



ISTA GMO Proficiency Tests: strategies and results after 9 rounds

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ISTA GMO Proficiency Test

- PT concept
- PT programme so far
- Rating
- Units
- Future rounds



Elements of accreditation

- Accreditation Standard/ISTA Rules
 - Scope of accreditation
1. Participation in proficiency testing
 2. Performance data evaluation
 3. On-site assessment

ISTA Seminar for Specified Trait Testing, 16 June 2008, Bologna, Italy

INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)



ISTA GMO Proficiency Test

- **Participants demonstrate their competence**
 - ✓ **detection**
 - ✓ **quantification**
- **additional features: genetic purity, stacked events**



PT programme until 2007



PT-Round	Species	Event	spiking levels	# samples	# detection results	# quant results
PT01	Maize	T25 MON810	1.0	30	43	-
PT02	Maize	MON810	0.7, 1.4	10	48	22
PT03	Maize	T25 MON810	0.2, 2.0, 4.0	12	40	25
PT04	Soybean	GTS40-3-2	0.1, 0.5, 1.0	12	51	12+36
PT05	Soybean	GTS40 A2704	0.2, 0.5, 1.0, 1.5	12	58	55
PT06	Canola	GT73	0.3, 0.6	10	49	35
PT07	Maize	MON863 NK603	0.4, 0.8, 2.0	12	57	50
PT08	Soybean	GTS40-3-2	0.13, 0.5, 1.0, 2.3	14	52	50
PT09	Maize	MON863 NK603	0.1, 0.8, 90, 96	12+2	56	52



Rating: general

- **A** No problem has been detected in this test
- **B** There are small problems, but no specific action is suggested to the participant
- **C** Indicates the need for follow-up activities and corrective measures
- **BMP** Below Minimum Performance, indicates that the laboratory should investigate to find explanations and improve/correct



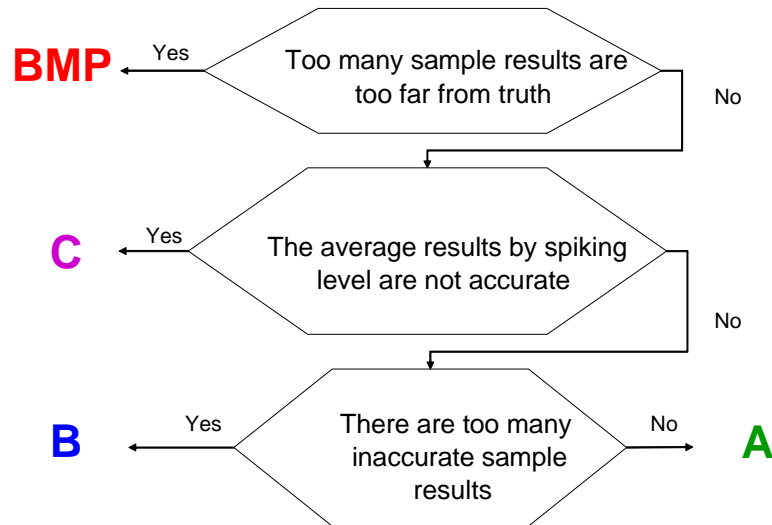
Rating: detection



Rate	Percent of missclassified samples	Ex.: 12 samples
A	from 0% to 5%]	0
B	>5% to 10%]	1
C	>10% to 20%]	2
BMP	more than 20%	3 and more



