Module 5:

Introduction to Quality Assurance/Quality Control Standards

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**Introduction**

An important aspect when establishing capacity for the detection, identification and quantification of LMOs is the implementation of a laboratory management system that ensures the provision of minimum performance criteria with respect to quality control and quality assurance (QA/QC) to confirm the adequate handling and processing of samples, as well as to ensure the quality of and confidence in the results obtained. While there is a wide variety of methodologies and instruments that can be used in a molecular biology laboratory for the detection and identification of LMOs, it is important to select and implement methods that will produce reliable and consistent results that meet the regulatory requirements of the Party, while, at the same time, meeting minimum performance criteria.

The implementation of minimal performance criteria ensures that differences in methodology do not bias the results of laboratory analysis and form part of the QA/QC system that is required for laboratory accreditation. It is important that the analyte being measured is defined and that the unit of measurement is standard and understood in simple and internationally accepted language. Furthermore, it is important to use certified reference materials where available to confirm harmonization between laboratories and methods as well as for method validation and verification.

This section of the training manual provides an introduction to the minimum performance criteria that may be considered by laboratories when implementing their selected methods for the detection and identification of LMOs as well as information on the establishment of a QA/QC system and how to validate such methods within a laboratory. A laboratory may be certified to a QA standard such as ISO 17025 or related standards required by the Party. The following information is consistent with such a standard.

**Laboratory Documentation System**

An overarching aspect of QA/QC is the need to substantiate critical aspects of all laboratory activities with documentation. Establishing sound documentation practices contributes to ensuring that minimum performance criteria are implemented and maintained in the laboratory.

Documentation of laboratory activities including the minimum performance criteria include but are not limited to the following:

* Standard operating procedures for the different methods used to process samples;
* Standard operation procedures for equipment used to process samples;
* Standard operating procedures for the preparation of reagents used to process samples;
* Safety manual for the laboratory.

In addition, information relating to the sample as it is processed should also be documented in order to allow for complete traceability the procedure. This should include, but is not limited to, documentation of the following:

* reagent preparation;
* reagent lot numbers used during sample processing including controls;
* temperature controlled equipment including fridges/freezers and incubators;
* maintenance and verification/calibration of equipment.
* raw data for samples and controls throughout the analysis process;
* the final report for the result of the analysis.

Additional information on specific documentation requirements are provided under relevant sections in this chapter.

**Laboratory set-up requirements and environment**

A well-organized laboratory is one of the most important considerations in an LMO testing laboratory in order to produce reliable and consistent results as well as minimize the possibility of sample contamination. Separate rooms or areas for each processing step should be used while maintaining a unidirectional forward workflow. This should include, dedicated areas for sample reception and/or sample homogenization, analyte extraction, reagent preparation, PCR set up, PCR, agarose electrophoresis and/or data analysis. Such considerations are particularly important in multipurpose laboratories where shared spaces are used for diagnostic as well as research purposes.

Each separate laboratory area should ideally have dedicated equipment that is not moved from one laboratory or area to another without carrying out the necessary cleaning and decontamination steps to prevent contamination. Furthermore, each laboratory area should ideally be furnished with dedicated laboratory wear, stationary and personal protective equipment, such as laboratory coats and gloves to facilitate the implementation of good laboratory practice by personnel.

Good laboratory practice[[1]](#footnote-1) includes implementing a cleaning routine of laboratory areas before and after use to minimize the risk of contamination. This includes:

1. Cleaning and decontamination of the working area using detergent and decontamination reagent. It is important that when decontamination reagent is used that the area also be rinsed with water to remove any residual decontamination reagent from working surfaces.
2. Cleaning and decontamination of the sampling and homogenization equipment using detergent and decontamination reagent. It is important that when decontamination reagent is used that the equipment also be rinsed with water to remove any residual decontamination reagent.
3. The sample preparation work area can also be vacuumed after receiving a sample to remove particulate matter.
4. ***Sample reception and Homogenisation***

Samples that are submitted for analysis need to be received, catalogued, and where appropriate, homogenized. Samples should be analysed as soon after receipt as possible. Samples may be split so as to furnish both the test sample and that retention (or retained) samples. Once prepared they are stored in a dedicated space that provides an appropriate storage environment and is secure. Retained samples can be stored at room temperature provided that the sample integrity is maintained for the period of storage. For example, high temperatures and/or high humidity may lead to the destruction of the sample as a result of the growth of microbes and result in subsequent DNA degradation. Under the latter conditions, it would be necessary to store retained samples in a dedicated fridge. Some retained samples may not store well at room temperature or in a fridge, for example seed potatoes, such samples may therefore be stored in a freezer. It is important that precaution is taken not to dispose of any remaining non-homogenized sample in a way that can result in its accidental introduction into the environment.

Sample homogenization should be performed in a dedicated room where no other laboratory procedures are performed and does not share ventilation with other rooms to avoid the spread of possible dust resulting from sample homogenization. Good laboratory practice must be implemented to ensure that dust particles produced by the sample do not contaminate other laboratory samples considering the high sensitivity of subsequent analytical methods. Ideally, the procedure should be carried out in a dedicated fume hood or dust extraction cabinet.

It is important that standard operating procedures include the cleaning and decontamination of the sample reception and homogenization area as well as any equipment. To validate whether a cleaning and decontamination method is effective, either of the following tests can be performed:

1. After processing an LMO sample, a non-LMO sample is processed and then tested for the presence of LMO material; or a sample of one species (for example corn) is processed followed by a second sample from another species (for example soybean). The second sample is then tested for the presence of the first species.
2. Working surfaces and equipment can be swabbed with sterile PCR grade water and the swab placed in a tube with a minimal amount of PCR grade water. Following a brief incubation period, including vigorous shaking using a vortex and centrifugation, the same volume of liquid of water from the swab is added to a PCR reaction to test for the presence and contamination that my result in DNA amplification.
3. ***Protein extraction***

Protein-based methods are in general less succesptible to contamination than PCR-based methods, as proteins are more labile than DNA. Protein extraction is normally carried out by grinding the sample directly in the extraction buffer. A portion of that liquid is either left to settle, or filtered to provide the test portion. Care should be taken that liquid extract does not splash or come into contact with gloves and surfaces, such that it can contaminate other samples.

