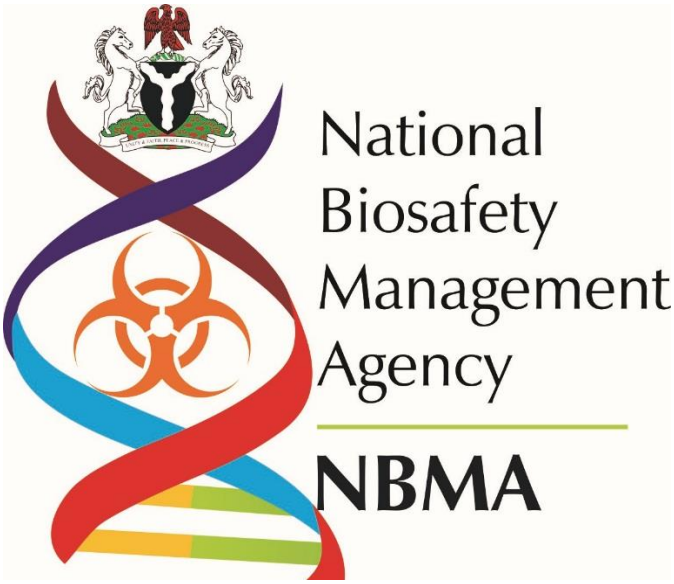

BIOSAFETY LABORATORY MANUAL

August, 2018

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Chapter 1: Biosafety Laboratory Guidelines

PART 1: INTRODUCTION

The National Biosafety Management Agency (NBMA) recognizes safety in the practice of modern biotechnology in containment facilities. Biosafety Laboratory manual guides Scientists in the correct laboratory work ethics. Biosafety Laboratory manual serves as the national code of practice for the safe handling of Genetically Modified Organisms (GMOs) in laboratories.

This manual has been developed, with the view of reviewing on standard operational basis, for handling organisms in all BL2 laboratories which are unlikely to pose serious hazards to laboratory workers, the community, livestock or the environment. The laboratory facilities contained herein are designated as basic – Biosafety Level 2 based on a composite of the design features, containment facilities, practices and operational procedures.

All agents in Biosafety Level 2 laboratory have been assigned to Risk Group 2 and generally require Biosafety Level 2 practices and procedures for safe conduct of work. The assignment of biosafety level for specific work to be carried out in this laboratory is driven by professional judgement based on a risk assessment. This laboratory is appropriate to provide the necessary degree of work, following its provision for state of the art equipment in GMO analysis.

However, the availability of state-of-the-art equipment in a containment facility does not guarantee safety during laboratory operations. It is therefore necessary to adhere to all laboratory safety measures detailed herein to ensure safe conduct of experiments.

PART 2: BASIC LABORATORY – BIOSAFETY LEVEL 2

For the purposes of this manual, the guidance and recommendations are given as minimum requirements pertaining to Level 2 laboratories.

The guidelines for Basic Biosafety Level 2 presented here are comprehensive and detailed, as they are fundamental to this level of Biosafety laboratory.

Code of Practice

This code is a listing of the most essential laboratory practices and procedures that are basic. This laboratory has adopted a safety or operations manual that identifies known and potential hazards, and specifies practices and procedures to eliminate or minimize such hazards.

Specialized laboratory equipment is a supplement to but can never replace appropriate procedures. The most important concepts are listed below:

Access

1. Only authorized persons should be allowed to enter the laboratory working areas.
2. Laboratory doors should be kept closed.
3. Children should not be authorized or allowed to enter laboratory working areas.
4. No animals should be admitted other than those involved in the work of the laboratory.

Personal Protection

1. Laboratory coveralls, gowns or uniforms must be worn at all times for work in the laboratory.
2. Appropriate gloves must be worn for all procedures. After use, gloves should be removed and hands must then be washed.
3. Personnel must wash their hands after handling materials and before they leave the laboratory working areas.
4. Safety glasses, face shields or other protective devices must be worn when it is necessary to protect the eyes and face from splashes, impacting objects and sources of artificial ultraviolet radiation.
5. It is prohibited to wear protective laboratory clothing outside the laboratory, e.g.in canteens, offices, libraries, staff rooms and toilets.
6. Open-toed footwear must not be worn in this facility.
7. Eating, drinking, smoking, application of cosmetics and handling contact lenses is prohibited in the laboratory working areas.
8. Storing human foods or drinks anywhere in the laboratory working areas is prohibited.
9. Protective laboratory clothing that has been used in the laboratory must not be stored in the same lockers or cupboards as street clothing.

Procedures

1. Pipetting by mouth is strictly forbidden in the laboratory.
2. Materials must not be placed in the mouth. Labels must not be licked.
3. All technical procedures should be performed in a way that minimizes the formation of aerosols and droplets.
4. The use of hypodermic needles and syringes should be limited. They must not be used as substitutes for pipetting devices or for any purpose other than aspiration of fluids.

5. All spills and accidents or potential exposures to infectious materials must be reported to the laboratory supervisor. A written record of such accidents and incidents should be maintained.
6. A written procedure for the clean-up of all spills must be developed and followed.
7. Contaminated liquids must be decontaminated (chemically or physically) before discharge to the sanitary sewer. An effluent treatment system may be required, depending on the risk assessment for the agent(s) being handled.
8. Written documents that are expected to be removed from the laboratory need to be protected from contamination while in the laboratory.

Laboratory Working Areas

1. The laboratory should be kept neat, clean and free of materials that are not pertinent to the work.
2. Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of the working day.
3. All contaminated materials, specimens and cultures must be decontaminated before disposal or cleaning for reuse.
4. Packing and transportation must follow applicable national and/or international regulations.
5. When windows can be opened, they should be fitted with arthropod-proof screens.

