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ISTA’s work with regards to GMOs: putting it into context

- ISTA’s policy statements over time, for example in position papers (since 2001)
- Proficiency Tests (since 2002)
- Accreditation of laboratories (since 2006)
- Development of statistical tools (since 2001)
- A performance based approach (no specified methods)
ISTA’s work with regard to GMOs: putting it into context

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- Publication in 2014 of a rules chapter
- A future Handbook will offer more precise guidelines to laboratories and auditors

Chapter 19: Testing for seeds of GMO

GMO Handbook
Development and content of GMO chapter

The objective:
- To have a chapter dealing with GMO testing in ISTA’s rules
- To maintain uniformity in GM seed testing

The need for a chapter
- ISTA is involved in GMO testing on seeds
- GMO testing is an increasing activity for seed trade
- Laboratories need guidance about ISTA’s principles and technical aspects of GMO testing.
- Answer ISTA’s strategy decision (2001: to establish a GMO chapter)

Characteristics
- This chapter stays general on all aspects of GMO seed testing
- This chapter is based on already existing ISTA’s papers
- Doesn’t provide protocols (according to PBA)
- Is linked to a Handbook for the detailed technical considerations
- Is not creating new ISTA position / statement about GMO analysis

In charge of ISTA GMO TCOM (technical committee)
- Constitution of a Working Group (2012-2013)

Voted during 2013 ISTA congress Antalya (TU)
Structure of ISTA’s Rules Chapter 19

“Testing for Seeds of Genetically Modified Organisms”

- 19.1 Object
- 19.2 Definitions
- 19.3 General principles
- 19.4 Procedure
- 19.5 Testing approaches
- 19.6 Calculation and expression of results
- 19.7 Reporting results
- 19.8 References
19.1 Object

- The object of testing for seeds of genetically modified organisms (GMOs) is to give guidelines to detect, quantify or confirm the presence of GMO seeds in seed lots

- These guidelines can be applied to testing adventitious presence (AP) of genetically modified organisms (GMOs) and GMO trait purity testing

Main points:

- It is about seed testing

- It covers adventitious presence and purity
• 19.2 Definitions

- Adventitious presence ; Analyte ; Certified reference material ; Genetically modified organism ; GMO event ; GMO trait ; Limit of detection ; Limit of quantification ; Performance-based approach ; Proficiency test ; Seed bulk ; Seed group ; Transgenic ; Reference material

• Main points:
  - Internationally used and accepted terminology in the field of GMOs
19.3 General principles

- The ISTA strategy regarding methods for the detection, identification and quantification of genetically modified seeds in conventional seed lots is available on the ISTA website at: https://www.seedtest.org/upload/cms/user/42Int-M-I200142ISTAPositionPaperonGMOapproved14112001-update1.pdf

- This chapter describes testing for adventitious presence of GM seeds and GMO trait purity. Currently there is no universal threshold for GM seeds in conventional seed lots, or of regulated GM seed in deregulated GM seed, or a specified level of GMO purity in a seed lot; the establishment of reliable methods for the detection, identification and quantification of GMO content is therefore very important. Different technologies, strategies and methods for GMO testing are continuously evolving and new methods being developed. The quality of these test results depends much more on methodology, equipment and training than in other classical seed testing methods. This makes the standardization of GMO testing very difficult. The ISTA approach has targeted the uniformity in GMO testing results, not by the uniformity in testing methodology, but by using a performance-based approach (PBA). The PBA requires that laboratories demonstrate that the GMO detection, identification or quantification methods that they are using on seed samples for reporting results on ISTA Certificates meet acceptable standards set by ISTA. These standards include, among others, sampling, testing and reporting. In order for a laboratory to be recognised as ISTA accredited for GMO testing, it will need to ensure that documented evidence of validation and reliability of the laboratory is available to the ISTA auditors. The evidence must include:
  - performance data based on seed samples for the event and species for which the laboratory is seeking ISTA accreditation, and
  - participation in an ISTA GMO proficiency test including the specific event and species, if available.
19.3 General principles

• This requirement will ensure the reliability of the analysis and the final test result reported on the ISTA Certificate. The PBA gives seed testing laboratories the choice to use different technological approaches, e.g. bioassays, protein-based methods and DNA-based methods.

• For further information, see the ISTA Principles and Conditions for Laboratory Accreditation under the Performance Based Approach.

• Generally, GMO tests that are used to assess GMO trait purity are identical to the tests used for testing for AP of GM seeds. However, there are differences in the testing steps as well as in the objectives. This chapter addresses these distinctions whenever they apply.