1. ***DNA Extraction***

DNA is extracted from seed, grain or plant parts using a combination of approaches as listed in module 3. The DNA extraction area is considered a “dirty” environment, meaning that there is potential to for contamination to take place. Care must therefore be taken to ensure that good laboratory practice is followed to minimize potential cross contamination between samples as they are processed in this area.

1. ***PCR mastermix set-up***

This procedure must be carried out in a clean environment that is free of any nucleic acid. In this area or dedicated room, also known as the “clean room”, reagents, such as primers and buffers, are prepared, stored, and aliquoted.. Aliquoting stock solutions into smaller working volumes is an important step in managing the spread of contamination and prevents excessive waste of unused reagents if contamination occurs. Furthermore, making aliquots minimizes the frequency of freeze/ thaw cycles that stock solutions may be exposed to therefore helping to maintain the quality of reagents that may be sensitive to temperature changes. Other procedures that take place in this area include the preparation of PCR master mixes prior to the addition of the sample DNA.

1. ***PCR Sample Preparation***

This area is dedicated to the addition of the extracted and positive control DNA to the PCR master mixes. The addition of sample and positive control DNA to the PCR reaction can also take place in the PCR amplification area.

1. ***PCR Amplification***

This area is dedicated to the amplification of the PCR target and contains the PCR machines. The PCR area can also be combined with post-PCR if the laboratory area allows a suitable separation of work activities. The isolated DNA as well as PCR controls can be added to the PCR master mix in a dedicated area in the PCR amplification room if a separate PCR sample addition area is not available.

1. ***Post PCR***

This area is dedicated to post-PCR activities but can also be combined with the PCR amplification area depending on the available laboratory space. Post-PCR primarily deals with gel electrophoresis as well as well as other possible activities such as DNA sequencing. Ideally, PCR machines should be in separate areas to that of other post PCR activities including gel electrophoresis.

**Sample Tracking**

All samples that are received by the laboratory must be assigned a unique sample tracking number. This unique tracking number is used to identify the sample as it is processed. The use of a tracking number helps maintain traceability and confidentiality of the sample information. Furthermore, the unique sample number can be used to facilitate the systemic storage of samples and/or extracted DNA.

**Minimal Performance Criteria and Requirements for Quality Assurance**

Quality assurance is defined by ISO9000:2015 as “part of quality management focused on providing confidence that quality requirements will be fulfilled”[[2]](#footnote-2). ISO17025 is the most applicable version of this standard series for laboratories. It is a multifaceted procedure that is put in place by the laboratory to ensure that the results of testing are precise, accurate and reproducible. The laboratory quality assurance system also outlines the necessary corrective action to be taken if required.

The following section presents an overview of specific areas where quality assurance measures are applied and the impact they have on maintaining quality. Understanding these parameters and being able to detect any deviations from set criteria during routine testing ensures that results are consistent and remain within acceptable ranges of variability.

1. ***Personnel***

Similar to other fields of molecular diagnostics, LMO detection requires that it is performed by competent and trained personnel. New personnel should undergo formal training and demonstrate competency that is documented before being allowed to work unsupervised. Trained laboratory personnel should periodically undergo retraining such that they remain up to date on testing methodologies. Furthermore, the roles of personnel should be clearly defined with a clear reporting structure.

1. ***Analytical Controls***

There are several potential sources of contamination or DNA degradation as a sample is processed, such as:

1. Contamination of extracted DNA or controls by PCR amplified target DNA;
2. Cross-contamination between samples during DNA extraction and addition of DNA to PCR reactions;
3. Degradation of DNA due to the use of decontamination reagents to clean equipment and bench surfaces.

The first two can lead to false positive results whereas the third will produce a possible false negative result. In order to obtain reliable results the use of analytical controls, which are tested in parallel with test samples, must always be used to monitor the performance of the DNA extraction and PCR for the presence of contaminants (ISO 24276[[3]](#footnote-3)) (Table 4).

**Table 4**: Common controls that can be used to monitor reagent and environmental conditions during DNA extraction and PCR

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Description** | **Purpose** | **Expected result** |
| **Environmental / PCR blank control** | Nucleic acid free water | Monitor contamination during PCR setup and in the PCR reagent | Negative |
| **Extraction blank control** | Control in which the sample is substituted by nucleic acid-free water and that follows all the extraction steps | Monitor contamination during the extraction procedure and in the DNA extraction reagent | Negative |
| **Positive extraction control** | Control sample positive for the target analyte (it can be certified reference material) | Monitor the extraction and PCR amplification of a sample containing the target analyte | Positive |
| **Positive PCR control** | DNA positive for the target analyte that can be used at an amount of DNA or copy number that reflects the limit of detection or limit of quantification | Monitor that the PCR assay can amplify the target analyte at the limit of detection or limit of quantification | Positive |
| **Negative LMO control/positive taxon control** | Positive for the taxon specific target DNA but negative for the LMO target | Monitor the absence of false LMO positives during the extraction method and PCR and confirm that the taxon specific target can be PCR amplified | Positive for the taxon specific target but negative for the LMO target |
| **PCR inhibition control** | Dilution of the sample DNA, detection of a taxon specific target or the addition of positive target DNA to the sample DNA | Monitor PCR inhibition in the sample | Positive |

***Positive control***

The purpose of the PCR positive control is to confirm that the testing procedure was performed correctly and can also be used to monitor that the target analyte is detected at the necessary limit of detection (LOD) or quantification. A sample containing the target DNA can be included as extraction control and analyzed with other samples to ensure that the extraction method and PCR assay is acceptable.