Biosafety Management

1. It is the responsibility of the Head of the laboratory (the person who has immediate responsibility for the laboratory) to ensure the development and adoption of a biosafety management plan and a safety or operations manual.
2. The laboratory Head should ensure that regular training in laboratory safety is provided.
3. Personnel should be advised of special hazards, and required to read the safety or operations manual and follow standard practices and procedures.
4. The Head of the laboratory should ensure that all personnel understand these practices.
5. Appropriate medical evaluation, surveillance and treatment should be provided for all personnel in case of need, and adequate medical records should be maintained.

Essential Biosafety Equipment

Equipment available in Biosafety Level 2 laboratory include the following:

1. Pipetting aids – to avoid mouth pipetting.
2. Autoclaves to decontaminate materials.
3. Real Time Quantitative Thermal Block
4. Water Bath
5. Gradient Thermal Block
6. Automatic Nucleic Acid Extractor and Desk Top
7. Incubator
8. PH Meter
9. Spectrophotometer
10. Vortex Mixer
11. Measuring cylinders, Flasks, Reagent Bottles and Porcelain Crucibles
12. Oven
13. Water distiller
14. Biosafety cabinets, to be used whenever:
    - potentially infectious materials are handled. Such materials may be centrifuged in the open laboratory if sealed centrifuge safety cups are used and if they are loaded and unloaded in a biological safety cabinet.
    - there is an increased risk of airborne infection
    - procedures with a high potential for producing aerosols are used. These may include centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption, opening of containers of infectious materials whose internal pressure may be different from the ambient pressure.

Equipment such as autoclaves and biosafety cabinets must be validated with appropriate methods before being taken into use. Recertification should take place at regular intervals, according to the manufacturer's instructions.

Waste Handling

Waste is anything that is to be discarded. In this laboratory, decontamination of wastes and their ultimate disposal are closely interrelated. In terms of daily use, all contaminated materials will require actual removal from the laboratory or destruction. Glassware, instruments and laboratory clothing may be reused or recycled. Appropriate waste containers and labelling should be maintained in the laboratory. The overriding principle is that all infectious materials should be decontaminated, autoclaved or incinerated within the laboratory.

The principal questions to be asked before discharge of any objects or materials from this laboratory should include:

1. Have the objects or materials been effectively decontaminated or disinfected by an approved procedure?
2. If not, have they been packaged in an approved manner for immediate on-site incineration or transfer to another facility with incineration capacity?
3. Does the disposal of the decontaminated objects or materials involve any additional potential hazards, biological or otherwise, to those who carry out the immediate disposal procedures or who might come into contact with discarded items outside the facility?

Decontamination

Steam autoclaving is the preferred method for all decontamination processes. Materials for decontamination and disposal should be placed in containers, e.g. autoclavable plastic bags that are colour-coded according to whether the contents are to be autoclaved and/or incinerated. Alternative methods may be envisaged only if they remove and/or kill microorganisms.

PART 3: SAFETY MEASURES IN THE LABORATORY

The use of safety equipment is no assurance of protection unless the operator is trained and uses Standard Operating Procedure (SOP). Equipment should be tested regularly to ensure its continued safe performance. Some safety equipment available in Biosafety laboratory includes:

Pipetting Aids

A pipetting aid must always be used for pipetting procedures. Mouth pipetting is strictly forbidden in the laboratory. The most common hazards associated with pipetting procedures are the result of mouth suction. Oral aspiration and ingestion of hazardous materials have been responsible for many laboratory-associated infections but pathogen ingestion can be prevented by the use of pipetting aids. Pipettes with cracked or chipped suction ends should not be used as they damage the seating seals of pipetting aids and so create a hazard.

Personal Protective Equipment and Clothing

Personal protective equipment and clothing may act as a barrier to minimize the risk of exposure to aerosols, splashes and accidental inoculation. The clothing and equipment selected is dependent on the nature of work performed. Protective clothing should always be worn when working in the

laboratory. Before leaving the laboratory, protective clothing should be removed, and hands should be washed. Some personal protective equipment that must be used in the laboratory include:

Laboratory coats, gowns, coveralls, aprons

Laboratory coats should be worn fully buttoned. Aprons may be worn over laboratory coats or gowns where necessary to give further protection against spillage of chemicals or biological materials such as culture fluids. Laundering services should be provided at or near the laboratory.

Laboratory coats, gowns, coveralls, or aprons should not be worn outside the laboratory areas.

Goggles, safety spectacles, face shields

The choice of equipment to protect the eyes and face from splashes and impacting objects will also depend on the activity performed. Safety spectacles do not provide for adequate splash protection even when side shields are worn with them. Goggles for splash and impact protection should be worn over normal prescription eye glasses and contact lenses (which do not provide protection against biological or chemical hazards).

Goggles, safety spectacles, or face shields should not be worn outside the laboratory areas.

Gloves

Contamination of hands may occur when laboratory procedures are performed. Hands are also vulnerable to “sharps” injuries. Disposable microbiologically approved latex, vinyl or nitrile surgical-type gloves are used widely for general laboratory work, and for handling infectious materials.

Gloves should be removed and hands thoroughly washed after handling infectious materials, working in a biosafety cabinet and before leaving the laboratory. Used disposable gloves should be discarded with laboratory wastes. Stainless steel mesh gloves should be worn when there is a potential exposure to sharp instruments e.g. during dissections. Such gloves protect against slicing motion but do not protect against puncture injury.

Gloves should not be worn outside the laboratory areas.

Respirators

Respiratory protection may be used when carrying out high-hazard procedures (e.g. cleaning up a spill of infectious material). The choice of respirator will depend on the type of hazard(s).

It is important that the respirator filter is fitted in the correct type of respirator. To achieve optimal protection, respirators should be individually

fitted to the operator's face and tested. Fully self-contained respirators with an integral air supply provide full protection. Some single-use disposable respirators have been designed for protection against exposures to biological agents.

Respirators should not be worn outside the laboratory areas.

