

Methods **verification**

Transfer of **validated**
methods into
laboratories working
routine

1. Introduction

2. Definitions and differences
validation – verification

3. How to perform verification in
GMO detection laboratories

ISO/IEC 17025:2005

General requirements for the competence of testing and calibration laboratories

5.4.2 Selection of methods

5.4.5 **Validation** of methods

Advantages of validated methods

Providing

- reliable and accurate test results
- comparable results between different laboratories

requirement for the accreditation according to
ISO/IEC 17025 Chapter 5.4.5 'validation of methods'

ISO/IEC 17025: 2005

Section 5.4.2 Selection of methods

1. Methods published in international, regional or national standards shall (i.e. must) preferably be used.
Latest valid edition
2. Published by
reputable technical organisations,
in relevant scientific texts or journals or
as specified by the manufacturer of the equipment
3. Laboratory-developed methods or methods adopted by the laboratory

Regulation (EC) No 882/2004

Article 11

Methods of sampling and analysis

1. Sampling and analysis methods used in the context of official controls shall comply with relevant Community rules or,
(a) if no such rules exist, with internationally recognised rules or protocols,
for example those that the European Committee for Standardisation (CEN) has accepted or those agreed in national legislation;
or,
(b) in the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols.

1. Methods fixed in legislation
2. ISO methods / CEN methods
3. Other appropriate methods (“fit for purpose”)

- [ISO](#) 24276:2006 Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – General requirements and definitions
- [ISO](#) 21571:2005 Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Nucleic acid extraction
- [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 Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Quantitative nucleic acid based methods

ISO EN Standards

- [ISO](#) 21572:2004 Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Protein based methods

ISO EN Standard

- [EN](#) 15568:2005 Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Sampling



EN Technical Specification

Method validation

Method validation is a process

**Development of
a new method**



**Optimisation of the
method**



**Establishment
of the method**

Method **validation** is a process

In-House
(single)-
validation of
the method



**Interlaboratory
Validation** of the
method per
interlaboratory
(collaborative) study



Standardisation
Publication



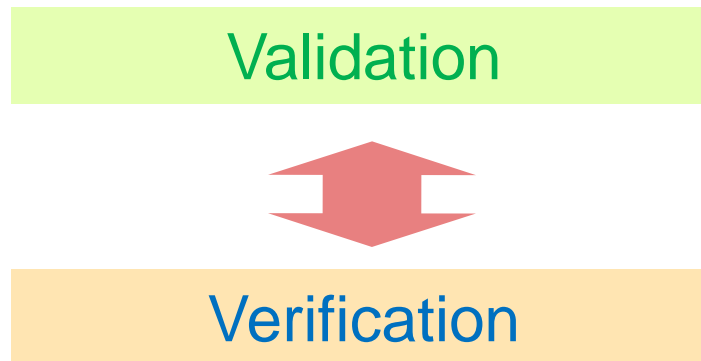
Verification,
introduction in
different
laboratories

Definitions (1)

Validation is the confirmation by examination and provision of objective evidence that the

(ISO 9000:2005 section 3.8.4) ...requirements for a specific intended use or application have been fulfilled

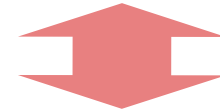
(ISO 17025:2005 section 5.4.5.1)... particular requirements for a specific intended use are fulfilled



Verification is the confirmation, through the provision of objective evidence that specific requirements have been fulfilled (ISO 9000:2005 section 3.8.4)

Definitions (2)

Validation



Verification

“Full” validation “

usually by interlaboratory method performance study
(collaborative study)

A laboratory using a collaboratively studied method, which has been found to be fit for the intended purpose, needs only to demonstrate that it can achieve the performance characteristics stated in the method by **verification**.

