TECHNICAL REPORT

STUDENT'S INDUSTRIAL WORK EXPERIENCE SCHEME (SIWES)

AT

FEDERAL COLLEGE OF ANIMAL HEALTH AND PRODUCTION TECHNOLOGY, MOOR PLANTATION, IBADAN.

BY

AJEKIIGBE FUNMILOLA RUTH

MATRIC NUMBER: 21/05BLL003

SUBMITTED TO

THE DEPARTMENT OF BIOLOGICAL SCIENCE

FACULTY OF COMPUTING AND APPLIED SCIENCES

THOMAS ADEWUMI UNIVERSITY, OKO, KWARA STATE.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B. SC) DEGREE IN MICROBIOLOGY

NOVEMBER, 2024.

CERTIFICATION

This is to certify that, the three months students industrial work experience scheme at **FEDERAL COLLEGE OF ANIMAL HEALTH AND PRODUCTION TECHNOLOGY, MOOR PLANTATION, IBADAN** was carried out by **AJEKIIGBE FUNMILOLA RUTH** with matriculation number **21/05BLL003** of the department of Microbiology, **THOMAS ADEWUMI UNIVERSITY, OKO, KWARA STATE**

STUDENT	DATE
SUPERVISOR	DATE
SIWES COORDINATOR	DATE

4

ACKNOWLEDGMENT

My profound gratitude goes to Almighty God, who in his infinite mercies and abundant grace has given me all the protection, grace, courage, strength, knowledge, understanding, divine support, and endurance needed to start and finish my industrial training in spite of all difficulties.

My continuous indebtedness and sincere appreciation go to my beloved parent MR and MRS AJEKIIGBE and my sibling for their undying love, care, prayers and support they gave to me during my industrial training program.

My sincere appreciation goes to my efficient, brilliant, and hardworking lecturers in the department of Microbiology, Thomas Adewumi University, Oko, Kwara State, Nigeria for impacting a lot of knowledge in me. The knowledge acquired from them helped me greatly in my attachment program

I acknowledge the efforts of the Mrs Adetayo and Mrs Mary, my supervisors for adding to my body of knowledge and for the love showed towards me during my industrial training at Federal College of Animal Health and Production Technology.

DEDICATION

This report is dedicated to the Almighty God for the grace and strength given to me for the completion of the report, My Parents, My Friends and colleagues for their great support.

ABSTRACT

This report gives a detailed account of my three (3) months of SIWES carried out at Federal College of Animal Health and Production Technology. The major test carried out in the laboratory includes: the preparing a bacterial smear, making a wet mount, making a dry mount and examination of live bacteria using a microscope is done. Chapter one contains the history and function of SIWES. Chapter two contains the history of Federal College of Animal Health and Production Technology, various units in the department. Chapter three contains experience gained at federal college of animal health and production technology and so on. Chapter four contains the problems encountered, conclusion and recommendations.

TABLE OF CONTENT
CERTIFICATION
ACKNOWLEDGMENT
DEDICATION
TABLE OF CONTENT
BACKGROUND11
STUDENT INDUSTRIAL WORK EXPERIENCE (SIWES)11
OBJECTIVES OF SIWES12
ADMINISTRATIVE STRUCTURE OF FEDERAL COLLEGE OF ANIMAL HEALTH AND PRODUCTION TECHNOLOGY
UNITS/SECTIONS IN FEDERAL COLLEGE OF ANIMAL HEALTH AND PRODUCTION TECHNOLOGY
2.2.1 Introduction To Animal Microbiology Lab17
2.2.2 Objectives of the Animal Microbiology Lab17
2.2.3 Key Areas of Study17
2.2.4 Typical Equipment and Techniques
TECHNIQUES FOR STUDYING BACTERIA CHARACTERISTICS AND BEHAVIORS
3.1.1 Preparing A Bacterial Smear
3.1.2 Making A Wet Mount20
3.1.3 Making A Dry Mount
STAINING TECHNIQUES USED FOR BACTERIAL SAMPLES
3.2.1 Gram Staining
3.2.2 Acid-Fast Staining
3.2.3 Simple Staining
3.2.4 Negative Staining
3.2.5 Endospore Staining
3.2.6 Giemsa Staining
3.2.7 Hematoxylin and Eosin (H&E) Staining
3.2.8 Silver Staining

