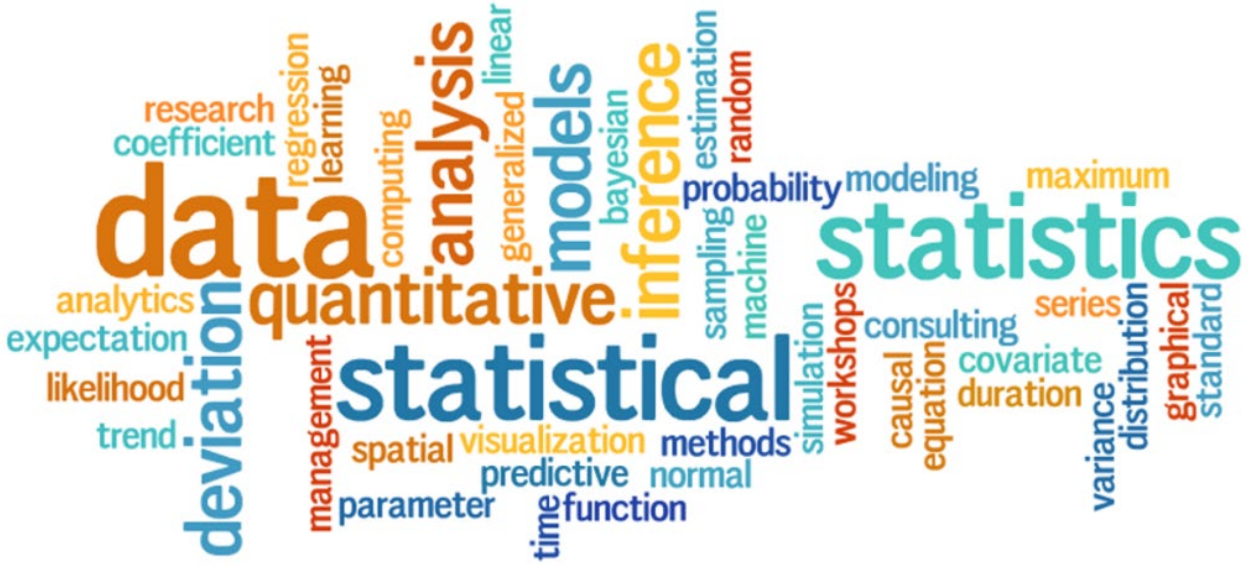
ISTA Statistics Committee and statistical seed testing tools



ISTA / ISSS / INSR Webinar – December 14, 2022

Overview

1. ISTA Statistics Committee Activities

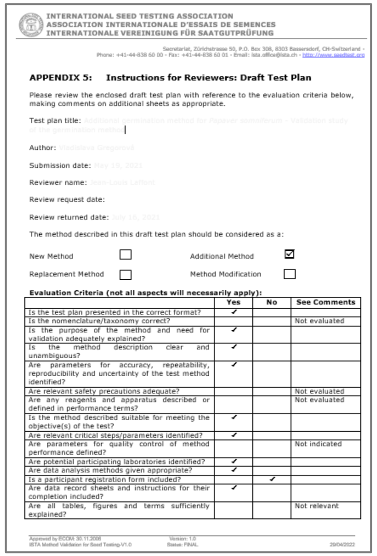
2. Sampling of statistical tools available on the ISTA website

Kirk and Jean-Louis at face-to-face working meeting in Johnston, Iowa (US) in Spring 2022

Jean-Louis Laffont, Kirk Remund and the ISTA Statistics Committee have delivered statistical solutions and tools to ISTA Technical Committees, ISTA membership and the seed testing industry for over 17 years and counting…

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

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

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

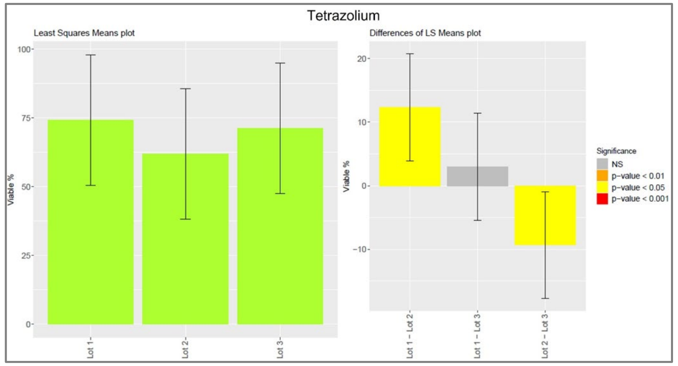
Approved by ECOM: 30.11.2006 — Version: 1.0 — ISTA Method Validation for Seed Testing-V1.0 — Status: FINAL — 29/04/2022

```
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
```



Sampling of theoretical contributions

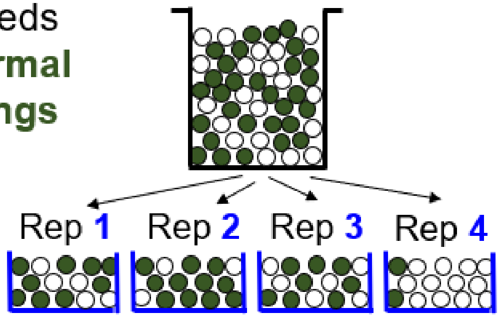
Seed Science Research
cambridge.org/ssr

Received: 11 July 2018
Revised: 21 December 2018
Accepted: 13 January 2019

Exact theoretical distributions around the replicate results of a germination test

