28th ISTA Congress

XV Congresso Brasileiro de Sementes

STATISTICS COMMITTEE

May 10th 2007 13:30 14:00

Iguassu falls, Brazil

Sylvain Gregoire

List of members

to the very active members

TCOM / STA

Statistics Committee

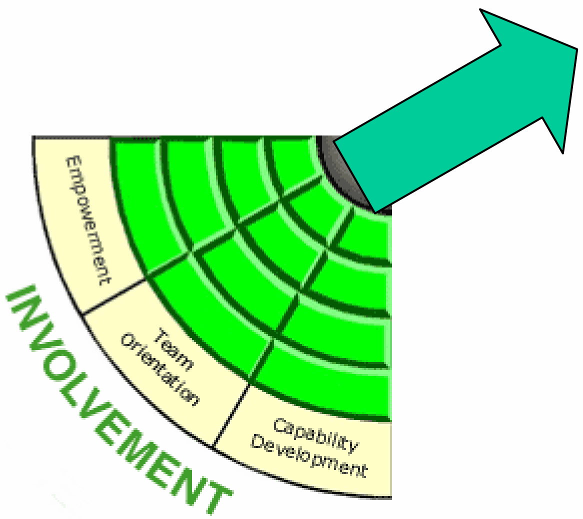
Working Programme   Activity Report   Stats Tool Box   Stats Links   Workshops   Documents

Chair	Sylvain Grégoire
Vice	Kirk Remund
Member	Julianna Bànyai
	Julia Barabas
	Olfat H. El Bagoury
	Winfried Jackisch
	Michael Kruse
	Jean-Louis Laffont
	Andrew Peace
	Petra Remeeus
	Erhard Thomas
	Mohamed Tourkmani

New chair JL Laffont

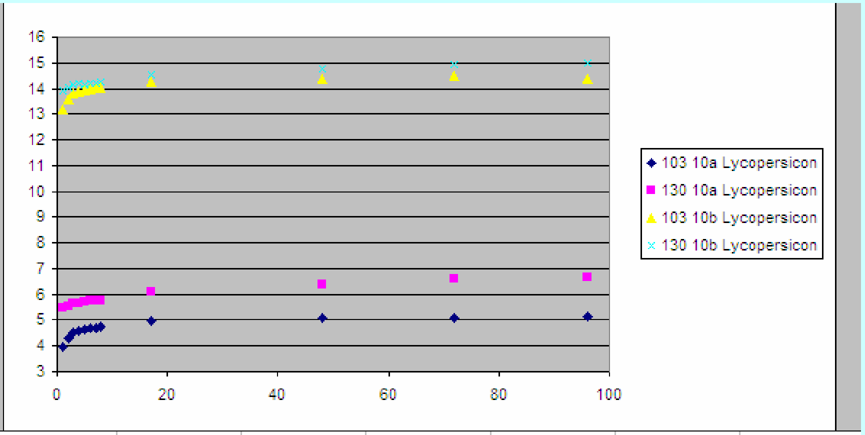
Alphabetic order

Can be improved

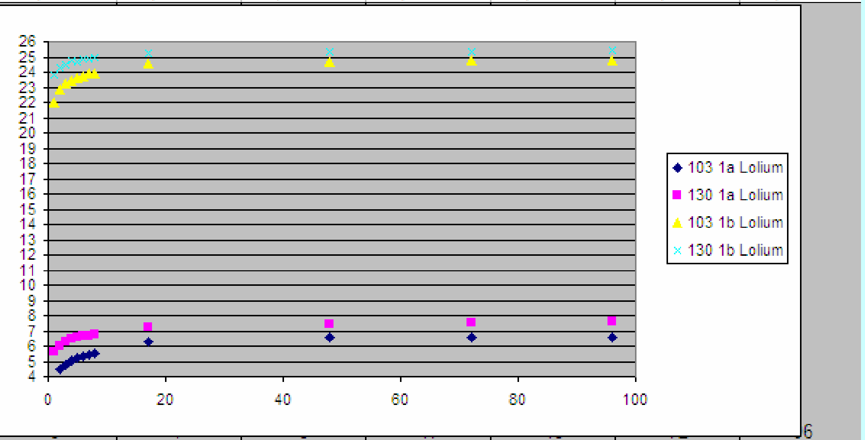


method validation/ comparative tests

On request by committees (SHC, VAR, TEZ, MOI…) STA help in planning experiments, analyse data, draw conclusions



4,682293693	4,709032416	4,728857557	4,989156067	5,093877554	5,099221289	5,112876813
5,729902486	5,736311456	5,763411383	6,086850107	6,395705819	6,599372864	6,660301268
13,98232901	14,01503054	14,04462663	14,27989161	14,35407305	14,49297557	14,37330914
14,2120707	14,20498004	14,24399797	14,51137038	14,78535024	14,9385705	14,99718267



130°

1-4h

103°

17h

. Compute the (lab x sample) means for the reference method (103°C-17h) and the 130°C methods.
. Compute the sample means for the reference method.
. Compute the differences between the (lab x sample) means for the candidate method and the sample means for the reference method.
. If 75% or more of these differences are within the tolerance range +/- 0.3%, keep the corresponding 130°C method.
. Select the 130°C method from the retained ones which have the shortest duration test.

