ISTA Statistics Committee 2025-2026 Activity Report

Kirk Remund & Jean-Louis Laffont

ISTA Statistics Committee

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ISTA ECOM Liaison Officers: Vanessa Sosa & Ruel Gesmundo

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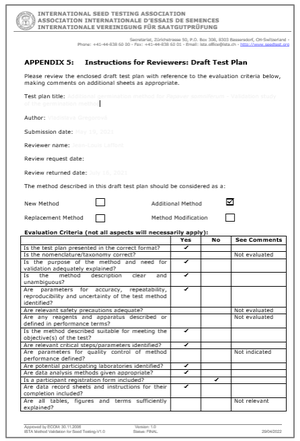
ISTA Statistics Committee Activities

- Testing plan and method validation report reviews
- ISTA rules proposals
- Statistical analysis & simulation
- Seed Science & Technology reviews
- Theoretical contributions
- Seed testing tools development
- ISTA & industry workshops
- ISTA & industry collaborations
- ISTA tech. committees and member questions
- Develop next generation (Young@ISTA)



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

APPENDIX 5: Instructions for Reviewers: Draft Test Plan

Please review the enclosed draft test plan with reference to the evaluation criteria below, making comments on additional sheets as appropriate.

Test plan title:

Author:

Submission date:

Reviewer name:

Review request date:

Review returned date:

The method described in this draft test plan should be considered as a:

New Method ☐ Additional Method ☑

Replacement Method ☐ Method Modification ☐

Evaluation Criteria (not all aspects will necessarily apply):

	Yes	No	See Comments
Is the test plan presented in the correct format?	✓		
Is the nomenclature/taxonomy correct?			Not evaluated
Is the purpose of the method and need for validation adequately explained?	✓		
Is the method description clear and unambiguous?	✓		
Are parameters for accuracy, repeatability, reproducibility and uncertainty of the test method identified?	✓		
Are relevant safety precautions adequate?			Not evaluated
Are any reagents and apparatus described or defined in performance terms?			Not evaluated
Is the method described suitable for meeting the objective(s) of the test?	✓		
Are relevant critical steps/parameters identified?	✓		
Are parameters for quality control of method performance defined?			Not indicated
Are potential participating laboratories identified?	✓		
Are data analysis methods given appropriate?	✓		
Is a participant registration form included?		✓	
Are data record sheets and instructions for their completion included?	✓		
Are all tables, figures and terms sufficiently explained?			Not relevant

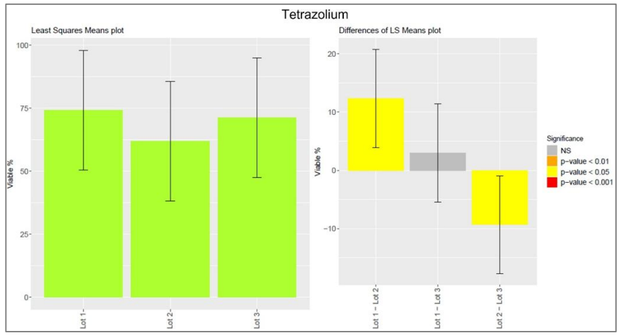
Approved by ECOM 30.11.2008 Version: 1.0
ISTA Method Validation for Seed Testing-V1.0 Status: FINAL 20Oct2022

```
Mean s_repeatability disp s_Reproducibility s_Lab s_LotxLab
  69              6.28 0.96                12.19 10.26     1.98
```

```
ANOVA Table of type III with Satterthwaite approximation for degrees of freedom:
-------------------------------------------------------------------------------
 Source of variation Sum of Squares Mean Square Num DF Den DF  F value     Pr(>F)
         Lot            710.1356     355.0678      2      4 9.008063 0.03300944

Least Squares Means Table:
-------------------------
      Estimate Std. Error    lower    upper
Lot 1 74.25000   6.299437 50.59267 97.90733
Lot 2 61.91667   6.299437 38.25933 85.57400
Lot 3 71.25000   6.299437 47.59267 94.90733

Differences of Least Squares Means Table:
----------------------------------------
           Estimate Std. Error     lower     upper
Lot 1 - Lot 2 12.333333   3.030707  3.918741 20.7479255
Lot 1 - Lot 3  3.000000   3.030707 -5.414592 11.4145922
Lot 2 - Lot 3 -9.333333   3.030707 -17.747926 -0.9187411
```