***Negative controls***

Contamination may occur during the extraction and purification of the target DNA, as well as during the preparation of the amplification reaction mixture. The negative control, therefore, confirms that no contaminants were introduced into the testing reagents during the testing process and thus assures that the results obtained from the test samples actually reflect the content of the test sample. An extraction control blank containing sterile PCR grade water can be included in each batch of extractions to monitor if contamination of the extraction and PCR reagent occurs. Furthermore, a negative LMO target/positive taxon specific DNA control and be used to: 1) confirm the absence of the LMO target and 2) confirm that the taxon target is amplifiable.

**Inhibition controls**

An inhibition control can be used to ensure that the PCR test result is not a false negative due to sample inhibition. The degree to which a PCR is inhibited is proportional to the concentration of inhibitors that may have been co-extracted with the DNA from the sample. Sample inhibition can be determined by either a dilution of the sample, the detection of an endogenous taxon specific target in the sample or by spiking the sample by the addition of a known amount of positive control DNA.

**Controls in Protein methods**

Similarly, there are several potential sources of contamination or Protein degradation as a sample is processed, such as:

1. Contamination of protein extracts or controls by other samples;
2. Cross-contamination between samples during protein extraction and by positive controls;
3. Degradation of proteins due to the use of decontamination reagents to clean equipment and bench surfaces

The first two can lead to false positive results whereas the third will produce a possible false negative result. In order to obtain reliable results the use of analytical controls, which are tested in parallel with test samples, may be used to monitor the performance of the test. In addition, standardized protein samples may be used as controls,. Especially when performing ELISA analysis (ISO 21572[[4]](#footnote-4)).

1. ***Equipment***

The maintenance and/or verification/calibration of laboratory equipment is an essential component in ensuring the precision, accuracy and reproducibility of laboratory results. The routine maintenance and calibration of equipment is vital to maintaining the QA/QC programme within a laboratory. The interval for maintenance and/or verification/calibration of equipment as well as the results of such verification/calibration should be well documented.

The equipment verification/calibration procedure must be appropriate for the intended use of the equipment and performance criteria and can often be obtained from the manufacturer’s operating manuals. Equipment that is not performing as required should either be removed from the laboratory area where it is used or labelled as “out of order” and not used in any further sample processing. Equipment can be calibrated using external service providers, however, this may be cost prohibitive for some laboratories. Where the appropriate expertise is available in the laboratory, an internal verification/calibration system can be adopted.

Equipment must be regularly cleaned and checked to ensure that it is performing as expected. Where the measurement of units is critical for the performance of equipment, such as the accuracy of an analytical balance or the temperature of a heating block, verification can be carried out using a calibrated measure of the unit. For example, a calibrated thermometer can be used to check the accuracy of temperature sensitive equipment such as heating blocks. In addition, equipment used to measure the intensity of color development (eg for ELISA) or fluorescence should also be regularly calibrated. Calibrated weights can be used to check the accuracy of an analytical balance. In turn, the verified analytical balance can be used to check the accuracy of a pipette by weighing the required volume of pipetted water by weight

**Table 1:** Sample checklist of information that can be kept in an instrument maintenance log-book. (Adapted from UNOCD 2009).

|  |
| --- |
| Checklist of information to be kept in the instrument maintenance log-book |
| * Name of the equipment and associated software |
| * Name of the manufacturer, model and/or type Serial number |
| * Date of receipt of equipment in laboratory/Date equipment was placed in service by the laboratory |
| * Details and date of checks made for compliance with relevant calibration or test standard specification |
| * Current location in the laboratory, if appropriate |
| * Copy of the manufacturer’s operating instruction(s) |
| * Details of maintenance carried out and records of the subsequent performance check |
| * Maintenance/verification/calibration intervals * Maintenance/verification/calibration record |
| * Identity of the individual performing maintenance check and/or verification/calibration. |

1. ***Reagent Quality Control***

Reagents can either be prepared in the laboratory and/or commercially acquired as appropriate. Reagents that are prepared in the laboratory should be labelled as applicable with the following information as needed: identity of the reagent, molarity or concentration, date of preparation, the lot number of the reagent and whom it was prepared by. Additionally, the expiration date can also be included on the label. Commercially acquired reagents should also be labeled as applicable with the date opened and by whom. Quality control documentation, especially of laboratory prepared reagents, should be used to maintain a written record of the information listed above. It is also important to document the safety information regarding reagents as applicable regarding managing reagent spills and disposal.

New batches of laboratory prepared and commercially acquired reagent must be tested to ensure correct functionality. For example, new batches of reagent can be tested in parallel with an existing batch of the same product. The functionality of reagent used for DNA extraction or PCR, can be tested for functionality using appropriate controls.

Lateral flow strips have a built-in reagent quality control in the form of a control line, which should always show up for the test to be considered acceptable.

1. ***Reference materials***

ISO Guide 30[[5]](#footnote-5) defines a reference material as “material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process”. Reference materials (RMs) play a key role in the calibration of laboratory instruments by providing precise reference values and data”[[6]](#footnote-6). RM is an important tool in the verification and/or validation of methods. However it should be noted that “A single RM cannot be used for both calibration and validation of results in the same measurement procedure” (ISO guide 30 2.1 Note 4).

There are different forms of reference material that can be used. These include: reference material containing a known analyte including certified material, DNA that has been extracted from a sample containing a known analyte, plasmid copies of the target analyte or a PCR amplicon of a target analyte. It is also possible to use synthetic oligomers as a target analyte if its sequence is known. It is important that reference materials are stored under the correct conditions as specified by the manufacturer in order to minimize target analyte degradation. For example, reference material in matrix format or extracted DNA can ideally be stored at -20oC. Reference materials, like any other reagent, must be tested for reliability every time a new lot is used in the laboratory.

