Animal Science

Veterinary Laboratory Technology

Government of Nepal
Ministry of Education, Science and Technology
Curriculum Development Centre
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Grade 10

Technical and Vocational Stream
Learning Resource Material

Veterinary Laboratory Technology

(Grade 10)

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Preface

The curriculum and curricular materials have been developed and revised on a regular basis with the aim of making education objective-oriented, practical, relevant and job oriented. It is necessary to instill the feelings of nationalism, national integrity and democratic spirit in students and equip them with morality, discipline, self-reliance, creativity and thoughtfulness. It is essential to develop linguistic and mathematical skills, knowledge of science, information and communication technology, environment, health and population and life skills in students. It is also necessary to bring the feeling of preserving and promoting arts and aesthetics, humanistic norms, values and ideals. It has become the need of the present time to make them aware of respect for ethnicity, gender, disabilities, languages, religions, cultures, regional diversity, human rights and social values to make them capable of playing the role of responsible citizens with applied technical and vocational knowledge and skills. This learning resource material for Animal Science has been developed in line with the Secondary Level Animal Science Curriculum with an aim to facilitate the students in their study and learning on the subject by incorporating the recommendations and feedback obtained from various schools, workshops, seminars and interaction programs attended by teachers, students and parents.

In bringing out the learning resource material in this form, the contribution of the Director General of CDC Mr. Yubaraj Paudel and members of the subject committee Dr. Manraj Kolakshpati, Madhukumari Tiwari, Lavdev Bhatta is highly acknowledged. The learning resource material is written by Dr. Ganesh Gautam Dr. Shibalal Bhandari and Dr. Asis Mahat the subject matter of the materials, was edited by Mr. Badrinath Timsina and Mr. Khilanath Dhamala and language was edited by Mr. Bijaya Kumar Ranabhat. CDC extends sincere thanks to all those who have contributed to developing this material in this form.

This learning resource material contains a wide coverage of subject matters and sample exercises which will help the learners to achieve the competencies and learning outcomes set in the curriculum. Each chapter in the material clearly and concisely deals with the subject matters required for the accomplishment of the learning outcomes. The Curriculum Development Centre always welcomes constructive feedback for the betterment of the material.

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Table of Content

Guidelines to Teachers

A. Facilitation Methods

The goal of this course is to combine the theoretical and practical aspects of the contents needed for the subject. The nature of contents included in this course demands the use of practical or learner focused facilitation processes. Therefore, the practical side of the facilitation process has been focused much. The instructor is expected to design and conduct a variety of practical methods, strategies or techniques which encourage students engage in the process of reflection, sharing, collaboration, exploration and innovation new ideas or learning. For this, the following teaching methods, strategies or techniques are suggested to adopt as per the course content nature and context.

Brainstorming

Brainstorming is a technique of teaching which is creative thinking process. In this technique, students freely speak or share their ideas on a given topic. The instructor does not judge students' ideas as being right or wrong, but rather encourages them to think and speak creatively and innovatively. In brainstorming time, the instructor expects students to generate their tentative and rough ideas on a given topic which are not judgmental. It is, therefore, brainstorming is free-wheeling, non-judgmental and unstructured in nature. Students or participants are encouraged to freely express their ideas throughout the brainstorming time. Whiteboard and other visual aids can be used to help organize the ideas as they are developed. Following the brainstorming session, concepts are examined and ranked in order of importance, opening the door for more development and execution. Brainstorming is an effective technique for problem-solving, invention, and decision-making because it taps into the group's combined knowledge and creative ideas.

Demonstration

Demonstration is a practical method of teaching in which the instructor shows

or demonstrates the actions, materials, or processes. While demonstrating something the students in the class see, observe, discuss and share ideas on a given topic. Most importantly, abstract and complicated concepts can be presented into visible form through demonstration. Visualization bridges the gap between abstract ideas and concrete manifestations by utilizing the innate human ability to think visually. This enables students to make better decisions, develop their creative potential, and obtain deeper insights across a variety of subject areas.

Peer Discussion

Peer conversation is a cooperative process where students converse with their peers to exchange viewpoints, share ideas, and jointly investigate subjects that are relevant or of mutual interest. Peer discussion is an effective teaching strategy used in the classroom to encourage critical thinking, active learning, and knowledge development. Peer discussions encourage students to express their ideas clearly, listen to opposing points of view, and participate in debate or dialogue, all of which contribute to a deeper comprehension and memory of the course material. Peer discussions also help participants develop critical communication and teamwork skills by teaching them how to effectively articulate their views, persuasively defend their positions, and constructively respond to criticism.

Peer conversation is essential for professional growth and community building outside of the classroom because it allows practitioners to share best practices, work together, and solve problems as a group. In addition to expanding their knowledge horizon and deepening their understanding, peer discussions help students build lasting relationships and a feeling of community within their peer networks.

Group Work

Group work is a technique of teaching where more than two students or participants work together to complete a task, solve a problem or discuss on a

given topic collaboratively. Group work is also a cooperative working process where students join and share their perspectives, abilities, and knowledge to take on challenging job or project. Group work in academic contexts promotes active learning, peer teaching, and the development of collaboration and communication skills. Group work helps individuals to do more together than they might individually do or achieve.

Gallery Walk

Gallery walk is a critical thinking strategy. It creates interactive learning environment in the classroom. It offers participants or students a structured way to observe exhibition or presentation and also provides opportunity to share ideas. It promotes peer-to-peer or group-to-group engagement by encouraging participants to observe, evaluate and comment on each other's work or ideas. Students who engage in this process improve their communication and critical thinking abilities in addition to their comprehension of the subject matter, which leads to a deeper and more sophisticated investigation of the subjects at hand.

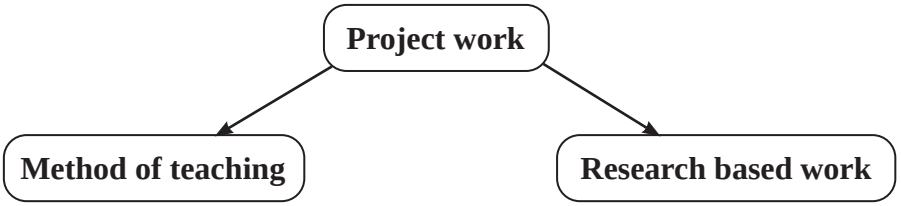
Interaction

The dynamic sharing of ideas, knowledge, and experiences between people or things is referred to as interaction, and it frequently takes place in social, academic, or professional settings. It includes a broad range of activities such as dialogue, collaboration or team work, negotiation, problem solving, etc. Mutual understanding, knowledge sharing, and interpersonal relationships are all facilitated by effective interaction. Interaction is essential for building relationships, encouraging learning, and stimulating creativity in both in-person and virtual contexts. Students can broaden their viewpoints, hone their abilities, and jointly achieve solutions to difficult problems by actively interacting with others.

Project Work

Project work is a special kind of work that consists of a problematic situation which requires systematic investigation to explore innovative ideas and solutions.

Project work can be used in two senses. First, it is a method of teaching in regular class. The next is: it is a research work that requires planned investigation to explore something new. This concept can be presented in the following figure.



Project work entails individuals or teams working together to achieve particular educational objectives. It consists of a number of organized tasks, activities, and deliverables. The end product is important for project work. Generally, project work will be carried out in three stages. They are:

- Planning
- Investigation
- Reporting

B. Instructional Materials

Instructional materials are the tools and resources that teachers use to help students. These resources/materials engage students, strengthen learning, and improve conceptual comprehension while supporting the educational goals of a course or program. Different learning styles and preferences can be accommodated by the variety of instructional resources available. Here are a few examples of typical educational resource types:

- Daily used materials
- Related Pictures
- Reference books
- **Slides and Presentation:** PowerPoint slides, keynote presentations, or other visual aids that help convey information in a visually appealing and organized manner.
- **Audiovisual Materials:** Videos, animations, podcasts, and other

multimedia resources that bring concepts to life and cater to auditory and visual learners.

- **Online Resources:** Websites, online articles, e-books, and other web-based materials that can be accessed for further reading and research.

Maps, Charts, and Graphs: Visual representations that help learners understand relationships, patterns, and trends in different subjects.

Real-life Examples and Case Studies: Stories, examples, or case studies that illustrate the practical application of theoretical concepts and principles.

C. Assessment

Formative Test

Classroom discussions: Engage students in discussions to assess their understanding of concepts.

Quizzes and polls: Use short quizzes or polls to check comprehension during or after a lesson.

Homework exercises: Assign tasks that provide ongoing feedback on individual progress.

Peer review: Have students review and provide feedback on each other's work.

Summative Test

Exams: Conduct comprehensive exams at the end of a unit or semester.

Final projects: Assign projects that demonstrate overall understanding of the subject.

Peer Assessment

Group projects: Evaluate individual contributions within a group project.

Peer feedback forms: Provide structured forms for students to assess their peers.

Classroom presentations: Have students assess each other's presentations.

Objective Test

Multiple-choice tests: Use multiple-choice questions to assess knowledge.
True/False questions: Assess factual understanding with true/false questions.

Matching exercises: Evaluate associations between concepts or terms.

Portfolio Assessment

Compilation of work: Collect and assess a variety of student work samples.

Reflection statements: Ask students to write reflective statements about their work.

Showcase events: Organize events where students present their portfolios to peers or instructors.

Observational Assessment

Classroom observations: Observe students' behavior and engagement during class.

Performance observations: Assess practical skills through direct observation.

Field trips: Evaluate students' ability to apply knowledge in real-world settings.

Common Laboratory Equipment and Their Functions

Unit 1

1.1 Microscope: Simple and Compound

Microscope: A microscope is an optical instrument with one or more lens systems that are used to get a clear, magnified image of minute objects or structures that can't be viewed by the naked eye. Microscope are used for observing microscopic items such as cells, crystals and cell organelles. It has a dual function of magnification and resolution for routine microbiological works, bright field compound microscope with oil immersion objective is adequate.

Type of microscope

a. **Simple Microscope:** It consists of single lens and single magnifying glass. With the help of simple microscope the image of an object can be magnified up to 10x.

b. **Compound Microscope:** Generally compound microscopes are used in the lab because it consists of different types of lens. Compound microscope consists of two types of lens. The magnified image of an object by one lens is further magnified by the second lens. Due to this the objects can be identified easily. The lenses used in the compound microscope are known as eyepiece and objective lens.

i. **Monocular Microscopes:** Monocular microscope has one eyepiece and eye tube that can magnify samples up to 1,000 times, commonly use to view objects mounted onto glass slides with no depth.

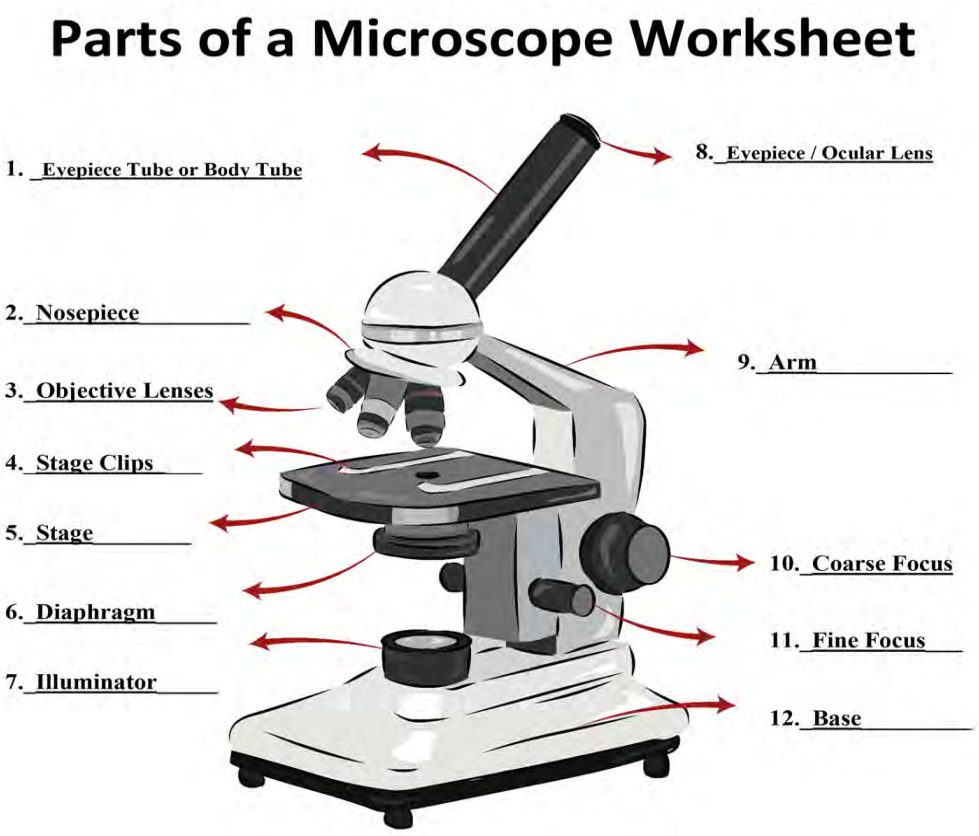


Figure 1. Monocular Microscope

ii. **Binocular Microscope:** The term binocular refers to 'relating to or involving the use of both eyes'. Binocular microscope provides us the ability to focus both eyes on an object at the same time so that a single image is seen. This enables judgment of distance and depth of an image.

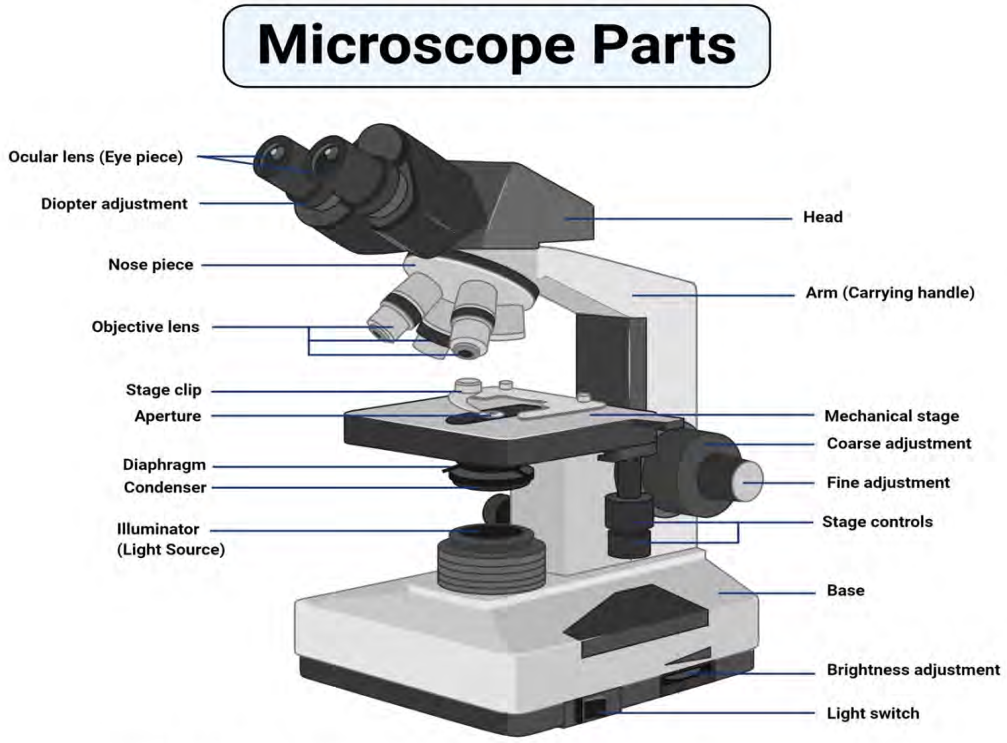


Figure 2. Binocular Microscope

Parts of microscope and their functions:

1. **Eyepiece or Ocular Lens**

Eyepiece lens magnifies the image of the specimen. This part is also known as ocular. Most school microscopes have an eyepiece with 10X magnification.

2. **Eyepiece Tube or Body Tube:** The tube holds the eyepiece.
3. **Nosepiece:** Nose piece holds the objective lenses and is also called a revolving turret. It is connected to the body tube and lies just above the stage. It can be rotated clockwise or counterclockwise to increase or decrease the magnification. The change in magnification results due to a change in the objective lens.
4. **Objective Lenses:** Most compound microscopes come with three or four

objective lenses that revolve on the nosepiece. The most common objective lenses have power of 4X, 10X and 40X. Combined with the magnification of the eyepiece the resulting magnification is 40X, 100X and 400 X magnifications. Total magnification is calculated by multiplying the power of the eyepiece by the power of the objective lens. (10X Eyepiece X 40X Objective = 400X Total Magnification) Some more advanced microscopes have an additional objective lens with 100X power. This results in 1,000X magnification.

5. **Arm:** The arm connects the base to the nosepiece and eyepiece. It is the structural part that is also used to carry the microscope.
6. **Stage:** The stage is where the specimen is placed. This place is for observation.
7. **Stage Clips:** Stage clips are the supports that hold the slides in place on the stage.
8. **Diaphragm (also called the Iris):** The diaphragm controls the amount of light passing through the slide. It is located below the stage and is usually controlled by a round dial. How to set the diaphragm is determined by the magnification, transparency of the specimen and the degree of contrast you wish to have in your image also called the condenser diaphragm.
9. **Illuminator:** Most light microscopes use a low voltage bulb which supplies light through the stage and onto to the specimen. Mirrors are sometimes used instead of a built-in light. If your microscope has a mirror, it provides light reflected from ambient light sources like classroom lights or sunlight if outdoors.
10. **Coarse focus:** Coarse focus moves the stage to provide general focus on the specimen. When bringing a specimen into focus, the course dial is the first one used.
11. **Fine Focus:** Fine focus moves the stage in smaller increments to provide a clear view of the specimen. When bringing a specimen into focus, the fine focus dial is the second one used.

12. **Base:** The base is the main support of the microscope. The bottom, all the other parts of the microscope stand.

Uses of Microscope

Micrope are used in different fields for different purposes. Some of their uses are tissue analysis, the examination of forensic evidence, to determine the health of the ecosystem, studying the role of protein within the cell, and the study of atomic structure.

1. To observe tiny things like cells and bacteria that are too small for our eyes.
2. To study plant and animal cells and understand their structure.
3. To observe changes in experiments, like how cells react to different substances.
4. To identify microorganisms that can cause diseases.
5. To learn how science works by exploring the hidden world around us.

1.2 Hot Air Oven

It is the square box shaped laboratory equipment . It consists of double layered internal and external wall of steel or aluminum. It also consists of a door. It consists of a switch for maintaining the temperature. It also consists of a thermometer. It consists of a hole on both sides for the entrance and exit of the air. It consists of racks for placing different materials. On the lower inner side it consists of heating element for heating purpose. Hot air oven consists of a fan for maintaining the

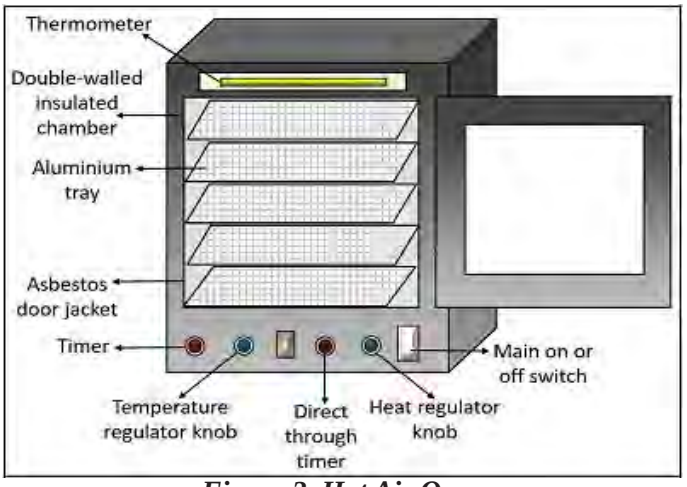


Figure 3. Hot Air Oven

air temperature inside it. The maximum temperature of 180 ° Celsius can be maintained inside the hot air oven.

Use of Hot Air Oven

1. Sterilization of metals and glassware's can be carried out inside the hot air oven. For example surgical equipment's glass bottles, pipettes, petridis etc.
2. Glassware's, tubes, sterilized equipment's can be air dried inside the hot air oven.
3. The slides contains different bacteria can be fixed by keeping inside the hot air oven. For e.g. Slides to be used for the staining of bacillus anthracis.

Procedure to Use Hot Air Oven

1. Connect the plug of hot air oven to the electrical supply and close the door.
2. Switch on the electrical supply.
3. Maintain the temperature using the temperature knob. Be careful whether the temperature increases or not.
4. Keep the equipment to be kept inside it.
5. After certain time, switch off the electrical supply and open the door when the temperature goes down.

Handling of Hot Air Oven

1. Always be sure to perform earthing of the wires to be used on hot air oven
2. Plastics, papers, clothes, cottons etc. should never be dried on hot air oven.
3. If water, medias, plastics, etc. fall inside the hot air oven they should be immediately removed and cleaned.
4. Always close the door before running the hot air oven.
5. Never keep the edibles, clothes etc. inside the hot air oven.
6. Never set the high temperature and go away for another work.
7. Clean the hot air oven after the completion of work.

1.3 Autoclave

An autoclave is a machine that provides a physical method of sterilization by killing bacteria, viruses, and even spores present in the materials put inside in the vessel using steam under pressure. Autoclave sterilizes the materials by heating them up to a particular temperature for a specific period. The autoclave is considered as a more effective method of sterilization. It is based on moist heat sterilization.

Working Principle of Autoclave

The autoclave works on the principle of moist heat sterilization where steam under pressure is used to sterilize the material present inside the chamber. The high pressure increases the boiling point of water and thus helps achieve a higher temperature for sterilization. Water usually boils at 100°C under normal atmospheric pressure (760 mm of Hg); however, the boiling point of water increases if the pressure is to be increased. Similarly, the high pressure also facilitates the rapid penetration of heat into deeper parts of the material, and moisture present in the steam causes the coagulation of proteins causing an irreversible loss of function and activity of microbes.

This principle is employed in an autoclave where the water boils at temperature of 121°C (775mm of Hg) for 15-psi pressure at least 15 minutes by using saturated steam.

Working

In general, an autoclave runs at a temperature of 121° C for at least 15 minutes by using saturated steam under at least 15 psi of pressure.

Steps to be Followed while Running an Autoclave

- Before beginning to use the autoclave, it should be checked for any items left from the previous cycle.
- A sufficient amount of water should be put inside the chamber.
- Now, the materials to be sterilized are placed inside the chamber.

- The lid is then closed, the screw are tight to ensure an airtight condition, and the electric heater is switched on.
- The safety valve are adjust to maintain the required pressure in the chamber.
- Once the water inside the chamber boils, the air-water mixture is allowed to escape through the discharge tube to let all the air inside to be displace. The complete displacement can be ensured once the water bubbles cease to come out from the pipe.

Figure 4. Autoclave

- The drainage pipe is then closed, and the steam inside is allowed to reach the desired levels (15 lbs in most cases).Once the pressure is reached, the whistle blows to remove excess pressure from the chamber.
- After the whistle, the autoclave runs for a holding period, which is 15 minutes in most cases.
- Now, the electric heater is switched off, and the autoclave is allowed to cool until the pressure gauge indicates the pressure inside has lowered down to that of the atmospheric pressure.
- The discharge pipe is then opened to allow the entry of air from the outside into the autoclave.
- Finally, the lid is opened, and the sterilized materials are out of the chamber.

1.4. Incubator

An incubator is based on the principle that microorganisms require a particular set of parameters for their growth and development. All incubators are based on the concept that when organisms are provided with the optimal condition of temperature, humidity, oxygen, and carbon dioxide levels, they grow and divide to form more organisms. In an incubator, the thermostat maintains a constant temperature that can be reads from the outside via the thermometer.

The temperature is maintained by utilizing the heating and no-heating cycles. During the heating cycle, the thermostat heats the incubator, during the no-heating period, the heating is stopped, and the incubator is cooled by radiating heat to the surrounding. Insulation from the outside creates an isolated condition inside the cabinet, which allows the microbes to grow effectively. Similarly, other parameters are humidity and airflow maintained through different mechanisms that create an environment similar to the natural environment of the organisms. Similarly, they are provided with adjustments for maintaining the concentration of CO2 to balance the pH and humidity required for the growth of the organisms. Variation of the incubator like a shaking incubator is also available, which allows for the continuous movement of the culture required for cell aeration and solubility studies.

