**REPORT OF**

**STUDENT INDUSTRIAL WORK EXPERIENCE SCHEME (SIWES)**

**DONE AT**



**NATIONAL AGENCY FOR FOOD AND DRUG ADMINISTRATION CONTROL**

**(NAFDAC)**

**ZONAL LABORATORY AGULU, AWKA-EKWULOBIA ROAD,**

**AGULU, ANAMBRA STATE, NIGERIA.**

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**DEPARTMENT OF PHARMACOLOGY & THERAPEUTICS**

**STUDENT INDUSTRIAL WORK EXPERIENCE SCHEME (SIWES)**

**PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE B.Sc. in PHARMACOLOGY**

**AFE BABALOLA UNIVERSITY, ADO-EKITI,**

**EKITI STATE,**

**NIGERIA.**

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 NAFDAC ORGANISATIONAL CHART

**GOVERNING COUNCIL**

**DIRECTOR GENERAL**

**ADMIN. AND HUMAN RESOURCES**

**REGISTRATION AND REGULATORY**

**LABORATORY SERVICES**

**ESTABLISHMENT INSPECTION**

**FINANCE AND ACCOUNT**

**PLANNING, RESEARCH AND STATISTICS**

**ENFORCEMENT**

**NARCOTICS AND CONTROLLED SUBSTANCES**

**PORTS INSPECTION**

**AREA LABORATORY, KADUNA**

**CENTRAL LABORATORY, YABA, LAGOS**

**CENTRAL LABORATORY, OSHODI, LAGOS**

**CENTRAL LABORATORY, MAIDUGURI**

**ZONAL LABORATORY, AGULU**

**AREA LABORATORY CALABAR (IN PROGRESS)**

**AREA LABORATORY, PORTHACOURT**

**HEAD OF LABORATORY**

**FOODLAB**

**WATER LAB**

**INSTRUMENT LAB 1& 2**

**MEDICAL DEVICES LAB**

**PHARMACOGNOSY LAB**

**MICROBIOLOGY LAB**

**COSMETICS LAB**

**PHARMACEUTICAL CHEMISTRY LAB**

**PHARMACEUTICAL CONTROL LAB**

**DIFFERENT LABORATORY IN ZONAL LAB AGULU**

**Food laboratory unit:**

 This is the unit where food samples are analysed. Examples of such samples are bread, custard, fruit juice, cashew nut, milk, tea, etc.

**Water laboratory unit:** Water samples are analysed in this unit of the zonal laboratory.

**Medical Devices unit:**

In this unit, medical devices like condoms, gloves, syringes, needles, cotton wool, diapers, plasters, infusion sets, blood bags, brushes etc. are being analysed.

**Cosmetics laboratory unit:**

Soaps, powder, eye pencils, eye shadows, perfumes, air fresher, detergents, creams, tooth pastes, etc. are all examples of cosmetics samples being analysed in the cosmetics laboratory unit.

**Pharmaceutical chemistry laboratory unit:**

In this unit, the quantitative and qualitative analyses of orthodox drugs are done.

**Pharmaceutical control laboratory unit:**

The hardness, friability, disintegration and dissolution/bioavailability tests are done on orthodox drugs.

**Pharmacognosy laboratory unit:**

This is the unit where analyses on herbal drugs are done.

**Microbiology laboratory unit:**

This unit carries out microbiological analysis on food, drug, water, cosmetics and medical device samples. This is to make sure that these samples are free from pathogenic microorganisms.

 **GENERAL LABORATORY RULES AND SAFETY MEASURES**

All employees and students working in the laboratory are required to learn and understand the properties of the chemicals and harmful microorganisms they work with as well as operational features of laboratory equipment and to follow all precautions applicable to each task.

 Prevention, they say is better than cure, in this wise, some laboratory preventive and first aid measures are listed as follows:

