NATIONAL BIOSAFETY AUTHORITY

GUIDELINES FOR THE TESTING OF GENETICALLY MODIFIED ORGANISMS IN KENYA

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FOREWORD

The National Biosafety Authority (NBA) is a state corporation mandated (under the Biosafety Act, 2009) to exercise general supervision and control over the transfer, handling and use of Genetically Modified Organisms (GMOs) with a view to ensuring safety of human and animal health and provision of adequate protection to the environment. As part of its mandate, the Authority conducts routine market surveillance and inspection of consignments at border points to ensure that no unauthorized GMOs are imported into the country or placed in the market.

The Authority is committed to ensuring that samples collected during market surveillance and from consignments at entry and exit points are representative of the entire consignment. This is critical as results obtained from the sample should warrant a generalization of the entire consignment. To fulfill these commitments, the NBA will comply with all statutory rules and accepted codes and practices relating to sample collection and processing of samples for laboratory analysis.

In line with this, the NBA has developed testing guidelines that will guide laboratory personnel during analysis of the collected samples from routine market surveillance, inspection of consignments at the ports of entry and exit points as well as privately submitted samples from commodities of interest using DNA and protein-based testing methods. This guideline provides the basis for setting up valid testing protocols to be used during the analysis of the samples for possible GMOs detection. The guidelines are compliant with national and other internationally accepted Codes of Practice.

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DEFINITION OF TERMS

The following terms and definitions apply for the purpose of this guideline;

Analytical/working sample: Homogenized laboratory sample, consisting either of the whole laboratory sample or a representative portion thereof.

Consignment: quantity of some commodity delivered/dispatched at one time and covered by one set of documents. The consignment may consist of one or more lots or part(s) of lots.

Detection means determination of whether a product is GM or not. For this purpose, a general screening method can be used and the result is either positive or negative.

Genetically modified organism: means an organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology techniques;
'Modern Biotechnology' includes the application of-

a) in-vitro nucleic acid techniques including the use of recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles; or
b) fusion of cells beyond the taxonomic family, that overcome natural physiological, reproductive and recombinant barriers and which are not techniques used in traditional breeding and selection.

Identification: means finding out which GM events or products are present and whether they are authorized or not in the country.

Laboratory/submitted sample: Quantity of product taken from the bulk sample intended for laboratory inspection and testing.

Lot: An identifiable quantity of a commodity delivered/dispatched at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor or markings.

Sample: means One or more sampling units selected from a population according to some specified procedure

Quantification means determining the amount of GM threshold in each product.

ACRONYMS

DNA	Deoxyribonucleic acid
GMO	Genetically Modified Organism
ISO	International Organization for Standardization
NBA	National Biosafety Authority
SD	Standard Deviation
LFD	Lateral Flow Device
ELISA	Enzyme Linked Immunosorbent Assay
LAMP	Loop-Mediated Isothermal Amplification
PCR	Polymerase Chain Reaction
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
qPCR	Real Time Polymerase Chain Reaction

CHAPTER ONE

1.0 NATIONAL BIOSAFETY AUTHORITY

1.1 Background information

The National Biosafety Authority (NBA) is a state corporation in Kenya mandated to ensure safety of human and animal health and provide adequate protection of the environment from harmful effects that may result from genetically modified organisms (GMOs).

The Authority was established pursuant to the provisions of the Biosafety Act, 2009 to regulate all activities involving GMOs in food, feed, research, industry, trade and environmental release and it fulfills its mandate by ensuring and assuring safe development, transfer, handling and use of GMOs in Kenya.

NBA has made great strides in establishing strong Biosafety framework in Kenya by developing and publishing the implementing Biosafety Regulations. These regulations laid down a clear procedure on handling GMOs whether plants, animals or microorganisms. NBA is the National Focal Point for the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD) and is mandated to implement the provisions of the Cartagena Protocol on all Biosafety matters pertaining to GMOs.