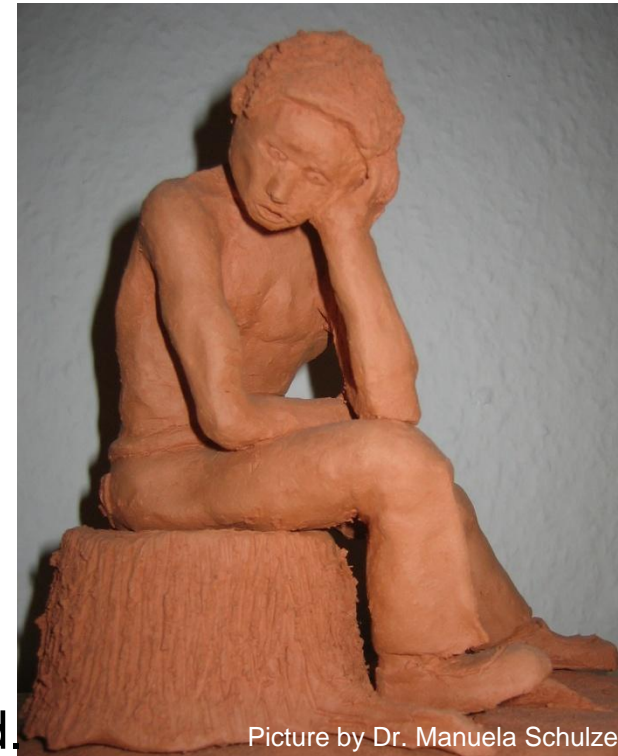
Regulation (EC) No 882/2004

Annex III

CHARACTERISATION OF METHODS OF ANALYSIS

1. Methods of analysis should be characterised by the following criteria:

- (a) accuracy;
- (b) applicability (matrix and concentration range);
- (c) limit of detection;
- (d) limit of determination;
- (e) precision;
- (f) repeatability;
- (g) reproducibility;
- (h) recovery;
- (i) selectivity;
- (j) sensitivity;
- (k) linearity;
- (l) measurement uncertainty;
- (m) other criteria that may be selected as required.



Picture by Dr. Manuela Schulze

Validation

(ISO 17025, 5.4.5.3 note 3)

Validation is always a **balance** between

costs,
risks and
technical possibilities.

There are many cases in which the range and uncertainty of the values can only be given in a simplified way due to lack of information.

HARMONIZED GUIDELINES FOR SINGLE LABORATORY **VALIDATION** OF METHODS OF ANALYSIS

(IUPAC Technical Report)

Prepared for publication by

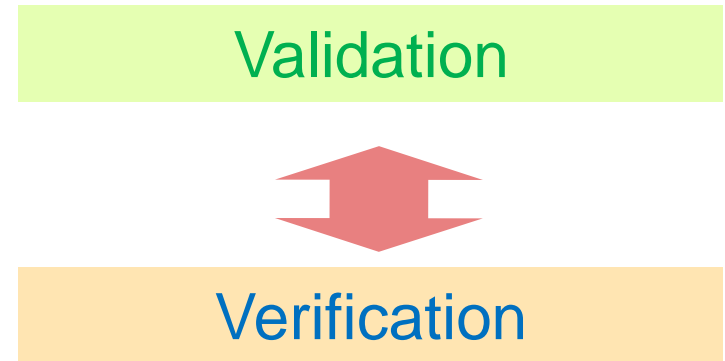
MICHAEL THOMPSON, STEPHEN L. R. ELLISON AND ROGER WOOD

In: Pure Appl. Chem., Vol. 74, No. 5, pp. 835–855, 2002.

© 2002 IUPAC

Guideline which provide minimum recommendations on procedures that should be employed to ensure adequate **validation** of analytical methods.

Definitions (3)



Validation and **verification** apply
to a defined protocol
for the determination of a specified analyte
for a specific range of concentrations
in a particular type of test material
for a specific purpose

Validation / Verification



Realistic operation situations

- matrix
- analyte concentration
- staff
- equipment
- reagents
- environment

Verification

Verification is applicable only for standard/accepted methods which have been validated before.

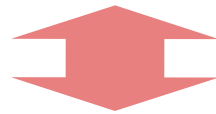
The laboratory has to

- demonstrate that the performance parameters specified in the method have been met with the matrices to which the method is being applied.

Most often the critical parameter are the trueness and the precision (ALACC Guide 2007).