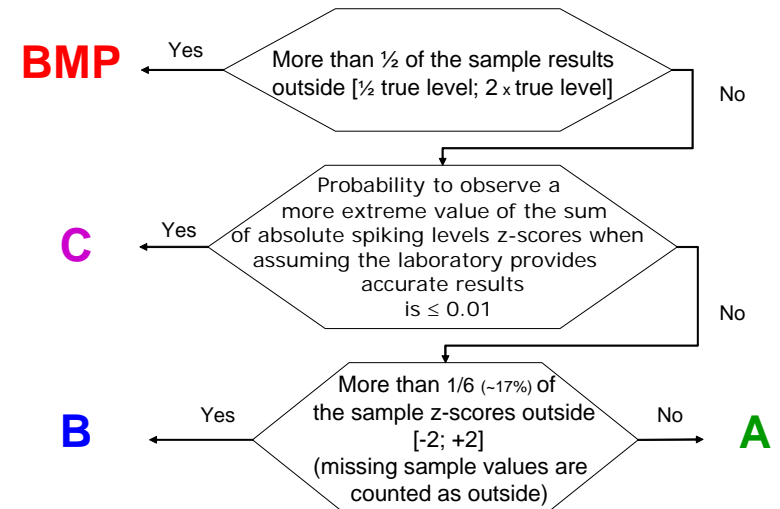
Rating: quantification



Principles



Decision rules





Rating: quantification

Reference values for evaluation of the quantitative results are:

- the true number of GM seeds in percent
- the true mass of the GM seeds in percent
- the median of the results reported by the participants in the unit ‘%DNA copies’



Example: PT09



PT-Round	Species	Event	spiking levels	# samples	# detection results	# quant results
PT09	Maize	MON863 NK603	0.1, 0.8, 90, 96	12+2	56	52



PT09 design



Spiking level	0%	0.1%	0.1%	0.8%	0.8%	0.8%	90%	96%
Event		Mon863	NK603	MON863+ NK603	MON863+N K603	MON863+N K603	MON863	MON863
Lot No	1	2-4	5, 6	7, 8	9, 10	11, 12	13	14
Number of samples	1	3	2	2	2	2	1	1
Number of non-GM seeds	2000	1998	1998	1984	1984	1984	5	2
Number of GM seeds	0	2	2	8+8	4+12	12+4	45	48



Rating PT09



Results PT09

Species: Maize

Event(s): MON863, NK603

Participant # 4

Rating detection (qualitative) **A**
 Rating quantification **A** reference for rating: % number

Sample	% mass GMO			% number GMO		median	qual result reported*	quant result reported			Unit: % number	Note
	Total	MON863	NK603					Total	MON863	NK603		
1	0.12	0.12	0.00	0.1	0.125	1	0.1	0.1	0			
2	0.10	0.00	0.10	0.1	0.125	1	0.1	0	0.1			
3	0.86	0.66	0.20	0.8	0.73	1	0.8	0.6	0.2			
4	0.09	0.00	0.09	0.1	0.125	1	0.1	0	0.1			
5	0.11	0.11	0.00	0.1	0.125	1	0.1	0.1	0			
6	0.11	0.11	0.00	0.1	0.125	1	0.1	0.1	0			
7	0.78	0.44	0.34	0.8	0.73	1	0.8	0.4	0.4			
8	0.85	0.49	0.36	0.8	0.73	1	0.7	0.3	0.4			
9	0.80	0.21	0.59	0.8	0.73	1	0.9	0.2	0.7			
10	0.85	0.63	0.22	0.8	0.73	1	0.9	0.7	0.2			
11	0.84	0.21	0.63	0.8	0.73	1	0.9	0.2	0.7			
12	0.00	0.00	0.00	0		0	0	0	0			
13	96.61	96.61	0.00	96								
14	91.02	91.02	0.00	90								

* 1=positive, 0=negative



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Results PT09

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8	0.85	0.49	0.36	0.8	0.73	1	0.7	0.3	0.4			
9	0.80	0.21	0.59	0.8	0.73	1	0.9	0.2	0.7			
10	0.85	0.63	0.22	0.8	0.73	1	0.9	0.7	0.2			
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Results PT09

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Rating PT09



Results PT09

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PT09 Results



Detection

rate	Misclassified samples	Misclassified samples absolute numbers	Number of laboratories
A	0% - 5%	0	48 (86%)
B	>5% - 10%	1	2 (4%)
C	>10% - 20%	2	3 (5%)
BMP	>20%	3 and more	3 (5%)



