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MEETING OF THE PARTIES TO THE CARTAGENA
PROTOCOL ON BIOSAFETY

Seventh meeting

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Item 16 of the provisional agenda*

**SUMMARY OF THE ACTIVITIES UNDER THE ELECTRONIC NETWORK
OF LABORATORIES FOR THE DETECTION AND IDENTIFICATION OF
LIVING MODIFIED ORGANISMS (2012-2014)**

INTRODUCTION

1. In paragraph 5 of decision BS-V/9, Parties were invited to nominate national and international reference laboratories with the view to establishing, through the Biosafety Clearing-House (BCH), an electronic network of laboratories to facilitate the identification of living modified organisms (LMOs) as well as the sharing of experiences.
2. Furthermore, paragraph 1 (c) of the same decision requested the Executive Secretary to organize regional workshops for heads of laboratories involved in the detection of LMOs to exchange information and experiences on the implementation of relevant standards and methods.
3. The Network of Laboratories for the Detection and Identification of Living Modified Organisms (hereinafter the “Network”) was launched in March 2012. A report of the activities of the Network was submitted to the sixth meeting of the COP-MOP summarizing the series of discussion groups held with the aim of (i) sharing information and experiences; and (ii) identifying challenges in the detection of LMOs.¹
4. Section I of this document presents an overview of the Network and section II summarizes the activities of the electronic network of laboratories during the 2012 to 2014 intersessional period.

* UNEP/CBD/BS/COP-MOP/7/1.

¹ The 2012 discussions are summarized in document UNEP/CBD/BS/COP-MOP/6/INF/9, available at <http://www.cbd.int/doc/meetings/bs/mop-06/information/mop-06-inf-09-en.pdf>.

I. OVERVIEW OF THE NETWORK

5. Following the sixth meeting of the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol, taking into account that the detection and identification of LMOs is a cross-cutting issue with relevance to several provisions of the Protocol, a new section on “Sampling, Detection and Identification” was created under the key Protocol issues in the BCH. The Network was moved from the “Portal on the Handling, Transport, Packaging and Identification of LMOs”,² where it was originally created, to the new section on “Sampling, Detection and Identification”.

6. In February 2013, the Secretariat issued a notification³ announcing that the Network would resume its activities and inviting Parties, other Governments and relevant organizations to send nominations of representatives of laboratories to participate in the Network.

7. The number of participants in the Network has since increased from 30 to 88, and nominations continue to be accepted on an ongoing basis.

II. SUMMARY OF ACTIVITIES OF THE NETWORK

A. *Compilation of tools and guidance on the detection and identification of LMOs*

8. The Network resumed its activities with a view to making progress toward the implementation of the Strategic Plan for the Cartagena Protocol on Biosafety for the period 2011-2020, in particular its operational objectives 1.6, 1.8 and 2.3 addressing the issue of LMO detection and identification.⁴

9. The first series of online discussions during this intersessional period was held from 20 May to 28 July 2013 with the specific objectives to (i) develop a strategy to address Parties’ needs regarding detection and identification of LMOs, to be presented as a set of recommendations for consideration of the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety (COP-MOP) at its seventh meeting; and (ii) compile laboratory methods and tools for the detection and identification of LMOs, in particular LMOs that are unauthorized or unintentionally released into the environment.

10. With a view to initiating the discussions towards achieving the objectives above, the following topics were selected by the Secretariat for discussion by the Network as relevant to the detection and identification of LMOs:

- (a) National regulatory context and current capacity for detecting LMOs;
- (b) Overview of existing networks for LMO detection and identification;
- (c) Compilation of laboratory methods for the detection and identification of LMOs;
- (d) Access to DNA sequence information and reference material;
- (e) Specificity, sensitivity and costs of established methods for LMO detection;
- (f) Emerging techniques for LMO detection;

² http://bch.cbd.int/protocol/cpb_art18.shtml.

³ Notification SCBD/BS/CG/KG/da/77120 available at <http://www.cbd.int/doc/notifications/2013/ntf-2013-015-bs-en.pdf>.

⁴ Available at http://bch.cbd.int/protocol/issues/cpb_stplan_txt.shtml.

(g) Challenges and progress in the detection of LMOs unintentionally released into the environment and unauthorized LMOs.

11. The discussions and their summaries are available through the BCH at http://bch.cbd.int/onlineconferences/portal_detection/discussions.shtml.

12. Further to the initial series of online discussions, the “Workshop of the Network of Laboratories for the Detection and Identification of Living Modified Organisms” was held at the European Commission’s Joint Research Centre (JRC), Institute for Health and Consumer Protection in Ispra, Italy, from 25 to 27 November 2013, with view to developing the following:

(a) A detailed implementation strategy for the detection and identification of LMOs consisting of a plan of action to assist Parties in making progress toward the outcomes of the Strategic Plan; and

(b) A set of recommendations identifying possible key players and specific activities to assist in the implementation of the plan of action in (a) above.

13. In line with Operational Objectives 1.6, 1.8 and 2.3 of the Strategic Plan, among their conclusions and recommendations, the participants of the workshop agreed on the urgent need to compile technical tools and guidance that could assist Parties in the detection and identification of LMOs in the context of the Protocol.

14. Furthermore, the participants of the workshop also agreed that the online Network could start compiling tools and guidance prior to the seventh meeting of the COP-MOP on the following topics:⁵

(a) Overview of available detection methods, including validated methods;

(b) Overview of available databases for methods and sequence information, and available screening matrixes;

(c) Minimum performance criteria for sample handling, extraction, detection and identification methodology;

(d) Experience and case-studies on detection and identification.

15. Participants of the workshop also elected moderators from among themselves to draft introductory texts for each of the topics above and moderate the online discussions for the submission of tools and guidance.

16. Following the workshop, a second series of online discussions was held from 20 January to 7 April 2014 where participants of the Network were invited to share resources for the development of a compilation of appropriate tools and guidance for each of the topics referred to in paragraph 14 above.

17. A total of 122 resources representing a wide array of tools and guidance on the detection and identification of LMOs were proposed during the online discussions.

18. The introductory texts produced by the moderators and the resources submitted by the Network during the online discussions were compiled by the Secretariat as “Technical Tools and Guidance for the Detection and Identification of LMOs” available through the BCH at

⁵ The report of the workshop is published as UNEP/CBD/BS/COP-MOP/7/INF/8 and can be found at <http://www.cbd.int/doc/meetings/bs/mop-07/information/mop-07-inf-08-en.pdf>

http://bch.cbd.int/protocol/cpb_detection/toolsandguidance.shtml both in an online format as well as in an offline format for download.

B. Conclusions and recommendations

19. The final series of online discussions in this intersessional period was held from 30 June to 11 July 2014 in which the Network was invited to consider and provide feedback on the (i) resulting compilation of tools and guidance, and (ii) recommendations the “Workshop of the Network of Laboratories for the Detection and Identification of Living Modified Organisms”.

20. The participants of the Network were supportive of the technical tools and guidance compiled so far on the topics identified in paragraph 14 above. They also provided some comments and proposals for improving the tools and guidance.

21. Furthermore, the Network endorsed the recommendations from the “Workshop of the Network of Laboratories for the Detection and Identification of Living Modified Organisms” for consideration by the Parties at their seventh meeting.

22. The recommendations, as endorsed by the Network, include suggestions for further development and compilation of tools and guidance, as well as capacity building activities on the detection and identification of LMOs. The recommendations are available in document UNEP/CBD/BS/COP-MOP/7/INF/8.

*Annex I***TECHNICAL TOOLS AND GUIDANCE FOR THE DETECTION AND IDENTIFICATION OF LIVING MODIFIED ORGANISMS*****Introduction and strategic umbrella***

Moderators: Joachim Kreysa, Angela Lozan, Lilian Odongo, and Nisreen Al-Hmoud

Background

Since its invention, the genetic engineering of plants has led to an increasing number of Living Modified Organisms (LMOs) (commonly known as Genetically Modified Organisms or GMOs) being developed. Currently, there are more than 300 LMOs in the research and development pipeline with more than 100 having entered the global market and/or been released into the environment.

In 2000, the Conference of the Parties to the Convention on Biological Diversity (CBD) adopted the Cartagena Protocol on Biosafety (CPB) as an international legally binding instrument that sets the minimum requirements for regulating the transboundary movement of LMOs. The Cartagena Protocol came into force in 2003 and, by May 2014, it was ratified or accessioned by 167 countries⁶. Its objective is to *contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.*

In addition to their obligations vis-à-vis the Protocol, Parties require the capacity to monitor, detect and identify LMOs in such a manner that enables them to meet the requirements of trade agreements, as appropriate.

At its fifth meeting, the Conference of the Parties serving as the Meeting of the Parties to the Protocol (COP-MOP) acknowledged the importance of the detection and identification of LMOs by including the following three outcomes in the Strategic Plan for the Cartagena Protocol on Biosafety to be achieved by 2020:

- Easy to use and reliable technical tools are available for the detection of unauthorized LMOs;
- Guidance is available to assist Parties to detect and take measures to respond to unintentional releases of LMOs; and
- Personnel is trained and equipped for sampling, detection and identification of LMOs⁷.

At its fifth meeting, in decision BS-V/9, the COP-MOP requested the establishment, through the Biosafety Clearing-House (BCH), of an electronic network of laboratories involved in the detection and identification of LMOs and the organization of workshops for heads of detection laboratories. The COP-MOP also requested Parties and encouraged other Governments and relevant organizations to make available to the BCH, inter alia, methods for the detection and identification of LMOs.

To implement the above, the Network of Laboratories for the Detection and Identification of Living Modified Organisms was launched through the BCH⁸. The Network held several series of online

⁶ <http://bch.cbd.int/protocol/parties/>

⁷ bch.cbd.int/protocol/decisions/decision.shtml?decisionID=11066

⁸ Available at http://bch.cbd.int/onlineconferences/portal_detection/discussions.shtml

discussions on various topics that are relevant to the detection and identification of the unintentional transboundary movement of LMOs with the view to facilitate the sharing of information and experiences, identifying challenges in the identification of LMOs and to make progress toward the implementation of the Strategic Plan for the Cartagena Protocol on Biosafety for the period 2011-2020. Furthermore, based on these discussions, a workshop was held in November 2013 and focused on the following objectives:

- (a) A detailed implementation strategy for the detection and identification of LMOs consisting of a plan of action to assist Parties in making progress toward each of the outcomes in the Strategic Plan for the Cartagena Protocol on Biosafety for the period 2011-2020; and
- (b) A set of recommendations identifying possible key players and specific activities to assist in the implementation of the plan of action in (a) above.