Definitions

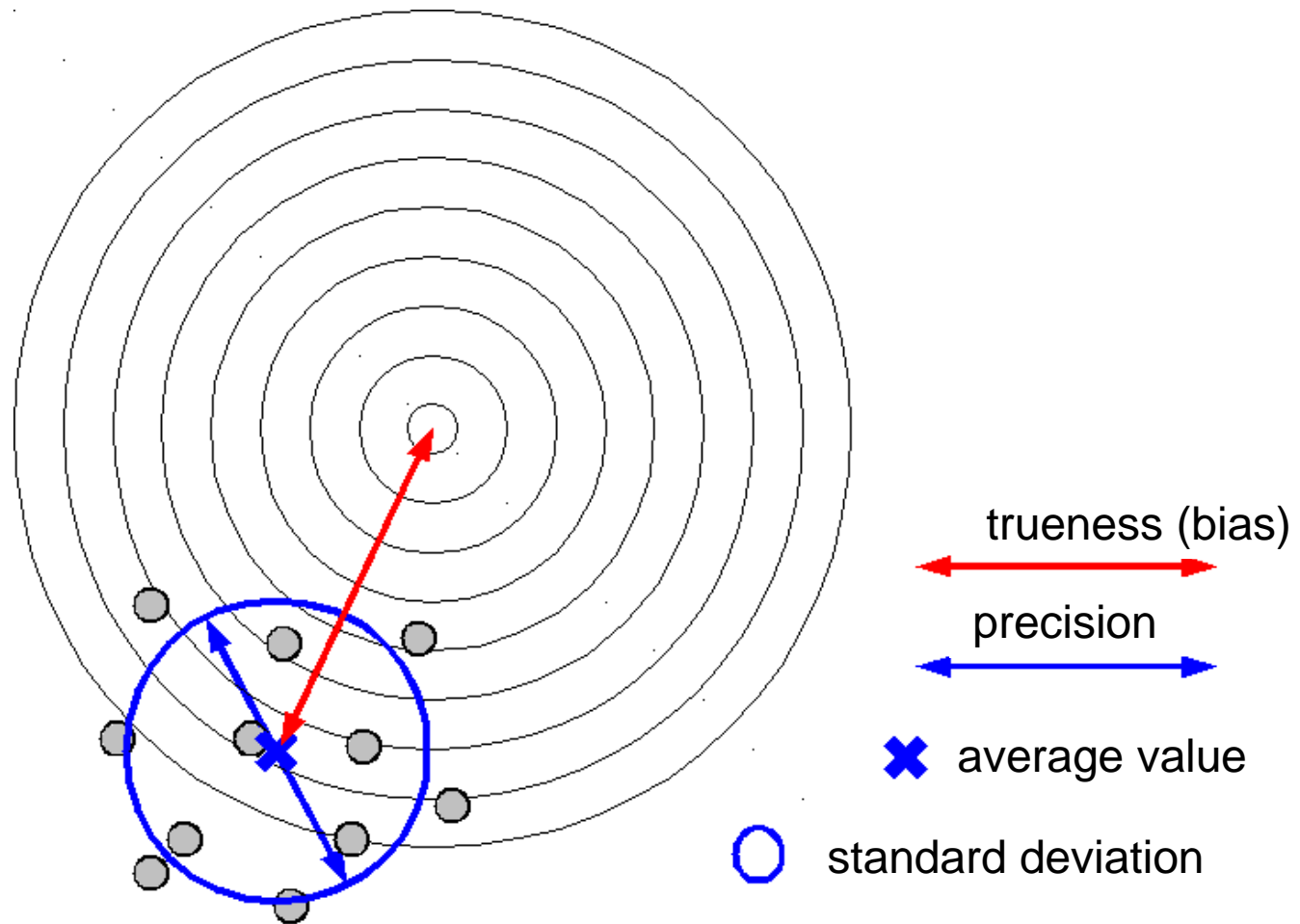
accuracy



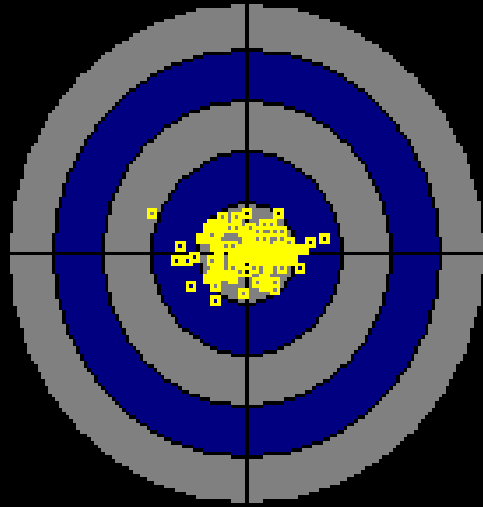
trueness and precision

Trueness: Closeness of agreement between the average value obtained from a series from test results and an accepted value

