Review Article

International development of methods of analysis for the presence of products of modern biotechnology

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Methods of analysis for products of modern biotechnology are required for national and international trade in seeds, grain and food in order to meet the labeling or import/export requirements of different nations and trading blocks. Although many methods were developed by the originators of transgenic events, governments, universities, and testing laboratories, trade is less complicated if there exists a set of international consensus-derived analytical standards. In any analytical situation, multiple methods may exist for testing for the same analyte. These methods may be supported by regional preferences and regulatory requirements. However, tests need to be sensitive enough to determine low levels of these traits in commodity grain for regulatory purposes and also to indicate purity of seeds containing these traits. The International Organization for Standardization (ISO) and its European counterpart have worked to produce a suite of standards through open, balanced and consensus-driven processes. Presently, these standards are approaching the time for their first review. In fact, ISO 21572, the "protein standard" has already been circulated for systematic review. In order to expedite the review and revision of the nucleic acid standards an ISO Technical Specification (ISO/TS 21098) was drafted to set the criteria for the inclusion of precision data from collaborative studies into the annexes of these standards.

Key Words: biotechnology, methods of analysis, international standards organizations, GMO

INTRODUCTION

Analytical methods are necessary to determine the level of any analyte in a material or matrix. Routine use of a method in a laboratory will lead to a level of proficiency that results from the specificity of the test for this application, the suitability of the chemicals and equipment used and the skills of the operator. These factors may be assessed to give the measurement uncertainty.[1] The reliability of those measurements can be assured in an organization where there are defined quality control and quality assurance procedures in place.

However, a method must also give consistent results when the same sample is tested in multiple laboratories. Testing a method in multiple locations requires considerable time and resources and is often left to a standards development organization (SDO). Such studies are usually undertaken only when there are sufficient data available to judge the ruggedness and applicability of the method. This process of method testing is termed a collaborative study or round robin. Two sets of guidelines are defined for the conduct of a collaborative study and are defined by ISO[2] and jointly by AOAC International and the International Union for Pure and Applied Chemistry (IUPAC).[3]

Analytical measurements should be made to meet a defined objective, for example, the detection of a particular constituent, or the detection of a contaminant or unwanted substance and may be used to detect the presence or absence of an analyte at a particular level. Thus, the methods developed by an SDO in response to industry need may be used by regulatory and enforcement agencies to test the conformity of a product to trade, contractual or other specifications.

USE OF METHODS OF ANALYSIS

Methods of analysis developed by SDOs may become the recommended testing procedures used to assure product quality in Codex Alimentarius specifications and international consensus standards are the tests preferred by the World Trade Organization (WTO) in the Technical Barriers to Trade (TBT) agreement.

Preference is given to methods of analysis developed in an open, transparent and consensus-driven manner. Since many SDOs manage their methods programs in this way, there is a wide choice of methodology available from a variety of competing sources. Hence Codex Alimentarius lists methods of analysis from numerous organizations in its commodity and procedural specifications. All methods found in Codex Alimentarius commodity specifications are endorsed by the Codex Committee for Measurement and Sampling (CCMAS). The SDOs providing methods for CCMAS endorsement are also recognized international non-governmental organizations (INGO) or

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inter-governmental organizations. Methods providers are also participants in an InterAgency Meeting that reports to CCMAS the results of its deliberation on relevant issues.

Listed amongst the standards endorsed by Codex Alimentarius are those of the International Organization for Standardization (ISO) and, less frequently, the European Committee for Standardization (CEN – Comité Européen de Normalisation).

Generally, for worldwide acceptance, ISO standards have been adopted as trade standards. The process of standard development laid out by ISO occurs in 6 phases (see Figure 1). Each country nominates a single standards organization to vote at each stage of development and all participating countries (member bodies) are encouraged to act in an open, transparent and consensus-driven manner. Additionally, an important criterion in the ISO process is global relevance. Global relevance has been defined by ISO as "the required characteristic of an International Standard [is] that it can be used/implemented as broadly as possible by affected industries and other stakeholders around the world".