• Main points:
  • Refers to ISTA’s principles for laboratory accreditation under PBA (performance based approach)
19.4 Procedure

- **Main points:**
  - Similar workflow for adventitious presence (AP) or purity
  - Different “levels” of analysis:
    - DNA, Protein, Organism
  - AP and purity testing will require different testing schemes, ex:
    - Seed bulk testing is more common in testing AP GMO
    - Purity tests are usually performed on a representative sample of individual seeds
19.4 Procedure

19.4.1 Sample size

- Chapter 2: Sampling gives definitions of various sample types, including primary, composite, submitted and working samples, as well as guidelines for obtaining seed lot samples that represent the properties of the seed lot. These definitions and guidelines apply also to GMO testing. The working sample is the portion of the submitted sample that is actually tested by the testing method (as defined in Chapter 2). The size of the working sample depends on given threshold requirements, the method capability and the degree of required statistical confidence, and can be determined using appropriate statistical tools (e.g. SeedCalc (19.6.3)). The sample submitted to the laboratory must therefore be at least the size of the working sample, but more realistically larger than the working sample. For more information regarding sampling, see Chapter 2.

- The sizes of seed bulks and groups must be consistent with the performance of the analytical method in terms of limit of detection, in order to allow the detection of even one GM seed. For quantitative methods, if a laboratory aims at quantifying the presence of a single seed in the working sample then the size of the sample must be consistent with the limit of quantification.

19.4.2 Personnel and equipment

- Many of the procedures used for GMO testing are composed of several stages (e.g. seed planting or grinding, DNA or protein extraction, detection of the target analyte, and reporting of results) which can be carried out by different personnel in the laboratory (see Figure 1). The laboratory must show that personnel are adequately trained in the procedures that they are carrying out, and that they understand the overall workflow of the procedures and their contribution to that workflow. Each part of the workflow and the equipment must be adequately validated, verified or calibrated before use. Appropriate equipment and facilities must be provided for the use of the chosen methods. For biomolecular assays (DNA and protein), apparatus for grinding and analyte extraction are necessary, as well as equipment dedicated to the detection of the target analyte.

- For DNA-based detection, it is important to prevent contamination, and the use of separate rooms for certain manipulations is preferred.

- For protein-based detection, care must be taken to avoid degradation of the matrix and the extracted analyte.

- For bioassays, care must be taken to ensure the provision of controlled germination conditions adequate to allow the expression of the trait. 19.4.3 Test conditions

- Tests must be carried out under conditions of the ISTA Accreditation Standard quality framework. This includes, but is not limited to the following:
  - Analysts involved in this testing must have the documented skills and training in the corresponding procedures.
  - All equipment must be appropriate to the techniques used. Scheduled maintenance, verification, and calibration of the instrumentation used must be carried out.
  - The spatial arrangements and organization of the testing area must prevent contamination.
  - Reagents of appropriate grade and certified reference materials (when available) must be used.
  - Appropriate controls must be used to validate the testing results.

Main points:

- Chapter 2 definitions and guidelines apply also to GMO testing
  - Ex: working sample
- Working sample size:
  - Threshold; Method; Statistical confidence
- The laboratory must show that personnel are adequately trained
- Appropriate equipment and facilities must be provided for the use of the chosen methods
Content of ISTA’s Rules Chapter 19 on GMO (7/10)

- **19.5 Testing approaches**
  - **19.5.1 DNA-based methods**
    - 19.5.1.1 General principles of DNA-based testing
    - 19.5.1.2 End-point qualitative PCR
    - 19.5.1.3 Real-time PCR
    - 19.5.1.4 Other technologies
  - **19.5.2 Protein-based methods**
    - 19.5.2.1 General principles of protein-based testing
    - 19.5.2.2 Lateral flow strip test
    - 19.5.2.3 Enzyme-linked immunosorbent assay
  - **19.5.3 Bioassays**
    - 19.5.3.1 General principles of bioassays
    - 19.5.3.2 Scoring of GMO presence

- **Main points:**
  - Explains the principle of the techniques
  - Lists the necessary technical considerations related to the different steps of the test
  - Whatever the type of method chosen, its performance will always be evaluated using PBA
• 19.6 Calculation and expression of results
  • 19.6.1 Consideration of the testing objective

• The applicant must clearly state the specific testing objective, as this is critical in defining the testing approach and in calculating and expressing results. Possible testing objectives include:

  • reporting the presence or absence of a GMO in the seed lot;
  • estimating the proportion of the GMO present in the seed lot with the associated measurement uncertainty.

• The methods described in 19.5 produce either qualitative, i.e., detected (GM trait observed) or not detected (GM trait not observed), or quantitative results. Both types of results can be statistically analysed to meet the testing objective, but the data analysis methods and associated calculation tools differ.

• To assess for the presence of two or more stacked events in the same seed, testing individual seed is the appropriate approach. When seed are tested in bulk, the presence of stacked events cannot be demonstrated. However, some statistical tools such as the one proposed by ISTA in SeedCalc Stack9 can estimate the percentage of seeds that could have two or three stacked events.

