			
Anaemia			
Ectoparasite, vector prevalent			
According to you, which of these actions farmers in your area do when they notice a contagious			
Quarantine of newly introduced goats			
Isolation Sick animals			
Treat sick animals			
Sale healthy animals without signs			
Sale of diseased animals only			
Slaughters diseased animals			
Slaughters all animals			
Consumption of dead/slaughtered animals			
Burying of carcass in the farm			
Cleansing and disinfection of pen			
Owner sales meat			

Pig Herd & Management

Variables	Number herd/ Farm/Unit (Frequency %)		
	Semi-commercial Farms (n=)	***Backyard Farms (n=)***	***Scavenging Farms (n=)***
Farm Attributes			
Dry/Plain			
Wet land			
Hills			
Forest			
Production system			
All – in- all out			
Continuous cycle			
Breeding Farm			
Animal raising			
Indoor			
Partially Outdoor			
Scavenging			
Tethering			
Pen type			
Breeds of Pigs			
Local			
Exotic			
Cross-breed			
Origin of stock			
Own Farm bred			
Live market			
Breeder - other farm			
Breeding			
Natural service			
Artificial insemination			
Total Herd size / Categories			
0—3 months			
3 Month—1 Year			
1 Year—2 Years			
Above 2 Years			
Boar			
Feeds			
Commercial			
Industrial and agricultural by products (e.g Rice Beer)			
Hotel/ house hold waste			

Pig Health & Farm Hygiene



Variables	Number herd/ Farm/Unit (Frequency %)		
	Semi-commercial Farms (n=)	***Backyard Farms (n=)***	***Scavenging Farms (n=)***
According to you which are the common vaccines used			
Swine fever			
FMD			
HS			
Anthrax			
Regular deworming			
Others			
According to you which are the top ten infectious diseases in your locality			
Swine fever			
FMD			
Swine pox			
Pneumonia			
Diarrhoea			
Abortion/still birth			
Nervine symptoms			
Coccidiosis			
Enteric Parasites			
Skin infection			
Anaemia			
Ectoparasite, vector prevalent			
According to you, which of these actions farmers in your area do when they notice a contagious disease			
Quarantine newly introduced pigs			
Isolation Sick pigs			
Treat sick animals			
Sale healthy animals without signs			
Sale of diseased animals only			
Slaughtes diseased animals			
Slaughters all animals			
Consumption of dead/slaughtered animals			
Burying of carcass in the farm			
Cleansing and disinfection of pig sty			
Owner sales pig meat/pork			

Poultry Flock & Management

Variables	Number Farm/Unit (Frequency %)		
	Semi-commercial Farms (n=)	***Backyard Unit (n=)***	***Free grazing Unit (n=)***
Farm Attributes			
Dry Plain			
Wet land			
Hills			
Forest			
Production system			
Broiler			
Layer			
Dual type			
Breeding Farm			
Raising of birds			
Intensive			
Semi-intensive			
Free ranging			
Cage type			
Breeds of Poultry			
Local			
Exotic			
Cross-breed			
Origin of stock			
Own Farm bred			
Live market			
Breeder - other farm			
Breeding			
Natural service			
Artificial insemination			
Total Flock size / Categories			
Less than 1 week			
1 wk-2wks			
2 wks – 3 wks			
3 wks- 4 wks			
Above 4 wks			
Spent bird			
Feeds			
Commercial			
House hold waste			