The level of 75% is based on past experience, and is safe and practicable. It is still applicable when only three labs participate.

Example with three participating labs, two moisture levels (low and high), 3 samples per modality (1,2,3):
The table shows the check of 130°C 1 hour, the same apply for 2 3 and 4 hours.

sample	Moisture level	Lab	Reference Results 17hrs-103°C	Results 1 hr-130°C	Difference with reference sample mean
1	low	A	9.9	10.0	0.10
		B	10.0	9.9	0.00
		C	9.8	9.6	-0.30
Reference 1 low sample mean			9.90		
2	low	A	7.9	8.1	0.10
		B	8.1	8.6	0.60*
		C	8.0	8.4	0.40*
Reference 2 low sample mean			8.00		
3	low	A	8.9	9.2	0.37*
		B	9.1	9.2	0.37*
		C	8.5	8.9	0.07
Reference 3 low sample mean			8.83		
1	high	A	12.8	12.7	-0.23
		B	12.9	12.9	-0.03
		C	13.1	13.4	0.47*
Reference 1 high sample mean			12.93		
2	high	A	13.5	13.6	-0.07
		B	13.7	13.7	0.03
		C	13.8	14.0	0.33
Reference 2 high sample mean			13.67		
3	high	A	13.0	13.3	0.03
		B	13.3	13.4	0.13
		C	13.5	13.5	0.23
Reference 3 high sample mean			13.27		

STA GMO TF

STA has supported the work of the GMO TF on rules, proficiency testing, performance data evaluation, accreditation of laboratories

ACCREDITATION / Specified Traits

Accreditation of Laboratories for the Testing of Specified Traits

Relevant Accreditation Documents for the Accreditation of laboratories for the testing of specified traits under a performance based approach

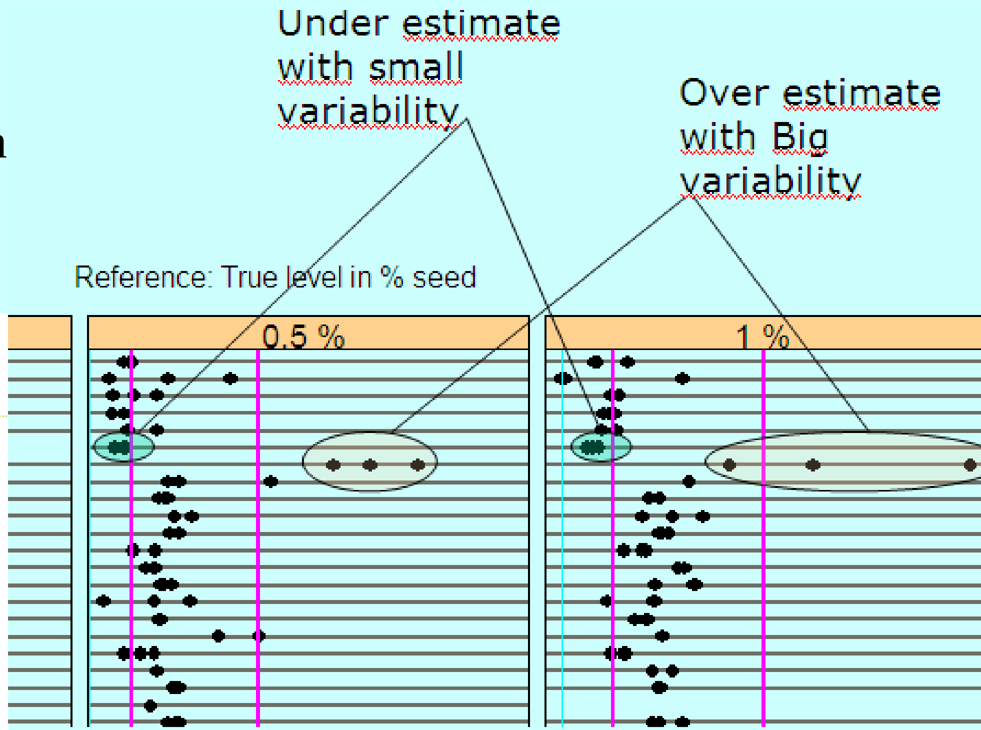
During the Ordinary Meeting 2005 in Bangkok, Thailand, the proposed **new version of the ISTA Rules Chapter 8** was adopted by the ISTA voting delegates after an intensive discussion during the Ordinary Meeting. The Ordinary Meeting gave the Executive Committee of the Association the mandate to finalise the relevant accreditation documents in all details and decided that the new version of Chapter 8 will come into force 6 months after the publication of these relevant accreditation documents.
After intensive discussions on the level of the Executive Committee, this process could be finalised on July 29, 2005.

