

Sampling and Detection Methods for Products of Modern Agricultural Biotechnology in NAFTA Countries

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Sampling and Detection Methods for Products of Modern Agricultural Biotechnology in NAFTA Countries

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Foreword

A number of different entities within Canada, Mexico, and the United States are involved in developing, assessing, and utilizing detection methods for products of modern biotechnology. Each of these organizations specializes in specific analytes or matrices and their approaches, processes, and methods are not always consistent. Recognizing these inconsistencies and the need to take steps to foster harmonization, the ILSI International Food Biotechnology Committee (IFBiC) commissioned the development of this paper to describe:

1. The need for harmonization of test methods for biotech products;
2. Recently concluded or current activities involving method development/ validation for products of modern biotechnology; and
3. Future directions/needs in this area.

Information on the need for harmonization, current analytical method activities (and implementation), and an indication of the various organizations' short and long-term research activities was gathered primarily by interviewing representatives within each of the agencies and laboratories with a responsibility for and ongoing activities in the development of GM methods, standardization, international communications, and implementation of regulations. Active private groups in Canada, Mexico, and the US were also invited to contribute.

This report represents a unique survey of the present status of these activities in the North American Free Trade Agreement (NAFTA) region. It also served as background to a workshop organized by IFBiC on October 11-12, 2007 to foster discussions among the regulatory bodies within NAFTA countries on approaches to the validation and harmonization of analytical methods for crops derived from modern biotechnology. This meeting provided the opportunity for different government agencies to share the latest information and published data on these methods. Meeting participants were asked to consider the challenges and barriers to harmonization and to propose recommendations for how these may be overcome to encourage scientific harmonized approaches for the validation or performance of methods used for testing products derived through biotechnology. It is hoped that this dialogue can serve as a model for other regions of the world.

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Introduction

The NAFTA countries have the opportunity to be leaders in developing both the policies and the technical detection capabilities to manage the products of agricultural biotechnology across the food supply chain. The agencies responsible for regulating biotechnology-derived (also known as genetically modified or GM) crops, and the foods derived from them, are expected to provide this leadership. Some NAFTA government agencies have developed capabilities in the area of testing for new genetic traits to facilitate the management of regulations, but most of those interviewed in preparing this document reported that there has been limited opportunity to discuss or develop a harmonized approach to sampling and testing. This document provided background information for an October 11-12, 2007 ILSI IFBiC discussion workshop on specific issues and viewpoints related to harmonization of sampling and detection methods for GM foods in NAFTA countries.

The impact of GM crops on agricultural trade

International activity in the development and acceptance of agricultural biotechnology (GM) crops has affected all levels of the food supply chain. However, differences in national regulations of GM crops have had negative effects on international trade. To manage the movement of GM crops through the supply chain, a number of management and technical tools are needed by both industry and governments to ensure and enforce compliance with regulatory frameworks and legal requirements. Some tools are as straightforward as a paper trail with complementary computer tracing

software, while others require representative sampling plans and testing methods. Where events are either allowed with thresholds or are not approved, a sampling and detection analysis scheme is essential for effective management and regulation of the food in the supply chain.

Analytical methods for detection of GM foods

The three main reasons for developing and using analytical methods to detect GM traits in crops are outlined below. All of these require that the methods are validated to published procedures including the representative sampling plan, each of the analysis steps, and the final report. Consideration of methods for GM animals (e.g. transgenic salmon) is outside this paper's scope, though it is recognized as significant and the analytical methods for detection may have similar issues.

1. Research and development of the new GM trait(s), the subsequent seed verification programs and field trial releases

Research into biotechnology traits for food crops is carried out by the companies that first commercialized the events 10 years ago, and by university and government laboratories around the world. The first commercialized GM crops came from the private sector, which is not surprising given the time and significant costs necessary to research, develop, register, and introduce a GM crop. More recently, partnerships between the public and private sectors brought GM crops through all the development steps to field trials and some are now heading towards commercialization.¹