CEN is a regional standards organization representing the member countries of the European Economic Region. Regional (EN) standards are less effective than global standards in their acceptance by trading partners.

STANDARDS FOR THE DETECTION OF PRODUCTS OF MODERN BIOTECHNOLOGY

The following discussion outlines the history of the current international standards for the detection of the products of modern biotechnology, the potential for their revision and the opportunity for the development of future standards.

In the year 2000, following the decision by ISO/TC 34 SC02 (Oleaginous Seeds and Fruits and Oilseed Meals) that work on genetically modified organisms (GMO) should be led by the parent Technical Committee (TC 34 – Food Products), a new Working Group was formed by CEN (CEN/TC 275/WG 11) to work on standards for the detection of GMO in food products. Subsequently, a mirror group, WG 7, was formed under ISO/TC 34. Therefore, the development of the initial suite of standards (Figure 2) was led by CEN/TC 275/ WG 11.

The suite of GMO standards contains the following ISO standards:

ISO 21569, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods*[4]

ISO 21570, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods*[5]

ISO 21571, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction*[6]

ISO 21572, *Foodstuffs — Methods for the detection of genetically modified organisms and derived products — Protein based methods*[7]

The first published, ISO 21572, has already been subjected to systematic review. Included in Figure 2 is a requirement for a sampling protocol:

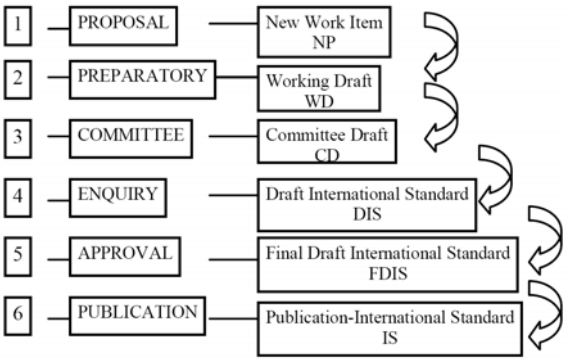

Figure 1. The six stages of ISO standard development

EN/TS 21568, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Sampling strategies*[8]

This requirement was considered necessary by the CEN Working Group but was discontinued at ISO after Working Group 7 determined that current ISO standards were sufficient for GMO sampling and the proposed protocol was both experimental (not used in regular trade) and not practicable (could not be implemented without undue impact on trade). Further revision of all bulk commodity sampling standards is expected after the delivery of an International Workshop Agreement on Sampling in the future.

Revision of the GMO measurement standards is of particular concern because they each contain a “general” descriptive section together with a number of informative annexes that detail experimental protocols. Inspection of the annexes reveals that a number of them contain incomplete validation data and most do not conform to the requirements of ISO 5725:1994. Although the use of annexes was thought to simplify the addition, removal and amendment of the descriptive methods, no process was published by the CEN Working Group. Therefore, after evaluation, a Working Group was formed separately under the auspices of ISO/TC 34 to develop the Technical Specification: *ISO/TS 21098 Foodstuffs - Nucleic acid based methods of analysis of genetically modified organisms and derived products - Information to be supplied and procedure for the addition of methods to ISO 21569, ISO 21570 or ISO 21571*[9]. It was published in late 2005.

The introduction to ISO/TS 21098 states “ISO has an obligation to ensure that the international standards it develops, adopts and publishes are globally relevant.…. [and] should be performance based as opposed to design prescriptive….” “For a standard to be performance based, a clear definition of performance characteristics must be available.” “Although a number of specific methods have been proposed as part of the proposed standards (ISO 21569, ISO 21570 and ISO 21571) and associated general document (ISO 24276), there is not sufficient clarity for submitters to be able to judge whether a method meets the standard, and no mechanism is in place to govern acceptability and/or adoption of such method or for retaining methods in the standards.”