**Method Validation/Verification**

As previously mentioned, any method that is being used in an LMO detection laboratory must meet minimum performance criteria.

New methods that have been developed by the laboratory need to undergo a validation process, which involves a comprehensive analysis of the methods, to ensure they meet these performance criteria.

On the other hand, methods that have already been validated by other laboratories and are being implemented in another laboratory need to undergo a method verification process. Verification of an already validated method is carried out to ensure that the method functions according to validated performance criteria in the hands of the implementing laboratory.

The performance criteria for validated/verified methods should include an analysis of the following criteria, as applicable: accuracy, precision, specificity, limit of detection, limit of quantification, reproducibility and linearity/range of detection. Descriptions of the parameters to test and the criteria regarding method validation and verification are given in the documents in the following table

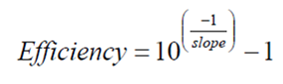
Table XXX: International Documents relating to criteria for methods used for the detection, identification and quantitation of LMOs

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| --- |
| Codex 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” (also available for download from http://files.foodmate.com/2013/files\_1810.html) |
| [ISO 21569:2005](https://www.iso.org/standard/34614.html?browse=tc" \o "ISO 21569:2005)  Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- Qualitative nucleic acid based methods |
| [ISO 21570:2005](https://www.iso.org/standard/34615.html?browse=tc" \o "ISO 21570:2005)  Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- Quantitative nucleic acid based methods |
| [ISO 21571:2005](https://www.iso.org/standard/34616.html?browse=tc" \o "ISO 21571:2005)  Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- Nucleic acid extraction |
| [ISO 21572:2013](https://www.iso.org/standard/51005.html?browse=tc" \o "ISO 21572:2013)  Foodstuffs -- Molecular biomarker analysis -- Protein-based methods |
| [ISO 24276:2006](https://www.iso.org/standard/37125.html?browse=tc" \o "ISO 24276:2006)  Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- General requirements and definitions |

Below is a brief definition of each of the criteria and an overview of how they can be determined.

1. **Dynamic range, R2 coefficient and PCR efficiency:**

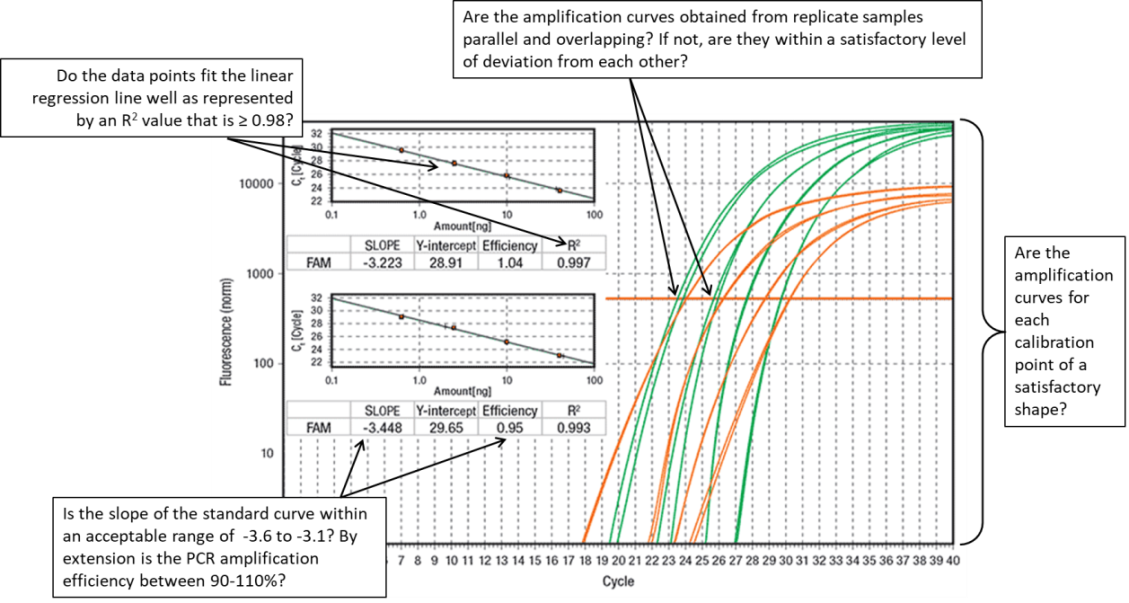
*Amplification Efficiency for Real-time PCR:* The rate of amplification that leads to a theoretical slope of –3.32 with an efficiency of 100% in each cycle. The PCR efficiency is calculated by determining the slope of a standard curve for the target analyte. The standard curve can be obtained by making a serial dilution of a known amount of target analyte. The efficiency of the reaction can be calculated by the following equation.



Acceptance Criterion: The slope of the standard curve should be in the range of -3.1 ≥ slope ≥ -3.6)

*R2 Coefficient for standard curves:* The R2 coefficient is the correlation coefficient of a standard curve obtained by linear regression analysis. Acceptance Criterion: The R2 should be ≥0.98.

