# ISTA Statistics Committee 2023-2024 Activity Report

Jean-Louis Laffont and Kirk Remund

# ISTA Statistics Committee

| | | |
|---|---|---|
| **Chair:** | **Kirk Remund** | **USA** |
| **Vice:** | **Jean-Louis Laffont** | **France** |
| **Members:** | **Gabriel Carré** | **France** |
| | **Mustapha El Yakhlifi** | **France** |
| | **Kelly Evans** | **New Zealand** |
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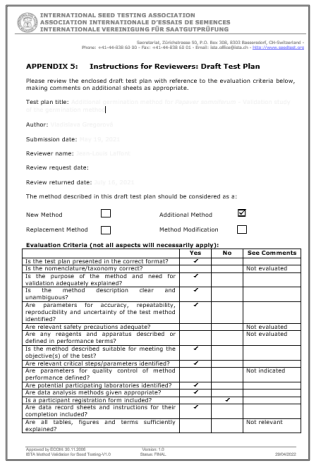
| | **Bonnie Hong** | **USA** |
| | **Bo-Jein Kuo** | **Separate Custom Territory of Taiwan, Penghu, Kinmen and Matsu** |
| | **Thomas Michelon** | **Brazil** |
| | **Oluseyi Odubote** | **USA** |
| | **Nicholas Syring** | **USA** |

**ISTA ECOM Liaison Officer: Vanessa Sosa**

# 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:

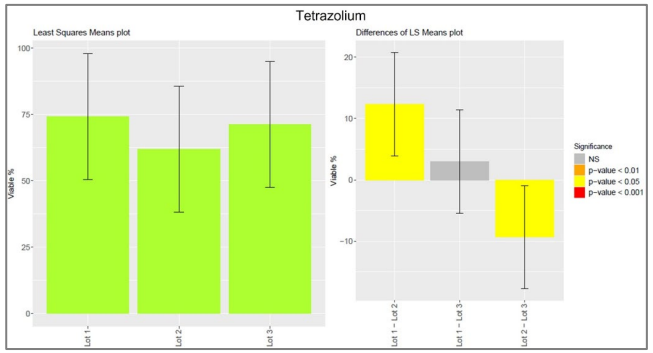
| | | | |
|---|---|---|---|
| New Method | ☐ | Additional Method | ☑ |
| Replacement Method | ☐ | Method Modification | ☐ |

**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 |

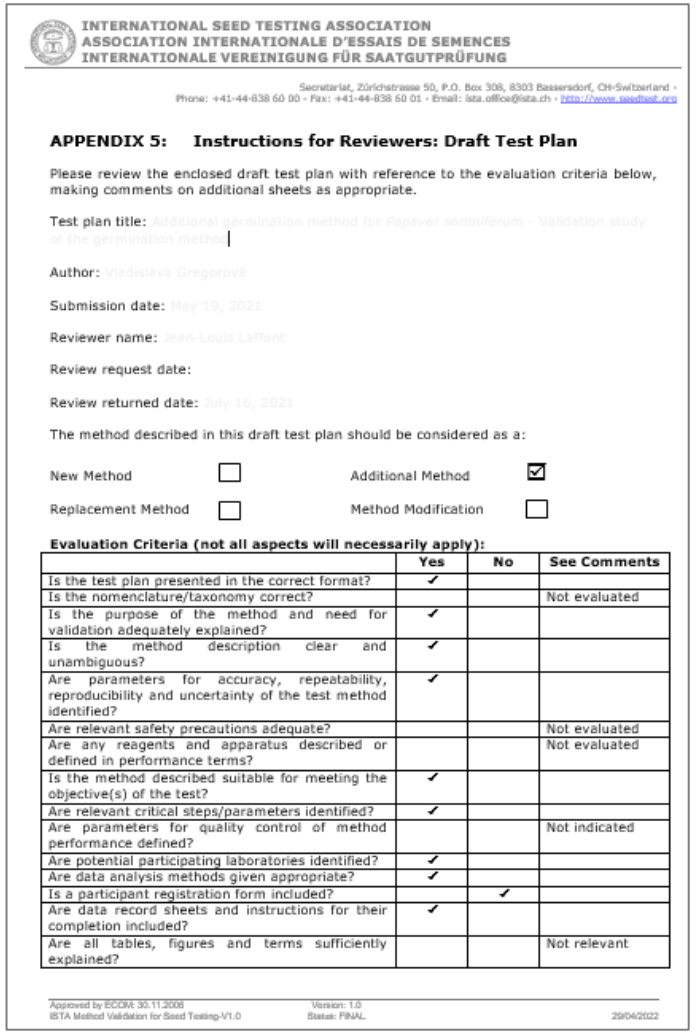
```
Mean s_repeatability disp s_Reproducibility s_Lab s_LotxLab
  69             6.28 0.96                12.19 10.26      1.98
```

```
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 -5.414392 11.4143922
Lot 2 - Lot 3 -9.333333   3.030707 -17.747926 -0.9187411
```