Jean-Louis Laffont[1], Bonnie Hong[2], Bo-Jein Kuo[3] and Kirk M. Remund[4]

400 seeds
360 normal seedlings

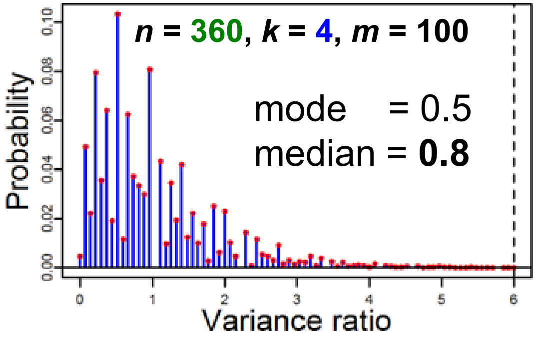


Random assignment of the seeds in the **4** replicates

# of **normal seedlings**	90	90	90	90
	99	70	94	97
	...			

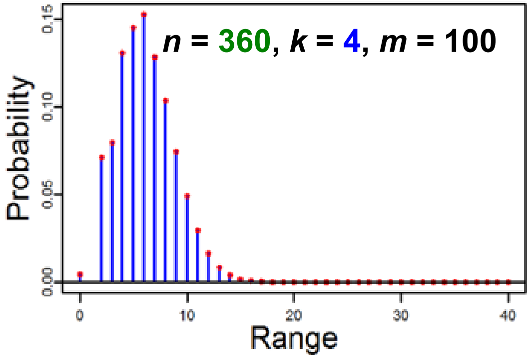
➡ Distribution of the number of normal seedlings in the reps: **multivariate hypergeometric** distribution

➡ Exact distribution of the **variance ratio** $F = \frac{\mathrm{Var(reps\_of\_germ\_test)}}{\mathrm{Binomial\_variance}}$



The variance ratio is likely to be well below unity just by chance

➡ Exact distribution of the **observed range**



Checking ISTA Tolerance Tables (Miles, 1963) elaborated using a normal and binomial approximation: approximation is good

Sampling of theoretical contributions

Unpublished:

- Laffont, J-L., Remund, K.R., R. Shillito, R. and T. Perez (2015). **Probabilities associated to proportions for subsequent samples**

Posterior predictive.xlsx

Probability of observing no more than a given proportion of GM seeds in a subsequent sample for large seed lots, in the remaining seeds for a small seed lot

The seed lot can be of infinite size or finite size. In the later case, the sum of the size of the 1st sample with the size of the 2nd sample is equal to the lot size. Computations of the posterior predictive density assume a uniform distribution as a prior distribution for the proportion of GM seeds in the lot.

Size of the 1st sample	3000	
Number of GM seeds observed in the 1st sample	0	
Size of the 2nd sample or number of remaining seeds	1000	
Upper specification (%)	0.10%	(1 seeds)
Probability to have no more than 0.1% GM seeds in 1000 seeds	0.937578	

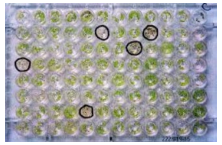
Change any value in a yellow cell

- ISTA Statistics Committee — April 25, 2020

  GMO purity through bioassays

  Jean-Louis Laffont and Kirk Remund – ISTA Statistics Committee

  → Estimate of purity taking into account germination



- Model for generating over-dispersed binomial data (e.g. tests made by different laboratories): Beta-binomial model with parameters k, α and β:

$$\alpha = p\left(\frac{k-1}{f^2-1} - 1\right) \qquad \beta = \alpha\left(\frac{1}{p} - 1\right)$$

where k is the # of seeds, p is the true germination and f is the over dispersion factor