Procedure for Running an Incubator

Once the cultures of organisms are created, the culture plates are to be placed inside an incubator at the desired temperature and required period. In most clinical laboratories, the usual temperature is maintained is 35–37°C for bacteria.

Steps to be followed while running an incubator

- Before using the incubator, it should be made sure that no remaining items are presented in the incubator from the previous cycles. However, in some cases, if the same incubator is being used for multiple organisms, and they require the same set of parameters, they can be placed together in the same incubator.

- The door of the incubator is closed, and the incubator is switched on. The incubator has to be heated to the desired temperature for the growth of the particular organism. The thermometers used to see. The temperature has reach.
- In the meantime, if the organism requires a particular concentration of CO_2 or a specific humidity, those parameters should also be set in the incubator.
- Once all the parameters are met, the petridish cultures is placed on the perforated shelves upside down, i.e., media uppermost. This is necessary because if the plates are incubated normally, condensation collects on the surface of the medium and prevents the formation of isolated colonies.
- If it is necessary to incubate Petri dish cultures for several days, the plates are sealed with adhesive tapes or are place in plastic bags or plastic food containers.
- Now, the door is locked, and the plates are kept inside for the required time before taking them out.

1.5 Water bath

A water bath is laboratory instrument. It is a container or vessel filled with heated water. The temperature of water is maintained at a constant level. It is used to incubate samples over a period at a constant temperature.

The Main Parts of Water Bath

- Container or tank bath
- Heater
- Thermometer
- Thermostat or regulator

Type of Water Bath

- Circulating water bath

- Non-circulating water bath
- Shaking water bath

Principle of Water Bath

Principle of water bath is based on the fact that this device depends on the heat applied to the sample using the heater.

Use of Water Bath

- It is used to improve the solubility of poorly soluble substances.
- It is used for melting of some substances.
- It is used for warming of chemical reagents.
- It is used for facilitating of some chemical reactions.
- It is used for incubation of cell cultures.

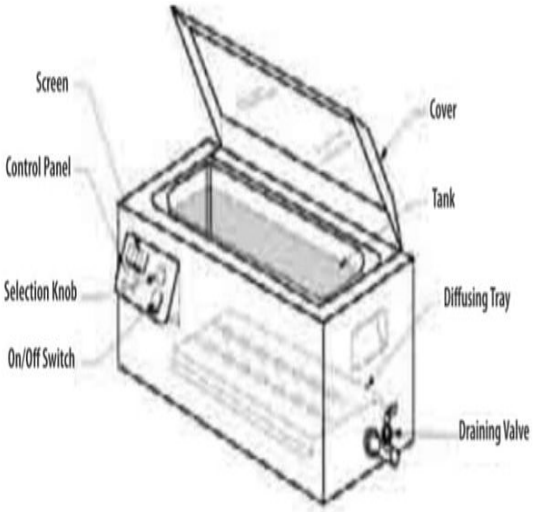


Figure 6. Water bath

- It is used as a heat source for some substance such as flammable chemicals.

1.6 Distillation Set

Distillation is a common operation in many laboratories to separate and purify components of a liquid mixture. The apparatus used consists of three major parts: a distillation flask to heat the mixture and vapourized the components, a condenser to cool the vapours back to a liquid state, and collection vessels to collect the liquid.

The conversion of substance into vapour and then converting then liquid is called distillation. In other words, the process of evaporation follow by condensation

known as distillation. The liquid, which is received after condensation is called distillate. The following apparatus are required to prepare distillation set:

1. Glass retort with stopper : to take sample
2. Conical flask; to receive distillate
3. Stand and clamp: to hold apparatus
4. Water trough: To keep conical flask cold, (it may be obtaining by wet cloth/ filter paper on the outer wall conical flask.)

Parts of the Distillation Set

- Burner
- Vapours
- Condenser
- Water in tube
- Distillation flask
- Thermometer
- Water out tube
- Receiving flask

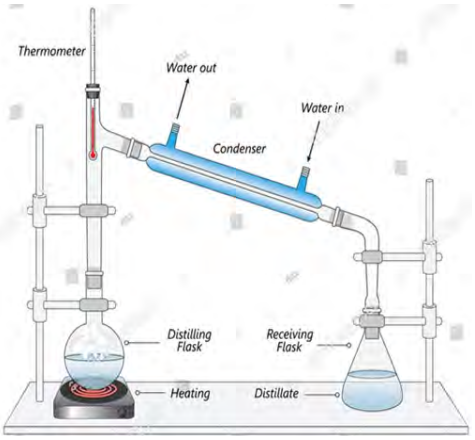


Figure 7. Distillation set

The Procedure of Distillation Set

- First place the water up to the half region of the distillation flask placed over the burner.
- The after the vapors will start to form, allow the vapours to pass through the condenser.
- The tap water cools the condenser continuously. The vapour will cool down, change into water, and get stored at the reservoir.
- After the work has been completed, switch off the Bunsen burner.
- At last, close the water supply.

1.7 Colorimeter

A colorimeter is a device used in colorimetry that measures the absorbance of particular wavelengths of light by a specific solution. This is used for biochemistry analysis in the laboratory.

Procedure to Use Colorimeter

- First, switch the colorimeter 10-15 minutes before use.
- Maintain the filter and wavelength according to the need.
- Now, place the bank solution on the cuvette and place it on the cuvette holder.
- The Optical Density (OD) should be adjusted to zero by coarse and fine adjustment. We should maintain the OD of the blank solution to zero for two times.
- The blank solution is removed from the cuvette and the test solution placed. Now, observe the OD, and similarly observe the OD of the standard solution.
- After the procedure is finished, remove the solution from the cuvette and clean it. The colorimeter can be switched off.

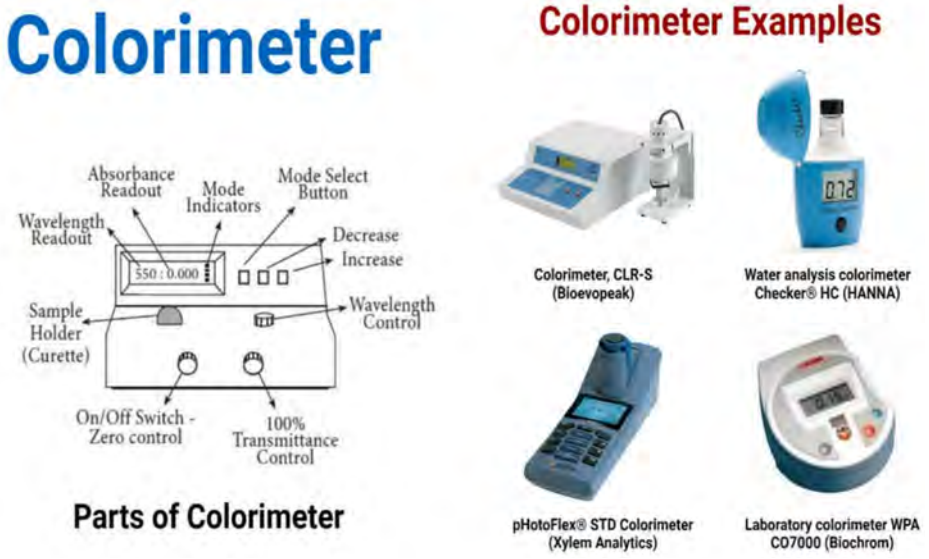


Figure 8. Parts of colorimeter

1.8 Refrigerator

A laboratory refrigerator is a common laboratory apparatus that consists of a thermally insulated compartment and a heat pump. The heat pumps transmit the heat from the inside of the unit to the external environment. They are used to cool and store samples or specimens, for preservation. They include refrigeration units for storing blood plasma and other blood products, as well as vaccines and other medicines at a specific temperature. The temperature to be maintained inside the refrigerator should be 2-8^{0}c.

Cold Chain Maintenance

- The samples, reagent, vaccines, and other substances that can be degraded by high temperatures should be kept at a low temperature for their efficacy. These substances will be in effective. When they are kepted at room temperature or higher temperature. In the laboratory, the refrigerator containing these substances should not be turned off.

Precautions while Using Refrigerator

- Voltage stabilizers always should be used as the refrigerator can get damage due to voltage fluctuations.
- The temperature should not be lowered in the refrigerator as it may cause ice formation.
- One should only open the refrigerator when there is a need. The unnecessary opening and closing of the door should be avoided.

Figure 9. Refrigerator

- The edibles, food, and other household materials should not be kept inside the laboratory refrigerator.
- Always clean the refrigerator regularly.

1.9 Centrifuge

- A centrifuge is a device that uses centrifugal force for the separation of two liquids in a mixture. This is achieved by spinning the fluid at high speed in a centrifuge machine, which separates fluids of different densities.
- In this process, the denser components of mixture migrates away from the axis (bottom) and the lighter component migrates toward the axis (top). Example during centrifugation of blood, the plasma components are at the top, and red blood cells are at the bottom.

Procedure of Using a Centrifuge

- First, open the lid of the centrifuge.
- The sample that is loaded inside the test tube should kept inside the centrifuge.
- The tubes of the centrifuge must contain an equal amount of the sample. If there is an odd number of samples, then it should balance by keeping tubes with water/saline.
- Now, close the lid and switch on the electricity supply.
- The motion of the centrifuge should increase simultaneously from lower to higher.
- Rotate the centrifuge as required. This depends upon the sample, and the time can be from 5-15 minutes.
- After the required time has been met, rotate the knob to zero.
- As the centrifuge stops completely, open the lid.
- Now, remove the centrifuge tubes and close the lid after all the tubes are taken out.
- Switch off the lid of the centrifuge.

Precautions while Using the Centrifuge

- Always balance the buckets of the centrifuge.

- Never spill the liquids /salts on the centrifuge.
- The motion should be increased simultaneously from lower to higher.
- Do not seal the mouth of the centrifuge tube.
- After the completion of the rotation cycle, one should not stop the rotation by using unnecessary force.

Figure 10. Centrifuge

1.10 pHmeter

pH meter is an electronic instrument used for the measuring hydrogen ion concentration of solution and mixtures in microbiology lab. It is used for maintaining pH of the medium and diluents. Then pH meter must be standardized with buffer solution before operation. Since the instrument is very sensitive, it must not be dipped in hot or very cold solution. The electrodes must bealways kept immersed in suitable solution. Read the manual carefully before using the instrument.

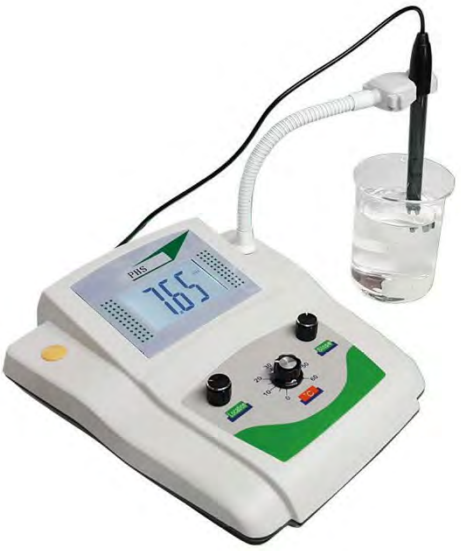


Figure11. pH meter

It is used for measuring the PH of solution on the lab. Different types of pH meter are available on the market. Some pH meters run through battery while some run with direct current. It consists of sensitive voltmeter. Ti measures the current influence of measuring electrode and reference electrode.

pH of solution

All the solution contains the mixture of hydroxide ion and hydrogen ion. For

example some solution contains the equal amount of hydroxide ion and hydrogen ion which is known as neutral solution. Distilled water ionizes one molecule of hydrogen ion and one molecule of hydroxide ion. If the solution contains more amount of hydrogen ion it is called acidic solution and if the solution contains more amount of hydroxide ion it is called to be alkaline solution.

The value of PH ranges from 0-14. If the pH values goes 6,5,4 it is acidic and if the pH increases from 7 and goes to 9,10 it is called alkaline solution.

Acidity	Neutrality	Alkalinity
1,2,3,4,5,6	7	8,9,10,11,12,13,14

The help of pH value can measure the acidity and alkalinity of a solution. If the solution contains low amount of hydrogen ion it contains more amount of hydroxide ion therefore the solution is alkaline. If there is low amount of hydroxide ion and more amount of hydrogen ion the solution is acidic.

pH can be measured by using the pH paper or pH meter.

pH value of some chemical solution and other solution

Measuring of pH

- pH value is compared with the pH of a standard solution, which can be directly known from electric amplifier, digitally.
- The temperature of standard solution and the sample solution which pH is to be measured should be equal.

Calibration of PH meter

Before using the pH meter it should be checked on the buffer solution containing the pH of 4, 7 and 9.

Procedure to use pH Meter

- Switch on the pH meter.
- Electrode should be cleaned with distilled water and wiped with tissue paper

- Dip the electrode on pH 4 buffer solution and maintain the pH of 4. Clean the electrode with distilled water.
- Dip the electrode on pH 9 buffer solution and maintain the pH of 9. Clean the electrode with distilled water.
- Dip the electrode on the solution, which pH is to be measured to record the value of pH seen on pH meter.
- Clean the electrode with distilled water after measuring the pH of every solution.
- After the completion of measuring, wash the electrode with distilled water or dip it on distilled water.

Taking Care of PH Meter

- The electrode of pH meter should be dipped on water.
- Never measure the pH of extremely hot solution.
- Always keep the electrode of pH meter clean.
- While measuring the pH of different solution the electrode should be immediately cleaned after measuring the pH of one solution.

1.11 Weighing Balance

Weighing balance is the most important equipment of laboratory. While preparing the reagents, solutions, staining solution etc the chemical reagents should be measured on exact amount. Weighing balance on lab should be very sensitive and have the capacity to differentiate the weight of 0.01 gm (10mg)

Balance is needed in microbiology lab for weighing chemicals, sample, media, etc. digital balance is fast to work with but needs frequent calibration.

The triple-beam-balance and 4-beam-balance are robust equipment that need little care and maintenance. Beam balance runs on mechanical principle on which electric balance run is quite complicated.

Different Types of Weighing Balance Used on Laboratories

- Manually operated balance
- Electronic balance
- Analytical balance

Manually Operated Balance

It contains pans on both sides. On the one side, weight is placed and on the other side the sample to be measured is placed. Manually operated balances may contain 2 pans, one pan or may be of sliding type. This type of balance weighs up to 10 gm.

Figure 12. Manually operated balance

Electronic Balance

Figure 13. Electronic balance

It runs with electricity. It is small, square, flat and box like in shape. It contains of a measurement to see whether it is on balance or not. The height of the measurement can be increased or decreased to balance the weighing balance. It consists of single pan and the weight is displayed digitally. It is expensive and can not be used on the place where there is no electrical supply.

Analytical Balance

It also runs through electricity and is used to measure the extremely smaller weight. It also consists of measurement of balance. It is affected by air too so ,it is kept inside the glass chamber. It consists of single pan and weight is displayed on digital screen. Extremely low weight can be measured.

Figure 14. Analytical Balance

Taking Care of the Weighing Balance

- While measuring the weight, do not directly place the samples on the pan. Plastic or paper pieces should be placed over the pan before measuring the sample.
- It should be kept on a sturdy surface that does not get destroyed easily.
- Equipment such as centrifuge, vortex should not be kept near the balance. It may alter the weight.
- It should not be kept on the sunlight and an open area.
- Liquid solution, extremely cold samples should not be measured on balance.
- Always keep the pans clean.
- Never weigh samples that are heavier than the capacity of the balance.
- If something spills over the balance, clean it immediately.
- Before measuring the weight, take care whether the pans are on balance or not.
- After completion of work, the weighing balance should be kept covered by cleaning.

1.12 Thermometer

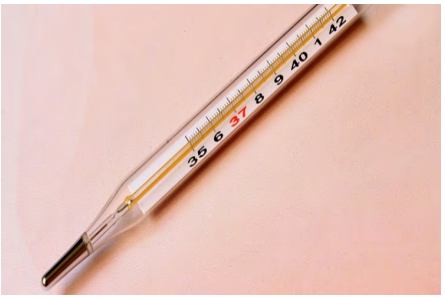


Figure 15. Thermometer

Thermometers are required to ensure the heating equipment is running at the correct temperature. The temperature of the medium, incubators, etc. needs to be frequently checked. Mercury glass thermometers are standard thermometers, and the temperature measurement is based on the expansion of mercury present in the bulb. Digital thermometer are also used for measurement of temperature.

Exercise

Choose the correct answer from the given alternatives.

1. Determine the name of the following piece of lab equipment.....

   a. Crucible b. Beaker

   c. Evaporating dish d. Watch glass

2. Determine the name of the following piece of lab equipment.....

   a. Buret b. Funnel

   c. Watch glass d. Well plate

3. Determine the name of the following piece of lab equipment.....

   a. Graduated cylinder b. Buret

   c. Erlenmeyer flask d. Beaker

4. Determine the name of the following piece of lab equipment.....

   a. Buret b. Thermometer

   c. Scooopula d. Stirring rod

5. Determine the name of the following piece of the lab equipment.....

   a. Electronic balance b. Well plate

   c. Centrifuge d. Hot plate

6. Determine the name of the following piece of lab equipment.....

   a. Buret b. Beaker

   c. Erlenmeyer flask d. Graduated cylinder

7. Determine the name of the following piece of lab equipment.....

   a. Filter paper b. Ceramic wire guaze

   c. Funnel d. Weigh boat

8. Determine the name of the following piece of lab equipment.....

a. Pipet holder
b. Watch glass
c. Weigh boat
d. Well plate

9. Determine the name of the following piece of lab equipment.....

a. Watch glass
b. Weight boat
c. Funnel
d. Ceramic wire guaze

10. Determine the name of the following piece of lab equipment.....

a. Beaker
b. Graduated cylinder
c. Buret
d. Erlenmeyer flask

11. Determine the name of the following piece of lab equipment.....

a. Stirring rod
b. Thermometer
c. Scoopula
d. Pipet

12. Determine the name of the following piece of lab equipment.....

a. Pipet
b. Thermometer
c. Stirring rod
d. Scoopula

13. Determine the name of the following piece of lab equipment.....

a. Evaporating dish
b. Watch glass
c. Well plate
d. Crucible

14. Determine the name of the following piece of lab equipment.....

a. Buret
b. Graduated cylinder
c. Pipet
d. Bunsen burner

15. Determine the name of the following piece of lab equipment.....

a. Test tube
b. Test tube rack
c. Test tube holder
d. Test tube brush

16. The laboratories and medical industries set the temperature of the refrigerator as.....

a. -2-8°C
b. 0°C
c. 0-10°C
d. Upto-60°C

17. What kind of image is formed by stereo microscope?

a. 2-D image
b. 3-D image
c. Magnified image
d. Both b & c

18. Which of the following microscope uses electrons to magnify an image?

a. Simple microscope
b. Compound microscope
c. Stereo microscope
d. Electron microscope

19. Which of the following material cannot be autoclaved?

a. Acids
b. Explosive material
c. Chlorine based products
d. All of the above

20. What can be placed inside the incubator?

a. Food
b. Edible material
c. Bacterial culture
d. Acids

21. What cannot be placed inside the incubator?

a. Food
b. Edible material
c. Acids
d. All of the above

22. What happens if the cold chain is not maintained?

a. The efficacy of the vaccine will decrease
b. The sample will degrade
c. The medicine will not work
d. All of the above

23. What kind of water can be placed inside the water bath?

a. Normal water
b. Distilled water
c. Any clean water
d. Both a and b

24. What kind of water can be used to clean the electrodes of the pH meter?

a. Normal water
b. Distilled water
c. Any clean water
d. Both a and b

25. What is the best instrument for measuring the volume of the equipment listed below?

a. Beaker
b. Erlenmeyer flask
c. Buret
d. Evaporating dish

26. What piece of equipment do you need each time you work with acid, bases and open flames?

a. Beaker
b. Safety goggles
c. Electronic balance
d. Buret

27. What is the equipment that you need to kill all the spores, bacteria, viruses and fungi?

a. Hot air oven
b. Boiling water
c. Water bath
d. Autoclave

28. Which of the following is necessary for the cultivation of microorganisms under artificial conditions?

a. Hot air oven
b. Water bath
c. Incubator
d. Graduated cylinder

29. The microscope that uses multiple lenses to enlarge the image of a sample is known as.....

a. Simple microscope
b. Compound microscope
c. Stereo microscope
d. Electron microscope

30. Which of the following microscope is used for the dissection of insects?

a. Simple microscope
b. Compound microscope
c. Stereo microscope
d. Electron microscope

31. Which of the following instrument can be used for the fixation of the slides?

a. Microscope
b. Autoclave
c. Hot air oven
d. All of the above

32.. What is the use of a colorimeter in the laboratory?

a. Analysis of Blood
b. Analysis of water
c. Biochemistry analysis
d. All of the above

33. Which is the most common weighing balance used in the laboratory?

a. Electronic balance
b. Analytical balance
c. Manual balance
d. Any of the above

34. What is the use of the distillation apparatus in the laboratory?

a. Purification of liquids mixture
b. Boiling of water
c. For hot water
d. To make local alcohol

35. Which of the following will prevent exposure to hazardous fumes in the laboratory?

a. Fume hood
b. Ventilation
c. Filter
d. AC Filter

36. What is the equipment used to draw the required amount of blood in the laboratory?

a. Syringe
b. Vacutainer
c. Blood collection tube
d. All of the above

37. The range of concentrated Hydrochloric Acid is.....

a. 1-7 b. 7-14

c. 7 d. Any of the above

38. During the process of Centrifugation of Blood, Red blood cells are found ……..

a. At top b. At the bottom

c. In the middle d. Nowhere

39. When the temperature of the Hot Air Oven is 160°C, the time required is

a. 150 minutes b. 60 minutes

c. 30 minutes d. 15 minutes

40. What is the standard temperature for Bacterial growth?

a. 35-37°C b. 20-23°C

c. 37-40°C d. 30-35°C

41. The readings of the Microscope should be done as.....

a. Lower power to higher power

b. Higher power to lower power

c. Can do any of them

d. None of the above

42. Which of the following is the commonly used temperature of an Hot air oven?

a. 170°C b. 160°C

c. 150°C d. All of the above

43. The denser component of the incubator will migrate……

a. Bottom b. Top

c. Middle d. Anywhere

44. The temperature used inside the refrigerator is.....

a. 2-8°C b. 4-8°C

c. 6-8°C d. 8-12°C

45. The temperature used inside the deep freezer can be set as.....

a. 0°C b. 5°C

c. 0-10°C d. 8°C

Write short answer to the following questions.

1. Enlist the name of any five laboratory equipment's.
2. What is microscope?
3. What are the three parts of the distillation flask?
4. Differentiate between autoclave and hot air oven.
5. Write objectives for use of compound microscope.
6. What are the instruments that cannot be autoclaved?
7. How does autoclave sterilization work?
8. How is the compound microscope different from a simple microscope?
9. How does a forced air hot air oven work?
10. Differentiate between the fine and the coarse adjustment knobs.
11. How does the centrifuge machine separate the liquids?
12. Describe different temperatures for sterilization.
13. Write down the working principle of hot air oven?

Write long answer to the following questions.

1. Write the principle of autoclave. Differentiate between hot air oven and incubator.
2. Enlist the name of any five-laboratory equipment and mention their one function. Mention the type of microscope and write the name of different parts of compound microscope.

3. Write about the precautions while using:
   a. Autoclave
   b. Water bath
   c. Distillation apparatus
   d. Colorimeter
   e. Refrigerator
4. Differentiate between hot air oven and autoclave. How is an autoclave different from a hot air oven.
5. Describe different types of microscope according to their application.
6. Write about the procedure of using the following.
   a. Colorimeter b. pH meter
   c. Autoclave d. Water bath
   e. Hot air oven
7. Enlist the name of any 5-laboratory equipment and mention their one function. Mention the types of microscope and write the name of different parts of compound microscope.
8. How does deep freeze differ from refrigerator?
9. Diagrammatically, identify the various parts of a microscope**.** Write down the working principle of autoclave.

General Laboratory Procedure

Unit 2

Laboratory procedures are detailed documents, checklists, or guidelines that instruct you how to safely act in a laboratory environment. These checklists guide the lab operator to carry out procedures related to tests and diagnoses in veterinary medicine. The laboratory procedure is important to use common laboratory equipment, proper quality control methods, parasitology, blood analysis, and laboratory diagnosis. By following the guidelines and rules of the procedure, we can easily complete the procedure and obtain the desired results.

