32nd ISTA Congress
June 26th - July 3rd 2019
Hyderabad - India

Annual Meeting 2019
GMO Committee Report

Enrico Noli - Chair

René Mathis - Vice Chair

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Membership

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

GMO Committee Members:

1. Elizabeth Bates (Belgium)
2. Sofia Ben Tahar (France)
3. Tajinder Grewal (Canada)
4. Lutz Grohmann (Germany)
5. Andrea Jonitz (Germany)
6. Jean-Louis Laffont (France)
7. Marco Mazzara (Italy)
8. Dwarkesh Parihar (India)
9. Elena Perri (Italy)
10. Kirk Remund (U.S.A.)
11. Ray Shillito (U.S.A.)
12. Ana Laura Vicario (Argentina)
13. Bruno Zaccomer (France)
14. Dabing Zhang (China)

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GMO Committee Activities

- Rules development and maintenance
- Handbook on GMO testing in seeds
- List of standardised terms
- Website
- Proficiency tests
- Workshops

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GMO Handbook Development

Contents

- Generalities of GMO Testing
- Objectives of GMO Testing
- Analytical Approaches
- Statistical methods
- Laboratory accreditation
- Best Practices/QA

8 WG members + 1 staff
from the ISTA Secretariat

GMO Handbook Meeting,
Bologna 28-31 January 2019

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List of Standardised Terms for GMO Testing

Why we need it

- labs use the **same term** to indicate **different things** (omonyms)
  - e.g. epsps for gene and protein
- labs use **different terms** to indicate the **same thing** (synonyms)
  - e.g. CaMV p35S or 35S

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  - e.g. CaMV p35S or 35S

Who will benefits

- **Laboratories**: it will contribute to clarify the accreditation for a method
- **Accreditation Department**: it will help Auditors in assessing applications of accreditation and in requiring labs the obligatory participation to PTs
- **ISTA Stakeholders**: the "Scope of Accreditation" of different labs (published on the ISTA website) will be more uniform and transparent