- …

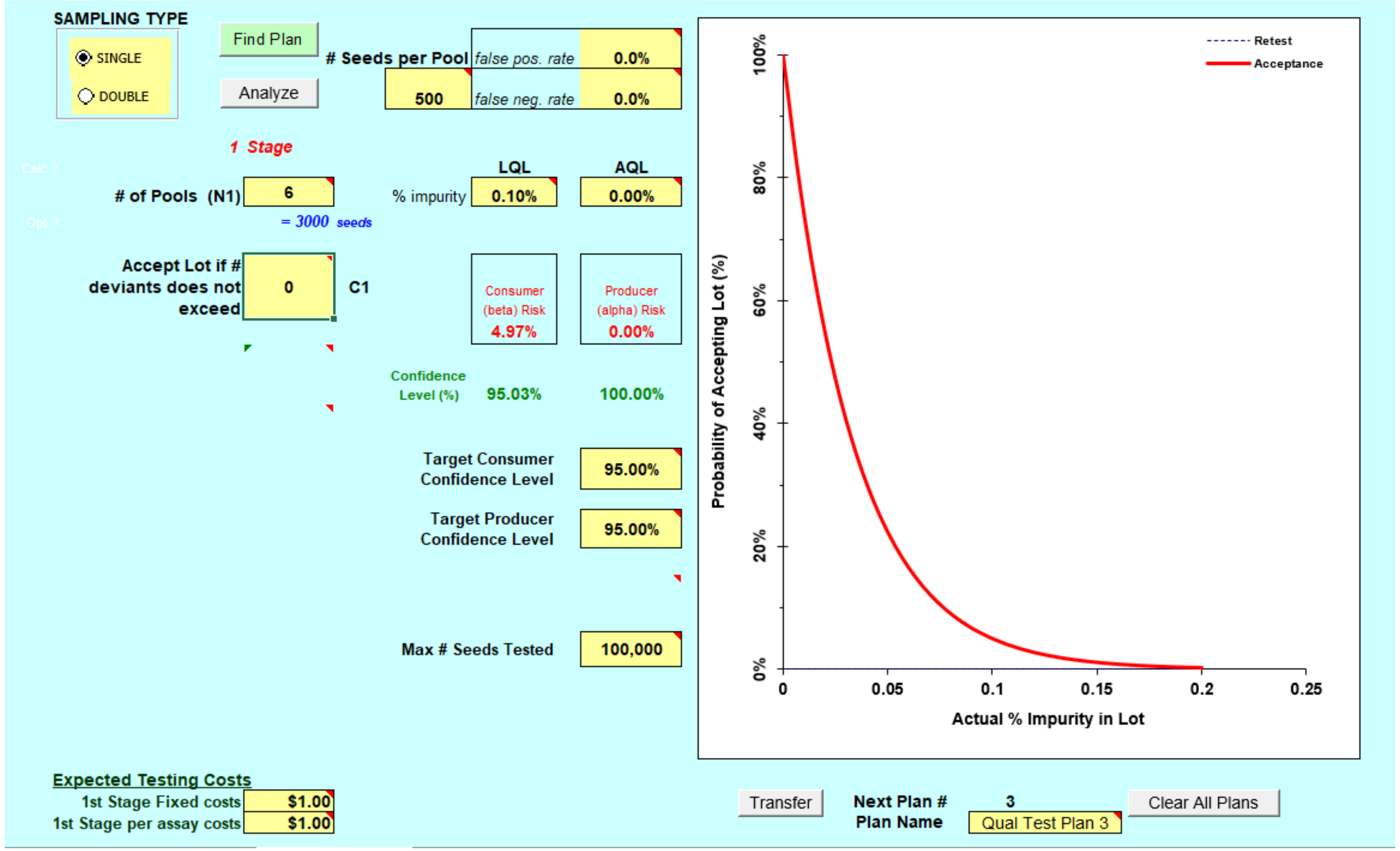
Sampling of statistics tools available on ISTA website

Statistics toolbox: https://www.seedtest.org/en/services-header/tools/statistics-committee/statistical-tools-seed-testing.html
Germination toolbox: https://www.seedtest.org/en/services-header/tools/germination-committee/germination-toolbox.html

- **SeedCalc8**
- Qualstat
- Statistical tests for seed mixtures tool
- Box plots calculator
- **Germination test tolerance and confidence interval calculator**
- SeedCalcStack9
- Heterogeneity Testing Calculator
- **Purity/OSD minimum working sample weights calculator** NEW

SeedCalc8

(testing plan design for qualitative assay)