PART 4: GOOD LABORATORY SAFETY TECHNIQUES

Human error, poor laboratory techniques and misuse of equipment cause the majority of laboratory injuries and work-related infections. A compendium of technical methods that are designed to avoid or minimize the most commonly reported problems of this nature is highlighted.

GMO sample containers

Sample containers may be of glass or preferably plastic. They should be robust and should not leak when the cap or stopper is correctly applied. No material should remain on the outside of the container. Containers should be correctly labelled to facilitate identification. Sample request or specification forms should not be wrapped around the containers but placed in separate, preferably, waterproof envelopes.

Transport of GMO samples within the facility

To avoid accidental leakage or spillage, secondary containers, such as boxes, should be used, fitted with racks so that the sample 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.

Opening of GMO packages

Personnel who receive and unpack samples should adopt standard precautions particularly when dealing with broken or leaking containers. Sample containers should be opened in a biosafety cabinet. Disinfectants should also be available.

Use of pipettes and pipetting aids

In pipetting, the following precautions should be adopted:

1. A pipetting aid must always be used. Pipetting by mouth must be prohibited.
2. All pipettes should have cotton plugs to reduce contamination of pipetting devices.

3. Air should never be blown through a liquid containing infectious agents.
4. Infectious materials should not be mixed by alternate suction and expulsion through a pipette.
5. Liquids should not be forcibly expelled from pipettes.
6. Mark-to-mark pipettes are preferable to other types as they do not require expulsion of the last drop.
7. Contaminated pipettes should be completely submerged in a suitable disinfectant contained in an unbreakable container. They should be left in the disinfectant for an appropriate length of time before disposal.
8. A discard container for pipettes should be placed within the biosafety cabinet, not outside it.

Use of biosafety cabinets

The biosafety cabinet does not protect the operator from spillage, breakage or poor technique. Therefore, care must be taken while working in a biosafety cabinet.

The following precautions are necessary:

1. The cabinet must not be used unless it is working properly.
2. The glass viewing panel must not be opened when the cabinet is in use.
3. Apparatus and materials in the cabinet must be kept to a minimum. Air circulation at the rear plenum must not be blocked.
4. Bunsen burners must not be used in the cabinet. The heat produced will distort the airflow and may damage the filters. An electric micro incinerator is permissible but sterile disposable transfer loops are better.
5. All work must be carried out in the middle or rear part of the working surface and be visible through the viewing panel.
6. Traffic behind the operator should be minimized.
7. The operator should not disturb the airflow by repeated removal and reintroduction of his or her arms.
8. Air grills must not be blocked with notes, pipettes or other materials, as this will disrupt the airflow causing potential contamination of the material and exposure of the operator.
9. The surface of the biosafety cabinet should be wiped using an appropriate disinfectant after work is completed and at the end of the day.
10. The cabinet fan should be run for at least 5 minutes before beginning work and after completion of work in the cabinet.
11. Paperwork should never be placed inside biosafety cabinets.

Avoidance of chemicals ingestion and contact with skin and eyes

1. Large particles and droplets (> 5 μm in diameter) released during analysis settle rapidly on bench surfaces and on the hands of the operator. Disposable gloves should be worn. Laboratory workers should avoid touching their mouth, eyes and face while working.
2. Food and drink must not be consumed or stored in the laboratory.
3. No articles (pens, pencils, chewing gum) should be placed in the mouth while working in the laboratory.

Use of centrifuges

Satisfactory mechanical safety performance is a prerequisite in the use of centrifuges in the laboratory. In the use of centrifuges therefore, the following precautions should be adopted:

1. Centrifuges should be operated according to the manufacturer's instructions.
2. Centrifuges should be placed at such a level that workers can see into the bowl to place buckets correctly.
3. Centrifuge tubes and sample containers for use in the centrifuge should be made of thick-walled glass or preferably of plastic and should be inspected for defects before use.
4. Tubes and sample containers should always be securely capped (screw-capped if possible) for centrifugation.
5. The buckets must be loaded, equilibrated, sealed and opened in a biosafety cabinet.
6. Buckets and trunnions should be paired by weight and, with tubes in place, correctly balanced.
7. The amount of space that should be left between the level of the fluid and the rim of the centrifuge tube should be given in manufacturer's instructions.
8. Distilled water or alcohol (propanol, 70%) should be used for balancing empty buckets. Saline or hypochlorite solutions should not be used as they corrode metals.
9. When using angle-head centrifuge rotors, care must be taken to ensure that the tube is not overloaded as it might leak.
10. The interior of the centrifuge bowl should be inspected daily for staining or soiling at the level of the rotor. If staining or soiling is evident then the centrifugation protocols should be re-evaluated.
11. Centrifuge rotors and buckets should be inspected daily for signs of corrosion and for hair-line cracks.
12. Buckets, rotors and centrifuge bowls should be decontaminated after each use.

13. After use, buckets should be stored in an inverted position to drain the balancing fluid.
14. Infectious airborne particles may be ejected when centrifuges are used. Good centrifuge technique and securely capped tubes offer adequate protection against infectious aerosols and dispersed particles.

Care and use of refrigerators and freezers

1. Refrigerators, deep-freezers and solid carbon dioxide (dry-ice) chests should be defrosted and cleaned periodically, and any tubes that have broken during storage removed. Face protection and heavy duty rubber gloves should be worn during cleaning. After cleaning, the inner surfaces of the cabinet should be disinfected.
2. All containers stored in refrigerators should be clearly labelled with the name of the contents, the date stored and the name of the individual who stored them. Unlabeled and obsolete materials should be autoclaved and discarded.
3. An inventory must be maintained of the freezer's contents.
4. Flammable solutions must not be stored in a refrigerator unless it is explosion proof. Notices to this effect should be placed on refrigerator doors.

PART 5: DISINFECTION AND STERILIZATION

A basic knowledge of disinfection and sterilization is essential for biosafety in the laboratory. It is important to understand the rudiments of cleaning prior to disinfection. The requirements for decontamination depend on the type of experimental work and the nature of materials being handled in the laboratory.