TECHNIQUES USED FOR STAINING FUNGI	24
3.3.1 Gomori's Methenamine Silver (GMS) Staining	24
3.3.2 Periodic Acid-Schiff (PAS) Staining	24
3.3.3 Calcofluor White Staining	24
3.3.4 Lactophenol Cotton Blue Staining	25
3.3.5 Gram Staining	25
3.3.6 Hematoxylin & Eosin Staining	25
3.3.7 Fluorescent Antibody Staining	25
METHODS FOR SPORE STAINING	26
3.4.1 Schaeffer-Fulton Method	26
3.4.2 Dorner Method	26
3.4.3 Negative Staining	26
3.3.4 Fluorescent Staining	27
TECHNIQUES FOR NUCLEAR STAINING	27
3.5.1 Hematoxylin Staining	27
3.5.2 Giemsa Staining	27
3.5.3 DAPI Staining (4',6-Diamidino-2-phenylindole)	27
3.5.4 Propidium Iodide Staining	28
3.5.5 Toluidine Blue O Staining	28
3.5.6 Feulgen Staining	28
ZIEHL-NEELSEN METHOD	28
HOW TO CONDUCT SELECTIVE STAINING EFFECTIVELY	30
3.7.1 Safety Considerations	31
SECTIONING ANIMAL TISSUE	31
3.8.1 Materials Needed	31
3.8.2 Method of Sectioning Tissue	31
3.8.3 Safety Considerations	32
GUIDELINES FOR THE EFFECTIVE STORAGE OF PREPARED SLIDES	32

CHAPTER	FOUR:	PROBLEMS	ENCOUNTERED,	CONCLUSION	AND
RECOMME	ENDATIC)N		••••••	34
4.1 Problem	ms Encou	ntered			34

4.2 Conclusion	34
4.3 Recommendation	34

CHAPTER ONE

INTRODUCTION TO SIWES PROGRAM BACKGROUND

Industrial training is a program that aims to provide the best practical training in a student's personal career and academic course of study. It converts students' theoretical knowledge into practical experiments within a particular time frame. Industrial training is pre-professional work of experience that offers the student great skills and practical knowledge and encourages their self-confidence. To achieve this, the twelve (12) weeks training program with specific tasks was carried out at Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State. The various test was aimed at studying the various techniques used in culturing, preparation of bacteria smear and introduction to animal microbiology.

STUDENT INDUSTRIAL WORK EXPERIENCE (SIWES)

The Student Industrial Work Experience Scheme (SIWES) was established through the Industrial Training Fund (ITF) as a result of the realization by the Federal Government in 1973, of the need to introduce a new dimension to the quality and standard of university education obtained in the country in order to achieve the much needed technological advancement because it has been shown that a correlation exists between a country's level of economic and technological advancement and it's level of investment in man power development. The scheme exposes students to industrial based skills required for a smooth transition from the lecture room to the outside world. It affords students of tertiary institutions the opportunity of being familiarized and exposed to the needed experience in handling machinery, equipment, and skills that are usually not available in their educational institutions. Participation in SIWES has become an essential pre-condition for the award of Diploma and Degree Certificates in specific disciplines, Food Science and Technology are included, in most institutions of higher learning in the country in accordance with the educational policy of the Nigerian government. The operators and coordinators of this scheme are the; ITF, National University Commission (NUC), National Board for Technical Education (NBTE), employers of labour, and the institutions.

The scheme is funded by the Federal Government of Nigeria. The functions of the bodies mentioned above, among others are as follows:

- i. Ensure adequate funding of the scheme.
- ii. Establish SIWES and accredit units in the approved institutions.
- iii. Formulate policies and guidelines for participating bodies and institutions as well as appointing SIWES
- iv. Coordinators and supporting staff.
- v. Supervise students at their places of attachment, and sign their log books and Industrial Training Fund (ITF) forms.
- vi. Vet and process students' log books and forward them to the ITF area office.
- vii. Ensure payment of allowances to the students and supervisors.

Hence, the success or failure of the Students Industrial Work Experience Scheme (SIWES) depends on the efficiency of the ministries, ITF, Higher institutions; employers of labor, and of course the students partaking and the general public involved in the articulation and management of the program.

OBJECTIVES OF SIWES

The objectives of SIWES are as follows: To provide an avenue for students in higher learning institutions to acquire industrial skills and experience in their respective courses of study.

