GUIDELINE FOR THE ACCREDITATION OF LABORATORIES TESTING FOR GENETICALLY MODIFIED ORGANISMS (GMO)

ESYD G-GMO

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This document gives guidance to and is intended for laboratories conducting testing for the presence of GMOs in foodstuffs, feeding stuffs and seeds. At the same time it aims to provide information for assessors involved in the accreditation process of these laboratories. The scope of this document is currently restricted to DNA detection and quantification methods. Accreditation of laboratories in a flexible scope should be granted according to the document "European technical guidance document for the flexible scope accreditation of laboratories quantifying GMOs" EUR 26547 EN.

Applicant and accredited facilities must also comply with the ISO/IEC 17025 standard and the Accreditation Body's *Procedures for Accreditation.*

Testing for DNA sequences derived from GMOs relies on the ability to test for the specific DNA sequences associated with modifications and/or other regulating sequences associated with the inserted sequences.

Accreditation covers qualitative and quantitative analysis of GMOs through the use of DNA extraction and Polymerase Chain Reaction (PCR) methods.

1. Staff qualifications and experience (ISO/IEC 17025:2005 §5.2.1)
Staff releasing results must be approved on the basis of their demonstrated ability to evaluate the validity of test results as well as ability to communicate orally the technical aspects.

2. Accommodation and environmental conditions (ISO/IEC 17025:2005 §5.3) According to ISO 24276:2006 (§5.3)

3. Assuring the quality of reagents and consumables (ISO/IEC 17025:2005 §4.6.2)
According to ISO 24276:2006, §5.3)

The quality of the standard samples and reagents, must be adequate for the procedure used e .g.
- Oligonucleotide primers,
- Taq polymerase enzymes
- Deoxynucleotide triphospates
- Enzyme buffers
- Magnesium solutions
- Sterile water
- Kits and related reagents

Batch numbers of all critical reagents are to be recorded.

Standards and reagents must be labelled with:
• name of the reagent/standard;
• concentration where appropriate;
• preparation date or expiry date;
• identity of preparer.

Where necessary, the following must be included on the labels:
• storage conditions
• hazard warnings.

In order to avoid freeze/ thaw cycle's aliquots of critical reagents (e.g. primers, probes etc) should be prepared.

4. Method validation (ISO/IEC 17025:2005 §5.4.5)
Methods must be validated / verified for each group of products to establish the method's applicability and limitations. The range of matrices that require individual validation includes raw and processed materials, also depending on the technique used (conventional PCR and gel electrophoresis or Real Time PCR), as well as on the scope of the method (qualitative or quantitative).

If possible, Certified Reference Materials or Reference Materials should be used for the validation. If CRMs or RMs are not available, samples from proficiency tests with a known assigned value, Standard (plasmid or genomic) DNA solutions with a known concentration as secondary RMs or samples with a known concentration, could be used.

Records of validation work must cover the entire range of products for which accreditation is sought.

Validation/Verification of **DNA extraction modules** includes evaluation of the following parameters depending on the technique used (conventional or Real Time PCR) and the scope (detection or quantification):

- DNA concentration, determined in the same way that applies to routine samples and inhibition test in case of use of Real Time PCR.
- *DNA structural integrity (size and damage status)*
- *Quality - suitability for PCR* / q-PCR (reaction efficiency and absence of PCR inhibitors in a DNA sample).
  Acceptance criterion: The method should provide appropriate DNA for the intended analysis (at least enough to meet the desired practical LOD/LOQ).
- Robustness for the incubation temperature (especially in cases where the method for DNA extraction has been developed by the laboratory and it is not a part of a validated extraction kit) to be checked at least at 2 levels, one below and one above the nominal temperature of the extraction.
- Matrix effect. Matrix (plant species – level of processing) control covering the scope is required.

DNA working solution (same DNA concentration with routine analysis) should be used for the validation study.

Validation of **PCR module**
For Qualitative GMO methods the following parameters should be evaluated:

- Specificity. Expressed by the % ratio (correct negative / correct negative+ false negative results (sum of total certified non –GM samples tested). Acceptance criteria: ≥95%. In case of in house designed primers and probes,

specificity should be evaluated theoretically performing a sequence similarity search and experimentally using DNA from other GM plants/target sequences. The PCR module should only produce amplification products with the target sequence for which the module was developed.

- Sensitivity. Expressed by the % ratio (correct positive / correct positive+false positive results) (sum of total tested samples at the minimum concentration detected (LOD) Acceptance criteria: ≥95%.