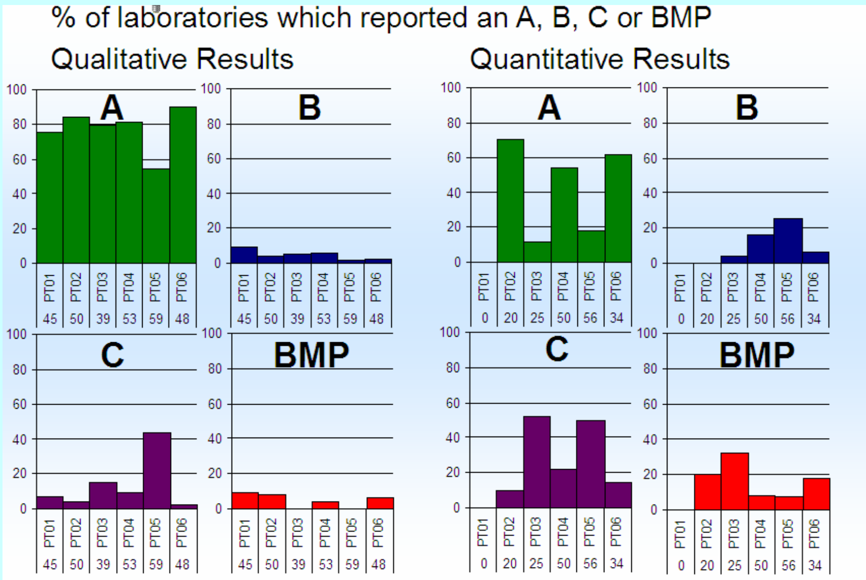
Consequently, the new version of Chapter 8 of the ISTA Rules will come into force on February 1, 2006. From that date on it will be possible for laboratories to become ISTA accredited for the testing of seeds with specified traits under the performance based approach.

Relevant accreditation documents:

- Principles and Conditions for Laboratory Accreditation under the performance based approach (Version 2.1) (102 KB)
- Performance data evaluation for the presence of seeds with specified traits in seed lots (Version 2.1) (73 KB)
- Performance Data Evaluation for Specified Trait Purity (Version 1.1) (48 KB)
- The ISTA Seed Testing Laboratory Accreditation Standard (Version 4.0)

Performance data evaluation documents available on ISTA web site

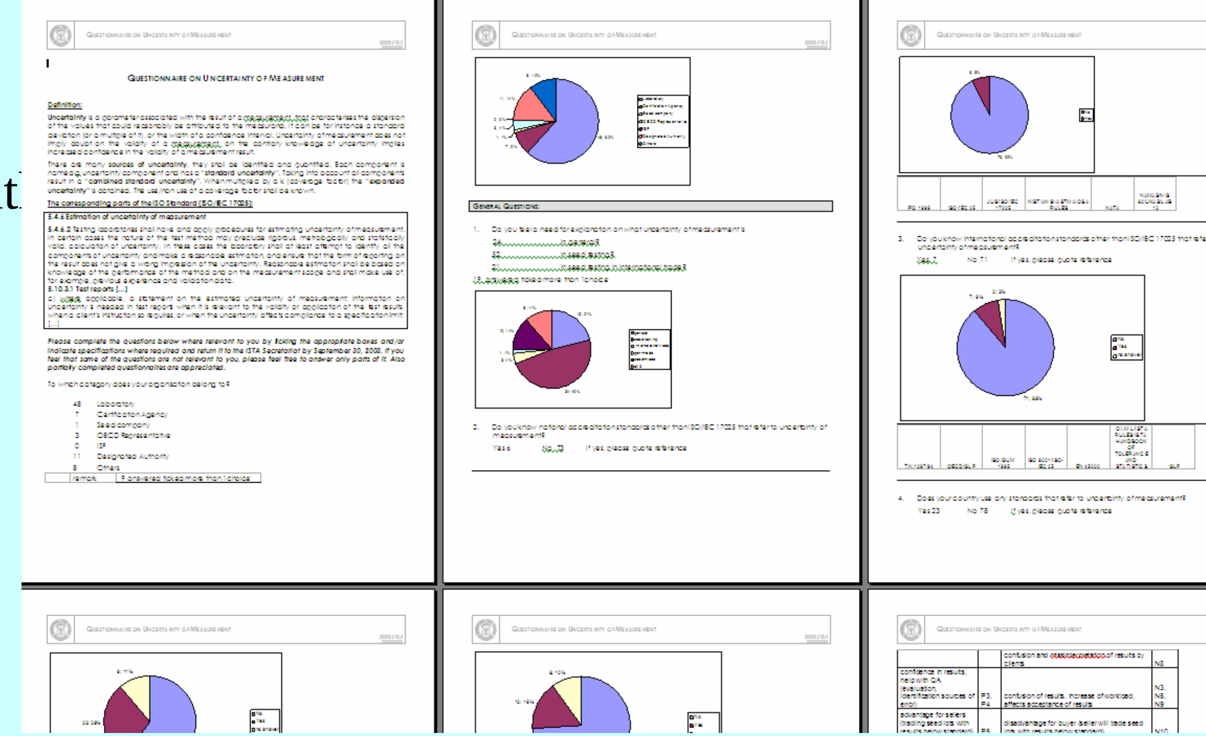




Uncertainty

Upon request by ISTA laboratories, in cunjuction wit ISO 17025 accreditation.

The enquiry made by ISTA on uncertainty was reviewed



INTERNATIONAL SEED TESTING ASSOCIATION
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

Document 09/2007/OM
18.02.2007

Draft ISTA Position Paper on Quantifying and Reporting Uncertainty of Measurement in Seed Testing

This document was prepared by the ISTA Statistics Committee and has been endorsed by the ISTA Executive Committee to be submitted as proposal to the ISTA Ordinary Meeting 2007 for voting by the nominated ISTA Designated Members voting on behalf of their respective Government.

