CHUKA UNIVERSITY

WORK INSTRUCTION

FOR

ANIMAL HEALTH PRACTICAL SESSIONS (CU/WI/ANHE/05)

DOCUMENT REVIEW SHEET

The signatures below certify that thisWork Instruction (WI) has been reviewed and accepted, and demonstrate that the signatories are aware of all the requirements contained herein and are committed to ensuring their provision.

Name	**Signature**	**Date**
Revised By: COD/COORDINATOR		15.1.2018
Controlled By: COD/COORDINATOR		15.1.2018
Approved By: DEAN		15.1.2018

CONTENTS

1.0 AMENDMENT RECORD SHEET

DATE	ISSUE NO.	REVISION NO.	PAGE NO.	SUBJECT OF REVIEW /MODIFICATION	REVISED BY	APPROVED BY
18.3.13	02	00	ALL	Changed Chuka University College to Chuka University and inserted a new Logo	Technologist	Dean
18.3.13	02	00	ALL	Changed CUC to CU	Technologist	Dean
18.3.13	02	00	ALL	Changed QMR to MR everywhere it existed	Technologist	Dean
18.3.13	02	00	ALL	Changed Principal to Vice-Chancellor everywhere it existed	Technologist	Dean
18.3.13	02	00	ALL	Replaced Quality Management Representative with Management Representative all over the footer	Technologist	Dean
15.1.2018	03	00	ALL	Changed reference Standard to ISO 9001:2015	COD	ISO M.R.
15.1.2018	03	00	1	Changed revised/controlled by to COD	COD	ISO M.R.

2.0 GENERAL

2.1 Purpose

The purpose of this work Instruction is to ensure that laboratory procedures are followed for the identification of microorganisms,safety of the workers and the environment.

2.2 Scope

This work instruction applies to bacteriology,protozoology, and mycology practicals.

2.3 References

1. ISO 9001:2015 Clause 4.4.2
2. Quality Manual
3. Manuals for operation of various laboratory apparatus and equipment

2.5 Definitions and Abbreviations

Definitions

In addition to the relevant common definitions of terms given in ISO 9000:2005, the following specific definitions shall apply:

Abbreviations

AMR: Assistant Management Representative
MR: Management Representative
QMS: Quality Management System
WI: Work Instruction
BSL: Biosafety Level

2.5 Responsibility

The HOD has the principal responsibility for ensuring that this procedure remains adequate for its intended purposes.

3.0 GENERAL ANIMAL HEALTH PRACTICAL REGULATIONS

3.1 Tools and Equipment

- Incinerator
- A trained animal safe and easy to handle in farm unit
- Mounted skeletons of farm animals (bovine, equine, porcine, sheep/goat, canine, avian) and detached bones in anatomy laboratory.
- Dissection tables and large animal postmortem kit.

3.2 Safety Requirements

(i) The safety guidelines are provided before any procedure is started in the laboratory.
(ii) The laboratory technologist is responsible for assessing the risks and applying the recommended biosafety levels.
(iii) A trained animal safe and easy to handle in farm unit
(iv) Biohazard warning signs listing responsible personnel are posted on all laboratory access doors.
(v) Sandals and open-toe shoes are not appropriate footwear in the laboratory
(vi) All laboratory materials (lab coats, gloves, eye wear etc.) remain in the laboratory unless properly decontaminated.
(vii) Never place laboratory waste in office containers.
(viii) Place all sharps in 'sharps' containers.
(ix) Place all laboratory waste into appropriate discard pans or discard/autoclave bag
(x) Decontaminate discard pans before leaving laboratory.

3.3 Standard Practices

(i) The access to the laboratory is controlled by the technologist or Lecturer in charge.
(ii) Persons should wash their hands after handling samples and animals, after removing gloves, and before leaving laboratory.
(iii) Eating, drinking, smoking, handling contact lenses and applying cosmetics are not permitted in work areas.
(iv) Mouth pipetting is prohibited.
(v) All procedures are performed to minimize aerosol or splash production.
(vi) Work surfaces should be disinfected always after working.
(vii) All culture and regulated wastes are decontaminated before disposal by an approved method such as autoclaving.
(viii) An insect or rodent control to be instituted.
(ix) Biosafety chambers to be used whenever there is potential for aerosol/splash creation or when high concentrations of infectious agents used.
(x) Face protection should be used for anticipated splashes or sprays to the face.
(xi) Lab coats should be worn in the laboratory at all times.
(xii) Gloves should be worn when handling infected animals and when hands may come in contact with infectious materials. All infectious or regulated waste is decontaminated via autoclave, chemical disinfection, incinerator or other approved method.