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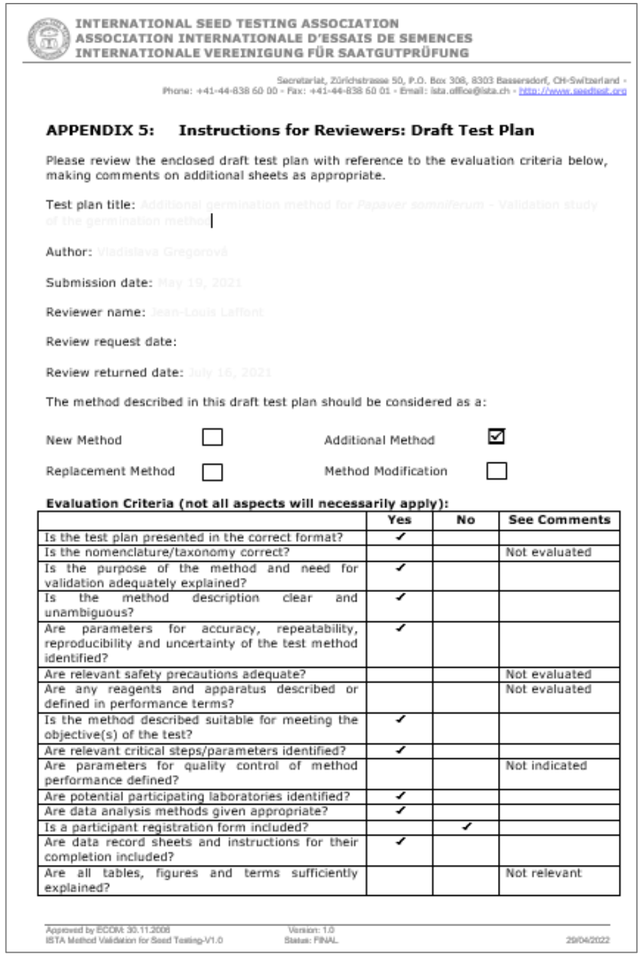
Support of TCOMs – Report Reviews

- **6 test plan** reviews
- **8 validation study** reviews
- **4 validation study** analyses
- **GM PT 24**: quantitative rating computations



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

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APPENDIX 5: Instructions for Reviewers: Draft Test Plan

Please review the enclosed draft test plan with reference to the evaluation criteria below, making comments on additional sheets as appropriate.

Test plan title: Additional germination method for *Papaver somniferum* - Validation study of the germination method

Author: Vladislava Gregorová

Submission date: May 19, 2021

Reviewer name: Jean-Louis Laffont

Review request date:

Review returned date: July 16, 2021

The method described in this draft test plan should be considered as a:

New Method ☐ Additional Method ☑

Replacement Method ☐ Method Modification ☐

Evaluation Criteria (not all aspects will necessarily apply):

	Yes	No	See Comments
Is the test plan presented in the correct format?	✓		
Is the nomenclature/taxonomy correct?			Not evaluated
Is the purpose of the method and need for validation adequately explained?	✓		
Is the method description clear and unambiguous?	✓		
Are parameters for accuracy, repeatability, reproducibility and uncertainty of the test method identified?	✓		
Are relevant safety precautions adequate?			Not evaluated
Are any reagents and apparatus described or defined in performance terms?			Not evaluated
Is the method described suitable for meeting the objective(s) of the test?	✓		
Are relevant critical steps/parameters identified?	✓		
Are parameters for quality control of method performance defined?			Not indicated
Are potential participating laboratories identified?	✓		
Are data analysis methods given appropriate?	✓		
Is a participant registration form included?		✓	
Are data record sheets and instructions for their completion included?	✓		
Are all tables, figures and terms sufficiently explained?			Not relevant

Support of TCOMS – Consulting & Questions

- 21 questions answered from TCOMs, Labs, and others (Young@ISTA committee members are helping)
- Some questions have required significant time to answer (days to weeks)
- A few questions received where not answered (we cannot answer everything)
- Examples of question areas

  sampling intensities, seed disease transmission, seed lot heterogeneity calculation, maximum group size in GMO testing, method validation, tolerances, image analysis

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Sampling of 2025-2026 Efforts

- SANSOR/ISTA Webinar on Statistics – Fall 2025

- Support for a Rules change submitted by the GER Committee: abandonment of the "100-Seed Grouping Rule" and derivation of new tolerance tables
- Sample size for the heterogeneity test applied in the preparation of PUR, GER, TZ and SH Proficiency Tests
- Actively engaged in revising the 66-page Method Validation document, including reviewer forms, seed health guidelines (in collaboration with ISHI), sample preparation, and analysis
- Development of a Bayesian predictive framework for the design and evaluation of GM proficiency test samples
- Development of a new ISTA paradigm integrating biological (seed physiological quality) variability into tolerance tables and heterogeneity tests for physiologically heterogeneous species (e.g., tree and wild species), using a Beta–Binomial framework grounded in de Finetti's representation theorem.