Yellow cells are inputs
Other cells are outputs

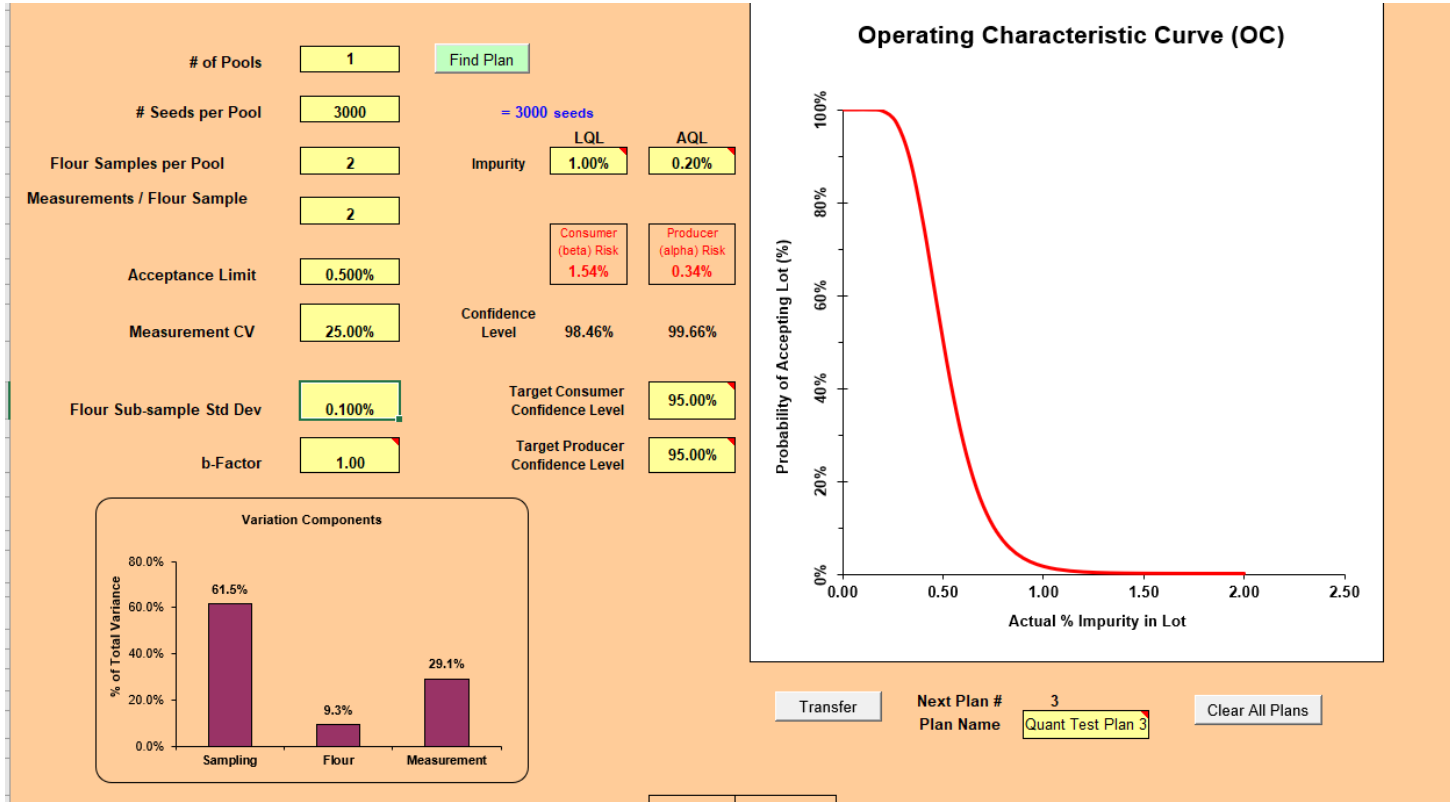
Much utility for biotech trait purity and adventitious presence (AP) impurity seed testing.

Also, utility for seed health and even physiological seed testing.

<u>Testing Plan Design</u>
Determine the number of individual seeds or seed pools (groups) to test and the maximum number of deviants to accept a seed lot.

SeedCalc8

(testing plan design for quantitative assay)



Much utility for biotech adventitious presence (AP) impurity seed testing.

<u>Testing Plan Design</u>

Determine the number of seed pools (groups) to test and the maximum quantitative acceptance limit in the working sample.

Yellow cells are inputs
Other cells are outputs

SeedCalc8

(qualitative assay estimation)

Impurity Estimation & Confidence Intervals (Assay measures impurity characteristic)

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

# of Seed Pools	6	Computed % in sample	0.1385 %
# of Seeds per Pool	500		
Total Seeds Tested	3000	*Measured property on seed pools*	
# Deviants Pools	3		
		Desired Confidence Level	95 %

Upper Bound of True % Impurity	0.3745		
(95% confident that the lot impurity is below 0.37%.)			
2-sided CI for True % Impurity	*0.0251*	*to*	*0.4263*
Lower Bound of True % Purity	99.6255		
(95% confident that the lot purity is above 99.63%.)			
2-sided CI for True % Purity	*99.5737*	*to*	*######*

The confidence intervals are an F-distribution approximation. The validity of these calculations are based on the following assumptions:

1) A random sample of seed is taken from the seed lot for testing.

2) The deviant seeds are evenly distributed across the lot of seed.

3) The number of seed sampled is no more than one-tenth the total number in the lot.

Much utility for biotech trait purity and adventitious presence (AP) impurity seed testing.

Also, utility for seed health and even physiological seed testing.