The outcomes of the workshop include a list of topics for the compilation of technical tools and guidance to facilitate the detection and identification of LMOs in the context of the Protocol. Participants of the workshop prioritized five of the topics from the list and agreed to develop the technical tools and guidance on these topics prior to the seventh meeting of the COP-MOP. They also agreed that the remaining technical tools and guidance could be addressed after the seventh meeting of the COP-MOP, as appropriate. In addition, the workshop also put forward a set of recommendations for consideration by the COP-MOP at its seventh meeting⁹.

The resources in this portal build on the outcomes of the workshop with the aim to provide Parties with a set of tools and technical guidelines to facilitate the implementation of their obligations *vis-a-vis* the Protocol and their national regulatory requirements regarding the detection and identification of LMOs.

Introduction

A strategy for an efficient sampling, detection and identification of LMOs may start by defining:

1. The national monitoring and control objectives/targets;

Possible targets include the monitoring of (transboundary) movements of authorised LMOs and/or the detection and control of unauthorised LMOs.

2. The perceived, potential, or known parameter that shall be monitored and controlled;

Possible parameters include maintaining a paper trail of any (trans-boundary) movement of material that could be LMO or contain (traces of) LMOs. Details of this paper trail can be agreed upon at the international level. In addition, or alternatively, the parameter of interest could also be the presence or absence of LMO material in sampled consignments. This would require first an appropriate capability of statistically representative sampling and second an appropriate capability to analyse the samples. Depending on the intended control level, the required analytical capability may be qualitative (presence/absence) or quantitative (e.g. if certain threshold require different measures.) The main text of this report relates to the issue of adequate analytical capability – assuming that representative samples of good quality reach the laboratories.

3. The resources (financial, human) that can be made available in the foreseeable future.

Besides the resources required for laboratory testing capacities, countries also need to develop adequate capacities to sample LMOs, including training of their border control officers and field inspectors. The role of sampling is critical in that it determines the quality of the results and the overall outcomes of a system to monitor, detect and identify LMOs. Taking the importance of sampling into account, where

⁹ The report of the workshop is available at: <http://www.cbd.int/doc/meetings/bs/mop-07/information/mop-07-inf-08-en.pdf>.

resources are limited, efforts may be more efficiently allocated if focused on sound field sampling combined with basic, qualitative laboratory capacities.

In facilitating Parties' implementation of the relevant objectives of the Strategic Plan for the Cartagena Protocol on Biosafety the Network of Laboratories for the Detection and Identification of LMOs agreed to develop a set of technical tools and guidance for the detection of unauthorized LMOs and unintentional releases. This involves a living compilation of resources on four prioritised topics, specifically: 1) Overview of available detection methods, including validated methods; 2) Overview of available databases for methods and sequence information, and available screening matrixes; 3) Minimum performance criteria for sample handling, extraction, detection and identification methodology; and, 4) Experience and case studies on detection and identification with the view to assist Parties that are currently building their capacities in this area.

Overview of available detection methods, including validated methods
Moderators: Chris Viljoen, Sarah Agapito-Tenfen, and Gretta Abou-Sleymane

General introduction into GM detection methods

A number of methodologies and techniques are available to detect, identify and quantify living modified organisms (LMOs). These methodologies range from those that are fast and more cost-effective, such as lateral flow tests and endpoint PCR, to those that can be more complex, such as quantitative real-time PCR. When planning and setting up a laboratory for the detection and identification of LMOs a choice must be made regarding which methodologies and protocols will be adopted. For service-oriented laboratories, particularly those servicing regulatory authorities, the selection of methods is guided by, amongst other things, the country's specific regulatory requirements in accordance with national biosafety laws. The methodologies may therefore range from qualitative methods to detect the presence of LMOs, to tests that identify individual LMOs, to quantitative tests that measure the percentage of LMOs present in a sample. Below is a brief overview of some of the more commonly used methodologies for the detection, identification and quantification of LMOs, their strengths and limitations.

Introduction to protein versus DNA approaches to GM detection

LMOs are often developed by inserting one or more “genes of interest” which are DNA molecules encoding proteins that confer particular traits of interest, such as insect resistance or herbicide tolerance. Either the DNA or the protein can be targeted for the detection or identification of such LMOs. Both approaches, i.e. protein- or DNA-based methods, have advantages and disadvantages and the adoption of one over the other, or both, will depend largely on the available expertise, infrastructure to handle samples, laboratory equipment and regulatory requirements.

Protein-based methods for LMO detection

LMO 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 method of protein testing is either in the form of a lateral flow strip test, a micro-well format as an enzyme-linked immunosorbent analysis (ELISA) or a gel electrophoresis protein immunoblot (also known as western blot)

Protein detection using lateral flow strip tests, ELISA or western blot is performed through a simple procedure of extracting total crude proteins from a sample by adding water or buffer followed by sample homogenization. Protein-based detection methods require the use of antibodies to detect the transgenic protein. Since the process of antibody production is extremely complex and costly, detection using these methods typically relies on the availability of commercial antibodies.

For lateral flow strip testing, the strip is placed in the crude protein extract and a positive result is indicated by the appearance of a test line due to the antibody recognition of the transgenic protein. 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 point of sampling. Electronic devices have also been developed that allow a semi-quantitative interpretation of the result. A disadvantage of this method is that its sensitivity is dependent on the binding affinity between the antibody and the protein.

The ELISA based approach to LMO detection follows the same crude protein extraction step as the lateral flow strip. However, the antibody is used to pre-coat the inside of a micro-well plate. Following a series of steps which allow the target protein to bind to the antibody, the cell debris, including other proteins, are removed from the plate through a series of wash steps. The bound protein is detected

through a colour reaction that can be read through visual inspection or by an optical plate reader. This method produces a qualitative result if read through visual inspection. However, a quantitative result can be obtained if the necessary protein standards are included on the plate and an optical plate reader is used to evaluate the intensity of the colour reaction resulting from antibody recognition of the target protein in the LMO. The advantages of ELISA LMO testing are that it can produce a qualitative or quantitative result and is more sensitive than the lateral flow strip method.

For western blotting, the extracted crude proteins are separated according to their size by gel electrophoresis. Following this, the proteins are transferred from the gel to a membrane for detection of the target protein. Usually this step of the process involves two antibodies: firstly a primary antibody that is specific to the target protein followed by a secondary antibody, which is linked to a reporter molecule that binds to the primary antibody. After the excess antibody is removed from the membrane, the secondary antibody is typically visualized by colorimetric, chemiluminescent or fluorescent methods performed by either colouring the membrane itself or exposing it to a light sensitive film, such as x-ray film. Once the membrane or film is developed, the presence of the transgenic protein is indicated as a distinct band on the membrane or film. The advantage of this method is that it is sensitive and may detect different isoforms of the target protein. A disadvantage of this method is that the primary antibody may cross react with native forms of the same protein that may be present in the organism.

DNA-based methods for LMO detection

DNA-based methods for LMO detection and identification are based mainly on the use of the polymerase chain reaction (PCR). PCR is a method that employs synthetic DNA oligonucleotides, so called “primers”, to replicate or “amplify” targeted regions of an inserted DNA sequence that is present in the LMO. The amplified product can then be detected to determine whether or not DNA originating from an LMO is present in a sample.

DNA-based methods require the extraction of DNA prior to PCR detection. It is important to determine which DNA extraction method is appropriate for a particular LMO. While most extraction methods employ a cetyltrimethylammonium bromide (CTAB) protocol following mechanical homogenization of the LMO, different crop types may require additional steps for optimal DNA extraction. It is also important to note that the pre-analytical sampling regime employed and the amount of laboratory sample used will also impact on the accuracy and sensitivity of LMO detection.

Following the extraction of DNA from a sample, target sequences only found in the LMO are amplified using primers that have been designed to specifically bind the target sequence during the PCR reaction. The resulting PCR product can either be detected in real-time during the amplification process or after the PCR is completed.

Detection for the presence of the target sequence post PCR, also known as end-point PCR, is done through the process of gel electrophoresis where the amplified target sequence is separated based on its size through a gel matrix under the influence of an electric current. The fragment of amplified DNA corresponding to the inserted DNA in the LMO can be visualised through colour detection using a dye that binds to double stranded DNA and fluoresces under ultraviolet light

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 LMO event in a sample can be determined. The overall applicability of this method must be evaluated in terms of the availability of equipment, its practicability and cost efficiency.

PCR has the advantage that it can be used to screen a sample for the presence LMOs by using primers that target sequences that are commonly found in a variety of different LMOs. 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 LMO content, identify the specific LMO gene or event present and, when used in a real-time PCR platform, quantify the amount of LMO present in the sample. The disadvantage of a PCR based approach is that it requires specialized expertise and equipment.

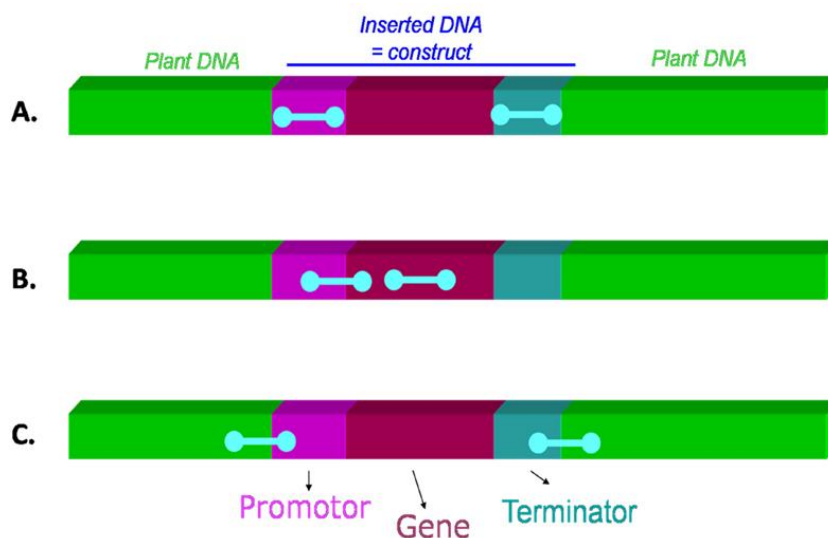


Figure 1: Different types of PCR target regions A) common regulatory elements (such as promoters, terminators) B) gene-specific or construct-specific (junction between two genetic elements within the construct) C) event-specific (junction between the inserted construct and the plant genome)¹⁰

Comparison between protein- and DNA-based methods for LMO detection

Both protein- and DNA-based approaches to LMO detection are useful and each serves different purposes. Protein-based methods, such as strip testing and ELISA, are simple, time efficient (several minutes to a few hours). Strip testing is useful for LMO testing at the point of sampling. However, protein detection of LMOs requires a different test for the detection of individual LMO traits and cannot be used to distinguish between different LMO events that may be present in a single sample. In contrast, western blots and PCR approaches require more steps and time (several hours to days) and rely on specialized laboratory equipment and expertise.