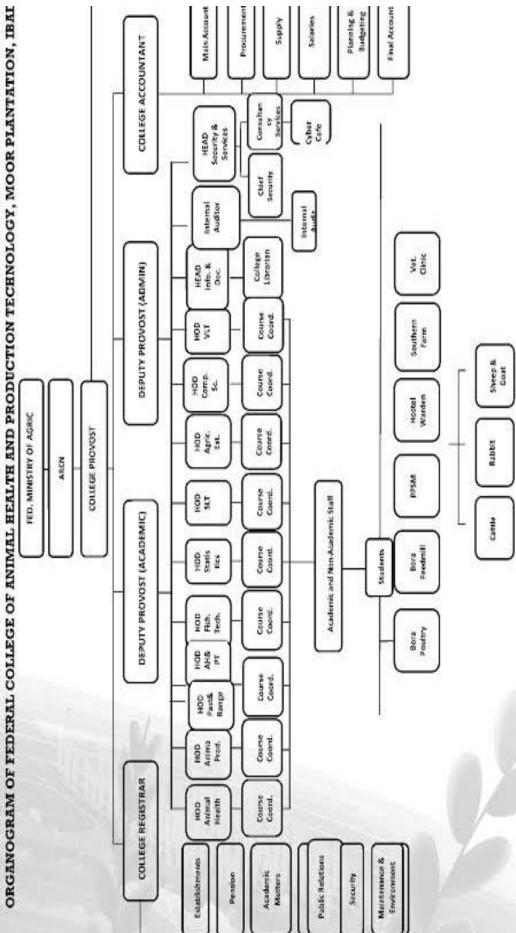
- i. To provide the students with the opportunities to be involved in the practical aspect of their respective disciplines; thus, creating more understanding of the theoretical aspect taught in their lecture rooms.
- To keep students abreast of the latest developments and innovations in their disciplines.
- iii. To expose students to sophisticated machinery they don't have access to in their institutions.
- iv. To prepare students for the likely challenges they will face in the labor market.
- v. To enable students, to make reasonable choices of their fields of specialization.
- vi. Also, to brings students of different institutions, ethnic backgrounds, mentalities, and of course religions under the same umbrella in which they learn to tolerate one another, work together, be of their best behaviours, share ideas, and make good friends with each other, within a very short period.

CHAPTER TWO

BRIEF HISTOIRY OF FEDERAL COLLEGE OF ANIMAL HEALTH AND PRODUCTION TECHNOLOGY

The Federal College of Animal Health and Production Technology (FCAHPT) in Moor Plantation, Ibadan, Nigeria was established in 1964. The college was founded to train Livestock Assistants to meet the growing demand for veterinary assistance. In 1970, the college became part of the Institute of Agricultural Research and Training (IAR&T) of Obafemi Awolowo University, Ile-Ife. In 1985, the National Board for Technical Education (NBTE) was given the power to accredit the college's programs. The college's mission is to improve expertise in animal health, production, and technology. It's a hub for education and research in these areas, and its community of learners, researchers, and practitioners work together to address challenges in the animal health, production, and technological sectors. In 2024, the college celebrated its 60th anniversary. The celebrations included the commissioning of a new central building, a medical center, a block of classrooms, and an office complex.

ADMINISTRATIVE STRUCTURE OF FEDERAL COLLEGE OF ANIMAL HEALTH AND PRODUCTION TECHNOLOGY



UNITS/SECTIONS IN FEDERAL COLLEGE OF ANIMAL HEALTH AND PRODUCTION TECHNOLOGY

Presently, there are many units at Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan , Oyo State, where I carried out my industrial training program. I was opportune to work in Animal Microbiology Lab in relative of it's importance to my Course of study, Microbiology.

2.2.1 Introduction To Animal Microbiology Lab

Animal microbiology is a branch of microbiology that focuses on the study of microorganisms associated with animals, including their roles in health, disease, and the environment. An animal microbiology lab provides a controlled environment for investigating these interactions, helping researchers understand the complex relationships between animals and microbes.

2.2.2 Objectives of the Animal Microbiology Lab

- i. Understanding Microbial Diversity: Investigating the various microorganisms found in different animal species, including bacteria, viruses, fungi, and parasites.
- ii. Studying Host-Microbe Interactions: Exploring how microbes affect animal health, including beneficial roles in digestion, immunity, and disease pathogenesis.
- iii. Disease Diagnosis: Developing techniques for isolating and identifying pathogens responsible for animal diseases.
- iv. Antimicrobial Resistance: Investigating resistance patterns in animal-associated pathogens, which can impact both animal and human health.
- v. Biotechnology Applications: Applying microbiological knowledge to improve animal health, nutrition, and production through probiotics, vaccines, and other interventions.