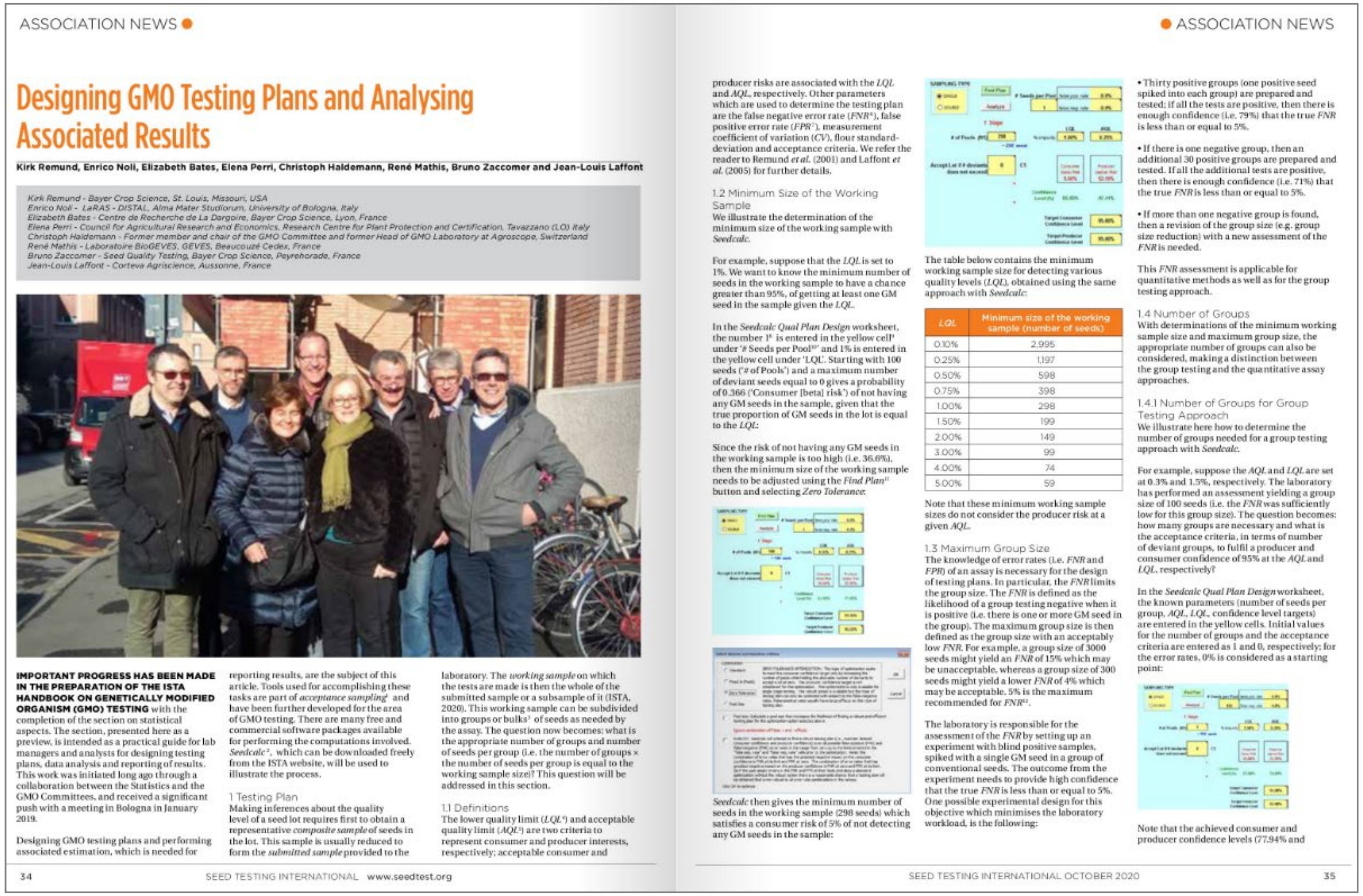
Impurity/purity estimation and confidence interval

Estimates the seed lot impurity/purity and a one-sided and two-sided confidence interval

Yellow cells are inputs
Other cells are outputs

SeedCalc8

User’s guide in
October 2020 Issue of Seed Testing International



ASSOCIATION NEWS

Designing GMO Testing Plans and Analysing Associated Results

Kirk Remund, Enrico Noli, Elizabeth Bates, Elena Perri, Christoph Haldemann, René Mathis, Bruno Zaccomer and Jean-Louis Laffont

Kirk Remund - Bayer Crop Science, St. Louis, Missouri, USA
Enrico Noli - LaRAS - DISTAL, Alma Mater Studiorum, University of Bologna, Italy
Elizabeth Bates - Centre de Recherche de La Dargoire, Bayer Crop Science, Lyon, France
Elena Perri - Council for Agricultural Research and Economics, Research Centre for Plant Protection and Certification, Tavazzano (LO) Italy
Christoph Haldemann - Former member and chair of the GMO Committee and former Head of GMO Laboratory at Agroscope, Switzerland
René Mathis - Laboratoire BioGEVES, GEVES, Beaucouzé Cedex, France
Bruno Zaccomer - Seed Quality Testing, Bayer Crop Science, Peyrehorade, France
Jean-Louis Laffont - Corteva Agriscience, Aussonne, France

IMPORTANT PROGRESS HAS BEEN MADE IN THE PREPARATION OF THE ISTA HANDBOOK ON GENETICALLY MODIFIED ORGANISM (GMO) TESTING with the completion of the section on statistical aspects. The section, presented here as a preview, is intended as a practical guide for lab managers and analysts for designing testing plans, data analysis and reporting of results. This work was initiated long ago through a collaboration between the Statistics and the GMO Committees, and received a significant push with a meeting in Bologna in January 2019.

Designing GMO testing plans and performing associated estimation, which is needed for reporting results, are the subject of this article. Tools used for accomplishing these tasks are part of *acceptance sampling*[1] and have been further developed for the area of GMO testing. There are many free and commercial software packages available for performing the computations involved. *Seedcalc*[2], which can be downloaded freely from the ISTA website, will be used to illustrate the process.

1 Testing Plan

Making inferences about the quality level of a seed lot requires first to obtain a representative *composite sample* of seeds in the lot. This sample is usually reduced to form the *submitted sample* provided to the laboratory. The *working sample* on which the tests are made is then the whole of the submitted sample or a subsample of it (ISTA, 2020). This working sample can be subdivided into groups or bulks[3] of seeds as needed by the assay. The question now becomes: what is the appropriate number of groups and number of seeds per group (i.e. the number of groups × the number of seeds per group is equal to the working sample size)? This question will be addressed in this section.