Protein-based methods are only suitable for detecting LMOs developed by inserting DNA fragments that produce a protein, i.e. transgenes, but not for detecting inserted genetic elements that do not produce a

¹⁰ Source: <http://www.nib.si/eng/index.php/aktualno/project/272-gmoseek-better-control-of-genetically-modified-organisms-gmos-for-an-improved-european-food-safety.html>

protein, such as regulatory sequences. Protein-based methods rely on the specific recognition of an antigen in the transgenic protein by an antibody and therefore, any changes in the tertiary structure of the protein renders the method ineffective. Such conformational changes are sometimes induced during sample processing where the samples are subjected to heat and/or chemical treatment. The detection capability in protein-based methods is also affected by the expression level of the transgenic protein that can vary between different parts of the LMO or different stages of its life cycle, and can be influenced by external factors such as climate and soil conditions. In addition, some LMOs have been specifically designed to express the transgenic protein in a specific tissue and may not be necessarily present in the part of the organism being tested.

In contrast, DNA has a greater chemical stability as compared to proteins which allows it to withstand chemical and heat treatments. DNA is also present in all cells and therefore any part of the organism can be used for testing. Furthermore, PCR methods are more versatile than protein methods and PCR can be used to screen a sample for the presence of several potential LMOs simultaneously with relative ease. In addition, PCR can be applied qualitatively, to detect specific genes or events, and quantitatively, to determine the percentage level of a particular LMO event in a sample.

Challenges in LMO detection and new technology developments

There are several considerations and challenges for the application of LMO detection. These include the use of validated methods, availability of reference material for specific LMO events and access to information on the LMO event including sequence data for detection method development. With the development of each new LMO, their sampling, detection and identification becomes more challenging and complex. However, progress in the field of LMO detection technologies will lead towards making LMO detection more accessible.

Method validation and quality control

An important consideration in the application of LMO detection is that standardized operating procedures (SOPs) for validated methods and equipment should be used to ensure that results are reproducible and accurate. It is therefore important that criteria to test the performance of the methods be harmonized internationally to make results comparable throughout the world taking into account the availability of resources in different countries. The development of harmonized performance criteria should aim at simplifying and increasing the accessibility to LMO detection technologies by countries with less capacity and fewer resources. Thus a challenge for LMO detection is to ensure that methods are validated and meet the necessary minimum performance criteria for quality control purposes. This means that the LMO detection laboratory is required to develop and maintain the necessary quality control measures to ensure the reliability, sensitivity and reproducibility of its methods.

Access to information and reference material for method design and validation

Another challenge in LMO detection is the difficulty for LMO detection laboratories to gain access to sequence data for genetic elements inserted into LMOs in order to design detection systems that are specific to a transformation event. While many countries require notifiers to provide a method to detect the LMO, in many cases validation of the method is not required. In addition, most regulatory LMO detection laboratories do not have access to reference material for the specific LMO event in order to validate the detection method. It may be worthwhile for regulatory systems to consider requiring LMO developers to provide reference material to the regulatory laboratory performing LMO detection for each LMO being notified to the regulatory authorities. Furthermore, since it is costly to develop and validate a new method to detect an LMO, developers could be required to provide a method for the detection of an

LMO event, the necessary sequence information so that the method can be verified and to cover, as appropriate, the costs of method validation by the laboratory performing LMO detection as part of the regulatory requirements of a country.

Novel approaches for simultaneous detection of multiple LMOs

The increase in LMO research and commercialization is resulting in a continuous increase in the number of LMO events that detection laboratories must detect. As a result, the potential presence of unapproved LMO events is also increasing and detection laboratories are faced with having to potentially detect multiple LMOs, some of which may be approved while others may be unauthorised or illegal within the same sample. One approach to this challenge is to follow a matrix approach in detecting multiple genetic elements commonly used in the transgene construct in an attempt to widen the screening capabilities. When used in conjunction with bioinformatics software, the matrix results can be used to identify which potential LMOs are present in a sample. This approach can be customized to include as many LMOs as the laboratory is required to detect but is only applicable to PCR-based methods.

Emerging technologies for LMO development and detection methods

New and emerging technologies for developing LMOs are proving to be a challenge to the detection and identification of the LMOs. For example, new gene silencing technologies increasingly include using double stranded RNA (dsRNA) molecules to confer desirable traits in LMOs. In such cases, a transgenic protein is not produced therefore only DNA-based detection methods can be used.

On the other hand, the continued development of new technologies is making LMO detection more readily accessible, especially in countries with fewer resources. For example, digital PCR and other chip-based technologies will soon enable the routine detection of LMOs in the field with hand held devices. While many countries may consider the development of a framework for LMO detection to be a burden, the technology may also be applied to other purposes such as human, plant and animal pathology, amongst others.

Resources

A High-Throughput Multiplex Method Adapted for GMO Detection

A high-throughput multiplex assay for the detection of genetically modified organisms (GMO) was developed on the basis of the existing SNPlex method designed for SNP genotyping. This SNPlex assay allows the simultaneous detection of up to 48 short DNA sequences (70 bp; “signature sequences”) from taxa endogenous reference genes, from GMO constructions, screening targets, construct-specific, and event-specific targets, and finally from donor organisms.

<http://pubs.acs.org/doi/abs/10.1021/jf801482r>

A new dual plasmid calibrator for the quantification of the construct specific GM canola Oxy-235 with duplex real-time PCR

To overcome the difficulties of obtaining the Certified Reference Material (CRM) and according to the key documents of the European Union Reference Laboratory (EU-RL), a new standard reference molecule containing the construct specific of the canola event Oxy-235 (3'-junction Nitrilase/Tnos) and the canola endogenous reference gene (acety-CoA-carboxylase) was constructed and used for duplex real-time quantitative analysis.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=838>

A simple DNA extraction method suitable for PCR detection of genetically modified maize

<http://onlinelibrary.wiley.com/doi/10.1002/jsfa.3026/pdf>

A Strategy for Designing Multi-Taxa Specific Reference Gene Systems. Example of Application—ppi Phosphofructokinase (ppi-PPF) Used for the Detection and Quantification of Three Taxa: Maize (*Zea mays*), Cotton (*Gossypium hirsutum*) and Rice (*Oryza sativa*)

Description of a new strategy for the development of a plant reference gene system that can be used for genetically modified organism (GMO) analysis.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=834>

Advances in molecular techniques for the detection and quantification of genetically modified organisms

Review of new and emerging techniques in the development of analytical methods for GMO screening and quantification.

<http://link.springer.com/article/10.1007%2Fs00216-008-1868-4>

An accurate real-time PCR test for the detection and quantification of cauliflower mosaic virus (CaMV): applicable in GMO screening

A new qualitative and quantitative method based on real time polymerase chain reaction (PCR) techniques was developed for the detection and quantification of CaMV.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=833>

An innovative and integrated approach based on DNA walking to identify unauthorised GMOs

Developed a strategy to identify unauthorised GMOs containing a pCAMBIA family vector, frequently present in transgenic plants.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=845>

Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food products

This review summarises the status of the most widely used GMO analysis technologies, identifies new areas of analytical investigation and discusses current needs and future challenges.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=813>

Applicability of Three Alternative Instruments for Food Authenticity Analysis: GMO Identification

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3065168/pdf/BTRI2011-838232.pdf>

Aspecte metodologice în testarea plantelor modificate genetic (Romanian)

Methodological aspects in testing genetically modified plants (in the Romanian language)

http://www.biosafety.md/public/209/ro/Aspecte_.pdf

Compendium of reference methods for GMO analysis

This "Compendium on Reference Methods for GMO Analysis" aims at providing a technical state of the art of the detection methods applied in GMO analysis that have been validated according to international standards.

http://publications.jrc.ec.europa.eu/repository/bitstream/11111111/22754/1/gmo-jrc_reference%20report_2011_publ.pdf

Detecting un-authorized genetically modified organisms (GMOs) and derived materials

<http://www.sciencedirect.com/science/article/pii/S0734975012000377>

Detection and traceability of genetically modified organisms in the food production chain

Discusses current detection and identification methodologies as well as highlights of new diagnostic methodologies.

<http://www.sciencedirect.com/science/article/pii/S0734975012000377>

Detection of genetically modified plants – methods to sample and analyse GMO content in plants and plant products.

The aim of this report is to uncover and overview existing methods for sampling and analysing GMOs.

http://www.sns.dk/erhvogadm/biotek/REPORT_rev_maj.pdf

Detection of Living Modified Organisms (LMOs) and the Need for Capacity Building

The paper emphasizes on the need of implementing the Biosafety Protocol for the developing countries and suggests to establish capacity building measures. It identifies various ways to detect GMOs and discuss their relevance in the context of developing countries. The paper has elaborated the concept of transgene and provides an overview of GMO regulations across various countries.

http://ris.org.in/images/RIS_images/pdf/vol7no3_article4.pdf

Development and validation of a multiplex real-time PCR method to simultaneously detect 47 targets for the identification of genetically modified organisms

Based on simultaneous 24 multiplex RTi-PCR running on a ready-to-use 384-well plate, this new procedure allows the detection and identification of 47 targets on seven samples in duplicate.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=853>

Development and validation of duplex, triplex, and pentaplex real-time PCR screening assays for the detection of genetically modified organisms in food and feed.

Herein we describe the development and validation of a pentaplex, as well as complementary triplex and duplex real-time PCR assays, for the detection of the most common screening elements found in commercialized GMOs: P-35S, T-nos, ctp2-cp4-epsps, bar, and pat.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=850>

Development and validation of real-time PCR screening methods for detection of cry1A.105 and cry2Ab2 genes in genetically modified organisms

Primers and probes were developed for the element-specific detection of cry1A.105 and cry2Ab2 genes, based on their DNA sequence as present in GM maize MON89034

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=851>

Development of 10 new screening PCR assays for GMO detection targeting promoters (pFMV, pNOS, pSSuAra, pTA29, pUbi, pRice actin) and terminators (t35S, tE9, tOCS, tg7)

In the present study, new real-time PCR screening assays were developed targeting 10 promoter and terminator elements used in genetically modified constructs: pFMV, pNOS, pSSuAra, pTa29, pUbi, pRice actin, t35S, tE9, tOCS, and tg7.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=852>

Development of a Molecular Platform for GMO Detection in Food and Feed on the Basis of “Combinatory qPCR” Technology

Specifically discusses methodologies that are geared towards the development of a testing system that is based on qPCR to detect and identify LMOs.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=754>

Development of a Real-Time PCR Method for the Differential Detection and Quantification of Four Solanaceae in GMO Analysis: Potato (*Solanum Tuberosum*), Tomato (*Solanum Lycopersicum*), Eggplant (*Solanum Melongena*), and Pepper (*Capsicum Annuum*)

This study designs four highly specific TaqMan-MGB probes. A duplex real time PCR assay was developed for simultaneous quantification of tomato and potato. For eggplant and pepper, only simplex real time PCR tests were developed.