  Come to Statistics Committee open session and learn more about modeling biological variation!
- Increased participation of Young@ISTA members in committee efforts
- Planning for ISTA Statistics Workshop at 2027 ISTA meeting in Angers, France!
  - Venue: GEVES
  - Multiple topics including method validation

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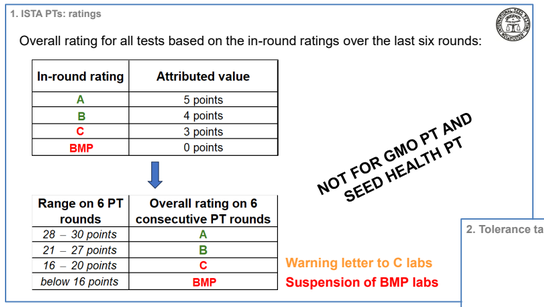
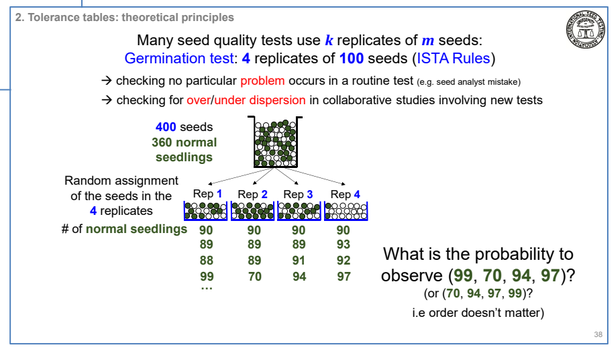
SANSOR/ISTA Workshop of Statistics – Fall 2025





1. ISTA Proficiency Tests (PTs)

- Overview of the ISTA Accreditation Programme
- Rating calculations for the standard PTs
- Examples: Germination and Purity
- Other tests: brief overview

2. Tolerance Tables

- Rationale
- Theoretical principles underlying the construction of tolerances
- Tolerance tables for germination and tetrazolium tests
- Other tolerance tables: brief overview

More than 100 attendees, including SANSOR seed analysts as well as international participants from ISTA and the wider seed testing community.

Due to popular demand ISTA Statistics Committee will have another **ISTA Webinar with similar content in Fall 2026 with Young@ISTA members as presenters**.

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Support for a Rules change submitted by the GER Committee

CURRENT VERSION	PROPOSED VERSION
5.11 Tolerance tables	**5.11 Tolerance tables**
Table 5B gives the maximum tolerated differences between the highest and lowest germination percentages of the replicates of a germination test, allowing only for random sampling variation at a probability of 0.025. ~~To determine whether a test is reliable, calculate the average germination percentage over all replicates, to the nearest whole number. If necessary, in tests of 400 or 200 seeds, four or two replicates, respectively, of 100 seeds each can be formed by combining the subreplicates of 50 or 25 seeds which were closest together in the germinator. In tests of 100 seeds, two replicates of 50 seeds each can be formed by combining the subreplicates of 25 seeds which were closest together in the germinator, and multiplying the results of each of the two replicates by 2 to obtain an average germination percentage.~~ ….	Table 5B gives the maximum tolerated differences between the highest and lowest germination percentages of the replicates of a germination test, allowing only for random sampling variation at a probability of 0.025. To determine whether a test is reliable, calculate the average germination percentage over all replicates, to the nearest whole number. ….