2.1 Concept, Needs/Importance and Application of Bio-safety

Biosafety refers to the procedures, policies, and principles to be adopted to safeguard the environment, animal, and human populations. It includes principles, strategies, and practices that are adopted to prevent exposure to pathogens and toxins. The main objective of biosafety is to keep a check on harmful biological agents, toxins, chemicals, and radiation.

'Laboratory bio-safety' is the term used to describe the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release.

“Laboratory biosecurity” refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins.

Bio-safety protection is to protect laboratory workers, clinical specimens and the environment. Diagnostic and health-care laboratories (public health, clinical or hospital-based) must all be designed for at least Bio-safety Level 2 or above if

required. As no laboratory has complete control over the specimens it receives, standard precautions should always be adopted and practiced.

The basic objective of a biosafety program is the containment of potentially harmful biological agents. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. The use of vaccines may provide an increased level of personal protection. The term “containment” is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The appropriate combination of the elements of containment required in a laboratory is determined on the basis of the risk assessment of the work to be done with a specific agent. The application of biosafety can be done in many laboratory settings, which include.

- Human and veterinary clinical and diagnostic laboratories
- Biological research and production laboratories (academia, industry, government, etc.)
- Environment research and analytical laboratories
- Academic and teaching laboratories
- Laboratory dealing with chemicals, and radiation

2.2 Bio-safety measures in laboratory

Safety in the laboratory requires every employee's participation and cooperation. Non-compliance with safety precautions not only endangers the individual, but also compromises the health and safety of fellow workers. All staff of the laboratory shall follow the Good Microbiological Techniques (GMTs) which include the following:

- Wear gloves, face mask and laboratory coat when opening lyophilized vials
- Eating, drinking, smoking and application of cosmetics are prohibited in the laboratory.

- Sandals and open style shoes which do not afford proper foot protection are not to be used.
- As far as possible lenses should not be worn in eyes instead one should wear spectacles.
- Laboratory and work tables should be scrupulously cleaned with liquid detergents and disinfectants. Laboratory work surface should be decontaminated once a day after completion of day's activity and immediately after spill of viable material with disinfectant.
- Paper work should not be done on potentially contaminated surface.
- Clean / dirty areas must be clearly demarcated
- All work surfaces in daily use such as bench tops, sinks, and trolleys etc. must be disinfected with a disinfectant designed for laboratory use at the end of each work shift. (0.5%) dilution of sodium hypochlorite or bleach is used as disinfectant if specimens of blood, blood products or body fluids have been handled.
- Cuts in hands should be properly covered with waterproof adhesive bandage.
- In case of a highly precious specimen if the container shows evidence of breakage, leakage or soiling, it should be transferred with a gloved hand into a second sterile container. Any important information should be rewritten from the old to the new container.
- Mouth pipetting should be strictly prohibited. Mechanical pipetting devices should be used for pipetting of all liquids in the laboratory.
- Personal Protective Equipment (PPE) may act as barrier to minimize the risk of exposure. The clothing and equipment selected are dependent on the nature of work performed, type of the pathogen and its transmissibility. PPE should be worn when working in the laboratory. It should be removed and hands should be washed before leaving the laboratory.

2.3 Safety and First Aid in Laboratory

First aid is the term used for the immediate medical care provided to an individual after an injury occurs, usually at the same location where the event takes place.

First aid can be crucial in minimizing injuries and preventing disabilities or long-term effects. In extreme situations, first aid can be the difference in keeping the injured person alive.

Common examples of first aid treatment include cleaning minor scrapes, cuts, scratches, or bruises, applying bandages, drinking fluids, or providing asthmatic care, such as an inhaler.

The Importance of Administering First Aid

Injuries can occur suddenly in dynamic work site, which is why first aid is so important and a vital aspect of an emergency response plan. Companies are legally obligated to ensure that they keep first aid kits in different, easily accessible locations, and train employees on how to administer it.

There are several reasons why administering first aid is so important.

Importance of First Aid

1. **First Aid Training Saves Lives**

   Employees with proper first aid training are able to administer first aid more confidently and can take charge of the situation with more confidence, allowing them to act quickly.

   This split-second difference could save the victim's lives and prevent a person from long-term disability.

2. **Relieve Pain Immediately**

   A severe injury often causes sharp, searing pain. Administering first aid can help relieve the pain quickly and prevent the patient from suffering even more.

The tools and equipment within a first aid kit can be used to mitigate the harmful effects. For instance, in case of a knock, an employee can use an ice pack to massage the point of impact and relieve pain.

3. Prevent Serious Infections or Diseases

- In many cases, injuries that aren't immediately treated can result in serious infections or diseases, which can ultimately result in debilitating long-term effects.
- First aid kits include all the required materials that a person may need to treat an injury and prevent an infection. They include cleaning agents and bandages for cleaning the wound and preventing contaminants from causing an infection.
- Staff with appropriate first aid training can quickly clean and treat the wound to prevent the infection from spreading until medical assistance arrives.

4. Improve Employee Morale and Productivity

- Providing first aid kits in the workplace is mandatory and ensures legal compliance. However, it also has another effect: it improves employee morale and productivity.
- Employees feel motivated when they know that management cares for their well-being and is taking steps to ensure their safety. Furthermore, first aid provisions ensure that minor injuries can be quickly treated.

5. First Aid Training in the Workplace

- First aid training is vitally important in the workplace. Companies often bring on third-party safety officials to give presentations and provide in-depth training to employees.
- However, basic principles generally involve the use of applying adhesive bandages or teaching employees how to apply pressure on bleeding wounds.

- In most cases, companies leverage practical training, often using safety dummies to show how to provide first aid. Common treatment methods include administering CPR, applying bandages, or massaging techniques.
- Those who receive the training are granted a certification. However, due to regular updates to first aid training methods, primarily because of updates in our clinical and medical knowledge, refresher courses are held regularly in most organizations.

Common Contents in First Aid Kits

- It is pertinent to mention that there is universally accepted list of contents that must be included within a first aid kit. The HSE states that workplace first aid kits often contain different items depending upon the needs of the organization.
- In general, common items found in first aid kits include:

1. Safety pins
2. Adhesive bandages Cotton balls
3. Scissors
4. Face shields and masks
5. Foil blankets
6. Burn treatment creams
7. Safety pins
8. Fabric shears
9. Burn dressings
10. Sterile wipes
11. Antiseptic cream

- As mentioned, this list is not exhaustive, and companies can add different items based on their risk assessment.

First Aid for Lab Accidents

Even when all safety precautions are in place, accidents can still happen, which is why its essential to have a well-stocked first aid kit and know first aid for lab settings.

1. Bleeding and Wound Care

- Breakage of glass pipettes, beakers, and other containers can lead to cuts. If you are with someone who has a minor cut:
- Wear clean gloves.
- Cover the area with gauze (or clean paper towels).
- Apply pressure to the bleeding area – have the injured person sit or down.
- If the wound is large or the person is dizzy or weak, call an emergency.

2. Burns–Heat/Chemical

- Burns is one of the more common injuries in the lab. Not only chemical Wear clean gloves.
- Cover the area with guaze (or clean paper towels).
- Apply pressure to the bleeding area – have the injured person sit or down.
- If the wound is large or the person is dizzy or weak, call an emergency.

Treat Heat Burns

- Run cool water over the area for 5 to 10 minutes.
- If minor (first degree or less), apply burn cream and a sterile bandage.
- If more serious, seek medical treatment, but if the burn is large or the victum appears to be in shock call emergency.

Treat Chemical Burns (either acid or alkaline)

- Put on gloves and remove the dry chemicals if any.
- Remove contaminated clothing and rinse the area with large amounts of cool, running water for 20 minutes.
- Wrap the wound with a clean bandage to avoid putting pressure on burned skin.
- If more sensation that is burning is felt, rinse the area again for several minutes, and seek emergency care.

Eye Splash Chemical

- In dust, liquid, or a chemical makes contact with the eye area, follow these steps:
- If a chemical splashes in the eye, flush with lukewarm (room temperature) running water.
- Make sure the victim turns their head from side to side and allows water to run across both eyes.

Eye: Foreign Body (dust or metal, paint, wood chips)

- If dust or solid material is in the eye, cover or close the eye and seek medical treatment.

2.4 Techniques for Washing and Cleaning of Glassware

Introduction

Glasswares need to be cleaned and sterilized even for those that have been not used or used. It is essential to clean and sterilize for killing or destroying microorganisms and to prevent contamination.

Cleaning of new glass-wares

New glassware also require to be cleaned because there may be the resistant spores in the packing materials. It is not that the new glassware are ready for direct use due to dirt stains etc.

Materials Required

- 5% HCL
- Autoclave
- Hot air oven
- Distilled water
- Wire bucket

Procedure

- Dip new glasswares in 5% HCL solution overnight. Place into a

container having tap water and rinse at least for 2 times. Then rinse in warm distilled water.

- Place the glasswares in wire basket and dry in hot air oven and use as required or after wrapping in craft paper, which can be autoclaved. Clean the used glasswares
- After use, glasswares must be dipped immediately in water.
- Dip in 3% Lysol or 1% sodium hypo-chloride solution for overnight.
- Rinse the glasswares in tap water properly.
- Dip the glassware in 5% soapy solution. (Detergent water) for 1 hour.
- Using brush, scrub each glasswares and place in container containing clean water.
- Wash in tap water to remove soap.
- Wash in warm water.
- Ultimately, rinse in distilled water.
- Then dry at 100^{o}c and use the dried glasswares as required or sterilize in hot air oven at 160^{0} c for 1 hour.

Washing of Laboratory Glassware

The type of glassware, i.e. new and dirty or used, is subjected to washing for further use. The method used for each type is described below.

New Glassware

Purpose: Usually new glassware is slightly alkaline in nature. Before washing, this alkaline nature has to be neutralized for final use.

Material Required

- 2% hydrochloric acid
- Big plastic basin
- Demineralized water
- Hot air oven for drying purpose only

Procedure

- Prepare sufficient quantity of 2 % hydrochloric acid (e.g. 98 ml of water & 2.0 ml hydrochloric acid) as per the requirement in a big plastic basin
- Wash the newly received glassware under running tap water to remove the visible dust sticking inside and/or outside surface of the article
- Soak the already washed articles in 2% hydrochloric acid solution
- Leave them there overnight
- Take the articles from 2 % hydrochloric acid and rinse in clean water twice
- Finally wash using demineralized water. Allow to dry using hot air oven
- Pass on for packing & sterilization for further use Note
- Care should be taken while using HCl
- Add acid to water drop by drop by constant stirring (and not vice versa)

Dirty Glassware

Material Required

- 1 % detergent solution
- Cotton or aluminum foil for plugging
- Washing brush
- Good quality water supply
- Hot air oven
- Draining rack
- Wire basket for drying
- Demineralized water.

Procedure

- Take material, glassware etc. already decontaminated (chemically/

autoclaving) and rinse twice in lukewarm water to remove any dirty stain sticking on them

- Put the material to be washed in bowl containing 1% detergent solution.
- Allow to boil.
- While in solution, scrub inside & outside surface of the glassware with the help of the brush
- Leave the glassware in the solution for 2 - 3 hours.
- Take out each article one by one and rinse under running tap water till no trace of detergent is left, which otherwise may lead to false results when used.
- Drain the water by putting each article on a draining rack or by keeping articles upside down in a wire basket.
- Put articles in wire basket and keep in hot air oven at 160°c for drying purpose only.
- Take out each article and plug using non-absorbent cotton/aluminium foil.
- Sterilization of glassware can be done using dry heat or by autoclaving.

2.5 Sterilization

Sterilization is the process of destruction of all forms of micro-organisms. An instrument is considered sterilized when it is free from living micro-organisms. The very word sterile, sterilize and sterilization in a microbiological stand point indicates total absence or killing of all micro-organisms.

Sterilization is carried out by steam under pressure, dry heat, gas or liquid chemicals. The choice of the methods like autoclaving, use of hot air oven etc. depends on a number of factors including type of material of the object, number and types of organisms involved and risk of infection to patients or staff. Any sterilization procedure should be monitored routinely by mechanical, chemical and biological techniques. Sterilized items should be protected against

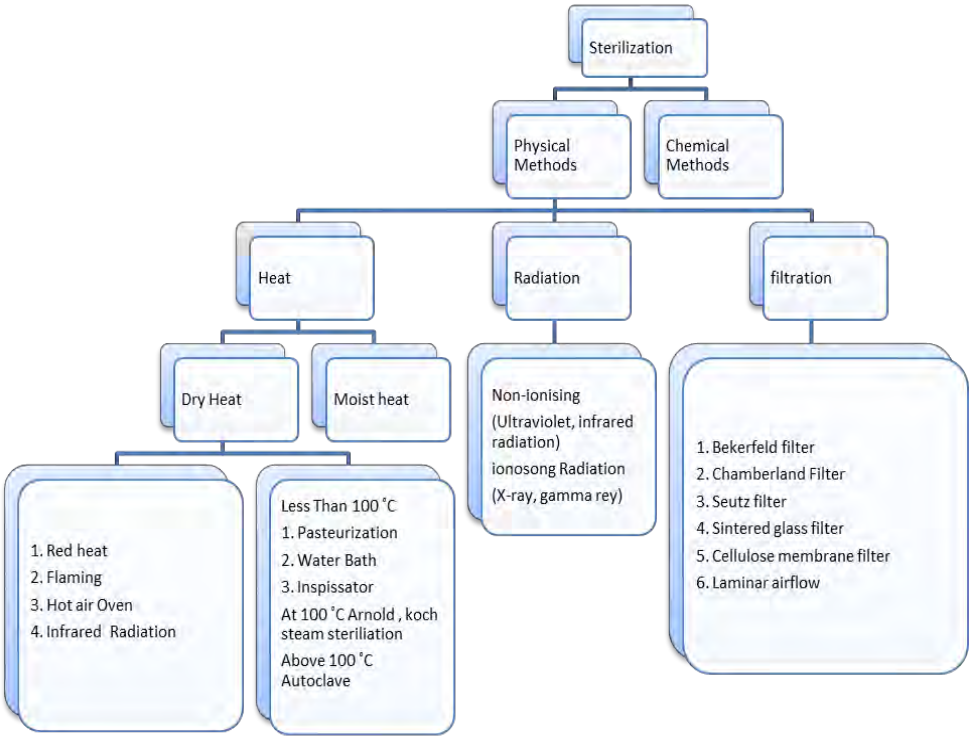
recontamination. Depending upon the nature of the material to be sterilized, sterilization procedures used in a microbiology laboratory can be divided into the following categories.

- Dry heat
- Moist heat
- Filtration

Procedures of the Sterilization of some Important Materials

Method of Disinfectant/ Antiseptic	Materials
Burning Incineration	Infective agent like bedding, animal carcasses, soiled dressing etc.
Hot air oven	Glassware, syringe, petri dishes, test tube, conical flask etc.
Autoclaving	Metal instrument, most of culture media, dressing equipment, aprons, gloves, catheter, surgical instrument except, sharp instrument, surgical material except catgut
Formaldehyde	Ward and laboratory or operation theater, floor place
Chlorine as Hydrochloride (0.2%)	Water (H20)
Tincture iodine, sprite (70% alcohol), savlon (phenol derivation)	Skin

Method of Sterilization



Flowchart 2.4 : Method of sterilization

Sterilization are done by

a. Physical Method

b. Chemical Method

Physical Method

1. **Dry Heat:** Dry heat sterilization has limited value. Prolonged exposure may cause damage.

It is done by the following.

i. **Red heat:** Inoculating wire, forceps, spatula etc are sterilized at the blue zone of the Bunsen burner up to heated red heat.

ii. **Flaming:** Scalpel, needle, cultural tube, cotton wool plug, glass slide

etc are sterilized by passing the articles through a Bunsen flame without allowing them to become red hot.

iii. **Hot air oven:** Here heating is done by electricity above chosen temperature, which is maintained by thermostat. Here sterilization is done at 160 °Celsius for 1 hour. Materials to be sterilized through this are all glassware's, surgical instruments, glass syringe etc. Should be taken before sterilization by this method are.

a. All glass articles must be wrapped with craft paper.

b. All articles must be free from moisture.

c. After the switch is put off, the whole system is allowed to be cool.

d. Dry materials in sealed container with petroleum jelly that are impermeable to microbes can be sterilized by this method.

2. Moist Heat

a. Autoclave: Moist heat in the form of saturated steam under pressure is the most practical method for sterilization. The laboratory apparatus used with stem under regulated pressure is called autoclave. Both pressure and temperature for a definite period kills the organisms. Autoclave is an essential instrument in every diagnostic microbiological laboratory. Cotton, wools, various cultural media, solutions are sterilized with the instrument. In autoclave, most of the materials are sterilized under 15 lb pressure at 121^0 Celsius temperature.

b. Tyndallization: Tyndallization is also a process of moist heat application. Some materials like amino acids, sugar solutions etc are required to be heated at 90-100 ° Celsius temperature on three successive days with interval in between. During the interval or incubation period, resistant spore germinate and on subsequent exposure to heat the vegetative stage are destroyed. This Method of heating at successive heating is called fractional sterilization or Tyndallization.

c. **Boiling:** Through boiling, it is possible to destroy most of the vegetative forms of the microorganisms, but spores cannot be destroyed with this method. Boiling water can be considered as a true method of sterilization. In field conditions, surgical instruments are frequently put under boiling water. Through this method instrument can be brought under disinfection but not to the extent of sterilization.

d. **Pasteurization:** Pasteurization is the process by which food and food products (milk, fruits, juices, wine etc) are protected from putrefaction and fermentation. It involves a short exposure to heat at a lower temperature than that employed in ordinary sterilization. In this process, the product is subjected to a controlled heat treatment, which kills microorganisms of certain types but not all. Milk is commonly pasteurized and then marketed. The temperature selected for pasteurization is based on a thermal death time of most resistant type of microorganisms to be destroyed by this process.

Two methods of pasteurization are used commercially low temperature long time method (LTLT) and high temperature short time method (HTST). In LTLT method milk is exposed to 62.8 ° Celsius for 30 minutes and in case of HTST method milk is exposed to a temperature of 71.1 ° Celsius for 15 seconds.

1. **Membrane filter** are Millipore filters. Filters with the pore of 0.22 micrometer are sufficient for the bacteria.
2. **Seitz filter** are disposable asbestos pad filter.
3. **Flaming** when the material is wetted by alcohol and then flamed. This method is rapid.
4. **Ultraviolet** light causes damage to bacteria.
5. **Radiation** in from of beta and gamma x-rays used for the surgical pads.
6. **Supersonic and ultrasonic** waves 9000 cycles per seconds or above are used to rupture and disintegration of the cells.

2.6 Antiseptics

Antiseptic is an agent that prevents sepsis i.e. prevents the growth of infective agents like bacteria, virus, protozoa etc. These substances are substantially non-toxic for superficial application to living tissues. Therefore, these agents can be applied externally on animals to kill or prevent the growth of microbial population. The antiseptics belong to a variety of chemical substances e.g. alcohol, phenol, variety of metallic salt, acridine dyes etc. They are used on the intact skin before surgical operation and injection. They are applied to break skin following wounds, burns etc. They are also used on mucous membrane e.g. conjunctiva or bladder to prevent or treat the superficial infection. They are grouped as

a. Oxidizing agent's e.g. Hydrogen Peroxide (H_2O_2), Potassium Permanganate ($KMNO_4$), Chlorine, Iodine, Iodophore etc.
b. Reducing agents e.g. Sulphur Dioxide and Formaldehyde
c. Metallic compounds e.g. Mercuric Chloride and Iodide
d. Acids and alkalis e.g. Boric acid and various sodas etc
e. Alcohol -70% ethyl alcohol
f. Phenol and cresol
g. Various dyes- acriflavine, brilliant green, proflavin
h. Detergents- various soap
i. Cetrimides
j. Quaternary ammonium compound
k. Antimicrobial agents- they include various antibiotics and Chemotherapeutics. They are called as therapeutic agents.

2.7 Disinfectants

Disinfectants are agents that are too toxic to be applied to the tissues of the host but which can be used in destroying contaminating inanimate objects e.g. drains, fecal matter, building, vehicles, cooking materials, surgical instruments

and apparatus, and the rooms or sheds.

Disinfectants are the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects (non- living things). Disinfectant can be achieved by physical or chemicals methods. Chemicals used in disinfection are called disinfectants.

Common Disinfectants Include

- Alcohols
- Formaldehyde and Glutaraldehyde, bleach
- Chloramine
- Chlorine dioxide
- Ozone
- TH_4
- Virkone S
- Sodium hypochlorite
- Hydrogen peroxide
- Non- chemical disinfectant is UV light

Properties of Ideal Disinfectant

An ideal disinfectant or antiseptic has the following characteristics:

1. Ideally, the disinfectant should have a wide spectrum of antimicrobial activity. It must be effective against a wide variety of infectious agents (Gram- positive and Gram-negative bacteria, acid-fast bacteria, bacterial endospores, fungi, and viruses) at high dilutions.
2. It should act in the presence of organic matter.
3. It should not be toxic to human or corrosive. In practice, this balance between effectiveness and low toxicity for animals is hard to achieve. Some chemicals are used despite their low effectiveness, because they are relatively nontoxic.

4. It should be stable upon storage and should not undergo any chemical change.
5. It should be odorless or with a pleasant odor.
6. It should be soluble in water and lipids for penetration into microorganisms.
7. It should be effective in acidic as well as in alkaline media.
8. It should have speedy action.
9. If possible, it should be relatively inexpensive.

Common Disinfectants and their Use

Disinfectant	Articles	Comments
Sodium hypochlorite	Disinfection of glassware contaminated with blood and body fluids	• Should be used in well ventilated areas • Protective clothing must be worn while handling undiluted • Not to be mixed with strong acids to avoid release of chlorine gas • Corrosive to metals
Bleaching powder (7gm/ liter of water with 70% available chlorine, may be used in place of liquid bleach if liquid bleach is not available)	Toilets, bathrooms	Same as above
Chlorhexidine Combined with alcohol or detergents	Disinfection of skin and hands	• Most commercially available preparations contain large amounts of alcohol (70%) and are flammable. • Do not use them or store them near a flame, heater, or electrical device.

		• Apply in a well-ventilated place
Alcohol (70%) Isopropyl alcohol, ethyl alcohol, methylated spirit	• Smooth metal surfaces, table tops and table tops on which sodium hypochlorite cannot be used	• Flammable, toxic, to be used in well ventilated areas, avoid inhalation • To be kept away from heat sources, electrical equipment, flames, hot surfaces • Should be allowed to dry completely

2.8 Storage of Chemicals, Reagents and Vaccines

Chemicals, reagents and vaccines need to be stored at a recommended temperature for its effectiveness. **Many vaccines must be stored at low temperature, some below -15^{0}C and other "between" 2-8^{0}C. The ideal temperature for storing vaccine is 2-8^{0}C.** If vaccine is not stored correctly, they can lose their effectiveness. Failure in storage to recommend specification for storage and handling of immunobiologics can reduce or destroy their potency, resulting in adequate or no immune response in recipient. Maintenance of vaccine quality is the shared responsibility of all handlers, from the time a vaccine is manufactured until administration (cold-chain- maintenance).

All vaccines should be stored in a refrigerator or freezer that is designed specifically for the storage of biologics or alternatively in a separate dedicated unit. Accurate and uniform temperature in a refrigerator plays a key role in ensuring the life of vaccines reagents and other biological. Research has shown that the minors' variation in temperature such as those in a household refrigerator can comprise that effectiveness of your biological. Within the refrigerator and freezer, vaccines are also comprised thought excess warm air in, and cold air out of the refrigerators or freezers. Over time this causes the compressor to work harder and eventually leads to failure. This can be remedied by using probe access ports, found on most clinical refrigerators and freezer. These are easy to

open up and drastically reduce air intake and loss from inside the units.

Vaccine Storage and Handling

There are few immunization issues more important than the appropriate storage and handling of vaccines. Vaccine decreases preventable disease rates because of proper storage and handling of vaccines. Exposure of vaccines to temperatures outside the recommended range can decrease their potency and reduce the effectiveness and protection they provide. Storage and handling errors can cost thousands of dollars in wasted vaccine and re-vaccination. Errors can also result in the loss of patient confidence when repeat doses are required. It is better not to vaccinate than to administer a dose of vaccine that has been mishandled. Vaccine management, including proper storage and handling procedures, is the basis on which good immunization practices are built.