<http://pubs.acs.org/doi/abs/10.1021/jf073313n>

Development of a Seven-Target Multiplex PCR for the Simultaneous Detection of Transgenic Soybean and Maize in Feeds and Foods

In this paper, we propose a method for the simultaneous detection of four transgenic maize (MON810, Bt11, Bt 176, and GA21) and one transgenic soybean (Roundup Ready), which allows routine control analyses to be sped up.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=857>

Development of real-time PCR method for the detection and the quantification of a new endogenous reference gene in sugar beet “*Beta vulgaris L.*”: GMO application

Describing a newly developed quantitative real-time PCR method for the detection and quantification of a new specific endogenous reference gene used in GMO analysis

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=837>

Four new SYBRGreen qPCR screening methods for the detection of Roundup Ready, LibertyLink, and CryIAb traits in genetically modified products

In this study, four novel SYBR Green qPCR methods that perform at equal efficiency were developed to allow the detection of the most common GM traits present in genetically modified crops to date

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=844>

ISO 21569:2005 Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- Qualitative nucleic acid based methods

ISO 21569:2005 describes the procedure to qualitatively detect genetically modified organisms (GMOs) and derived products by analysing the nucleic acids extracted from the sample under study. The main focus is on polymerase chain reaction (PCR) based amplification methods.

http://www.iso.org/iso/catalogue_detail.htm?csnumber=34614

ISO 21570:2005 Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- Quantitative nucleic acid based methods

ISO 21570:2005 provides the overall framework of quantitative methods for the detection of genetically modified organisms (GMO) in foodstuffs, using the polymerase chain reaction (PCR).

http://www.iso.org/iso/catalogue_detail.htm?csnumber=34615

Methodological Guidelines 1 (Russian)

Methodical Guidelines for detection and identification developed and approved in the Russian Federation and Belarus (in the Russian Language)

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=818>

Methodological Guidelines 2 (Russian)

Methodical Guidelines for detection and identification developed and approved in the Russian Federation and Belarus (in the Russian Language)

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=819>

Methods for detection of GMOs in food and feed

This paper reviews aspects relevant to detection and quantification of genetically modified (GM) material within the feed/food chain. The GM crop regulatory framework at the international level is evaluated with reference to traceability and labelling. Current analytical methods for the detection, identification, and quantification of transgenic DNA in food and feed are reviewed.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=814>

Minimum cost acceptance sampling plans for grain control, with application to GMO detection

This paper describes a practical way to get the least expensive acceptance sampling plan keeping both the consumer's and the producer's risks below a predetermined threshold. The method is more specially illustrated by examples in GMO detection.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=815>

Molecular Identification of Four Genetically Modified Maize (Bt11, Bt176, Mon810 and T25) by Duplex Quantitative Real-Time PCR

The development of four duplex polymerase chain reaction (PCR) tests for the identification and the quantification of four maize transformation events from which commercial lines have been authorised in Europe namely, Bt11 and Bt176 (Syngenta, DE, USA), Mon810 MaisGard™ (Monsanto, MO, USA) and T25 Liberty Link™ (Bayer CropScience, Monheim, Germany).

<http://link.springer.com/article/10.1007%2Fs12161-013-9667-8>

Monitoring of Genetically Modified Food and Feed in the Tunisian Market Using Qualitative and Quantitative Real-time PCR

The present study aims to check the status of GMO in Tunisian market using qualitative and quantitative real time-PCR (QRT-PCR).

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=835>

New approaches in GMO detection

This paper reviews the latest progress made in GMO analysis, taking examples from the most recently developed strategies and tools, and addresses some of the critical aspects related to these approaches.

<http://link.springer.com/article/10.1007%2Fs00216-009-3237-3>

New approaches in GMO Detection: Detection of unapproved GMOs

Presentation on the detection of unapproved GMOs from the Second International Workshop on Harmonisation of GMO Detection and Analysis, 7-8 February 2012, Nelspurit, South Africa

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=817>

PCR-Based Detection of Genetically Modified Foods

http://ucbiotech.org/resources/methods/GMO_detection.pdf

PCR-Based Detection of Genetically Modified Soybean and Maize in Raw and Highly Processed Foodstuffs

Touches upon the isolation of DNA from processed food samples for the purposes of PCR analysis and detection as well as suggestions for circumventing potential sources of co-extracted PCR inhibitors.

http://www.biotechniques.com/multimedia/archive/00011/01312pf01_11584a.pdf

Practical Experiences with an Extended Screening Strategy for Genetically Modified Organisms (GMOs) in Real-Life Samples

For the present study, the application of an informative detailed 24-element screening and subsequent identification strategy was applied in 50 animal feed samples. Almost all feed samples were labeled as containing GMO-derived materials. The main goal of the study was therefore to investigate if a detailed screening strategy would reduce the number of subsequent identification analyses.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=848>

Production of Certified Reference Materials for the Detection of Genetically Modified Organisms

Discusses some steps in the production of reference materials. This information may be useful for those colleagues who are involved in in-house production of RM of, for example, regionally specific LMOs.

[http://lib3.dss.go.th/fulltext/Journal/J.AOAC%201999-2003/J.AOAC2002/v85n3\(may-jun\)/v85n3p775.pdf](http://lib3.dss.go.th/fulltext/Journal/J.AOAC%201999-2003/J.AOAC2002/v85n3(may-jun)/v85n3p775.pdf)

Qualitative and Quantitative Polymerase Chain Reaction Analysis for Genetically Modified Maize MON863

Qualitative and quantitative analytical methods were developed for the new event of genetically modified (GM) maize, MON863.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=849>

Real-time PCR Verfahren zum Nachweis gentechnisch veränderter Rapslinien mit dem bar/T-g7-Genkonstrukt (German)

Real-time PCR method for the detection of genetically modified oilseed rape lines with the bar/T-g7-gene construct

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=856>

Real-Time PCR zur quantitativen Bestimmung gentechnisch veränderter Rapslinien mit dem 35S/pat-Genkonstrukt (German)

Real-time PCR assay for the quantitation of genetically modified oilseed rape lines with the 35S/pat-gene construct

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=855>

Relative quantification in seed GMO analysis: state of art and bottlenecks

Critical points that affect the expression of the GMO content in seeds are discussed in this paper.

<http://link.springer.com/article/10.1007/s11248-012-9684-1>

Report on the Validation of a DNA Extraction Method for Maize seeds and Grains

http://gmo-crl.jrc.ec.europa.eu/summaries/MON88017_DNAExtr_report.pdf

Sampling and analysis guidelines for the detection of genetically modified flax

http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/Fachmeldungen/GVO_Flax.pdf;jsessionid=6F3DC99F30ECEFE822332E7609D76721.1_cid332?_blob=publicationFile&v=2

Sampling and detection of living modified organisms: Sampling methodology

A presentation on sampling methodologies from the Central and Eastern European Regional Training of Trainers' Workshop on the Identification and Documentation of Living Modified Organisms under the Cartagena Protocol on Biosafety“, Ljubljana, Slovenia, 11-15 April 2011

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=816>

Statistical considerations in seed purity testing for transgenic traits

This paper discusses factors that should be considered when designing and implementing seed purity testing procedures to manage this misclassification risk – especially with regard to the presence or absence of transgenic traits.

<http://www.seeds.iastate.edu/publications/stat/Seed%20Purity%20Testing.pdf>

Statistical Tools for Seed Testing

A collection of statistical programs and modules to assist ISTA members in their routine work and method development and validation.

<http://seedtest.org/en/stats-tool-box-content--1--1143.html>

Testing for adventitious presence of transgenic material in conventional seed or grain lots using quantitative laboratory methods: statistical procedures and their implementation

In this paper, we present the details of procedures specific to quantitative laboratory methods.

<http://www.seeds.iastate.edu/publications/stat/SSR01500197.pdf>

Training Manual on the Analysis of Food Samples for the Presence of Genetically Modified Organisms

The scope of the training courses is to assist staff of control laboratories to become accustomed with molecular detection techniques, and to help them adapt their facilities and work programmes to include analyses that comply with worldwide regulatory acts in the field of biotechnology.

<http://gmo-crl.jrc.ec.europa.eu/capacitybuilding/manuals/Manual%20EN/User%20Manual%20EN%20full.pdf>

Training Manual on the Analysis of Food Samples for the Presence of Genetically Modified Organisms (Arabic)

In 2008, FAO approved a two-year Technical Cooperation Programme (TCP) project in the Near East and North Africa region entitled "Strengthening capacities towards the establishment of a regional platform for the detection of genetically modified organisms", with Jordan, Lebanon, the Sudan, Syria, United Arab Emirates and Yemen as the six participating countries. As part of this TCP project, an advanced training course on "Detection of genetically modified organisms and biosafety for food and agriculture" took place in Aleppo, Syria on 19-24 June 2010, jointly organized by FAO, the International Center for Agricultural Research in the Dry Areas (ICARDA) and the General Commission for Scientific and Agricultural Research (GCSAR). In the context of this training course, a laboratory manual on GMO detection was prepared, edited by A.M. Abdul Kader et al, which is now available on the web.

<http://www.fao.org/biotech/biotech-add-edit-section/biotech-add-edit-news/biotech-news-detail/en/c/168577/>

Validation and collaborative study of a P35S and T-nos duplex real-time PCR screening method to detect genetically modified organisms in food products

In this work the intra and inter-laboratory validation of a duplex real-time PCR screening method for the detection of genetically modified (gm) plants is described.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=846>

Validation of a newly developed hexaplex real-time PCR assay for screening for presence of GMOs in food, feed and seed

Here we describe the development and validation of a hexaplex real-time polymerase chain reaction (PCR) screening assay covering more than 100 approved GMOs containing at least one of the GMO targets of the assay

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=854>

Overview of available databases of methods, and screening matrices for the detection of living modified organisms

Moderators: Bjørn Spilberg, and Lutz Grohmann

Databases of methods for the detection of living modified organisms

Databases containing accurate and reliable information on methods, reference materials and DNA sequences, if available, are an important tool to enable countries to effectively detect and identify Living Modified Organisms (LMOs). To be useful to the public at large, such databases must, at a minimum, be available online and open to the public, contain accurate and up-to-date information, and have user-friendly mechanisms for searching and retrieving information. The scope of the database may vary in the type of methods (e.g. DNA and/or protein; validated or not), type of LMOs (e.g. crops, vaccines, etc) or geographic area (e.g. local or global).

Below is information on databases that meet these criteria. They include databases of detection methods and reference materials.

*European Union Database of Reference Methods for GMO Analysis*¹¹

This database was developed by the Joint Research Centre's European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF). It is maintained by the EURL-GMFF in collaboration with the European Network of GMO Laboratories (ENGL). This database contains fully validated detection methods for LMOs using, mostly, real-time PCR methods as well as some gel-based PCR methods. The collection includes methods that are event-specific, construct-specific and element-specific methods for screening as well as plant taxon-specific methods for species identification. For each method a complete protocol with the information needed to conduct the test and all the validation data are provided.

Event-specific detection methods validated by the EURL-GMFF can also be found by searching the "Status of Dossiers" list on the EURL-GMFF website.¹²

*GMO Detection Method Database (GMDD)*¹³

GMO Detection Method Database (GMDD) was developed and is maintained by the GMO Detection Laboratory at Shanghai Jiao Tong University in China. It contains information on event-specific detection methods for many LMOs, as well as real-time PCR and gel-based PCR methods that are element-specific, gene-specific or reference gene specific. Some protein-based methods are also included. Not all the methods in the database are validated through collaborative trial studies, however, if available, information on the validation status of a method is provided. The primer and probe sequences are provided with citations to the relevant publications. The database also includes a large collection of publicly available LMO sequence information. In addition, links to GenBank and other publications containing information on the sequence of the inserts is provided. Finally, there is also information on relevant certified reference materials (CRM), if available.

¹¹ The event-specific detection methods are available at: <http://gmo-crl.jrc.ec.europa.eu/gmomethods/>

¹² The compendium of detection methods is available at: <http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>

¹³ The GMO Detection Method Database is available at: <http://gmdd.shgmo.org/>

*CropLife International Detection Methods Database*¹⁴

This database is maintained by CropLife International, a global federation of the plant biotechnology industry, and contains both DNA- and protein-based methods. It currently contains approximately 40 methods related to about 27 LMOs. The detection methods available have been developed and validated by the technology providers for their own proprietary technologies and products. Not-for-profit laboratories have free access to the methods, whereas laboratories that undertake “fee-for-service” testing must request a license to access the methods.

Screening matrices for the detection of living modified organisms

The number of commercially available LMOs is huge and is growing at the global scale. It is therefore not efficient to only employ event-specific methods when testing for LMO presence. Fortunately, there is a considerable body of knowledge in the scientific literature that contains valuable information on strategies that have been developed for the identification of LMOs. The most cost efficient alternative strategy to detect the potential presence of an LMO in a sample is to use a screening approach which employs the combination of element-specific detection methods selected on the basis of information on presence or absence of the target in a list of LMOs.

There are two different types of commonly used matrices as described in the recently drafted European Technical Specification for the application of PCR-based screening strategies using the so called “matrix approach”. According to this document, the first type of screening matrix is the “GMO method matrix”, is defined as a relational presentation, for example a table, of symbols or numbers, where the genetic elements and genetic constructs that are detected by a defined PCR method and the corresponding LMOs are tabulated. The symbols (“+” or “-”, or numbers) indicate whether or not the target sequence is detectable by the specified method in a given LMO.

The second type of screening matrix, the “GMO target matrix”, tabulates only information about the presence of genetic elements or genetic constructs in LMOs. The entered symbols or numbers indicate the presence or absence of the target sequence and copy number, if available, in a given LMO event. In contrast to “GMO method matrices”, “GMO target matrices” are independent from a particular detection method.

*German laboratory network Screening Table*¹⁵

The German laboratory network has developed a “GMO method matrix” (also known as the Waiblinger Table), which is based on five methods targeting specific genetic elements and constructs that are most frequently present in commercialized LM crops. It comprises a set of real-time PCR methods to detect:

1. the Cauliflower Mosaic Virus 35S promoter (P-35S);
2. the *nos* terminator derived from *Agrobacterium tumefaciens* (T-nos);
3. the *ctp2-cp4epsps* junction of the chloroplast-transitpeptide (CTP2) from *Arabidopsis thaliana* and the *epsps* gene from *A. tumefaciens* strain CP4 (*epsps*);
4. the *bar* gene from *Streptomyces hygroscopicus*; and
5. a sequence from the P35S-pat junction of the CaMV P-35S promoter and the synthetic pat gene.

¹⁴ CropLife International Detection Methods Database is available at: www.detection-methods.com

¹⁵ The Waiblinger Screening Table is available at:

http://www.bvl.bund.de/SharedDocs/Downloads/09_Untersuchungen/screening_tabelle_gvoNachweis.xls?blob=publicationFile&v=2

All five methods have been fully validated and are included in the EURL-GMFF method database. Furthermore a table listing the accessibility of publicly available reference materials and their sources is provided. The Screening Table is maintained by the German NRL and the Excel spreadsheet with implemented filter-functionalities and a list of available LMO reference materials can be downloaded from the internet.¹⁶.

*GMOseek matrix*¹⁷

The GMOseek matrix is a ‘GMO target matrix’ and provides a comprehensive and user-friendly overview of 273 genetic elements and their occurrence in 328 LMOs⁵. The GMOseek matrix is freely available online as an Excel spreadsheet. Filtering functions allow users to search for events that fit into a defined pattern of genetic elements based on their absence or presence in an LMO. It also helps users in identifying genetic elements that could be targeted during the screening phase of LMO analysis.

*JRC GMO-Matrix*¹⁸

The JRC GMO-Matrix is a “GMO method matrix”. It takes advantage of the DNA sequence information compiled in the JRC’s Central Core DNA Sequence Information System (CCSIS). The JRC receives DNA sequence information on the insertions in LMOs from plant biotechnology companies, as part of their legal obligations in the EU. In addition they extract sequence information from nucleotide or patent sequence databases, as well as the primer and probe sequences of the detection methods compiled in the EURL-GMFF reference method database.

The user selects a set of plant species and/or LMOs and a set of detection methods and after in silico simulations of PCR amplification using bioinformatics tools, the results are displayed in a table with predictions of possible amplification(s).

*GMOfinder*¹⁹

The GMOfinder is a compilation of data to construct a combination of both, a ‘GMO target matrix’ and a ‘GMO method matrix’. It is based on an MS Access database and has integrated algorithms that facilitate the interpretation of the results of screening analyses. The tabular matrix provides information on selected genetic elements originating from the literature, LMO notifications and other sources. This information is integrated in a tabular format for 15 real-time PCR methods partly targeting the same genetic element but having different ranges of specificity. The recording of the sources of information facilitates a careful evaluation of the screening results and the tracing back of possible errors in the conclusions of the screening analysis. The GMOfinder is available free of charge upon requests addressed to the authors.

*Combinatory qPCR SYBR® Green screening*²⁰

Combinatory qPCR SYBR® Green screening (CoSYPS) is a “GMO method matrix” based on the SYBR® Green qPCR analysis method for detecting the presence of the following genetic elements in LMOs: the *Cauliflower Mosaic Virus* 35S promoter and terminator, the nos promoter and terminator

¹⁶ The compilation of reference materials is available at:

http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/nachweis_kontrollen/referenzmaterialien.pdf?__blob=publicationFile&v=6

¹⁷ GMOseek matrix is available at: <http://www.gmoseek.com/gmoseek>

¹⁸ The JRC GMO-Matrix is available at <http://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/>

¹⁹ GMOfinder matrix is available at: <http://link.springer.com/article/10.1007%2Fs12161-012-9378-6>

²⁰ The CoSYPS matrix is available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2836468/pdf/216_2009_Article_3286.pdf

derived from *Agrobacterium tumefaciens*, the *Figwort Mosaic Virus* promoter, the rice actin promoter, the nptII gene from *Escherichia coli*, the epsps gene from *Agrobacterium tumefaciens* CP4, the epsps gene from *Zea mays*, the pat gene from *Streptomyces viridochromogenes*, the bar gene from *Streptomyces hygroscopicus*, the barnase gene from *Bacillus amyloliquefaciens* and several cry genes (cry1Ab, cryAc, cryF, cry3Bb) from *Bacillus thuringiensis*. In addition, a set of plant taxon-specific method is included in the CoSYPS testing platform.

The analytical results obtained with the CoSYPS matrix are interpreted and evaluated in combination with a “prime number”-based algorithm, by which the nature of the subsets of corresponding LMOs in a sample can be determined.

*GMO Checker*²¹

This screening application was developed as a real-time PCR array and is a ‘GMO method matrix’. The platform can be used for the comprehensive and semi-quantitative detection of LM crops. It is a combination of 14 event-specific and 10 element specific methods. The specificity and sensitivity of the PCR assays were evaluated experimentally and are tabulated in the publication. An Excel spreadsheet application for the evaluation of analytical results concerning the presence of LM crops has been developed and can be downloaded from the internet.

Other relevant databases

*Biosafety Clearing-House*²²

The Biosafety Clearing-House (BCH) central portal contains an LMO Registry with detailed descriptions for each LMO, including a unique identifier, if available, and detailed information on the transformation method, modified genetic elements, and vector as well as links to other registries in the BCH such as for risk assessments and countries’ decisions. Each entry in the LMO registry also contains links to relevant detection methods for many of the commercialized LMOs.

The BCH also contains two other registries which are closely related to the LMO registry: the Organism Registry and the Genetic Element Registry. The Organism Registry includes information on the donor organisms and the recipient or parental organisms for the registered LMOs.

The Genetic Element Registry contains records of the genes and other genetic elements that were modified in the LMOs. For each entry there is a brief description of the element and links to associated LMOs. Due to the confidential nature of the information, actual sequence information is available for only a few of the genetic elements.

*BioTrack Product Database*²³

The Biotrack Product database is maintained by the Organisation for Economic Co-operation and Development (OECD). It compiles a list of Unique Identifiers (UIs) for LM plants that have been approved for commercial application in at least one country, in terms of food, feed or environmental safety. UIs are codes of a fixed length of 9 alphanumeric digits specific for a single transformation event and are intended to be used as "keys" to access and share information on a particular LMO.

²¹ The GMO Checker is available at: http://cse.naro.affrc.go.jp/jmano/UnapprovedGMOChecker_v2_01.zip

²² The LMO, Organism and Genetic Element Registries of the BCH can be found at: <http://bch.cbd.int/database/organisms/>

²³ The BioTrack Product Database is available at: <http://www2.oecd.org/biotech/default.aspx>

*BIOTradeStatus*²⁴

The BIOTradeStatus database is maintained by the Biotechnology Industry Organization and contains information on approvals, commercialization and seed sale of common commercially available LMOs.