¹ FAO BioDeC database: www.fao.org

OECD database: webdomino1.oecd.org/ehs/bioproduct.nsf

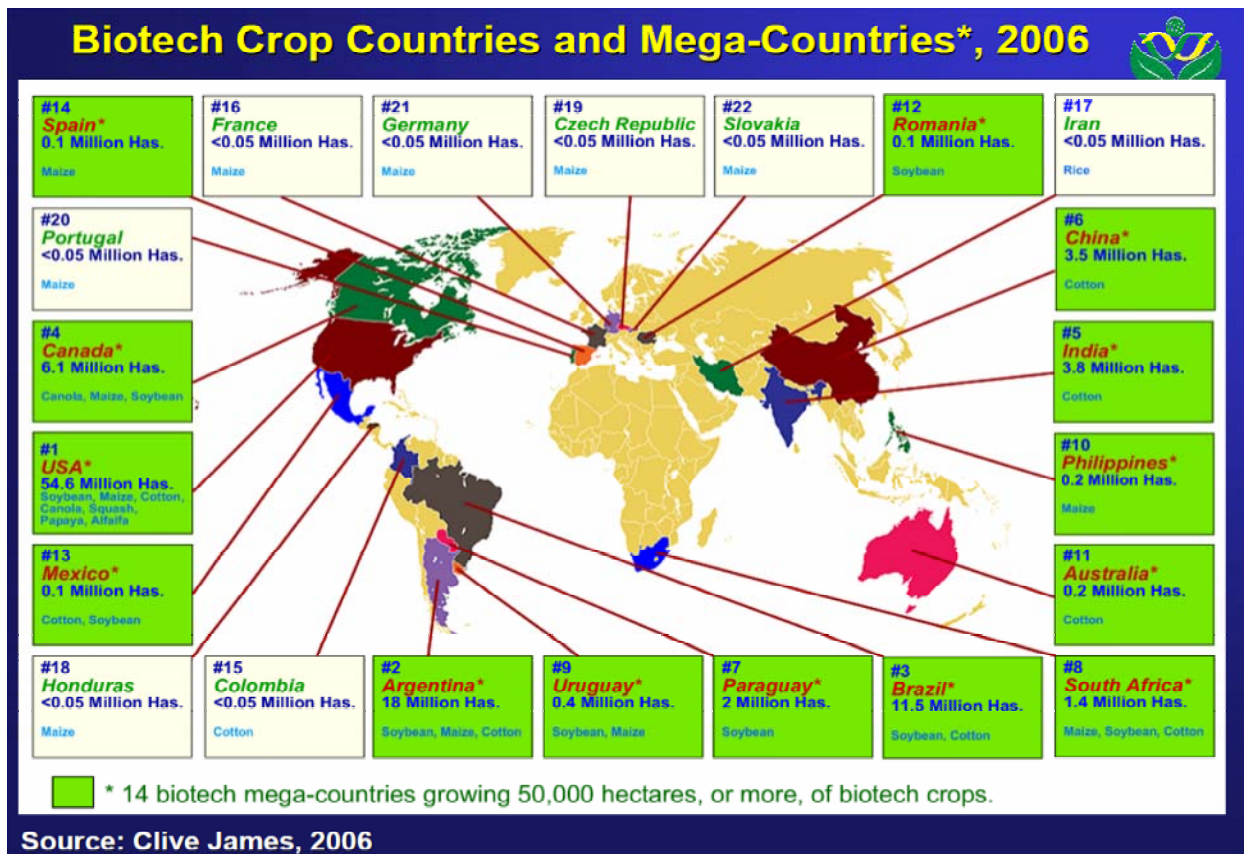


Figure 1: Biotechnology derived crop adoption

Soy and corn (maize) have the most total acreage and there are increasing areas planted with biotechnology-derived cotton and canola. The commercialization of GM rice in Iran has placed biotechnology even closer to the food supply chain and added more urgency and complexity to testing considerations.

2. Grain export and import trade, quality assurance and quality control

Producers in all of the major agriculture exporting countries (*Figure 1*) have adopted agricultural biotechnology. As such, GM crops are inevitably moving through the commodity trading systems around the world.

A recent International Service for the Acquisition of Agri-biotech Applications (ISAAA) update on the specific crops, traits, adopting countries, and area of commercial

planting claims that, “annual hectareage of biotech crops exceeded 100 million hectares (250 million acres); for the first time, the number of farmers growing biotech crops (10.3 million) exceeded 10 million; the accumulated hectareage from 1996 to 2006 exceeded half a billion hectares at 577 million hectares (1.4 billion acres).”²

In 2006, the number of countries that adopted GM crops increased to 22; the number of hectares (acres) planted and the number of types of crops also increased (*Figure 1*). 2007 marks the eleventh year that biotech crops have been planted commercially, and it is timely to consider the opportunities and implications for analytical testing.

² ISAAA Briefs No. 35-2006: Executive Summary can be found at www.ISAAA.org.

Most of the corn and soybeans in developed countries is grown for feed. By comparison, a small percentage is grown for food use. Because both end uses share a common handling infrastructure, there is an expectation that both will be unintentionally commingled as the grain moves through the supply chain. Some effort has been made to develop identity preserved (IdP) supply systems to minimize commingling and meet specific compliance and enforcement standards (approved events) or special market demands (like non-GM).

Grains grown in separate channels usually provide an opportunity for premium pricing to the supplier because they are more difficult to originate and more expensive to supply. Identifying and confirming the quality of these premium crops requires the ability to sample and test the products that are traded. In addition, compliance and enforcement testing by government agencies is implemented by specific regulatory agencies, generally those closer to the movement of grains. Several may have responsibility to ensure successful trade for their governments in terms of compliance and enforcement. To ensure uninterrupted grain trade, it is critical that sampling and analytical methods are harmonized at the international level, and that communication mechanisms are developed among exporters, importers, and the relevant regulatory agencies.

3. Food regulation, labeling laws, regulatory compliance of approved and unapproved events in different geographic regions and countries³

³ USA-approved corn events were delayed in Europe by the European Union (EU) moratorium on approvals of agricultural crops derived from biotechnology, as were several Canola and Soybean events. The World Trade Organization (WTO) ruled in favor of US, Argentina and Canada's challenge to this moratorium. (WT/DS291/INTERIM, WT/DS292/INTERIM, WT/DS293/INTERIM; 2006)

Different countries have different labeling requirements for GM crops. Some countries require mandatory labeling that covers all aspects of the food chain from crop planting to finished food products, while labeling of GM products is voluntary in other countries. In addition, some countries or trading blocks require provision of detection methods. In many cases, countries are concerned about the capability of the management tools currently available including testing. This concern has resulted in many types of regulations at different stages in the worldwide supply chain and a corresponding challenge for the industry to understand and stay current when considering international management of crops and products.