2.2.3 Key Areas of Study

- i. Microbial Ecology: Examining the composition and function of microbial communities in the gut, skin, and other habitats of animals.
- ii. Pathogen Identification: Utilizing culture techniques, molecular methods, and biochemical tests to identify pathogenic microorganisms in diseased animals.

- iii. Vaccination and Immunology: Studying how vaccines can be developed to protect animals from infectious diseases.
- iv. Antibiotic Use and Resistance: Investigating the effects of antibiotics in veterinary medicine and the emergence of resistant strains.

2.2.4 Typical Equipment and Techniques

- i. Culturing Equipment
- Incubators: For growing bacterial cultures at optimal temperatures.
- Autoclaves: For sterilizing media and instruments.
- ii. Microscopy
- Light Microscopes: For observing microorganisms and their morphology.
- Electron Microscopes: For studying viruses and other fine structures.
- iii. Molecular Biology Tools
- PCR Machines: For amplifying DNA from microbial samples
- Gel Electrophoresis: For analyzing DNA and RNA.
- iv. Biochemical Tests
- Various assays to determine microbial characteristics, such as enzyme production and metabolic pathways.



MICROBIOLOGY LABORATORY EQUIPMENT

EQUIPMENTS	FUNCTION
AUTOCLAVE	 Neutralize or decontaminates biological waste and sterilize media, instruments, and lab wares.
BACTERIAL INCUBATOR	 a medical device designed with heating temperature for storage of bacterial plate and growth of bacterial culture.
HOT AIR OVEN	 sterilized equipment and other materials in the laboratory by using dry heat.
CENTRIFUGE	 used to separate particles suspended in a liquid based on particle size and density, rotor speed, etc.
REFRIGERATOR	 cools samples and specimens for preservation.
TRIPLE BEAM BALANCE	 a weighing device to accurately gauge the mass of an object in grams.

CHAPTER THREE

EXPERIENCE GAINED AT FEDERAL COLLEGE OF ANIMAL HEALTH AND PRODUCTION TECHNOLOGY

TECHNIQUES FOR STUDYING BACTERIA CHARACTERISTICS AND BEHAVIORS

3.1.1 PREPARING A BACTERIAL SMEAR

Materials Needed

- i. Clean glass microscope slides
- ii. Sterile inoculating loop or swab
- iii. Bacterial culture (broth or solid)
- iv. Bunsen burner (for sterilization)
- v. Staining reagents (if applicable)

Procedures

- i. Sterilize the Loop by heating the inoculating loop in a Bunsen burner until it's red hot. Let it cool.
- ii. Inoculate the Slide using a broth culture, dip the cooled loop into the broth and place a small drop on the center of the slide. For solid culture, touch the loop to the colony and then transfer to the slide.
- iii. Spread the Sample using the loop to spread the drop into a thin film over the slide's surface.
- iv. Allow the smear to air dry completely at room temperature.

3.1.2 MAKING A WET MOUNT

Materials Needed

- i. Clean microscope slides
- ii. Cover slips
- iii. Liquid sample (e.g., broth culture, water with organisms)
- iv. Dropper or pipette

Procedures

- i. Place a Drop using a dropper, place a small drop of the liquid sample on the center of the slide.
- ii. Gently place a cover slip over the drop at an angle to avoid air bubbles.
- iii. Observe under a microscope, focusing on live organisms.

3.1.3 MAKING A DRY MOUNT

Materials Needed

- i. Clean microscope slides
- ii. Cover slips (optional)
- iii. Specimens (e.g., dry plant material, insect parts)

Procedures

- i. Position the specimen in the center of the slide.
- ii. Carefully place a cover slip over the specimen.
- iii. Observe under a microscope.

3.1.4 EXAMINATION OF LIVE BACTERIA

Materials Needed

- i. Prepare a wet mount slide or bacterial smear
- ii. Microscope
- iii. Staining reagents (if needed for contrast)

Procedures

- i. Set up the Microscope by adjusting the light source and stage for optimal viewing.
- ii. Start with the lowest power objective to locate the sample, then switch to higher magnifications for detailed observation.
- iii. Note the morphology (shape, arrangement) and any motility observed.

STAINING TECHNIQUES USED FOR BACTERIAL SAMPLES 3.2.1 Gram Staining

This is one of the most widely used techniques to differentiate bacterial species based on the properties of their cell walls.