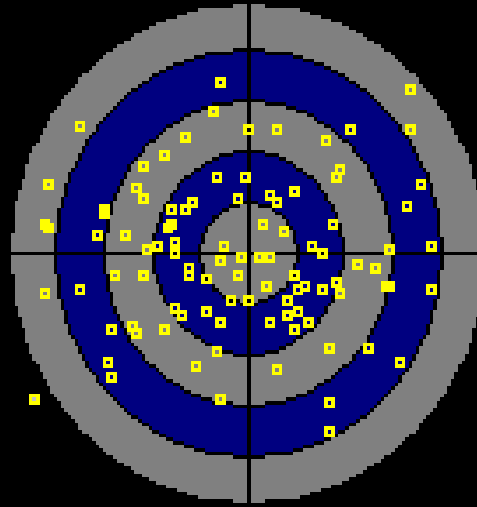
Accuracy: Closeness of agreement between a test result and the accepted reference value



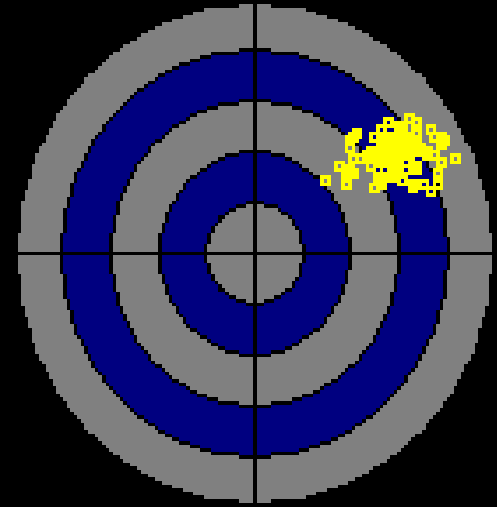
[Source: wikipedia.com]



high trueness
high precision



high trueness
low precision



low trueness
high precision

(note: no picture for low trueness and low precision)

Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between the mean and the accepted true value, together with confidence intervals.

Accuracy involves a combination of
random components and
a common systematic error or bias component.

The **validation/verification** parameter which have to be considered are dependent on

- the data already available.

The extent of **validation/verification** is dependent on

- the parameters which have already been tested.

Laboratory own methods need the “**whole validation**”.

Procedures and responsibilities for **validation/verification** have to be described in the quality management system of the laboratory and records about the process of **validation/verification** have to be kept.

Strategy

Selection of method(s) needed

Check of available methods

- fit for purpose for the laboratory
- **validation** status of the methods

Consider the modular approach

- step by step
- extraction method
- PCR method
- real time PCR



Picture by Dr. Manuela Schulze

Available methods

- Booklet: Compendium of reference methods for GMO analysis, developed by EURL-GMFF in collaboration with the ENGL



On-line GMO detection method database

<http://gmo-crl.jrc.ec.europa.eu/gmomethods/>

[Legal Notice](#) [Privacy statement](#) [English \(EN\)](#)



JOINT RESEARCH CENTRE

European Union Reference Laboratory for GM Food and Feed

[European Commission](#) > [JRC](#) > [IHCP](#) > [EU-RL GMFF](#) > [GMOMETHODS](#)

[Home](#) [Legal basis](#) [Guidance documents](#) [Status of dossiers](#) [Methods database](#) [Capacity building](#) [ENGL](#) [Contacts](#)

Main **Search** **for** **Select by GMO Unique**
Identifier:

GMOMETHODS: EU Database of Reference Methods for GMO Analysis

Quantitative GMO detection PCR methods

- GMO specific
 - Event specific
 - Maize
 - Soybean
 - Cotton
 - Oilseed rape
 - Potato
 - Rice
 - Sugar beet
 - Construct specific
 - Element specific
- Taxon specific
 - Validated independently
 - Validated in combination with other method(s)

Qualitative GMO detection PCR methods

- GMO specific
 - Event-specific
 - Construct-specific
 - Element-specific
 - Cauliflower Mosaic Virus 35S promoter (CaMV P-35S)
 - Figwort Mosaic Virus 35S promoter (P-FMV)
 - Neomycin phosphotransferase II gene (nptII)
 - Nopaline synthase terminator (T-nos)
 - Phosphinothricin N-acetyltransferase gene (bar)
- Taxon specific
 - Validated independently
 - Validated in combination with other method(s)
 - Plant-specific

Released the GMOMethods app for iPad on 20-12-2011.