To date, most commercial GM crops traded internationally originated from the Northern Hemisphere, but that situation is expected to change. For instance, Iran introduced commercial rice and several other Asian countries are expected to follow suit. Other food crops are also progressing through the development process in various countries and their appearance in international trade is only matter of time.

Agencies involved in food and feed regulation need to be able to enforce their laws; harmonization of sampling and detection methods at both national and international levels can simplify this task. For example, the US Department of Agriculture (USDA), Food and Drug Administration (FDA), and the US Environmental Protection Agency (EPA) may all be responsible in some manner for compliance and enforcement testing of foods containing GM traits that are imported into the United States.

Present status of harmonization

In the food supply chain it has become expected that government agencies will provide leadership and guidance.⁴ While recognizing national differences and local priorities, there is a similar expectation for consistency in the regulatory requirements including analytical methods. This is far from being accomplished globally for GM crops and foods derived from them. Food manufacturing companies that sell to a global market need to know and understand the regulatory environment on both ends of the process. They need to know and understand the origin of the ingredients they are using. Most important is to determine whether they are sourcing the ingredients in the local area or in a broader marketplace. International pricing advantages have made sourcing ingredients a global activity, which requires a rigorous effort to understand the origin and subsequent steps to delivery.

Disruptions have arisen for so-called non-GM products. For example, unexpected positive test results for the presence of GM material have been obtained in products that claimed to be GM-free but contained soy lecithin. The supplier was sourcing soy lecithin from Brazil and the Brazilian government has since acknowledged the situation and introduced limited regulations.⁵ In addition, some manufacturers expected that processing would remove all traces of the modified DNA, which was also a false assumption. Unless a product is highly processed, it is unlikely that all traces of the modified plant DNA or proteins will be removed. Detection methods can be designed to be both sensitive and

capable of detecting even trace levels of the DNA sequences.

Preferred situation

The best situation from the food industry perspective might be to have regulations and detection methods that are 100% harmonized in all geographic regions. This is far from the present situation and with so many diverse approaches already in place globally, it is unlikely to be accomplished. Most regions have implemented regulations that follow local activities and requirements and only a few appear to consider the concerns of trading partners. In the European Union (EU), the focus is on retail product labeling regulations set at low %GM levels measured as total modified DNA in the sample. In some Asian countries, where significant levels of commodity crops are imported, there are labeling regulations but the focus is on bulk shipment requirements set at %GM levels that take into account the capability of the trading partners and existing systems. In some instances, bilateral trade agreements have helped balance and manage the regulations.

Sampling

Sampling plans for food analysis are well documented.⁶ Different protocols are recommended for different types of analytes, the type of distribution within the bulk material, and the concentrations. A number of recommended protocols are commonly used in the grain trading industry. An example of these can be found at the Grain Inspection, Packers and Stockyards Administration (GIPSA) website.⁷ Recent discussions in the EU have suggested a new sampling protocol;

⁴ Codex Alimentarius: www.codexalimentarius.net

⁵ www.gmo-guidelines.info/public/regions/brazil/regres.html;
www.ictsd.org/biores/02-02-21/story3.htm

Some companies do grow non-GM soy in Brazil and maintain segregation.

⁶ ISO standards: www.iso.org

ISO 7002:1986 Agricultural food products, Layout for a standard method of sampling from a lot; ISO/DIS 664 Oilseeds, Reduction of laboratory sample to test sample; ISO/DIS 24333 Cereals and cereal products, Sampling.

⁷ GIPSA: www.gipsa.usda.gov/GIPSA/documents/GIPSA_Documents/sampling.pdf

however, this has been rejected at the international level by the International Standards Organization (ISO).

Methods of analysis

Intellectual property considerations have restricted the open distribution of some GM trait analytical testing methods. Methods are typically developed during the research phase of the trait development and were designed to work with single plants or seeds. In many cases, they were the only method(s) available when the introduction of labeling regulations in the EU caused an immediate need for analytical methods.

To meet this early demand, agreements were made to share the technology between the method developer (usually the trait provider⁸) and the competent authorities. More recently, the EU published the submitted methods on the internet. As a rule, methods are only validated on DNA (EU) or in the raw material matrices due to the complexity of validating any method for multiple processed materials. Sometimes methods cannot be shared even within a single government agency or competent authority.

The proprietary nature of sequence information to both the commercial laboratories and the event developers means access to some of their methods is also limited. Many laboratories develop their own analysis methods. Although some laboratories claim to have “back engineered” the original method, there are also cases where the methods have become available by “unknown circuitous routes.” The current EU regulations require that a validated method of analysis is included with the trait approval documentation that is subsequently published.⁹ Recently, there appears to be more interest on the

part of method developers in having methods collaboratively validated by organizations such as AACC International,¹⁰ which would mean that the approved method becomes publicly and widely available even though a license may be needed for commercial use.

Food labeling regulations require analytical methods that have been validated in processed food matrices, or the ability to trace food back to a grain sample that has been tested and/or grown under identity preserved conditions and kept segregated. Since the proteins are often denatured during the processing steps, detection of the DNA from the introduced trait is the preferred target analyte in processed foods.