1.1 Definitions

The lower quality limit (*LQL*[4]) and acceptable quality limit (*AQL*[5]) are two criteria to represent consumer and producer interests, respectively; acceptable consumer and producer risks are associated with the *LQL* and *AQL*, respectively. Other parameters which are used to determine the testing plan are the false negative error rate (*FNR*[6]), false positive error rate (*FPR*[7]), measurement coefficient of variation (*CV*), flour standard-deviation and acceptance criteria. We refer the reader to Remund *et al.* (2001) and Laffont *et al.* (2005) for further details.

1.2 Minimum Size of the Working Sample

We illustrate the determination of the minimum size of the working sample with *Seedcalc*.

For example, suppose that the *LQL* is set to 1%. We want to know the minimum number of seeds in the working sample to have a chance greater than 95%, of getting at least one GM seed in the sample given the *LQL*.

In the *Seedcalc Qual Plan Design* worksheet, the number 1[8] is entered in the yellow cell[9] under '# Seeds per Pool[10]' and 1% is entered in the yellow cell under 'LQL'. Starting with 100 seeds ('# of Pools') and a maximum number of deviant seeds equal to 0 gives a probability of 0.366 ('Consumer [beta] risk') of not having any GM seeds in the sample, given that the true proportion of GM seeds in the lot is equal to the *LQL*:

Since the risk of not having any GM seeds in the working sample is too high (i.e. 36.6%), then the minimum size of the working sample needs to be adjusted using the *Find Plan*[11] button and selecting *Zero Tolerance*:

Seedcalc then gives the minimum number of seeds in the working sample (298 seeds) which satisfies a consumer risk of 5% of not detecting any GM seeds in the sample:

The table below contains the minimum working sample size for detecting various quality levels (*LQL*), obtained using the same approach with *Seedcalc*:

LQL	Minimum size of the working sample (number of seeds)
0.10%	2,995
0.25%	1,197
0.50%	598
0.75%	398
1.00%	298
1.50%	199
2.00%	149
3.00%	99
4.00%	74
5.00%	59

Note that these minimum working sample sizes do not consider the producer risk at a given *AQL*.

1.3 Maximum Group Size

The knowledge of error rates (i.e. *FNR* and *FPR*) of an assay is necessary for the design of testing plans. In particular, the *FNR* limits the group size. The *FNR* is defined as the likelihood of a group testing negative when it is positive (i.e. there is one or more GM seed in the group). The maximum group size is then defined as the group size with an acceptably low *FNR*. For example, a group size of 3000 seeds might yield an *FNR* of 15% which may be unacceptable, whereas a group size of 300 seeds might yield a lower *FNR* of 4% which may be acceptable. 5% is the maximum recommended for *FNR*[12].

The laboratory is responsible for the assessment of the *FNR* by setting up an experiment with blind positive samples, spiked with a single GM seed in a group of conventional seeds. The outcome from the experiment needs to provide high confidence that the true *FNR* is less than or equal to 5%. One possible experimental design for this objective which minimises the laboratory workload, is the following:

- Thirty positive groups (one positive seed spiked into each group) are prepared and tested; if all the tests are positive, then there is enough confidence (i.e. 79%) that the true *FNR* is less than or equal to 5%.
- If there is one negative group, then an additional 30 positive groups are prepared and tested. If all the additional tests are positive, then there is enough confidence (i.e. 71%) that the true *FNR* is less than or equal to 5%.
- If more than one negative group is found, then a revision of the group size (e.g. group size reduction) with a new assessment of the *FNR* is needed.

This *FNR* assessment is applicable for quantitative methods as well as for the group testing approach.

1.4 Number of Groups

With determinations of the minimum working sample size and maximum group size, the appropriate number of groups can also be considered, making a distinction between the group testing and the quantitative assay approaches.

1.4.1 Number of Groups for Group Testing Approach

We illustrate here how to determine the number of groups needed for a group testing approach with *Seedcalc*.

For example, suppose the *AQL* and *LQL* are set at 0.3% and 1.5%, respectively. The laboratory has performed an assessment yielding a group size of 100 seeds (i.e. the *FNR* was sufficiently low for this group size). The question becomes: how many groups are necessary and what is the acceptance criteria, in terms of number of deviant groups, to fulfil a producer and consumer confidence of 95% at the *AQL* and *LQL*, respectively?

In the *Seedcalc Qual Plan Design* worksheet, the known parameters (number of seeds per group, *AQL*, *LQL*, confidence level targets) are entered in the yellow cells. Initial values for the number of groups and the acceptance criteria are entered as 1 and 0, respectively; for the error rates, 0% is considered as a starting point:

Note that the achieved consumer and producer confidence levels (77.94% and

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SEED TESTING INTERNATIONAL OCTOBER 2020 35

Germination Tolerance Calculator

(tolerances for tests in one lab)

Reproduces standard tolerance in ISTA Rules

from ISTA Rules Table 5B Part 1

Germination Tolerances for tests in 1 laboratory

2-way test equivalent at 2.5% significance level

# of tests or replicates	4
# of seeds/test or replicate	100
Average germination	92
Reported germination	92
Maximum range	11