Cleaning of Laboratory Materials

Cleaning is the removal of dirt, organic matter and stains. Cleaning includes brushing, vacuuming, dry dusting, washing or damp mopping with water containing soap or detergent. Dirt, soil and organic matter can affect the results of analysis.

Pre-cleaning is necessary to achieve proper disinfection or sterilization. Pre-cleaning must be carried out with care to avoid exposure to infections.
Materials chemically compatible with disinfectants to be applied later must be used. It is quite common to use the same disinfectant for pre-cleaning and disinfection.

Disinfectants

Many types of chemicals can be used as disinfectants and/or antiseptics. As there is an ever-increasing number and variety of commercial products, formulations must be carefully selected for specific needs.

Some disinfectants can be harmful to humans or the environment. They should be selected, stored, handled, used and disposed of with care, following manufacturers' instructions. For personal safety, gloves, aprons and eye protection are recommended when preparing dilutions of disinfectants. Disinfectants are generally required for regular cleaning of floors, walls, equipment and furniture.

Proper use of disinfectants will contribute to workplace safety. The number of disinfectants to be used should be limited to reduce environmental pollution

Autoclaving

Saturated steam under pressure (autoclaving) is the most effective and reliable means of sterilizing laboratory materials. For most purposes, the following cycles will ensure sterilization of correctly loaded autoclaves:

1. 3 min holding time at 134°C
2. 10 min holding time at 126°C
3. 15 min holding time at 121°C
4. 25 min holding time at 115°C.

While loading autoclaves, materials should be loosely packed in the chamber for easy steam penetration and air removal. Bags should allow the steam to reach their contents. In the operation of autoclaves, the following precautions must be adhered to:

1. The steam should be saturated and free from chemicals (e.g. corrosion inhibitors) that could contaminate the items being sterilized.
2. All materials to be autoclaved should be in containers that allow ready removal of air and permit good heat penetration; the chamber should be loosely packed so that steam will reach the load evenly.
3. For autoclaves without an interlocking safety device that prevents the door being opened when the chamber is pressurized, the main steam valve should be closed and the temperature allowed to fall below 80 °C before the door is opened.
4. Slow exhaust settings should be used when autoclaving liquids, as they may boil over when removed due to superheating.
5. Operators should wear appropriate gloves and visors for protection when opening the autoclave, even when the temperature has fallen below 80 °C.

6. In any routine monitoring of autoclave performance, biological indicators or
1. thermocouples should be placed at the centre of each load. Regular monitoring
2. with thermocouples and recording devices in a “worst case” load is highly desirable to determine proper operating cycles.
7. The drain screen filter of the chamber (if available) should be removed and cleaned daily.
8. Care should be taken to ensure that the relief valves of pressure cooker autoclaves do not become blocked by paper, etc. in the load.

Incineration

Incineration is useful for disposing of laboratory waste, with or without prior decontamination. Proper incineration requires an efficient means of temperature control and a secondary burning chamber. Ideally, the temperature in the primary chamber should be at least 800°C and that in the secondary chamber at least 1000°C.

Materials for incineration, even with prior decontamination, should be transported to the incinerator in bags, preferably plastic. It is worth noting that the efficient operation of an incinerator depends heavily on the right mix of materials in the waste being treated.

Disposal

Autoclaved waste should be disposed of by off-site incineration or in approved landfill sites.

PART 6: CONTINGENCY PLANS AND EMERGENCY PROCEDURES

A written contingency plan for dealing with laboratory accidents is a necessity in any Biosafety laboratory.

Contingency Plan

The contingency plan should provide operational procedures for:

1. Precautions against natural disasters, such as fire and explosion
2. Biohazard risk assessment
3. Incident-exposure management and decontamination
4. Emergency evacuation of people from the premises
5. Emergency medical treatment of exposed and injured persons
6. Medical surveillance of exposed persons
7. Clinical management of exposed persons
8. Post-incident continuation of operations.

In the development of this plan the following items should be considered for inclusion:

1. Identification of at-risk personnel
2. Identification of responsible personnel and their duties, e.g. biosafety officer, safety personnel, local health authority, clinicians, microbiologists, fire and police services.
3. Lists of treatment and isolation facilities that can receive exposed or infected persons
4. Transport of exposed or infected persons
5. Lists of sources of immune serum, vaccines, drugs, special equipment and supplies
6. Provision of emergency equipment, e.g. protective clothing, disinfectants, chemical and biological spill kits, decontamination equipment and supplies.

PART 7: EMERGENCY PROCEDURES FOR BIOSAFETY LABORATORIES

Puncture wounds, Cuts and Abrasions

The affected individual should remove protective clothing, wash the hands and any affected area(s), apply an appropriate skin disinfectant, and seek medical attention as necessary. The cause of the wound and the organisms involved should be reported. Appropriate and complete medical records should be kept.

Ingestion of Potentially Infectious Material

Protective clothing should be removed and medical attention sought. Identification of the material ingested and circumstances of the incident should be reported. Appropriate and complete medical records should be kept.

Potentially Infectious Aerosol Release (outside a biosafety cabinet)

All persons should immediately vacate the affected area and any exposed persons should be referred for medical advice. The laboratory Head should be informed at once. No one should enter the room for an appropriate amount of time (at least 1 hour), to allow aerosols to be carried away and heavier particles to settle. Entrance should be delayed for at least 24 hours following the absence of a central air exhaust system.

Signs should be posted indicating that entry is forbidden. After the appropriate time, decontamination should proceed, supervised by the

laboratory Head. Appropriate protective clothing and respiratory protection should be worn.

Breakage of Tubes in Centrifuges not having Sealable Buckets

If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (e.g. for 30 mins) to allow settling. If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed (e.g. for 30 mins). In both instances, the Head of the laboratory should be informed. Thick rubber gloves covered, if necessary, with suitable disposable gloves, should be worn for all subsequent operations. Forceps, or cotton held in the forceps, should be used to retrieve glass debris. All broken tubes and glass fragments and the rotor should be placed in a noncorrosive disinfectant.

Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered. The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried. All materials used in the clean-up should be treated as infectious waste.

Breakage of Tubes inside Sealable Buckets (safety cups)

All sealed centrifuge buckets should be loaded and unloaded in a biosafety cabinet. If breakage is suspected within the safety cup, the safety cap should be loosened and the bucket autoclaved. Alternatively, the safety cup may be chemically disinfected.

Fire and Natural Disasters

Fire and other services should be involved in the development of emergency preparedness plans. It is beneficial to arrange for these services to visit the laboratory to become acquainted with its layout and contents.

Emergency Services: Whom to Contact

The telephone numbers and addresses of the following are displayed in this facility:

1. The National Biosafety Management Agency (NBMA)
2. Office of the Director General/Chief Executive Officer
3. Laboratory Head
4. Fire services
5. Hospitals
6. Police
7. Responsible technician
8. Water, gas and electricity services
9. National Emergency Management Agency (NEMA).

CHAPTER 2 : TESTING PROTOCOL FOR GMOS

PART 1 INTRODUCTION

Modern biotechnology offers the opportunity to use living systems and organisms to develop, make or modify useful products or processes for specific use with the aim of improving living conditions and socio-economic development across the world. The engineered organisms or Genetically Modified Organisms (GMOs) are also referred to as Living Modified Organisms (LMOs).

GMOs are organisms such as bacteria, yeast, insects, plants, fish and mammals whose genetic materials have been altered using genetic engineering techniques.

GMOs have been applied in four major industrial areas, including medical, agriculture, industrial uses of crops and other products (e.g. biodegradable plastics, vegetable oil, biofuels), and environmental bio-remediation. The applications of GMOs can also be used to provide alternative approaches to addressing poverty- related challenges using widely applicable innovative tools at the community and national levels. In this respect, the use of GMOs in improving agricultural productivity has particularly been widely reported.

Currently in agriculture, existing genetically modified crops have traits such as pest resistance, herbicide tolerance, drought tolerance, increased nutritional value, or production of valuable goods such as drugs.

Despite the obvious advantages in the development, handling and use of plant GMOs for FFP, these GMOs are sometimes considered as alien species by critics, since they may have no defined geographical distribution in the natural environment until they are released from the generate sources. Concerns therefore arise as to whether their release could have harmful effects on living organisms, biodiversity and environmental safety (IUCN 2000). Also, concerns may be raised about food and feed safety such as altered composition, nutritional bio-availability and allergenic risks.

In order to address these concerns, the Cartagena Protocol on Biosafety adopted by the Conference of the Parties (COP) of the

Convention on Biological Diversity (CBD) on 29th January 2000, sought to protect biological diversity from the potential risks posed by GMOs resulting from modern biotechnology.

The Cartagena Protocol on Biosafety (CPB) provides an Advance Informed Agreement (AIA) procedure that ensures that countries have the information necessary to make an informed decision before permitting the import and production of such organisms into their territory. The Protocol also establishes regulations for developing biosafety frameworks in member countries, as well as Biosafety Clearing House (BCH) to facilitate the exchange of information on GMOs for direct use for food, feed or processing.

In accordance with the CPB, Nigeria has developed a comprehensive biosafety framework with policy, administrative and regulatory instruments in place. This specifically includes the National Biosafety Policy and the National Biosafety Bill of 2006. The National Biosafety Bill was subsequently passed by the National Assembly and assented to by the President on the 18th of April, 2015 as the National Biosafety Management Agency Act 2015. The Act established the National Biosafety Management Agency in 2015, charged with the responsibility for providing regulatory framework, institutional and administrative mechanism for safety measures in the application of modern biotechnology in Nigeria with the view to preventing any adverse effect on human health, animals, plants and environment. The agency also develops capacity for biosafety risk assessment including testing and detection of GMOs.

In view of the above, Nigeria has developed these testing protocols to cover the following areas:

1. Comprehensive list of potential GMOs to test for in FFP.
2. Specific approved protocols for detecting selected GMOs
3. Standard Operating Procedures (SOPs)
4. Location of resources such as equipment and institutions that can carry out GMO testing
5. Safety measures for handling of samples and disposal of specimens.

PART2: LIST OF POTENTIAL GMOS TO TEST FOR IN FOOD, FEED AND FOR PROCESSING (FFP)

Nigeria has a vibrant local agricultural and horticultural sectors known for the production of local food crops and cash crops. The food crops include cereals (maize, millet, sorghum, rice, etc.), roots and tubers (yam, cassava, potato, cocoyam, etc.), fruits and vegetables (pepper, tomato, egg-plant, onion, carrot, cabbage, mango, orange etc.) and legumes and nuts (beans, soya beans, groundnuts, cashew nuts, etc.). Some major cash crops for export are: cocoa, shea butter, rubber, cotton and oil palm. Other exports include forestry resources such as timber, wood products and some wildlife.

Nigeria also imports various food crops and other plant resources. These include wheat, rice, tomatoes, cowpea, and soybean. The importation of plant resources, food, feed and products for processing is monitored and promoted by several regulatory agencies namely: National Agricultural Seed Council (NASC), Nigeria Customs Service (NCS), Nigeria Agricultural Quarantine Service (NAQS), National Agency for Food and Drug Administration and Control (NAFDAC) and Standards Organization of Nigeria (SON). With the enactment of the National Biosafety Management Agency (NBMA) Act 2015, all GMOs imported into the country are regulated by the NBMA.

Building capacity for testing and detection of GMOs is essential for reliable monitoring and evaluation of the safe development, handling, use, and trans-boundary movement. GMO testing capacity is critical to building confidence among scientists, regulators and the general public.

Below is a list of crops that have been modified by modern biotechnology using genetic engineering to generate plant GMOs with unique identifiers that can be tested by ELISA and/or PCR methods.