The submission of new methods of analysis to ISO/TC 34/WG 7 has triggered the creation of the expert panel

required by ISO/TS 21098 to evaluate the availability of precision data.

It contains the following information in the Scope; "It also specifies the process for adding, amending and retaining methods annexed to these standards. This Technical Specification is necessary in order to attain consistency in methods that are to be employed as part of the standards. It does not cover the specifics of the development of a method or laboratory set-up. The operation of laboratories is covered in ISO/IEC 17025. Method validation is instrumental [critical] in assessing the reliability of a test method. Its central role is to establish numerical values for the performance criteria that are to be established. ISO 24276 includes details on method validation, taking into consideration specific technical issues related to the detection of genetically modified organisms and derived products."

The TS requires that the following aspects of the method are presented for evaluation: Scientific basis; Scope/Applicability; Selectivity; Reference Materials; Analytical controls; Trueness and Precision; Instrument Specificity; Specification of the prediction model/ mathematical model needed for the method; Criteria for Acceptance of Data; Sensitivity and Range, Robustness Testing; Performance Requirement; Intellectual Property and Related Issues. In addition, the TS specifies the timeline for consideration of additional and current annexes.

ISSUES FOR LABORATORIES

Laboratories must choose methods to implement that meet their intended purpose. Many discussions have focussed on the "Fitness for Purpose" of a method. In deciding to use a particular method the laboratory must evaluate the precision data derived from collaborative studies and determine if the method performance matches the requirements of the analysis. A laboratory must also ensure that it can reproduce the levels of accuracy in the precision data. The use of reference materials, certified reference materials, control charting and proficiency testing all contribute to the understanding of a laboratory's capabilities. Although the precision of a method is provided in the published standard, individual bias and method uncertainty must be determined by the laboratory.

In considering methods of analysis for the products of modern biotechnology, it must be appreciated that detection relies on an amplification system for either specific protein(s) by immunological reaction or nucleic acid by polymerase chain reaction (PCR). Collaborative study and proficiency testing indicate that the coefficient of variation in these methods may be in the region of 25%. Individual laboratories perform with much greater precision, but tend to be biased high or low. Such findings confuse the interpretation of the possible detection of GMO at low levels, especially in geographic regions with stringent regulations. Since the determination of method precision

