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National Bioengineered Food Disclosure Standard Guidance on Testing Methods

The regulations implementing the National Bioengineered Food Disclosure Standard (the Standard) identify the standards of performance for detectability testing at 7 CFR 66.9(c). In the final rule, AMS indicated it would provide guidance regarding acceptable testing methods to satisfy that a food does not contain detectable modified genetic material.

Any regulated entity that is using a food on the AMS List of Bioengineered Foods and does not want to include a bioengineered food disclosure because the food or ingredient is highly refined and does not include detectable modified genetic material, should use the following guidance to ensure compliance with 7 CFR 66.9(c).

These instructions address the following:

- (1) General considerations in selecting a test method
 - a. Fit for purpose
- (2) DNA-based methods
- (3) Emerging technologies and other methods
- (4) General considerations in selecting a laboratory
- (5) Recordkeeping requirements

1. General considerations in selecting a test method

When choosing a testing method, regulated entities should ensure the method is fit for purpose. Regulated entities should assess the method's performance to determine its suitability to provide an answer to a given question or requirement, such as a regulatory requirement or limit, in an effective manner. The following factors are critical for ensuring the method is fit for purpose:

- Specific to the analyte of interest (i.e., define what is being measured);
- Appropriate (validated) for the product/commodity being tested;
- Accurate, precise, robust, reliable, and reproducible;
- Applicable range for the measurement value (i.e., appropriate sensitivity); and



- Accessible and practical for testing needs.

Regulated entities should use validated methods accepted by international bodies (e.g., ISO, Codex Alimentarius Commission, AOAC International) and/or validate their own methods to detect the modified genetic material. Method validation should consider sensitivity, specificity, accuracy, robustness, probability of detection (POD), limit of detection (LOD), limit of quantification (LOQ), applicability, selectivity, species specificity, repeatability, and reproducibility.

Internationally-accepted standards-producing bodies have established criteria that provide a basis for developing an analytical scheme to determine the presence and nature of recombinant DNA (rDNA) in a food or food ingredient. The International Organization for Standardization's (ISO) International Standard 24276 *Foodstuffs -Methods of analysis for detection of genetically modified organisms and derived products- General requirements and definitions*, specifies how to use standards for nucleic acid extraction, and both qualitative and quantitative nucleic acid analysis for food matrices. The standards referenced within include:

- ISO 21571 *Nucleic Acid Extraction*,
- ISO 21569 *Qualitative Nucleic Acid Analysis*, and
- ISO 21570 *Quantitative Nucleic Acid Analysis*.

Additionally, guidelines on performance criteria and validation criteria are also covered in Codex Alimentarius Commission document CAC/GL 74-2010 *Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods*.

2. DNA-based test methods

At this time, polymerase chain reaction (PCR) is the most widely used and commercially accepted test method for determining whether modified genetic material is detectable in a food or ingredient. PCR can be used to detect known or unknown genetic modifications, single or multi-genetic modification events, and qualitative or quantitative results. PCR includes both qualitative and quantitative measurements. Qualitative PCR testing will detect the presence or absence of modified DNA. Quantitative PCR testing will reveal how much modified DNA is detectable in a product.

Certain DNA sequences are often incorporated into many different products, which enables broad-spectrum PCR testing that may be capable of detecting many different events. For some



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matrices, these broad-spectrum tests may be sufficient to detect plant DNA and both known and unknown genetic modifications in a food or ingredient. However, such tests may not be capable of detecting all events and/or may be susceptible to false positives.

In some instances, event-specific or construct-specific tests may be necessary to identify specific genetic modifications. When used with appropriate reference materials, PCR testing can detect specific single and multi-genetic modification events.

While PCR is widely used, it may be limited by PCR-inhibiting compounds and is dependent on isolation of high-quality DNA from a sample. The laboratory must ensure that the method is validated for the specific matrix and adequately extracts DNA for each ingredient/matrix and should monitor PCR inhibition.

3. Emerging technologies and other methods

Any DNA-based method that meets the criteria and is fit for purpose for detecting modified genetic material may be acceptable. Technology continually progresses and advanced technology, such as DNA sequencing (e.g., whole genome, target enrichment), may become more commercially and economically accessible. If these emerging technologies meet the requirements of 7 CFR 66.9(c), they would be sufficient to comply with the Standard.

4. General consideration in selecting a laboratory

When choosing a testing laboratory, USDA strongly encourages adherence to the ISO 17025 standard. The laboratory must be able to meet the following standards, as required by the regulations at 7 CFR 66.9(c): (1) laboratory quality assurance must ensure the validity and reliability of test results; (2) analytical method selection, validation, and verification must ensure that the testing method used is appropriate (fit for purpose) and that the laboratory can successfully perform the testing, (3) the demonstration of testing validity must ensure consistent accurate analytical performance (i.e. performance testing and use of appropriate reference materials); and (4) method performance specifications must ensure analytical tests are sufficiently sensitive for the purposes of the detectability requirements of Part 66.

5. Recordkeeping requirements

Under 7 CFR 66.302(a), regulated entities must maintain customary or reasonable records to demonstrate compliance with the Standard. This includes maintaining records in electronic or paper format for at least two years beyond the date the food or food product is sold or distributed



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for retail sale. Examples of customary or reasonable records that could be used to demonstrate compliance with the disclosure requirements, specifically as it relates to testing to detect modified genetic material, include: supply chain records, supplier attestations, third party certifications, laboratory testing results, validated process verifications, and other records generated or maintained by the regulated entity in the normal course of business. As allowed by 7 CFR 66.9, regulated entities may also maintain certificates of analysis or other records of testing appropriate to the specific food that confirm the absence of modified genetic material. These records should include details listed above in section 1.

In the event of an audit or examination under 7 CFR 66.402, AMS intends to look at a regulated entity's ingredient-specific records and does not intend to conduct independent testing of food products or ingredients on its own.

Useful Resources

ISO 16393. (2019). Molecular biomarker analysis – Determination of the performance characteristics of qualitative measurement methods and validation of methods.

ISO 17025. (2017). General requirements for the complete testing and calibration laboratories.

ISO 24276 (2006). Foodstuffs- Method of analysis for the detection of genetically modified organisms and derived products-General requirements and definitions.

ISO 21568 (2005). Foodstuffs- Method of analysis for the detection of genetically modified organisms and derived products-Sampling.

ISO 21569 (2005). Foodstuffs- Method of analysis for the detection of genetically modified organisms and derived products-Qualitative nucleic acid based methods.

ISO 21570 (2005). Foodstuffs- Method of analysis for the detection of genetically modified organisms and derived products-Quantitative nucleic acid based methods.

ISO 21571 (2005). Foodstuffs- Method of analysis for the detection of genetically modified organisms and derived products-Nucleic acid extraction.

Codex Alimentarius Commission. (2009). Guidelines on analytical terminology, CAC/GL 72-2009.



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Codex Alimentarius Commission. (2010). Guidelines on performance and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods. CAC/GL 74-2010