Problem:
grouping the reps into pairs of 100 seeds or 50 seeds can lead to combinations of seedlings that may obscure the reading issue in one or more reps of 50 or 25 seeds

➡ Creation of new tables for tests with 4 reps of 50 seeds, 8 reps of 50 seeds, 4 reps of 25 seeds, 8 reps of 25 seeds, and 16 reps of 25 seeds

Table 5B Part 4. Four replicates of 50 seeds

Average germination percentage of test		Tolerance
51-100%	0-50%	
99	2	7
98	3	8
97	4	10
96	5	11
95	6	13
94	7	14
92-93	8-9	15
91	10	16
90	11	17
88-89	12-13	18
87	14	19
85-86	15-16	20
83-84	17-18	21
80-82	19-21	22
78-79	22-23	23
75-77	24-26	24
71-74	27-30	25
66-70	31-35	26
59-65	36-42	27
51-58	43-50	28

Table 5B Part 5. Eight replicates of 50 seeds

Average germination percentage of test		Tolerance
51-100%	0-50%	
99	2	8
98	3	10
97	4	12
96	5	13
95	6	15
94	7	16
93	8	17
92	9	18
91	10	19
89-90	11-12	20
88	13	21
87	14	22
85-86	15-16	23
83-84	17-18	24
81-82	19-20	25
79-80	21-22	26
77-78	23-24	27
74-76	25-27	28
71-73	28-30	29
67-70	31-34	30
62-66	35-39	31
51-61	40-50	32

Table 5B Part 6. Four replicates of 25 seeds

Average germination percentage of test		Toleran
51-100%	0-50%	
99	2	9
98	3	12
97	4	14
96	5	16
95	6	18
94	7	19
93	8	21
92	9	22
91	10	23
90	11	24
89	12	25
88	13	26
87	14	27
86	15	28
84-85	16-17	29
83	18	30
81-82	19-20	31
79-80	21-22	32
77-78	23-24	33
75-76	25-26	34
73-74	27-28	35
70-72	29-31	36
67-69	32-34	37
62-66	35-39	38
52-61	40-49	39
51	50	40

Table 5B Part 7. Eight replicates of 25 seeds

Average germination percentage of test		Tolerance
51-100%	0-50%	
99	2	11
98	3	14
97	4	17
96	5	19
95	6	21
94	7	22
93	8	24
92	9	25
91	10	27
90	11	28
89	12	29
88	13	30
87	14	31
86	15	32
85	16	33
84	17	34
82-83	18-19	35
81	20	36
80	21	37
78-79	22-23	38
76-77	24-25	39
74-75	26-27	40
72-73	28-29	41
69-71	30-32	42
66-68	33-35	43
62-65	36-39	44
56-61	40-45	45
51-55	46-50	46

Table 5B Part 8. Sixteen replicates of 25 seeds

Average germination percentage of test		Tolerance
51-100%	0-50%	
99	2	12
98	3	16
97	4	19
96	5	21
95	6	23
94	7	25
93	8	27
92	9	28
91	10	30
90	11	31
89	12	33
88	13	34
87	14	35
86	15	36
85	16	37
84	17	38
83	18	39
81-82	19-20	40
80	21	41
79	22	42
77-78	23-24	43
76	25	44
74-75	26-27	45
72-73	28-29	46
69-71	30-32	47
67-68	33-34	48
63-66	35-38	49
59-62	39-42	50
51-58	43-50	51

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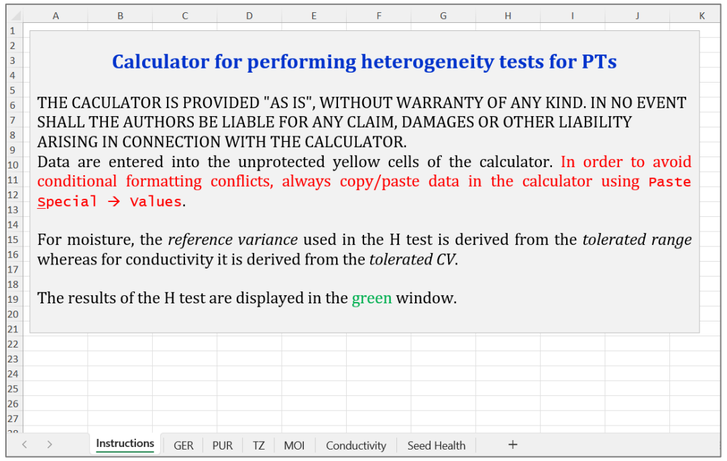
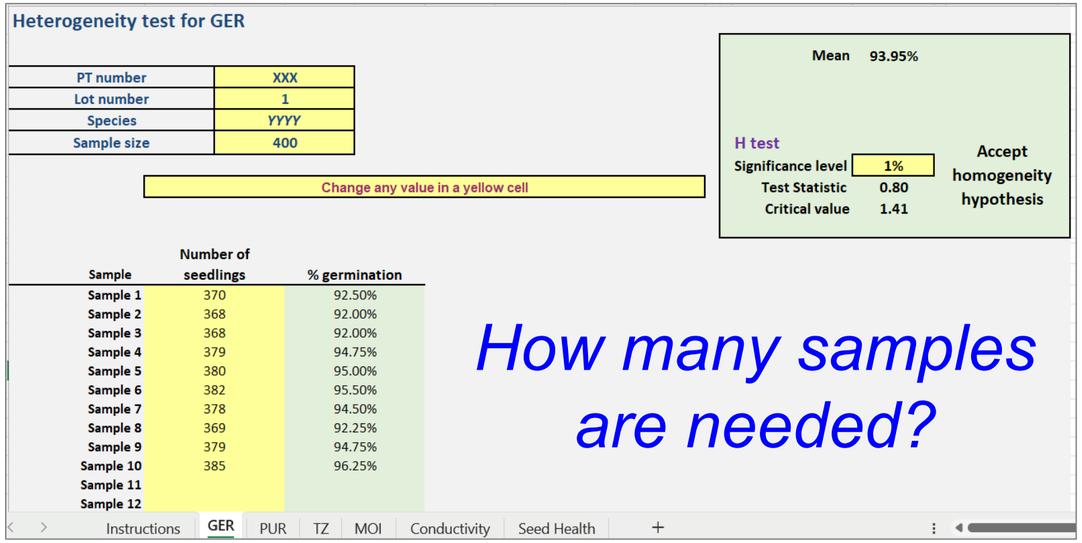
Sample size for the heterogeneity test