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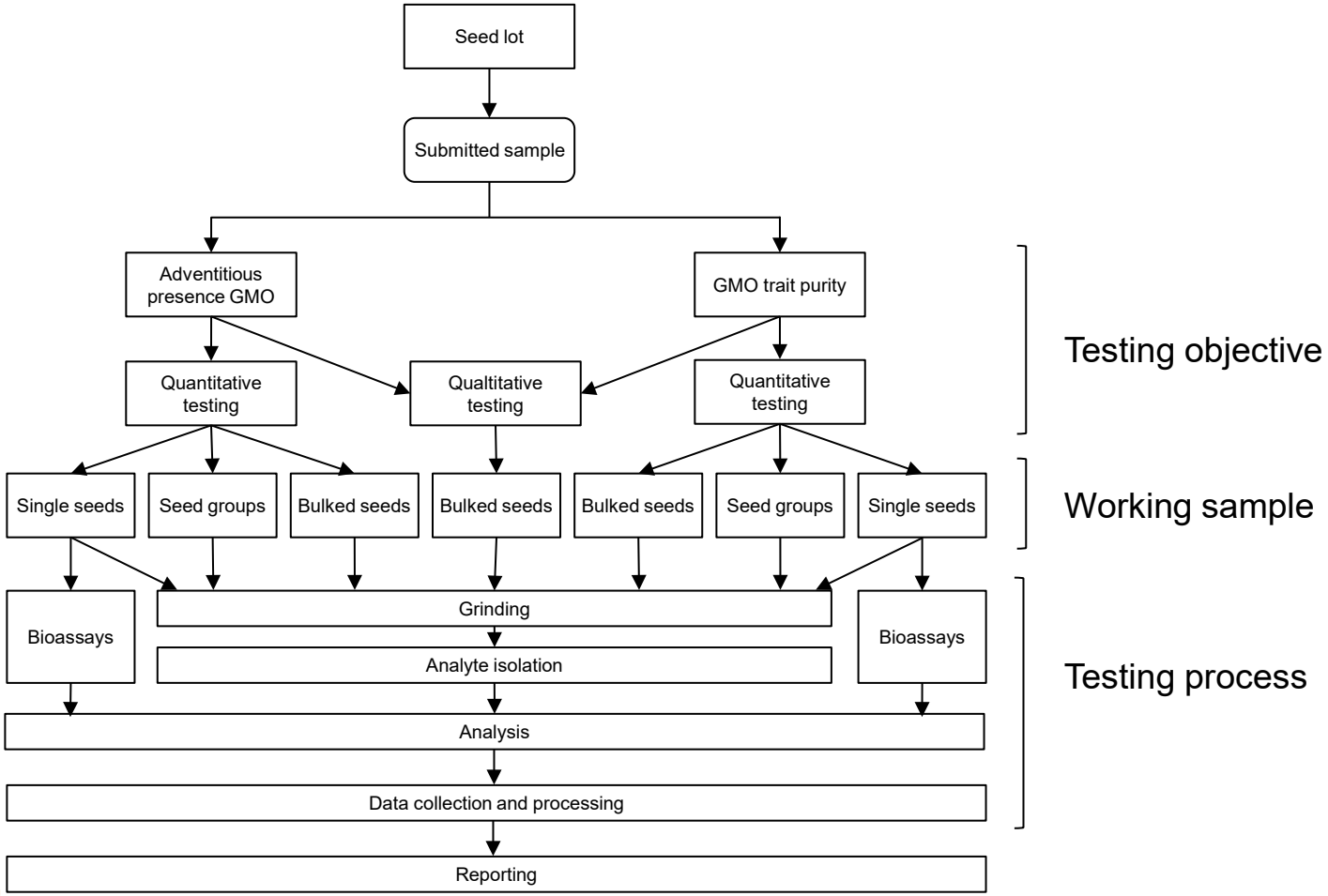
List of Standardised Terms for GMO Testing

ISTA Rules Fig. 19.1 - The different approaches to GMO testing and corresponding workflows.



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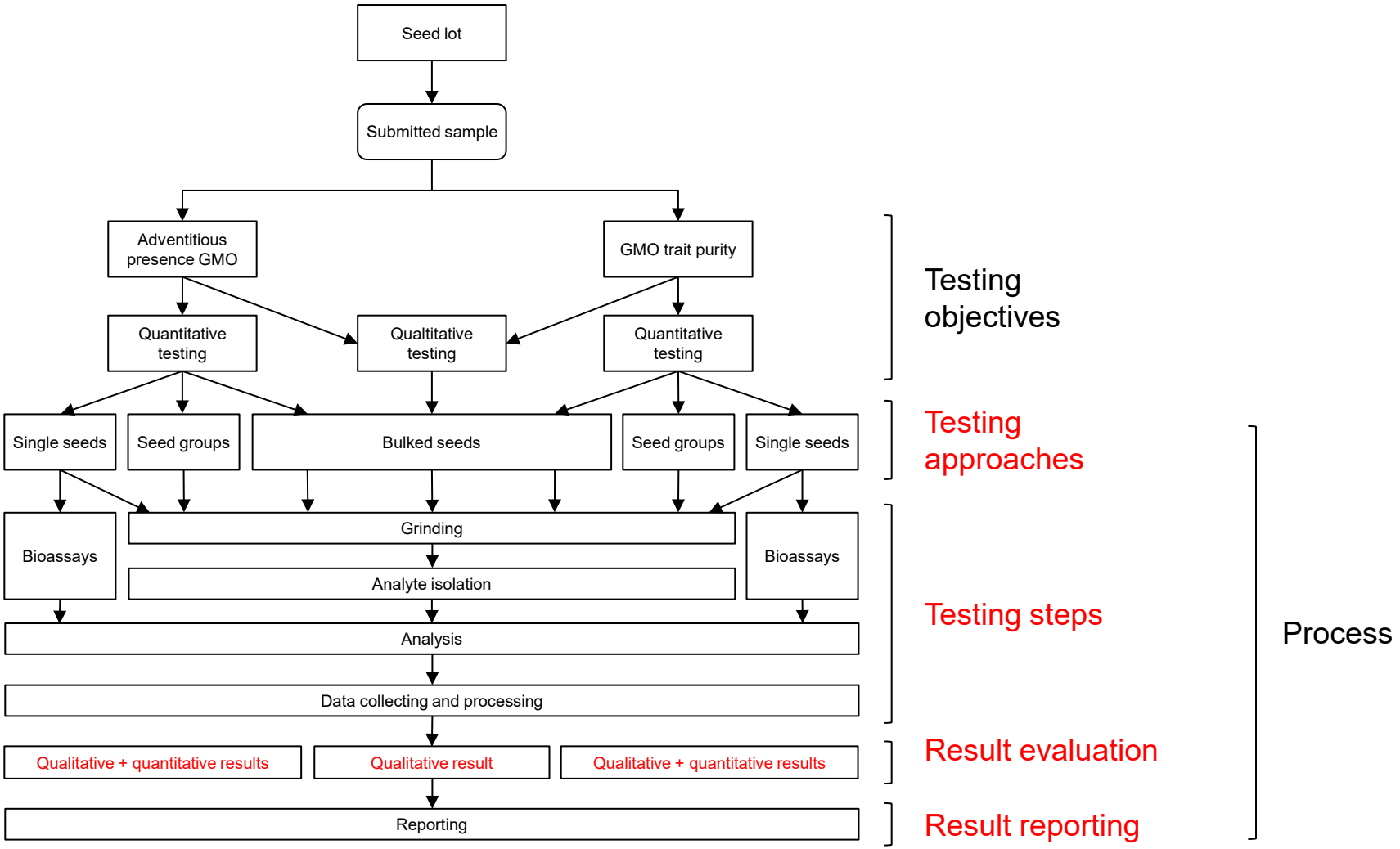
List of Standardised Terms for GMO Testing

ISTA Rules Fig. 19.1 - The different objectives and approaches to GMO testing and corresponding workflows.



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List of Standardised Terms for GMO Testing

Scope of accreditation

Method	Species	Objective/ Aim of testing	Objective/ Testing approach	Testing technology	Target	Event(s)
	Can be: *- Brassica napus* *- Glycine max* *- Gossypium spp.* *- Zea mays* *…..*	*Can be:* *- AP* *- TP* *- AP/TP*	*Can be:* *- Detection* *- Quantification IT (individual testing)* *- Quantification GT (group testing)* *- Quantification BT (bulk testing)*	*Can be:* *- Bioassay* *- Lateral Flow Strip (LFS)* *- ELISA* *- end point PCR (epPCR)* *- real time PCR (rtPCR)* *- digital PCR (digPCR)…*	*Can be:* *- trait (eg HT)* *- protein (eg EPSPS)* *- element (CaMV p35S)* *- gene (eg CP4epsps)* *- construct (eg CaMV p35S/CTP* *- event (eg SYN-Ø53Ø7-1)*	*Can be:* *SYN-Ø53Ø7-1; SYN-BTØ11-1; ACS-ZMØØ4-3; SYN-E3272-5; MON-ØØØ21-9…..*