Procedure

Make a bacterial smear on a glass slide and air dry. Heat-fix the slide by passing it through a flame. Apply crystal violet stain for about 1 minute, then rinse with water. Add iodine solution for another minute, which acts as a mordant. Rinse with water. Carefully apply 95% ethanol or acetone until the runoff is clear. Rinse immediately with water. Apply safranin for about 30 seconds, then rinse with water and air dry. Observe under a microscope. The result shows that Gram-positive bacteria will appear purple, while Gram-negative bacteria will appear pink.

3.2.2 Acid-Fast Staining

Used primarily for identifying mycobacteria (e.g., Mycobacterium tuberculosis).

Procedure

Prepare and heat-fix a bacterial smear. Stain with carbol fuchsin and heat gently for 5 minutes. This helps penetrate the waxy cell wall. Rinse with water. Rinse with acidalcohol (3% HCl in ethanol) until no more color runs off. Rinse with water. Apply methylene blue for about 30 seconds, then rinse and air dry. The result shows that Acid-fast bacteria will appear red, while non-acid-fast bacteria will appear blue.

3.2.3 Simple Staining

A straightforward method to observe cell shape, arrangement, and size.

Procedure

Prepare a bacterial smear and heat-fix. Apply a single stain (e.g., methylene blue, crystal violet, or safranin) for about 1 minute. Rinse with water and air dry. Observe under a microscope.

3.2.4 Negative Staining

This technique is used to visualize the morphology of bacteria without heat-fixing, preserving their natural shape.

Procedure

Place a drop of nigrosin or India ink on a slide using a sterile loop, mix a small amount of the bacterial culture into the dye. Use another slide to spread the mixture evenly. Allow the slide to air dry without rinsing. The result shows that the background will be dark, and the bacteria will appear as clear areas against it.

3.2.5 Endospore Staining

This technique is used to identify bacterial endospores.

Procedure

Prepare and heat-fix a bacterial smear. Stain with malachite green and steam for about 5 minutes to allow penetration. Rinse with water. Apply safranin for about 30 seconds, then rinse and air dry. The result shows that Endospores will appear green, while the vegetative cells will be pink.

3.2.6 Giemsa Staining

This technique differentiates cells and microorganisms based on their morphology and staining characteristics.

Procedure

Fix cells to the slide. Apply Giemsa stain for 20-30 minutes. Gently rinse with buffer. Stains cellular components; useful in identifying parasites and blood cells.

3.2.7 Hematoxylin and Eosin (H&E) Staining

It is commonly used in histology to differentiate tissue structures.

Procedure

Apply nuclei blue stains for 5-10 minutes. Apply cytoplasm pink stain for 30 seconds to 1 minute. Rinse and process for microscopy. The result shows that Nuclei appear blue; cytoplasm and extracellular matrix appear pink.

3.2.8 Silver Staining

Highlights specific structures, such as fungi or reticular fibers.

Procedure

Incubate tissues in silver solution. Apply to develop color. The result shows that the structures of interest appear dark brown or black against a lighter background.

TECHNIQUES USED FOR STAINING FUNGI

3.3.1 Gomori's Methenamine Silver (GMS) Staining

Highlights fungal cell walls, particularly useful for detecting molds and yeast in tissue sections.

Procedure

Fix tissue samples and section them. Immerse sections in Gomori's methenamine silver solution for 30-60 minutes. Rinse with distilled water. Optionally, use a counterstain (e.g., eosin) to visualize surrounding tissues. The result shows that Fungi appear black against a green or yellow background.

3.3.2 Periodic Acid-Schiff (PAS) Staining

It detects polysaccharides, including chitin in fungal cell walls.

Procedure

Treat sections with periodic acid for 10-30 minutes to oxidize carbohydrates. Apply Schiff's reagent for 15-30 minutes. Wash with water. The result shows that Fungal elements appear bright pink or magenta.

3.3.3 Calcofluor White Staining

This binds to chitin in fungal cell walls, useful for rapid identification.

Procedure

Apply a solution of Calcofluor White to the sample for 5-10 minutes. Wash with buffer or water. The result shows that Fungi fluoresce blue under UV light, making them easy to identify.

3.3.4 Lactophenol Cotton Blue Staining

It is commonly used for mounting and observing fungal cultures.

Procedure

Prepare a suspension of fungal hyphae or spores. Add a drop of lactophenol cotton blue to the slide and mix. Place a cover slip on top. The result shows that Fungi appear blue against a clear background, allowing for easy visualization of structures.