Poultry Flock & Farm Hygiene

Variables	Number Farm/Unit (Frequency %)		
	Semi-commercial Farms (n=)	***Backyard Unit (n=)***	***Free grazing Units (n=)***
According to you which are the common vaccines used			
RD			
IBD			
FP			
Mareks			
ILT			
IB			
According to you which are the top ten infectious diseases in your locality			
RD			
AI			
Coryza			
DP			
Pox			
Coccidia			
Nervine symptoms			
Coccidiosis			
Fungal infection			
Mange			
Deficiency			
Ectoparasite, vector prevalent			
According to you, which of these actions farmers in your area do when they notice a contagious			
Quarantine of newly introduced birds			
Isolation Sick birds			
Treatment of ailing birds			
Sale of birds without signs			
Sale of in-contact birds			
Slaughtering of diseased birds and sale			
Slaughtering of all birds on farm			
Consumption of dead/slaughtered animals			
Burying of birds /slaughtered in farm			
Cleansing and disinfection of poultry shed			
Owner sales birds in market			
Visitors allowed on your farm			

OFFICE OF THE CORELAB-1
ADVANCED ANIMAL DISEASE DIAGNOSIS & MANAGEMENT CONSORTIUM
COLLEGE OF VETERINARY SCIENCE, AAU, KHANAPARA, GUWAHATI-781022

File No: **Date.....**

Owner's name: *Ph No.*

Address:

Ref.no: *species:*

Clinical symptoms:

Post mortem findings:

Type of sample: *Date of collection :*

Date of received in laboratory: *Sample ID no:*

TYPE OF EXAMINATION	
BACTERIAL/FUNGAL AGENT	*Cultural examination:* *Microscopic:* *Biochemical:* *Molecular:* *Serological:*
ANTIBIOGRAM	*Sensitive to:* *Resistant to:*
VIRAL AGENT	*Isolation of virus:* *Detection of antigen:* *Detection of antibody (with titre):* *Molecular:*
RESULT: SUGGEESTION/COMMENT:	

Signature of the Investigator **Signature of the In-charge**

OFFICE OF THE CORELAB 1
ADVANCED ANIMAL DISEASE DIAGNOSIS & MANAGEMENT CONSORTIUM
COLLEGE OF VETERINARY SCIENCE, AAU, KHANAPARA, GUWAHATI-781022

File No: **Date.....**

Owner's name: *Ph No.*

Address:

Ref.no: *species:*

Clinical symptoms:

Post mortem findings:

Type of sample: *Date of collection:*

Date of received in laboratory: *Sample ID no:*

TYPE OF EXAMINATION	
Helminths	*Microscopic:* *Molecular:* *Serological:*
Blood protozoa	*Microscopic:* *Molecular:* *Serological*
Ectoparasite/ Arthropod	*Microscopic:* *Molecular:*
RESULT: SUGGEESTION/COMMENT:	

Signature of the Investigator

Signature Of the In-charge

OFFICE OF THE CORE LAB I
ADVANCED ANIMAL DISEASE DIAGNOSIS & MANAGEMENT CONSORTIUM
COLLEGE OF VETERINARY SCIENCE, AAU, KHANAPARA, GUWAHATI-781022

File No: **Date.....**

Owner's name: *Ph No.*

Address:

Ref.no: *species:*

Clinical symptoms:

Post mortem findings:

Type of sample: *Date of collection:*

Date of received in laboratory: *Sample ID no:*

TYPE OF EXAMINATION	
Gross Change	*External :* *Lymphoid organs:* *Non-lymphoid organs:* *Brain & Nervous system:* *GI organs:*
Histopathological changes	*Microscopic:* *Histochemical:*
RESULT: SUGGEESTION/COMMENT:	