- Efficiency. Expressed by the % ratio of the sum of (correct positive + correct negative) / sum of total samples tested.

- Limit of Detection
  Relative LOD (LOD_{rel}). Acceptance criteria: For conventional PCR and approved GMOs should be ≤ 0,1% with a level of confidence of 95%, ensuring ≤ 5% false negative results. For Real Time PCR, in case of non approved GMOs the LOD should be at lower levels, depending on the GMO and the levels of GM contamination (scientific literature and EURL-GMFF documents should always be taken into consideration).
  or Absolute.LOD (LOD_{abs}) expressed in copy number.
  The practical LOD (LOD_{prac}, the lowest relative quantity of the target DNA that can be reliably detected, given a known number of target taxon genome copies) could be determined for each individual sample tested.

- Robustness (tested for parameters as thermal cycler, final concentration of master mix, reaction volume, primers concentration, probe concentration & annealing temperature). The target amount/concentration to be tested should be at the LOD. Acceptance criteria: all replicates should give positive results.

  In case of use of interlaboratory validated methods the verification procedure should include all above mentioned except robustness.

For Quantitative GMO methods the following parameters should be evaluated:

- Specificity. Acceptance criteria: The PCR module should only produce amplification products with the target sequence for which the module was developed.

- Limit of Detection. Acceptance criteria: For combined modules the LOD should be < 0.045% with a level of confidence of 95%, ensuring ≤ 5% false negative results. For individual modules the LOD should be < 25 copies with a level of confidence of 95%, ensuring ≤ 5% false negative results.

- Limit of quantification. Acceptance criteria: The LOQ should be ≤ the lowest amount or concentration included in the dynamic range (i.e. 0.09% or 50 copies).

- Dynamic Range. Amplification Efficiency. Linearity (Coefficient R^2). For evaluation of these parameters standard curves (at least 5) are prepared on a minimum of four concentration levels evenly distributed each one analyzed in duplicate. Acceptance criteria: For the combined PCR modules (i.e. GM assay

and taxon-specific assay) the dynamic range expressed in relative concentration should comprise at least 0.09% and 4.5%. The average value of the slope of the standard curves should be in the range of - 3.1 ≤ slope ≤ - 3.6, corresponding to amplification efficiencies of 110% to 90%. The individual values of R^2 of the standard curves should be ≥0.98.

- Trueness. Acceptance criteria: The trueness should be within ±25% of the accepted reference value over the whole dynamic range of the PCR modules individually (i.e. GM assay and taxon-specific assay) and in combination.

- Precision - Relative Repeatability Standard Deviation (RSDr). Acceptance criteria: RSDr ≤25% over the whole dynamic range of the PCR modules individually (i.e. GM assay and taxon-specific assay) and in combination.

- Intermediate Precision $RSD_{ip.}$ Acceptance criterion: RSD_{ip} <35% over the whole dynamic range. However, at relative concentrations <0.2% or at an amount <100 copies RSD_{ip} values <50% are deemed acceptable.

- Robustness (tested for parameters as thermal cycler, final concentration of master mix, reaction volume, primers concentration, probe concentration & annealing temperature, if necessary). The target amount/concentration to be tested should be at the LOQ. Acceptance criteria: the RSD_r and trueness calculated for a combination of changes should not exceed 30%.

- Uncertainty of measurement should be determined according to the document "Guidance Document on Measurement Uncertainty for GMO Testing Laboratories" EUR 22756 EN/2 - 2009

In the case of an inter-laboratory validated quantification method, without any modifications, the method is verified according to the document "Verification of analytical methods for GMO testing when implementing interlaboratory validated methods" (EUR 24790 EN – 2011), for the following parameters:
- Dynamic Range .
- Linearity (coefficient R^2)
- Amplification Efficiency
- Trueness
- Precision – Relative Repeatability Standard Deviation (RSDr).
- LOQ
- Measurement Uncertainty

5. Equipment (ISO/IEC 17025:2005 §5.5 & ISO 24276:2006 §5.3)
Specialised equipments are required.

The following calibrations / checks are required for various instruments:
1. Analytical balance. Calibration at least once a year (if a different frequency is applied, it should be justified), as well as daily control.

2. Incubator for DNA extraction (water bath, thermoblock etc): annual calibration for working temperature (if a different frequency is applied, it should be justified).