Last update

The European Union Reference Laboratory for GM Food and Feed (EU-RL GMFF) is responsible for GMO detection methods for food, animal feed and seeds in the EU’s authorisation process.

The EU-RL GMFF regularly publishes information on the status of **validation** dossiers on the internet. <http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm>

Last updated 29/03/2012  Watch this page for changes

Event	Crop	Unique identifier	Applicant	Status/Progress	Reports	Validated Method
Bt10	maize	Not applicable	Not applicable	Validation completed	Validation report Published on: 13/07/2005	Validated method Published on: 13/07/2005
Bt11	sweet maize	SYN-BT011-1	Syngenta Crop Protection AG	Validation completed	Validation report Published on: 05/08/2004	Validated method Published on: 05/08/2004
NK603	maize	MON-00603-6	Monsanto Company	Validation completed	Validation report Published on: 10/01/2005 Validation report	Validated method Published on: 10/01/2005

Once the **validation** process is complete, the full **validation** report and the protocol of detection and quantification are published on the EU-RL GMFF web site.

Preparation of the verification (1)

Prerequisite: An appropriate **validated** method is available!

1. Do you have the same matrix to analyse?

Extraction: Sometimes different materials from the same plant need additional steps



Picture by Dr. Manuela Schulze

e.g. potato leaf: CTAB extraction method without CTAB precipitation step works

potato tuber: a CTAB precipitation step should be included

Preparation of the verification (2)

Prerequisite: An appropriate **validated** method is available!

2. Check the possibility to introduce the validated method without changes in your laboratory
 - do you have all the equipment?
 - If not, do you intend to introduce the equipment?
 - If not, how can you achieve the result by a modification or adaptation?
 - Do you have all chemicals needed?
 - If not, which chemicals are mandatory for a first test?
 - How can you achieve the result by a modification or adaptation?

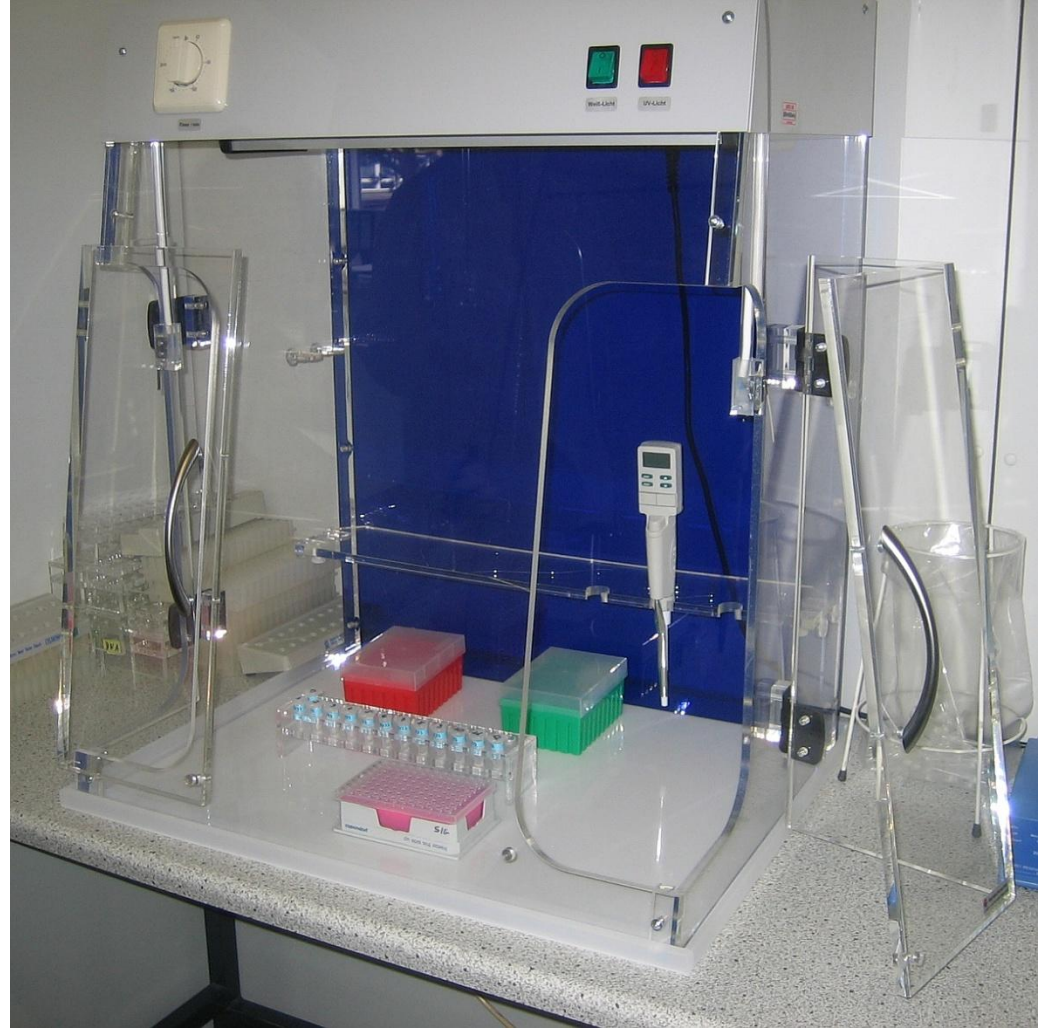
Also the price can be a reason for a modification.