Vaccines must be stored properly from the time they are manufactured until they are administered. Assuring vaccine quality and maintaining the cold chain is a shared responsibility among manufacturers, distributors, public health staff, and health-care providers. A proper cold chain is a temperature-controlled supply chain that includes all equipment and procedures used in the transport and storage and handling of vaccines from the time of manufacture to administration of the vaccine. By following a few simple steps and implementing best storage and handling practices, providers can ensure that patients will get the full benefit of vaccines they receive.

Vaccine Storage and Handling

- Vaccine decreases preventable disease rates because of proper storage and handling.
- Storage and handling errors
- Decrease potency and reduce effectiveness and protection
- Cost thousands of dollars in wasted vaccine and re-vaccination
- Loss of patient confidence

- It is better to not vaccinate than to administer a dose of vaccine that has been mishandled.

Cold Chain (a temperature-controlled supply chain)

- Vaccines must be stored properly from the time they are manufactured until they are administered
- Shared responsibility among manufacturers, distributors, public health staff, and healthcare providers

Important Points to be Noted During Vaccination

- Animals should be in good health at the time of vaccination.
- The cold chain of the vaccines wherever prescribed should be maintained till the time of administration to the animal.
- The manufactures instruction on the route and dosage should be strictly followed.
- A minimum vaccination coverage of 80% of population is required for proper control of the disease.
- It is beneficial to deworm the animals 2-3 weeks before vaccination is carried out for better immune response.
- Vaccination should be carried out at least a month prior to the likely occurrence of the disease.
- Vaccinations of animals in advanced pregnancy may be avoided even through in most cases nothing untoward may happen.

Common Reasons for Vaccination Failure

- Lack of maintenance of cold chain from the time of manufacture till vaccination
- Poor immune response in weak and improperly fed animals
- Lack of herd immunity due to only a few animals being vaccinated
- Poor quality of vaccine- quality will deteriorate if repeatedly thawed

and cooled

- Low efficiency or ineffective vaccine- may occur in case of strain variation (eg.FMD)

2.9 Collection, Storage, Labelling and Dispatch of Samples to Laboratories

Improper collection, transport and handling of specimens in the laboratory not only carry a risk of infection to the personnel involved but also will not be useful for testing/diagnosing infectious organism.

For the diagnosis of disease, different type of samples from different parts of the body of the animal must be collected. After collecting the sample, the samples should be stored at appropriate media before sending it to the lab for examination. While sending the samples to the lab, following information must be provided for the diagnosis of different type of diseases.

Sample no:	Collection date:	Name of the owner:
Address:	Species of animal:	Breed of animal:
Age of animal:	Sex of animal:	Tag of the animal:

Brief information about the suspected disease:

Chemicals used to preserve the samples:

Contact no of the owner:

Collection of samples

- Specimens, especially blood and body fluids, should be collected in pre-sterilized screw-capped plastic containers properly sealed to prevent spillage or leakage.
- Specimen containers should be robust and should not leak when the cap or stopper is correctly applied. Always grasp the tube or outside of the specimen container, not the stopper or cap, when picking up tubes or specimen containers to prevent spills and breakage.

- Ensure tops are tightly secured on all specimen containers, blood-collection tubes, and specimen tubes before advancing for analysis or storage.
- Request a new specimen if a specimen container is broken or has spilled its contents
- Document the incident, and notify the supervisor if an exposure occurred.
- In case of a highly precious specimen, if the container shows evidence of breakage, leakage, or soiling, it should be transferred with a gloved hand into a second sterile container. Any important information should be rewritten from the old to the new container.
- Containers should be correctly labelled to facilitate identification.
- Do not keep the specimens on requisition forms.
- If the requisition slip is contaminated with blood, it should be rejected. In case of emergency, the contaminated slip may be handled using gloves.
- Hands should be thoroughly washed with soap and water before and after handling specimens.
- If the outside of the container is visibly contaminated with blood, it should be cleaned with disinfectant. All blood specimens should be placed in small leak proof impervious plastic tubes for transportation to the laboratory. Preferably, blood specimens should be collected in vacutainer tubes.
- Specimen request or specification forms should not be wrapped around the containers but placed in separate, preferably waterproof envelopes/ zip locks whenever the specimen needs to be transported.

Specimen Transport within the Facility

- To avoid accidental leakage or spillage, secondary leak proof containers, should be used so that the specimen containers remain upright.

- The secondary containers may be of metal or plastic, should be autoclavable or resistant to the action of chemical disinfectants, and the seal should preferably have a gasket. They should be regularly decontaminated.
    - The outer container should be rigid and sturdy.

Receipt of Specimens in the Laboratory

- Laboratories that receive large numbers of specimens should have a designated room or area for this purpose. It is preferable to have computerized system for record maintenance.
- Leaking specimen containers, requisition forms smeared with specimens, and improperly labeled specimen containers should not be accepted.

Opening Specimen Packages

- Personnel who receive and unpack specimens should be aware of the potential health hazards involved, and should be trained to adopt standard precautions, particularly when dealing with broken or leaking containers.
- Primary specimen containers should be opened preferably in a biological safety cabinet if not available must be opened while wearing proper PPE.

Dispatch of the Samples to Laboratories

Collected samples should be dispatched to the lab for the diagnosis of bacterial, viral and fungal diseases. Similarly, the isolated bacteria from the cultures should be sent to the reference labs for examination. While sending samples to the lab, information about the samples, sample size, information about the animals, general information about the symptoms, and information about the sample collector, address of the sample collector should also be included. The collected samples should be sent to the lab within 10-24 hours. If it is not possible to send

the samples to the lab immediately, the collected samples should be preserved on the refrigerator at 40℃ but should not be kept at deep fridge. While sending the sample to bacteriological lab, the sample should be preserved at on cooked meat media. While sending the samples to virological lab, the samples should be kept on virus transport media. The samples should be well labeled and kept on icepack or thermocool vials. “Sample handle with care” should be written on the external part of the sample containing vials.

Exercise

Choose the correct answer from the given alternatives.

1. Which of the following type(s) of Personal Protective Equipment (PPE) is frequently used?

   a. Safety glasses b. Gloves

   c. Lab coats d. All of the above

2. Good work practices include.....

   a. Smelling and tasting chemicals

   b. Not washing hands before and after lab

   c. Confining long hair and loose clothing

   d. Using damaged equipment and glassware

3. What is the name of the procedure performed under sterile conditions to eliminate contamination in hopes to obtain a pure culture of one type of microorganism?

   a. Sterilization technique

   b. Disinfectant technique

   c. Aseptic technique

   d. Pathogen technique

4. _____ is needed as a source of nutrients for the growth and reproduction of microbes.

   a. Pathogens b. Reagents

   c. Bacteria d. Media

5. The main objective of Biosafety is.....

   a. To keep check on harmful biochemical agents

   b. To keep check on pathogens and toxins

c. To prevent harmful action of chemicals and radiation

d. All of the above

6. Which of the following activities are prohibited inside the laboratory?

a. Eating, drinking, and smoking

b. Bringing handbags and spectacles

c. Not wearing lab coats and gloves

d. All of the above

7. To avoid contamination in the laboratory, the gloves should be removed while.....

a. Answering the phone

b. Using computer keyboard

c. Opening the door

d. All of the above

8. Which of the following is PPE that should be worn inside the laboratory?

a. Safety goggles b. Jacket

c. Earrings d. Bags

9. What are the possible accidents that can occur in the laboratory?

a. Cuts and Burns b. Fire

c. Electric shock d. All of the above

10. Which of the following is an absolute method for removing the microorganism in the laboratory?

a. Disinfection b. Antiseptics

c. Sterilization d. Both a and b

11. Which of the following is the most effective method of Physical sterilization?

a. Boiling b. Steaming c. Autoclaving d. Hot air oven

12. Which of the following is the method for dry heat?

a. Red heat
b. Flaming
c. Hot air oven
d. All of the above

13. Which of the following is a Physio-chemical method for sterilization?

a. Steam-formaldehyde
b. Fumigation
c. Flamization
d. Autoclave

14. The chemicals that reduce the number of microorganism in the skin is known as.....

a. Disinfectant
b. Sterilizer
c. Antiseptics
d. Both a & b

15. The antiseptics should be avoided on.....

a. Large wounds
b. Burns
c. Eye infection
d. All of the above

16. Which of the following is the chemical disinfectant?

a. Chlorhexidine
b. Chlorxylenol
c. Betadine
d. Glutaraldehyde

17. How long does chemical splash in the eye rinse for_____?

a. 10 seconds
b. 5 minutes
c. 30 seconds
d. 15 minutes

18. Chemical, reagents or broth cultures should be pipetted by _____?

a. Mouth
b. Pipetter
c. Ear
d. Nose

19. To prevent the contamination of microscopes and surrounding areas disinfectant/clean used slides, prepared by student with .....

a. 70% ethanol and lens paper

b. acetone and lens paper

c. 5% methylene blue and lens paper

d. water and lens paper

20. Where should we store large and heavy chemicals?

a. High shelves

b. High cabinets

c. At the floor

d. At shoulder lever or below

21. Which of the following methods is used for the sterilization of surgical gloves?

a. Autoclave

b. Hot air oven

c. Radiations

d. Chemicals

22. How can we prevent lab accidents?

a. By forgetting to wear Personal Protective Equipment (PPE)

b. By not following any laboratory rules

c. By haphazardly playing with chemicals

d. By following the instructions of the laboratory

23. What should you wear in the laboratory?

a. Jewelry

b. High heels

c. Both a and b

d. None of the above

24. How can you be cautious with the chemicals inside the laboratory?

a. By working in a well – ventilated area

b. By using a Fume-hood

c. Always storing chemicals safely

d. All of the above

25. Which of the following are the general rules for handling and cleaning glassware?

a. Cleaning the apparatus immediately after use

b. Cleaning the used apparatus after some time

c. Handling cracked glassware

d. Pouring hot liquids into glassware with abrasion

26. Which of the following glassware requires a brush to be cleaned?

a. Test tubes b. Burettes

c. Volumetric glassware d. Both a & b

27. Which method can be used for the sterilization of surgical gloves?

a. Red heat b. Radiation

c. Hot air oven d. Sunlight

28. Which method can be used for the sterilization of syringes?

a. Red heat b. Radiation

c. Sunlight d. Hot air oven

29. Which of the following is not used for sterilization?

a. Copper b. Phenols

c. Dettol d. 70% ethyl alcohol

30. Steam formaldehyde is commonly used in .....

a. Poultry Farm b. Livestock Farm

c. Hatcheries d. All of the above

31. Which of the following chemical is commonly used for scrubbing before surgery?

a. Chlorxylenol b. Betadine

c. Chlorhexidine d. Both b and c

32. Which of the following chemical is commonly used to treat minor cuts and wounds?

a. Chlorxylenol
b. Betadine
c. Chlorhexidine
d. Both b and c

33. Which of the following chemical is used for scrubbing before surgery in Cats?

a. Chlorxylenol
b. Betadine
c. Chlorhexidine
d. Both b and c

34. Which of the following should be done while storing the chemicals/ reagents?

a. Labelling the name, manufactured and expiry date of the chemical
b. Storing chemicals in approved flammable liquid storage cabinets
c. Sealing the containers tightly
d. All of the above

35. Which of the following should be avoided while storing the chemicals/ reagents?

a. Storing the chemicals in large and heavy containers
b. Storing the bottles on the floor
c. Using expired chemicals/reagents/vaccines
d. All of the above

36. What is the standard temperature, pressure and time required for the operation of Autoclave?

a. 121°C, 15lb. 15 m
b. 120°C, 21lb. 15m
c. 121°C, 51lb. 5m
d. 121°F, 15lb. 15s

37. When the temperature of the hot air oven is 150°C, the required time is.....

a. 150 minutes
b. 60 minutes
c. 30 minutes
d. 15 minutes

38. What is the desired temperature for vaccine storage in the refrigerator?

a. -50 to 15°C
b. 2-8°C
c. 2-4°C
d. 1-2°C

39. Which of the following is effective for disinfection of the Foot and Mouth Disease virus?

a. Phenols
b. Sodium hydroxide
c. Calcium carbonate
d. Ethanol

40. Which is the most common disinfectant used in Poultry farms?

a. Quick lime
b. Soda ash
c. Virkon-S
d. Formaldehyde

41. Which of the following are the properties of an ideal disinfectant?

a. Broad spectrum
b. Narrow spectrum
c. Slow acting
d. Ineffective against Om

42. Which of the following is not the properties of an ideal disinfectant?

a. Broad spectrum
b. Fast acting
c. Explosive
d. Effective against Om

43. Which of the following is the properties of ideal disinfectant?

a. Broad spectrum
b. Fast acting
c. Economical
d. All of the above

44. Which of the following disinfectants is considered as the most effective for Foot and Mouth disease?

a. Sodium carbonate
b. Ammonium hydroxide
c. Sodium hydroxide
d. Calcium oxide

Write short answer to the following questions.

1. Define sterilization. Write down the working principle of autoclaving.

2. Explain Bio-safety measures in laboratory in brief.
3. Write about different glassware used in laboratory settings.
4. What is sterilization? Describe the rules and guidelines that students must follow in the laboratory.
5. Explain techniques for washing and cleaning glassware in the lab.
6. Write short notes on :

a. Write first aid procedure in the laboratory

b. Procedure collection and dispatch of the sample

7. How can you prevent contamination inside the laboratory?
8. Write the important points to be noted during vaccination.
9. Write common reasons for vaccination failure
10. Differentiate between :
    a. Sterilization and Disinfection
    b. Antiseptics and Disinfectant
11. Write about different types of antiseptics and their uses.

Write long answer to the following questions.

1. Why is first aid important when working in a laboratory? Describe possible laboratory accidents and their corresponding first aid measures.
2. Explain in brief about vaccine storage. Write about the things to take care of and avoid during chemical storage.
3. Why is it important to clean glassware? Describe the different methods used to clean various types of laboratory glassware in a laboratory.
4. Write the different method of sterilization. What are the general laboratory procedures?
5. What do you mean by Biosafety? Explain the importance of laboratory procedures.
6. What is disinfection and explain the different methods used for disinfectants.

7. What are the properties of an ideal disinfectant? Mention the names of different chemical disinfectants along with their uses.
8. What are the properties of an ideal antiseptic? Describe the use of different antiseptics for various conditions.
9. How should anthrax samples be collected, stored, labeled, and sent to the laboratory? Write in detail.

Sample Collection Procedure

Unit 3

3.1 Fecal Sample and External Parasite Collection and Tool for Examination

Parasite: An organism that lives in or an organism of another species (its host) and benefits by driving nutrients at the other.

Parasitology: The branch of biology or medicine concerned with the study of organism.

Ectoparasites: The parasites, which live on the body surface of the host are called ectoparasites. e.g., Lice, tick, mange, Bed-bug

Endoparasite: The parasites, which live inside the body of the host are called endoparasites. e.g., malarial parasite, ascaris, tapeworm

Common internal (endoparasite) infection in animals:

1. Toxocare Canis (ascarid worm)

Symptoms

- Anorexia
- Diarrhea
- Vomiting
- Intestinal obstruction
- Malabsorption
- Anemia
- Larvae migrations produce dyspnea and cough

Diagnosis

- Clinical finding: Detection of ova/egg on feces

Treatment

- Piprazine 100mg/kg body weight
- Mebendazole 10 mg/kg body weight
- Pyrental pamoate 10 mg daily for 3 days.

2. Rumen Fluke of Ruminants

- Amphistome (*Paramphistomum* spp)
- Anorexia
- Fetid diarrhea
- Malabsorption
- Edema of sub-mandibular region

3. Liver Fluke (Faciola spp)

- Symptoms
- Anorexia
- Depression
- Loss of body weight
- Anemia
- Hepatitis
- Potbelly
- Bottle jaw condition
- Constipation followed by diarrhea

Diagnosis of Rumen and Liver Fluke

- Presence of egg of parasites in feces in fecal examination

Clinical Finding

- Available drugs:
- Nilzan 1ml/3kg body weight

- Matemar 1 bolus /100 kg
- Livertonic 40ml for 3 days
- Broton
- Hepatonic
- K-liv
- Liv-52
- livoferal etc
- Treatment:
- Oxyclozanidae 3.4% 1ml/kg body weight.
- Niclosan 75mg/kg body weight.

4. Nematodes

- Hemonchus
- Trichuris
- Oesophagostomum
- Toxocara

Clinical Finding

- Progressive loss of body weight
- Emaciation
- Malabsorption
- Diarrhea
- Anaemia
- Feces may contain mucus and blood

Monezia

- Monezia expansa
- Monezia benidini

Clinical Finding

- Dull and depressed
- Malabsorption
- Stunted growth
- Rough body coat
- Potbelly
- Poor body condition

Diagnosis

- Egg in feces
- Segment may be seen in feces in tape worm infestation
- Larvae culture

Treatment

- Albendazole 10 mg /kg body weight
- Febendazole 10 mg/kg body weight

Available Drugs

- Albomar liquid 1ml/3kg body weight (L.A)
- Fenbendazole bolus 1 bolus /50kg (L.A)
- Albendazole bolus 1bolus /100 kg (L.A)
- Albendazole 1 tab/13 kg body weight (S.A)
- Febendazole 1 tab/13 kg body weight (S.A)
- Albomar liquid 1ml/3kg body weight (S.A)
- Note: Albendazole should not be given to pregnant animal
- Mixed drugh available in market for Endoparasite infection are:
- Oxyliv bolus 1 bolus /100 kg body weight (L.A)
- Nilzan 1ml/3kg body weight
- Zanide –L bolus 1 bolus /100kg body weight (L.A)
- Ivermectin inje ction 1ml/50kg body weight

Routine Fecal Examination, Collection and Preservation of Fecal Sample:

Objectives (why to do)

a. To acquire knowledge of fecal examination, collection and preservation of fecal sample
b. To be able to do fecal examination
c. To be familiar with method and procedures

Material Required

- Fecal sample
- Beaker
- Collection pouches/vial
- Bamboo stick/glass rod
- Sieve
- Funnel
- Compound microscope
- Tissue paper
- Centrifuge machine
- Standard solution
- Mortar and pestle
- Slide and cover slip
- Mac-master slide

Chemical Required

- 10%normal saline
- Formal dehyde (formalin)
- Ether solution
- NaCl (common salt), $ZnS0_4$ (Zinc Sulphate), $MgS0_4$ (Magnesium Sulphate)

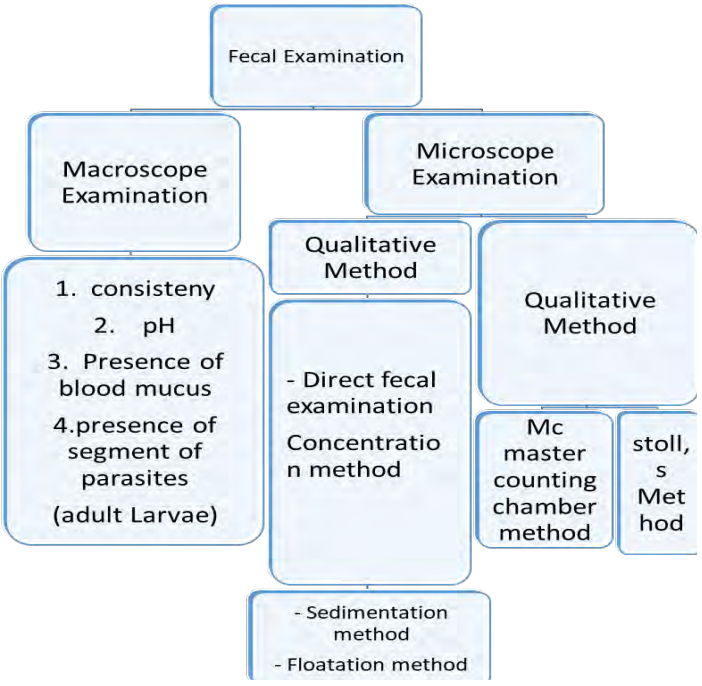
Fecal Sample Collection

The sites and methods of collection vary among farm animals. Fecal samples are typically collected from different farm animals using the following methods.

Animal	Collection site and procedure
Dogs and cats	Mostly fecal sample (feces) are collected per rectum with the help of a glass rod or thermometer.
Large animals (cattle, buffalo, horse)	-Entering hand inside the rectum -In field condition, a middle portion of a freshly excreted fecal sample may be taken.
Small animals (sheep and goat)	Inserting the index finger into the rectum
Birds	Glass rod is inserted into cloaca.

Feces are collected in sterilized wide vials with screw or sterilized pouches. Fecal sample should be fresh, examined within 4 hours to avoid fungal growth and the hatching of eggs.

Fecal Examination Method



Flowchart: Different Method of Fecal Examination

Generally, there are two type of fecal examination method

Macroscopic Examination

- It is an examination of physical characteristics done with the naked eye. It includes characters such as consistency, pH, presence or absence of blood and mucus, odor of feces, and presence of segments of parasites.

Microscopic Examination

- Qualitative method
- Quantitative method

Qualitative Examination

It includes

- Direct fecal Examination
- Concentration method
  a. Sedimentation method
  b. Floatation method

Direct Fecal Examination

For this method, take a clean slide, put one drop of normal saline, and take one pinch of fecal sample to make smear with the glass rod. Staining may be done with staining solution either Lugol's iodine or Methylene blue or examination done without staining.

A small quantity of fresh feces is placed on a glass slide, mixed with few drops of water and spread it evenly on the slide, then place a cover slip over it and examine under the microscope.

Sedimentation Method

The sedimentation technique is qualitative methods for detection of trematode egg in feces of domestic animals. The majority of trematode eggs are too large

and heavy to float reliably in the flotation fluids normally used for nematode eggs. They however sink rapidly to the bottom of a faecal/water suspension and this is the basis of the faecal sedimentation technique.

Materials required and some reagent required:

- Plastic containers or two beakers
- Gauze or tea strainer, double layer of cheese cloth
- Measuring cylinder
- Strirring rod
- Mortar and pestle
- Test tube
- Centrifuge
- Test tube rack
- Methylene blue
- Pipettes
- Balance
- Tea spoon
- Microscope
- Slide and cover slide

Procedures

- Weigh or measure 3 g of faeces and grinded it, then transfer it into cylinder.
- Pour 40-50 ml of tap water into cylinder.
- Mix faeces and water thoroughly.
- Filter the suspension through a tea strainer or double layer of cheesecloth into cylinder.
- Pour the filtered material into a test tube. In addition, allow to sediment for 5 minutes

- Remove the supernatant with a pipette very carefully.
- Re-suspend the sediment in 5ml of water.
- Allow to sediment for 5 minutes.
- Discard the supernatant carefully.
- The eggs of liver flukes are heavy and do not float on water and settle in sediment.
- Stain the sediment by adding one drop of methylene blue
- The dyes stain the faecal particles a deep blue or green leaving the trematode eggs unstained. The egg of trematode is revealed in figure
- Transfer a small drop of the stained sediment to a microscope slide using a pipette.
- Cover droplet with a coverslip and examine under a microscope at 10 x 10 magnifications.

Flotation Examination

Examination by the flotation method is more complex and requires more time, but is usually more accurate than the direct smear. The feces is mixed with either saturated sugar solution, saturated salt solution, saturated sodium nitrate solution, 41% magnesium sulfate solution or 33% zinc sulfate solution. The flotation method uses the principle that most fecal particles fall to the bottom of the tube or vial. Parasite eggs and cysts rise to the top of the salt or sugar solution, which is the result of a weight difference between feces, parasite eggs, and cysts within the solution. In pure water, parasite eggs and cysts settle to the bottom rather than float; whereas in the salt or sugar solution, they float due to the higher density of the solution.