Change any value in a yellow cell

In accordance with Miles (1963)
Tables G1 and G2, columns D, H and L, 100 and 400 seed tests

Germ Tol - 1 lab | Germ Tol - Different labs | CI for Germ

Ready

Tolerance between test replicates in <u>standard</u> germination test on a seed lot

Yellow cells are inputs
Blue cells are outputs

Flexibility to derive other useful tolerances

uses same Miles(1963) logic as ISTA tolerances

Germination Tolerances for tests in 1 laboratory

2-way test equivalent at 2.5% significance level

# of tests or replicates	2
# of seeds/test or replicate	50
Average germination	92
Reported germination	92
Maximum range	12

Change any value in a yellow cell

In accordance with Miles (1963)
Tables G1 and G2, columns D, H and L, 100 and 400 seed tests

Germ Tol - 1 lab | Germ Tol - Different labs | CI for Germ

Ready

Tolerance between test replicates in 100 seed germination test on a seed lot

Germination Tolerance Calculator

(tolerances for tests between labs)

Reproduces standard tolerance in ISTA Rules

from ISTA Rules Table 5F

Germination Tolerances for tests in different laboratories

2-way test equivalent at 5% significance level

# of tests	2
# of seeds/test	400
Average germination	95
Reported germination	95
Maximum range	5

Change any value in a yellow cell

In accordance with Miles (1963) Table G5, columns C, F and I, 400 seed tests

Germ Tol - 1 lab | Germ Tol - Different labs | CI for Germ

Tolerance of standard germination test in two labs on same seed lot

Yellow cells are inputs
Blue cells are outputs

Flexibility to derive other useful tolerances

uses same Miles(1963) logic as ISTA tolerances

Germination Tolerances for tests in different laboratories

2-way test equivalent at 5% significance level

# of tests	3
# of seeds/test	200
Average germination	95
Reported germination	95
Maximum range	8

10 maximum

Change any value in a yellow cell

In accordance with Miles (1963) Table G5, columns C, F and I, 400 seed tests

Germ Tol - 1 lab | Germ Tol - Different labs | CI for Germ

Tolerance of 200 seed germination test in three labs on same seed lot

Germination Tolerance Calculator

Confidence Intervals for percent germination

# of seeds in sample tested	**400**
Level of confidence (%)	**95%**
Average germination	**94.25**
Reported germination	**94**
Confidence Interval	**91 - 96**

Change any value in a yellow cell

In accordance with Miles (1963) Tables G9 and G10

Note: due to the way the results are rounded, there might be some differences with Miles' tables

Germ Tol - 1 lab | Germ Tol - Different labs | **CI for Germ**

Enter number of seeds in working sample, statistical confidence level and average germination from working sample in yellow cells and the reported germination and germination confidence interval is returned in blue cells.

Yellow cells are inputs
Blue cells are outputs

Purity/OSD minimum working sample weights calculator

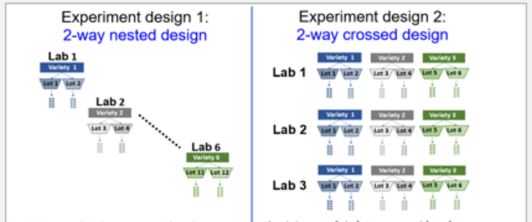
Flexibility in design and robustness of calculations within a MS Excel spreadsheet

Calculator for adding working weights to Table 2C of the ISTA Rules

THE CACULATOR IS PROVIDED "AS IS", WITHOUT WARRANTY OF ANY KIND. IN NO EVENT SHALL THE AUTHORS BE LIABLE FOR ANY CLAIM, DAMAGES OR OTHER LIABILITY ARISING IN CONNECTION WITH THE CALCULATOR.

Experiment designs

Two types of experiment designs are considered in the calculator:



A minimum of 12 lots are considered across a minimum of six varieties represented in the experiment as a general rule. These 12 lots will be evaluated by a minimum of two labs however six labs are preferred. A minimum of eight 100 seed reps are weighed per lot.

A minimum of six lots are considered across a minimum of three varieties represented in the experiment as a general rule. These six lots will be evaluated each by a minimum of two labs however six labs are preferred. A minimum of eight 100 seed reps are weighed per lot.

Calculations

The rep weights are entered into the unprotected yellow cells of the calculator. If experiment design 1 is used, data for the different lots from each lab are entered in different columns. In order to avoid conditional formatting conflicts, always copy/paste data in the calculator using `Paste Special → Values`.

. For each lot in a given laboratory, outliers are highlighted in red using Grubbs's method at the 5% significance level (Grubbs, 1969). These outliers are then excluded manually from the computations.

. The linear random effects models used for the analysis of the two experiment designs are:

. **Experiment design 1**:

$$y_{ijk} = \mu + \alpha_i + \beta_{ij} + e_{ijk}$$

in which:
. y_{ijk} is the observed 100-seeds weight of lot j ($j = 1, 2, ..., b_i$) in lab i ($i = 1, 2, ..., a$) and replication k ($k = 1, 2, ..., n_{ij}$);
. μ is the intercept;
. α_i is the random effect of lab i ($\alpha_i \sim$ i.i.d. $N(0, \sigma^2_{Lab})$);
. β_{ij} is the random effect of lot j within lab i ($\beta_{ij} \sim$ i.i.d. $N(0, \sigma^2_{Lot})$);
. e_{ijk} is the residual ($e_{ijk} \sim$ i.i.d. $N(0, \sigma^2_{Res})$).