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1	Zea mays	AP	Detection/Quantification BT	rtPCR	t-NOS	MON-ØØØ21-9; MON-ØØ6Ø3-6, DP-Ø9814Ø-6, MON-88Ø17-3
2	Zea mays	AP	Detection/Quantification GT	rtPCR	SYN-Ø53Ø7-1	SYN-Ø53Ø7-2
3	Zea mays	AP	Detection/Quantification BT	rtPCR	MON-ØØ81Ø-6	MON-ØØ81Ø-6
4	Zea mays	AP	Detection/Quantification GT	epPCR	t-NOS	SYN-Ø53Ø7-1; SYN-BTØ11-1; MON-ØØØ21-9; SYN-IR604-5; PH-MON8Ø9-2; MON-87427-7; MON-87460-4; ACS-ZMØØ5-4 ; SYN-ØØØ98-3 ; MON-ØØ6ØØ3-6
5	Zea mays	AP	Detection/Quantification GT	ELISA	Cry2Ab	MON-89Ø34-3
6	Zea mays	TP	Detection/Quantification IT	ELISA	Cry2Ab	MON-89Ø34-4

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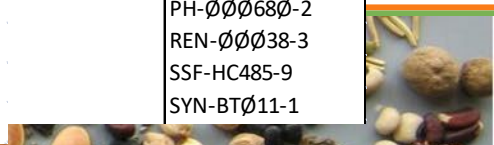
List of Standardised Terms for GMO Testing

Test Targets – Example for *Zea mays*

Traits	Proteins	Gene	Promoters	Terminators	Constructs	Events
2,4-D herbicide tolerance	AAD-1	*aad-1*	P-2xOCS35S-SYNTH	t-35S	p-35S/CTP	ACS-ZMØØ1-9
ALS-inhibitors herbicide tolerance	2mEPSPS	*2mepsps*	p-35S	t-Ara5	PG/t-nos	ACS-ZMØØ2-1
Bromoxynil herbicide tolerance	AAD-12	*aad-12*	p-5126del	t-barstar	IVS2/adh2	ACS-ZMØØ3-2
Dicamba herbicide tolerance	AHAS	*amy797*	p-Actin1	t-GBL1	P-CDPK/cry1Ab	ACS-ZMØØ4-3
Glufosinate herbicide tolerance	AMY797E	*bar*	p-AHAS	t-Hsp17		ACS-ZMØØ5-4
Glyphosate herbicide tolerance	BARNASE	*barnase*	p-CA55	t-In21		DAS-4Ø278-9
Imidazolinone herbicide tolerance	BARSTAR	*barstar*	p-CMP	t-Ms45		DAS-59122-7
Isoxaflutole herbicide tolerance	Cry1A.105	*cordapA*	p-e35S	t-NOS		DAS-Ø15Ø7-1
Mesotrione herbicide tolerance	Cry1Ab	*cp4epsps*	p-eFMV	t-ORF25		DAS-Ø6275-8
Sulfonylurea herbicide tolerance	Cry1Ac	*cry1A.105*	p-eTMV	t-PinII		DKB-89614-9
	Cry1F	*cry1Ab*	p-FMV	t-tr7		DKB-8979Ø-5
	cry1Fa3	*cry1Ac*	p-GBL1	t-TubA		DP-32138-1
	Cry2Ab	*cry1F*	p-gz27	t-Ubi		DP-Ø9814Ø-6
	Cry34Ab1	*cry1Fa2*	p-Ltp2	t-ZMPer5		DP-ØØ4114-3
	Cry35Ab1	*cry2Ab2*	p-MTL			MON-87411-9
	Cry3Bb1	*cry34Ab1*	p-PCDK			MON-ØØ863-5
	Cry9C	*cry35Ab1*	p-PCISV			MON-87419-8
	CSPB	*cry3Bb1*	p-Pg47			MON-87427-7
	DAM	*cry9C*	p-pIIG			MON-8746Ø-4
	DHPS	*cspB*	p-POX			MON-874Ø3-1
	DMO	*dam*	p-TA29			MON-88Ø17-3
	eCry3.1Ab	*dmo*	p-tac			MON-89Ø34-3
	EPSPS	*dvsnf7*	p-TubA			MON-8Ø2ØØ-7
	EPSPS	*ecry3.1Ab*	p-Ubi			MON-ØØ6Ø3-6
	GAT	*epsps*	p-UbiSO4			MON-ØØ81Ø-6
	GOX	*gat*	p-Ubq			MON-ØØØ21-9
	mCry3A	*gm-hra*				PH-MON8Ø9-2
						PH-ØØØ676-7
						PH-ØØØ678-9
						PH-ØØØ68Ø-2
						REN-ØØØ38-3
						SSF-HC485-9
						SYN-BTØ11-1

SOURCES

OECD Biotrack Database	https://biotrackproductdatabase.oecd.org/
BCH Gene and DNA Sequence Registry	https://bch.cbd.int/database/gene-registry/
ISAAA	http://www.isaaa.org/gmapprovaldatabase/geneslist/default.asp
GENBIT Database	https://www.genbitgroup.com/en/gmo/gmodatabase/

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GMO Proficiency Tests

- **PT22 – Canola – *Brassica napus* – Completed**

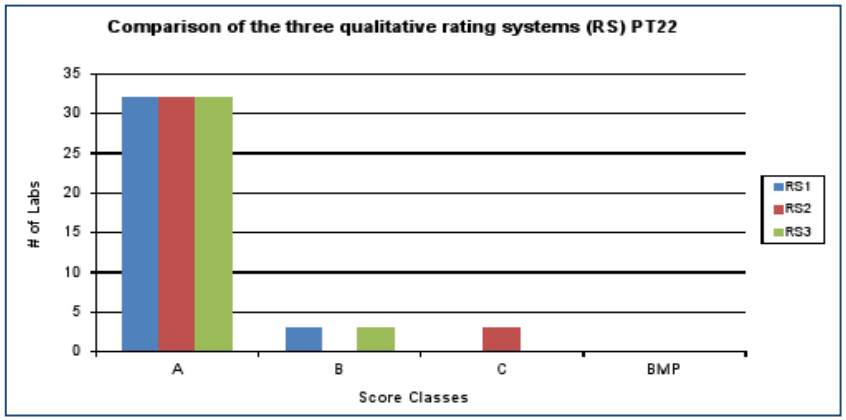
Regular part: Event: Rf3 (ACS-BNØØ3-6)

N. blind samples: 8 GMO Levels: 0 – 0.3 – 0.8 – 1.3

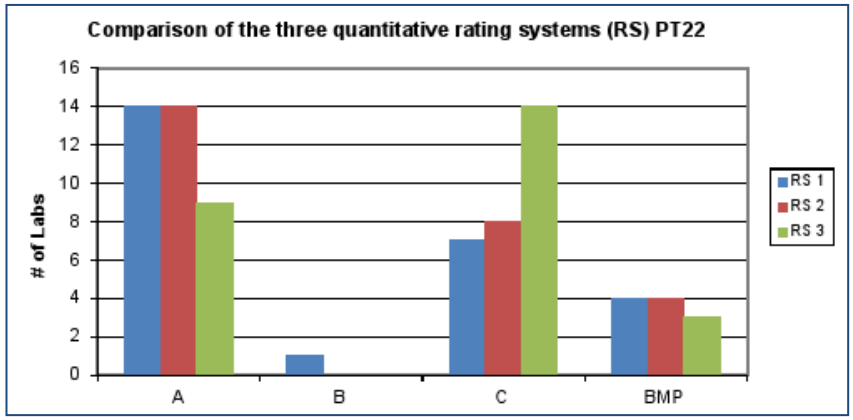
N. of Laboratories: 36 - of them 35 sent back their results

Final report sent May 15 2019

Rf3: Qualitative part: 35 labs



Rf3: Quantitative part: 26 labs



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GMO Proficiency Tests

- **PT22 – Canola – *Brassica napus***

  Voluntary part: Event: Ms8 (ACS-BNØØ5-8)

  - counterpart of Rf3 in male-sterility system
  - not tested before in PTs

NOT TO BE RATED (since material is only 50% genetically pure it is impossible to know exactly the level of spiking)

N. blind samples: 6

GMO Levels: 0 – 0.8 – 1.6

Final report still due

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GMO Proficiency Tests
Some issues

- Issues related to the shipping and delivery GMO PT samples had emerged over the years and during PT22
- Changes in national phytosanitary regulations or requirements for import permits for GMO-containing samples have made sometimes challenging, the timely and safe delivery of samples to the participating labs (difficulties in shipping, samples delayed, blocked, lost)
- The Provider of the Material (GM and non-GM), in the framework of its collaboration with ISTA, asked to review the Material Transfer Agreement and the stewardship provisions therein in order to improve the system and adopt measures to prevent such occurrences

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GMO Proficiency Tests
Some issues

- Review procedure initiated in December 2018, just ended
- Amendments to documents to be finalized and accepted by ISTA
  - Material Transfer Agreement
  - Material Shipment Destination and Documentation
  - Pre-Shipment Checklist for the Export of Biological Materials and GMO
  - Incident Response Plan
- The new documents will be used for next PT
- Possibility of using de-vitalized seeds for AP testing whenever applicable

Very constructive dialogue, collaboration with industry confirmed

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Collaboration with other Organisations

With SCST for preparation of GMO PTs

Expected benefits from the collaboration:

- Decreasing costs of organization and maximize benefits
- Increasing n. of labs, higher prestige of ISTA GMO PT program
- Widening the scope of lab proficiency monitoring toward trait purity
- Supporting training of analysts

– Discussion between the two Organizations has slowed down due to difficulties arisen during PT22

– Identify the model of a possible collaboration considering the complexity of the management of movement of GM materials across borders

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Collaboration with other Organisations

With ISO for development of new standard

ISO TC 34/SC 16/WG9 Horizontal method for molecular biomarker analysis – Subsampling seed and grains

The GMO Committee has been invited as liaison C organisation to make a technical contribution to the activity of the working group

Recently an upgrade to liaison A status has been proposed to make an effective contribution at the level of the technical committee or subcommittee

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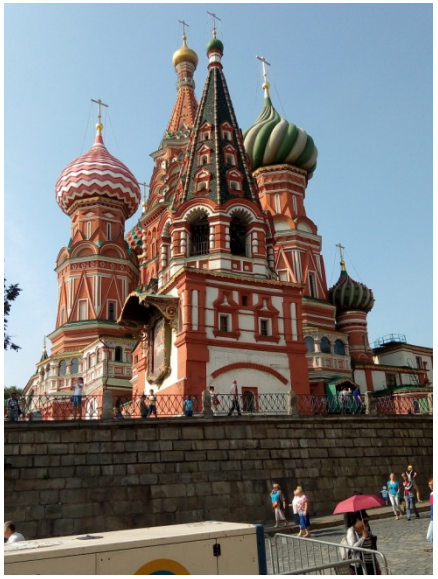
Workshops



ISTA Biotechnology Trait Detection Workshop

Moscow, Russian Federation, 28-31 August 2018

ISTA GMO Com: Kirk Remund, Bruno Zaccomer, Enrico Noli
ISTA Secretariat: Olga Stoeckly

- Organised in collaboration with FSBE "The Russian Agricultural Center“

- Held at the premises of GenBit LLC (private company) at “Slava Technopark”

- prof. Alexander Golikov of GenBit and his collaborators took care of the running of the practical part

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Workshops

ISTA Biotechnology Trait Detection Workshop

- 31 participants from public ad private institutions
- Theoretical Part : generalities on GMO; testing objectives, technologies and approaches; statistical aspects of GMO testing
- Practical Part: DNA extraction, qualitative and quantitative real time PCR; test plan design and data analysis

The GMO Committee is available for planning of future workshops upon request

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Acknowledgements

- Christoph Haldemann
- Benoit Maes
- Nadine Ettel

- Andreea Militaru
- Branislava Opra
- Andreas Wais

- Statistics Committee
- ECOM liaisons: Rita Zecchinelli, Berta Killermann

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Thank You
for Your Attention!