Materials Required and some Reagent Required

- Plastic containers or two beakers
- Saturated sugar,
- Saturated salt,

- Saturated Sodium Nitrate,
- Magnesium Sulfate
- Zinc sulfate solutions.
- Gauze or tea strainer, double layer of cheesecloth
- Measuring cylinder
- Centrifuge
- Stirring rod
- Mortar and pestle
- Test tube
- Test tube rack
- Pipette
- Balance
- Teaspoon
- Microscope
- Slide and cover slide

Procedures

- The flotation method involves the use of a flotation solution that has a specific gravity, greater than 1.2, such as a salt or sugar solution.
- Simple floatation, about one gram of feces is taken and grinded and mixed with 42 ml of saline water.
- Then filter it through a fine sieve or muslin cloth or gauze in to test tube or cylinder until it form meniscus (up to top of tube).
- A clean glass slide or cover slide is placed on the mouth of test tube or cylinder. Then leave it for 10- 15 minute at room temperature without disturbance, then remove cover slide/ slide and examine under10 x of microscope.
- In centrifugation floatation, the first step as simple floatation is

similar, except this method use centrifugation. Mixed the contents and centrifuge at 1500 rpm for 5 minute, the tube is taken out and placed without disturbance. Transfer the small amount of superficial contents of the tube on a clean and dry glass slide.

- Place the cover slip on a slide and examine it under a microscope and the parasite ova may be observed under microscope.

McMaster Egg Counting Technique

McMaster is the quantitative method for determining the number of nematode eggs per gram of feces in order to estimate the worm burden in an animal. Advantage of this technique is quick as the eggs are floated free of debris before counting.

Equipment Required

- Beakers or plastic containers
- Balance
- Tea strainer or cheesecloth
- Measuring cylinder
- Stirring device (fork)
- Pasteur pipettes and rubber teats
- Flotation fluid
- McMaster counting chamber
- Microscope

Procedure of McMaster Techniques

- Weigh out 2 gm of feces and transfer in to container.
- Add 60 ml of saturated salt solution into container.
- Mix feces with saturated salt solution by stirring device.
- Filter feces from container 1 into container 2 or cylinder by gauze or sieve.

- Take a sub- sample with a Pasteur pipette from container 2.
- Fill both side of the McMaster counting chamber with the sub sample.
- Allow the counting chamber to stand for 3-5 minutes.
- Examine the sub sample of filtrate under a microscope at 10x 10 magnification.
- Count all eggs and coccidia oocytes within the engraved area of both chambers.
- Focus first on the etched lines of the grid, then go down a tiny bit, the egg will be floating just below the top of the chamber.
- The calculation of eggs from chambers is: Multiply the total number of eggs in the 2 chambers by 100= eggs per gram (EPG) or multiply the total by 50. This gives the EPG (egg per gram of feces) of feces. (Example: 50 eggs seen in chamber 1 and 100 eggs seen in chamber 2 = (50+ 100) x 50 = 25,000 epg.)

Stoll's Method

It is also called sedimentation method. In this method, 3gm of fecal sample is dissolved in 42ml of distilled water. The solution is then screened. Then 0.5ml of dissolved fecal sample is kept in glass slide. A special type of cover lining (20x30) mm is used. The eggs are counted line by line serially.

We dissolved 3gm of fecal sample with 42ml distilled water.

No of egg in 0.15ml of solution = X

45ml of solution contain egg = $(x * 45)/ (0.15 * 3)$

Egg per gram = $(x * 45)/0.15$

= $x*100$

3.2 Skin Scrapping Test

This is the method of examination of presence of external parasites in the animal body when mites are suspected. Skin scrapping can be collected and forwarded

to laboratory by placing them in glycerin in a tightly sealed vials for transport to laboratory. Vials should be placed carefully to prevent breakage or leakage. Skin scrapping technique is described as follows.

- Add several drops of mineral oils or glycerin to area to be scrapped. The area should be at the periphery of the cutaneous lesions or in the center of the lesions.
- With the scalpel blade, scrape the area to the depth that blood being to ooze from the wound.
- Transfer the bloody material, that was scrapped to the laboratory.
- Add additional minerals oil and cover with cover slip.
- Examine under low power of microscope (40x) of the microscope first, and if nothing seen increase the magnification.

Note:

- Scrapping should be sent dry and unpreserved in the plastic bottle.
- Scrap deep at the periphery of the lesion until blood oozes.
- If ringworm is suspected, pull the hair of the edge of a lesion with forceps and add to the sample
- Large amounts of hair and skin can be digested by 10% KOH for 12-24 hours, and the sediment can be examined for mites either directly after centrifuging or the sediment can be mixed with sugar floatation solution.

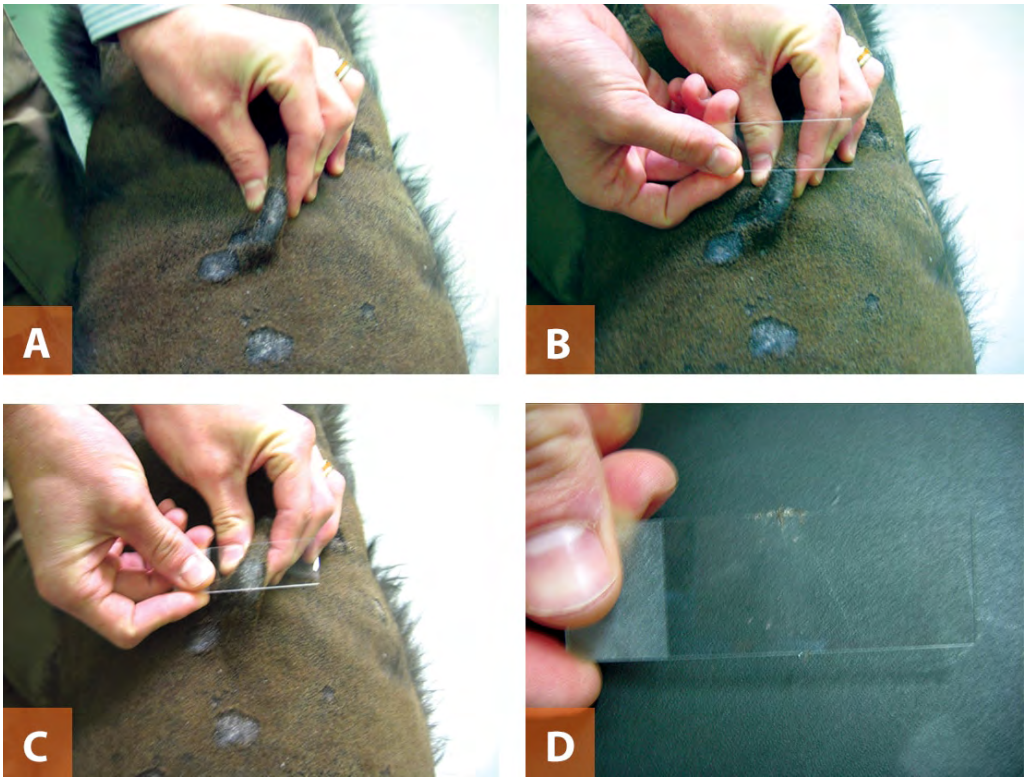


FIGURE 3. Skin scraping

Identification of parasites

Kingdom – *Animalia*

Phylum - *Nemathelminthes*

Class – 1. *Nematodes* (Round worm)

1. **Trematodes (Fluke)**
2. **Cestodes (Tapeworm)**

1. Nematodes of livestock and poultry

Snoring disease / Schistosomiasis (*Schistosomo nasalis*)

Oesophagostomiasis (*Oesophagostomum* spp)

Lungs worm *(Dictyocaulus Spp)*

Different Roundworm of livestock and poultry are:

a) Round Worm of Stomach

- Hemonchus contortus /wire worm
- Ostertagia/Brown worm
- Trichostrongylus

Round Worm of small Intestine

- Cooperies/Bankrupt Worm
- Nematodirus
- Round Worm of large Intestine
- Charbetia
- Oesophagostomum/Nodular worm

Large Round Worm

Ascaris lumbricoids – Human

Ascaris sum – pig

Toxocara vitllurum – Cattle

Others Important Roundworm

- Oxyrus equi – Horse
- Heteractis gallinarum-Poultry
- Hemonchus cantrotus- Sheep
- Strongyloides _ Horse
- Thelazia _ Eye worm of livestock
- Dirofilariasis iritis – Dog

Trematodes

- Fasciola spp
- Paramphistomum Spp

Cestodes

- Monezia- cattle, sheep, Goat
- Taenia spp- cattle, sheep, Goat, Human

Common Ectoparasite (external) Infection in Animals

Tick

Tick are of Two Types

- Hard tick (ixodidae)
- Soft tick (argacidae)

Name of the Ticks and their Host

- Ixodes: sheep, goat, dog
- Boophilus : cattle
- Rhipicephalus: cattle, horse, sheep, goat
- Dermacentor: cattle, dog

Clinical Finding

- Ticks produce cutaneous injury which support the infestation by fly and bacteria
- Tick consume considerable amount of blood through sucking and thereby make animal unthrifty and anemic.

Extensive Damage of Skin, which Reduce Value of Skin.

- Ticks may also transmit disease.
- They produce irritation and dermatitis.

Mites

- Sheep: Psoroptes scabies
- Cattle: Sarcoptes scabies
- Dog: Demodex canis

Clinical Finding

- Sarcoptes mites produce scabs, alopecia, itching, erythema (superficial reddening of skin, usually in patch, which result injury and irritation) and hyper keratitis lesion are mostly in the ear, face, elbow and root of tail. Animal become emaciated and in goat there is death even.
- Psoroptes mites produce lesion in all part of the body, mostly the wool of the sheep is lost and there is formation of scab known as sheep scab.
- Demodex mites produce pustular, popular lesion. There is nodules formation. Itching is less evident.

Diagnosis of ticks and mites

- History of itching

Clinical finding

- Recovery of ticks and mites from skin (mites in skin scrapping)

Treatment and Control

- Hair should be clipped before a caricidal treatment
- A course of antibiotics should be given to inhibit bacterial infection.
- Malathion 0.5%,cypermethrine 0.4% solution may be usesd for dipping.
- Ivermectin s/c injection 200 mg/kg body weight i.e 1ml/50kg body weight.

Lice

- Biting
- Sucking

Name of Lice

- Bovicola : cattle, sheep
- Linognathus : cattle, sheep, goat.
- Heterodocus : dog

Clinical Finding

Marked itching, irritation, formation of erythematous macules, dermatitis lesion and anemia.

Diagnosis

- Detection of lice.

Treatment

- Through grooming is an effective remedy
- Clipping of hairs
- Ivermectin injection
- Butox is effective against lice.

Flea

- Ctenocephalides canis : Dog
- Ctenocephalides felis : cat

Clinical Finding

Itching flea allergy, dermatitis, self-inflected injury hyper keratinization and corrugated (fold, wrinkle) appearance of skin.

Treatment

- D.D.T- 5% dust or solution
- B.H.C- 0.01% dust
- Malathion- 0.5%

Fly

- Musca domestica (housefly) – cattle ,buffalo, sheep, goat, pig, dog
- Stomoxys stable fly – cattle buffalo sheep goat horse
- Hippobosca (blood sucker) – cattle buffalo
- Lucila (green bottle fly) – sheep goat

Clinical Finding

- Irritation
- Larvae may produce septic and holes in the skin
- Maggot infection (myiasis)
- Annoyances and worry

Diagnosis

- Detection of larvae on the wound

Treatment

- Fly repeilent
- Oil turpentine
- Oil eucalyptus

Mixed Drugs Available in Market for Ectoparasite Inection are:

- Amitraz – 2ml/lit of water (don't use for horse,cat and dog's younger)
- Butox – 2ml/lit of water
- Poron – back line application

Spray

- D-may spray
- Topicure spray
- Exoheal spray

Ointment

- Himax
- Charmil
- Charmil plus

Shampoo for Dog (external use only)

- Tick out
- No tick
- Clinar

Precaution

- Prevent to entry for natural opening.
- Animals should not lick and drink the medicine solution.

3.3 Blood Sample Collection Methods for Different Species of Animal

Introduction

Hematology is a medical science, which deals with the study of the blood, blood forming organs and blood diseases.

Blood

Blood is thick red fluid. It is liquid connective tissue which is seen everywhere of the body. It consists of cell and fluids. Cells are the RBC, WBC and platelets; fluid is called plasma, which contains mineral, protein and nutrients. Serum is that factor portion of blood plasma in which fibrinogen clotting factor is not present.

Types of Blood Cell

Blood cells are about 45% of total volume. It consists of:

1. Red Blood Cell (RBC)/Erythrocytes
2. White Blood Cell (WBC)/Leukocytes
3. Platelets/Thrombocytes

Red Blood Cell (RBC)/Erythrocytes: RBC is flexible and resembles bi-concave disks. They lack nucleus. They are formed in red bone marrow are mostly present in the end of long bone. The lifespan of RBC is 120 days. It contains hemoglobin which carries oxygen. Iron is one of the hemoglobins. Decrease in number of RBC leads to anemia.

White Blood Cell (WBC)/Leukocytes: It is also known as leukocytes. They are defense cell of animal body. They contain nucleus whichvary size. They are the largest blood cell, lifespan of WBC about a week. Nucleus of WBCs increases during infection. They are divided into two groups according to presence or absence of granules in the cell. Granulocytes are Neutrophil, Basophil and Eosinophil where Agranulocytes are Monocytes and Lymphocytes.

Function of WBC

1. They produce the antigen and antitoxins.
2. They help in ingestion of living germs.
3. They help to remove injured tissue.
4. They help in healing process.

Platelets

They are smallest cell of blood. They are irregular in shape. They live only few days. They help to prevent blood loss by clotting blood. It contains several clotting factor.

Function of Platelets

- It helps in healing of wound.
- It prevents blood loss by clotting.

Function of Blood

1. It carries nutrients.
2. It carries O_2 from lungs to tissue and CO_2 from tissue to lungs.
3. Waste products from various tissues which are carried to the kidney for excretion.
4. Hormones are carried from endocrine glands.
5. It maintains temperature, water balance in the body.
6. It maintains acid, base balance.
7. It contains clotting factor, which prevents from blood loss in trauma.
8. It contains agent, which protects body from infections.

Flow Chart of Blood Components: Blood Cell (45%) Plasma Cell (55%)

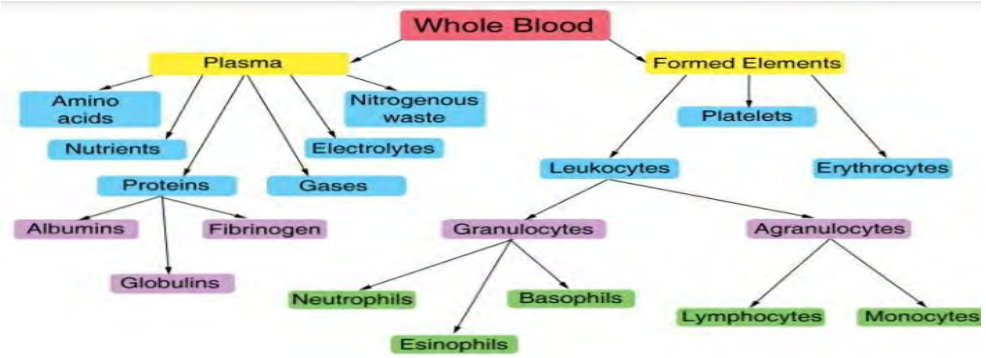


Figure: flow chart of blood components

Clinical Significance of Blood Cell Numbers

1. Erythrocytes

a. Erythrocytosis/polyerythemia: It is increase in number of erythrocytes in circulation. This may occur in some disease like Brucellosis, H.S, Leptospirosis, R.P, Poisoning, Chronic Heart Disease, Shock, Vomiting, Diarrhea, etc.

b. Erythropenia/Oligoerythemia: This is decrease in erythrocytes count in circulation. This may occur in disease like anemia, leukemia, babesiosis, anaplasmosis, coccidiosis, fascioliasis, etc.

3. Leukocytes/WBC

i. Increase in number of leukocytes in circulation is leukocytosis. It is temporary useful to animal and is reversible.

ii. **Leukemia:** It is considered as cancer of leucocytes and is harmful. Leucocytes are also increased in circulation.

iii. **Leukopenia:** It is decrease in the number of white blood cells. It is due to diminishing production, increase distraction and altered distribution.

iv. **Neutrophilia:** Increase in neutrophils number in circulation. Neutrophil is the first defense cell of body. It is increase in infectious diseases like E. coli, Pox, Leptospirosis, etc.

v. **Eosinophila:** It is a condition where eosinophils are increased in circulation. It may occur in allergic and parasitic condition.

vi. **Lymphocytosis:** It is an increase in circulating lymphocytes in the blood. It may occur in viral disease and chronic bacterial disease.

vii. **Monocytes:** It is a condition of increasing circulating monocytes. It mainly occurs in protozoan infection.

Normal Hematological Value in Domestic Animals

Animal species		**Differential leukocytes Count (DLC)**						
	Hb gm%	Lymphocyte	Neutrophil	Eosinophil	Monocytes	Basophil	TEC in 1000/ ml	TLC in 1000/ ml
Cattle	8-14	48-75	15-45	2-15	2-7	0-2	5-8	5-9
Sheep	8-16	40-75	10-15	1-8	1-5	0-3	8-15	5-9
Goat	8-14	50-70	30-48	3-8	1-4	0-2	8-17.5	12
Horse	4-18	15-50	35-75	1-10	2-10	0-3	7-13	8-9
Pig	10-16	39-60	28-47	1-11	2-10	0-2	5-8	11-32
Dog	12-18	12-30	60-75	2-10	3-9	0-1	6-9	8-13
Cat	8-15	20-35	35-75	2-10	1-4	0-1	5-10	8-25
poultry	9-13	76	13	3	6	2	3-4	29-39

- DLC: Differential leucocytes count
- TLC: Total Erythrocytes Count
- TLC: Total Leucocytes Count

Blood Sample Collection Method

Blood travels in blood vessels, i.e arteries, veins, and capillaries. Blood consist of cells and plasma. Cell are RBC, WBC and Platelets. Plasma contain fibrinogen, globulins, and albumins.

1. Blood Percentage of Body Weight in Different Animals

Animal Species	Quantity of Blood
Cattle, Buffalo	8% of body weight
Horse	10% of body weight
Pig	6-7% of body weight
Sheep	8% of body weight
Goat	6% of body weight
Cat	6.5% of body weight
Dog	7% of body weight

Collection of Blood

The blood is collected from animals through the puncture of a vein using a syringe and needle. In a laboratory, animal or poultry blood may be directly collected from the heart. The sites of blood collection in different animal species are as follows:

Animal Species	Collection Sites
Cattle, Buffalo	Jugular vein
Horse	Jugular vein
Camel	Jugular vein
Sheep, Goat	Jugular vein
Pig	Ear vein / Anterior venacava
Dog, Cat	Cephalic vein, Recurrent Tarsal vein /saphenous vein/ Femoral vein/Jugular vein
Poultry	Wing vein/ Heart puncture
Lab animals	Heart puncture

Common Blood Collection Tubes

Color	Tube contains	Purpose
Purple top	Contains Ethylene diamine tetra acetic acid (EDTA), an anticoagulant.	1. Complete blood count CBC 2. Mammalian blood smear 3. Blood typing
Red top	Contains a clot activator and no anticoagulant	1. To obtain serum 2. Sterile urine collection
Green top	Lithium heparin/sodium heparin (anticoagulant)	1. Routine biochemistry test
Grey top	Fluoride oxalate (clot activator)	1. Serum glucose and lactate
Yellow top	Clot activator/ separation gel	1. Urea and electrolytes 2. Liver function test 3. Serology

Things to Remember while Blood Collection

1. Animals should always be properly restrained before the collection of blood.
2. The site of blood collection should be sterilized with an antiseptic solution such as savlon, spirit, etc.
3. Always draw the blood in the accurate amount into the sterile tube.
4. Tests requiring whole blood or plasma should be collected in tubes with anticoagulant, while tests requiring serum should be collected in tubes without anticoagulant.
5. Anticoagulant blood tubes should be gently inverted several times immediately after collection.
6. Avoid vigorous shaking of the sample to prevent hemolysis.
7. It is always advisable to start the examination as soon as it is collected. In case of any delay, the blood sample must be kept in the refrigerator at 4^0c for 24 hours.

Procedure of Blood Sample Collection

Materials Required

- Blood collection vials (e.g, syringes, vacutainer tubes)
- Needles 18,19,20 ,22,23,24 guaze
- Restraint materials (e.g., squeeze chute, halter, casting ropes)
- Muddy ground for casting
- Clippers
- Antiseptic
- Guaze etc.

The procedure of blood collection from farm animals (cattle)

Procedure for blood collection via the jugular vein in cattle are described below.

1. Restrain the animals with a casting rope or halter or travis, and secure it for personal safety with a quick-release knot.
2. Clip and swipe with antiseptic gauze to remove superficial dirt and debris. This may also assist in visualizing the raised jugular vein.
3. The jugular vein by applying pressure at the base of the jugular groove and visualize raised vein.
4. If using a vacutainer, once the needle is inserted, stabilize the needle and push the vacutainer tube into the hub. If you have hit the vein, blood will flow freely into a tube. Multiple tubes can be filled by removing the filled tube and replacing in with a fresh tube.
5. Once the collection is complete, remove the vacutainer tube, then, applying pressure over the injection site, remove the needle.
6. Apply pressure with gauze for 30-60 seconds to stop bleeding.

Jugular Vein

It is situated in the jugular groove over the trachea on both side of the neck. Before collection of blood, the hairs of the area are removed with the help of

scissors and the area is shaved with razor. Then antiseptics are applied like sprit. Animal is properly restrained. The vein is raised by applying pressure at the ventral point of puncture. The vein is left by finger tapping. Blood is collected by 18G, 16G needle in small and large animals respectively. The needle is inserted in the vein by force in an angle, the blood can be collected directly in the collection tube or apply syringe over needle to suck the blood. Press the site of penetration with cotton pierce after collection. After the collection, pressure is removed and needle is removed.

Pull head to the side and secure lid to hold it	Occlude jugular vein by applying pressure at base of jugular groove and insert needle	Aspirate syringe to collect blood

Figure 1. Pull Head to the Side, and Secure Lead with Quick Release Knot

Figure 2. Occlude Jugular Vein by Applying Pressure at Base of Jugular Groove and Insert Needle

Figure 3. Aspirate Syringe to Collect Blood

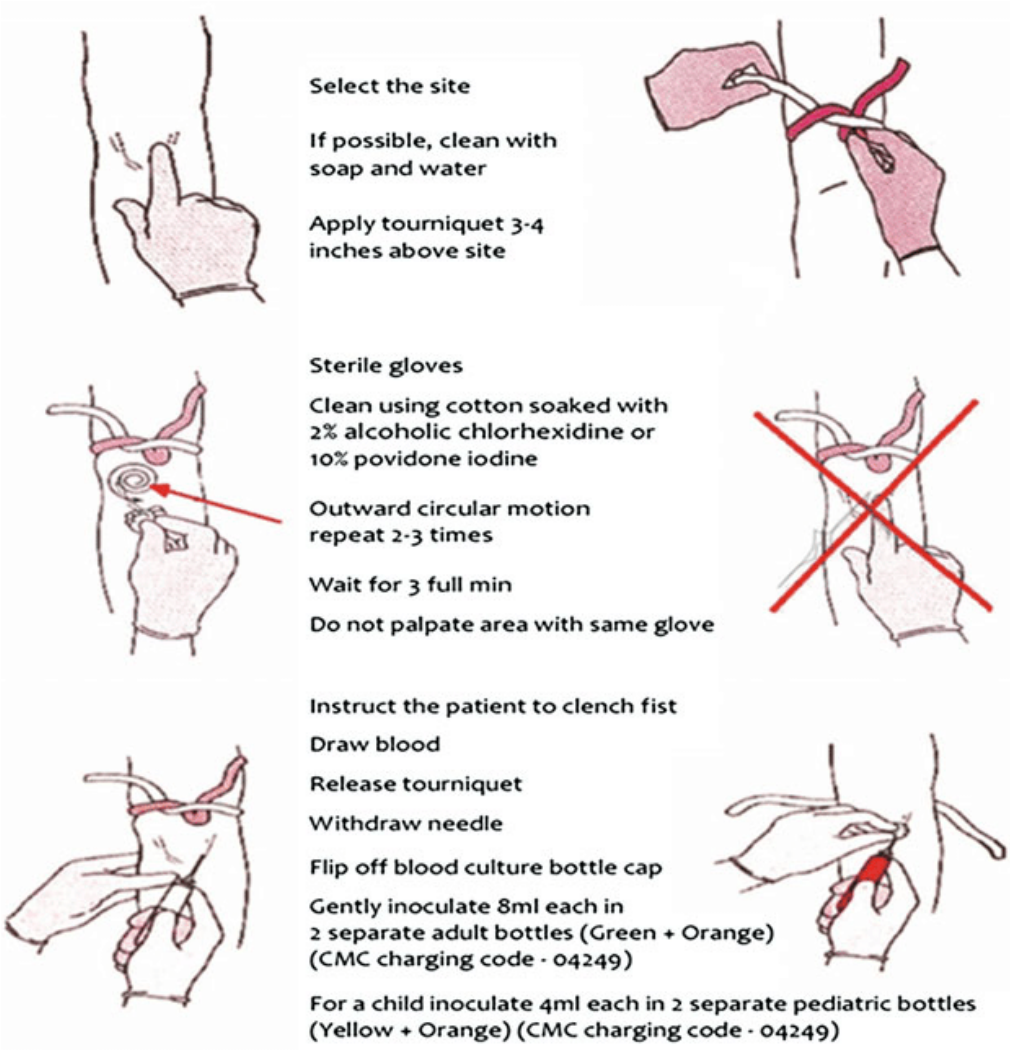


Figure: Schematic diagram of specimen collection from blood

Storage of Blood

Blood should be stored in 4^0c temperature without alteration for 24hrs. Anti-coagulant is used to prevent clotting. They are EDTA, sodium fluoride, sodium citrate, mixture of potassium oxalate and ammonium oxalate.