PT09 Results



Quantification

rate	Number of labs	% number	%mass	% DNA copy numbers
A	27 (52%)	7	15	5
B	11 (21%)	1	5	5
C	1 (2%)	-	-	1
BMP	13 (25%)	1	2	10



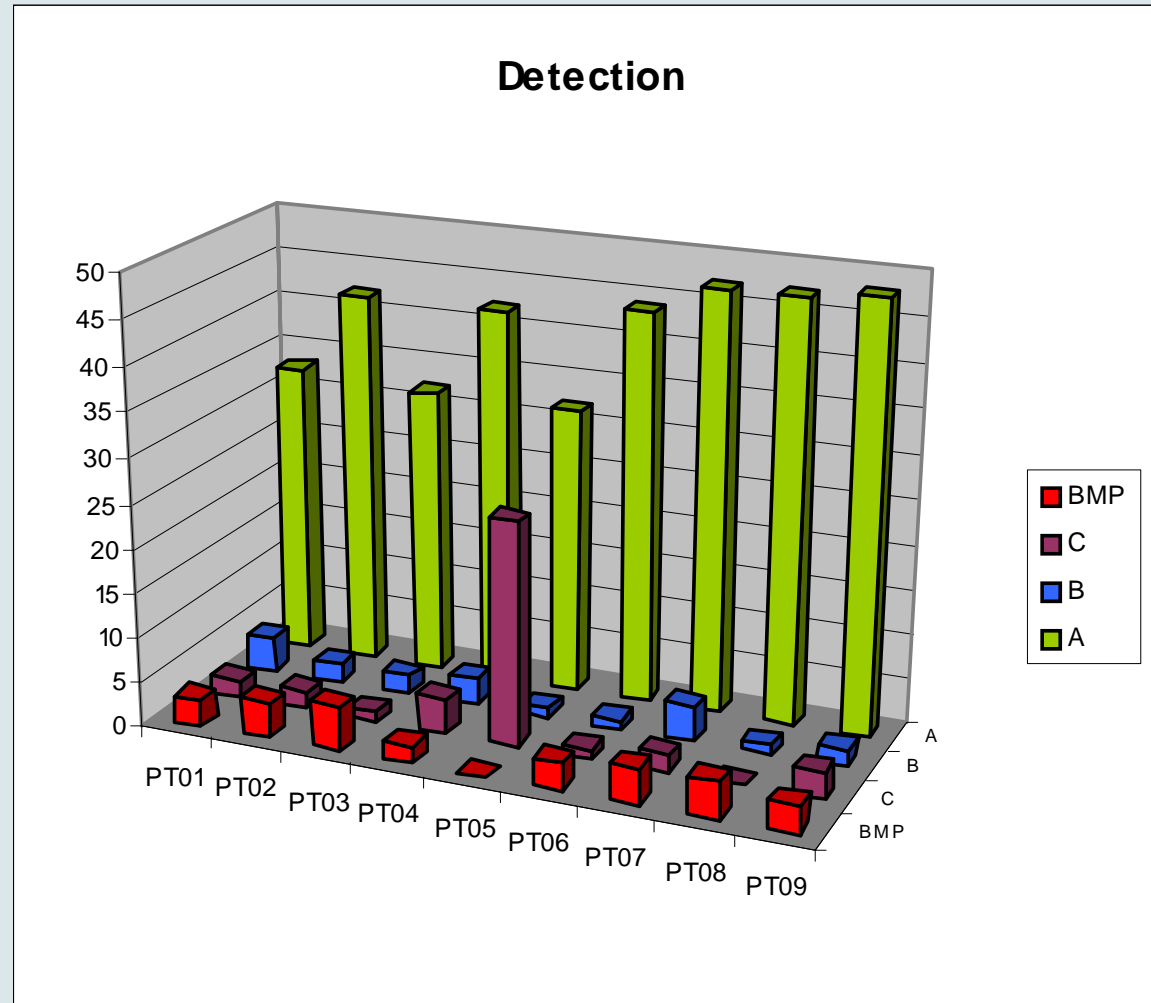
Rating PT01-PT09



Percentage Rating	Detection				Rating	Quantification			
	A	B	C	BMP		A	B	C	BMP
PT01	79	10	5	7	PT01	-	-	-	-
PT02	84	4	4	8	PT02	68	0	14	18
PT03	80	5	3	13	PT03	16	4	52	28
PT04	82	6	8	4	PT04	56	17	19	8
PT05	55	2	43	0	PT05	18	24	51	7
PT06	90	2	2	6	PT06	62	6	12	21
PT07	82	7	4	7	PT07	28	6	46	20
PT08	90	2	0	8	PT08	52	14	26	8
PT09	86	4	5	5	PT09	52	21	2	25
ALL	81	4	9	6	ALL	43	13	28	16
PT06-PT09	87	4	3	7	PT06-PT09	48	12	21	18