New calculator released in December 2025 for the heterogeneity test applied in the preparation of PUR, GER, TZ, MOI, Conductivity and SH Proficiency Tests



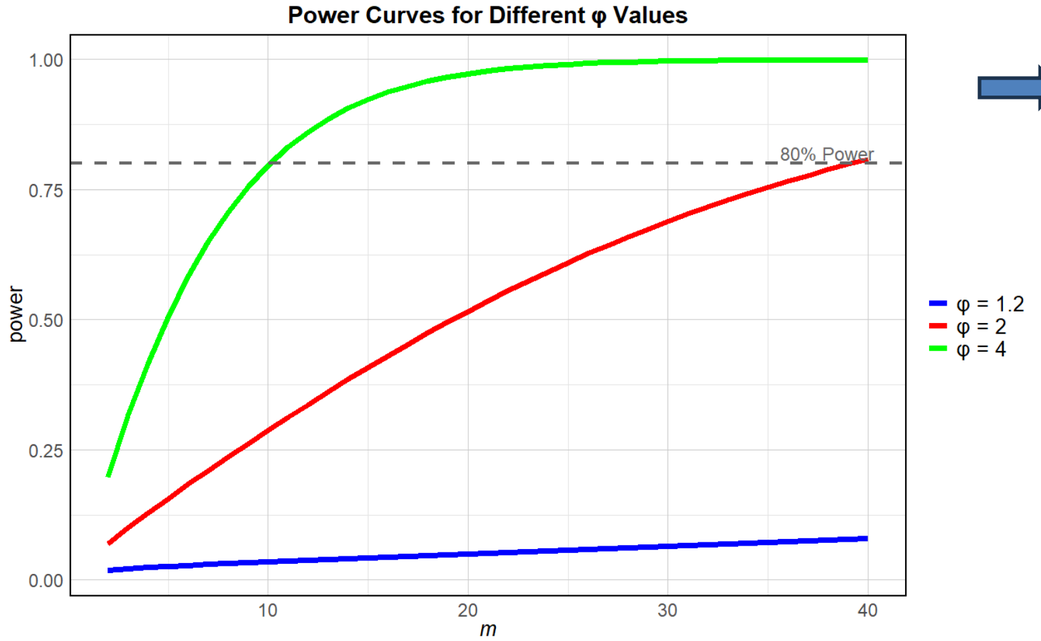


How many samples are needed?