1.2 Vision Statement

A World-class Biosafety Agency

1.3 Mission Statement

To ensure and assure safe development, transfer, handling and use of genetically modified organisms (GMOs) in Kenya.

1.4 Our Core Values

a) Good governance & Integrity,
b) Professionalism,
c) Customer Focus
d) Inclusiveness.

1.5 Our Objectives

a) To facilitate responsible research and minimize risks that may be posed by genetically modified organisms;
b) To ensure adequate level of protection in the development, transfer, handling and use of genetically modified organisms that may have an adverse effect on the health of the people and the environment; and
c) To establish a transparent, science-based and predictable process for reviewing and making decisions on the development, transfer, handling and use of genetically modified organisms and related activities.

1.6 Our Core Functions

The Biosafety Act no.2 of 2009 lists the functions of NBA as follows:

a) Consider and determine applications for approval for the development, transfer, handling and use of genetically modified organisms, and related activities in accordance with the provisions of the Biosafety Act;
b) Co-ordinate, monitor and assess activities relating to the safe development, transfer, handling and use of genetically modified organisms in order to ensure that such activities do not have adverse effect on human health and the environment.
c) Co-ordinate research and surveys in matters relating to the safe development, transfer, handling and use of genetically modified organisms, and to collect, collate and disseminate information about the findings of such research, investigation or survey;
d) Identify national requirements for manpower development and capacity building in biosafety;
e) Advise the Government on legislative and other measures relating to the safe development, transfer, handling and use of genetically modified organisms;
f) Promote awareness and education among the general public in matters relating to biosafety; and
g) Establish and maintain a Biosafety clearing house (BCH) to serve as a means through which information is made available to facilitate exchange of scientific, technical, environmental and legal information on, and experience with, Genetically Modified Organisms;
h) To exercise and perform all other functions and powers conferred on by the Act.

CHAPTER TWO

2.1 OVERVIEW OF TECHNIQUES USED IN MODERN BIOTECHNOLOGY

Introduction

Modern Biotechnology or genetic modification (GM) is one of the available tools that can increase agricultural production. The increased productivity using this tool has led to a rapid global adoption of biotechnology. According to the 2018 International Service for the Acquisition of Agribiotech Applications (ISAAA) biotech crops report, more than 17 million farmers, in 26 countries planted 191.7 million hectares (479.25 million acres) of biotech crops in 2018. The four major biotech crops globally are soybeans, maize, cotton, and canola (ISAAA Brief No 54). Information on approved GMOs can be obtained in the Biosafety Clearing House (BCH) of the Convention of Biological Diversity (CBD) via link www.https://bch.cbd.int/.

At the national level, by end of 2022, Kenya had approved 40 contained use experiments and 15 confined field trials. The Authority has also given approval for full commercialization of Bt. Cotton and Bt maize and limited approval for environmental release Virus resistant Cassava for conducting national performance trials (NPTs) in readiness for commercialization. Updated information on approved GMOs in Kenya is available via link http://ke.biosafetyclearinghouse.net/approvedgmo.shtml.

Transgenic organisms are characterized by the insertion of a new gene or sets of genes into their genome. The new genes translate and new protein is expressed and this gives the organism new characteristic. Genetically modified organisms (GMOs) are indistinguishable from their non-GM counterparts to the naked eye.

The basis of testing is to exploit the differences between conventional and transgenic organism. Testing methods focuses on the genes (DNA) introduced or the proteins produced by the introduced gene. Importance of detection methods to producers of GMO is to assure purity and segregation of products and to enable the tracing of genetic modification in breeding. To food and feed industry, it is important for seed companies to assure purity and segregation of products and ensure compliance with legislation. On the other hand, to Competent (enforcement) authorities, testing is vital in product control, compliance with legislation and to be able to retrieve specific products e.g. if marketing permission withdrawn.

The general testing procedure entails:

1. **Detection** which entails determination of whether a product is GM or not. For this purpose, a general screening method can be used and the result is either positive or negative.