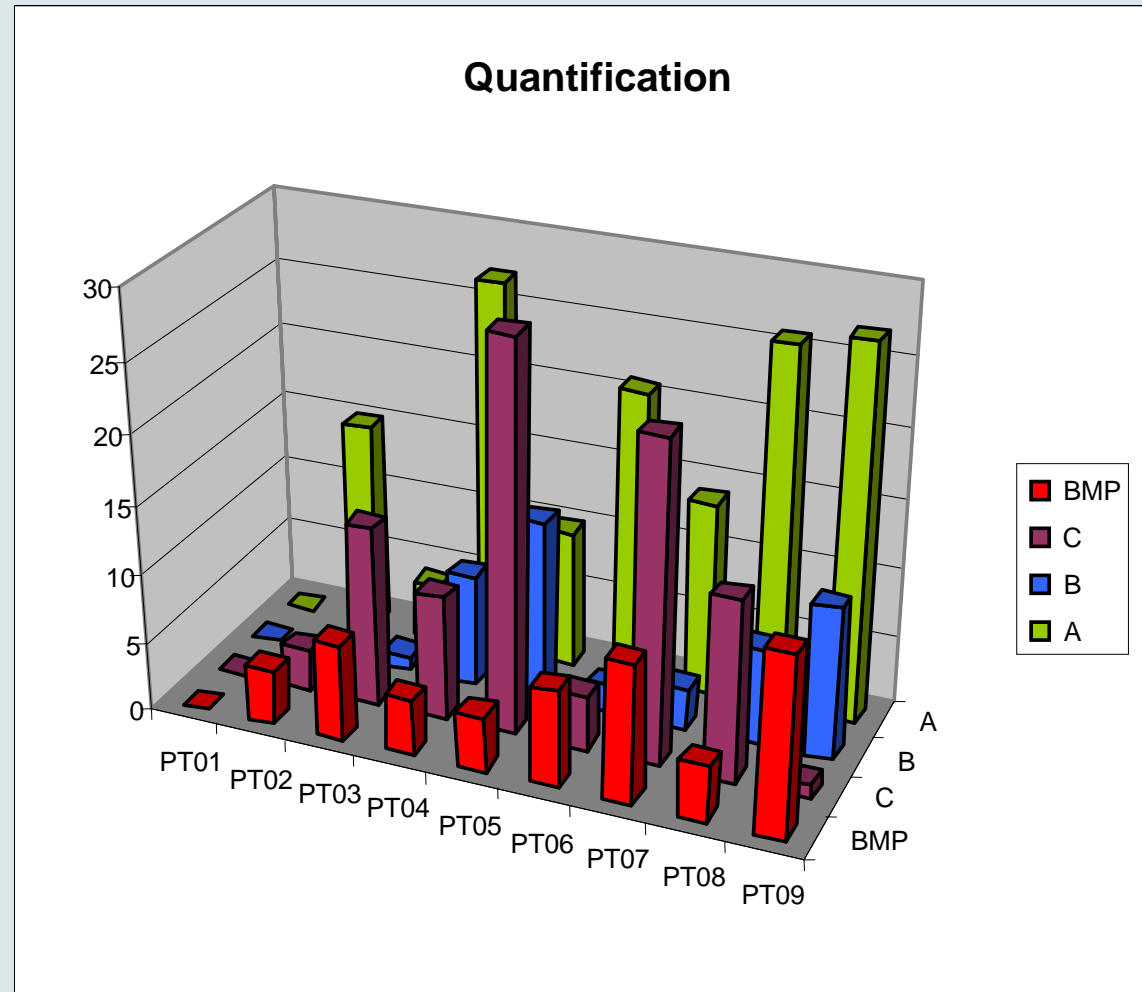


PT01-PT09 Ratings



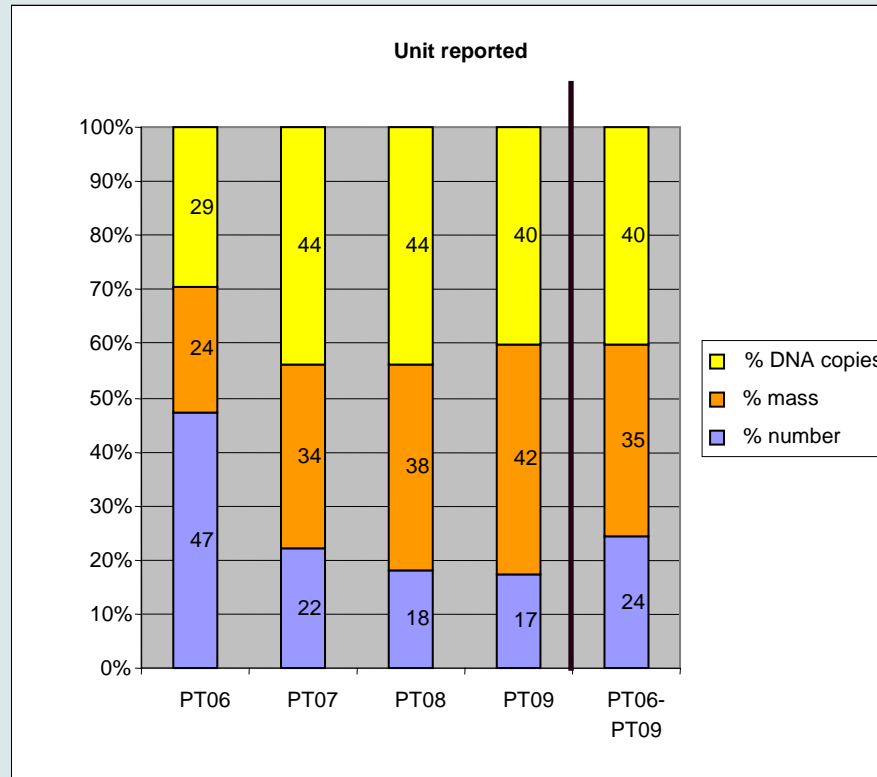


PT01-PT09 Ratings



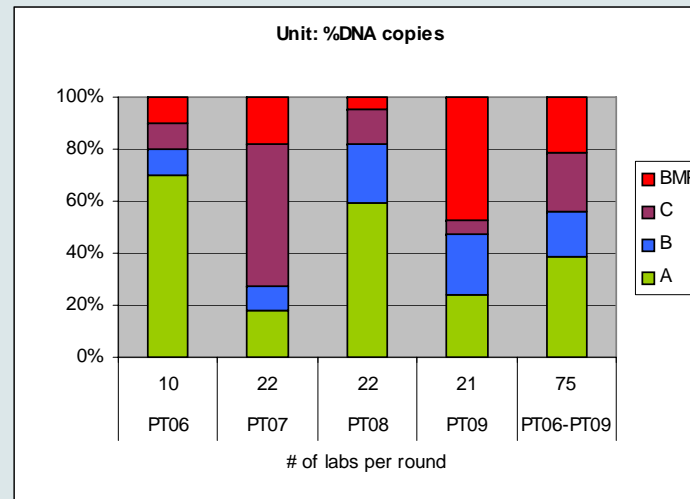
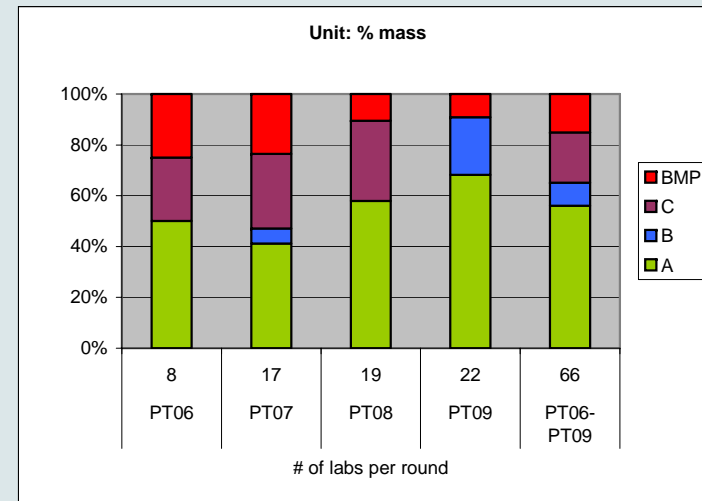
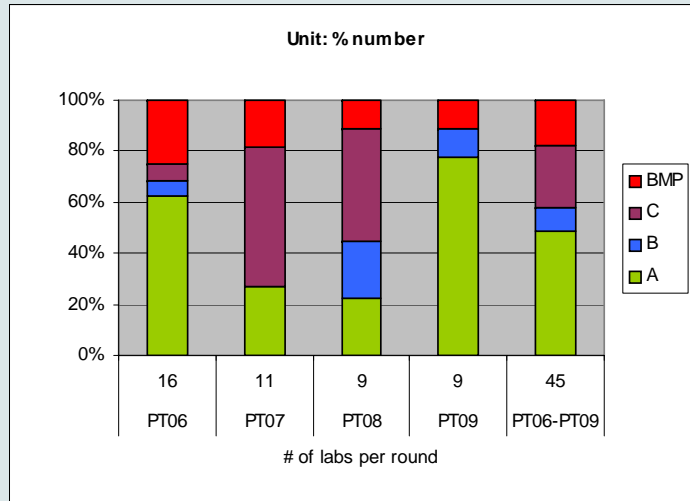


Units for quantitative results





Units reported





Issues to be addressed

- **Preferential units for reporting results:**
 - % number of seeds
 - % mass of seeds

Reference values based on known proportions of GM and non GM seeds



Issues to be addressed

- **Lack of clarity among participants on the definitions used for the different units**



**Clarification document for
rt PCR quantifications**



Calibration curves (Standards)

by mass fraction

- The standards are produced from Certified Reference Material (CRM), typically flour. These certified samples consist of conventional seed flour fortified with genetically modified seed flour at a given w/w proportion

→ unit of measurement: % mass



Calibration curves (Standards)

by haploid genome copy number:

- standard curve prepared by haploid genome copy number for both GM and taxon PCR targets (taking into account the genetic structures of the hemizygous seeds)

→ unit of measurement: % DNA copies



Future PT rounds



PT-Round	Species	Event	spiking levels	# samples	Year
PT10	Cotton	MON531 MON1445 MON531* MON1445		12	2008
PT11	Canola	Ms8 T45		12	2008
PT12	Maize				2009
PT13	Soy				2009
PT14	Canola				2010
PT15	Maize				2010



Future PT rounds

- **Two rounds per year**
- **Samples consisting of seeds**
- **Focussed on internationally important species and traits**
- **Reference to the clarification document (units for reporting)**