Types of Vials to store Blood

i. EDTA vials

This is vials with blue cap. Blood for hematological test are store in the vials. These prevent clotting of blood.

ii. Bio-chemistry Vials

This is vial with yellow cap it contains gel for clotting of blood. Blood store in this vial are used for bio-chemical test.

Collection of Serum from Blood

Blood is fluid connective tissue which consists of blood cell and plasma. Blood cells are RBC, WBC, and platelets. Plasma and serum are two frequent terms, which are used alternatively. Serum is that portion of blood, which is similar in composition of plasma but exclude clotting factor of blood. Fibrinogen is a protein that is involved in blood coagulation.

Serum collection

Procedures

- Whole blood is collected in tube with standard procedure.
- Allow sample to clot for 1hrs in room temperature.
- Centrifuge for 1min at about 6000rpm.
- Collect the serum, which is supernatant fluid and blood cell, are remaining sediment in tube.

Extra

Blood=Cell +Plasma

Plasma= Serum + Clotting Factor (Fibrinogen)

Serum= Plasma – Clotting Factor (Fibrinogen)

3.4 Urine Sample Collection

Methods of urine collection are the most important. The urine can be collected by gently tickling of the perineum around the vulva with a piece of straw or the

fingers may encourage a cow or heifer to urinate. In male animals, similar handling of the prepuce may be followed by urination. The cattle very often will urinate during examinations, so have a suitable container ready. Continuous stroking of the skin just below the vulva of cows will usually induce urination. Once collected, the urine sample should be inspected, smelled and its contents tested. Urine can be collected by catheter methods, in cow and heifers plastic catheter 0.5cm in diameter and 40 cm long may passed into the bladder. The methods how catheter is inserted into bladder for collection of blood, first disinfection of vagina and placed gloved for finger into sub-urethral diverticulum and insert catheter over the finger into urethra. Then urine may flow freely from the bladder into a sterile syringe via catheter.

Horses of both sexes urinate only while resting and cease feeding for the time, and cows urinate similarly to mares, male cattle on the other hand urinate not only while feeding but also while walking, old dogs and pigs male void the urine in the interrupted jerky stream. Since bulls and steers cannot be catheterized, longer observation may be needed to obtain a sample. A collecting urinal may be strapped on male swine, cattle, sheep, or goats to obtain a sample. Catheters can be used on both male and female horses. In male horses, manual pressure on the bladder via the rectum will sometimes induce urination. Close observation will enable collection from dogs and cats, but catheterization can be used successfully. Other collection methods from dogs and cats include applying pressure on the bladder and using a collection cage.

Storage of Sampled Urine

Urine sample can be examined immediately after collection. However, the sample is expected to delayed, the urine sample should be kept at 4oc without adding any preservative. For long preservative toluene can be added in urine to form a layer over urine, this suit for chemical examination. One drop of 40% formalin can be used as preservative in urine; however it may give false reaction for sugar examination.

1. **Voided sample collection/Free Catch**

   - This is the easiest method for the patient. But this method may be very challenging for the urine collector depending on the animals. A clean dry collection container is placed into the urine stream and urine is collected directly into the container. Ideally, sample would be free from the mid -stream, clean collection to decrease potential bacterial cellular and artificial contamination. Bacterial contamination may result false positive result.

2. **Catheterization**

   - This technique required quite knowledge about anatomy of urinary system. In this system, cather is used to collect urine directly from the bladder. Catheter is small tube, which is used to collect fluid from the body cavity.

3. **Cystocentesis**

   - This is the method of obtaining urine sample by punchuring the bladder through the abdominal wall.

Urine Sample Collection in Cattle and Buffalo

1. **Voided Sample Collection/Free Catch**
2. **Induced Method**

   - Gentle tickling of the perineum around the vulva with the piece of straw or the fingers may encourage a cow or heifer to urinate. In some males, similar handlings of the prepuce may be followed by urination. This is the safe method.

3. **Catheterization**

   - Catheterization is the method of urine sample collection. It is seldom possible in bull, in cow and heifers, a sterile, rigid metal or plastic catheter of approximately 0.5cm in a diameter and 40cm long may be passed into the bladder. The vulva is carefully wiped clean with

mild disinfectant. A gloved fore finger is placed into sub-urethral diverticulum, and the catheter is passed over the finger into urethra. Slight resistance is experienced as the catheter passed. Urine may follow freely from the bladder or it may be necessary to aspirate urine from the bladder via catheter into sterile syringe.

Urine Sample Collection in Sheep and Goat

1. **Voided Sample Collection/Free Catch**
2. **Induced Method/Induced Micturation**
    - To collect urine sample in ewe two people are required one person restrains the sheep in standing position and temporarily close the nostrils and second person must be ready at the rear end to collect the urine in a specimen bottle. If no urine is produced and sheep is becoming distressed after 30 seconds, the nostrils may be released. In male animals, gently tickling around the preputial opening may be induced urination.
3. **Catheterization**
4. **Cystocentesis**

Urine Sample Collection in Pig

1. **Voided Sample Collection/Free Catch**
2. **Catheterization**
3. **Induced Micturation**
    - The sow can be encouraged to pass a urine sample by stimulation the vulva skin into standing position, if not possible catheterization may be done.

Urine Sample Collection in Dog and Cat

1. **Voided Sample Collection/Free Catch**
2. **Catheterization**
3. **Cystocentesis**

General Properties of Urine

- Urine is liquid excrement consisting of water, salt and urea, which is made in the kidney, stored in bladder and then released through the urethra. Urine is most significant way of elimination of volatile substances from body of animals. These included nitrogenous substances of protein and nucleic acid metabolism such as urea, uric acid and creatinine, ingested substances such as excess glucose and water and substances of cellular metabolism produce in excess such as water and electrolyte. The composition of urine reflects the kidney function in maintaining homeostasis of the organism urine composition is widely varying among the species. Urine is varying in different age, diet and physical activity.

Physical Properties of Urine

1. colour
2. Odour
3. Specific gravity
4. PH or reaction
5. Turbidity/Physical condition/Transparency
6. Volume
7. foam
8. Viscosity

Chemical Properties of Urine

1. Proteinuria (Protein)
2. Glycosuria (Glucose)
3. Ketonuria (Ketone body)
4. Bilirubinuria (Bile)

3.5 Excision of Cyst, Pus, Abscess

Definition: A cyst is an epithelial lined, pathological cavity having fluid, semi-fluid or gaseous contents, and surrounded by connective tissue.

Abscesses are collections of pus in confined tissue spaces, usually caused by bacterial infection. Symptoms include local pain, tenderness, warmth, and swelling (if abscesses are near the skin layer) or constitutional symptoms (if abscesses are deep). Imaging is often necessary for diagnosis of deep abscesses. Treatment is surgical drainage or percutaneous needle aspiration and often antibiotics.

Pus is a thick yellowish, whitish, or greenish fluid made up of dead white blood cells, dead tissues, and dead bacteria or fungi. Also called liquor purist or purulent exudate, it is produced as part of the body's response to an infection. Pus is usually odorless but can emit a foul odor if an infection is severe.

Cyst

A cyst is a sac-like pocket of membranous tissue that contains fluid, air, or other substances. The content of the cyst can be natural bodily secretions (e.g. sebum or sweat) or abnormal breakdown products such as dead cells or keratin. The most common cyst that occurs is in the hair follicle and sebaceous glands.

Causes of Cyst

- Local injury to the follicles
- Blockage of the opening of the pore or follicle
- Sun damage
- Breed exposure in hairless breeds, boxer dogs, basset hounds, etc.
- Drugs such as Glucocorticoids (steroids)
- Site of injection, trauma, hemorrhage etc.

Clinical signs of Cysts

- Appears as single round nodules on or underneath the skin.

- If filled with keratin, they appear bluish and contain thick, yellowish, or gray cheesy materials.
- Secondary infection with bacteria or yeast can occur, which can produce a foul smell.
- Cysts appearing in the sweat gland cause hair to fall out and produce a yellow substance.
- Cysts appearing in the sebaceous gland appear as a single raised bump that seems white or slightly blue and produce grayish-white, brownish, or cheese-like discharge.
- Cysts can be filled with blood and look dark. These are called false cysts.

Diagnosis

- Biopsy
- Histopathology

Procedures for the excision of a Cyst is given below

Materials Required

- Cleansing solution (Betadine or Chlorhexidine)
- General anesthesia for small animals
- Hair clipper/Razor
- Local anesthetic (1% Lidocaine)
- Adrenaline/Hemostatic forceps
- Sterilized cotton gauze
- Sterilized surgical pack
- Surgical scalpel blade
- Culture swab
- 4*4 gauze squares and tapes
- Sterile surgical gloves.

Procedures

- First confirm the location of the cyst and the surrounding area.
- The surgery should be performed by a registered veterinarian with general and local anesthesia.
- Clip the hair around the cyst, and clean it with an antiseptic solution containing chlorhexidine or betadine.
- After the area is cleaned, provide a drape over the cyst.
- Make an eclipse-shaped incision through the subcutaneous tissue.
- Identify the plane between the cyst and subcutaneous tissue. After that remove 25% of the cyst circumference using the blunt dissection technique.
- Use thumbs to put pressure on the surrounding tissue to move the cyst toward an upper direction.
- Grasp the cyst with forceps and surgical scissors to separate the cyst from the tissue.
- Manage the bleeding by hemostatic forceps / adrenaline / absorbable cotton guaze.
- Close the incision with non-absorbable sutures.
- A saline solution will be used to clean the surgical area after closure.

Post-operative Care

- Antibiotics will be prescribed to prevent infection.
- E-collar should be put around the neck of the dog /cat. This will prevent the animals from licking the wound.
- In the case of horse / cattle / buffalo, tie the animals head with short ropes so that they cannot reach the operated area. Also, put the animal in a clean shed to prevent any infection.
- Follow-up treatment for cleaning the wound and checking the infection should be done every 1-2 days.

Abscess

Abscesses are a collection of pus in tissue spaces, which is caused by bacterial infection. It can occur anywhere in the body where pyogenic (pus-forming) bacteria can establish and multiply. After some time, the pus in the abscess is mostly replaced by fibrous tissue. The most common site for abscess formation is lymph nodes and subcutaneous tissue.

Causes of Abscess

- Bacterial infection
- Vaccination of subcutaneous tissue
- Fluid accumulation in the tissues
- In cattle, the infection with Actinomyces sp. leads to the growth of large abscesses on the head and neck of infected animals.
- Nails, screws, and glass damage the hoof of the animals leading to the trapping of bacteria inside the hoof.

Symptoms and Signs of Abscesses

- Early symptoms include Pain, Heat, Swelling, Tenderness, and Redness.
- Presence of whitish or yellowish pus with the thin center tissue in case of superficial abscesses.
- Anorexia and weight loss due to pain.
- Deep abscess shows local pain, tenderness, fever, and loss of function of the organ involved.
- Later stages include cold skin surrounded by a fibrous capsule.

Diagnosis of Abscesses

1. Physical Examination

- Presence of signs and symptoms mentioned above
- Palpation of the affected area reveals hot, soft, fluctuating mass.

2. Ultrasonography, CT Scan

- Diagnosis of a deep abscess like lungs, kidneys, liver, etc requires imaging.
- USG can detect soft-tissue abscesses, while CT is more accurate.

3. Microbiological culture

a. Inserting needle and finding pus

b. Incising of swelling at its most fluctuant or raised point

c. The culture of the contents reveals bacterial infection

Excision of Abscess

Material Required

- Cleansing solution (betadine or chlorhexidine)
- 21 and 25 gauge needles
- 10 ml syringe
- 11 number scalpel blade
- Culture swab
- Packing materials, such as 0.5 to 1 cm sterile gauze stripe
- 4×4 gauze squares and tapes
- Non sterile gloves etc.

Procedure

1. First, ensure the abscess is ripe. If the abscess is not ripe, we have a use magnesium sulfate (MgSo4) salt along with warm water. Apply the solution with a towel to the abscess twice a day for 4-5 days for ripening the abscess.
2. Once the abscess is ripe, we can do surgical drainage or percutaneous needle aspiration to drain the abscess.
3. Restrain the animals by casting in case of cattle and buffalo, halter in case of the horse, and muzzle in case of dogs and cats.

4. Inject the local anesthetic lidocaine around the affected area.
5. Clean the site of incision or aspiration by clipping the surrounding hair, and using a cleaning solution (Betadine or chlorhexidine).
6. Pierce the needle into the abscess or provide an S-shaped/straight incision the full length of the abscess.
7. Gently squeeze the wound to express the pus with a clean-gloved hand.
8. Irrigate the abscess cavity with a normal saline solution to remove all the pus.
9. Place an absorbent gauze pad over the wound and secure the pad with bandaging.

Post-operative Care

1. Dressing of the wound should be done in every 1-2 days.
2. The pain of the abscess goes along with the drainage, but postoperative analgesics may be required.
3. The use of antibiotics should be done after Antibiotic Sensitivity Test by taking a culture on the swab.

Exercise

Choose the correct answer from the given alternatives.

1. The study of parasites is known as.....
   a. Virology b. Parasitology
   c. Bacteriology d. Biology

2. Which of the following is not an Endo parasite?
   a. Ticks b. Tapeworm
   c. Liver fluke d. Roundworm

3. Which liver fluke does lives in cattle?
   a. Brain b. Kidney
   c. Bile ducts d. Pancreas

4. Which of the following is a common roundworm which infects domestic animal?
   a. Ascaris b. Enterobius
   c. Ancylostoma d. Rhabditis

5. Coccidiosis is an infection of …….
   a. Brain b. Intestine
   c. Eyes d. Tongue

6. Tick fever is also known as ……
   a. Fascioliasis b. Ascariasis
   c. Babesiosis d. Coccidiosis

7. Serum differs from blood as it lacks.....
   a. Antibodies b. Clotting factor
   c. Albumins d. Globulins

8. Which of the following is correct?

   a. Serum contains blood and fibrinogen

   b. Plasma is blood without lymphocytes

   c. Blood comprises plasma, RBC, WBC and platelets

   d. Lymph is plasma with RBC and WBc

9. Which plasma protein is responsible for blood coagulation?

   a. Fibrinogen b. Globulin

   c. Serum amylase d. Albumin

10. Which of the following are components of blood?

   a. Plasma

   b. Blood cells and platelets

   c. Gases and other dissolved substances

   d. All of the above

11. Which of the following is usually not found in the urine?

   a. Magnesium b. Urea

   c. Uric acid d. Glucose

12. Which is the most sterile method for Urine sample collection?

   a. Cystocentesis b. Free catch

   c. Bladder expression d. Catheterization

13. When protein is present in the urine, it doesn't means .....

   a. High consumption of food b. Fever

   c. Infection d. It's normal

14. The color of the feces of Buffalo is .....

   a. Dark brown to Dark green b. Yellowish brown to black

   c. Yellowish brown to dark green d. Dark yellow

15. The shape of the feces of Cow/Buffalo is .....

a. Pellet b. Bolus

c. Cylindrical d. Cake like

16. The shape of the feces of Pigs is .....

a. Pellet b. Bolus

c. Cylindrical d. Cake like

17. The shape of the feces of Horses is.....

a. Pellet b. Bolus

c. Cylindrical d. Cake like

18. The shape of the feces of Sheep/Goats is .....

a. Pellet b. Bolus

c. Cylindrical d. Cake like

19. The shape of the feces of Dogs/Cats is .....

a. Pellet b. Bolus

c. Cylindrical d. Cake like

20. The blood is composed of .....

a. Blood cells suspended in Blood plasma

b. Blood cells suspended in Blood serum

c. Body fluids suspended in Blood vessels

d. Connective tissue throughout the body

21. Which of the following is the function of the blood?

a. Carrying oxygen and Carbon dioxide to different cells of the body

b. Transports hormones and nutrients

c. Maintenance of internal body temperature

d. All of the above

22. Which is the common site of blood collection for Cattle/Buffaloes?

a. Jugular vein
b. Ear vein
c. Both a and b
d. Anterior venacava

23. Which is the common site of blood collection for Goat/Sheep?

a. Jugular vein
b. Ear vein
c. Heart puncture
d. Anterior venacava

24. Which is the common site of blood collection for pigs?

a. Jugular vein
b. Ear vein
c. Anterior venacava
d. Both b and c

25. Which is the common site of blood collection for Horses?

a. Jugular vein
b. Ear vein
c. Heart puncture
d. Anterior venacava

26. Which is the common site of blood collection for dogs?

a. Jugular vein
b. Cephalic vein
c. Recurrent tarsal vein
d. Both b and c

27. Which is the common site of blood collection for cats?

a. Saphenous vein
b. Cephalic vein
c. Femoral vein
d. All of the above

28. Which is the common site of blood collection for Rabbits?

a. Marginal vein
b. Heart puncture
c. Recurrent tarsal vein
d. Both a and b

29. Which is the common site of blood collection for Poultry?

a. Winy vein
b. Heart puncture
c. Recurrent tarsal vein
d. Both a and b

30. Which is the common site of blood collection for Lab animals?

a. Jugular vein b. Cephalic vein

c. Heart puncture d. Both b & c

31. How does the fresh infection of Ascaris occur in various animals?

a. Contaminated feed b. Contaminated clothes

c. Contact with infected animals d. Eating healthy food

32. Which method of fecal examination cannot detect eggs of Roundworms?

a. Direct Smear method

b. Saturated Sodium Chloride method

c. Sedimentation method

d. Saturated Zinc Chloride method

33. Which method of fecal examination can detect eggs of Tapeworms?

a. Direct Smear method

b. Saturated Zinc Chloride method

c. Sedimentation method

d. Saturated Sodium Chloride method

34. Which method calculates Egg per gram of feces?

a. Mac Master technique

b. Saturated Sodium Chloride method

c. Sedimentation method

d. Direct method

35. What is the indication of the Skin Scrapping Test?

a. Demodecosis b. Skin cancer

c. Fungal infection d. All of the above

36. The site of the operation should not be cleaned with.....

a. Chlorhexidine b. Betadine

c. Alcohol d. Salvon

37. What should be done when the abscess is not ripe?

a. Use Magnesium sulfate solution with warm water

b. Drain the abscess

c. Use antibiotics

d. Operate on the animal

38. The blood collected in the Purple top EDTA tube is used to.....

a. Complete blood count b. Blood smear

c. Blood typing d. All of the above

39. How do you collect fecal samples in small animals?

a. Inserting the whole hand into the rectum

b. Inserting a moistened finger into the rectum

c. Picking up the stool from the ground

d. Picking up the feces from the road

40. How much of the feces should be collected?

a. 5gm b. 7gm

c. 10gm d. Any of the given

41. Which of the following things should not be missed while sampling collection?

a. Collection of 5-10gm of sample

b. Storing sample in refrigerator

c. Observing the consistency of sample

d. All of the above

42. Which of the following things should not be done while observing a fecal sample?

a. Checking whether the sample is solid, semi-solid or watery

b. Checking for the presence of parasitic segment

c. Checking for the smell of the feces

d. Checking for flavor and taste of feces

43. The gross examination of the feces is done to check.....

a. Consistency of feces b. Color of feces

c. Presence of parasitic segment d. All of the tube

44. The purpose of blood collection in an EDTA tube is to.....

a. Complete Blood Count (CBC)

b. Blood smear

c. Blood typing

d. All of the above

45. The purpose of the clot activator yellow top tube is for.....

a. Determination of Urea & Electrolytes

b. Liver Function Test

c. Serology

d. All of the above

46. For the collection of the blood, the needle should be inserted at an angle of.....

a. 20° b. 30° c. 40° d. 50°

47. Where do the embryonated eggs of Ascaris hatch out in the animal?

a. Heart b. Brain c. Kidney d. Intestine

48. Which of the following is not caused by the movement of larvae through the body of animal?

a. Diarrhea
b. Anemia
c. Typhoid
d. Constipation

49. Which of the following should not be used for the preservation of Ecto-parasite?

a. Ethanol
b. Formalin
c. Saline
d. None

50. *Heterakis gallinarum* is the ……worm of Poultry.

a. Intestinal
b. Tracheal
c. Cecal
d. Brain

51. Which are called the Gapeworm of poultry?

a. *Syngamus trachea*
b. *Hetarakis gallinarum*
c. *Hemonchus contortus*
d. Oxyuris equi

52. The use of the KOH in the Skin scrapping test is .....

a. To digest the cells and debris
b. To provide ease for the vision
c. To make the microscopic slide clear
d. Stick the coverslip to the slide

53. The ..... produces red blood cells which transport ..... and some .....

a. Liver; oxygen; mineral ions
b. Liver; oxygen; carbon dioxide
c. Bone marrow, oxygen; hormones
d. Bone marrow; oxygen ; carbon dioxide

54. The purple top blood collection tube contains.....

a. EDTA
b. Clot activator
c. Lithium heparin
d. Sodium heparin

55. The red-top blood collection tube contains.....

a. EDTA
b. Clot activator
c. Lithium heparin
d. Fluoride oxalate

56. Which of the following part of the urine is the best for Sample collection?

a. First part
b. Midstream
c. Last part
d. Any part

57. Which of the following is not a gastrointestinal nematode?

a. *Cooperia sp.*
b. *Toxocara vitulorum*
c. *Dictyocaulus sp.*
d. *Ostertagia sp.*

58. *Dictyocaulus sp.* is found in.....

a. Liver
b. Bronchi
c. Trachea
d. Both b & c

59. Which is known as nodular worm?

a. Cooperia sp.
b. Toxocara vitulorum
c. Oesophagostomum sp.
d. Ostertag*ia sp.*

60. Which of the following is known as an Eye worm?

a. Thelezia sp.
b. Fasciola hepatica
c. Dictyocaulus sp.
d. Oestertagi*a sp.*

61. Liver fluke is commonly found in.....

a. Liver
b. Bile duct
c. Gall bladder
d. All of the above

62. The other name of *Echinococcus granulosus* is.....

a. Muscle worm
b. Nervous worm
c. Hyatadid worm
d. Cyst

63. *Schistosoma sp.* is also known as.....

a. Blood fluke
b. Liver fluke
c. Vessel fluke
d. Both a & c

64. Which of the following is known as a Rumen fluke?

a. Fasciola hepatica
b. Paramphistomum sp.
c. Moniezia sp.
d. Schistosoma s*p.*

65. Which of the following is known as the Threadworm of poultry?

a. Syngamus sp.
b. Oxyspiura sp.
c. Strongyloides sp.
d. Capillaria sp.

66. Which of the following is known as the Hairworm of poultry?

a. Capillaria sp.
b. Syngamus sp.
c. Oxyspiura sp.
d. Strongyloides sp.

67. Prosthogonimus sp. is found in …… of …… and known as ……

a. Liver, Poultry, Liver fluke
b. Oviduct, Cattle, Oviduct fluke
c. Liver, Cattle, Liver fluke
d. Oviduct, Poultry, Oviduct fluke

68. For the Mac master technique, we will require……ml of water in a bowl.

a. 42 ml
b. 45ml
c. 47ml
d. 50ml

69. Biting insects are also known as .....

a. Hemophagous b. Hematophagous

c. Hetophagous d. Heterophagous

70. The percentage of blood in a Cow out of its body weight is.....

a. 8% b. 10%

c. 12% d. 14%

71. The percentage of blood in a Sheep out of its body weight is.....

a. 8% b. 10%

c. 12% d. 14%

72. The percentage of blood in a Goat out of its body weight is.....

a. 6% b. 10%

c. 12% d. 14%

73. The percentage of blood in a Horse out of its body weight is.....

a. 8% b. 10%

c. 12% d. 14%

74. The percentage of blood in a Dog out of its body weight is .....

a. 6% b. 7%

c. 8% d. 9%

Write short answer to the following questions.