1. Cotton 2. Maize 3. Rice 4. Cowpea 5. Sorghum 6. Canola	7. Tomato 8. Soybean 9. Potato 10. Apple 11. Banana

	12. Egg-Plant

List of important plants that may be modified for improved productivity in the near future.

Bambara beans	Okra
Cabbage	Onion
Carrot	Citrus
Cassava	Pepper
Cocoyam	Pineapple
Oil Palm	Plantain
Cowpea	Yam
Lettuce	Cocoa
Mango	Millet

PART 3: TESTING METHODS AND CAPACITY IN NIGERIA

Technical Expertise

In the testing for GMOs in food and feed, it is important to have technical experts to ensure proper running of the laboratory, handling of samples and testing methodology. A few of such expertise are listed below:

1. Laboratory technician
2. Biomedical scientist
3. Geneticist
4. Molecular biologist
5. Biochemist
6. Nutritionist
7. Biotechnologist
8. Agronomist
9. Food Scientist
10. Laboratory Scientist

Equipment/Apparatus required for Genetically Modified Organism (GMO) Testing/ Screening

Testing for GMOs requires the use of specific equipment, a few of which are listed below.

I. Molecular (DNA) Testing

1. Polymerase Chain Reaction (PCR) machine (Exicycler 96)
2. Electrophoresis machine
3. Autoclave
4. Vortex
5. Centrifuge
6. Micropipettes and Tips
7. Microcentrifuge tubes
8. Biosafety cabinet
9. Water bath
10. DNA detection kit
11. ExiPrep 16 Plus
12. Weighing Balance
13. Draining Rack
14. Safety Goggles
15. Thermometer
16. Porcelain Crucible
17. Thermal Block
18. Petri Dishes
19. Hand Gloves
20. Spectrophotometer
21. Glass Troughs

GMO Detection Procedure

The general procedure that would be adopted for the detection of GMOs in the Biosafety laboratory include the following

1. Preparation of work place

Before the commencement of work, work place is cleaned with absolute ethanol to avoid contamination. If work is to be performed inside the Biosafety cabinet, the cabinet is wiped with absolute ethanol and the ultraviolet light turned on for some minutes. The UV light is then turned off after which, work commences.

2. Sample preparation

During sample preparation, significant errors can occur in the absence of appropriate care. To avoid the occurrence of such errors, a representative composite sample must be crushed to appropriate particle size. Crushed sample must be thoroughly mixed prior to analysis. As sample preparation is a crucial step in the testing of GMOs, care must be taken to avoid sample carryover during grinding which is a potential source of error that can be introduced into the final result.

3. DNA Extraction

Three factors (quantity of DNA extracted, quality and purity of DNA) determine the success of DNA extraction. DNA extraction is often the most time-consuming step in DNA detection and can form a bottleneck for high throughput detection.

DNA isolation would involve a number of steps. First, chemical agents will be used to disrupt cells for the release of DNA into a solution. Second, proteins and other cellular components will be largely removed by a protein precipitation step after which DNA will be maintained in solution. Finally, DNA will be selected from most of the contaminants by precipitation in alcohol.

4. Polymerase Chain Reaction (PCR)

PCR is a technique for the amplification of a number of copies of a specific DNA sequence. PCR must be very sensitive to always amplify the sequence of interest if present so as to prevent false negative result, and highly selective to only amplify the intended target sequence to prevent false positive results.

Most GMOs currently approved worldwide contain any of the three genetic elements that can be targets for GMO screening. These elements are the Cauliflower Mosaic Virus (CaMV) 35S promoter, the NOS terminator from the soil bacterium, *Agrobacterium tumefaciens* and the Kanamycin resistance marker gene (NPTII). Naturally, these sequences occur in plants and soil microorganisms. A positive result therefore will not necessarily confirm presence of GMOs but will only suggest they are probable.

To confirm definitively the presence of a GMO, a sample with positive signal in 35S and/or NOS screening will be further analysed using a construct-specific or event-specific method. Alternatively, the sample could be further analysed for the presence of naturally occurring CaMV or *A. tumefacien*s infection.

The CaMV promoter is preferred above other potential promoters because it is a more powerful promoter than others and is not greatly influenced by environmental conditions or tissue types. CaMV has two Promoters 19S and 35S, of these two the 35S promoter is most frequently used in biotechnology because it is most powerful. Where samples are recorded in large numbers, protein immunoassays would be utilized for testing.

5. Electrophoresis

The success of PCR amplification is assessed by agarose gel electrophoresis at 94 Volts for 60 seconds by observing the following procedure.

100 mL of Tris–acetate–EDTA (TAE) solution would be mixed with 1g of agarose in a conical flask. The mixture will be heated for one minute followed by cooling while it will constantly be shaken to prevent solidification on one side of the mixture. On the completion of cooling, SYBR Green I (SG) nucleic acid stain will be added to the mixture after which, the mixture will be poured into a flat tray and allowed to set. While still in liquid form, four combs will be placed astride the tray to form wells which following gel setting would form the wells. Individual wells will be loaded with the PCR product and gel loading buffer. The first wells of gel will be filled with ladder standard solution. Electrophoresis of the gel will follow after which the DNA in the gel will be visualized under an ultraviolet illumination

Immunological (Protein) Testing

Immunological analysis, or immuno-analysis for short, is a GMO testing method that detects proteins. Currently, there are two types of GMO tests that use this method: The Strip Test and ELISA Methods.

1. Lateral Flow Devices (LFDs)/Strip tests also known as "dipsticks"

This is a rapid antibody-based method used for measuring GMO protein in unprocessed materials such as seed, grain, or leaves. This method is appropriate for qualitative or semi-quantitative testing of GMOs. The method is suitable for field testing.

2. ELISA Test (Enzyme-Linked Immunosorbent Assay)

This is a rapid antibody-based method used for measuring GMO protein in unprocessed materials such as seed, grain, or leaves. ELISA is appropriate for qualitative or quantitative testing and is performed in a laboratory setting. For insect resistance analysis, Near Infrared Spectroscopy can be used.