2. **Identification** serves to find out which GM events or products are present and whether they are authorized or not in the country.

3. **Quantification**: If a crop or its product has been shown to contain GM, then it is necessary to assess compliance with the threshold regulation determining the amount in each product.

2.2 Scope

This guideline provides guidance for detection, identification and quantification of genetic modifications in samples submitted from routine market surveillance, inspection of consignments at the ports of entry and exit points as well as privately submitted samples from commodities of interest using DNA and protein-based testing methods.

2.3 Objectives

The main purpose of this guideline is to guide the Authority on how to process and analyze samples collected during routine surveillance and cargo clearance or any other sample submitted to the molecular laboratory by a client for analysis. The specific objectives include:

i). To ensure representative samples are appropriately prepared for genetic modification testing;

ii). To inform selection of a suitable technique for testing of genetic modification for valid and reproducible results;

CHAPTER THREE

3.1 SAMPLE MANAGEMENT AND PREPARATION

3.1.1 Sample Management

All samples submitted to the Laboratory should be accompanied by the official sample submission form (Annex 2 of sampling guidelines) requesting an examination in either hardcopy or electronic format.

The laboratory personnel will verify the documentation for completeness and conformance to the sample submitted. The laboratory personnel should inventory the sealed sample packages and compare the number and descriptions of the samples received with the information in the official submission form to ensure that all samples are present. If there is a discrepancy in the number or description of the samples, the submitter should be contacted to clarify and reconcile the discrepancy.

Furthermore, the condition of the sample packaging should be evaluated. If the sample packaging has been compromised or damaged during shipping, for example a tear in the packaging that resulted in the cross-contamination of samples, then this must be recorded. If the compromised packaging affects the suitability of a sample to be tested then laboratory personnel should contact the submitter to inform them. This information should also be recorded.

Following the verification of documentation, a verification of the sample size has to be made. The sample is weighed and a note is made if the weight of the sample is in accordance with the specifications set out by the laboratory. If the sample size is too small, this will be documented and the submitter will be informed for remedial measures. If the sample size is larger than the specifications set out by the laboratory, then a representative mass reduction may need to be carried out in accordance with laboratory manual to obtain a suitable laboratory sample. In either case, a note would have to be issued in the laboratory sample register.

3.1.2 Sample Preparation in the laboratory

Samples are submitted to the laboratory for testing in their raw form, therefore processing have to take place in order to prepare the sample for analysis. The procedures for sample preparation is important in obtaining reliable results regarding the presence of GMOs in a sample. Therefore, proper procedures have to be followed in order to ensure that the sample obtained is homogeneous and representative of the original submitted sample.

3.2 Preparation of the analytical sample

The laboratory sample shall be thoroughly homogenized prior to any division procedure intended to obtain the analytical sample according to Sample Homogenization Standard

Operating Procedure). The analytical sample is prepared from the laboratory sample by grinding, if necessary, and homogenization. Grinding should be performed in a physically separated area to prevent contamination of other rooms or analytical laboratories.

In the case of material composed of discrete entities, care should be taken to ensure that the particle size is reduced to the appropriate size.

Appropriate apparatus for grinding and reduction of the particle size, such as grinders, laboratory mills and blenders and dispersing devices, should be used prior to homogenization. Apparatus and equipment used to prepare the analytical samples shall ensure that the analytical sample is homogenous. Test portions shall be prepared according to applicable SOP of the analytical technique

CHAPTER FOUR

4.0 OVERVIEW OF DETECTION, IDENTIFICATION AND QUANTIFICATION OF GENETICALLY MODIFIED ORGANISMS

General introduction to GM detection methods

The methodologies for detection and identification of GMOs range from qualitative methods that detect the presence of GMOs (detection), to tests that identify individual GMOs (identification), to quantitative tests that measure the percentage of GMOs present in a sample (Quantification).