➡ Power analysis

$$\text{power} = 1 - F_{\chi^2_{m-1}}\left(\frac{\chi^2_{0.99,m-1}}{\varphi}\right)$$

where m is the number of samples,
$F_{\chi^2_{m-1}}(x)$ is the cdf of a chi-square distribution with $m-1$ df,
φ is the variance inflation factor



➡ 10 samples are sufficient to detect strong heterogeneity (power ≈ 80%),

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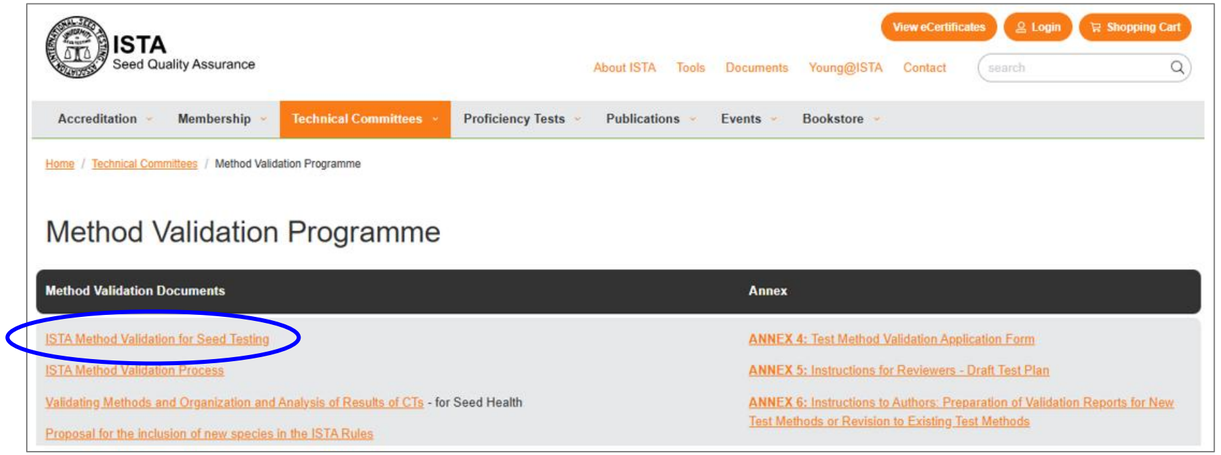
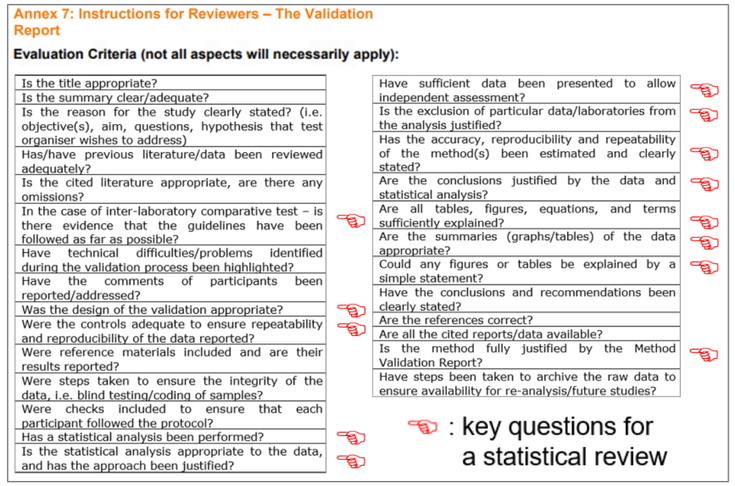
66-page Method Validation document revision: identification of areas for revision



Guidelines for sample preparation are lacking, particularly for verifying the homogeneity of the lots used to prepare samples (except in SH, where such guidelines already exist)

STA reviewer forms requires revision



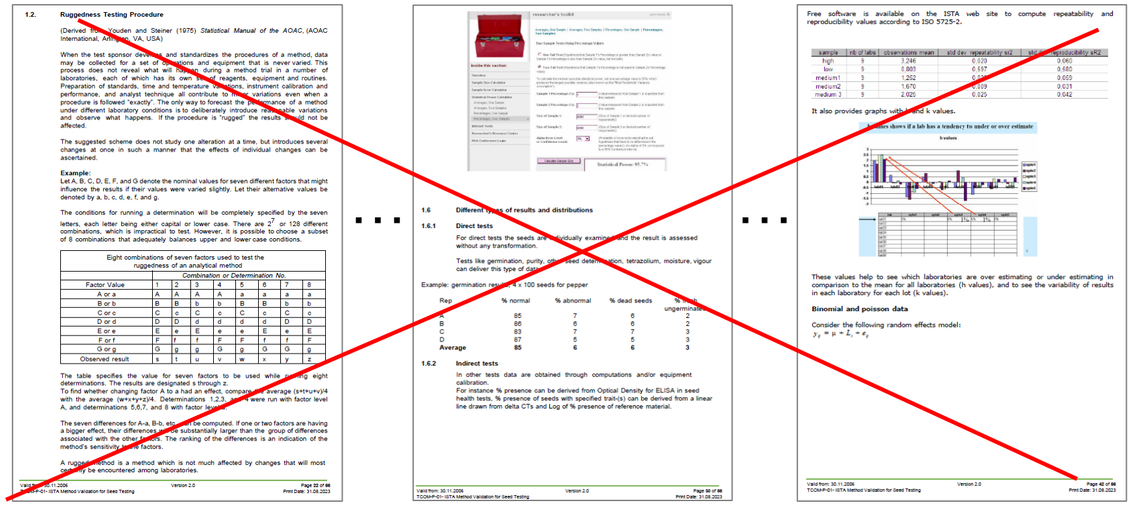
Annex 7: Instructions for Reviewers – The Validation Report