Chemicals

PCR-Mastermix: Compare especially the MgCl_2 - concentration of the validated method and your own mastermix.

The mastermix intended to be used must be compatible with your thermal cycler.

Note: Try to minimize the different numbers of mastermixes in your laboratory.



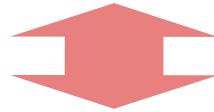
Probe: If necessary, adapt the detection wavelength if you have to modify the label of the probe

Preparation of the verification (3)

3. Check the availability of positive and negative material



Reference material

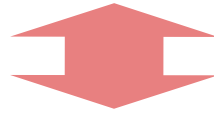


Certified Reference
material

RM and CRM **provide** essential **traceability** in measurements and are used for, e.g.

- demonstrate the accuracy of results
- calibration of equipment
- monitoring of laboratory performance (quality control)
- Validation** of methods
- Comparison of methods

Reference material



Certified Reference
material

ISO Guide 30:1992/Amd.1:2008

Certified reference material

CRM

Reference material

- characterized by a metrological valid procedure for one or more specified properties,
- **accompanied by a certificate** that provides
 - the value of the specified property,
 - **its associated uncertainty**, and
 - a statement of metrological traceability

Positive Materials

Check available certified reference materials

Check available reference materials

Check available positive material

For the verification of an extraction method a matrix matched material is useful, for the verification of a PCR method a DNA-Standard can be chosen.

For the verification of an extraction method there is no need for genetically modified material.

Sometimes you have to consider to use spiked material.

Controls (ISO/EN 24276:2006, drafted update/actual revision)

Control step	Environment control ^b	Extraction blank control ^c	Positive extraction control ^d
Homogenization	Mandatory		
Nucleic acid extraction	↓ ^a	one per series	mandatory at regular intervals
Assessment of nucleic acid quality	↓	↓	↓
Nucleic acid amplification	↓	↓	↓
Assessment of results of nucleic acid amplification	↓	↓	↓
Interpretation		↓	↓
Test report		↓	↓

Controls (ISO/EN 24276:2006)

Control step	Positive DNA target control ^e	Negative DNA target control ^f	Amplification reagent control ^g	PCR inhibition control ^h
Homogenization				
Nucleic acid extraction				
Assessment of nucleic acid quality				
Nucleic acid amplification	mandatory	recommended	mandatory	recommended, but mandatory in certain cases ⁱ
Assessment of results of nucleic acid amplification	↓	↓	↓	↓
Interpretation	↓	↓	↓	↓
Test report	↓	↓	↓	↓



SAMPLE SHOP

Picture by Dr. Manuela Schulze

2.2 MATRIX MATERIALS

2.2.1 CERTIFIED FOR GMO CONTENT

The materials were prepared by quantitative mixing of non genetically modified powder and genetically modified powder, produced from ground seed with the help of a dry-mixing technique, and are intended for the calibration of methods for the detection of genetically modified food.