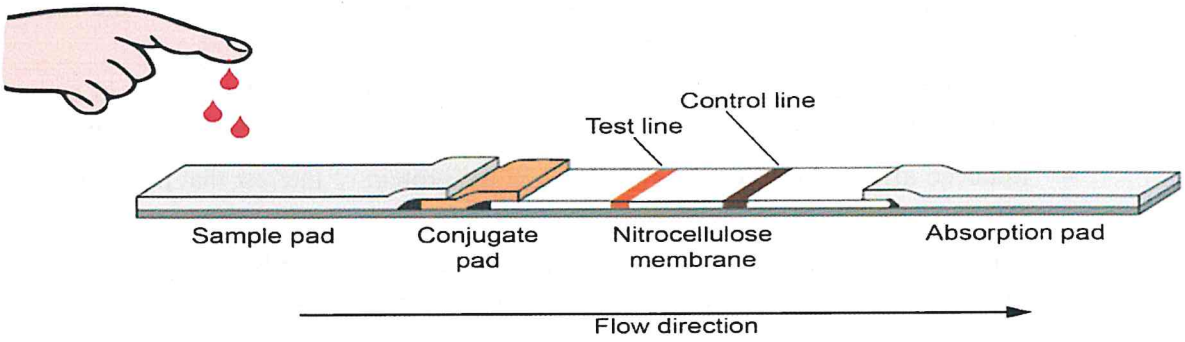
Below is a brief overview of some of the commonly used DNA and protein-based detection methods.

4.1. Protein-based methods for GMO detection

GMO specific proteins (i.e., those produced by the inserted genes) can be detected by antibody recognition of an epitope specific to the transgenic protein. The methods of protein testing are either in the form of a lateral flow strip test, a micro-well format as an enzyme-linked immunosorbent analysis (ELISA) or a polyacrylamide gel electrophoresis protein immunoblot (also known as western blot).

4.1.1 Lateral Flow Assay/ Rapid Testing Kits

A lateral flow assay is a simple qualitative detection method that is based on the use of immunochromatography to confirm the presence or absence of a specific analyte. The assay is available in the form of a test strip impregnated with antibodies that recognize and bind to a specific antigen that is the target of the testing.



Example of a Lateral Flow strip

The advantages of this qualitative method are that it is simple to perform, requires little technical expertise or equipment and can be performed at the site of sampling. Electronic devices have also been developed that allow a semi-quantitative interpretation of the result.

The disadvantages of this test are i) It does not detect inserted genetic elements that do not produce a protein, such as regulatory sequences, ii) genetic modification may not always lead

to a production of a new protein i.e., RNAi, iii) it is a qualitative test that doesn't quantify the protein, iv) high chances of false positives due to antibodies binding to antigens to structurally similar to target antigens in a sample.

4.1.2 ELISA:

The Enzyme-Linked Immunosorbent Assay (ELISA), is qualitative and quantitative detection method based on antigen-antibody reaction that has been widely applied to evaluate the expression level of the protein(s) synthesized by the newly introduced gene. Given the nature of the ELISA test, it is possible to quantify the amount of antigen present in a sample since the intensity of the colour change observed within the sample is directly proportional to the concentration of the antigen. The advantages of ELISA are simplicity, reasonable sensitivity, cost-effective, process large number of samples, rapid, and can be automated. The disadvantages of ELISA are similar to those of lateral flow assay.

4.1.3 Western blot:

Western blot is another protein-based assay that can be used for GMO detection that is however not commonly used in regulatory settings due to its lengthy hands-on procedure. This method is sensitive and may detect different isoforms of the target protein. However, it has a disadvantage of the primary antibody potentially cross-reacting with native forms of the same protein that may be present in the organism.