* You should acquaint yourself with the layout of the building and the location of emergency exits.
* Avoid working alone in the laboratory as much as possible. If you must work alone, inform somebody of your presence.
* Use appropriate personal protection and wash your hands regularly when working with chemical reagents, especially before any meal.
* Do not place personal items such as clothing, bags, etc on work benches.
* Always use pipette filler or other pipetting devices instead of using your mouth.
* Before starting an experiment, make sure you are familiar with all the procedure and the potential hazards of the materials.
* If you must leave the laboratory before the completion of an experiment, put a warning sign beside your set up to indicate that experiment is in progress.
* Remember to turn off the water supply, gas, bulbs and disconnect all electric appliances from their sources except the refrigerator at the end of the day.
* Operations involving flammable solvents, fuming acids, sieving powders or using gaseous chemicals must be carried out in the fume cupboard.
* Avoid pouring water into acid, rather pour acid into water.
* All containers containing chemical reagents must be labelled properly.
* Flammable solvents should be stored in an approved storage cabinet or well ventilated area away from burners, hot plates, power sources, etc.
* One should examine all glass wares before use and discard any broken glass apparatus.
* All gas cylinders should be labelled with their name and date in use.
* Compressed gas cylinders in the upright position should be supported and secured.
* Always move large gas cylinders on an approved cylinder trolley. Do not drag, roll or slide cylinder.
* Pressurized gas cylinders should be kept in a cool and ventilated place.
* Naked flames should only be used under critical and close monitoring.
* You should know the position of fire extinguishers in the laboratory and learn the working principle.
* Sterilize used media and cultures before discarding.
* If your clothing catches fire in the laboratory, lie horizontally while another person extinguishes the fire with the fire blanket.
* Never use a fire blanket on any apparatus.
* You must wear suitable eye protection in the laboratory at all time.
* Wear hand gloves and nose marks when necessary.

**PHARMACOLOGY LABORATORY**

 **WHAT IS PHARMACOLOGY**

**Pharmacology** is the branch of biology concerned with the study of drug or medication action, where a drug can be broadly defined as any man-made, natural, or endogenous (from within the body) molecule which exerts a biochemical or physiological effect on the cell, tissue, organ, or organism (sometimes the word pharmacon is used as a term to encompass these endogenous and exogenous bioactive species). More specifically, it is the study of the interactions that occur between a living organism and chemicals that affect normal or abnormal biochemical function. If substances have medicinal properties, they are considered pharmaceuticals.

 **WHAT HAPPENS IN PHARMACOLOGY LABORATORY**

The laboratory is one of the 9 regulatory analytical laboratories in zonal laboratories Agulu where the quality of medical products or drugs mostly are ascertained using a corresponding assay. The methods used could involve either the use of a mammalian (Rats or Rabbits) or the use of Lymulose Amebocyte Lysate (LAL) in analysis of the possible drug effects on human beings exposed to the drugs.

This analysis is carried out in other to check the presence of Lipopolysaccarrides LPS in other words ENDOTOXINS. And hence BACTERIAL ENDOTOXIN TEST as the name of the analysis.

**ENDOTOXINS**

Lipopolysaccharides (LPS), also known as lipoglycans and endotoxin, are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core and inner core joined by a covalent bond; they are found in the outer membrane of gram-negative bacteria. *(e.g E.coli)*. These endotoxins when they are left carelessly inside samples that get directly into the blood stream are highly dangerous and increase mortality rate in the body of the med consumers.

**BACTERIAL ENDOTOXIN TEST**

**Endotoxin testing** (LAL test) ensures that sterile pharmaceutical products are safe for human use.

Endotoxins are bacterial structural components that are released when such a cell is lysed. These components are toxic if administered to humans and/or animals, causing a pyrogenic response (rise in body temperature). For this reason it is important that drugs and medical devices which are either injected or implanted must be tested for their endotoxin content.

There are several methods available for conducting the endotoxin test, which includes the in vivo rabbit pyrogen test and several in vitro alternatives that utilize the Limulus Amebocyte Lysate (LAL) system.

The most common approach to endotoxin testing is the limulous amoebocyte lysate test (**LAL test**). This can be accomplished by various options including gel clot, kinetic chromogenic and kinetic turbidimetric assays. This methodology is also used for the evaluation of medical devices such single- use disposable equipment and implants. This is done by extracting the test product with pyrogen -free water (PFW) and testing for the presence of endotoxin in the extracts.

 **METHODS IN BACTERIAL ENDOTOXIN TEST**

* **CHROMOGENIC METHOD**
* **GEL-CLOTHING METHOD**
* **TURBIDIMERTRIC METHOD**

 **CHROMOGENIC METHOD**

This method is used to quantify the amount of endotoxins in a sample, when Lymulose Amebocyte Lysate LAL reacts with the suspected sample if there is a presence endotoxin it is indicated by colour change.

 **GEL-CLOTHING METHOD**

This method is use to identify endotoxin using a formation of gel when the suspected sample is reacted with Lymulose Amebocyte Lysate LAL.

 **APPARATUS USED IN BACTERIAL ENDOTOXIN TEST**

* LAL Reagent with a label sensitivity of 0.125 or 0.06 EU/ml
* LAL Reagent Water
* Disposable sterile micro-plates
* Reagent reservoir
* Eight-channel multi-pipette
* Kinetic QCL micro-plate reader
* Vortex mixer
* Calibrated adjustable micropipette 100-200µl
* Control Standard Endotoxin (CSE) Standard
* 10 x 75 de-pyrogenated soda lime test tubes
* Disposable (pyrogen free) pipette Tips
* Test tube rack
* Heating Block
* Blocks
* Calibrated thermometer
* Stop Watch

**METHOD A (CHROMOGENIC TECHNIQUE)**

* Switch the micro-plate reader ON
* Log in using User ID and Password.