# Support of TCOMs – Report reviews

- **3 test plan reviews**
- **2 validation study reviews**
- **2 validation study analyses**



INTERNATIONAL SEED TESTING ASSOCIATION
ASSOCIATION INTERNATIONALE D'ESSAIS DE SEMENCES
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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 |

Approved by ECOM 30.11.2008 Version: 1.0
ISTA Method Validation for Seed Testing-V1.0 Status: FINAL 29/04/2022

# Support of TCOMs – Consulting

| | Subject of Consulation/Question | From | Date |
|---|---|---|---|
| 1 | CV guidannce for TSW | Japan | July |
| 2 | Method Validation | Argentina | July |
| 3 | Repeatability / Reproducibility for GLMM model | France | August |
| 4 | Vigor Test  vs Field Emergence Question | Rwanda | August |
| 5 | ISTA Tool Macro Security Issue | Netherlands | August |
| 6 | Tolerance for comparing TSW reps | Canada | August |
| 7 | Basis of Working and Submitted Sample Weight | South Africa | August |
| 8 | ISO 22753 Question on LOD for group testing | France | September |
| 9 | Z-score Quantiles for PT Tests | Africa | September |
| 10 | Table 5B Part 1 Tolerance Question | Iran | November |
| 11 | ISTA Rules Table 4A Use Question for Purity | Netherlands | November |
| 12 | ISTA Tolerances Question for 4 x 50 Seed Test | France | March |
| 13 | Dormancy Breaking Germination Method Validation | France | March |
| 14 | Forest and TZ Committee Question on Validaiton Study | Italy | April |
| 15 | Heterogentiy Test Question | US | April |
| 16 | ISTAgermMV Question on Version | France | May |
| 17 | Germination Tolerances | Denmark | June |

**70% increase in consultations & questions from 2022-2023**

**Requires significant time from Statistics TCOM to address**

# Support of TCOMs – Tool Development

**Calculator for performing heterogeneity test for continuous data**

**Checking homogeneity for moisture and conductivity tests**

Jean-Louis Laffont and Kirk Remund
ISTA Statistics Committee

For germination, the heterogeneity test is based on a chi-square test which tests if the variance between samples is not greater than the binomial variance.
For moisture and conductivity, we will follow the same principle: a chi-square test is used to test if the variance between samples ($\sigma^2$) is not greater than a *reference variance* ($\sigma_0^2$). The null and the alternative hypotheses of the test are:

$H_0: \sigma^2 \leq \sigma_0^2$
$H_1: \sigma^2 > \sigma_0^2$

The test statistic is:

$$H = \frac{(m-1)s^2}{\sigma_0^2}$$

where $m$ is the number of samples and $s^2$ is the sample variance of $m$ sample means. $s^2$ is calculated through fitting the following random effects model:

$$y_{ij} = \mu + a_i + e_{ij}$$

in which:

- $y_{ij}$ is the test result for sample $i$ and replication $j$;
- $\mu$ is the intercept;
- $a_i$ is the random effect of sample $i$ ($a_i \sim$ i. i. d. $N(0, \sigma^2_{Sample})$);
- $e_{ij}$ is the residual ($e_{ij} \sim$ i. i. d. $N(0, \sigma^2_{Res})$).