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ISTA GMO Accredited Labs

1	Argentina	ARDL0100
2	Austria	ATDL0300
3	Denmark	DKML0800
4	France	FRML0300
5	Germany	DEDL0400
6	Hungary	HUDL0100
7	Italy	ITDL0300
8	Italy	ITDL0100
9	Japan	JPDL0100
10	Japan	JPML0700
11	Japan	JPDL0300
12	Serbia	RSDL0200
13	Uruguay	UYDL0200
14	USA	USML1100

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GMO Proficiency Tests

Rating		Percent of participating laboratories		
		Pt11 (2009)	Pt13 (2011)	Pt22 (2019)
Qualitative	A	95,3	96,7	91,4
	B	2,3	0,0	0,0
	C	0,0	0,0	8,6
	BMP	2,3	3,3	0,0
		43 labs)	*(30 labs)*	*(35 labs)*

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Rating		Percent of participating laboratories		
		Pt11 (2009)	Pt13 (2011)	Pt22 (2019)
Qualitative	A	95,3	96,7	91,4
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	C	0,0	0,0	8,6
	BMP	2,3	3,3	0,0
		43 labs)	*(30 labs)*	*(35 labs)*
Quantitative	A	16,1	22,7	53,8
	B	3,2	22,7	3,8
	C	58,1	36,4	26,9
	BMP	22,6	18,2	15,4
		(31 labs)	*(22 labs)*	*(26 labs)*

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GMO Proficiency Tests

PT 11

Spiking level	**0%**	**0.4%**	**0.6%**	**1.2%**	**1.2%**	**2.0%**
Event	None	T45	RF3	T45	RF3	T45 + RF3
Lot No.	1, 2	3, 4	5, 6	7, 8	9, 10	11, 12
Number of samples	2	2	2	2	2	2
Number of non- GM seeds	3000	2988	2982	2964	2964	2940
Number of GM seeds	0	12	18	36	36	30 + 30

PT 13

Spiking level	**0%**	**1.6%**	**2.0%**	**1.0%**	**2.6%**	**2.0%**
Event	None	T45	RF3	RF3	RF3 + T45	T45
Lot Nos	1, 2	3, 4	5, 6	7, 8	9, 10	11, 12
Number of samples	2	2	2	2	2	2
Number of non-GM seeds	3000	2952	2940	2970	2922	2940
Number of GM seeds	0	48	60	30	48 + 30	60

PT22

Spiking level	**0%**	**0.3%**	**0.8%**	**1.3%**
Event	None	RF3	RF3	RF3
Lot numbers	1, 2	7, 8	5, 6	3, 4
Number of samples	2	2	2	2
Number of non-GM seeds	3000	2991	2976	2961
Number of GM seeds	0	9	24	39

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PT13

Rate	Misclassified samples	Misclassified samples absolute numbers	Number of laboratories
A	0% - 5%	0	29
B	>5% - 10%	1	0
C	>10% - 20%	2	0
BMP	>20%	>2	1

Rate	Misclassified samples	Misclassified samples absolute numbers	Number of laboratories
A	0% - 5%	0	32
B	>5% - 10%	-	-
C	>10% - 20%	1	3
BMP	>20%	>1	-

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PT13

Reporting unit	Number of laboratories
% number	6
% mass	10
% DNA copies	6

Rate	Number of laboratories
A	5
B	5
C	8
BMP	4

PT22

Reporting unit	Number of laboratories
% number	7
% mass	16
% DNA copies	3

Taking this into account, the quantitative ratings av

Rate	Number of laboratories
A	14
B	1
C	7
BMP	4