Evaluation Criteria (not all aspects will necessarily apply):

Is the title appropriate?	Have sufficient data been presented to allow independent assessment?
Is the summary clear/adequate?	Is the exclusion of particular data/laboratories from the analysis justified?
Is the reason for the study clearly stated? (i.e. objective(s), aim, questions, hypothesis that test organiser wishes to address)	Has the accuracy, reproducibility and repeatability of the method(s) been estimated and clearly stated?
Has/have previous literature/data been reviewed adequately?	Are the conclusions justified by the data and statistical analysis?
Is the cited literature appropriate, are there any omissions?	Are all tables, figures, equations, and terms sufficiently explained?
In the case of inter-laboratory comparative test – is there evidence that the guidelines have been followed as far as possible?	Are the summaries (graphs/tables) of the data appropriate?
Have technical difficulties/problems identified during the validation process been highlighted?	Could any figures or tables be explained by a simple statement?
Have the comments of participants been reported/addressed?	Have the conclusions and recommendations been clearly stated?
Was the design of the validation appropriate?	Are the references correct?
Were the controls adequate to ensure repeatability and reproducibility of the data reported?	Are all the cited reports/data available?
Were reference materials included and are their results reported?	Is the method fully justified by the Method Validation Report?
Were steps taken to ensure the integrity of the data, i.e. blind testing/coding of samples?	Have steps been taken to archive the raw data to ensure availability for re-analysis/future studies?
Were checks included to ensure that each participant followed the protocol?	
Has a statistical analysis been performed?	
Is the statistical analysis appropriate to the data, and has the approach been justified?	

: key questions for a statistical review

Annex 1, *Statistical Aspects of Method Validation* (23 pages) requires a complete revision



1.2. **Ruggedness Testing Procedure**

(Derived from Youden and Steiner (1975) *Statistical Manual of the AOAC*, (AOAC International, Arlington, VA, USA))

When the test sponsor documents and standardizes the procedures of a method, data may be collected for a set of conditions and equipment that is never varied. This process does not reveal what will happen during a method trial in a number of laboratories, each of which has its own brand of reagents, equipment and routines. Preparation of standards, time and temperature variations, instrument calibration and performance, and analyst technique all contribute to these variations even when a procedure is followed "exactly". The only way to forecast the performance of a method under different laboratory conditions is to deliberately introduce reasonable variations and observe what happens. If the procedure is "rugged" the results should not be affected.

The suggested scheme does not study one alteration at a time, but introduces several changes at once in such a manner that the effects of individual changes can be ascertained.

Example:
Let A, B, C, D, E, F, and G denote the nominal values for seven different factors that might influence the results if their values were varied slightly. Let their alternative values be denoted by a, b, c, d, e, f, and g.

The conditions for running a determination will be completely specified by the seven letters, each letter being either capital or lower case. There are 2^7 or 128 different combinations, which is impractical to test. However, it is possible to choose a subset of 8 combinations that adequately balances upper and lower case conditions.

Eight combinations of seven factors used to test the ruggedness of an analytical method

	Combination or Determination No.							
Factor Value	1	2	3	4	5	6	7	8
A or a	A	A	A	A	a	a	a	a
B or b	B	B	b	b	B	B	b	b
C or c	C	c	C	c	C	c	C	c
D or d	D	D	d	d	d	d	D	D
E or e	E	e	E	e	E	e	E	e
F or f	F	f	f	F	F	f	f	F
G or g	G	g	g	G	g	G	G	g
Observed result	s	t	u	v	w	x	y	z

The table specifies the value for seven factors to be used while running eight determinations. The results are designated s through z.
To find whether changing factor A to a had an effect, compare the average (s+t+u+v)/4 with the average (w+x+y+z)/4. Determinations 1,2,3, and 4 were run with factor level A, and determinations 5,6,7, and 8 with factor level a.