Open the WinKQCL® Software and select TEMPLATE which contains the name of the analyst, type of assay, lot numbers of reagents, the number and concentration of endotoxin standards, number of replicate, and how standards and samples will be organized on the micro-plate. The Default Template Parameters that follow must not be changed.

* Print the TEMPLATE for use as a guide in placing standards and samples into the micro- plate “Run” the TEMPLATE, following the WinKQCL® Software prompts.
* Prepare the endotoxin standard dilutions.
* Take one ampoule of the endotoxin standards and reconstitute with the volume of LAL Reagent water indicated in the certificate of analysis to make a solution of 50EU/ml stock Solution and vortex at high speed of 15 minutes.
* Take 0.1ml of the stock solution and add 0.9ml LAL reagent water to give a 5EU/ml Solution. Vortex for 1 minute at high speed.
* Take 0.1ml of the 5EU/ml solution and add 0.9ml LAL reagent water to give a 0.5EU/ml Solution. Vortex for 1 minute at high speed.
* Take 0.1ml of the 0.5EU/ml solution and add 0.9ml LAL reagent water to give a

 0.05EU/ml solution. Vortex for 1 minute at high speed.

* Take 0.1ml of the 0.05EU/ml solution and add 0.9ml LAL reagent water to give

 0.005EU/ml solution. Vortex for 1 minute at high speed.

 ***NOTE 1:*** There must be at least 3 dilution places in a micro-plate to form a standard curve for every new test kit.

* Carefully dispense 100µl of the LAL Reagent water blank, endotoxin standards, and product Samples, into the appropriate wells of the micro-plate.
	+ - Add endotoxin to wells designated on the template for PPC in the following order as required.
* Add 10µl of the 50 EU/ml, 5 EU/ml or 0.5 EU/ml endotoxin standards into each of the PPC wells containing 100µ of the product sample as directed by the assay template. Each well of the product sample will now contain a 5 EU/ml, 0.5 EU/ml or 0.05 EU/ml solutions respectively.
* Place filled plate in the micro-plate reader and close the lid. Pre-incubate the plate for 10minutes at 370C ± 10C.
* Near the end of the pre-incubation period reconstitute sufficient number of kinetic-QCL® Reagent vials with 2.6ml LAL Reagent Water per vial. Mix gently but thoroughly.
* Pool the reagents into a reagent reservoir and mix gently rocking the reservoir and mix gently rocking the reservoir from side to side.
* Open the kinetic- QCL® Reader and using an 8-channel multi-pipette dispense 100µl of the kinetic- QCL® Reagent into all the filled wells on the micro-plate beginning with the first column (Al-Hl) and proceeding in sequence to the last column used.
	+ - Add reagent as quickly as possible. (Avoid causing bubbles)
		- Immediately click on the OK button on the computer keyboard to initiate the test.

The result is displayed on the computer monitor indicating whether the product is within the

Endotoxin release limit or not and subsequently printed out.

**NOTE 2:** Thekinetic-QCL® assay is performed with the micro-plate cover removed.

**METHOD B (GEL- CLOT TECHNIQUE)**

* CSE and the Lysate should be Store at between 20C-80C before and after reconstitution
* All reagents and samples should be at room temperature before testing.
* All the glassware to be used for analysis should be depyrogenated,
* Reconstitute the CSE with Pyrogen free water as given in the steps below;
* Remove the metal seal from the vial and aseptically remove the stopper.
* Add LRW to the vial, recommended reconstitution volume is 5mL; however, alternate volumes may be used to achieve desired concentration of stock solution.
* Vortex vigorously for one minute at 5 minute intervals over a 30 minute period at room temperature.
* Store reconstituted CSE at 2-80C for not more than four weeks and should not be frozen.
* Vortex the CSE for at least 30 seconds immediately before making the first dilution and then make appropriate dilutions to achieve desired concentrations.
* Prepare Endotoxins dilution equivalent labeled sensitivity (λ), One half the labeled Sensitivity (λ/2), twice the labeled sensitivity (2 λ) and four times the labeled sensitivity (4 λ).

**NOTE 4**: The values for Lambdas (λ) can be determined following the given labeled Lysate sensitivity by serially diluting the endotoxin Reference standard.

For instance if a stock concentration of CSE = 20Eu/ml and the lysate sensitivity = 0.06 EU/ml, the reconstitution table Will look like the one below: