GMO Committee
Open Meeting

Enrico Noli and René Mathis

Verona, 31/05/2023

Outline

- Membership
- PDE document
- GMO Hand Book
- Proficiency Tests
- Alternative seed materials for PDE and PTs
- Discussion

Membership

Chair: Enrico Noli (Italy) **Vice-Chair:** René Mathis (France)

GMO Committee Members:

1. Laura Bowden (United Kingdom) new
2. Tajinder Grewal (Canada)
3. Andrea Jonitz (Germany)
4. Jean-Louis Laffont (France)
5. Benoit Maes (Belgium) new
6. Dwarkesh Parihar (India)
7. Elena Perri (Italy)
8. Kirk Remund (U.S.A.)
9. Sophie Séoane (France) new
10. Ray Shillito (U.S.A.)
11. Ana Laura Vicario (Argentina)
12. Bruno Zaccomer (France)
13. Dabing Zhang (China)

ECOM liaison officer: Vanessa Sosa (Uruguay)

Working Areas

- Handbook
- Publications
- Website
- Proficiency Tests
  - Expert Group
  - Material Procurement
- Workshops
- List of Standadised Names

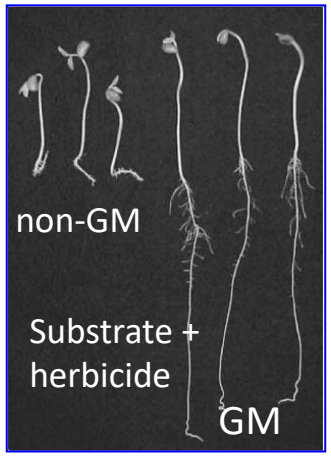
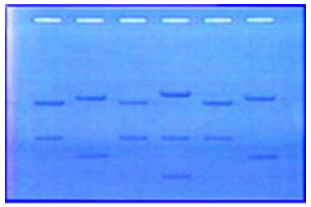
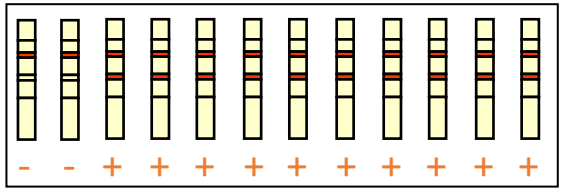
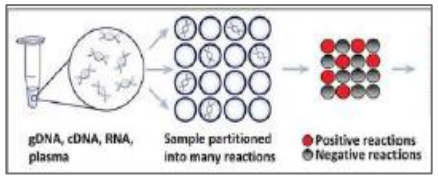
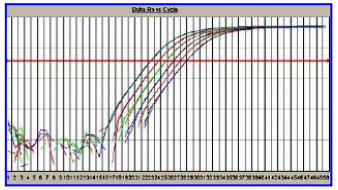
Performance Based Approach (PBA)

19.2.20 Under the PBA individual laboratories can choose the test method, as long as it has been validated as fit for purpose and complies to given performance standards.

- **Laboratories can choose**
  - the testing technology (bioassay, ELISA, LFSs, end-point-, real-time-, and digital-PCR, etc.)
  - the assay target (a given protein, or sequence, or phenotype e.g. herbicide tolerance)
  - the testing approach (i.e. type of assay + unit of observation)

- **Methods must be validated/verified** (satisfy acceptance criteria)

Possible approaches to estimation of level in TP and AP testing

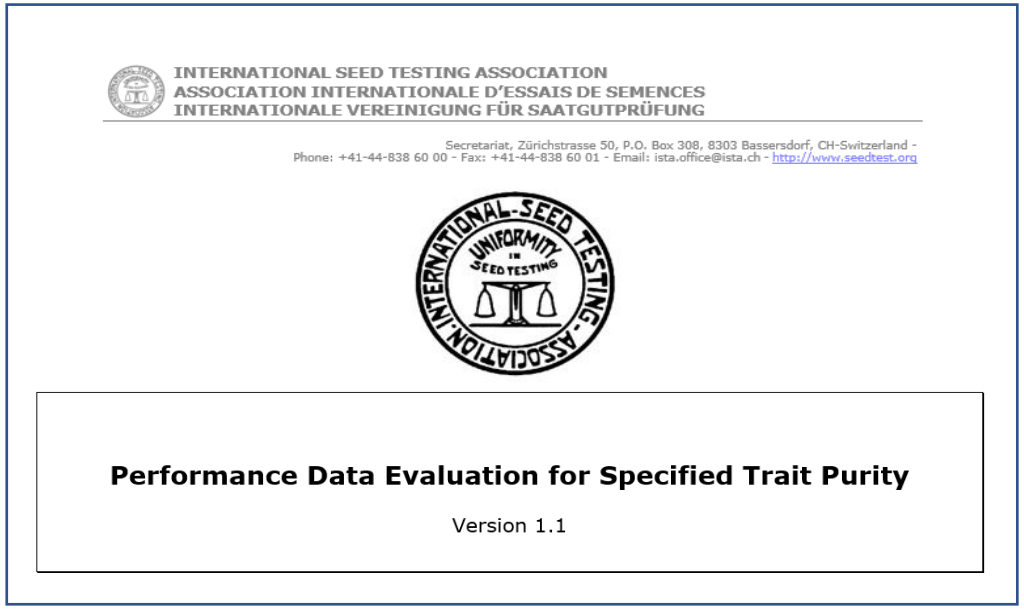
Assay type	Qualitative	Qualitative	Quantitative
Unit of observation	 individual seeds	 n. seed groups (subsamples)	 1-few seed groups/bulks
Testing technology	 Bioassay	 *ep* PCR ↑ or immunoassay ↓ 	 ddPCR ↑ or rt PCR ↓ 
Unit	**% seeds**	**% seeds**	**% mass or % copies**

Performance Based Approach (PBA)

Key items for accreditation

- Production of **Performance Data** produced from **seed samples**
- Participation to **Proficiency Test** conducted on **seed samples**
- **On-site assessment** (audit)

PDE for TP

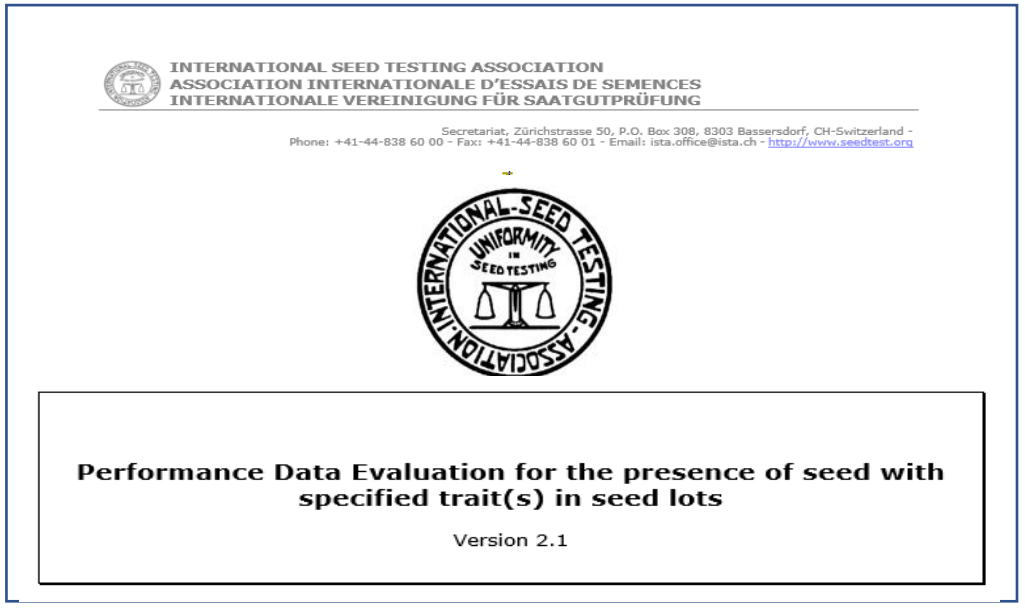

INTERNATIONAL SEED TESTING ASSOCIATION
ASSOCIATION INTERNATIONALE D'ESSAIS DE SEMENCES
INTERNATIONALE VEREINIGUNG FÜR SAATGUTPRÜFUNG

Secretariat, Zürichstrasse 50, P.O. Box 308, 8303 Bassersdorf, CH-Switzerland -
Phone: +41-44-838 60 00 - Fax: +41-44-838 60 01 - Email: ista.office@ista.ch - http://www.seedtest.org

Performance Data Evaluation for Specified Trait Purity

Version 1.1

PDE for AP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Performance Data Evaluation for the presence of seed with specified trait(s) in seed lots

Version 2.1

Part 1 Assessment of presence	Part 2 Estimation of level

Which PDE to run?

What Performance Data is a lab expected to produce?

That will depend on the objectives and approach that it is going to use in testing.

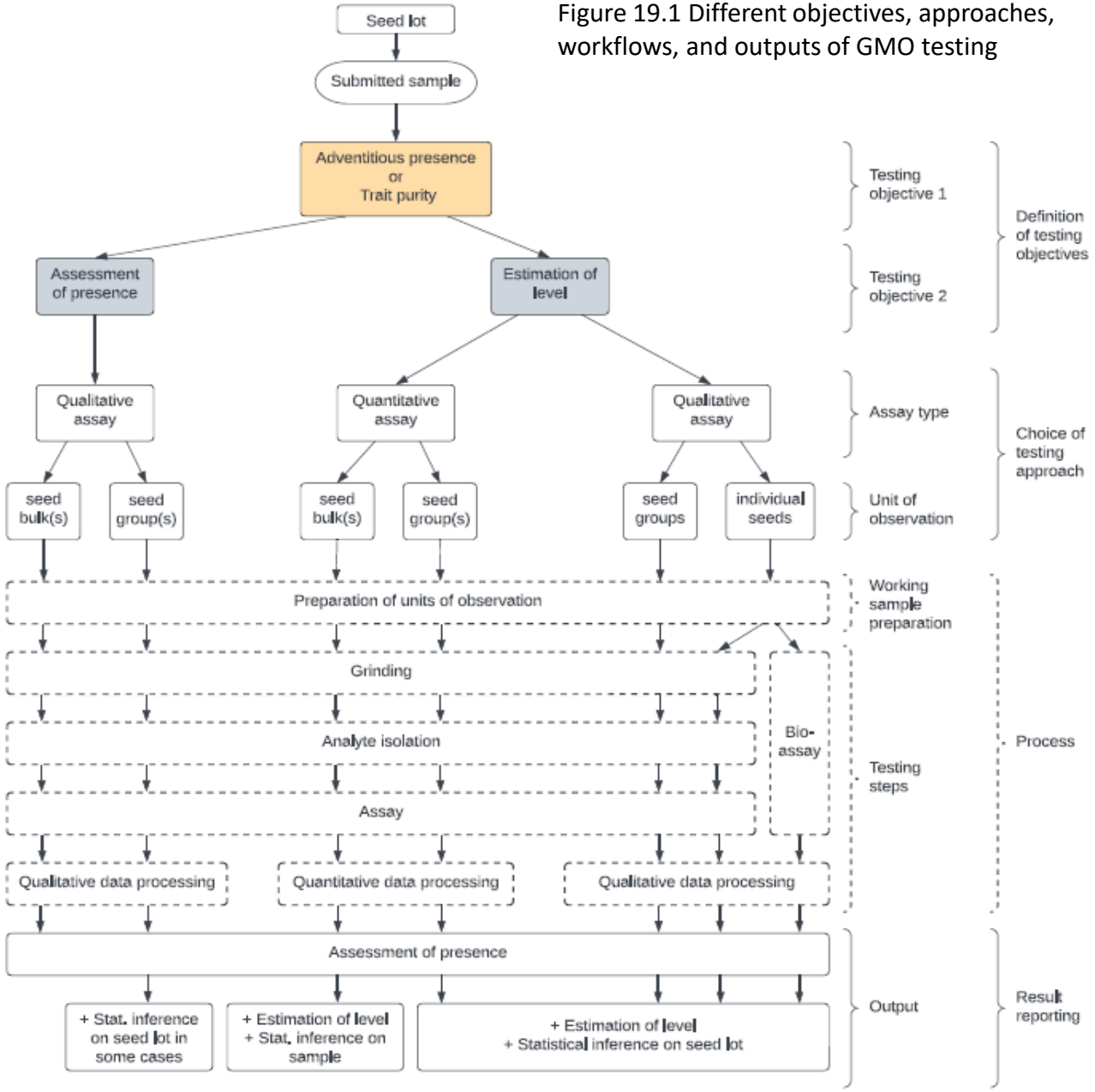

Figure 19.1 Different objectives, approaches, workflows, and outputs of GMO testing

Which PDE to run?

What Performance Data is a lab expected to produce?

That will depend on the objectives and approach that it is going to use in testing.

Case 1. TP-estimation of trait level by testing individual seeds/seedlings with a qualitative assay (e.g. herbicide bioassay, ELISA, PCR) - PDE-TP

Case 2. AP-assessment of trait presence by testing groups or bulks with a qualitative assay (e.g. LFS, ELISA, PCR) - PDE-AP-1

Case 3. AP-estimation of trait level by testing groups with a qualitative assay (e.g. LFS, ELISA, PCR) - PDE-AP-1

Case 4. AP-estimation of trait level by testing groups or bulks with a quantitative assay (e.g. real time PCR, digital PCR) - PDE-AP-1+2.

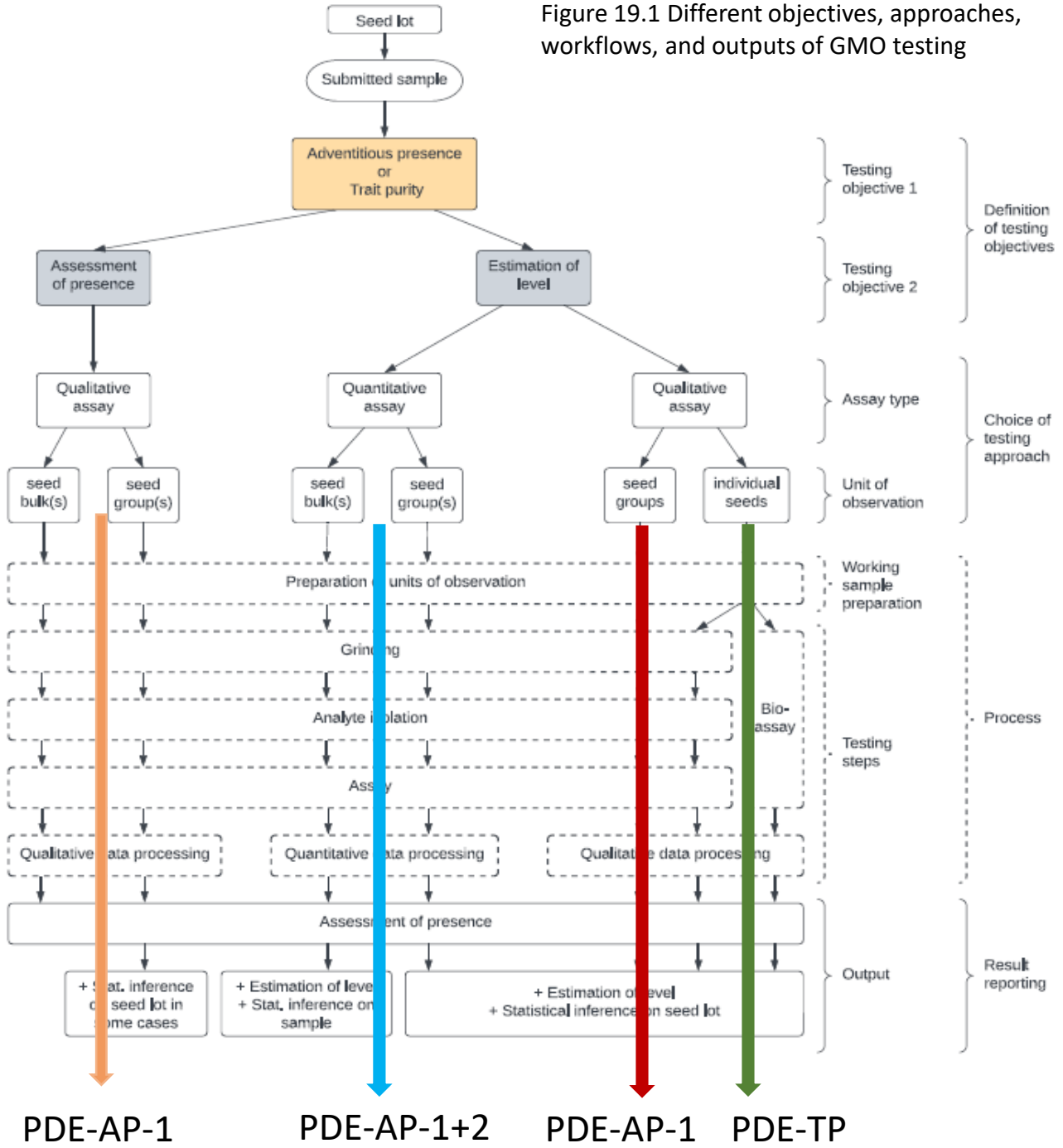

Figure 19.1 Different objectives, approaches, workflows, and outputs of GMO testing

2023 Revision of Chapter 19

- Clarifications on different **testing approaches and their outputs**

		Unit of observation			
		Individuals	**Group (known # seeds)**		**Bulk (unknown # seeds)**
			One Group	**Multiple Groups**	**One or more bulks**
Assay type	**Qualitative**	- assessment of presence - estimation of level - statistical inference on seed lot level	- assessment of presence - statistical inference on seed lot level (only in case of negative result)	- assessment of presence - estimation of level - statistical inference on seed lot level	- assessment of presence
	Quantitative	Not performed in routine testing	- assessment of presence - estimation of level - statistical inference on seed lot level		- assessment of presence - estimation of level - statistical inference on sample level, but not on seed lot level

PDE for AP - Content

1. Summary
2. Object of evaluation: species and testing process
3. Seed material to prepare the samples
4. Ability to assess the presence of specified traits by qualitative methods
5. Ability to estimate the level of specified traits by quantitative methods
6. Evaluation of the data submitted by the laboratory

Must be documented for all PD package

Either only 4 o 4+5

PDE for AP – Revisions
Version 3.0

Revisions concern:

- **Adoption of standardized terms** as in Chapter 19 current version
- Preparation of **seed material** (in case no prior- or prior-information available)
- Requirement for determining **maximum group size** for detection (with 2-stage option)
- **Increased n. of samples** for determination of both false positive/false negative rates
- Introduced **flexibility in the choice of levels**
- **Decreased n. of levels** for assessing precision and trueness (from 7 to 4)
- Introduced grading for evaluating performance (1, 2, and BMP)

PDE for AP – Revisions - Version 3.0

Performance Data Evaluation for the assessment of presence and estimation of level of seed with specified traits in seed lots

1 SUMMARY

This document describes how to obtain, present and evaluate performance data (PD) in the context of laboratory accreditation for testing of GMOs or specified traits under the Performance Based Approach. According to Chapter 19 of the ISTA Rules for the detection of seeds with GMOs or specified traits in seed lots, as no standardised method is included in the Rules for this type of test. The content will be later integrated into the GMO handbook.

For the sake of simplicity, throughout this document the expression "specified trait" will also be used to indicate GMO, since the first has a broader meaning than the latter. For the general terminology refer to definitions provided in Chapter 19.

For each **species** and **testing process** the laboratory shall provide performance data demonstrating its competence and proficiency starting from seeds. Therefore, experimental samples must be made up of seeds.

PDE for AP – Revisions - Version 3.0

1. Summary

The laboratory shall describe the whole analytical process (working sample preparation and, in particular, the testing steps) providing technical details regarding seed grinding, analyte extraction, assay (testing technology), and data processing (Section2). The description shall be sufficiently detailed to allow auditors understanding how results are obtained.

In addition, the laboratory shall indicate the target (e.g. genetic element, construct, event-specific sequence, protein, or trait) and provide information on the assay (including validation/verification data) that it will use to test the experimental samples.

The laboratory shall show evidence for the ability to assess the presence (Section 4) or also for the ability to estimate the level (Section 5) of the specified trait, when present, in the content range for which they intend to obtain analytical results to be reported on ISTA certificates (e.g. from 0.1% to 3%, or from 1% to 10%).

The PDE document produced by the laboratory should be simple and clearly organised in order to allow its evaluation by the auditors. In the present document, the essential pieces of information that need to be included in the PDE document (where applicable) are reported in boxes. Text of examples on how to provide the information requested is in italic.

2 OBJECT OF EVALUATION: SPECIES AND TESTING PROCESS

Species	e.g. *Glicyne max*

Details about the material used to produce Performance Data	
Material with the specified trait (seeds) (event UID if available and/or trade name; Variety name (optional), TSW)	e.g. *MON-Ø4Ø32-6 (GTS 40-3-2, Roundup Ready™ soybean), TSW: 158.6 g*
Material without the specified trait (seeds) (Variety name (optional), TSW)	e.g. *Cv. 'Pacific' (optional), TSW: 160.0 g*

2. OBJECT OF EVALUATION: SPECIES AND TESTING PROCESS

Description of the testing process under evaluation	
Working sample preparation	e.g. *Working sample and seed groups prepared by counting (SOP XXX) from pure seed fraction*
Grinding	e.g. *grinding in 500 ml glass jars with homogenizer (SOP XXX)*
Analyte extraction	e.g. *Nucleospin columns (SOP XXX)*
Testing technology	e.g. *Real-time PCR with TaqMan probes* *Qualitative: duplex format (SOP XXX); (quantitative method used qualitatively)* *Quantitative: simplex format (SOP XXX); (quantitative method using dilution series)*
Data processing	e.g. *Cycle threshold (Ct) determination;* *for qualitative assessment: samples with Ct > 37 => negatives (SOP XXX);* *for quantitative assessment: regression line based on dilution series of reference standard (SOP XXX)*

2. OBJECT OF EVALUATION: SPECIES AND TESTING PROCESS

Details about the assay used to produce Performance Data	
Assay target	e.g. *P-35S-CaMV*
Name and origin of the assay	e.g. *ISO Standard; QT-ELE-00-004 (EU GMO Methods)*
Reference on assay validation and its fitness for the purpose	e.g. *https://gmo-crl.jrc.ec.europa.eu/gmomethods/docs/QT-ELE-00-004.pdf*
Statement made by the lab about the way the assay was verified and installed	e.g. *Method Verification Report N. XXXX*

3 SEED MATERIAL TO PREPARE THE SAMPLES

The material used must be pure seeds.

In case the seeds are ground, the ability to obtain fine and homogeneous flour from them is one of the key elements to obtain a result which is representative from the sample.

Samples are prepared from 2 sources of seeds which shall be in principle 100% seeds with the specified trait on one hand, and 100% seeds without the specified trait on the other hand.

[….]

Material	No prior data from outside – full purity check needed	Prior data available - reduced purity check needed
GM seed lot	400 seeds – all positive	10 seeds – all positive
Non-GM seed lot	30,000 seeds – all groups negative	2 groups of 200 seeds – both negative

4 ABILITY TO ASSESS THE PRESENCE OF SPECIFIED TRAITS BY QUALITATIVE METHODS

Performance Data Evaluation in assessing the presence of specified traits by qualitative methods (or even quantitative methods used qualitatively) consists in checking the False Negative Rate (FNR) and the False Positive Rate (FPR) at the level of 1 seed with the specified trait per sample (i.e. per seed group). For this purpose, seed groups should be of two types, either negative or positive (no different spiking levels are necessary).

The definition of the group size (N) (in terms of number of seeds) to be used in this section of the PDE is left to the lab, according to its own analytical practice. Since for the presence of a single seed with the specified trait in the group FNR increases with group size, N should be equal to the maximum group size that the lab is willing to use in routine testing for that species.

ISTA requires that at least 60 groups shall be prepared to check the ability to assess the presence of specified trait(s) (Step 1). Of them, 30 should contain no seeds with the trait and 30 should contain 1 seed with the trait.

They shall be randomly coded from 1 to 60 and given blind to the staff who will perform the tests for presence/absence.

4 ABILITY TO ASSESS THE PRESENCE OF SPECIFIED TRAITS BY QUALITATIVE METHODS

The laboratory shall indicate the result for each group, whether the method showed presence of the trait (+), or its absence (-). The person who prepared the blind groups shall also report the results.

If all 30 groups of a type (either with or without trait) are correctly classified, there is a 79% confidence that the upper limit of the specific false rate (either false positive or false negative) is ≤5%. [It is worth noting that if all groups of both types are correctly classified one can declare with 95% confidence that the upper limit of the overall false rate (false positive and false negative together) is ≤5%; however, this is less informative than estimating the two error rates distinctly]

In case among the 30 groups of a type (either positive or negative) there is one false result, then 30 additional groups of the same size and type are prepared and tested (Step 2). If all the additional groups are correctly classified, then there is still an acceptable confidence (i.e. 71%) that the specific true false rate is less than or equal to 5%.

In Figure 1 an example is shown on how to collect and report data for ability to assess the presence of specified traits (see also attached worksheet Tables for PDE 3.0.xlsx).

PDE for AP – Revisions - Version 3.0

4 ABILITY TO ASSESS THE PRESENCE

Species	*Glycyne max*														**Group size (N)**						2000
Step 1					Positive groups (n)			30									Negative groups (n)				30		
Group random #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
True value	-	-	+	+	-	-	-	+	+	+	-	+	-	-	-	-	+	-	+	+	
Test result	-	-	+	+	-	-	-	+	+	+	-	+	-	-	-	-	+	-	+	+	
Group #	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
True value	+	-	+	-	+	+	+	+	+	-	-	-	+	-	+	-	-	-	-	+	
Test result	+	-	+	-	+	-	+	+	+	-	-	-	+	-	+	-	-	-	-	+	
Group #	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	
True value	+	-	-	-	+	+	-	-	+	+	-	+	+	-	+	+	-	+	+	-	
Test result	+	-	-	-	+	+	-	-	+	+	-	+	+	-	+	+	-	+	+	-	

+	trait presence
-	trait absence
nt	not tested

Misclassified groups			
Positive classified as negative	1	of	30
Negative classified as positive	0	of	30

Step 2

Positive groups (n) 30

1	2	3	4	5	6	7	8	9	10
+	+	+	+	+	+	+	+	+	+
11	12	13	14	15	16	17	18	19	20
+	+	+	+	+	+	+	+	+	+
21	22	23	24	25	26	27	28	29	30
+	+	+	+	+	+	+	+	+	+

Negative groups (n) 30

1	2	3	4	5	6	7	8	9	10
nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
11	12	13	14	15	16	17	18	19	20
nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
21	22	23	24	25	26	27	28	29	30
nt	nt	nt	nt	nt	nt	nt	nt	nt	nt

Misclassified groups			
Positive classified as negative	1	of	30
Negative classified as positive		nt	

5 ABILITY TO ESTIMATE THE LEVEL OF SPECIFIED TRAITS BY QUANTITATIVE METHODS

This procedure does not apply to laboratories willing to gain accreditation for estimating the level of specified traits by means of qualitative tests applied to multiple groups. In such cases, in addition to the procedure previously described in Section 4, the laboratory will need to demonstrate its competence in the design of the appropriate testing plans using suitable statistical tools, such as Qualitative Testing Plan Design in Seedcalc.

Performance Data Evaluation in estimating the level of specified traits by quantitative methods consists in determining the Repeatability and Trueness of the estimates obtained when analysing seed samples over a range of spiking levels, defined by the laboratory and covering the targeted threshold(s) for the testing need of the laboratory.

Target (T) specified trait range (%) (laboratory's choice)	spiking levels to be tested (%)			
$0.1 < T \leq 0.5$	0.1	0.2	0.5	**1.0**
$0.2 < T \leq 1.0$	0.2	0.5	1.0	**2.0**
$1.0 < T \leq 3.0$	**0.5**	1.0	2.0	3.0
$T > 3.0$	**1.0**	3.0	5.0	7.5

PDE for AP – Revisions - Version 3.0

5 Ability to estimate the level by quantitative methods

Species	*Glycine max*
Trait seed levels (%)	0,2 - 0,5 - 1,0 - 2,0
Group size (N)	2000
Unit for reporting results	% by weight
Transformation of results	no transformation necessary

Spiking level		Sample #	Random #	Number of seeds		Weight (g)		Weight (%) / Copy (%)		Repeatability variance	Trueness
Level	%			trait	non-trait	trait	non-trait	Expected[1]	Measured		
1	0,20	1	4	4	1996	0,637	319,36	0,20	0,24	0,01530	0,20
1	0,20	2	16	4	1996	0,617	318,00	0,19	0,48		1,47
1	0,20	3	9	4	1996	0,617	320,00	0,19	0,22		0,14
1	0,20	4	6	4	1996	0,667	319,90	0,21	0,24		0,15
2	0,50	1	10	10	1990	1,535	318,90	0,48	0,52	0,00300	0,08
2	0,50	2	2	10	1990	1,564	319,30	0,49	0,51		0,04
2	0,50	3	15	10	1990	1,594	317,04	0,50	0,49		-0,03
2	0,50	4	14	10	1990	1,643	318,40	0,52	0,40		-0,22
3	1,00	1	11	20	1980	3,069	317,30	0,97	1,11	0,01849	0,15
3	1,00	2	13	20	1980	3,128	315,40	0,99	1,00		0,01
3	1,00	3	3	20	1980	3,188	316,80	1,01	1,23		0,22
3	1,00	4	1	20	1980	3,287	317,70	1,03	1,31		0,27
4	2,00	1	7	40	1960	6,376	314,00	2,03	2,29	0,03577	0,13
4	2,00	2	5	40	1960	6,277	313,00	2,01	2,40		0,20
4	2,00	3	12	40	1960	6,534	313,60	2,08	2,57		0,23
4	2,00	4	8	40	1960	6,237	315,10	1,98	2,12		0,07
Average variance (repeatability variance) (%)										0,01814	
Mean of the true levels (%)										0,93	
Repeatability std-dev in % of the mean (Coefficient of Variation)										14,4786	

[1] In case of % copies, expected values are calculated based on % by weight and using an appropriate conversion factor, taking into account zygosity of the trait and its parental origin, as well as tissue composition of the seed. The conversion factor applied must be clearly stated by the laboratory.

6 EVALUATION OF THE DATA SUBMITTED BY THE LABORATORY

The performance data are a part of the different elements of assessment for accreditation.

The grades given below to evaluate the performance data serve as a guide for the auditors.

Grade 1 and 2 indicate an acceptable level of performance, BMP (below minimum performance) indicates an insufficient level of performance for the species and testing process under evaluation.

6.1 Ability to assess the presence of the trait(s):

Grade 1: all 60 groups (30 positive and 30 negatives) were correctly classified

Grade 2: 1 group was misclassified in either the 30 positives or the 30 negatives or in both sets; 30 additional groups for each misclassified set were subsequently tested and correctly classified.

BMP: 2 or more groups were wrongly identified in one or in both sets, after 30 or 60 groups tested.

6 Evaluation of the data submitted by the laboratory

6.2 Trueness of trait(s) quantification:

Bias

[(observed value)-(true value)]/true value

Grade 1: all 16 samples have bias within -0.25 and +0.5 (inclusive of boundaries)

Grade 2: no samples have bias smaller than -0.5 or greater than 1

BMP: one or more samples have the bias smaller than -0.5 or greater than 1

6.3 Repeatability of trait(s) quantification:

Grade 1: Repeatability std-dev in % of the mean is below 20%

Grade 2: Repeatability std-dev in % of the mean is below 30%

BMP: Repeatability std-dev in % of the mean is above 30%

GMO Hand Book writing

Further progress on Chapter 6 : ISTA Accreditation for GMO Testing

6.1 Principles and Conditions for Laboratory Accreditation under the Performance Based Approach

6.2 Production of Performance Data and Evaluation of Data Package

6.3 Requirements for subsequent scope additions

6.4 Requirements when a method is changed

6.5 ISTA GMO Proficiency Tests

(Proposed finalization 2023)

Proficiency tests

- Proficiency is one of the pillars of accreditation based on the PBA
- Proficiency tests on GMO are not run at the frequency expected
- Collaboration with the Industry is essential and should be strenghten
- Committee members from the Industry should be more involved in material procurement
- Alternative/integrative solutions to sending vital GM seeds should be sought

Alternative/integrative materials for PDE and PT

- A major issue for laboratories wishing to perform PDE is the lack of suitable GM materials. Same difficulty, on a larger scale, encountered by the GMO Committee when organizing PTs.
- Can we circumvent this obstacle?
- A possible solution, limited to DNA-based detection: preparing PDE samples spiked with non-GM seeds of a selected genotype (preferably a homozygous line) having a «unique» or at list a «rare» sequence (possibly an insertion).
- For the detection of this sequence a PCR assay could be designed having performance characteristics similar to GMO validated assays. The assay should be made available.
- Also the line genotype could be made available for interested laboratories. It could be a special ISTA Reference Material.

Alternative/integrative materials for PDE and PT

- A major issue for laboratories wishing to perform PDE is the lack of suitable GM materials. Same difficulty, on a larger scale, encountered by the GMO Committee when organizing PTs.
- Can we circumvent this obstacle?
- A possible solution, limited to DNA-based detection: for each species of interest, preparing PDE samples spiked with non-GM seeds of a selected genotype (preferably a homozygous line) having a 'unique' or at list a 'rare' sequence (preferably an insertion). Let's call this line 'A'.
- The background material would be any line chosen by the lab, not harbouring the same 'rare' sequence (prior check is necessary).
- For the detection of the 'A' specific sequence, a PCR assay could be designed having performance characteristics similar to GMO validated assays. The assay could be made available.
- Also the 'A' line could be made available through/by ISTA for interested laboratories. It could be considered a special 'ISTA Reference Material'.
- The identification of the appropriate material and the design of the assay could be the objective of a funded ISTA project.

Thank you for your attention