*CERA LM Crop Database*²⁵

The Center for Environmental Risk Assessment (CERA), established by the food-industry funded, non-profit International Life Science Institute Research Foundation (ILSI), also maintains an LM crop database which includes not only plants produced using recombinant DNA technologies (e.g., genetically engineered or transgenic plants), but also plants with novel traits that may have been produced using more traditional methods, such as accelerated mutagenesis or plant breeding. The database provides information on the genetic elements construct, vector as well as the LMO characteristics (traits, common use etc.), risk assessments and regulatory decisions.

*GMOTrack*²⁶

This program generates cost-effective testing strategies for traceability of LMOs and computes the optimal set of screening assays for a two-phase testing strategy.

Resources

A practical approach to screen for authorised and unauthorised genetically modified plants

In this paper, it is shown that the combination of five DNA target sequences can be used as a universal screening approach for at least 81 GM plant events authorised or unauthorised for placing on the market and described in publicly available databases. *Anal.Bioanal.Chem.* 2010, 396, 2065-72.

<http://link.springer.com/article/10.1007%2Fs00216-009-3173-2>

BioTrack Product Database

The Biotrack Product database compiles a list of Unique Identifiers (UIs) for LM plants that have been approved for commercial application in at least one country.

<http://www2.oecd.org/biotech/default.aspx>

BIOTradeStatus

The BIOTradeStatus database is maintained by the Biotechnology Industry Organization and contains information on approvals, commercialization and seed sale of common commercially available LMOs.

<http://www.biotradestatus.com/>

²⁴ The BIOTradeStatus is available at: <http://www.biotradestatus.com/>

²⁵ The CERA LM Crop Database is available at: http://cera-gmc.org/index.php?action=gm_crop_database

²⁶ GMOTrack is available at <http://kt.ijs.si/software/GMOTrack/>

CERA LM Crop Database

CERA's LM crop database.

http://cera-gmc.org/index.php?action=gm_crop_database

Combinatory SYBR®Green qPCR Screening

“Combinatory qPCR SYBR®Green screening” (CoSYPS) is a matrix-based approach for determining the presence of GM plant materials in products. Anal Bioanal Chem 2010, 396, 2113-23.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2836468/pdf/216_2009_Article_3286.pdf

CropLife International Detection Methods Database

A global federation of private plant biotechnology developers, and contains both DNA- and protein-based methods

www.detection-methods.com

EU Database of Reference Methods for GMO Analysis

Maintained by the Joint Research Center of the European Commission

<http://gmo-crl.jrc.ec.europa.eu/gmomethods>

EU-RL GMFF validation process

A table listing the current status of dossiers within the EU-RL GMFF validation process carried out within the frame of the Regulation (EC) No 1829/2003.

<http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>

GMDD: a database of GMO detection methods

In this database, users can obtain the sequences of exogenous integration, which will facilitate PCR primers and probes design. Also the information on endogenous genes, certified reference materials, reference molecules, and the validation status of developed methods is included in this database. BMC Bioinformatics 2008, 9, 260.

<http://www.biomedcentral.com/1471-2105/9/260>

GMO Checker

This screening application was developed as a real-time PCR array and is a ‘GMO method matrix’. The platform can be used for the comprehensive and semi-quantitative detection of LM crops.

http://cse.naro.affrc.go.jp/jmano/UnapprovedGMOChecker_v2_01.zip

GMO Detection Method Database

Maintained by the GMO Detection Laboratory at Shanghai Jiao Tong University.

<http://gmdd.shgmo.org/>

GMOfinder—A GMO Screening Database

GMOfinder is a database for collection and interpretation of information related to the screening for genetically modified organisms (GMOs). *Food Anal. Methods*, 2012, 5, 1368–1376

<http://link.springer.com/article/10.1007%2Fs12161-012-9378-6>

GMOMETHODS: The European Union Database of Reference Methods for GMO Analysis

The GMOMETHODS database currently contains 118 different PCR methods allowing identification of 51 single GM events and 18 taxon-specific genes in a sample. *J. AOAC Int.* 2012, 95, 1713-9.

<http://www.ingentaconnect.com/content/aoac/jaoac/2012/00000095/00000006/art00024?token=0057177ebfb259639412f415d7678257345552b427a406342253048296a7c2849266d656c5b44372999a6b3>

GMOtrack: Generator of Cost-Effective GMO Testing Strategies

This program generates cost-effective testing strategies for traceability of LMOs and computes the optimal set of screening assays for a two-phase testing strategy.

<http://kt.ijs.si/software/GMOtrack/>

Practical experiences with an extended screening strategy for genetically modified organisms (GMOs) in real-life samples

A study of the application of an informative detailed 24-element screening and subsequent identification strategy was applied in 50 animal feed samples. *Agric Food Chem.* 2013, 61, 9097-109

<http://pubs.acs.org/doi/abs/10.1021/jf4018146>

Real-time PCR array as a universal platform for the detection of genetically modified crops and its application in identifying unapproved genetically modified crops in Japan

A novel type of real-time polymerase chain reaction (PCR) array with TaqMan chemistry as a platform for the comprehensive and semiquantitative detection of genetically modified (GM) crops. *J Agric Food Chem.*, 2009, 57, 26-37

<http://pubs.acs.org/doi/abs/10.1021/jf802551h?journalCode=jafcau>

Reference Materials for GMO Detection

A table listing the accessibility of publicly available reference materials and their sources is presented with this method matrix.

http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/nachweis_kontrollen/referenzmaterialien.html?nn=1404276

The GMOseek matrix a decision support tool for optimizing the detection of genetically modified plants

The program is designed to assist in selecting the optimal combination of screening methods and interpreting the data from screening and event-specific testing.

<http://kt.ijs.si/software/GMOtrack/GMOseek.html>

The GMOseek matrix: a decision support tool for optimizing the detection of genetically modified plants.

The GMOseek matrix was built as a comprehensive, online open-access tabulated database which provides a reliable, comprehensive and user-friendly overview of 328 GMO events and 247 different genetic elements. BMC Bioinformatics 2013, 14, 256

<http://www.biomedcentral.com/1471-2105/14/256>

Waiblinger Screening Table

A GMO method matrix which is based on 5 methods targeting specific genetic elements and constructs that are most frequently present in commercialized LM crops.

http://www.bvl.bund.de/SharedDocs/Downloads/09_Untersuchungen/screening_tabelle_gvoNachweis_2013.html

Minimum performance criteria for sample handling, extraction, detection and identification methodology

Moderators: Nathalie Campos Reales Pineda, and Mojca Milavec

An important aspect when building up capacity for the detection, identification and quantification of living modified organisms (LMOs) is to apply minimal standard criteria to ensure the adequate handling and processing of samples, as well as the quality and confidence in the results obtained. A wide variety of methodologies and instruments can be put in place by a molecular biology laboratory that wishes to undertake activities pertaining to the detection and identification of LMOs. Deciding which of the available methods is sufficiently reliable, accurate and more suitable to each laboratory's needs, including considerations such as costs of implementation, can sometimes be challenging. In addition, ensuring that the selected methods produce reliable and consistent results, while, at the same time, meeting minimum performance criteria, is essential.

Minimal performance criteria represent an advantageous set of parameters for selecting and implementing routine methodologies within each laboratory. Inconsistencies in results can have a major impact on the reliability of data produced. For example, the use of different measurement platforms, whether for the analysis of protein or nucleic acids involving the use of different instruments, or different algorithms to interpret the data, can cause serious discrepancy if no basic quality and performance criteria are established upfront. Understanding the relevance of minimal performance criteria, and monitoring some of the method's parameters on a routine basis, allows the lab-facility to establish a quality assurance and quality control system (QA/QC) and standard operating procedures (SOP) that can be used later on for corroborating laboratory proficiency. Additionally, when installing performance quality control measures, the procedure facilitates compliance with the requests from accreditation bodies. To ensure consistency, it is important that the analyte intended to be measured is first defined and that the units in which the measurement is expressed are common standard as well as ensuring the use of common vocabulary for better understanding. Use of certified reference materials is fundamental for protocol harmonization between laboratories and method validation. This is of particular interest when it becomes necessary to allow for result comparison and harmonized interpretation.

International guidance and useful standards about method performance criteria are well documented and available from several sources. These documents include ISO guidelines, *Codex Alimentarius* standards, guidelines from international associations (ISTA, AOAC), metrology institutions, as well as official and peer reviewed references regarding proficiency trials for method validation procedures. The following is an overview of some topics on requirements for minimal performance criteria that are needed in a laboratory performing detection and identification of LMOs:

General Performance Criteria

The performance of the selected methods is crucial for the production of reliable and reproducible data. This encompasses measuring, amongst other things, a method's robustness, precision, trueness, sensitivity and specificity and this can only be achieved in a well-organized laboratory. As such one of the most important precautions in an LMO testing laboratory is to prevent cross-contamination of the samples and organize the unidirectional flow of the sample. Separate rooms or chambers for each testing step should be assured wherever possible. This includes a separate area for the receiving samples, sample preparation including homogenization, analyte extraction, reagent preparation, addition of the analyte to the reagents, and analysis. A number of quality control measures can be applied during the testing procedure, including the use of (certified) reference materials, control samples and duplicates to monitor the performance of a method and ensure the validity of analytical results. All steps in the testing

procedure, from the first contact with the customer to issuing the test report should be documented in a traceable manner while ensuring data protection and confidentiality.

In working towards achieving these criteria, it is crucial to have competent and trained personnel to operate equipment and perform the technical steps of the testing procedure. Managerial and technical tasks and their associated responsible personnel should be clearly defined, including substitutes in the case the primary contact is absent. All personnel should be continuously educated and trained.

Understanding these parameters and being able to detect any deviations from set criteria during routine testing ensures that results are consistent and remain within acceptable ranges of variability.

Performance Criteria for the Detection and Identification of LMOs

Procedures can be regarded as the complete process from sampling to detection and analysis for data interpretation, or be approached in a modular manner. While method validation has relied on considering the process as a whole for determining the global uncertainty, a current trend to adopt a modular approach allows for setting step-wise criteria that permit corrective actions before proceeding to the next step if criteria are not met. Decisions over which of these approaches is to be taken into the lab depends on utility and particularities of the type of sample being analysed (e.g. food matrices, commodities, vegetative tissue, environmental, etc.). For facility, the following section describes procedures using a modular approach. The procedures include laboratory-related issues related to sample handling and homogenization and extraction for LMO detection. These steps are extremely important in the detection and identification of LMOs as significant analytical errors can be introduced at these stages. This section does not involve a description of sampling, which is an extremely important step of analytical procedure, but it is beyond the scope of this document.