Limitations of protein-based detection method

Protein based analysis can be a quick and easy tool that can be used to detect and identify GMOs. However, there are some limitations to its use including;

- It cannot distinguish between different GMO events that may be present in a single sample since different tests are required for detection of individual GMO traits.
- Reduced affinity between the antibody and the protein of interest that may arise from excessive sample processing;
- False positivity can arise due to antibodies binding to unintended antigen that may be structurally similar to antigen contained within the protein of interest; and
- Unavailability of commercial antibodies occasioned by lack of demand for such antibodies, are costly and have a short shelf-life.

4.2 DNA-based methods for GMO detection

DNA-based methods for GMO detection and identification are based mainly on the use of the Polymerase Chain Reaction (PCR). PCR is a method that employs synthetic DNA oligonucleotides, called "primers", to replicate or "amplify" targeted regions of an inserted DNA sequence that is present in the GMO. The amplified product can then be detected to determine whether the target sequence of an GMO is present in a sample.

DNA-based detection methods include; polymerase chain reaction (PCR) and Reverse transcriptase polymerase chain reaction (RT-PCR), and more recently quantitative real time PCR (qPCR), microarrays, and high-throughput sequencing. The testing methods mentioned above have revolutionized GMO detection as they are more sensitive, rapid or can detect many target sequences at the same time. However, they require lab facilities, specialized staff, costly reagents, and equipment. Alternatives advanced technologies for GMO detection includes micro-arrays, multiplex PCR, LAMP (loop mediated isothermal amplification), droplet digital PCR, NGS technology, and DNA fingerprinting.

Conventional or end-point PCR is a qualitative method that requires post-PCR step for the detection of the genetic modification through the process of agarose gel electrophoresis.

Real-time PCR technology allows the detection of the amplified target sequence during the PCR amplification process using either a fluorescent DNA binding dye or fluorescence tagged probe. The DNA binding dye simply detects the level of PCR amplification but does not discriminate between specific and non-specific amplification. In contrast, the use of a fluorescent probe can be used to verify that the specific target sequence was amplified during the PCR process. If real-time PCR technology is used in conjunction with the necessary standards, the percentage content of an GMO event in a sample can be determined.

PCR has the advantage that it can be used to screen a sample for the presence GMOs by using primers that target sequences that are commonly found in a variety of different GMOs. Depending on the combination of primers used, the PCR detection can be gene-specific, construct-specific or event-specific. The differences between each type of PCR target region is represented in Figure 1. The advantage of PCR technology is that it is versatile and can be used to simultaneously screen a sample for GMO content, identify the specific GMO gene or event present and, when used in a real-time PCR platform, quantify the amount of GMO present in the sample. The disadvantage of a PCR based approach is that it requires specialized expertise and equipment. Refer to the PCR SOP.