4.0 ANIMAL HEALTH PRACTICAL INSTRUCTIONS

4.1 Use of a Microscope

4.1.1. Care of microscope

(i) When carrying a microscope from one part of the room to another,use both hands.If it is carried by one hand and allowed to dangle at one side,there is always a risk of collission

with furniture or some other object.Under no circumstances should one attempt to carry two microscopes with one hand.

(ii) Keep your work station unclutterd while doing microscopy.Keep unnecessary books and other materials away from your work area.

(iii) Dont let the light cord in such a way as to risk foot entanglement.

a) At the beginning of each laboratory period,check the lenses to make sure they are clean.At the end of each laboratory session,be sure to wipe any immersion lens if it has been used.

b) If a dust cover is available,place it over the microscpe at the end of each period.

4.1.2 Operation of a microscope

(i) Position the slide on the stage with the material to be studied on the upper surface of the slide.

(ii) Turn on light source using a minimum amount of voltage.

(iii) Check the condenser to see that it has been raised to its highest point.

(iv) Rotate the low-power objective lens directly over the center stage until it clicks into its locked position.

(v) Turn the coarse adjustment knob to lower the objective until it stops.

(vi) While looking down through the ocular(s) bring the object into focus by turning the fine adjustment focusing knob.

(vii) Manipulate the diaphragm lever to reduce the light intensity to produce clearest, sharpest image. Microscopes that lack a voltage control on the light source rely on the diaphragm for controlling light intensity.

(viii) Once the image is visible, move the slide about to search out on what you are looking for.

4.2 Aseptic Technique

Note

The technique ensures that no contaminating organisms are introduced into culture materials when inoculating. It also ensures that organisms that are being handled do not contaminate the handler or others who may be present.

The procedure is:

(i) The work area is first treated with a disinfectant to kill any microorganisms that may be present.

(ii) The inoculating loop must be sterilized before transferring any culture. It is done over a Bunsen burner until it is red hot.

(iii) Before inserting a cooled loop into a culture tube, the cap is removed and the mouth of the tube is flamed.

(iv) After inoculating the loop is flamed to destroy any organisms that remain on these implements

(v) When all work area for the day is complete, the work area is treated to ensure that any organism that has been deposited during any procedures is killed.

Smear Preparation

(i) Wash the slide with soap or Bon Ami and hot water to remove all dirt and grease. Handle the clean slide by its edges.
(ii) Write the initials of the organism(s) on the left hand side with a marking pen
(iii) Transfer the organisms to the center of the slide.
(iv) Spread the organisms.
(v) Allow the organisms to dry by normal evaporation. Do not amply heat.
(vi) After the smear has become completely dry, pass it several times through the flame of a Bunsen burner. Avoid excessive heating as it can shatter from excessive exposure to heat.

4.3 Gram Staining

Note:

1) The gram staining is one of the most important of all the staining techniques used in microbiology.
2) It is important to use cultures that are 16-18 hours old. Gram positive cultures older than this can convert to gram negative and give variable results.
3) Prepare thin smears in order to allow the observation of individual cells.
4) Do not over decolorize to avoid removing the dye-mordant complex.

Staining procedure

1) Cover the smear with crystal violet and let stand for 20 seconds
2) Briefly wash off the stain, using a wash bottle of distilled water. Drain excess water.
3) Cover the smear with Gram's iodine solution and let it stand for one minute
4) Wash off the gram's iodine. Hold the slide at a 45-degree angle and allow the 95% alcohol to flow down the surface of the slide. Do this until the alcohol is colorless as it flows from the smear down the surface of the slide. This takes about 20 seconds.
5) Stop decolorizing by washing the slide with a gentle stream of water.
6) Cover the smear with safranin.
7) Wash gently for a few seconds, blot dry with bibulous paper, and air dry.
8) Examine the slide under oil immersion.

4.4 Anatomy Practical Instructions

4.4.1 Topographical anatomy practical

(i) Trained live animal is restrained in a crush, pen, or in animal arena in the farm animal units.
(ii) Lecturer/ technologist to provide copies of unlabeled animal diagram to students
(iii) Students to label the as various parts are identified by the lecturer / technologist.

4.4.2 Skeletal system

(i) Mounted skeleton well positioned in the laboratory
(ii) Detached bones prepared.
(iii) Students are given instructions on biosafety before starting of the practical.
(iv) Systematically each is identified and its location verified on mounted skeletons.

5.0 RECORDS

This section is used to identify records

Record ID	Owner	Location	Record Media	Retention/Disposition