These parameters are verified by testing a sample over 5-8 concentration points with 2-5 replicates per concentration. Acceptance criteria for these tests include assessing the quality specific data as shown in figure XX:



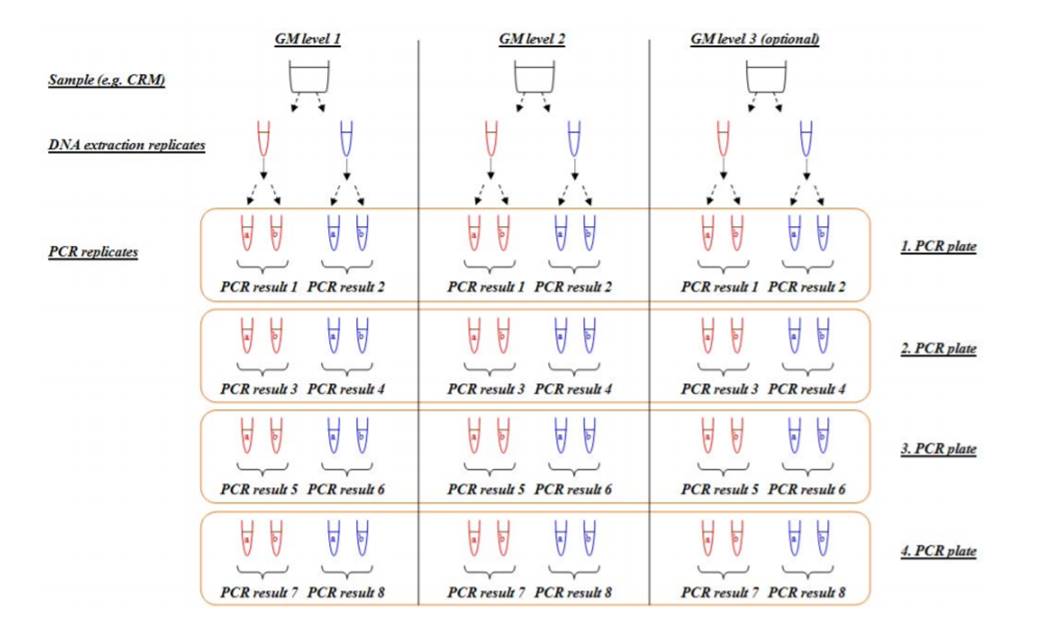
**Figure XX:** Considerations for acceptance criteria when verifying data for dynamic range, R2 coefficient and PCR efficiency. Source: adapted from <http://www.sigmaaldrich.com/technical-documents/articles/biology/roche/kapa-multiplex-rtpcr.html>.

1. **Trueness/Precision and Relative Repeatability Standard Deviation:**

*Precision- Relative Reproducibility Standard Deviation (RSDR):* The relative standard deviation of test results obtained under repeatability conditions. Repeatability conditions are conditions where test results are obtained with the same method, on identical test items, in the same laboratory, by the same operator, using the same equipment within short intervals of time. Acceptance Criterion: The relative repeatability standard deviation should be ≤25% over the whole dynamic range of the PCR method and ≤15% for an ELISA method.

*Trueness:* The closeness of agreement between the average value obtained from a large series of test results and an accepted reference value. The measure of trueness is usually expressed in terms of bias. Acceptance Criterion: The trueness for a PCR result should be within ±25% of the accepted reference value over the whole dynamic range. For protein-based methods, trueness should be within ±15% or better.

These parameters can be tested using RMs at 2-3 known LMO concentrations. For PCR they are typically analysed by comparing the data obtained from 2 sets of extracted material each of which is analysed using PCR that is tested in duplicates on each of 4 different PCR plates, therefore at least 16 data points are collected per concentration of CRM, as shown in figure XX.

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**Figure XX:** Schematic representation of the experimental design for the analysis of trueness/precision and relative repeatability standard deviation indicating the number of replicates for each sample. Source: <http://gmo-crl.jrc.ec.europa.eu/doc/ENGL%20MV%20WG%20Report%20July%202011.pdf>

The acceptance criteria for verifying trueness/precision is that the calculated concentration based on the tests conducted should be within 25% of the CRM concentration. Furthermore, the acceptance criteria for RSDr is less than 25%.

1. **Limit of Detection (LOD) and Limit of Quantification (LOQ):**

LOD and LOQ are applicable to both {PCR-based and Protein-based methods. LOD and LOQ are defeined in ISO 5725 and for biomolecular methods in ISO 16577[[7]](#footnote-7)

*LOQ:* The limit of quantification is the lowest amount of analyte in a sample that can be reliably quantified with an acceptable level of precision and accuracy. Acceptance Criterion: The LOQ should be less than 1/10th of the value of the target concentration with an RSDr ≤25%. The target amount should be intended as the threshold relevant for legislative requirements.

*LOD:* The limit of detection is the lowest amount of analyte in a sample, that can be reliably detected, but not necessarily quantified. Acceptance Criterion: The LOD should be less than 1/20th of the target amount. Experimentally, quantitative methods should detect the presence of the analyte at least 95% of the time at the LOD, ensuring ≤5% false negative results. The target amount should be intended as the threshold relevant for legislative requirements.

Similarly to measuring trueness/precision, LOD and LOQ are verified by testing multiple samples over a range of low concentrations of the analyte.

For PCR, they can be tested using certified reference materials that contain very low copy numbers of DNA of a LMO. in that case they may be analysed by comparing the data obtained from 10 replicates at each concentration that have been amplified using PCR, as shown in figure XX.

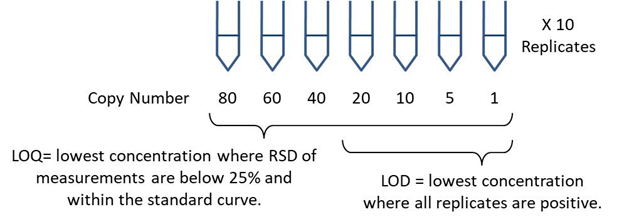


Figure XX: Outline of an approach for verifying LOD and LOQ, including guidance on the acceptance criteria.

The acceptance criteria for verifying LOD is the concentration at which 95% of the replicates are positive. With regards to LOQ the acceptance criteria for a PCR method is the lowest concentration at which the RSDr is less than 25% and the values are within the standard curve. For an ELISA method the criteria for the LOQ is a RSDr less than 15%.