3.3.5 Gram Staining

It can be used to differentiate between yeast (Gram-positive) and some fungi.

Procedure

Stain with crystal violet for 1 minute. Mordant with iodine for 1 minute. Use ethanol until the runoff is clear. Counterstain for 30 seconds with safranin. The result shows that Yeasts appear purple, while some fungal elements may appear pink.

3.3.6 Hematoxylin & Eosin Staining

It is useful for histopathological examination of fungal infections.

Procedure

Stain with Hematoxylin for 5-10 minutes. Counterstain with Eosin for 30 seconds to 1 minute. The result shows that Fungal elements may appear as pink or purple structures within tissues.

3.3.7 Fluorescent Antibody Staining

It is a specific identification of fungal pathogens using labeled antibodies.

Procedure

Fix the fungal sample on a slide. Apply fluorescent-labeled antibodies specific to the fungus. Rinse to remove unbound antibodies. The result shows that Fungi appear fluorescent under UV light, allowing for specific identification.

METHODS FOR SPORE STAINING

3.4.1 Schaeffer-Fulton Method

It is specifically designed to visualize endospores in bacteria.

Procedure

Prepare a bacterial smear and heat-fix it onto a slide. Stain the slide with malachite green and place it over steam for about 5 minutes to facilitate penetration. Rinse with water to remove excess stain. Apply safranin for about 30 seconds, then rinse again. Observe under a microscope. The result shows that Endospores appear green, while vegetative cells appear pink.

3.4.2 Dorner Method

It is another method for visualizing spores, particularly useful for spores that are more resistant to staining.

Procedure

Prepare a bacterial smear and heat-fix it. Stain the slide with carbol fuchsin and steam for about 5-10 minutes. Rinse with water to remove excess stain. Dip in acid-alcohol solution briefly. Apply methylene blue for about 30 seconds. Observe under a microscope. The result shows that Endospores appear red, while the vegetative cells take on a blue color.

3.4.3 Negative Staining

It is used to visualize the outline of spores without heat-fixing, preserving their natural shape.

Procedure

Place a drop of nigrosin or India ink on a slide. Add a small amount of the bacterial culture to the dye and mix. Use another slide to spread the mixture evenly. Allow the

slide to air dry without rinsing. Observe under a microscope. The result shows that Spores appear as clear areas against a dark background.

3.3.4 Fluorescent Staining

Uses fluorescent dyes to visualize spores under UV light.

Procedure

Prepare a smear and fix it. Apply a fluorescent stain specific for spores (e.g., fluorescein or acridine orange) and incubate for the recommended time. Rinse to remove excess stain. Observe under a fluorescent microscope. The result shows that Spores fluoresce brightly against a darker background.

TECHNIQUES FOR NUCLEAR STAINING

3.5.1 Hematoxylin Staining

It is commonly used in histology to stain nuclei.

Procedure

Fix tissue sections on slides. Immerse slides in hematoxylin solution for 5-10 minutes. Wash with running water for several minutes to remove excess stain. Optional treatment with acid alcohol to remove excess stain. Dehydrate and mount for microscopy. The result shows that Nuclei appear blue or purple.

3.5.2 Giemsa Staining

It is useful for staining blood smears and tissue sections, providing a differential stain for nuclei.

Procedure

Fix the slide with a sample. Stain with Giemsa solution for 20-30 minutes. Wash with buffer or distilled water. The result shows that Nuclei appear blue to purple, and cytoplasmic components vary in color.

3.5.3 DAPI Staining (4',6-Diamidino-2-phenylindole)

A fluorescent stain that binds specifically to DNA.

Procedure

Fix cells on slides. Incubate with DAPI solution for 10-30 minutes. Rinse with PBS or distilled water to remove excess dye. The result shows that Nuclei fluoresce bright blue under UV light.

3.5.4 Propidium Iodide Staining

A fluorescent dye that binds to DNA, used in flow cytometry and microscopy.

Procedure

Fix and permeabilize cells. Incubate with propidium iodide solution for 10-30 minutes. Rinse to remove excess stain. The result shows that Nuclei fluoresce red under fluorescence microscopy.

3.5.5 Toluidine Blue O Staining

It is used for staining nuclei and identifying certain cellular components.

Procedure

Fix tissue sections or cell smears. Stain with Toluidine Blue O Staining for 1-5 minutes. Rinse with distilled water. The result shows that Nuclei appear blue, and other cellular structures may be differentiated based on color intensity.