1. Write the method of fecal examination.
2. Enlist five external and internal parasites.
3. How do you collect fecal samples from different animal species?
4. Differentiate between normal and abnormal urine.
5. Define Hematuria.
6. Define blood. What are the consitituents of blood?

7. Define WBC and RBC.
8. Differentiated between cyst and Abscess.
9. Write down the importance of fecal examination.
10. Explain fecal sample examination methods in brief.
11. What is skin scrapping test? Explain the flotation method of fecal examination.
12. Write short notes on:

    a. cystocentesis b. catheterization c. free catch (voided sample)
13. Write down the procedure of sedimentation method of fecal examination.
14. List different blood collection tubes with their uses.
15. Give five name of poultry roundworm.
16. Explain the MacMaster technique in details.
17. How do you calculate the blood percentage of different animals species?
18. Which vein is used to collect blood in pig and poultry? Write five component of white blood cell.

Write long answer to the following questions.

1. Briefly describe the different methods of fecal sample collection. Explain the sedimentation method of fecal examination.
2. Define blood. Write about the properties and functions of blood.
3. What is anticoagulants? Give two examples of anticoagulants and write the function of platelets.
4. Briefly describe the different method of fecal sample collection. Write the procedure of simple floatation method.
5. Differentiate between serum and plasma. What is the indication of a skin scrapping test?
6. Write about different methods of urine sample collection.
7. What are the different sites of blood collection in animals? Write the name

and purpose of different tubes used in blood collection.

8. How do you drain an abscess? Write the steps and post-operative care in detail.
9. Write down the term for blood in urine. How do you examine feces in the veterinary laboratory for the detection of eggs of liver fluke? Explain it briefly.
10. How do you diagnosis a pus, cyst and abscess?
11. When in interpret, urine is acidic or alkaline? Please write this question.

Necropsy and Visceral Sampling Procedure

Unit 4

A necropsy, also known as a post mortem examination, is the examination of the body of an animal after its death. The word "necropsy" should not be inter changed or confused with the term "autopsy", which strictly means a human examining the body of another human following death of the latter, and as such does not apply to an animal postmortem. The goal of necropsy examination in veterinary medicine is to provide an analysis of dysfunction at the level of the entire animal or even the herd. Analysis of dysfunction only at the cell, organ or system level is inadequate.

Reasons for Conducting a Necropst

The information obtained from a complete necropsy is used primarily by veterinary clinicians and animal owners but in some instances more generally in society. There are many reasons to conduct a necropsy in veterinary medicine:

1. To determine the cause of death of an animal including the provision of a source of primary or corroborative information in cases of sudden, suspicious, or unexplained death, and to establish an etiology of disease
2. To confirm, clarify or correct a clinical diagnosis and to rule out other disease processes. In this way, the necropsy can serve as quality control for the clinician, monitoring the accuracy of interpretation of clinical signs and ante mortem diagnostic tests. Clarification of clinical signs, especially those that were unexpected or atypical of the disease condition and correlation of clinical signs and pathologic findings is there by provided
3. To increase the accuracy of diagnosis in any of a number of conditions which are very difficult to accurately diagnose clinically

4. To search for and to assess concurrent disease and management problems in order to establish causes of production loss
5. As an information-gathering device in research to assess the effectiveness of medical or surgical therapy, new medical and surgical techniques, and to determine the efficacy and toxicity of therapeutic agents
6. To provide accurate information, which can be used to compile provincial or national records of animal disease, to identify disease trends, to recognize, document and investigate diseases that are new to an area, emerging diseases, or novel disease processes. This is particularly important in veterinary medicine, because changing animal management systems may create entirely new diseases, or new opportunities for existing pathogens
7. As a method for education of veterinary students, animal health technicians and those who may need to deal with animal disease such as wildlife officers
8. To obtain forensic or legal information
9. To identify emerging diseases
10. To monitor the influence of environmental factors on physiologic processes
11. To serve as an indicator of the presence of zoonotic diseases. Very similar reasons are given for conducting autopsies in human medicine

4.1 Identify Materials Required for Necropsy

Necropsy

It is simply an examination of an animal after death. The term 'Necropsy' has been used to refer to the post-mortem examination of an animal species. In human patients, the post-mortem examination is denoted by the term 'Autopsy'. Necropsy is done to determine the cause of death or diagnose the disease that has caused the death. Necropsy involves careful examination through dissection, observation, interpretation, and documentation. Good knowledge of normal anatomy is a must for performing Necropsy. The color, shape, size, and consistency of the affected organ are noted with reports and photographs. The

diagnosis of the cause of death is done along with Serology, Histopathology, and Microbiology.

Importance of Necropsy

1. It contributes to our knowledge by understanding anatomy and physiology.
2. It helps in the diagnosis of disease.
3. It alerts us about diseases that are transmissible to humans and animals.
4. Necropsy serves as a legal function for the investigation of Animal cruelty, and violence.
5. It prevents the spread of disease and economic loss.

Materials Required for Necropsy

Necropsies are high-risk procedures because of potential contact with infectious body fluids, aerosols, and contaminated sharps. Personal protective equipment is a must to wear by the personnel performing Necropsy. This includes:

1. Protective outerwear (Lab coat or Scrubs)
2. Disposable gloves
3. Protective eyeglasses/goggles or a full-face shield
4. Cut-proof gloves (machine washable)
5. Face masks

The Necropsy kit designated for both small and large animals consists:

1. Mayo Scissors: It is used to cut tissues during the dissection process.
2. Bowel scissors: It is used for blunt dissection and cutting delicate tissues.
3. Dissection scissors: It is used to cut open body tissues.
4. Boning Knife Sharp: It is used to remove Muscles from bone or joints.
5. Skinning Knife curved: It is used to remove the skin of the carcass.
6. Autopsy knife curved: It is used in dissection.
7. Stainless chopper: It is also used in the dissection of tissue.

8. Bone saw: It is used to cut through a knife.
9. Postmortem Hammer: It is used to cut and divide the bones.
10. Knife sharpener: It is used to sharpen the knife.
11. Scalpel & Scalpel handle: It is used to dissect the tissue.
12. Measuring Tape 2 meters: It is used to measure the size of various organs.
13. Rib cutter: It is used to cut ribs.
14. Brain knife: It is used to slice thin sections of the brain during post-mortem.
15. Bone cutting Forceps: It is used to cut bones.
16. Bone chisel: It is also used to divide and cut bones during necropsy.

COMMON SURGICAL INSTRUMENTS

The operating room contains a multitude of instruments fit for accomplishing a number of procedures. Note that this is not an exhaustive list of instruments, but rather some that you will encounter frequently.

SCALPEL

Used for initial incision and cutting tissue. Consists of a blade and a handle. Surgeons often refer to the instrument by its blade number.

#10 Blade: Used primarily for making large skin incisions, e.g., in laparotomy.

#11 Blade: Used for making precise or sharply angled incisions.

#15 Blade: Smaller version of #10 blade used for making finer incisions.

SCISSORS

Used for cutting tissue, suture, or for dissection. Scissors can be straight or curved, and may be used for cutting heavy or finer structures.

Mayo Scissors: Heavy scissors available in multiple varieties. Straight scissors are used for cutting suture ("suture scissors"), while curved scissors are used for cutting heavy tissue (e.g., fascia).

Metzenbaum Scissors: Lighter scissors used for cutting delicate tissue (e.g., heart) and for blunt dissection. Also called "Metz" in practice.

Pott's Scissors: Fine scissors used for creating incisions in blood vessels.

Iris Scissors: Used for fine dissection and cutting fine suture. Originally for ophthalmic procedures, but now serves multipurpose role.

FORCEPS

Also known as non-locking forceps, grasping forceps, thumb forceps, or pick-ups. Used for grasping tissue or objects. Can be toothed (serrated) or non-toothed at the tip.

Tissue Forceps: Non-toothed forceps used for fine handling of tissue and traction during dissection.

Adson Forceps: Forceps toothed at the tip used for handling dense tissue, such as in skin closures.

Bonney Forceps: Heavy forceps used for holding thick tissue (e.g., fascial closure).

DeBakey Forceps: Used for atraumatic tissue grasping during dissection.

Russian Forceps: Used for atraumatic tissue grasping during dissection.

CLAMPS

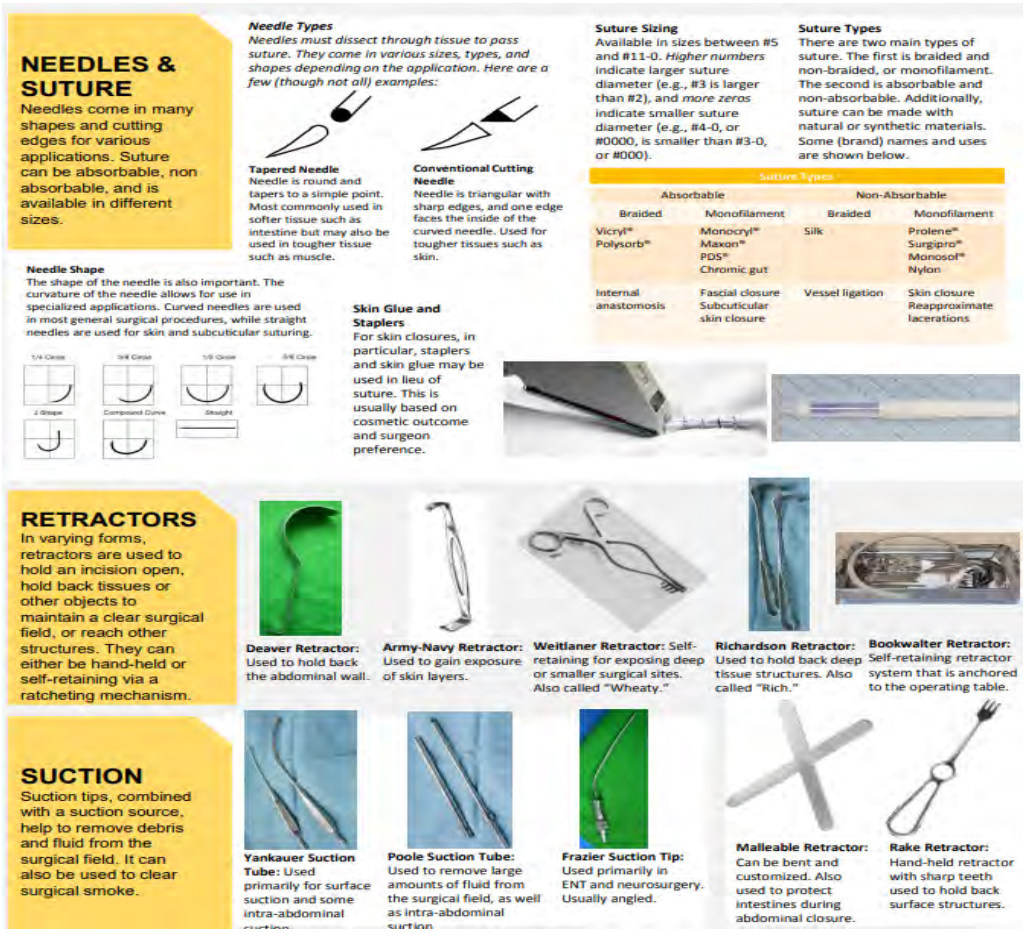
Also called locking forceps, these are ratcheted instruments used to hold tissue or objects, or provide hemostasis. Can be traumatic or atraumatic.

Crile Hemostat: aka "snap," atraumatic and non-toothed clamp used to grasp tissue or vessels that will be tied off. Also used in blunt dissection.

Kelly Clamp: Larger size variation of hemostat with similar function for grasping larger tissues or vessels.

Kocher Clamp: Traumatic toothed clamp used to hold tissue that will be removed.

Allis and Babcock Clamps: Slightly rounded jaws, both are used for grasping intestine.



4.2 Different organ Sample for Different Disease Diagnosis

Different Samples for Bacterial Disease Diagnosis

Disease/Condition	Causative agent	Sample type
Actinomycosis (Bacterial)	*Actinomyces bovis*	Swab of pus/exudates from the lesion
Anthrax (Bacterial)	*Bacillus anthracis*	Post-mortem blood from Ear or jugular vein Note: It is best not to open the carcass of anthrax suspected animal.

Black quarter	*Clostridium chauvoei*	Affected tissue/ Muscle sample (Muscles of the hind leg, hip, back neck, shoulder, etc)
Botulism	*Clostridium botulinum*	Serum, Wound/Tissue stool, Vomitus, Food etc
Brucellosis	*Brucella abortus, B. melitensis, B. canis, B. suis*	Placenta, Fetus stomach, Fetus lungs, Milk, Serum
Chronic Respiratory Disease (CRD)	*Mycoplasma gallisepticum*	Serum, Swabs from the sinus trachea, air sacs, lungs, or conjunctiva
Colibacillosis	*Escherichia coli*	Blood from the heart, liver, spleen, Bursal swab, Air sac
Coryza	*Avibacterium paragallinarum*	Nasal swabs, Oropharyngeal swabs, Blood, Serum, Exudates
Enterotoxaemia	*Clostridium perfringes Type A, B, C, D, E, F*	Blood Feces, Small intestine with its contents
Foot rot	*Fusobacterium necrophorum, Spherophorus necrophorum*	Pus, Scrappings taken from the lesion
Fowl cholera	*Pasteurella multocida*	Blood, Liver
Fowl typhoid	*Salmonella galllinarum*	Blood, Liver
Glanders	*Actinobacillus mallei*	Serum, Section of lesion
Hemorrhagic septicemia	*Pasteurella multocida*	AM : Blood smear from the lip of the ear PM : Smear from heart, Spleen
Johne’s Disease	*Mycobacterium paratuberculosis*	Rectum , Part of Small Intestine

Leptospirosis	*Leptospira sp.*	AM : Blood, Milk, Vaginal discharge PM : Placenta, Liver and stomach of an aborted fetus
Listeriosis	*Listeria monocytogens*	Blood, Cerebrospinal fluid (CSF)
Mastitis	*Escherichia coli, Staphylococcus, Salmonella sp, Kelbsiella, etc.*	Milk, Pus
Pullorum disease	*Salmonella pullorum*	Blood, Liver
Strangles	*Streptococcus equi*	Nasal swabs or washes
Tetanus	*Clostridium tetani*	AM : Smear from the wound PM : Muscle, Spinal cord, Brain
Tuberculosis	*Mycobacterium bovis*	AM : Sputum, Milk, Feces PM : Lymph node

Different Samples for Viral Disease

Disease/Condition	Causative agent	Sample type
African swine fever	*Asfivirus*	Blood, Kidney, Spleen, Lymph node and tonsils
Avian Leucosis Complex	*Retrovirus*	Blood, Serum, Tumors
Bird flu	*Avian influenza type A*	Oropharyngeal and Cloacal swabs, Blood, Serum
Blue tongue	*Reovirus*	Blood, Serum
Bovine Viral Diarrhoea (BVD)	*Paramyxo virus*	Blood, Serum, Nasal and ocular swab
Canine Parvovirus	*Parvovirus*	Feces, Blood
Classical swine fever (CSF)	*Pestivirus*	Whole blood, Serum, Tissues like Tonsils, Lymph nodes, Spleen, Kidney and intestine

Foot and Mouth Diseases (FMD)	*Aphthovirus*	Serum, Vesicular epithelium, Vesicular fluid, Oropharyngeal fluid or swab
Gumboro	*Birnavirus*	Serum, Cloacal bursa lesion
Marek' disease	*Herpes virus*	Dead bird
Pestis des Petits Ruminants (PPR)	*Paramyxo virus*	Swabs of nasal and ocular discharrges, Tonsils, Ileum and Large intestine
Pox	*Pox virus*	Scabs, Vesicular fluids
Rabies	*Rhabdovirus*	Brain
Ranikhet	*Paramyxo virus*	Blood, Nasal and Ocular swabs
Rinderpest	*Paramyxo virus*	Blood
Vesicular Stomatitis	*Rhabdovirus*	Vesicular fluid

Samples of other Important Diseases

Disease/Condition	Causative agent	Sample type
Aflatoxicosis (Mycotoxin)	*Aspergillus flavus Aspergilllus parasiticus*	Animal feed sample
Anaplasmosis (Rickettsial)	*Anaplasma marginale*	Blood/Serum
Coccidiosis (Protozoal)	*Eimeria sp.*	Fecal sample, Intestinal scraping
Diabetes mellitus	Metabolic disorder	Blood, Urine
Ketosis	Metabolic disorder	Urine
Mineral deficiency	Deficiency disease	Blood
Poisoning	Different poison	Feed, Gastric content, Vomitus, Blood , Serum
Skin disease	*Demodex, Scabies, Sarcoptes etc*	Skin scrapping
Vitamin deficiency	Deficiency disease	Feed/Blood

Type of Media

To study bacteria and other microorganisms, it is necessary to grow them in controlled conditions. Microbes are grown in substances that provide the nutrients necessary to sustain their metabolic activities and reproduction called growth media or simply media. Growth media can be either liquid, solid or semi-sold.

1. Liquid media (diffuse growth) is called a broth. Broths can be used to determine growth patterns

   In a liquid medium, and for certain types of inoculations and metabolic tests. They are also the method of choice for growing large quantities of bacteria, bacteria grow producing general turbidity.

   Disadvantages: It does not provide a pure culture from mixed inoculum and Identification of bacteria is not possible.

2. Solid media (discrete colonies) usually contains agar, which is a mixture of polysaccharides Derived from red algae. It is used as a solidification agent because

   a. It is not broken down by Bacteria

   b. Contains no nutrients that can be used by bacteria

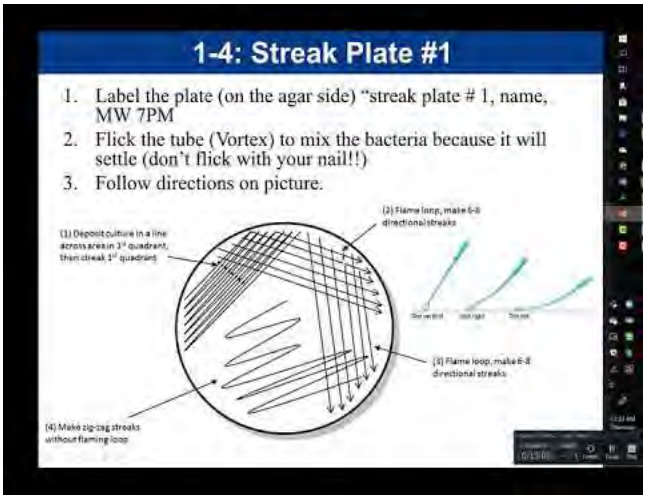
   c. Melts at high temperatures, and yet is solid at temperatures used for most bacterial growth.

      Solid growth media are used in the following forms: agar plates, agar slants and agar deeps. Melted agar is poured into a test tube and then allowed to solidify vertically for an agar deep, or at an angle for an agar slant. Agar plates are made by pouring melted agar into a petri dish.

3- Semi-solid media (motility medium): These media are useful in demonstrating bacterial motility and separating motile from non-motile strain.

Method of Bacterial Culture in the Laboratory

1. Streak Culture (Surface Plating)

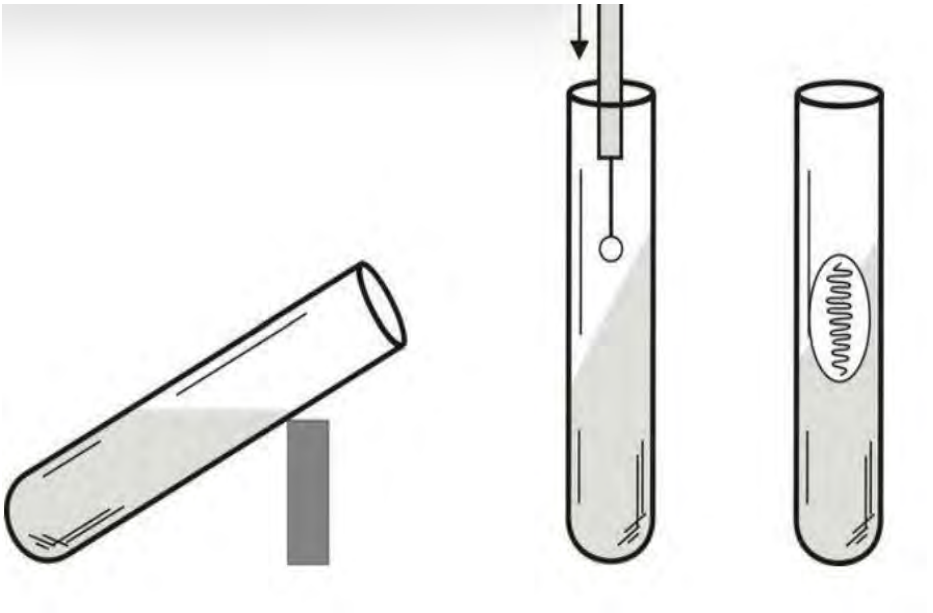


In this method, a microbial inoculum is transferred to the edge of an agar plate with an inoculating loop or swab and then streaked out over the surface in one of several patterns. This method is routinely employed for the isolation of bacteria in pure culture from clinical specimens. A platinum loop is charged with the specimen to be cultured. Owing to the high cost of platinum, loops for routine work are made of nichrome resistance wire. The loop is flat, circular, and completely closed with a 2-4 mm internal diameter mounted on a handle. One loopful of the specimen is smear thoroughly over an area, on the surface of a well-dried plate, to give a well inoculum or 'well'. The loop is re-sterilized and drawn from the well in two or three parallel lines onto the fresh surface of the medium. This process is repeated several times. At each step, the inoculum is derived from the most distal part of the immediately preceding strokes. Plates are incubated in the inverted position with the lid underneath. On incubation, growth may be confluent at the site of original inoculation (well) but becomes progressively thinner, and well-separated colonies are obtained over the final series of streaks.

2. Stroke Culture

Stroke culture is made in tubes containing agar slope or slant. Slopes are seeded by lightly smearing the surface of agar with a loop in a zigzag pattern taking care not to cut the agar. It is employed for providing pure growth of the bacterium for

slide agglutination and other diagnostic tests.



3. Stab Culture

In the preparation of the stab cultures, a suitable medium such as nutrient gelatin or glucose agar is punctured with a long, straight, charged wire into the center of the medium and withdrawing in the same line to avoid splitting the medium. The medium is allowed to set, with the tube in the upright position, providing a flat surface at the top of the medium.