1. **Robustness:**

Robustness is the capacity for a method to withstand small changes or deviations from experimental conditions. Verifying for robustness does not need to be carried out for methods that have already been formally validated. However, if deviations from the validated method have been introduced then verifying robustness may be necessary. Verifying robustness can be carried out by performing the method altered concentrations of primers and probes or by testing the method against DNA originating from different matrices. The acceptance criteria for this verification experiment are that the method performs equally well as the method as originally described in spite of the changes in the parameters.

1. **Specificity**:

Specificity is when the method being verified is capable of producing data that accurately and specifically determines the presence of the LMO that is being tested for in spite of the presence of other components within the sample. As with robustness, this parameter does not necessarily need to be verified if the method has already been formally validated. However, specificity may need to be verified for possible cross reactivity against newly developed LMOs that have been introduced into the market and may be found in samples.

**Proficiency testing**

Participation in proficiency testing schemes facilitates the independent assessment of laboratory performance in comparison with performance data from other laboratories when using approved technical procedures. Ideally, a laboratory should participate in at least one proficiency test per year. The choice of proficiency test depends on the methods that are in use in the laboratory and the type of samples that are commonly analyzed. While laboratories may use many methods for testing it may not be practical or feasible to assess all of them at the same time as each proficiency test covers only a limited number of LMOs and matrices. Therefore, it is recommended that the laboratory prepares a long-term plan for its participation in proficiency tests to ensure that it assesses a wide range of methods and matrices on a regular basis based on the laboratory’s needs and compliance with the relevant requirements of any accreditation bodies, as appropriate. There are various institutions that offer proficiency tests including:.

* BIPEA (<https://www.bipea.org/content/pt-programs)>
* FAPAS (<https://fapas.com)>
* ISTA Proficiency Test (PT) Programme (<https://www.seedtest.org/en/home.html)>
* EURL GMFF (<http://gmo-crl.jrc.ec.europa.eu/Comparative-Testing.html)>
* USDA (<https://www.gipsa.usda.gov/fgis/proficiencyprogram.aspx>)

**Non-Conformances, Root Cause Analysis and Corrective Actions**

Any activity in the laboratory that does not comply with standard operating procedures is referred to as a “non-conformance”. Non-conformances can be administrative/clerical or technical in nature.

As part of a laboratory’s QA/QC plan, a strategy has to be in place to direct staff towards taking appropriate action in the event a non-conformance is observed, for example contamination, and following root cause analysis, an outline of the steps for corrective action. The goals of this process is to document a non-conformance, identify the possible causes of the problem and the corrective action and implement a solution to avoid recurrence. The process of root cause analysis and corrective action should be consultative with the necessary staff to determine the most appropriate solution. Any non-conformance should be reported to the necessary authority in the laboratory, such as the laboratory manager, and documented, for example in a logbook for non-conformances. A root cause analysis should be performed to determine why the non-conformance occurred and the appropriate corrective action should be implemented as necessary and documented.

An administrative/clerical non-conformance can, for example, be an error in data transcription. The root cause analysis may identify that there has been an error due to inaccurate in data transcription. The corrective action may be to require that transcribed data be checked and signed off as being correct or to introduce a secondary check by another member of the laboratory. A technical non-conformance may occur when a method is not followed as described in the standard operating procedures or if a test result, for example of controls, do not meet minimum performance criteria. Repetitive failure of controls may indicate a systemic problem in sample processing.

**Databases of methods for LMO detection**

Databases containing accurate and reliable information on methods, reference materials and DNA sequences, if available, are an important tool to enable countries to effectively detect and identify LMOs. To be useful to the public at large, such databases must, at a minimum, be available online and open to the public, contain accurate and up-to-date information, and have user-friendly mechanisms for searching and retrieving information. The scope of the database may vary in the type of methods (e.g. DNA and/or protein; validated or not), type of LMOs (e.g. crops, vaccines, etc) or geographic area (e.g. local or global).

Below is information on databases that meet these criteria. They include databases of detection methods and reference materials.

***Databases of methods for the detection of living modified plants***

*European Union Database of Reference Methods for GMO Analysis*[[8]](#footnote-8)

This database was developed by the Joint Research Centre’s European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF). It is maintained by the EURL-GMFF in collaboration with the European Network of GMO Laboratories (ENGL). This database contains fully validated detection methods for LMOs using, mostly, real-time PCR methods as well as some PCR methods. The collection includes methods that are event-specific, construct-specific and element-specific methods for screening as well as plant taxon-specific methods for species identification. For each method a complete protocol with the information needed to conduct the test and all the validation data are provided.

Event-specific detection methods validated by the EURL-GMFF can also be found by searching the "Status of Dossiers" list on the EURL-GMFF website. [[9]](#footnote-9)

*GMO Detection Method Database (GMDD)*[[10]](#footnote-10)

GMO Detection Method Database (GMDD) was developed and is maintained by the GMO Detection Laboratory at Shanghai Jiao Tong University in China. It contains information on event-specific detection methods for many LMOs, as well as real-time PCR and PCR methods that are element-specific, gene-specific or taxon-specific. Some protein-based methods are also included. Not all the methods in the database are validated through collaborative trial studies, however, if available, information on the validation status of a method is provided. The primer and probe sequences are provided with citations to the relevant publications. The database also includes a large collection of publicly available LMO sequence information. In addition, links to GenBank and other publications containing information on the sequence of the inserts is provided. Finally, there is also information on relevant CRMs, if available.

*CropLife International Detection Methods Database*[[11]](#footnote-11)

This database is maintained by CropLife International, a global federation of the plant biotechnology industry, and contains both DNA- and protein-based methods. It currently contains approximately 40 methods related to about 27 LMOs. The detection methods available have been developed and validated by the technology providers for their own proprietary technologies and products. Not-for-profit laboratories have free access to the methods, whereas laboratories that undertake “fee-for-service” testing must request a license to access the methods.