3.5.6 Feulgen Staining

Specifically stains DNA and can quantify nuclear DNA content.

Procedure

Fix tissue or cells. Treat with hydrochloric acid to hydrolyze DNA. Stain with Schiff's reagent for 15-30 minutes. Rinse to remove excess stain. The result shows that Nuclei containing DNA appear bright magenta.

ZIEHL-NEELSEN METHOD

The Ziehl-Neelsen method is a specific staining technique used to diagnose tuberculosis (TB) by identifying acid-fast bacilli (AFB) in clinical specimens, particularly those from sputum. Here's how to conduct the Ziehl-Neelsen staining method for the diagnosis of tuberculosis

Materials Needed

- i. Reagents: Carbol fuchsin (primary stain), Acid-alcohol (decolorizer) and Methylene blue (counterstain)
- ii. Equipment: Glass slides, Bunsen burner or heat source, Staining trays, Microscope and Sputum samples or other clinical specimens

Procedure

Preparation of Smear

Obtain sputum or other clinical specimens suspected of containing Mycobacterium tuberculosis. Place a small drop of the sample on a clean glass slide. Use a sterile loop or stick to spread the sample evenly to create a thin smear. Allow the smear to air dry completely. Pass the slide through a flame to heat-fix the smear. This kills the bacteria and adheres the sample to the slide.

Staining Process

Flood the slide with Carbol Fuchsin and let it sit for about 5-10 minutes. The stain can be heated gently to enhance penetration but should not boil. Rinse the slide gently with water to remove excess carbol fuchsin. Apply acid-alcohol (a mixture of hydrochloric acid and ethanol) to the slide. Decolorize until the runoff is mostly clear (usually about 5-10 seconds). Rinse with water immediately to stop the decolorization process. Apply methylene blue to the slide for about 30 seconds to 1 minute. Rinse again with water and gently blot the slide dry.

Microscopic Examination

Examine the stained slide under a microscope using the oil immersion objective (100x magnification). Look for bright red or pink bacilli (Mycobacterium tuberculosis) against a blue background. These are the acid-fast bacilli.

Interpretation of Results

Presence of red or pink bacilli indicates a positive test for TB. Absence of acid-fast bacilli suggests no active TB, but further testing may be required.

Safety Considerations

Always wear appropriate personal protective equipment (PPE), including gloves and masks, when handling potentially infectious materials. Dispose of all biological waste according to your institution's safety guidelines.

Conclusion

The Ziehl-Neelsen method is a vital tool in the diagnosis of tuberculosis. It allows for the rapid identification of acid-fast bacilli in clinical samples, helping guide appropriate treatment and public health interventions.

HOW TO CONDUCT SELECTIVE STAINING EFFECTIVELY

Obtain the samples (tissues, cells, or microorganisms) using sterile techniques to prevent contamination. Fix samples using an appropriate fixative (e.g., formalin, methanol) to preserve cellular structures and enhance staining. Choose a stain that selectively binds to the component of interest (e.g., Gram stain for bacteria, PAS for polysaccharides). Review the staining protocol for the chosen dye to ensure proper application and timing. Prepare a thin smear of the sample on a glass slide. Allow it to air dry and then heat-fix (if applicable).

Apply the primary stain to the prepared sample. For example: **Gram Staining**, use crystal violet for bacteria and **PAS Staining**, use periodic acid followed by Schiff's reagent for polysaccharides. Then Incubate for the recommended time (usually 1-10 minutes). Rinse gently with a suitable solvent (e.g., water or buffer) to remove excess dye that does not bind to the target structures. Apply a counterstain to highlight other components without overshadowing the target structure. (If required). For example, use safranin after Gram staining. Rinse again to remove any unbound counterstain.

If necessary, mount the stained slides with a mounting medium and cover slip. Examine under a microscope, adjusting the light and focus to enhance visibility of the stained structures. Take notes and images of the stained samples for analysis and record-keeping.

3.7.1 Safety Considerations

- Always wear appropriate personal protective equipment (PPE) when handling chemicals and biological materials.
- Dispose of hazardous waste according to your institution's guidelines.

SECTIONING ANIMAL TISSUE

Sectioning tissue is a vital technique in histology that allows for the examination of thin slices of biological samples under a microscope.