Signature Of the Investigator

Signature Of the In-charge

19. Contact Details of Veterinary Disease Diagnosis Laboratories/ Institutes

Designation	Address	Phone No/ Email Id
National Institutes/Laboratories		
Director	Indian Veterinary Research Institute (IVRI) Deemed University, Izatnagar-243122(U.P)	+91-581-230096 dirivri@ivri.res.in
Director	ICAR -National Institute of High Security Animal Diseases, Anand Nagar, Bhopal- 462 022 (MP), India	0755-2759204 director.nihsad@icar.gov.in scd11@yahoo.in
Director	ICAR - National Institute of Veterinary Epidemiology and Disease Informatics(NIVEDI) Ramagondanahalli, Post Box No: 6450,Yelahanka, Bengaluru 560064, Karnataka State, India	080- 2309 3100 / 2309 3110 director.nivedi@icar.gov.in
Director	ICAR-National Research Centre on Equines, Sirsa Road, Hisar-125 001 (Haryana) India.	01662-275787 nrcequine@nic.in
Director	ICAR-Veterinary Type Culture Collection, Sirsa Road, Hisar-125 001 (Haryana) India	+91-1662-275787/ 278790 nrcequine@nic.in
Joint Director	Central Disease Diagnostic Laboratory, Centre for Animal Disease Research and Development (CADRAD) Indian Veterinary Research Institute. Izatnagar-243122(U.P)	0581-2302188 / 2310074 jdcadrad@rediffmail.com
North Eastern Institutes		
Director	ICAR Research Complex for NEH, Barapani, Umroi Road, Umiam, Meghalaya. Pin – 793103,Meghalaya	0364-2570257 director.icar-neh@icar.gov.in
Director	ICAR-National Research Centre on Pig, Rani (Near Airport), Guwahati- 781 131	0361-2847195, 2847221 nrconpig@rediffmail.com
Director	ICAR- National Research Centre on Yak,Dirang-790101, West Kameng District, Arunachal Pradesh.	+91-3780-242220,242387 yakdirector@gmail.com

Director	ICAR-National Research Centre on Mithun, Jharnapani, Medziphema Dist.: Dimapur,Nagaland 797106	03862 247340 director.nrcmithun@icar.gov.in
Central Institutes		
Dean	College of Veterinary Sciences & Animal Husbandry, Selesih, Aizawl - 796014, Mizoram, India	91-389-2361748 cvsc_dean@yahoo.co.in dean@cvsccauaizawl.org
Dean(i/c)	College of Veterinary Sciences & AH, Jalukie-797110. Peren. Dist. Nagaland	covcsccaujalukie@yahoo.com
State Institutes		
Vice-Chancellor	Assam Agricultural University Jorhat -785013, Assam, India	0376-2340001/ 2340013 vc@aau.ac.in
Director of Research(Vety)	Assam Agricultural University, Khanapara, Guwahati-781022	0361-2364941 drvetyaau@gmail.com
Dean	College of Veterinary Science, Khanapara, Guwahati-781022.	0361-2337700 dean.fvsc.aau@gmail.com
Associate Dean	Lakhimpur College of Veterinary Science, Saboti Rd, 9/6 Koilamari, Assam 787051	8011324088/7896376839
Principal	College of Veterinary Science & A.H, Agartala, Bodhjung Nagar, Agartala, Tripura 799008	0381 2391005/2391004 cvscrknagar@gmail.com
State Department, Assam		
Director	Directorate of AH & Veterinary Chenikuthi, Guwahati, Assam-781003	0361-2668609 assamvety@gmail.com
Deputy Director	North Eastern Regional Disease Diagnostic Laboratory Animal Husbandry & Veterinary Department Khanapara, Guwahati-781022	0361-2334177 nerddlguwahati@gmail.com