CRMs for genetically modified Roundup Ready™ soya beans (ERM-BF410)

Six CRMs of dried soya bean powder with different mass fractions of genetically modified (Roundup Ready™) soya beans were produced by IRMM.

	Certified value Roundup Ready mass fraction (g/kg)	Uncertainty (g/kg)
ERM-BF410a	< 0.3	-
ERM-BF410ak	< 0.7	-
ERM-BF410b	1.0	0.5
ERM-BF410bk	1.0	0.5
ERM-BF410c	5.0	1.0
ERM-BF410dk	10.0	1.0
ERM-BF410e	20.0	2.6
ERM-BF410gk	100	7

Availability: Vials containing about 1 g of soya bean powder.

Positive Materials

American Oil Chemists' Society (AOCS)

<https://secure.aocs.org/crm/index.cfm>



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Certified Reference Materials (CRM)

Prepared according to ISO Guidelines 30–35, are intended to serve as control material for third party qualitative testing of transformation events.

The tariff code for Certified Reference Materials is 3822.00.6000.

CRM's are available for:

- [Canola](#)
- [Cotton](#)
- [Maize](#)
- [Potato](#)
- [Rice](#)
- [Soybean](#)
- [Sugarbeet](#)

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Canola	Certificate (.pdf)	Report (.pdf)	Company	Event	Product Code
Leaf Tissue DNA 10 µg	0711A Certificate	Report	Bayer CropScience	Ms1	0711-A
Leaf Tissue DNA 10 µg	0711B Certificate	Report	Bayer CropScience	Rf1	0711-B
Leaf Tissue DNA 10 µg	0711C Certificate	Report	Bayer CropScience	Rf2	0711-C

Positive Materials

Possible sources:

- botanical garden

Millennium Seed Bank Kew Royal botanic garden www.kew.org
or DNA-bank e.g. botanical garden/German Science Foundation Berlin-Dahlem ww2.bgbm.org/herbarium/dna/default.cfm

- Commercial companies: e.g. divider of IRMM material

- Commercial companies producing genetically modified organisms

- Official authorities responsible for the control of deliberate release studies

- Research institutes

- EU-RL GMFF

Positive Materials?



Picture by Dr. Manuela Schulze



Picture by Dr. Manuela Schulze

Positive Materials?



Picture by Dr. Manuela Schulze



Picture by Dr. Manuela Schulze



Picture by Dr. Manuela Schulze

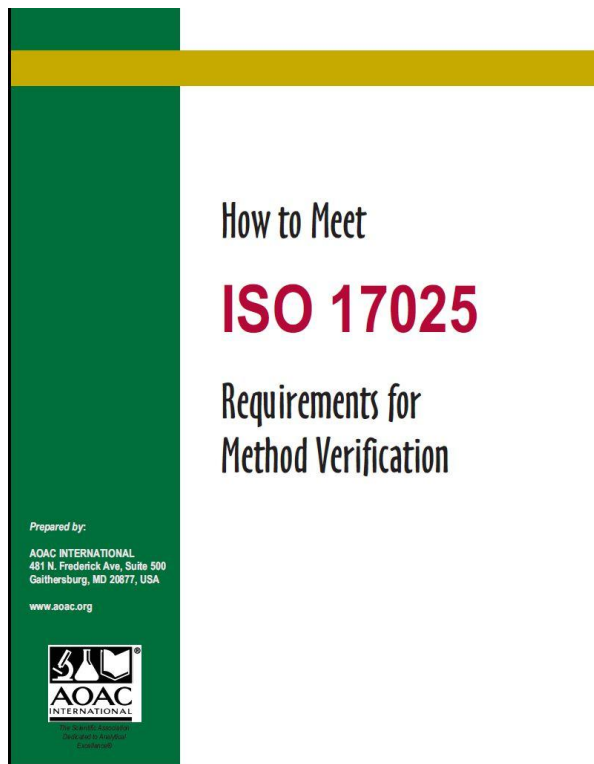


Picture by Dr. Manuela Schulze

Preparation of the verification (4)

4. Document all the changes you have to consider in your verification e.g. because you have a different thermal cyclers
5. Select the parameters which you have to verify