The most commonly used method is based on the polymerase chain reaction (PCR). The PCR method’s complexity precludes its rapid use outside of a laboratory facility, whereas methods most frequently used to manage day-to-day supply chain operations must be rapid and able to be performed in different kinds of facilities. Thus, methods based on protein detection technologies¹¹ are preferred in those situations. PCR methods can be used to confirm analytical results, but the cost of “holding” shipments while waiting for PCR results can add significant cost to the final products if not managed efficiently. Today there may be almost as many different PCR analysis methods for a particular analyte as there are laboratories performing them.

While many of the methods have been developed and used by laboratories that participate in the USDA/GIPSA proficiency trials, very few methods have been validated with international collaborative (ring) trials.

⁸ Trait providers in this case were mainly corporations, but may also be academic institutions or governments.

⁹ gmo-crl.jrc.it/statusofdoss.htm

¹⁰ AACC International Approved Methods: www.aaccnet.org

¹¹ The Starlink® issue in the United States resulted in the extensive use of protein analyses using lateral flow strips.

Ring trials provide validation data that set practical criteria for method performance and are an excellent basis for comparison. However, validating all of the presently available methods by international ring trials is not practical.

The ability to use the same detection methods effectively at all stages in the chain is a challenge that has not been fully resolved. This is a situation that would be unacceptable for measuring analytes such as sugars or vitamins for label claims or mycotoxins for safety in foods. At a minimum, the methods need to be fit for purpose and validated. While bias-free methods are preferable, if a bias exists between the methods used then it needs to be measured, understood, and consistent. To date, an understanding of the relationship between results obtained using protein and DNA detection technologies has not been attained.

Validation

Validation of methods for detecting analytes of interest in food has a long history.¹² Analytical methods are used as research development tools, manufacturing quality checks, and for regulatory compliance. Applying analytical methods for genetic traits is not functionally different, although there were some “miss-steps” as scientists unfamiliar with the usual food-matrix issues and literature suggested that radically new approaches to testing were required for these analytes.

The ultimate goal of validation is to yield test results that are accurate and precise, comparable from method to method, and predictable with the same results reported from one laboratory to another.

¹² Codex Alimentarius: www.fao.org/docrep/008/y7867e/y7867e00.htm

The following indicators should be considered in completing a validation process:

- Sensitivity (limit of detection, limit of quantification)
- Specificity
- Accuracy
- Ruggedness
- Precision (trueness)
- Applicability
- Practicability
- Fitness for purpose

Definitions and guidelines for measuring these indicators are published.^{13,14}

Analytical methods comprise a sampling step, a sample preparation step, an extraction or location of analyte step, an analysis step, and data collection and reporting. It is important to recognize that each of these steps can interact with other steps in the process. The method should be validated in its entirety first to ensure that it is fit for its intended purpose and secondly to avoid an underestimate of variability. It is important to include any possible interactions between the method's steps. Current practice in the laboratory responsible for validating methods in the EU has separated the extraction steps from the analysis steps (“modular validation”) in an apparent attempt to “lighten the workload.” To date, there is no published study that shows all steps in any one method are independent.¹⁵ No refereed publication exists as to how all PCR methods are similar enough to make this assumption comprehensive.

The key challenge to harmonization of methods is in the work that has already been

¹³ ISO 24276–2006 Foodstuffs—Methods of analysis for the detection of genetically modified organisms and derived products General requirements and definitions.

¹⁴ AOAC International OMA Program Manual Appendix X, May 2002

¹⁵ Holst-Jensen A, Berdal KG. The modular analytical procedure and validation approach and the units of measurement for genetically modified materials in foods and feeds. *JAOAC Int.* 2004;87:927–936.

done in each regional area. Methods were developed and validated to local regulatory focus and concerns. Even at a “broader regional level” there is little activity to study performance and harmonization of methods, with the exception of the ongoing work in Europe’s regulatory network (ENGL) and some exchange activities between Japan and Korea. The Codex Committee for Methods of Analysis and Sampling (CCMAS) is discussing agreement on the performance factors that a method must meet for consideration to be used in compliance questions.¹⁶

Development of new detection methods

Methods are first needed during the research phase of a new trait. The method that is developed will be appropriate for single plants and seeds and specific for the research study. This method may be improved to follow the product through the grain handling process.

Research on methods for biotech trait detection has been most active in those global regions where regulations requiring labeling of biotechnology-derived food materials were established. Some laboratories that were initially very active in method development and validation work have scaled back their efforts (often related to changing attitudes with respect to biotech crops in that geographic region) and others are expanding activities in response to uncertainties surrounding the interpretations for implementation of the Cartagena Protocol on Biosafety.¹⁷

The detection of biotech traits requires the application of known protein or DNA detection methods. The protein methods commonly used are variations of the enzyme

linked immunosorbent assay (ELISA); the lateral flow “strip tests” are a solid-phase application of the ELISA technique. Some manufacturers have produced strip readers that facilitate quantitation of the detected protein. Variations on the type and shape of the ELISA support substrate and detection technologies have allowed for development of multi-analyte methods (e.g. Luminex).¹⁸

The limitation of these methods is that the chemistry is specific to the particular protein’s three-dimensional structure (or close variations of it) in any given matrix. These methods are most valuable when used early in the food supply chain. Food processing leads to protein denaturation, which can cause the necessary antibody systems to not recognize the proteins. Testing of finished food products invariably requires using a DNA detection method such as PCR. Work is in progress to develop new chemistry for new traits and combinations of traits and on the chemistry delivery systems. DNA chip technology, which is used very successfully in medical and microbiological applications, has been explored as a possible alternative to the multi-array trays used with most of the current PCR methods. It has been shown that under the right conditions, chip technology is successful for the analysis and identification of DNA, but the significant time required for the preparation and extraction steps is not impacted.