• Main points:

  • Testing objective must by clear (from the applicant):
    • Testing approach is strictly associated with the nature of testing objective
    • Results are statistically analysed according to testing objective
Content of ISTA’s Rules Chapter 19 on GMO (9/10)

• 19.6 Calculation and expression of results
  • 19.6.2 Units of measurement

  • The calculation and expression of results depend on the testing objectives, testing methods and the associated units of measurement. The aim or request of the applicant will need to be carefully considered. In order to cope with the different objectives and circumstances where quantification of seeds with GMO traits is required, and in concordance with the PBA, it is acceptable to report quantitative test results using any one of the following units:

  a) % in number of seeds: the estimate of the percentage of GM seeds in the seed lot. In addition to individual testing, the percentage in number of seeds is the unit to be used when a group testing approach is chosen; e.g. with SeedCalc (see 19.6.3).

  b) % in mass of seeds: the estimate of the percentage of GMO content by mass. This unit should be used when a standard curve is prepared using certified reference material certified by % mass (g/kg).

  c) % DNA copies: the estimate of the percentage of GMO content by number of copies. This unit should be used when a standard curve is prepared using certified reference material certified by % DNA copies.

  All these three units are acceptable for preparing ISTA Certificates for reporting results by accredited laboratories. The acceptance of more than one unit can avoid raising the difficult question of converting factors. A simple mechanical conversion between units is complex or even impossible

  • Whatever the unit used to express results, the resulting GM estimate should be methodologically meaningful, that is, a laboratory using quantitative real-time PCR should not report a value that is lower than its validated limit of quantification.

  • Moreover, in quantitative real-time PCR, results should be biologically meaningful. The lab should pay attention to results that are lower than 1 divided by the size of the working sample.

  • 19.6.3 ISTA tools for calculation of results

  Remund et al. (2001) and Laffont et al. (2005) provided statistical tools for qualitative and quantitative testing methods which are implemented in the SeedCalc MS Excel workbook (available on the ISTA web site).
19.7 Reporting results

- The result of a genetically modified organism test must be reported under ‘Other determinations’ as follows:
  - the request of the applicant;
  - the name and scope (with reference to the target) of the method(s) used;
  - a description of the working sample (e.g. pure seed fraction, inert matter present, other seeds present, washed seed);
  - the number of seeds in the working sample;
  - a description and the source of the reference material used (e.g. certified reference material, provider);
  - the limit of detection of the method (when testing seed groups or seed bulk);
  - the limit of quantification of the method (when testing seed bulk with a quantitative method)

Main points

- As with other chapters there are guidelines on how to report the results
- Information provided must be complete
- Results can be:
  - Qualitative
  - Quantitative
    - from qualitative tests on multiple seed groups
    - from quantitative test on a bulk of seeds (mass or copie Nbr)
- Reporting results is detailed for each case
• **Conclusions**
  
  – The chapter on testing for GMO seeds reflects ISTA’s existing policy of presenting all relevant information in one document

  – GMO rule chapter was accepted by the vote of delegates at the 2013 ISTA congress in Antalya

  – Associated Technical details and guidelines will be placed in a Handbook
Development and content of ISTA’s GMO Hand Book

Objective:

- To give details of practical aspects on all subjects presented in Chapter 19 to the target audience of
  • Laboratory staff and technicians
  • Auditors
- To ensure that it is user friendly and easily understood

Status: Ongoing work
- Section 1: Introduction
- Section 2: Generalities on GMO
  - Information on GMO
- Section 3: Objectives of GMO testing
  - Context of seeds analysis (presence or purity check)
- Section 4: Analytical approaches
  - Details on the use of available techniques (at the level of the working sample)
- Section 5: Statistical approaches for results analysis
  - Computations and statistical tools corresponding to each type of analytical approach
- Section 6: Overview of method validation
  - Aims to harmonize the way laboratories validate (or verify) their methods
  - Allows a wide choice of approaches
  - Requires documented performance data
  - Covers determination of specified trait:
    - Detection of presence of GM seed / the ability to detect if present
    - Purity assessment of GMO trait / the ability to detect and quantify

- Section 7: Accreditation (Guidelines for laboratory)
  - Validated methods required to obtain accreditation
  - Scope of accreditation: method – species – event
  - Accreditation process:
    - How to present and evaluate performance data
    - ISTA GMO proficiency test (PT) program; requirements

- Section 8: Quality assurance (Guidelines for auditors)
  - Results not methods assessed (PBA, no standardised methods in the rules)
  - Audit checklist
The Chapter and the Handbook are interconnected
- ISTA’s policy position is clearly reflected in both
- Comprehensive approach (all necessary information included)

Provides written reference material to laboratories and auditors on ISTA’s performance requirements for GMO testing

ISTA’s position paper
(Strategy regarding Methods for GM seeds)

Chapter 19:
(since 2014)
Testing for seeds of GMO

Chapter 2

ISTA’s paper
Principles and Conditions for Lab Accreditation under PBA

GMO Handbook (In progress)

ISTA’s PD Evaluation (GM presence)

ISTA’s PD Evaluation (GM purity)

ISTA’s position paper
(View regarding the units for reporting)
The Chapter and the Handbook are interconnected
- ISTA's policy position is clearly reflected in both
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Provides written reference material to laboratories and auditors on ISTA's performance requirements for GMO testing

Chapter 19: (since 2014)
Testing for seeds of GMO

GMO Handbook (In progress)

The users will have access to the necessary information from these two documents
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