3.8.1 Materials Needed

- Fresh or fixed animal tissue
- Fixative (e.g., 10% neutral buffered formalin)
- Ethanol (for dehydration)
- Clearing agent (e.g., xylene)
- Paraffin wax
- Micro-tome
- Micro-tome blades
- Glass slides
- Slide warmer (optional)
- Water bath (optional)
- Staining reagents (e.g., hematoxylin and eosin)

3.8.2 Method of Sectioning Tissue

Obtain the tissue sample and keep it in a sterile environment. Immerse the tissue in fixative for an appropriate time (typically 24-48 hours), depending on the tissue type and size.Transfer the fixed tissue through a series of graded ethanol solutions (70%, 80%, 90%, 100%) for 1-2 hours each to remove water. Place the tissue in a clearing agent (like xylene) for 1-2 hours to make it transparent and prepare it for embedding. Immerse the tissue in molten paraffin wax (around 60°C) for several hours or overnight to allow complete infiltration. Pour fresh paraffin into a mold and place the infiltrated tissue inside.

Allow it to cool and solidify. Install a sharp, disposable micro-tome blade in the micro-tome. Secure the paraffin-embedded tissue block in the micro-tome holder. Set the micro-tome to the desired thickness (commonly 4-10 micrometers). Rotate the micro-tome handle to slice the block smoothly, producing thin sections. Carefully collect the sections using a brush or forceps and place them on clean glass slides or into a water bath for easier handling.

Allow the sections to air dry on slides. You can place them on a slide warmer at low temperature to assist in adherence. Some protocols may recommend fixing the sections again in formalin briefly. Stain the sections using appropriate reagents (e.g., hematoxylin and eosin) to visualize cellular structures. Examine the stained sections under a microscope and document findings.

3.8.3 Safety Considerations

- Always wear appropriate personal protective equipment (PPE) when handling chemicals and biological materials.
- Dispose of any hazardous waste according to institutional guidelines.

GUIDELINES FOR THE EFFECTIVE STORAGE OF PREPARED SLIDES

- i. Clean Slides: Ensure slides are free from dust and fingerprints. Clean with lens paper or a suitable cleaning solution if necessary.
- ii. Labeling: Clearly label each slide with relevant information (e.g., sample name, date, stain used) using a permanent marker or label maker.
- iii. Slide Boxes: Store slides in dedicated slide boxes that are designed to protect them from damage. These boxes typically have dividers to keep slides separated.
- iv. Material: Choose boxes made of acid-free materials to prevent chemical reactions that can damage the slides over time.
- v. Vertical Storage: Store slides vertically in the box to minimize the risk of scratching and breakage.
- vi. Orientation Marking: If the slides have specific orientations (e.g., a particular side facing up), ensure they are stored consistently.

- vii. Temperature and Humidity: Store slides in a cool, dry place to prevent moisture accumulation, which can lead to mold growth or damage to the slide and cover slip.
- viii. Light Protection: Keep slides away from direct sunlight or harsh artificial light to prevent fading of stains or degradation of the specimen.
- ix. Handle with Care: When retrieving slides, handle them by the edges to avoid contamination and fingerprints.
- x. Use Gloves: Consider wearing gloves when handling slides, especially if they contain biological samples.
- xi. Periodic Inspection: Regularly inspect stored slides for signs of deterioration, such as fading, mold growth, or damage to the cover slip.
- xii. Re-labeling: If labels fade or become illegible, re-label the slides to ensure information remains clear.
- xiii. Safe Disposal: If slides are no longer needed, dispose of them according to your institution's biological waste disposal guidelines.

CHAPTER FOUR

PROBLEMS ENCOUNTERED, CONCLUSION AND RECOMMENDATION PROBLEMS ENCOUNTERED

The problem of securing a placement for industrial attachment was one major challenge I was faced with. The federal government through SIWES and other private organizations should assist the student in this regard.

CONCLUSION

Federal College of Animal Health and Production Technology, Animal Microbiology section focuses on training student on research in biomedical sciences, molecular and microbiological studies, capacity building in the application of artificial intelligence to life sciences, research development and so on.

The efforts of SIWES are noted, but more is required of them. SIWES should make it mandatory to accommodate students for their training and also SIWES should encourage students by given allowances in form of emolument.

RECOMMENDATION

My students industrial work experience scheme (SIWES) has proved the fact without any doubt that what make up full knowledge is being married with practical. And indeed, this training program has boosted my confidence in my chosen field. I hereby recommended that students of higher institution should undergo this program.

I will advise the federal government through SIWES and university to develop a means of fixing students up for industrial training.