The method choice is largely governed by the location in the food chain that testing occurs. Early in the chain when the grain is raw in the field, protein testing is most likely the method of choice and certainly the most cost-effective. However, DNA detection methods are probably the only choice later in the food

¹⁶ Criteria for the Methods for the Detection and Identification of Foods Derived from Biotechnology CX/MAS 07/28/8

¹⁷ www.biodiv.org/biosafety/protocol.asp

¹⁸ www.luminexcorp.com/applications/index.html

supply chain. The relationship between the two methods is not a simple correlation and can vary from trait to trait.

Food company experience has shown it makes sense to test raw materials, which can be done most easily and cheaply, rather than attempting to test complex food matrices, which are composed of ingredients from many different plant sources.

Prevailing issues in method development

Trait sequence information

The problem most cited by those concerned about capacity building for GM detection and those laboratories consulted for this paper is access for the detection method developers, access to the trait sequence information from the trait provider, and access from commercial testing laboratories. Some competent authorities have obtained trait sequence information for regulatory purposes with the understanding that it is to be used under essentially confidential conditions depending on the particular situation. In other places, there is frustration within governments as they manage the proprietary trait sequence information and hold it strictly for a single purpose. Often, information that is not available openly or directly ultimately “leaks out into the public domain.” Alternatively, as commented earlier, laboratories have back-engineered the methods from the information gleaned from the literature or public filings.

Reference materials

Quantifying an analyte normally requires using a reference material as close to the unknown sample matrix as possible. This is a challenge for GM traits as reference materials are not available for all traits/events and may not be considered directly applicable to the processed foods being regulated. The Joint Research Commission Institute for Reference Materials and Measurements (IRMM JRC)¹⁹ has undertaken significant efforts to develop grain reference materials. This has not been without its problems but materials are available for some traits. The Canadian Food Inspection Agency (CFIA) has work ongoing to develop a canola reference material, and a joint effort between the Australian Government Analytical Laboratories (AGAL) and some Canadian research labs aims to develop alternative reference material approaches. The American Oil Chemists' Society (AOCS)²⁰ recently offered powder reference materials and certified DNA reference materials obtained from plants.

Some laboratories have used a DNA reference (plasmids) as an alternative to grain reference material. This limits the validation in omitting interactions of the matrix with the method extraction steps and the interaction of the matrix components with the quality of the DNA extracted. Plasmid reference materials are often used with correction factors and should be calibrated against a suitable reference material derived from the plant.

¹⁹ www.irmm.jrc.be/html/reference_materials_catalogue

²⁰ Certified Reference Materials for canola, corn, rice, cottonseed, and potato: www.aocs.org/tech/crm

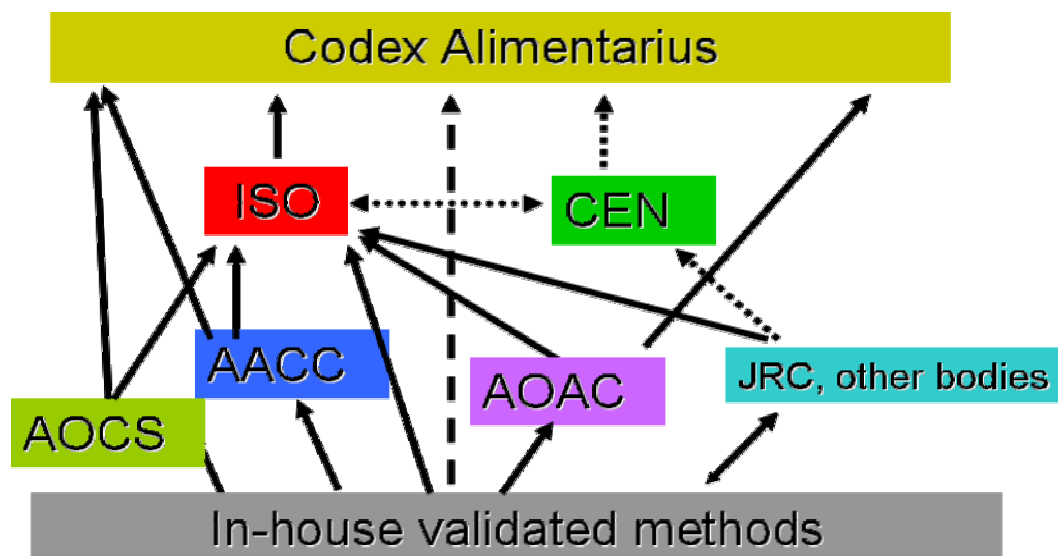


Figure 2: Relationship between bodies involved in standardization of detection methods

International validation and standards

The relationship and flow of methods between the parties interested in detection methods and standards organizations can be quite complex (Figure 2) and is not always consistent.