It is submitted to all ISTA Designated Authorities, ISTA Members and ISTA Observer Organisations for information two months prior to the ISTA Ordinary Meeting.

It will be discussed and voted on at the ISTA Ordinary Meeting 2007 to be held on Friday, May 11, 2007 at the Rafain Hotel, Iguaçu Falls, Parana, Brazil under Agenda point 14. Any other business raised by consent of the Executive Committee.

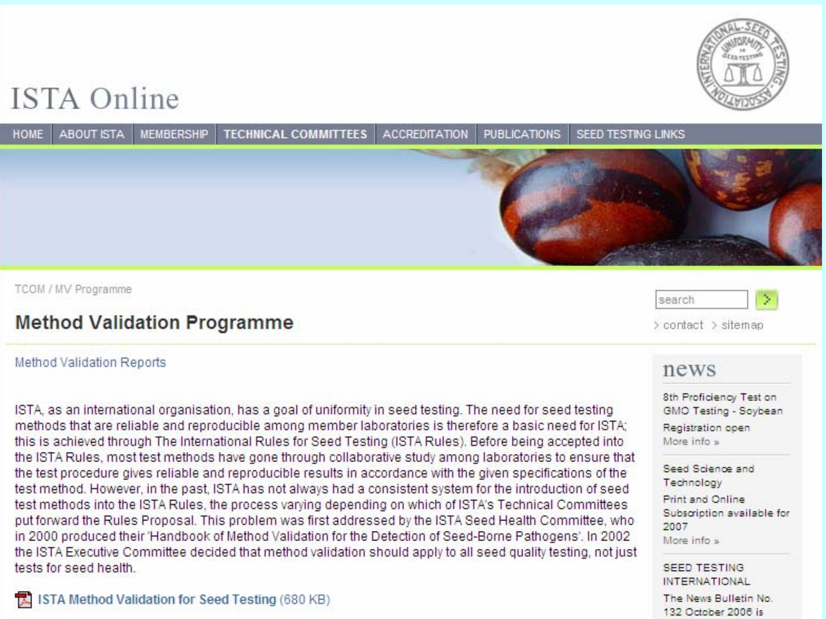
Approved by ECOM: November 29, 2006 Status: FINAL DRAFT Document No. 09/2007/OM
09-2007-OM Draft ISTA Position Paper on Quantifying and Reporting Uncertainty of Measurement in Seed Testing Version 1.0 18.02.2007

ECOM asked STA to establish a position paper on uncertainty in ISTA.

This position paper is presented at ordinary meeting 2007

Method Validation Program

STA has made comments on all MVP versions, from the first dedicated to seed health, to the current version



STA has provided simple statistics annexes for the MVP document and can add/improve in the future

APPENDIX 1: Statistical Aspects of Method Validation
(Note: This appendix is not yet completed)

1.1. Introduction

This appendix contains explanations and example of things that can be addressed in method validation from a statistical perspective. The methods described are simple compared to more sophisticated possibilities, but provide comparable and objective support for validation studies. If a test organizer wishes to use more sophisticated methods, this should be in addition to the simple methods.

Method validation for a test method involves a planned experiment, from which data are obtained. The aim of the experiment is to obtain the objective supporting evidence required to allow validation of the test method.

The method must be developed to the stage that it is ready for routine use with a protocol and identified parameters to control (for instance substrate, temperature and duration for germination).

a) The selection of appropriate parameters must have been done prior to submitting for method validation. If the test leader wishes to show evidence of ruggedness of the method, the simple experiment as described in **1.2 "Ruggedness testing procedure"** can be used.

b) When data are collected and a statistical analysis is performed, support from a statistician is required. See **1.3 "Statistical support to establish and review the test"**.

c) When planning the experiment it is important to consider **(1.4) "The number of samples, the number of repeats, the number of laboratories, and the true value of the samples prepared"**.

d) Among the things that should be considered before the experiment are the differences in results that are acceptable among laboratories, and if a statistical test will be able to show them as significant; this is introduced in **1.5 "Benefits of simulation for a test plan design"**.

e) The choice of statistical analysis will be driven in particular by **"The type of results and distributions"** (see **1.6**).

f) A first step of the statistical analysis is to explore and check the data set; simple graphical representations of data are usually very helpful, and **1.7 "Detection of outliers"** is one of the aspects for which statistical tests can be used.

g) There are a number of possibilities for statistical analysis. **1.8 "Statistical analysis, model and assumptions"** refers to applying a mathematical model to the data set, and **1.8.1 "ANOVA"** and **1.8.2 "GLM"** are given as classical statistical tools.

h) Because tests will be performed on an international basis, **1.9 "Repeatability-reproducibility"** are two important features; they quantify the expected variability of results respectively within a laboratory, and within a group of laboratories.

i) Accuracy, bias and uncertainty are also important features, but are not covered in this appendix.