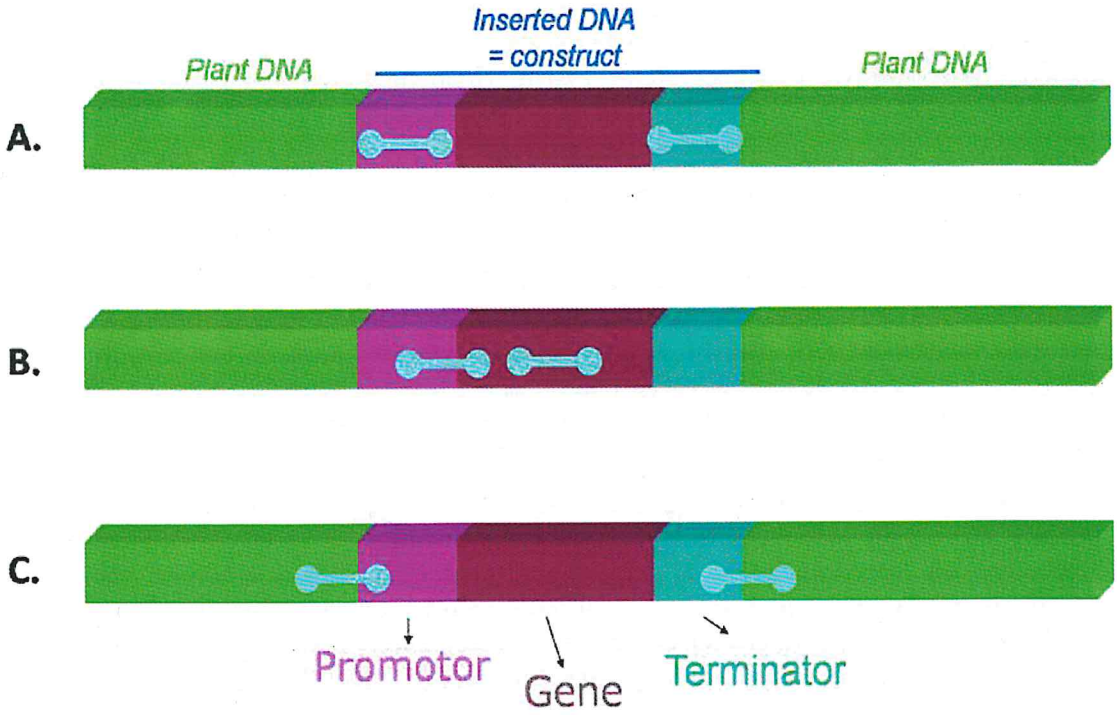


Figure 1: Different types of PCR target regions A) common regulatory elements (such as promoters, terminators) B) gene-specific or construct-specific (junction between two genetic elements within the construct) C) event-specific (junction between the inserted construct and the plant genome)

Table1: GMO Detection methods and their attributes

Detection method	**Target genes/protein**	**Sensitivity**	**Adaptable in the field environment**	**Sample type**	**Time to results**	**Cost**
LFD	single	Medium	Yes	protein	15-20 mins	Low
ELISA	single	Medium	No	protein	2 days	Low
Western Blot	single	High	No	protein	2 days	Medium
PCR	Single/multiplex	High	No	DNA	5 hours	High
QPCR	Single/Multiplex	High	No	DNA	3 hours	High
LAMP	Single/multiplex	High	Yes	DNA	30 mins	High
Microarray	Many	High	No	DNA/RNA	2 days	High

CHAPTER 5

QUALITY ASSURANCE AND QUALITY CONTROL STANDARDS

5.1. Introduction

An important aspect when establishing capacity for the detection, identification, and quantification of GMOs is the implementation of a laboratory management system that ensures the provision of minimum performance criteria with respect to quality control and quality assurance (QA/QC) to confirm the adequate handling and processing of samples, as well as to ensure the quality of and confidence in the results obtained. While there is a wide variety of methodologies and instruments that can be used in a molecular biology laboratory for the detection and identification of GMOs, it is important to select and implement methods that will produce reliable and consistent results, while, at the same time, meeting minimum performance criteria.

To conform with the international standards to ensure quality of the results of laboratory analyses, standard operating procedures (SOPs) have been put in place for the following aspects:

i. Validation of different protocols - all detection, identification and quantification methods should be validated before adoption.

ii. Lateral flow detection (LFD) assay

iii. ELISA (direct and indirect as a single document)

iv. Conventional PCR and Real-time qPCR and LAMP

v. Test result reporting procedure

vi. Laboratory safety manual

vii. Sample Homogenization

viii. Sample extraction procedure

5.2. Handling inconclusive and disputed test results

In cases where test results are inconclusive, the laboratory shall in the first instance conduct a re-test using the remnant laboratory sample.

In cases where test results are disputed by the client, the laboratory shall conduct a re-test using the duplicate/reference sample in instances where the sample was collected by NBA Biosafety Inspectors. If these results are further disputed, or instances of private samples, the

sample shall be sent to another accredited laboratory for confirmatory test at the expense of the client. Re-sampling of consignment shall not be conducted to confirm previous test results.

6.0. REVIEW OF THE GUIDELINES

These guidelines will be reviewed every three years or as need arises.

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