***Screening matrices for the detection of living modified organisms***

*German laboratory network Screening Table*[[12]](#footnote-12)

The German laboratory network has developed a “GMO method matrix” (also known as the Waiblinger screening Table), which is based on five methods targeting specific genetic elements and constructs that are most frequently present in commercialized LM crops. It comprises a set of real-time PCR methods to detect:

1. the Cauliflower Mosaic Virus 35S promoter (P-35S);
2. the nopaline synthase terminator derived from *Agrobacterium tumefaciens* (T-nos);
3. the ctp2-cp4epsps junction of the chloroplast-transitpeptide (CTP2) from *Arabidopsis thaliana* and the *epsps* gene from *A. tumefaciens* strain CP4 (epsps);
4. the bar gene from *Streptomyces hygroscopicus;*
5. a sequence from the P35S-pat junction of the CaMV P-35S promoter and the synthetic pat gene;
6. . the synthetic fusion gene cry1 Ab/Ac derived from *Bacillus thuringiensis*
7. the figwort mosaic virus 34S promoter (P-FMV)
8. the nopaline synthase promoter derived from *Agrobacterium tumefaciens* (P-nos)

All eight methods have been fully validated and are included in the EURL-GMFF method database. Furthermore a table listing the accessibility of publicly available reference materials and their sources is provided. The Screening Table is maintained by the German NRL and the Excel spreadsheet with implemented filter-functionalities and a list of available LMO reference materials can be downloaded from the internet.[[13]](#footnote-13).

*GMOseek matrix*[[14]](#footnote-14)

The GMOseek matrix is a ‘GMO target matrix’ and provides a comprehensive and user-friendly overview of 273 genetic elements and their occurrence in 328 LMOs5. The GMOseek matrix is freely available online as an Excel spreadsheet. Filtering functions allow users to search for events that fit into a defined pattern of genetic elements based on their absence or presence in an LMO. It also helps users in identifying genetic elements that could be targeted during the screening phase of LMO analysis.

*JRC GMO-Matrix*[[15]](#footnote-15)

The JRC GMO-Matrix is a “GMO method matrix”. It takes advantage of the DNA sequence information compiled in the JRC’s Central Core DNA Sequence Information System (CCSIS). The JRC receives DNA sequence information on the insertions in LMOs from plant biotechnology companies, as part of their legal obligations in the EU. In addition, they extract sequence information from nucleotide or patent sequence databases, as well as the primer and probe sequences of the detection methods compiled in the EURL-GMFF reference method database.

The user selects a set of plant species and/or LMOs and a set of detection methods and after in silico simulations of PCR amplification using bioinformatics tools, the results are displayed in a table with predictions of possible amplification(s).

*GMOfinder*[[16]](#footnote-16)

The GMOfinder is a compilation of data to construct a combination of both, a ‘GMO target matrix’ and a ‘GMO method matrix’. It is based on an MS Access database and has integrated algorithms that facilitate the interpretation of the results of screening analyses. The tabular matrix provides information on selected genetic elements originating from the literature, LMO notifications and other sources. This information is integrated in a tabular format for 15 real-time PCR methods partly targeting the same genetic element but having different ranges of specificity. The recording of the sources of information facilitates a careful evaluation of the screening results and the tracing back of possible errors in the conclusions of the screening analysis. The GMOfinder is available free of charge upon requests addressed to the authors.

*Combinatory qPCR SYBR®Green screening*[[17]](#footnote-17)

Combinatory qPCR SYBR® Green screening (CoSYPS) is a “GMO method matrix” based on the SYBR® Green qPCR analysis method for detecting the presence of the following genetic elements in LMOs: the *Cauliflower Mosaic Virus* 35S promoter and terminator, the nos promoter and terminator derived from *Agrobacterium tumefaciens*, the *Figwort Mosaic Virus* promoter, the rice actin promoter, the nptII gene from *Escherichia coli*, the epsps gene from *Agrobacterium tumefaciens* CP4, the epsps gene from *Zea mays*, the pat gene from *Streptomyces viridochromogenes*, the bar gene from *Streptomyces hygroscopicus*, the barnase gene from *Bacillus amyloliquefaciens* and several cry genes (cry1Ab, cryAc, cryF, cry3Bb) from *Bacillus thuringiensis*. In addition, a set of plant taxon-specific method is included in the CoSYPS testing platform.

The analytical results obtained with the CoSYPS matrix are interpreted and evaluated in combination with a “prime number”-based algorithm, by which the nature of the subsets of corresponding LMOs in a sample can be determined.

*GMO Checker*[[18]](#footnote-18)

This screening application was developed as a real-time PCR array and is a ‘GMO method matrix’. The platform can be used for the comprehensive and semi-quantitative detection of LM crops. It is a combination of 14 event-specific and 10 element specific methods. The specificity and sensitivity of the PCR assays were evaluated experimentally and are tabulated in the publication. An Excel spreadsheet application for the evaluation of analytical results concerning the presence of LM crops has been developed and can be downloaded from the internet.

***Other relevant databases***

*Biosafety Clearing-House*[[19]](#footnote-19)

The Biosafety Clearing-House (BCH) central portal contains an LMO Registry with detailed descriptions for each LMO, including a unique identifier, if available, and detailed information on the transformation method, modified genetic elements, and vector as well as links to other registries in the BCH such as for risk assessments and countries’ decisions. Each entry in the LMO registry also contains links to relevant detection methods for many of the commercialized LMOs.

The BCH also contains two other registries which are closely related to the LMO registry: the Organism Registry and the Genetic Element Registry. The Organism Registry includes information on the donor organisms and the recipient or parental organisms for the registered LMOs.

The Genetic Element Registry contains records of the genes and other genetic elements that were modified in the LMOs. For each entry there is a brief description of the element and links to associated LMOs. Due to the confidential nature of the information, actual sequence information is available for only a few of the genetic elements.