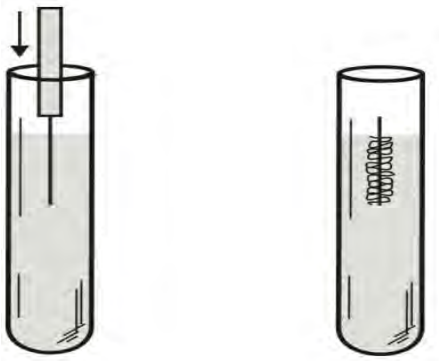
Uses

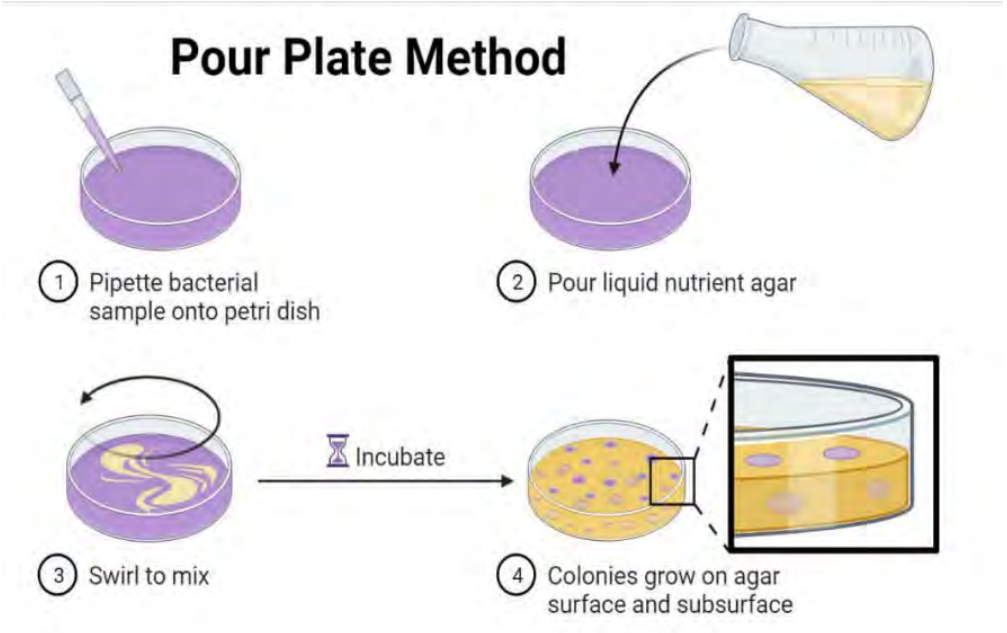
- Mainly for demonstration of gelatin liquefaction
- Demonstration of oxygen requirement of the bacterium under study
- For the maintenance of stock cultures
- To study the motility of bacteria in semisolid agar

3. Pour-Plate Culture

In the pour plate, the original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies after plating. This

method is used for counting the number of living bacteria or groups of bacteria in liquid culture or suspension. A measured amount of the suspension is mixed with molten agar medium in a Petri dish. Either 1.0 ml or 0.1 ml of dilutions of the bacterial suspension are introduced into a Petri dish. The nutrient medium, in which the agar is kept liquid by holding it in a water bath at 45-50°C, is poured over the sample, which is then mixed into the medium by gentle agitation of the plate. When the agar solidifies, the plate is incubated inverted at 37°C for 48 hours, or most suitable for the species examined. After incubation, colonies will grow within the nutrient agar (from cells suspended in the nutrient medium as the agar solidifies) as well as on the surface of the agar plate and can be enumerated using colony counters. Uses for: Gives an estimate of the viable bacterial count in a suspension & the recommended method for quantitative urine cultures.

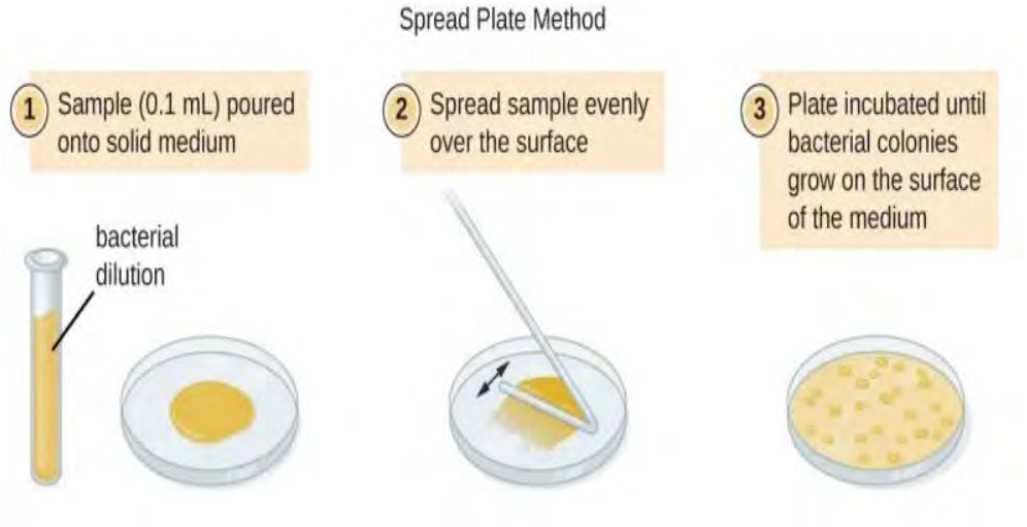




4. Spread Plate Culture

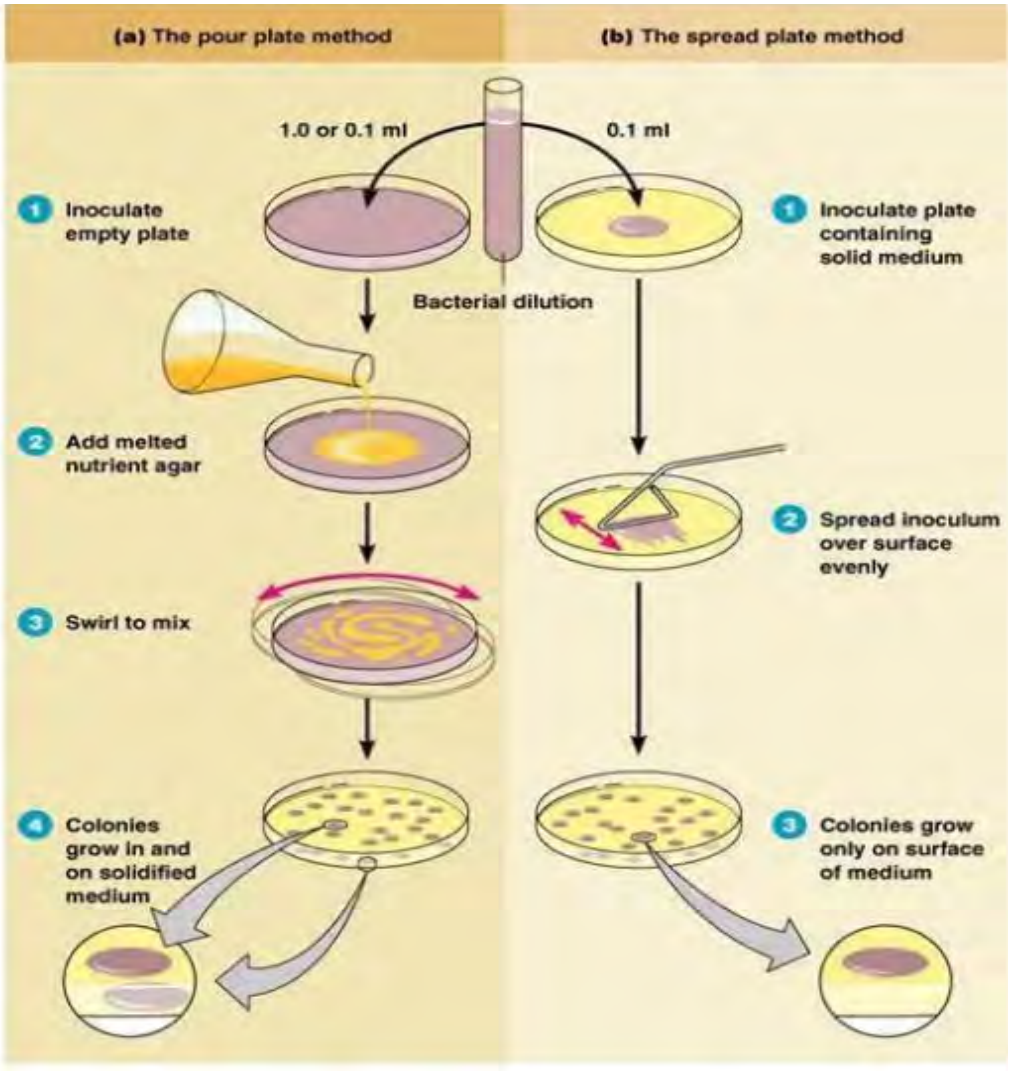
The spread plate method is a microbiological laboratory technique for isolating and counting the viable microorganisms present in a liquid sample by spreading a certain volume of the sample, is transferred to the center of an agar plate and spread evenly over the surface with a sterile bent-glass rod. The spread plate technique is a viable counting method employed to plate a liquid sample to isolate or count the bacteria in that sample. A perfect spread plate technique will result in visible and countable colonies of bacteria evenly distributed on the plate.

Uses: the spread plate technique can be performed quantitatively to determine the number of bacteria present in a sample. The spread plate technique most commonly applied for microbial testing of foods or any other samples or to isolate and identify a variety of microbial flora present in the environmental samples.



Benefits compared to pour plate technique

1. Only surface colonies develop
2. The organisms are not required to withstand the temperature of liquid agar



Figure……..

Adapted from ……

4.4 Milk Sampling and California Mastitis Test (CMT)

Collection and Preparation of Milk Samples for Microbiological Culturing

In developing individual farm mastitis control and treatment strategies, it is often necessary to characterize the types of bacteria that are present on your

farm. To answer this question, a microbiological analysis, or milk culture, must be performed on milk samples collected from cows showing clinical signs of mastitis or with elevated somatic cell counts. Results of the milk cultures will help identify which bacteria are causing the mastitis. In turn, this information can be used to alter mastitis control, prevention, and treatment options to fit your herd's conditions. During an investigation of a herd dealing with high somatic cell counts or a high incidence of clinical mastitis, milk culture results provide essential evidence for solving the problem. When managing a contagious mastitis problem (*Staphylococcus aureus* or *Streptococcus agalactiae*), milk cultures are even more important to help make individual cow treatment and culling decisions. Extra care and precaution are necessary during the collection process, using strict, clean, aseptic (without germs and bacteria) procedures to be sure that the bacteria originated from milk from the udder and not the teat end or hair, the sampler's hands, or the barn environment. If the samples are not collected, handled, and transported correctly, the bacteriological results will be of no diagnostic value.

Milk Sample Collection

Milk samples must be collected before the cow is treated with antibiotics. Samples for culture should be collected immediately before milking. When individual quarters show clinical signs of mastitis or positive California Mastitis Test results, individual samples should be collected from the affected quarters. If the entire herd is being sampled, composite samples (all four quarters in one collection vial) will provide reasonable results. To minimize contamination and maximize the chances of receiving useful information from the milk culturing process, adhere to the guidelines for the aseptic collection of clean milk. The steps for collection of Clean Milk Samples are:

1. Wear gloves
2. Remove (fore strip) three or four streams of milk from the quarters being sampled to minimize chances of sample contamination from bacteria in

the teat end.

3. Brush any dirt, debris, or bedding particles from the udder and teats. Use baby sanitary wipes to clean the tits.
4. Dry each tit thoroughly by using a single, dry paper or cloth towel per cow with particular emphasis on the teat end.
5. Double check to ensure that the teats and udder are clean and dry.
6. Collect milk in a 0.5-liter jug. Hold the collection jug at a 45° angle to keep debris (hair, manure, dirt) from accidentally falling into the jug. Turn the teat toward the jug, striving for direct streams of milk into the jug. The teat should never touch the jug. Sample as rapidly as possible, starting with the teats on the near side of the udder followed by the teats on the far side of the udder.
7. Combine the milk from the different teats.
8. Collect 100ml of milk.
9. Label the sample vials using a waterproof marker that will not come off during transport to the laboratory.
10. Fill the two 50ml tubes and mix by turning upside down 10 times.
11. Immediately place collection vial on ice and keep refrigerated or on ice until delivered to the lab.

Best results are obtained if samples are chilled or placed on ice during transport to the laboratory.

Figure: Collecting Clean Milk Samples

California Mastitis Test (CMT)

Mastitis continues to be one of the most costly problems in many dairy farms. Mastitis can manifest itself in either clinical or subclinical or Chronic form. Clinical mastitis is when milk appears abnormal with the presence of flakes,

clots, strings or watery. The mammary gland also may be warm or hard to the touch and may exhibit increased sensitivity. In severe cases, systemic signs may be apparent, such as, fever, cow off feed, and in shock.

Subclinical mastitis occurs when both milk and mammary gland appear normal but Somatic Cell Counts (SCC) are elevated to a level above 200,000 cells/ml.

Somatic cells are basically white blood cells (leukocytes) that migrate to the mammary gland in response to infection in both clinical and subclinical cases. This cell migration to the mammary gland is part of the inflammatory response to bacterial infection in the udder.

Cows that do not have mammary infections normally have SCC less than 200,000 cells/ml.

The CMT is performed to detect the presence of subclinical infections at the beginning of or during lactation as part of an udder health management program.

The California Mastitis Test (CMT) is a cow-side test that allows **farmers or veterinarians** to assess the**Somatic Cell Count (SCC)** of each quarter of a cow's mammary gland.

The test is very simple, can be performed at milking time, gives instant results and is economical.

CMT Principle

- CMT reagent (sodium lauryl sulphate) reacts and rupture leukocytes (WBCs) and thus deoxyribonucleic acid (DNA) is released from their nuclei, which result in gel formation. Thickness of gel indicates the severity of inflammation.
- The CMT will only trigger a visible reaction with a concentration of 400,000 cells/ml or more.
- The degree of gelling indicates the presence and severity of mastitis. The change in colour indicates the pH variation of the milk and therefore, the level of inflammation.

Procedure of CMT Test

Materials Required

a. CMT paddle
b. Four-compartment paddle with one compartment used per quarter
c. CMT reagent (3% sodium lauryl sulphate containing 1:10000 bromocresol purple pH 7.0 to 7.5)

Procedure of CMT Test

Step 1

Take about 2-3 ml milk from each quarter in each paddle compartment, after foremilk is removed.

Step 2

Add CMT solution to each cup in the paddle. CMT reagent is added to each compartment in volume equal to the milk quantity. The milk reagent mixture is swirled in a circular motion with presence of gel or slime being, recorded for each quarter.

Step 3

Rotate the CMT Paddle in a circular motion to thoroughly, mix the contents. Do not mix more than 10 seconds.

Step 4

Read the test quickly. Visible reaction disintegrates after about 20 seconds. The reaction is scored visually. The more gel formation, the higher the score.

CMT score

CMT score	Score	Total cell count	Visible reaction	Interpretation
O	Negative (-)	0-200,000 (0-25% neutrophils)	Normal milk fluid	Healthy Quarter

T	Trace (+)	200,000-400,000 (30-40% neutrophils)	Slight precipitation	Subclinical Mastitis
1	Weak positive (++)	400,000- 1200000/ 1,500,000 (40-60% neutrophils)	Distinct precipitation (thickening) but no gel formation	Subclinical Mastitis
2	Distinct positive (+++)	1,500,000-500,000 (60-70% neutrophils)	Mixture thickened immediately and a gel formation	Serious Mastitis Infection
3	Strong positive (++++)	Over 5,000,000	Viscosity greatly increased strong gel that is cohesive with a convex surface.	Serious Mastitis Infection

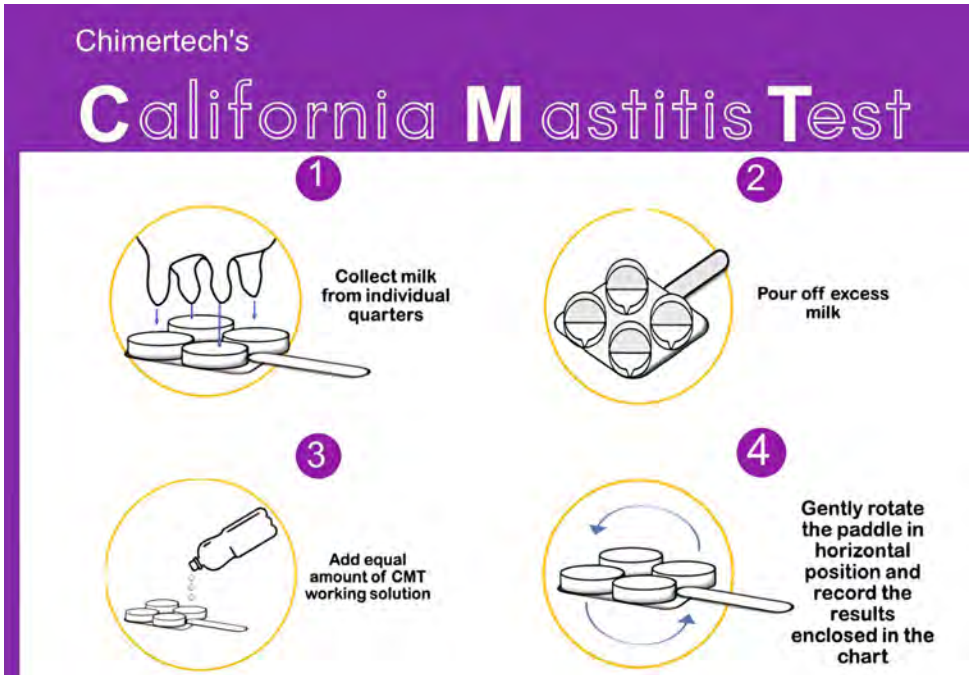


Figure source: https://www.amazon.in/CMT-Kit-California-Subclinicald Visceral Sampling Procedure

Exercise

Choose the correct answer from the given alternatives.

1. Somaticcellsoccurringinlargenumbersinmilkindicate_____.
   a. A cow has mastitis
   b. Cows consume too much protein
   c. Milk machines are dry
   d. Poor cooling of the milk in the bulk tank

2. The CMT test detects_____ in raw milk
   a. Bacteria
   b. Red blood cells
   c. Somatic cells
   d. Antibiotics

3. The sample taken for Fowl typhoid in poultry is…….
   a. Spleen
   b. Bursa
   c. Kidney
   d. Liver

4. Which of the following is a viral disease?
   a. Tetanus
   b. Black quarter
   c. Collibacillosis
   d. Rabies

5. In the Black quarter, the sample taken is……
   a. Muscle of affected organ
   b. Liver
   c. Bone of affected organ
   d. Nasal swab

6. Which of the following is a metabolic disease?
   a. Rabies
   b. Diabetes Mellitus
   c. Ketosis
   d. Both b and c

7. Skin disease is caused due to .....
   a. Demodex
   b. Scabies
   c. Psoroptes
   d. All of the above

8. Pus contains.....

a. Bacteria b. Tissue debris

c. Red blood cells d. All of the above

9. The examination of an animal after death is known as.....

a. Necropsy b. Biopsy

c. Autopsy d. Both a and c

10. The examination of humans after death is known as.....

a. Necropsy b. Biopsy

c. Autopsy d. Both a & c

11. Necropsy is important because.....

a. It contributes to knowledge of physiology and anatomy

b. It helps in the diagnosis of disease

c. It serves as a legal function for investigation

d. All of the above

12. Which of the following is a viral disease?

a. Brucellosis b. Rabies

c. Black quarter d. Botulism

13. Which of the following is a disease caused by a toxin?

a. Anthrax b. Botulism

c. Chronic respiratory disease d. Coryza

14. Foot and Mouth disease is a ……. disease.

a. Bacterial b. Parasitic

c. Viral d. Metabolic

15. In Ketosis, the sample should be taken from……

a. Urine b. Blood c. Vomitus d. Skin

16. Milk is a suitable medium for bacterial growth because it contains.....

a. Protein
b. Carbohydrates
c. Fat
d. All the essential nutrients for growth

17. What is the sample type in Black quarter disease?

a. Swab of pus/exudates
b. Post – mortem blood from Ear/Jugular vein
c. Affected muscle sample
d. Wound/Tissue

18. What is the sample type in Botulism disease?

a. Swab of pus/exudates
b. Post – mortem blood from Ear/Jugular vein
c. Affected muscle sample
d. Vomitus/Food/Serum/Wound

19. What is the sample to be taken for Foot rot disease?

a. Pus
b. Scrappings
c. Lesion
d. Any of the above

20. In Glanders, the sample to be taken is.....

a. Serum
b. Section of lesion
c. Both a and b
d. None of the above

21. The sample to be taken for Bird flu disease is.....

a. Oropharyngeal swab
b. Cloacal swab
c. Blood
d. All of the above

22. The sample to be taken in Classical swine fever is.....

a. Whole blood
b. Serum
c. Tonsil tissues
d. All of the above

23. The sample to be taken for Foot and Mouth disease is.....

a. Serum
b. Vesicular epithelium
c. Lymph node
d. Both a and b

24. The sample to be taken in Rabies is.....

a. Brain sample
b. Wound
c. Saliva
d. All of the above

25. In Aflatoxicosis the sample to be taken is .....

a. Vomitus
b. Mold
c. Serum
d. Animal Feed sample

26. In Demodicosis, the sample should be taken as....

a. Skin wounds
b. Blood
c. Serum
d. Skin scrapings

27. A dog is presented with difficulty in vision and requires to check of the Vitamin A level. How would you take the sample?

a. Feed history
b. Blood
c. Eye examination
d. All of the above

28. Which of the following is not used in Necropsy Examination?

a. Bone saw
b. Brain knife
c. Rib cutter
d. Brain screw

29. During California Mastitis Test, when the surface elevates in the middle due to thick gel formation, then the test is.....

a. Strongly positive
b. Positive
c. Trace
d. Negative

30. The slight formation of gel in the California mastitis test indicates.....

a. Strongly positive
b. Positive
c. Trace
d. Negative

31. Which of the following is true for a carcass that is suspected of Anthrax?

a. Perform a Necropsy of the carcass

b. Take the sample liver and kidney

c. Take a biopsy of the spleen

d. Best not to perform Necropsy

32. The most pathogenic Brucella species found in humans is....

a. *Brucellla abortus* b. *Brucella melitensis*

c. *Brucella canis* d. *Brucella suis*

33. The causative agent of Gumboro disease is ...

a. *Birnavirus* b. *Pestivirus*

c. *Apthovirus* d. *Parvovirus*

34. The causative agent of Foot and Mouth disease is.....

a. *Birnavirus* b. *Pestivirus*

c. *Apthovirus* d. *Parvovirus*

35. The causative agent of Classical swine fever is.....

a. *Birnavirus* b. *Pestivirus*

c. *Apthovirus* d. *Parvovirus*

36. The causative agent of Canine Parvovirus disease is.....

a. *Birnavirus* b. *Pestivirus*

c. *Apthovirus* d. *Parvovirus*

37. The causative agent of Mastitis is....

a. *Escherichia coli* b. *Staphlococcus*

c. *Salmonella sp.* d. *All of the above*

38. The causative agent of Tetanus is ....

a. *Clostridium tetani* b. *Clostridium chauvoei*

c. *Clostridium perfringes* d. *Clostridium botulinum*

39. The causative agent of the Black quarter is.....

a. *Clostridium tetani* b. *Clostridium chauvoei*

c. *Clostridium perfrigens* d. *Clostridium botulinum*

40. The causative agent of Fowl cholera is.....

a. *Pasteurella multocida* b. *Clostridium botulinum*

c. *Salmonella gallinarum* d. *Actinobacillus mallei*

41. The causative agent of Fowl typhoid is.....

a. *Pasteurella multocida* b. *Clostridium botulinum*

c. *Salmonella gallinarum* d. *Actinobacillus mallei*

42. The agar should be heated and cooled at …… before pouring it into Petri dishes.

a. 50°C b. 55°C c. 60°C d. 65°C

43. The incubation of bacterial plates should be done at.....

a. 37°C b. 47°C c. 55°C d. 57°C

44. The inoculation of the culture is done with the help of ……. in Stab culture.

a. Inoculation rod b. Inoculation loop

c. Both a and c d. None of the above

Write short answer to the following questions.

1. How long do we have to rotate paddle in a circular motion in CMT?
2. While taking the sample for Milk culture, we must scrub the teat by
3. How long do we have to wait for a reaction to occur in CMT?
4. The use of Mayo scissors is
5. The use of Skinning knife while performing a Necropsy is
6. The use of Post mortem hammer is
7. Which of the following is not used while Necropsy?

8. What is the sample type in *Actinomycosis* disease?
9. Write name of five organs seen during necropsy.
10. What are the different materials used for a necropsy? Write their function.
11. Enlist 5 bacterial,5 viral and 5 metabolic diseases.
12. Write the method of taking a sample in the following diseases.
13. Write down the indication of mastitis. Write down the interpretation of the California Mastitis Test.

Write long answer to the following questions.

1. What is the importance of Necropsy and Biopsy? Write the procedure of milk sampling.
2. Write different samples to be collected for different bacterial, viral and other important diseases.
3. Enlarged bursa of fibricius is suspected in which disease. Write the detailed procedure in Necropsy.
4. How do you perform CMT? Explain in detail.
5. What are the materials required for Necropsy?
6. What is milk sampling? Write the procedures in details.
7. Which temperature plate culture media should be stored?
8. Write about different organ sample collections in different bacterial, viral, and other important diseases for the diagnosis of their causative agents.
9. What are the methods bacterial culture in the laboratory? Write in brief.
10. Why is postmortem not conducted in suspection of anthrax?

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