3. Thermal Cycler (PCR instrument):
Annual Temperature calibration of the thermal block (e.g. accuracy, uniformity of temperature, rate of temperature change and temperature exceeded - overshoot). If a different frequency or procedure is applied, it should be justified. Periodical maintenance in accordance with the manufacturer's instructions required before calibration (e.g. cooling liquid used in Peltier system, filters etc.) For RT-PCR instrument depending on the specific RT-PCR system and the available procedures for calibration and performance check of the equipment: the Annual temperature calibration of the thermal block (e.g. accuracy, uniformity of temperature, rate of temperature change and temperature exceeded - overshoot).
Any other available procedure for checking optics and electronics performance depending on the equipment and according the recommendations of the manufacturer.
In the case of availability of specific kits or reagents checking the performance of a part of a system or of the whole system (e.g. dye calibration kit, Rnase plate P etc), the calibration program (temperature, optics etc) could be modified according to the instructions of the manufacturer. The laboratory should justify the calibration plan used for its specific instrument.

5. Micropipettes.: Regular calibration (usually annual) /check (e.g. every 3-6 months), depending on the use and the volume of the micropipette.

6. Spectrophotometer: Annual calibration (if a different frequency is applied, it should be justified).

6. Handling of test and calibration items (ISO/IEC 17025:2005 §5.8)
The high sensitivity of methods dictates a higher than usual awareness to the possibility of cross-contamination during transport, storage, preparation and analysis (See Accommodation).

7. Assuring the quality of test and calibration results (ISO/IEC 17025:2005 §5.9)
A quality control program that covers staff proficiency, infrequently performed matrices and performance in external proficiency testing programs, where available, is expected to be followed with appropriate analysis of data and corrective actions.

Controls that are incorporated with the GMO test system must address inhibition, sensitivity and contamination. The requirements of ISO 24276:2006 §5.2 should be followed.

In qualitative methods, the samples should be analysed in duplicate both in extraction and PCR steps. In quantitative methods the samples should be analysed in triplicate in PCR step.

Internal Quality control
Controls used in DNA extraction step:
1. Extraction blank control must be applied each time DNA is extracted from one or more samples. At least one extraction blank control per 10 parallel extractions should be included, last in the series.
2. Negative environmental control should be applied periodically, for the control of environmental contamination.
3. Positive extraction control should be applied periodically and always when a new batch of DNA isolation reagents (e.g. kits) is used.

Controls used in PCR amplification step:
1. Negative amplification reagent control must be included in each amplification reaction, at least one control after each 10 PCR wells.
2. Positive DNA target control must be included in each PCR reaction. The use of CRMs and /or PT samples as positive DNA target control is recommended, in particular with GM target content close to the method LOD, for qualitative assays, and close to the legal limit or any other target, for quantitative assays. This kind of control is also used for testing the quality of critical reagents to ensure their optimal performance, as well as the good performance of the PCR equipment.
3. Negative DNA target control could be included periodically.
4. Target – taxon specific PCR system should be included in qualitative methods in order to test the amplifiability (quality and quantity) of the isolated DNA. In quantitative methods the target –taxon specific method is a necessary step of analysis for the quantification.
5 PCR inhibition control must be applied if all PCR tests on the sample are negative both in GM and target- taxon specific systems, especially for matrices where the quality and the quantity of amplifiable DNA is completely unknown.

Control charts should be designed, especially in case of use of Real time PCR

Proficiency testing (PT) programs covering a period of 4 years should be developed. For the initial assessment, the laboratory must have at least one recent successful PT participation, for all tests of the requested scope of accreditation (analytical method / GM event/ substrate). On annual basis, at least one PT participation relating to each method of a laboratory's scope of accreditation is required.

8. Reporting of results (ISO/IEC 17025:2005 §5.10 ISO 24276:2006 §6.3, 6.4, 6.5 and 7)
Requirements for reporting test results are detailed in ISO/IEC 17025 clause 5.10. and in ISO 24276:2006 §6.3, 6.4, 6.5 and 7.

No affirmation shall be made stating that "*…there is no GMO present in the sample analysed as determined from test samples…"*.
The qualitative results should be expressed as detected or not detected, reporting the corresponding limit of detection for the matrix analysed or for the matrix used in the method validation.

The quantitative results should be expressed % mass/mass or % copies per copies.

Bibliography:

ISO 24276, Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions.
European technical guidance document for the flexible scope accreditation of laboratories quantifying GMOs, EUR 26547 EN, 2013.
Guidance Document on Measurement Uncertainty for GMO Testing Laboratories, EUR 22756 EN/2, 2009.
Verification of analytical methods for GMO testing when implementing interlaboratory validated methods, EUR 24790 EN, 2011.
Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing, JRC95544, 2015.