Sample handling

The laboratory sample is the portion of material to be used in the laboratory for the analyses. Sometimes mass reduction (sub-sampling) has to be done and the procedure has to be documented. The vast majority of samples must be homogenized prior to analysis. In general the whole laboratory sample is homogenized to obtain a test sample for the analysis. Homogenization is required for two reasons: to achieve sufficient efficiency of analyte extraction and, above all, to ensure homogeneity and an equal representation of LMO-derived particles in the test samples. The homogenization of samples may be achieved with mills, homogenizers, immersion blenders, coffee grinders, or a suitable equivalent device, depending on the size and the structure of the laboratory sample. After grinding, the laboratory sample should be thoroughly mixed to obtain a very homogeneous analytical sample. In cases where the laboratory sample is flour or liquid, homogenization is not needed but thoroughly mixing or shaking the sample is necessary. Homogenization is the step with the highest contamination risk. During grinding, a fine dust often appears that could contaminate subsequent laboratory samples, if appropriate laboratory practices are not in place. All the steps for sample preparation should be done under stringent conditions to avoid cross-contamination and minimize degradation of target analyte, be it DNA or proteins, in the test sample. Storage of the samples prior to and during sample preparation, as well as after its analysis should be performed under appropriate conditions (e.g. at room temperature, refrigerated, frozen).

Extraction

Analyte extraction implies that the processed amount of sample remains representative. The choice of extraction methods must ensure high yield and quality of the analyte, and must be carefully selected, since components of the extraction solutions can affect the efficiency of subsequent detection steps. The principal criteria for quality which should be taken into account are the analyte's integrity, quantity and the absence of inhibitors.

Validated methods of analysis

As mentioned in previous sections of this Guidance, there are several types of analytical methods that target different analytes and use a wide variety of chemistries and strategies. For instance, for DNA based molecular methods, detection may involve the amplification of a GM event or its genetic markers by simple PCR reaction, hydrolytic probes, isothermal amplification or high resolution melting strategies amongst others either in single reaction, arrays or in a multiplex. The laboratory may select from this wide array of test methods based on, among other things, each country's specific regulatory requirements, operational costs, infrastructure and technical capability as well as ensuring that the needs of the customer are met. The methods need to have been validated. The goal of the validation is to evaluate the performance characteristics and limitations of an analytical method. The validation process takes place either within each laboratory or, ideally, through inter-laboratory comparisons to ensure that they meet minimum performance criteria and that they provide accurate results that are, preferably, published in international, regional, or national standards. Specifically, during a validation process, laboratories participate in the study which is organized in accordance with internationally accepted requirements, such as the format described in ISO5725. Some of the parameters reported during the validation process are used to determine the acceptability of the method and include parameters such as applicability, specificity, dynamic range, robustness, limits of quantification, limits of detection and efficiency, amongst others. These elements also allow for measuring the method's level of uncertainties, determining its overall performance, for which, precision and trueness complement the set of measured parameters. It is important to mention that the method used is validated with appropriate reference samples, which have been critically evaluated and a proper value assigned by metrologically sound procedures, which is normally available through National Metrology Institutes (NMI) or Designated Institutes (DI).

Proficiency Testing and Validation

Participation in proficiency testing schemes is crucial for the independent assessment of laboratory performance. Participation in proficiency tests depends on the methods introduced in the laboratory and the type of samples analyzed. Laboratories can use many methods for testing however not all of them can be assessed as each proficiency test covers only a limited number of LMOs. Therefore it is recommended to prepare a plan for the participation in proficiency tests and to assess individual methods periodically. It is also important to cover different types of matrices that are subject to testing during routine analyses.

Resources

Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods

The goal of this document is to support the establishment of molecular and immunological methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, which produce results with comparable reproducibility when performed at different laboratories

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=867>

Validities of mRNA quantification using recombinant RNA and recombinant DNA external calibration curves in real-time RT-PCR

We have developed, optimised and validated an Insulin-like growth factor-1 (IGF-1) RT-PCR in the LightCycler, based on either a recombinant IGF-1 RNA (recRNA) or a recombinant IGF-1 DNA (recDNA) calibration curve. Above that, the limits, accuracy and variation of these externally standardised quantification systems were determined and compared with a native RT-PCR from liver total RNA. For the evaluation and optimisation of cDNA synthesis rate of recRNA several RNA backgrounds were tested.

<http://www.wzw.tum.de/gene-quantification/pfaffl-hageleit-bl-2001.pdf>

A new mathematical model for relative quantification in real-time RT-PCR

This study enters into the particular topics of the relative quantification in real-time RT-PCR of a target gene transcript in comparison to a reference gene transcript.

<http://www.gene-quantification.com/pfaffl-nar-2001.pdf>

Accreditation of facilities testing for genetically modified organisms (GMO)

This document provides additional interpretative criteria and recommendations for the application of ISO/IEC 17025 for both applicant and accredited facilities conducting testing for GMO.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=871>

Application of the Modular Approach to an In-House Validation Study of Real-Time PCR Methods for the Detection and Serogroup Determination of Verocytotoxigenic Escherichia coli

Here we applied a “modular approach” to the in-house validation of these PCR methods. The modular approach subdivides an analytical process into separate parts called “modules,” which are independently validated based on method performance criteria for a limited set of critical parameters.

<http://aem.asm.org/content/77/19/6954.full.pdf>

Biological Testing ISO/IEC 17025 Application Document NATA

This document provides interpretative criteria and recommendations for the application of ISO/IEC 17025 in the field of Biological Testing for both applicant and accredited facilities.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=872>

Coherence between Legal Requirements and Approaches for Detection of Genetically Modified Organisms (GMOs) and Their Derived Products

Here, key points where coherence is lacking are discussed. These include the definition of units of measurements, expression of GM material quantities, terminology, and inconsistent legal status of products derived from related but slightly different transformation routes.

<http://pubs.acs.org/doi/abs/10.1021/jf052849a>

Comparison of nine different real-time PCR chemistries for qualitative and quantitative applications in GMO detection

Provides a review of a number of different PCR chemistries and briefly touches on their performance criteria. This may be useful in the process of selecting specific methodologies that can be used in your laboratories.

<http://works.bepress.com/cgi/viewcontent.cgi?article=1031&context=torstein>

Criteria for Accreditation of Laboratories Testing Genetically Modified Organisms

This annex details criteria for accreditation of laboratories testing for the presence of Genetically Modified Organisms (GMOs) through the use of DNA extraction and Polymerase Chain Reaction (PCR) methods of detection.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=771>

Critical points of DNA quantification by real-time PCR – effects of DNA extraction method and sample matrix on quantification of genetically modified organisms

The purpose of the study was to analyse the effect of some of these parameters on GMO quantification. Five DNA isolation protocols were compared with respect to the influence of the extraction procedure on real-time PCR efficiency and, consequently, the GMO quantification. The effect of the sample matrix on PCR efficiency was studied on different soybean and maize matrixes. Additionally the first assessment of variability of PCR efficiency within sample matrixes is presented. PCR efficiency was considered as the primary criterion in assessing the quality of extracted DNA.

<http://www.biomedcentral.com/1472-6750/6/37>

Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing

Provides recommendations on how methods for GMO analysis shall be evaluated and validated by the community Reference laboratory for genetically modified food and feed.

http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf

Detection Methods and Performance Criteria for Genetically Modified Organisms

This review summarizes the value of these performance criteria approaches as applied to GMO detection methods.

[http://lib3.dss.go.th/fulltext/Journal/J.AOAC%201999-2003/J.AOAC2002/v85n3\(may-jun\)/v85n3p801.pdf](http://lib3.dss.go.th/fulltext/Journal/J.AOAC%201999-2003/J.AOAC2002/v85n3(may-jun)/v85n3p801.pdf)

Detection of genetically modified organisms (GMOs) using isothermal amplification of target DNA sequences

Here we have applied the loop-mediated isothermal amplification (LAMP) method to amplify GMO-related DNA sequences, 'internal' commonly-used motifs for controlling transgene expression and event-specific (plant-transgene) junctions.

<http://www.biomedcentral.com/1472-6750/9/7/>

Development of sampling approaches for the determination of the presence of genetically modified organisms at the field level

<http://link.springer.com/article/10.1007%2Fs00216-009-3406-4>

European technical guidance document for the flexible scope accreditation of laboratories quantifying GMOs

The aim of this guidance document is to facilitate harmonised flexible scope accreditation within Europe, according to ISO/IEC 17025:2005 related to quantitative testing of genetically modified organisms (GMOs) by quantitative real-time polymerase chain reaction (qPCR) for GM events authorised in the EU or which are in the authorisation process.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=873>

Guidelines for the validation and use of Immunoassays for determination of introduced proteins in biotechnology enhanced crops and derived food ingredients

Paper provides general background information and a discussion of criteria for the validation and application of immunochemical methods to the analysis of proteins introduced into plants and food ingredients using biotechnology methods.

http://www.aeicbiotech.org/WhitePapers/protein_doc.pdf

Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods

These guidelines provide appropriate criteria to validate the performance of methods developed to detect specific DNA sequences or specific proteins in foods.

http://www.fao.org/fileadmin/user_upload/gmfp/resources/CXG_074e.pdf

Immunoassay as an Analytical Tool in Agricultural Biotechnology

The document highlights the many areas to which attention must be paid in order to produce reliable test results when conducting immunoassay based tests. These include sample preparation, method validation, choice of appropriate reference materials, and biological and instrumental sources of error

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=870>

Increased efficacy for in-house validation of real-time PCR GMO detection methods

Provides an overview of the process of carrying out in-house validation of PCR methodologies with the view to determine and establish a more efficient and accurate validation procedure.

<http://link.springer.com/article/10.1007/s00216-009-3315-6>

Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits

This review assesses advantages and disadvantages of various commercially available DNA extraction kits, as well as modifications to published cetyltrimethylammonium bromide methods.

<http://link.springer.com/article/10.1007%2Fs00216-009-3150-9>

ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods

The purpose of this document is to provide a guideline for the validation of binary [a binary qualitative method is one that produces one out of two possible responses when it is used (e.g., end time PCR, visual inspection of a dip stick)] qualitative methods intended to detect biological and chemical compounds.

http://www.eoma.aoac.org/app_n.pdf

ISTA International Rules for Seed Testing

The International Rules for Seed Testing are ISTA's primary instrument to promote uniformity in seed testing.

http://www.seedtest.org/en/international-rules-_content---1--1083.html

Kernel Lot Distribution Assessment (KeLDA): a Comparative Study of Protein and DNA-Based Detection Methods for GMO Testing

The objective of this work was to assess the performance of a protein-based (lateral flow strips—LFT) and of a DNA-based (polymerase chain reaction—PCR) detection method for GMO analysis.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=875>

Laboratory Quality Assurance/Quality Control

This chapter specifies essential QA/QC activities to enable laboratories to achieve reproducible results on an ongoing basis.