The seven differences for A-a, B-b, etc., can be computed. If one or two factors are having a bigger effect, their differences will be substantially larger than the group of differences associated with the other factors. The ranking of the differences is an indication of the method's sensitivity to the factors.

A rugged method is a method which is not much affected by changes that will most certainly be encountered among laboratories.

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1.6 **Different types of results and distributions**

1.6.1 **Direct tests**

For direct tests the seeds are individually examined and the result is assessed without any transformation.

Tests like germination, purity, other seed determination, tetrazolium, moisture, vigour can deliver this type of data.

Example: germination results of 4 x 100 seeds for pepper

Rep	% normal	% abnormal	% dead seeds	% fresh ungerminated
A	85	7	6	2
B	86	6	6	2
C	83	7	7	3
D	87	5	5	3
Average	**85**	**6**	**6**	**3**

1.6.2 **Indirect tests**

In other tests data are obtained through computations and/or equipment calibration.
For instance % presence can be derived from Optical Density for ELISA in seed health tests, % presence of seeds with specified trait(s) can be derived from a linear line drawn from delta CTs and Log of % presence of reference material.

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Free software is available on the ISTA web site to compute repeatability and reproducibility values according to ISO 5725-2.

sample	nb of labs	observations mean	std dev. repeatability sr2	std dev. reproducibility sR2
high	9	3.246	0.030	0.060
low	9	0.883	0.157	0.582
medium1	9	1.202	0.049	0.669
medium2	9	1.670	0.109	0.021
medium 3	9	2.025	0.329	0.642

It also provides graphs with blend k values.

These values help to see which laboratories are over estimating or under estimating in comparison to the mean for all laboratories (h values), and to see the variability of results in each laboratory for each lot (k values).

Binomial and poisson data

Consider the following random effects model:

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Bayesian predictive framework for the design of GM PT

Given:

- A **GM lot** has been characterized through individual seed testing:
  - Number of seeds tested: $n = 141$
  - Number of GM seeds out of n: $y = 139$
- The **conventional lot** has previously been qualified as having a purity of at least 99.99% with 95% confidence

1. **What is the probability of a positive detection for a positive sample?**
2. **What is the uncertainty associated with the actual GM concentration in PT samples?**

Development of a new Bayesian framework to answer these questions

1. $P(X \geq 1) = 1 - \frac{B(y+1, m+n-y+1)}{B(y+1, n-y+1)}$

Number of seeds taken from the GM lot	Probability of at least 1 true GM seed in the sample
1	97.90%
2	99.94%
3	99.99%

The GM lot is highly suitable for the preparation of positive PT samples intended for qualitative detection

2. Bayesian predictive convolution framework

- $G = G_G + G_C$
- $P(G = g) = \sum_k P(G_G = k) P(G_C = g - k)$
- Compute: $P\ (|\ P - p^*\ | \leq \text{tol} \times p^*)$

Target concentration	GM seeds added	Probability within ±10% of target
0.20%	6	81.14%
0.50%	15	95.15%
1.00%	30	99.16%
1.50%	45	99.11%
2.00%	60	99.69%

Target concentrations between 0.5% and 2.0% provide reliable PT design for samples of 3000 seeds

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Development of a new ISTA paradigm integrating biological variability

Current ISTA framework

- Classical species
- Binomial assumption
- Tolerance tables well established
- χ^2 heterogeneity test appropriate

Limitation

- Some species show biological variability (e.g. tree species, wild species)
- Overdispersion observed
- Tolerances too stringent
- Classical tests reject valid lots

New paradigm

Distinguish biological variability from true heterogeneity

Implications

- Adapt tolerance tables
- Develop new heterogeneity tests

Proposed solution

- Use beta-binomial models
- Model biological overdispersion
- Test excess variability beyond biological level

ISTA ANNUAL MEETING 2026

Young@ISTA Statistics Committee Members are making a difference!

Statistics Committee workshop in Saint Louis – April 2025

Acknowledgments

- STA Committee members
- ECOM Liaison officers, Vanessa Sosa and Ruel Gesmundo
- ISTA Secretariat and ISTA ECOM
- TCOM Members
- Users of the tools developed by the STA Committee

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Thanks for your attention, support and patience as we work together!