. **Experiment design 2**:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

in which:
. y_{ijk} is the observed 100-seeds weight of lot j ($j = 1, 2, ..., b$) in lab i ($i = 1, 2, ..., a$) and replication k ($k = 1, 2, ..., n_{ij}$);
. μ is the intercept;
. α_i is the random effect of lab i ($\alpha_i \sim$ i.i.d. $N(0, \sigma^2_{Lab})$);
. β_j is the random effect of lot j ($\beta_j \sim$ i.i.d. $N(0, \sigma^2_{Lot})$);
. $(\alpha\beta)_{ij}$ is the random interaction effect between lab i and lot j ($(\alpha\beta)_{ij} \sim$ i.i.d. $N(0, \sigma^2_{Lab \times Lot})$);
. e_{ijk} is the residual ($e_{ijk} \sim$ i.i.d. $N(0, \sigma^2_{Res})$).

The calculator automatically selects which model to fit according to the dataset structure.

. Variance components for the two models are estimated from the data by the Henderson Method I (Searle *et al.*, 1992, Appendix F). When an estimate is negative, this estimate is reported as zero. Let $\hat{\sigma}^2_{Lab}$, $\hat{\sigma}^2_{Lot}$, $\hat{\sigma}^2_{Lab \times Lot}$ and $\hat{\sigma}^2_{Res}$ be these

Instructions | Calculator

Supporting Data of New Species Proposal to ISTA Rules Table 2C

Submitter Name:		Lab Full Name:	
Scientific Name of the Crop kind:	Genus Species	ISTA Member Code:	
		Contact Email:	

Change any value in a yellow cell

Number of observations	0	
Number of labs	0	
Number of lots	0	
General mean		
Lab variance		
Lot variance		
Lab x Lot variance		
Residual variance		Decision
2500 seed weight*		
25000 seed weight*		

* 95% Confidence

Rep weights in red are identified as outliers by Grubbs's method at the 5% significance level and needs to be suppressed (removed) manually

Lab	\ Seed lot	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Lab 1	Rep1																	
	Rep2																	
	Rep3																	
	Rep4																	
	Rep5																	
	Rep6																	
	Rep7																	
	Rep8																	
	Rep9																	
	Rep10																	
	Rep11																	
	Rep12																	
	Mean																	
	St. Dev.																	
	Number of reps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Grubbs critical values																	
Lab 2	Rep1																	
	Rep2																	
	Rep3																	
	Rep4																	
	Rep5																	
	Rep6																	
	Rep7																	
	Rep8																	
	Rep9																	
	Rep10																	
	Rep11																	

Instructions | **Calculator**

Purity/OSD minimum working sample weights calculator

Takes into account lab-to-lab, seed lot and within lot measurement variation to estimate conservative minimum working sample weight for high confidence of obtaining 2500 and 25000 seeds in sample

When needed, some warnings are displayed in red

Supporting Data of New Species Proposal to ISTA Rules Table 2C

Submitter Name:	XXX	Lab Full Name:	YYY
Scientific Name of the Crop kind:	Basella B. alba	ISTA Member Code:	ZZZ
		Contact Email:	AAA

Change any value in a yellow cell

Number of observations	232		
Number of labs	7		
Number of lots	5		6 lots are preferred for an accurate estimation
General mean	3.2584		
Lab variance	0.0020037		
Lot variance	0.1453077		
Lab x Lot variance	0.0963083		
Residual variance	0.0101622	Decision	
2500 seed weight*	103	100	Decision value should be greater than or equal to 103
25000 seed weight*	1022	1050	

* 95% Confidence

Rep weights in red are identified as outliers by Grubbs's method at the 5% significance level and needs to be suppressed (removed) manually

Lab	\ Seed lot	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Lab 2	Rep1	2.3672	3.4036	2.3585	3.1927	3.7473											
	Rep2	2.2734	3.4207	2.3530	3.0972	3.7309											
	Rep3	2.3198	3.5878	2.4268	3.2861	3.7818											
	Rep4	2.3866	3.4322	2.3827	3.2858	3.7380											
	Rep5	2.3600	3.3296	2.2917	3.2861	3.7908											
	Rep6	2.3720	3.3873	2.2663	3.1889	3.8542											
	Rep7		3.4601	2.2407	3.1103												
	Rep8			2.7779	3.2201												
	Rep9																
	Rep10																
	Rep11																
	Rep12																
	Mean	2.3465	3.4316	2.3872	3.2084	3.7738											
	St. Dev.	0.04225	0.08008	0.16963	0.07636	0.04613											
	Number of reps	6	7	8	8	6	0	0	0	0	0	0	0	0	0	0	0
	Grubbs critical values	1.89	2.02	2.13	2.13	1.89											

Outliers are automatically identified in red

We look forward to providing more statistical tools to ISTA and seed testing professionals in the future.

Thank you for your attention!

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