<http://www.env.gov.bc.ca/epd/wamr/labsys/lab-manual/pdf/2013/section-a-2013.pdf>

Polymerase Chain Reaction Technology as Analytical Tool in Agricultural Biotechnology

Document highlights the many areas to which attention must be paid in order to produce reliable test results. These include sample preparation, method validation, choice of appropriate reference materials, and biological and instrumental sources of error.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=868>

Principles for the use of sampling and testing in international food trade

http://www.codexalimentarius.org/download/standards/13276/CXG_083e.pdf

QMS For Laboratories Testing and Calibration ISO 17025

This document is a consolidation of information and resources on the process of Developing a Quality Management System for Testing Laboratories.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=886>

Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples

Manual is intended to serve as general guidance for the development laboratory and method specific QA/QC procedures for PCR analysis of environmental samples.

http://www.epa.gov/ogwdw/ucmr/ucmr1/pdfs/guidance_ucmr1_qa-qc.pdf

Selective control of primer usage in multiplex one-step reverse transcription PCR

Investigating the use of primers containing thermolabile protecting groups for improved multiplex one-step RT-PCR performance.

<http://www.biomedcentral.com/1471-2199/10/113>

Testing the interaction between analytical modules: an example with Roundup Ready® soybean line GTS 40-3-2

The modular approach to analysis of genetically modified organisms (GMOs) relies on the independence of the modules combined (i.e. DNA extraction and GM quantification). The validity of this assumption has to be proved on the basis of specific performance criteria.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2927498/pdf/1472-6750-10-55.pdf>

Testing the Robustness of Validated Methods for Quantitative Detection of GMOs Across qPCR Instruments

A study has been designed to evaluate the robustness of three validated methods for genetically modified organisms (GMO) quantification across six real-time platforms from four different suppliers.

<http://link.springer.com/article/10.1007%2Fs12161-012-9445-z>

The International Vocabulary of Metrology

The present Vocabulary pertains to metrology, the “science of measurement and its application”. It also covers the basic principles governing quantities and units.

http://www.bipm.org/utils/common/documents/jcgm/JCGM_200_2008.pdf

The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments

MIQE is a set of guidelines that describe the minimum information necessary for evaluating qPCR experiments.

<http://www.gene-quantification.com/miqe-bustin-et-al-clin-chem-2009.pdf>

Use of pJANUS™-02-001 as a calibrator plasmid for Roundup Ready soybean event GTS-40-3-2 detection: an interlaboratory trial assessment

The use of the pJANUS™-02-001 plasmid as a calibrator for the quantification of soybean event GTS-40-3-2 in food and feed products and its validation for such usage.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=877>

Verification of analytical methods for GMO testing when implementing interlaboratory validated methods

Guidelines on how to introduce and verify a validated method in the laboratory in a way that easily can be copied and implemented in practice

<http://gmo-crl.jrc.ec.europa.eu/doc/ENGL%20MV%20WG%20Report%20July%202011.pdf>

Verification of real-time PCR methods for qualitative and quantitative testing of genetically modified organisms

This article reports the experience of a university laboratory in accreditation of molecular biology methods for genetically modified (GM) organisms detection according to the International Organization for Standardization (ISO)/ International Electrotechnical Commission 17025 standard.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=874>

Experience and case studies on detection and identification

Moderators: Gurinder Randhawa and Ayako Yoshio

Detection and identification of living modified organisms (LMOs) is cross-cutting and relevant to a number of biosafety-related issues, such as risk assessment and management, detection of unauthorized or illegal LMOs, detection of unintentional introductions into the environment, and liability and redress. Thus, the capacity to detect and identify LMOs is arguably one of the main pillars for the effective implementation of the provisions of the Cartagena Protocol on Biosafety and national biosafety legislation. Nevertheless, monitoring, detecting and identifying LMOs remain a challenge to many Parties.

Unauthorised and/or unintentional transboundary movements of LMOs often found in shipments and products traded in the global market. There may be incidents of deliberate or accidental introduction of unauthorised LMOs into the environment.

As the development of LMOs expands in both number and geographic area, their different approval status in individual countries leads to “asynchronous approvals” where, for example, a particular LMO may be approved for introduction into the environment in one country, as food or feed in another country, while not being authorized in other countries. The analytically-based detection of unauthorised LMOs in consignments and of LMOs unintentionally introduced into the environment is a challenge due to the sheer volume of material, which may also include authorized LMOs and non-LMOs. The detection of any LMO is dependent on the availability of suitable detection method(s) and control materials to validate the method(s). Other information, e.g. describing the novel trait or introduced genetic elements, may also facilitate the detection, verification and identification of an LMO.

Depending on national biosafety policies and regulations, requirements for the detection and identification of LMOs may range from qualitative “yes/no” tests that detect the presence of an LMO, to more complex tests that allow for the identification of the LMO, to tests that are capable of quantifying an LMO. Further differentiation between different classes of unauthorised LMOs are sometimes referred to as *low-level presence* (LLP), where the LM product has been approved in at least one country, or *adventitious presence* (AP), occurring when the LM product is not approved in any market (i.e. is an experimental product or is cultivated under confined field trials).

The FAO defines LLP and AP as:

Low Level Presence (LLP): LLP refers to the detection of low levels of GM crops that have been approved in at least one country on the basis of a food safety assessment according to the relevant Codex guidelines. Readers should note that low level presence (LLP) is not specifically defined by Codex, however in the context of the Codex guidelines it is referred to as LLP.

Adventitious Presence (AP): AP refers to detection of the unintentional presence of GM crops that have not been approved in any countries on the basis of a food safety assessment according to the relevant Codex guidelines.

Source: The results of the FAO survey on low levels of genetically modified (GM) of the Technical Consultation on Low Levels of Genetically Modified (GM) Crops in International Food and Feed Trade Crops in International Food and Feed Trade (http://www.fao.org/fileadmin/user_upload/agns/topics/LLP/AGD803_4_Final_En.pdf)

The aim of this section is to provide laboratories with strategies and guidelines on how to handle unauthorised LMOs, as well as to interpret and report analytical results and related information to the competent authorities. It also captures the experience of some countries in addressing the presence of unauthorised transboundary movements and unintentional introduction into the environment using a scientific approach.

Examples on how countries establish the scope of their requirements relating to unauthorised transboundary movements and unintentional introductions into the environment can provide a blueprint of different options to Parties that are in the process of establishing new laboratories or updating their existing facilities. Additionally case studies providing examples of relevant technical and analytical issues, ranging from the setting up of laboratories to the actual sampling, detection and identification of LMOs, can be important tools for Parties that are currently building their capacities in this area.

Resources

A Scientific Framework for Assessing Transgenic Organisms in the Environment

Article provides examples for LMOs sampling methods and testing as part of monitoring and environmental risk assessment.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=804>

Detecting adventitious transgenic events in a maize center of diversity

Describes the approach and experience of Peru in working towards an approach to detect unintended presence of transgenic events within locally grown cultivars of maize.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=799>

Detection of Genetically Modified Organisms (GMOs) Using Molecular Techniques in Food and Feed Samples from Malaysia and Vietnam

This study employed PCR technology for detection and direct DNA sequencing for confirmation procedures respectively. The results demonstrated for the first time the presence of GM plants with glyphosate-resistant trait led by the control of P35S promoter and NOS terminator in either Malaysian or Vietnamese feed with high frequency.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=797>

Developing a Policy for Low-Level Presence (LLP): A Canadian Case Study

Canadian case study that iterates various considerations that can be taken into account when considering unintentional and unauthorized LMOs and their subsequent detection and identification.

<http://agbioforum.org/v16n1/v16n1a04-tranberg.pdf>

Development of an event-specific Real-time PCR detection method for the transgenic Bt rice line KMD1

Study describes an event-specific quantitative real-time PCR detection method for the transgenic rice line Kemingdao 1 (KMD1).

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=803>

Identification and detection method for genetically modified papaya resistant to papaya ringspot virus YK strain.

Identification of the transgenic vector construct generated based on resistance to infection with the papaya ringspot virus (PRSV) YK strain. A specific detection method to qualitatively monitor papaya products for contamination with the GM papaya was developed using the real-time polymerase chain reaction.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=809>

Indicated Detection of Two Unapproved Transgenic Rice Lines Contaminating Vermicelli Products

The study analysed DNA fragments extracted from four rice vermicelli products and detected two lines of unauthorized Bt rice harbouring the Bt toxin cry gene, one of which has a construct similar to the previously reported GM Shanyou 63 line (19-21) and the other is an unknown Bt rice line, which has a construct similar to the Kemingdao.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=802>

Japanese Experience of Developing of Detection Methods for Unapproved GM papaya

Outlines an experience of developing of detection methods for the unapproved GM papaya, imported for commercial seeds and seedlings.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=810>

Low Level Presence of Transgenic Plants in Seed and Grain Commodities: Environmental Risk/Safety Assessment, and Availability and Use of Information

The scope of this document covers low level presence situations in which seed [or certain commodities] contain low levels of transgenic seed that have been reviewed for environmental risk/safety and received authorisation for commercial cultivation (unconfined release) in one or more exporting countries but not in the country of import.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=805>

Overview on the detection, interpretation and reporting on the presence of unauthorised genetically modified materials

The aim of this document is to provide laboratories of the ENGL with harmonised strategies and guidelines on how to cope with unauthorised GM materials (UGM), as well as to interpret and report analytical results and related information to the competent authorities. It is the objective to facilitate detection of UGM, if present, without the requirements for a completely new detection paradigm. Therefore, the document also includes a review of presently available approaches for detection of UGM.

<http://gmo-crl.jrc.ec.europa.eu/doc/2011-12-12%20ENGL%20UGM%20WG%20Publication.pdf>

Post-Moratorium EU Regulation of Genetically Modified Products: Triffid Flax

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=801>

Practicality of detection of genetically modified organisms (GMOs) in food.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=808>

Technical Consultation on Low Levels of Genetically Modified (GM) Crops in International Food and Feed Trade

Several countries have requested FAO to facilitate international dialogue on the issue of trade disruptions involving low levels of GM crops in international food and feed trade.

<http://www.fao.org/food/food-safety-quality/a-z-index/biotechnology/LLP/en/>

Technical Guidance document from the EURL for GM food and feed on the Implementation of Commission Regulation (EU) No 619/2011

In the EU exists a Regulation (619/2011) on Low Level Presence. This guidance document was developed for laboratories on the execution of the LLP Regulation was developed by the EURL GMFF.

<http://gmo-crl.jrc.ec.europa.eu/doc/Technical%20Guidance%20from%20EURL%20on%20LLP.pdf>

The results of the FAO survey on low levels of genetically modified (GM) crops in international food and feed trade

This survey outlines international experiences and responses to incidents of unauthorised and unintended LMO presence.

<http://bch.cbd.int/cms/ui/collaboration/download/download.aspx?id=800>