Approved by ECOM: 30.11.2006 Version: 1.0
ISTA Method Validation for Seed Testing-V1.0 Status: FINAL 26/01/2007

Workshops, seminars

- Argentina, Buenos aeres
- Turkey, Izmir
- Germany, Hohenheim
- USA, Ames
- Brasil, Iguaçu

Workshops Reports 2005

5th ISTA Seed Health Symposium
Angers, France
10.05.2005 - 13.05.2005

7th Seminar on Statistics in Seed Testing
Location: University of Hohenheim, Stuttgart, Germany
Date: 29.08.2005 - 02.09.2005

3rd ISTA Workshop on Statistical Aspects of GMO Detection
Location: Buenos Aires, Argentina
Date: 31.10.2005 - 04.11.2005

ISTA Seminar on Statistics

The 8th ISTA Seminar on Statistics

is going to be held

from 22 to 26 September 2008

at Naktuinbouw in the Netherlands

Tools

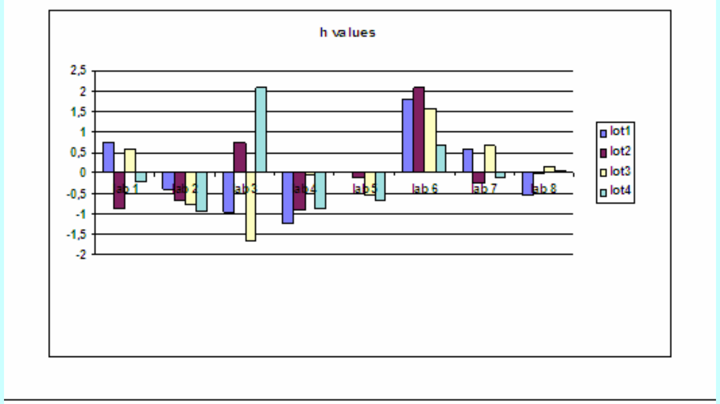
Successive versions of Seedcalc

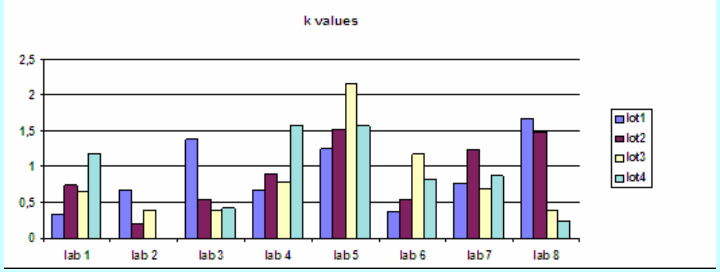
6 -> 7 -> 8 according to actual needs

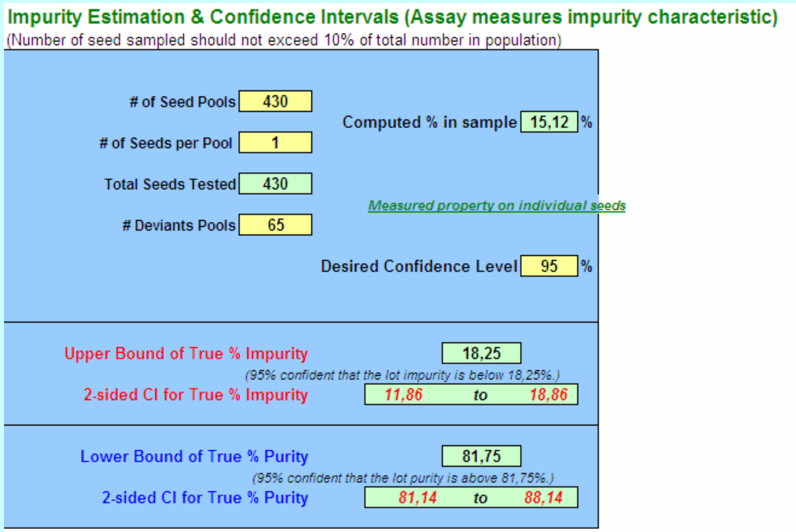
Stat. programs for ISTA secretariat and PT committee (PT evaluation)

For ISTA secretariat and GMO TF (Proficiency test, Performance data evaluation)

Repeatabilty/reproducibilty for binomial data (will complement ISO 5725-2 tool for measured data)







Impurity Estimation & Confidence Intervals (Assay measures impurity characteristic)

(Number of seed sampled should not exceed 10% of total number in population)

# of Seed Pools	430	Computed % in sample	15,12 %
# of Seeds per Pool	1		
Total Seeds Tested	430		
# Deviants Pools	65	Measured property on individual seeds	
		Desired Confidence Level	95 %

Upper Bound of True % Impurity: 18,25

(95% confident that the lot impurity is below 18,25%.)

2-sided CI for True % Impurity: 11,86 to 18,86

Lower Bound of True % Purity: 81,75

(95% confident that the lot purity is above 81,75%.)