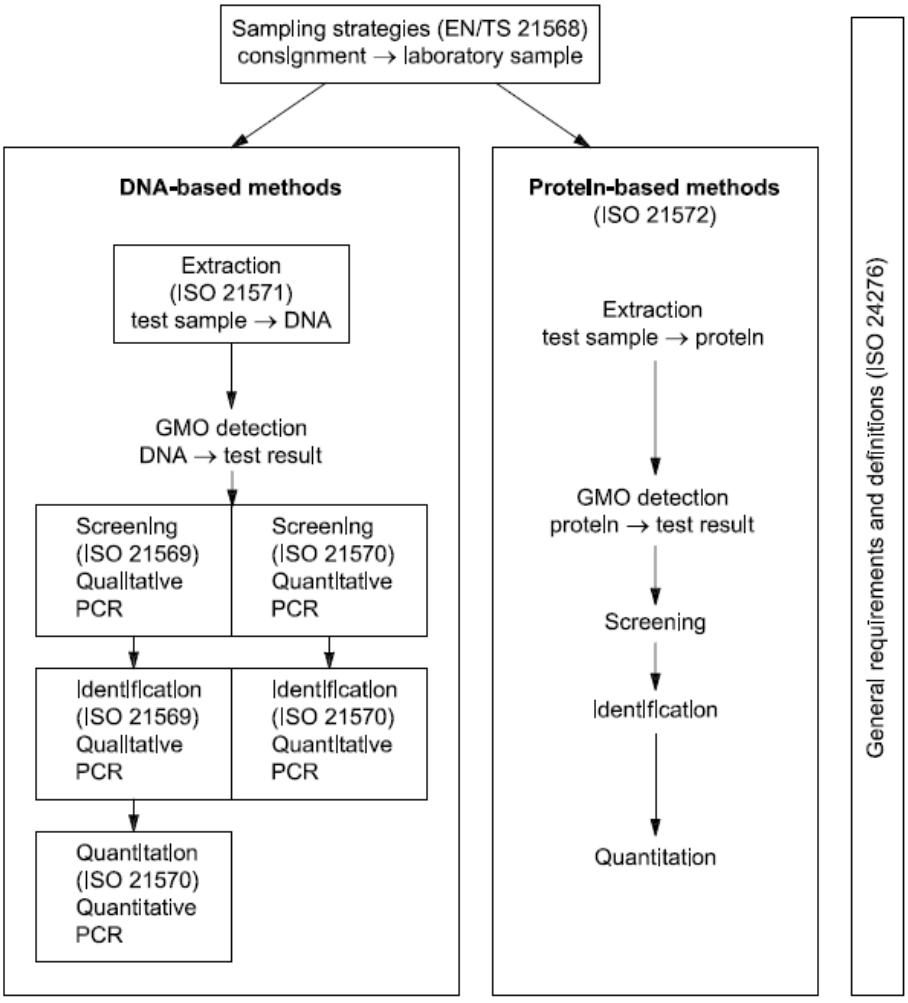

Figure 2. Interrelationship of the EN/ISO GMO standards, taken from ISO 24276

data requires the acceptance of all data within 2.8 standard deviations of the mean, this indicates that the variability in GMO determinations can be very large.

When applying these principles to the interpretation of results, care must be taken in assessing the risk to sellers and buyers when laboratory results fall in the region of statutory limits. If a statutory limit is 1%, then to be sure that a consignment is below this level, the seller must anticipate that the analytical result is 0.5% or less. Where there is zero-tolerance, the false positive rate of the method must be carefully determined.

FUTURE DEVELOPMENTS

At present ISO/TC 34 is considering the addition of a new Subcommittee entitled "Horizontal methods for the detection of molecular biomarkers in: foods; seeds and propagules of food crops; commodity food crops; fruits; vegetables and derived foods". The function of this subcommittee is to provide of focal point for biomolecular methods used in the determination of GMO levels and the authenticity of fruits and vegetables and varietal identification. In the structuring of this subcommittee, attention has been placed on the use of molecular biological techniques to measure different determinants and thus ensure optimal transfer of expertise and technologies. Current efforts of WG 7 are planned to be incorporated into the proposed subcommittee once established.

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AUTHOR DISCLOSURES

Richard C Cantrill, no conflicts of interest.

REFERENCES

1. Eurachem Guides The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics. 1998; http://www.eurachem.org/guides/valid.pdf
2. ISO 5725:1994 –Accuracy (trueness and precision) of measurement methods and results - Parts 1 – 6; 1994, International Organization for Standardization, Geneva, Switzerland
3. Horwitz W. Protocol for the design, conduct, and interpretation of method performance studies. Pure Appl. Chem. 1995; 67: 331-343. International Organization for Standardization, Geneva, Switzerland
4. ISO 21569:2005, Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods. 2005; International Organization for Standardization, Geneva, Switzerland
5. ISO 21570:2005, Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods. 2005; International Organization for Standardization, Geneva, Switzerland
6. ISO 21571:2005, Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction. 2005; International Organization for Standardization, Geneva, Switzerland
7. ISO 21572:2004, Foodstuffs — Methods for the detection of genetically modified organisms and derived products — Protein based methods. 2004; International Organization for Standardization, Geneva, Switzerland
8. EN/TS 21568:2006, Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Sampling strategies 2006; European Committee for Standardization, Brussels, Belgium
9. ISO/TS 21098:2005 Foodstuffs - Nucleic acid based methods of analysis of genetically modified organisms and derived products - Information to be supplied and procedure for the addition of methods to ISO 21569, ISO 21570 or ISO 21571. 2005; International Organization for Standardization, Geneva, Switzerland