Near Infrared Spectroscopy uses spectral properties of sample in Insect Resistance (IR) to detect GMOs. This method was developed for Roundup Ready soybean due to its specific characteristics. It is a non-invasive method and can be applied on-site, hence suitable for analysis of large sample lots of more expensive material, e.g. seeds.

PART 4: SAFETY ASSESSMENT OF PROTEINS USED IN GM CROPS

There are three key issues of consideration during GM testing. Amongst these issues are Allergenicity, Toxicity and Compositional Analysis.

Candidate proteins expressed in GM crops are usually compared and contrasted with proteins that are allergenic or toxic using a weight of evidence approach consisting of individual and independent studies. Recognizing that most of the early generation GM crops were developed to express proteins, documents authored by (*Codex alimentarius* Commission (Codex), 2001, 2003a, 2003b, 2007; European Commission[EC], 1997, 2003a, 2003b, 2004; European Food Safety Authority EFSA, 2006a, 2006b; FAO, 1996; FAO/WHO, 2000; International Life Sciences Institute ILSI, 1996, 1997, 2003, 2004; OECD, 1993, 1997, 2003; WHO, 1995) highlighted that proteins are an integral component of the diet. It is however, acknowledged that there are some proteins that exist in nature that can present hazard in the form of potential for allergenicity or toxicity. A weight of evidence approach therefore has been developed based on what is known about allergenic and toxic proteins to compare candidate proteins with known allergenic and toxic proteins before commercialization.

Allergenicity

The potential for allergenicity is assessed for proteins to ensure that they are not similar enough to cross react with the antibodies present in persons with food allergies. A key component in the allergenicity assessment is the source of proteins. This is one component of the safety assessment for individual proteins called History of Safe Use (HOSU; Constable *et al.*,2007). Another key component in the allergenicity assessment is a bioinformatics comparison of the amino acid sequence of the protein with the sequences of known allergenic proteins for similarity using computational methods. The identity and amino acid sequence of all known allergenic proteins is

available online (www.allergenonline.org) and up-dated annually. A physical property shared by numerous, but not all, allergenic proteins is resistance to degradation in the presence of digestive enzymes (Astwood *et al.*,1996). In vitro methods have been developed to evaluate the sensitivity of proteins to degradation in the presence of digestive enzymes (*pepsin* and *pancreatin*). The primary basis of these considerations is that proteins selected from sources that are not known to produce toxic proteins, are not similar in sequence to known protein toxins, and are readily degraded in the presence of digestive enzymes that are unlikely to be toxins.

Toxicity

Proteins used in GM crops need to be assessed for potential to cause adverse effects. There are overlaps in the methods used to assess the potential toxicity and allergenicity of proteins, specifically, consideration of history of safe use of the source of the protein, bioinformatics comparison to known protein toxins, and in vitro resistance to digestive enzymes (Delaney *et al.*, 2007). There exists a difference in the bioinformatics analysis compared with the allergenicity assessment. First, there are no pre-defined criteria that identify a "match" between two proteins. Second, there is currently no annotated and updated database in which the sequence of protein toxins is maintained. Rather, what is commonly conducted is a comparison to all known protein sequences in the NCBI BLAST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) followed by manual inspection to determine if sequence similarities are present.

Compositional Analysis

An important component of the data produced in the comparative safety assessment is a detailed compositional analysis of the key nutrients, antinutrients, secondary metabolites and toxins of a GM crop and a non-GM comparator. GM crops can be analyzed chemically to determine their chemical composition. In the case of maize, soybeans, canola, rice and cotton, the concentrations of the components are available at a publicly available website that is managed by the International Life Sciences Institute(https://www.cropcomposition.org/query/index.html).

Institutions with the capacity for GMO testing in Nigeria

There are several categories of institutions with varying capacity for GMO testing. These can be classified as regulatory, service, research, teaching, and capacity building. Institutions with infrastructure, equipment and human resources capacity to conduct GMO testing in Nigeria are as contained in Annex 1.

PART 5: PRECAUTIONS FOR HANDLING AND DISPOSAL OF GENETICALLY MODIFIED ORGANISMS (GMOS)

I. Plant GMOs

1. All Biosafety level 2 and 3 (BS2 and BS3) protocols should be adhered to.

2. All GM plants, non-GM plants inoculated with Genetically Modified Microorganisms (GMMs), pots, trays, compost, soil or other growth media must be decontaminated by validated means, on the same site as the contained use activity is taking place, prior to cleaning and / or disposal.

3. Containment of GMOs stored in cold storage facilities outside the main facility must be doubled in a sealed primary containment within a secondary containment.

4. Disposal of GMO samples can be done by incineration, chemical sterilization (commonly used for liquid samples) and pressure steam sterilization (autoclaving). Composting is a potentially new safe way of disposal. Chemical sterilization may be done using 0.5 % hypochlorite solution, 2 % glutaraldehyde (stock solution should be used within 24 hours), 10 % sodium hypochlorite, 70 % ethanol and 0.5 % iodine in 70 % ethanol. Virkon and Biogram may also be used.

5. In the event of unintentional spill or leakage or loss of GMOs, efforts must be implemented to retrieve the GMO and return it to containment to render them non-viable and the incident must be reported as soon as possible.

6. Polymerase Chain Reaction(PCR) reagents should be stored and handled in separate room and freezer where no nucleic acids (with exception of PCR primers or probes) or DNA degrading or modifying enzymes have been handled previously. All handling of PCR reagents and controls requires dedicated equipment, especially pipettes.

7. Powder-free gloves should be used and changed frequently.

II. Animal GMOs

1. All Biosafety level 1 and 2 (BS1 and BS2) protocols should be adhered to.

2. Microorganisms including liquid and solid cultures must be inactivated on-site prior to disposal. Common inactivation methods include autoclaving at 121 ^{0}C or treatment with bleach (refers to a number of chemicals which remove colour, or disinfect, often by oxidation) or other disinfecting agents.
3. Carcasses of animals infected with GM micro-organisms or GM animals infected with infectious agents must be decontaminated by pressure steam inactivation (autoclaving) or incineration.