In most instances, methods are developed in the local laboratory and will normally follow recognized validation guidelines to ensure the method is fit for purpose in that laboratory with its matrices, conditions, and equipment. Validation guidelines are available from organizations like The International Union of Pure and Applied Chemistry (IUPAC) and AOAC International.²¹

For methods that are used for commercial trade, additional validation of the method by a recognized organization is preferred. AACC International has conducted collaborative

trials to measure the fitness and robustness of some protein and DNA detection methods for

biotech crops.²² These can then be evaluated as approved methods and depended upon to perform reliably under the conditions specified by the approved method. Other organizations like USDA, GIPSA, and the UK Food Standards Agency (FSA) have conducted proficiency studies. In all instances, the matrices studied have been fairly limited in scope. Both approaches allow laboratories to measure and compare their performance with other laboratories doing similar work.

The increasing number of analytes, large number of potential matrices, diverse laboratory conditions and resources, and complexity of regulatory preferences and legislation made discussing standard methods in a global arena more challenging. Both the European Committee for Standardization (CEN) and ISO have prepared standards that provide guidelines for method performance rather than a single standard method.

²¹ IUPAC 1998. Compendium of Analytical Nomenclature The Orange Book - 3rd Edition J. Inczedy, T. Lengyel, and A.M. Ure Blackwell Science, 1998 [ISBN 0-632-05127-2] www.iupac.org/publications/analytical_compendium

AOAC: www.aoac.org/about/aoac.htm

²² AACC Methods 11-10, 11-20, 11-21:

www.aaccnet.org/ApprovedMethods/top.htm

One major area of conflict and disagreement is sampling. Plans to hold an international discussion on sampling under the experienced grain industry umbrella of the International Association for Cereal Science and Technology (ICC)²³ and AACC International, and the US ISO/TC 34/Working Group 7 technical advisory committee are in progress. While new concepts and ideas in science are always welcome and indeed necessary, developing standards requires consideration of the capacity to manage them in the field.

Codex Alimentarius had discussions on methods during the *Ad Hoc* Intergovernmental Biotechnology Task Force meetings. These discussions were referred on to CCMAS²⁴ and a working group was established. At this time, the group is discussing criteria for methods but has not reached international agreement to move forward.

Training programs and the status of capability in global regions

Training

ILSI (in collaboration with AACC International and AEIC²⁵) has provided training in some of its regional areas. The three-day workshop program covers the basics of detection technologies through hands-on laboratory activities and places emphasis on the application challenges. In each location, the training workshop is modified to meet the needs of local issues. The JRC has an advanced-training program for EU members, and although initially this program was

available to other countries, it is now delivered only in new EU accession countries.

Global regional activities

The technical complexity of methods for GM trait detection has stimulated extensive discussions among molecular biologists around the world. The early work comparing method performance between laboratories resulted in highly variable results. This appears to have resulted in a thought pattern that “it required a whole new framework of standards and regulations” to manage GM methods. The methods’ complexity has not diminished though it is now recognized to often be laboratory-specific or the method design may lack robustness; both are problems often associated with PCR methods.

Such problems are not generally found in protein detection methods that performed very well in collaborative trials²⁶ and have been verified in many cases by the USA GIPSA.²⁷ This may be related to the fact that protein tests are based on long-established technology, are fewer in number, and are offered as commercial kits. It may also be related to PCR signal generation’s logarithmic nature, which means small changes in the analytical efficiency lead to large changes in the signal developed. More recent work shows method problems can be addressed and variability reduced with additional training, changes in laboratory design and planning, and a broader understanding of food testing. In fact, many of the perceived and actual problems are now recognized as similar to those faced with other types of test methods in food matrices.

The European countries and Japan have been the most active in developing detection methods, training programs, reference materials, and discussion networks. The

²³ ICC website: www.icc.or.at/p-meth-sub.php

²⁴ Criteria for the Methods for the Detection and Identification of Foods Derived from Biotechnology CX/MAS 07/28/8.

²⁵ www.AEICbiotech.org

²⁶ AACCI: www.aaccnet.org/news/10_00bridges.asp

²⁷ www.gipsa.usda.gov

introduction of food labeling regulations in the EU and Japan was the driving force behind these activities. The effect has been to strongly influence activities in other global regions where the regulations may be different and a more cost-effective approach might be more appropriate.

In Asian countries, some method sharing and international cooperation is underway. The ASEAN countries have considered and are discussing the possibility of a “small scale network” to help build capabilities. There is also an early “expression of an interest” in an APEC network for the purpose of technical exchange. The need to cooperate often occurs under emergency situations and at minimum a communication link for technical exchange could be of value.

Status of validation and performance criteria

Europe

The EU countries have formed a network of regulatory authority laboratories that meets on a biannual basis to share ideas. The network is led by the JRC laboratory in Ispra, Italy. The JRC key responsibilities include: running the Community Reference Laboratory (CRL) in the context of the GM food and feed regulation, operating the European Network of GMO Laboratories (involving >120 members in the EU, Norway, and Switzerland), validating detection methods for GM traits, and participating in ring trial work.