*BioTrack Product Database*[[20]](#footnote-20)

The Biotrack Product database is maintained by the Organisation for Economic Co-operation and Development (OECD). It compiles a list of Unique Identifiers (UIs) for LM plants that have been approved for commercial application in at least one country, in terms of food, feed or environmental safety. UIs are codes of a fixed length of 9 alphanumeric digits specific for a single transformation event and are intended to be used as "keys" to access and share information on a particular LMO.

*BIOTradeStatus*[[21]](#footnote-21)

The BIOTradeStatus database is maintained by the Biotechnology Industry Organization and contains information on approvals, commercialization and seed sale of common commercially available LMOs.

*International Service for the Acquisition of Agri-biotech Applications Database[[22]](#footnote-22)*

The International Service for the Acquisition of Agri-biotech Applications (ISAAA) is a not-for-profit international organization that, amongst other things, maintains a GM approval database that draws on information from biotechnology clearing houses of approving countries and from country regulatory websites.

*CERA LM Crop Database*[[23]](#footnote-23)

The Center for Environmental Risk Assessment (CERA), established by the food-industry funded, non-profit International Life Science Institute Research Foundation (ILSI), also maintains an LM crop database which includes not only plants produced using recombinant DNA technologies (e.g., genetically engineered or transgenic plants), but also plants with novel traits that may have been produced using more traditional methods, such as accelerated mutagenesis or plant breeding. The database provides information on the genetic elements construct, vector as well as the LMO characteristics (traits, common use etc.), risk assessments and regulatory decisions.

*GMOtrack* [[24]](#footnote-24)

This program generates cost-effective testing strategies for traceability of LMOs and computes the optimal set of screening assays for a two-phase testing strategy.

**Overview of relevant accreditations and International standards**

International guidance and useful standards about method performance criteria are well documented and available from several sources. These documents include ISO guidelines, Codex Alimentarius standards, guidelines from international associations (ISTA, AOAC), metrology institutions, as well as official and peer reviewed references regarding proficiency trials for method validation procedures.

o ISO Standards (e.g. ISO17025)

o Codex Standards and guidelines (Methods and LLP)

o Role of Standards Developing Agencies

o ISTA guidelines

**References**

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<https://www.unodc.org/documents/scientific/validation_E.pdf>

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http://gmo-crl.jrc.ec.europa.eu/doc/ENGL%20MV%20WG%20Report%20July%202011.pdf

ISO/TC 34/SC 16 (Horizontal methods for molecular biomarker analysis) https://www.iso.org/committee/560239/x/catalogue/p/1/u/0/w/0/d/0

1. Good laboratory Practices are practices that are ideal for operation of a laboratory and should not be confused with ‘GLP’ requirements used for regulatory food safety purposes. [↑](#footnote-ref-1)
2. <https://www.iso.org/obp/ui/#iso:std:iso:9000:ed-4:v1:en:term:3.6.5> [↑](#footnote-ref-2)
3. ISO 24276:2006 Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- General requirements and definitions [↑](#footnote-ref-3)
4. ISO 21572:2013 Foodstuffs -- Molecular biomarker analysis -- Protein-based methods [↑](#footnote-ref-4)
5. ISO Guide 30:2015 Reference materials -- Selected terms and definitions [↑](#footnote-ref-5)
6. [↑](#footnote-ref-6)
7. ISO 16577:2016 Molecular biomarker analysis -- Terms and definitions [↑](#footnote-ref-7)
8. The event-specific detection methods are available at: <http://gmo-crl.jrc.ec.europa.eu/gmomethods/> [↑](#footnote-ref-8)
9. The compendium of detection methods is available at: <http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx> [↑](#footnote-ref-9)
10. The GMO Detection Method Database is available at: <http://gmdd.shgmo.org/> [↑](#footnote-ref-10)
11. CropLife International Detection Methods Database is available at: [www.detection-methods.com](http://www.detection-methods.com) [↑](#footnote-ref-11)
12. The Waiblinger Screening Table is available at: <http://www.bvl.bund.de/SharedDocs/Downloads/09_Untersuchungen/screening_tabelle_gvoNachweis.xls?__blob=publicationFile&v=2> [↑](#footnote-ref-12)
13. The compilation of reference materials is available at: <http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/nachweis_kontrollen/referenzmaterialien.pdf?__blob=publicationFile&v=6> [↑](#footnote-ref-13)
14. GMOseek matrix is available at: <http://www.gmoseek.com/gmoseek> [↑](#footnote-ref-14)
15. The JRC GMO-Matrix is available at <http://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/> [↑](#footnote-ref-15)
16. GMOfinder matrix is available at: <http://link.springer.com/article/10.1007%2Fs12161-012-9378-6> [↑](#footnote-ref-16)
17. The CoSYPS matrix is available at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2836468/pdf/216_2009_Article_3286.pdf> [↑](#footnote-ref-17)
18. The GMO Checker is available at: <http://cse.naro.affrc.go.jp/jmano/UnapprovedGMOChecker_v2_01.zip> [↑](#footnote-ref-18)
19. The LMO, Organism and Genetic Element Registries of the BCH can be found at: <http://bch.cbd.int/database/organisms/> [↑](#footnote-ref-19)
20. The BioTrack Product Database is available at: <http://www2.oecd.org/biotech/default.aspx> [↑](#footnote-ref-20)
21. The BIOTradeStatus is available at: <http://www.biotradestatus.com/> [↑](#footnote-ref-21)
22. ISAAA GM Approval Database is available at <http://www.isaaa.org/gmapprovaldatabase> [↑](#footnote-ref-22)
23. The CERA LM Crop Database is available at: <http://cera-gmc.org/index.php?action=gm_crop_database> [↑](#footnote-ref-23)
24. GMOtrack is available at <http://kt.ijs.si/software/GMOtrack/> [↑](#footnote-ref-24)