How to meet ISO 17025 requirements for method verification



prepared by

*Analytical Laboratory Accreditation
Criteria Committee
on request of AOAC*

Guideline available as

http://www.aoac.org/alacc_guide_2008.pdf

Table 1. Categories of Chemical Test Methods: Since the activities needed for method verification are a subset of those needed for validation, the required performance characteristics for validation are presented in this table

Performance Characteristic	Performance Characteristics Included in a Validation					
	Identification 1	Analyte at Low Concentration Quantitative 2	Analyte at Low Concentration Limit Test 3	Analyte at High Concentration Quantitative 4	Analyte at High Concentration Limit Test 5	Qualitative 6
Accuracy	No	Yes	No	Yes	Yes	No
Precision	No	Yes	No	Yes	Yes	No
Specificity	Yes	Yes	Yes	Yes	Yes	Yes
LOD	No	Yes	Yes	Yes/No	No	No
LOQ	No	Yes	No	Yes/No	No	No
Ruggedness	No	Yes	No	Yes	No	No
Linearity/Range	No	Yes	No	Yes	No	No

Table 2. Category 1: Confirmation of Identity—A method that ensures a material is what it purports to be or confirms the detection of the target analyte

Performance Characteristic	Verification	Verification Activities	Reason for Verification
Specificity	No—if the lab’s samples are identical to those in the standard method and if any differences in instrumentation do not impact specificity.	NA	If the samples have the same matrix, the specificity which is based on basic principles, will not be impacted. Basic principles are chemical reactions, e.g. reaction of Ag with Cl to create a precipitate.
	Yes—if the lab’s samples differ from those in the standard method.	Same as those required for validation.	
	Yes—if differences between instruments could affect specificity.	The activity need only deal with the unique aspect’s of the lab’s samples or instrumentation.	Specificity can be impacted by differences in instrumentation.

Example: Verification of validated real time PCR methods

Guidance document:

Verification of analytical methods for GMO testing when implementing interlaboratory validated methods

<http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm>



Verification of analytical methods for GMO testing when implementing interlaboratory validated methods

Guidance document from the European Network of GMO laboratories (ENGL)

Prepared by the ENGL working group on "Method Verification"



EUR 24790 EN - 2011

Example: Verification of validated real time PCR methods

Possible performance characteristics to be considered in the verification:

- Specificity
- Robustness
- Dynamic range
- Amplification efficiency
- R^2 coefficient
- Trueness
- Precision
- LOD
- LOQ

<http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm>

Preparation of the verification (5)

6. Set up a **verification** plan (documentation of the result of the considerations)
 - draft a version of the method as it will be performed in the laboratory
 - write down the persons foreseen
 - fix the number of experiments, replicates,
 - make sure that the word “replicates” or repetitions is unambiguous
(analytical sample, test sample, DNA-extractions replicates, PCR replicates, ...)

Determination of 1000 corn weight, documentation of subsamples



Documentation

Base for transparency, tracing back

- *When* did ?
- *Who* ?
- *What* ?
- By using
 - *Which* material, reagents ?
 - *Which* equipment ?
 - *Which* methods and *why* ?

Not documented means not done!

Collaborative study



Proficiency test

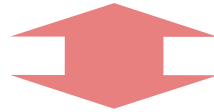
Test of the same method,
Ring trial



Test of the laboratory
proficiency, test of the
method used as in the
routine by the laboratory

- inter-laboratory studies
- analysis of unknown samples provided by an external source

Collaborative study



Proficiency test

Interpretation of PT outcome

Key evaluator: z-score

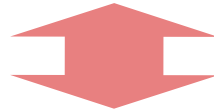
Acceptable: ± 2

Indicates that results are equal to those of the other laboratories participated in the PT

What if falling out of the score?

- Compare with other failing laboratories (similar methods, equipments...)
- Handle as “non-conformity”

Collaborative study



Proficiency test

Benefits for laboratories

Participation in proficiency testing programs allows laboratories to:

- identify areas where improvement in their testing and measurement methods is needed
- identify further training of their staff
- foster confidence in the performance of their testing and measurements
- assure laboratory performance in their accredited test and calibration results
- is mandatory for accredited laboratories
- is a confirmation of the correct **verification** of methods

After verification