Detection method activities in EU laboratories have been driven by labeling legislation (at the 0.9% level for approved events). More recent work has also considered the issues surrounding traceability. JRC at Ispra, the lead laboratory in the EU, is largely responsible for the method validation work.

CEN has also undertaken standards work in Europe, which led to a series of standards that were adopted as ISO standards. Concerns about possible regional approaches to standards have stimulated a lot of discussion in the international community and resulted in the proposed CEN sampling standard being rejected at the ISO level.

Extensive work on powdered reference materials is ongoing at the IRMM JRC laboratory. Particular challenges have related to the particle size (which is critical for the sampling considerations) and the stability of the material.

NAFTA²⁸

Excellent communication occurs among the policy departments of each of the competent authorities. There are high-level discussions that recognize the importance of analytical methods in managing biotech crops. However, the pre-workshop interviews suggest that these discussions do not appear to have translated to collaboration among those responsible for the laboratory activities.

Canada

Early activity was focused on developing the technical capability to handle any food-related emergency. CFIA manages programs to register varieties. The CFIA network of laboratories manages and identifies the movement of genetically modified plants with novel traits as defined in Canada, with each taking different responsibilities for crops and method types. These labs are capable of responding to “non regulated events” and made some progress towards new detection methods. The relaxation in political concerns regarding biotechnology in Canada has allowed laboratories to maintain their technical expertise by applying it to other areas. They

²⁸ See Appendix 1 for outline of agencies in each country with responsibility or other interest in GM crops.

are using the opportunity to explore other areas of research, such as DNA authentication studies and microbiological challenges. The western Canadian CFIA laboratory is preparing a canola standard reference material. The laboratories are all interested in building international technical partnerships.

Mexico

The identification of Mexico as a center of origin for a number of agricultural crops has increased interest in Mexico's position regarding testing. The different government agencies have been challenged to identify their best areas of interest. Through careful planning and World Bank funding, several agencies developed the technical capacity to participate in method development and testing. There is interest in the environment, agriculture and health ministries for technology and capacity building activities.

Mexico was concerned about help from other international agencies that has not been very fruitful, but the root cause appears to be the conflict with the proprietary agreements that are in place. Research in Mexico is very advanced and related to crops that are important to the local diet.

USA

The division of responsibilities in the USA is clearly defined. The FDA is responsible for food and has capacity and technical ability but no current activity. The EPA has a laboratory and trained staff and is requesting methods with all new registrations that require herbicide or insecticide regulations; these methods are focused on raw ingredients. The USDA Agricultural Marketing Service (AMS) is responsible for testing food crops and is also developing methods for detection; all methods are non-proprietary and a testing service is available as a fee-for-service program. The USDA GIPSA is using a combination of proprietary and non-

proprietary methods to monitor the grain trading programs. In addition, GIPSA manages a very widely used proficiency program for corn and soy. The National Institute of Standards and Technology (NIST) is looking at providing reference materials for DNA quantitation and attempting to improve methods for measuring the quality and quantity of the extracted DNA.

Commercial laboratories also operate in the USA to provide seed, grain, and food testing services and some of these are members of international laboratory testing companies and international testing associations. In addition, most states have seed certification laboratories that are members of the Association of Official Seed Certifying Agencies (AOSCA).

Asia

Most countries in the north Asia Pacific region have labeling regulations and extensive activities in method development.

Japan has well-developed detection method research capability and testing in a number of government laboratories to manage the 5% level for GM-related labeling. DNA detection methods and using DNA plasmids as reference materials have been the focus.

Korea has developed methods to manage the 3% level for GM-related labeling and is working in the local area to compare capability.

Both **Korea** and **Japan** are active participants in the ISO/TC 34/Working Group 7. They are also frequent participants in international collaborative trials.

China has held training programs (and participated in US-based technology exchange discussions) and is working to develop national methods. There is some

overlap in responsibility between different government agencies.

ASEAN countries have discussed their expertise and capacity (**Indonesia, Thailand, the Philippines** and **Singapore** have testing laboratories and some methods development capability). They held preliminary discussions to form a network in the region to share capability and perform proficiency testing. Thailand has been an active participant in international collaborative trials.

Discussion among Asia-Pacific Economic Corporation (**APEC**) countries on possible cooperative testing is ongoing.

South Asia

India and **Sri Lanka** have held training programs. Research capacity is advanced but there are limited facilities for routine testing.

South America

Argentina and **Brazil** have active government and commercial laboratories with testing capability. Because both countries are exporting countries, they have also established a comprehensive approach to handling international requirements. They have made commitments to training and other capacity building activities. Identity preserved programs are managed in each country, and both commercial and government testing laboratories are available. Argentina has been very active in ISO/TC 34/Working Group 7, particularly in the discussion of sampling issues.

Chile, Venezuela, Peru, and Columbia have started building expertise and technical capacity. Each of these countries is concerned about the as yet unresolved interpretation of possible responsibilities arising from the Cartagena Protocol.

Australia and **New Zealand** both have regulations affecting seeds, field trials,

commercial release, and food labeling. They have developed capability and there is a commercial laboratory capable of managing their regulations. There are discussions underway with respect to managing adventitious presence. Work on GM reference standards is ongoing at the Australian National Measurement Institute. It is currently based around a generic gene to develop the methodology but there are indications it could be useful for GM detection methods.