2-sided CI for True % Purity: 81,14 to 88,14

seeds tested: 3000

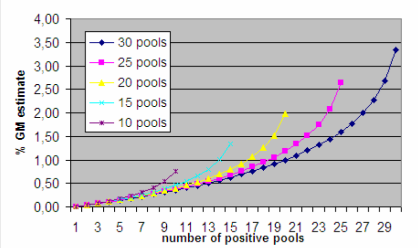
% GM Estimates Matrix

[A useful tool to help practitioners choose pool sizes that give the desired % GM

$$\%\ \text{GM est.} = (1-(1-d/n)^{1/m})\times 100$$

d= number of deviant pools (0 to 30)
n= number of pools (1 to 30)
m= number of seeds per pool (rounded to integer)

# of pools	seeds per pool	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15															
1	3000	0	----																													
2	1500	0	0,05	----																												
3	1000	0	0,04	0,11	----																											
4	750	0	0,04	0,09	0,18	----																										
5	600	0	0,04	0,09	0,15	0,27	----																									
6	500	0	0,04	0,08	0,14	0,22	0,36	----																								
7	428	0	0,04	0,08	0,13	0,20	0,29	0,45	----																							
8	375	0	0,04	0,08	0,13	0,18	0,26	0,37	0,55	----																						
9	333	0	0,04	0,08	0,12	0,18	0,24	0,33	0,45	0,66	----																					
10	300	0	0,04	0,07	0,12	0,17	0,23	0,30	0,40	0,54	0,76	----																				
11	272	0	0,04	0,07	0,12	0,17	0,22	0,29	0,37	0,48	0,62	0,88	----																			
12	250	0	0,03	0,07	0,12	0,16	0,22	0,28	0,35	0,44	0,55	0,71	0,99	----																		
13	230	0	0,03	0,07	0,11	0,16	0,21	0,27	0,34	0,41	0,51	0,64	0,81	1,11	----																	
14	214	0	0,03	0,07	0,11	0,16	0,21	0,26	0,32	0,40	0,48	0,58	0,72	0,91	1,23	----																
15	200	0	0,03	0,07	0,11	0,15	0,20	0,26	0,31	0,38	0,46	0,55	0,66	0,80	1,00	1,34	----															
16	187	0	0,03	0,07	0,11	0,15	0,20	0,25	0,31	0,37	0,44	0,52	0,62	0,74	0,89	1,11	1,47	----														
17	176	0	0,03	0,07	0,11	0,15	0,20	0,25	0,30	0,36	0,43	0,50	0,59	0,69	0,82	0,98	1,21	1,60	----													
18	166	0	0,03	0,07	0,11	0,15	0,20	0,24	0,30	0,35	0,42	0,49	0,57	0,66	0,77	0,90	1,07	1,31	1,73	----												
19	157	0	0,03	0,07	0,11	0,15	0,19	0,24	0,29	0,35	0,41	0,47	0,55	0,63	0,73	0,85	0,99	1,17	1,42	1,86	----											
20	150	0	0,03	0,07	0,11	0,15	0,19	0,24	0,29	0,34	0,40	0,46	0,53	0,61	0,70	0,80	0,92	1,07	1,26	1,52	1,98	----										
21	142	0	0,03	0,07	0,11	0,15	0,19	0,24	0,29	0,34	0,39	0,45	0,52	0,59	0,68	0,77	0,88	1,01	1,16	1,36	1,64	2,12	----									
22	136	0	0,03	0,07	0,11	0,15	0,19	0,23	0,28	0,33	0,39	0,44	0,51	0,58	0,66	0,74	0,84	0,95	1,08	1,25	1,45	1,75	2,25	----								
23	130	0	0,03	0,07	0,11	0,15	0,19	0,23	0,28	0,33	0,38	0,44	0,50	0,57	0,64	0,72	0,81	0,91	1,03	1,17	1,34	1,55	1,86	2,38	----							
24	125	0	0,03	0,07	0,11	0,15	0,19	0,23	0,28	0,32	0,38	0,43	0,49	0,55	0,62	0,70	0,78	0,88	0,98	1,10	1,25	1,42	1,65	1,97	2,51	----						
25	120	0	0,03	0,07	0,11	0,15	0,19	0,23	0,27	0,32	0,37	0,42	0,48	0,54	0,61	0,68	0,76	0,85	0,95	1,06	1,18	1,33	1,52	1,75	2,08	2,65	----					
26	115	0	0,03	0,07	0,11	0,15	0,19	0,23	0,27	0,32	0,37	0,42	0,48	0,54	0,60	0,67	0,75	0,83	0,92	1,02	1,13	1,27	1,42	1,61	1,86	2,21	2,79	----				
27	111	0	0,03	0,07	0,11	0,14	0,18	0,23	0,27	0,32	0,36	0,42	0,47	0,53	0,59	0,66	0,73	0,81	0,89	0,98	1,09	1,21	1,35	1,51	1,71	1,96	2,32	2,93	----			
28	107	0	0,03	0,07	0,11	0,14	0,18	0,23	0,27	0,31	0,36	0,41	0,47	0,52	0,58	0,65	0,71	0,79	0,87	0,96	1,06	1,16	1,29	1,43	1,60	1,80	2,07	2,44	3,07	----		
29	103	0	0,03	0,07	0,11	0,14	0,18	0,22	0,27	0,31	0,36	0,41	0,46	0,52	0,58	0,64	0,70	0,78	0,85	0,94	1,03	1,13	1,24	1,37	1,52	1,69	1,90	2,18	2,56	3,22	----	
30	100	0	0,03	0,07	0,11	0,14	0,18	0,22	0,27	0,31	0,36	0,40	0,46	0,51	0,57	0,63	0,69	0,76	0,83	0,91	1,00	1,09	1,20	1,31	1,44	1,60	1,78	1,99	2,28	2,67	3,34	----
n	m	0	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



Enter the total number or seeds to be tested and then the % GM estimates are calculated in the matrix for all possible combinations of d, n and m. Note: This matrix of estimates is appropriate for routine tests. This matrix is a good approximation of proficency test sample estimates when the proficency test samples are large (e.g., 3000 kernels).