4. Windows must be closed and locked while GM animals or animals containing GMOs are being processed in the laboratory. Also, all air supply and exhaust vents must be fitted with fly screens. However, where the laboratory is mechanically ventilated, a directional air flow into the laboratory must be maintained. Recirculation is permitted.

5. In the event of unintentional spill or leakage or loss of GMOs, efforts must be implemented to retrieve the GMO and return it to containment to render them non-viable and the incident must be reported as soon as possible.

6. Any material transported with the GM microorganism or GMO must either be retained with the organism under containment or decontaminated after transportation.

7. Disposal of GMO samples can be done by incineration, chemical sterilization (commonly used for liquid samples) and pressure steam sterilization (autoclaving). Composting is a potentially new safe way of disposal. Chemical sterilization may be done using 0.5 % hypochlorite solution, 2 % glutaraldehyde (stock solution should be used within 24 hours), 70 % ethanol and 0.5 % iodine in 70 % ethanol.

8. Where GMMs are being shed or not, waste materials such as bedding, faeces and urine should be decontaminated and properly disposed.

Things to Note

Records of all GMO inactivation events must be presented to the NBMA for inspection. This record or GMO contained use notification should include the following:

1. Nature of the waste (whether liquid or solid), amount of waste and the contaminating GMMs.

2. Details of the procedure e.g. procedure for the storage, removal of GM waste from the laboratory to the autoclave room, autoclave cycle parameters (temperature, time and pressure), validation procedures and disposal of decontaminated waste, should be clearly indicated where autoclaving is used in the inactivation of solid waste.

3. Details of the inactivating agent used (with reference chemical inactivation), the concentration of use, the duration of contact to inactivate liquid waste or spillages and how the waste is finally disposed.

4. Details of the validation procedure for the inactivation of waste where the waste is inactivated in-house.

5. Details of the registered waste contractor hired to remove the waste, including their waste license/permit number where GM/non-GM animals inoculated with Class 1 or Class 2 GMMs undergo off-site inactivation. The information should also include details of how the waste is treated and disposed of by the contractor.

6. The chosen method of inactivation (e.g. autoclaving or chemical inactivation) must be validated annually under normal working conditions. A copy of the validation protocol and the results of the validation exercise must be retained by the user.

Validation of inactivation

Where waste is being autoclaved, biological indicators (or similar) must be used at least monthly in order to validate inactivation. Decontaminated waste should not be removed off-site until such time as there is a positive inactivation result. A record of the validation must be maintained by the user.

CONCLUSION

This GM Testing protocol has highlighted important steps to be adopted during detection and necessary things to test for.

ANNEX1

Table 1: Institutions with resources to carry out GMO testing in Nigeria

NAME OF INSTITUTION	LOCATION	STATUS OF GMO TESTING	GMO TESTING EQUIPMENT AVAILABLE	GMO TESTING EXPERTISE	GMO TESTING ACCREDITATION	COLLABORATING INSTITUTIONS	CONTACT PERSON	CONTACT (EMAIL & PHONE
National Biotechnology Development Agency (NABDA)	NABDA, Umaru Musa Yar'adua Expressway Lugbe, Abuja	1. Have tested for LMO microorganisms and plasmid DNA from *E. coli* cells; mosquitoes (in the future) 2. Have tested for GMO plant	i) PCR ii)Gel Electrophoresis	Molecular Biologist	Yes	NONE	DG	
Institute of Agricultural Research (IAR)	Ahmadu Bello University Zaria	Have tested for GMO plants (Bt Cowpea, and Africa Bio fortified Sorghum ABS)			Yes		Executive Director (Principal Investigators)	
National	NCRI,	Have tested			Yes		Executive	

NAME OF INSTITUTION	LOCATION	STATUS OF GMO TESTING	GMO TESTING EQUIPMENT AVAILABLE	GMO TESTING EXPERTISE	GMO TESTING ACCREDITATION	COLLABORATING INSTITUTIONS	CONTACT PERSON	CONTACT (EMAIL & PHONE
Cereal Research Institute (NCRI	Badeggi. Niger State	for GMO plants (NEWEST RICE					Director (Principal Investigator)	
National Root Crops Research Institute (NRCRI)	NRCRI Ikot Ekpene road Umudike, Umuahia, Abia State	Have tested for GM Cassava resistant to *Cassava Mosaic Virus* and *Brown Streak Virus*			Yes		Executive Director (Principal Investigators)	
Federal University of Technology	FUTA Akure, Ondo State				Yes			

ANNEX 2

Table 2: List of crops that have been genetically modified, their events and unique modifiers and various protocols.

CROP	EVENT/UNIQUE IDENTIFIER	EVENT DESCRIPTION	NUMBER OF PCR PROTOCOLS	PROTOCOL DESCRIPTION	NUMBER OF ELISA PROTOCOL	PROTOCOL DESCRIPTION	NUMBER OF STRIP-TEST PROTOCOL	PROTOCOL DESCRIPTION
Maize	NK603 and MON 89034xNK603	Herbicide tolerance						
Cotton	MON 15895	Insect resistance						
Rice	NEWEST	(i) Nitrogen use efficiency (ii) Water efficiency (iii)salt tolerance						
Cassava	Stacked with Beta-Carotene trait	Vitamin A level increase						
Cassava	AMY3 RNAi lines	Modified to obtain storage roots with lower post-harvest physiological						

		degradation after harvest						
Cowpea	IT86D-1010	Insect Resistance						
Sorghum	ABS 321	Iron and zinc bioavailability						
			) is to reduce starch breakdown in storage roots of cassava after pruning the shoots, prior to harvest of the crop. The objective is					