Summary and topics for further discussion

It is clear that governments will continue to use GM testing methods to enforce regulation of agricultural biotechnology and ensure products comply with these regulations. Coordinating implementation of these compliance and enforcement methods to enhance testing predictability and addressing food and feed supply chain requirements can potentially minimize the disruptions associated with testing and ensure cost-effective food supply chain management. These requirements are:

1. Consistent test results for a product through the food supply chain

As a product moves through the food chain, it is subject to local and international food regulations that are managed by competent authorities. If sampling and testing are part of the process, then it is most important that both producers and regulators are able to report similar results. The methods used by third-party laboratories must also be validated and consistent to reduce the risk of costly failure in the food supply system. It is preferable to test a product at the beginning of the chain, although this is not always the situation that is regulated. For this reason, it is important to

recognize and use methods that are applicable to changes in the food matrix as it moves through the chain.

2. Recognition that the testing marketplace for GM needs standards and standardization

The food marketplace is ever more a global activity. The cost of transportation is high and it is imperative that false reports do not interrupt trade. Identity preserved products have relatively high management costs associated with them. This highlights the need for test methods to be validated on a global basis and for government support and perhaps oversight. International standards could provide a useful reference.

3. Recognition that all methods are NOT created equal

Numerous challenges to method results show that methods are not transferable without validation studies. It is important that test kit manufacturers acknowledge the limitations and validate the “entire method.” Validating each step separately may not be appropriate for DNA- based test methods. Similar concerns should be discussed with respect to using reference materials including the applicability of using grains as reference materials for processed foods. International recognition and acceptance of reference material definitions is an important issue to be resolved.

4. Recognition that sampling is critical

Sampling must be considered throughout the testing protocol. The scheme used for qualitative testing is not the same as that required for quantitative testing. One size does not fit all and the sample size must be reasonable and appropriate. A practical understanding of the process is essential. An important source of non-compliance occurs when the lot and/or samples are too large, too heavy, or too difficult to manage. The sample

size must consider the limit of detection (LOD) and the limit of quantitation (LOQ) required for any particular test situation. Sampling must also be considered within the laboratory. The test sample particle size is very important if testing for low levels of GM.

Recommendations

Interviews and discussions have drawn attention to the need for increased communication avenues for the technical community. At the same time, there were also specific concerns about available resources and remaining focused on the priorities of each respective regulatory community. With this in mind, it is suggested that all representatives and their advisers consider the goals below and the pros and cons of each. It would be timely to establish working goals with clear responsibilities and define the follow-up activities.

1. Network for technical capacity building, regular (annual) update meeting of technical level personnel

The power of information at the laboratory bench level cannot be underestimated. In addition, the opportunity to share and compare is a valuable learning and calibration tool that allows the technical support community to provide confident and timely support to policy decisions and food supply chain management. Participation of government agencies in scientific forums can also help share information.

2. Collaborative agreement between countries to develop guidelines for method performance and validation criteria

The most effective basis for information sharing and training would be a set of common guidelines and performance criteria.

3. Sampling given additional focus

Experiences in the global food supply chain indicated that sampling guidelines need to be kept in the forefront. It is envisaged that a separate goal should be developed to ensure there is a groundwork discussion that identifies a document (or documents) that will provide clear direction and can be referred to in both routine and crisis situations.

4. Reference materials, discussion and guidelines, and opportunity for more research in this area

The challenge of developing suitable reference materials is a global issue. However, some governments may have specific technical capabilities and be able to provide global leadership in developing criteria.

1 Appendix 1

List of NAFTA agencies and their responsibilities

Role	Mexico	Canada	USA
Regulation of Confined Trials	SAGARPA ²⁹ www.sagarpa.gob.mx	CFIA ³⁰ www.inspection.gc.ca/ english/toce.shtml	USDA-APHIS ³¹ www.aphis.usda.gov
Food	SSA (COFEPRIS) ³² www.salud.gob.mx/ www.cofepris.gob.mx	Health Canada www.hc-sc.gc.ca/	FDA-CFSAN ³³ www.foodsafety.gov/list.html
Feed	SAGARPA	CFIA	FDA-CFSAN
Grain		Canadian Grain Commission www.grainscanada.gc.ca/	USDA-GIPSA ³⁴ www.gipsa.usda.gov/
Environment	SEMARNAT ³⁵ www.semarnat.gob.mx	CFIA	USDA – APHIS EPA ³⁶ www.epa.gov for Plant pesticides
Biosafety Cartagena Protocol	CIBIOGEM ³⁷ www.cibiogem.gob.mx	Biosafety Guidelines	Biosafety Guidelines

29 SAGARPA Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación

30 CFIA Canadian Food Inspection Agency

31 USDA – APHIS United States Department of Agriculture Animal and Plant Health Inspection Service

32 SSA SECRETARÍA DE SALUD COFEPRIS Comisión Federal para la Protección contra Riesgos Sanitarios

33 FDA – CFSAN United States Food and Drug Administration Center for Food Safety and Applied Nutrition

34 USDA – GIPSA United States Department of Agriculture The Grain Inspection, Packers and Stockyards

35 SEMARNAT Secretaria de Medio Ambiente y Recursos Naturales

36 EPA Environment Protection Agency

37 CIBIOGEM La Comisión Intersecretarial de Bioseguridad y Organismos Genéticamente Modificados