Disease Investigation Officer (DIO)	North Eastern Regional Disease Diagnostic Laboratory Animal Husbandry & Veterinary Department Khanapara, Guwahati-781022	0361-2334177 nerddlguwahati@gmail.com
Director	Directorate Of Forensic Science Kahilipara, Guwahati, Assam 781019	0361 238 1385
State Department, Meghalaya		
Director	Directorate of Animal Husbandry and Veterinary, Govt. of Meghalaya, Lumdiengjri, Shillong – 793002, East Khasi Hills District,Meghalaya	91-364-2548388 ahvt-meg@nic.in/ ahvtmeg@nic.in
Disease Investigation Officer (DIO)	State Disease Diagnostic Laboratory, East Khasi Hills District, Shillong	dioahvety@bsnl.in
State Department, Tripura		
Director	Animal Resource Development Department, Prani Sampad Bikash Bhawan, Pandit Nehru Complex Gurkhabusti, P.o. –Kunjaban, PIN-799006, West Tripura.	0381-2323611 ardd.tripura@gmail.com
Disease Investigation Officer (DIO)	State Disease Investigation. Laboratory, Abhoynagar, West Tripura.	0381-232263
State Department, Nagaland		
Director	Directorate of Veterinary and Animal Husbandry Kohima-797001 Nagaland.	0370 – 2221320
Disease Investigation Officer (DIO)	Disease Investigation. Laboratory, Directorate of Veterinary and Animal Husbandry,Kohima-797001, Nagaland.	9862997615/9774292842 kedultu@yahoo.co.in

State Department, Manipur		
Director	Veterinary & A.H. Services, Manipur, Sanjenthong Imphal East-795001 Manipur	91-385-2450224
Disease Investigation Officer (DIO)	Disease Investigation. Laboratory, Directorate of Vety.AH Services Manipur, Imphal - Pin No. 795001.	Ibotombi7@gmail.com 09436890396
State Department, Arunachal Pradesh		
Director	Directorate of AH & Veterinary Department. Govt. of Arunachal Pradesh.	(0360)2257576 directorahvarunachal2016@gmail.com
Disease Investigation Officer (DIO)	Disease Investigation. Laboratory, Directorate of AH & Veterinary Department, Nirjuli, Papumpare, Arunachal Pradesh.	08729891694 yangtapir@gmail.com
State Department, Mizoram		
Director	Directorate of AH & Vety. Government of Mizoram Aizawl, Mizoram – 796001	0389-2333647
Disease Investigation Officer (DIO)	Disease Investigation Laboratory Directorate of AH & Vety. Government of Mizoram Aizawl, Mizoram – 796001	0389-2333647
State Department, Sikkim		
Director	Department Of Animal Husbandry Livestock, Fisheries Services Government Of Sikkim Krishi Bhawan, Tadong	09434110002
Disease Investigation Officer (DIO)	Dy. Director,DIC, Gangtok	zeruiah02@yahoo.co.in

20. CONTACT DETAILS OF COMPANIES/SUPPLIERS OF LABORATORY ITEMS AND REAGENTS

Items	Company	Phone No/ Email
Plastic wares	Tarson Products Pvt.Ltd.	011 43542761 info@tarsons.in
	Axygen Scientific Pvt. Ltd.	91-11-45501541/ 45501542/ 25534161/2553462
	Genexy Scientific Pvt. Ltd	01792-222 291 sales@genaxy.com
Glasswares	Borosil Glasswares Ltd.	91 (033) 2229 9166 / 2249 5574 calcutta@borosil.com
	Riviera Glass Pvt. Ltd.	+91-22-28475228 +91-22-28473297
Media (Chemicals)	HiMedia Laboratories Pvt. Ltd.	91-22-6147 1919, 2500 3747, 2500 0970, 2500 0278 info@himedialabs.com
	Merck Pvt. Ltd.	+91-22-6210 9000
	Sisco Research Laboratories Pvt.Ltd	+91-22-4268 5800
	Genetix Biotech Asia Pvt. Ltd.	011 4502 7000
	Bangalore Genei Pvt. Ltd.	098452 64202
ELISA Plate	Nunc (Thermo Fisher Scientific)	
D Vacutainer	Becton Dickinson	**Phone No.** 0361-2545282/2631752
Liquid Handling Pipette	Eppendorf	+91 44 66 312 222 info@eppendorf.co.in
	Finpipette (Thermo Fisher Scientific Ltd.)	
	Nichipet (Axygen Scientific)	91-11-45501541/ 45501542/ 25534161/2553462
	Tarson Products Pvt.Ltd.	011 43542761 info@tarsons.in