Suggested terms of reference 2007-2010

Tolerance tables: STA committee suggests to work with ISTA secretariat and Technical committees to define a process which will allow to check on a regular basis if tolerance tables are still appropriate to the current situation in laboratories. The way to obtain tolerance tables would also be formally (re)described.

Table 3.2 Tolerances for purity tests on two different submitted samples from the same lot when a second test is made in the same or a different laboratory (one-way test at 1% significance level)

This table gives the tolerances for purity results made on two different submitted samples each drawn from the same lot and analysed in the same or a different laboratory. It can be used for any component of a purity test when the result of the second test is poorer than that of the first test. The table is used by entering it at the average of the two test results (columns 1 or 2). The appropriate tolerance is found in columns 3 or 4, determined as to whether the seeds are chaffy or non-chaffy.

The tolerances in columns 3 and 4 are extracted from columns D and G respectively of Table P1 in Miles (1963).

Average of the two test results		Tolerance	
50-100%	Less than 50%	Non chaffy seeds	Chaffy seeds
1	2	3	4
99.95-100.00	0.00- 0.04	0.2	0.2
99.90- 99.94	0.05- 0.09	0.3	0.3
99.85- 99.89	0.10- 0.14	0.3	0.4
99.80- 99.84	0.15- 0.19	0.4	0.5
99.75- 99.79	0.20- 0.24	0.4	0.5
99.70- 99.74	0.25- 0.29	0.5	0.6
99.65- 99.69	0.30- 0.34	0.5	0.6
99.60- 99.64	0.35- 0.39	0.6	0.7

1963

Table 15.2 Tolerances for two conductivity tests on the same submitted sample when tests are made in the same laboratory (two-way test at 5 % significance level).

This table indicates the maximum difference in conductivity readings that is tolerable between tests completed on the same sample in the same laboratory. To determine if the two tests are compatible, calculate the average of the two test results and locate this in columns 1 or 2 of the table. The tests are compatible if the difference between the conductivity readings in the two tests does not exceed the tolerance given in column 3.

The tolerances take into account the experimental error between laboratories participating in comparative tests completed by the Vigour Committee 1998-2001.

Average conductivity ($\mu S\ cm^{-1}\ g^{-1}$)		Maximum range ($\mu S\ cm^{-1}\ g^{-1}$)	Average conductivity ($\mu S\ cm^{-1}\ g^{-1}$)		Maximum range ($\mu S\ cm^{-1}\ g^{-1}$)
from	to		from	to	
1	2	3	1	2	3
10	10.9	2.0	32	32.9	5.1
11	11.9	2.1	33	33.9	5.2
12	12.9	2.3	34	34.9	5.4
13	13.9	2.4	35	35.9	5.5
14	14.9	2.5	36	36.9	5.6
15	15.9	2.7	37	37.9	5.8
16	16.9	2.8	38	38.9	5.9
17	17.9	3.0	39	39.9	6.1

2001

Table 9.1 Tolerance levels for differences between the two duplicate determinations of moisture content of tree and shrub seeds (significance level not defined)

This table gives the maximum tolerated differences between the results of the two determinations. The table is used by entering it in column 2, 3, or 4 according to the average initial moisture content of the sample. The tolerated difference for the relevant seed size class (column 1) is selected.

The tolerances are from Table 3 in F.T. Bonner: Tolerance limits in measurement of tree seed moisture. Seed Science & Technology **12**, 789-794, 1984.

Seed size class	Average initial moisture content		
	<12%	12 to 25%	>25%
1	2	3	4
Small seeds*	0.3%	0.5%	0.5%
Large seeds	0.4%	0.8%	2.5%

1984

Suggested terms of reference 2007-2010

Proficiency tests: STA suggests continuing improvements on the statistical evaluation of existing proficiency tests, and help to install proficiency tests in areas where none are in place.

- Bulking and Sampling Committee > more
- Flower Seed Testing Committee > more
- Forest Tree and Shrub Seed Committee > more
- Germination Committee > more
- Moisture Committee > more
- Nomenclature Committee > more
- Proficiency Test Committee > more ←
- Purity Committee > more
- Rules Committee > more
- Seed Health Committee > more
- Statistics Committee > more
- Seed Storage Committee > more
- Tetrazolium Committee > more
- Variety Committee > more
- Vigour Committee > more
- GMO Task Force > more ←
- ← ISTA Secretariat

Arrows indicate where most of the work of STA occured in the past 3 years

STA is available where it's help is needed, could be Seed Health and other committees in next period

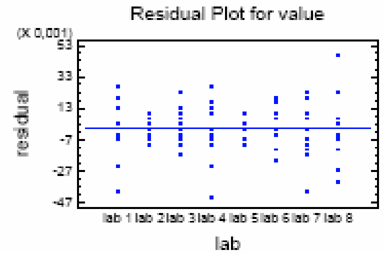
<u>Method validation program</u>:

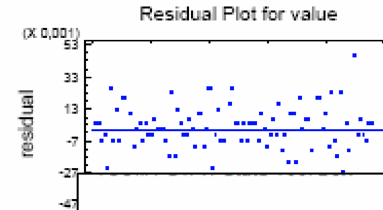
STA suggests reviewing the MVP document and improving it where necessary on statistics parts. STA suggest to help in defining per type of test, with each technical committee, how to implement method validation program in an efficient way with respect to defining testing plans and evaluating data.

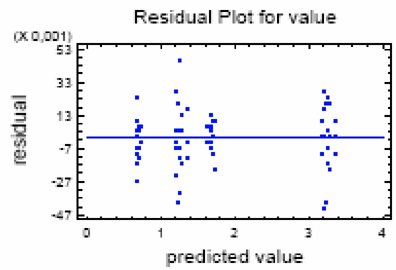
We selected simple features to help,

STA can do more,

Use only what is needed on the specific situation







Sometimes transformation of data can be sugge

There is no free tool to compute ANOVA on statistical package has this capability.

1.8.2 GLM

...to be inserted in the future

1.9 Repeatability-reproducibility according to ISO5725-2 (quantitative data)

a) Introduction

When the results of the tests are appropriate ISO 5725-2 is recommended to compute repeatability and reproducibility.

Repeatability quantifies the average variability of results within each laboratory, when repeats are made on samples from a given lot. Reproducibility is repeatability, increased by the variability of results from laboratory to laboratory.

Statistical Tools for Seed

A collection of statistical programs and r development and validation.

These programs were made for educati ISTA and the authors shall not be liable

Inter laboratory tests using ISO 5725

The Zip file contains a Access97® and a file, an example of file containing data in on the data from an experiment 3 types can be done. Descriptive statistics and described in ISO 5725-2 are computed, different labs test lots of seeds with the available for each lot in each laboratory. actions can cause the computations to b

Download ISO 5725 (823 KB)

Some topics of special interest, in cases where statistical computations will be perfomed

a) The selection of appropriate parameters must have been done prior to submitting for method validation. If the test leader wishes to show evidence of ruggedness of the method, the simple experiment as described in **1.2 "Ruggedness testing procedure"** can be used.

b) When data are collected and a statistical analysis is performed, support from a statistician is required. See **1.3 "Statistical support to establish and review the test"**.

c) When planning the experiment it is important to consider **(1.4) "The number of samples, the number of repeats, the number of laboratories, and the true value of the samples prepared"**.

d) Among the things that should be considered before the experiment are the differences in results that are acceptable among laboratories, and if a statistical test will be able to show them as significant; this is introduced in **1.5 "Benefits of simulation for a test plan design"**.

e) The choice of statistical analysis will be driven in particular by **"The type of results and distributions"** (see **1.6**).

f) A first step of the statistical analysis is to explore and check the data set; simple graphical representations of data are usually very helpful, and **1.7 "Detection of outliers"** is one of the aspects for which statistical tests can be used.

g) There are a number of possibilities for statistical analysis. **1.8 "Statistical analysis, model and assumptions"** refers to applying a mathematical model to the data set, and **1.8.1 "ANOVA"** and **1.8.2 "GLM"** are given as classical statistical tools.

h) Because tests will be performed on an international basis, **1.9 "Repeatability-reproducibility"** are two important features; they quantify the expected variability of results respectively within a laboratory, and within a group of laboratories.

i) Accuracy, bias and uncertainty are also important features, but are not covered in this appendix.

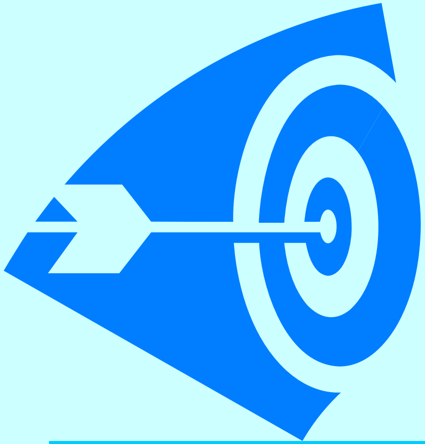
STA can also help to confirm :

no statistical computation

is required

Help to technical committees and ISTA secretariat : STA suggests to continue supporting technical committees, upon their request, on any matter where technical committees think STA can help.

Education: STA suggests to continue to organise workshops and seminars on statistics. The aim is to be fitted for actual needs on one hand, and to be understandable to people willing to apply the concepts and tools suggested by STA on the other hand. STA also suggests to continue to develop and to publish on the ISTA website free statistical software.



Target actual needs

STA committee Internal work

Anticipation of demands or needs

<=>be ready when question is decided as of importance, and

decided to be dealt within ISTA

- Stacked genes
- Uncertainty
- Tolerances
- Repeatability with non normal data
- Test design
- GLM
- R
- Bayesian
- …

$$P(A|B) = \frac{P(A) \times P(B|A)}{P(B)}$$

and thus in Seedcalc: Seedcalc8