Then:

$$s^2 = \widehat{\sigma^2_{Sample}} + \frac{\widehat{\sigma^2_{Res}}}{n}$$

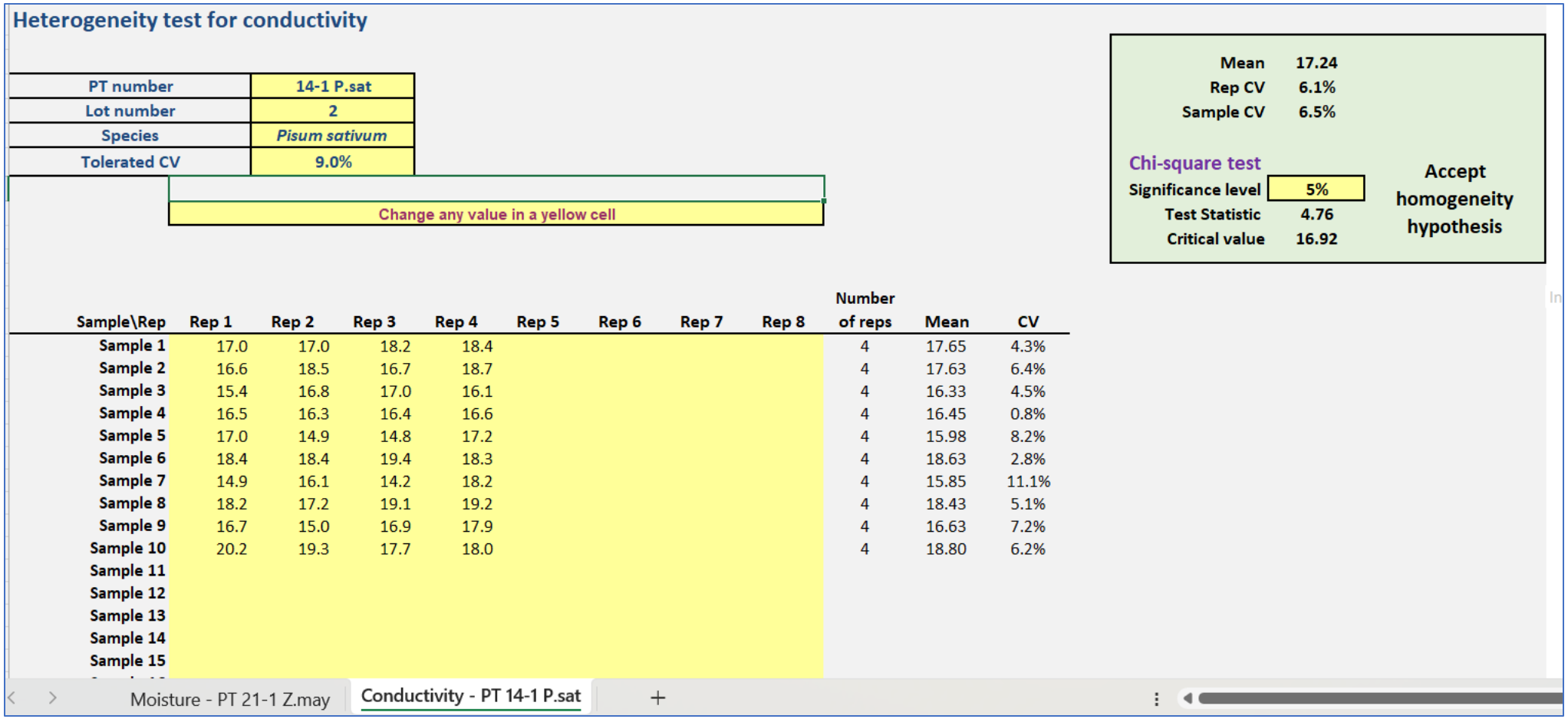
where $n$ is the number of reps for each sample. Note that for balanced data, $s^2$ is equal to the sample variance of the sample means.
The null hypothesis is then rejected if

$$H > \chi^2_{1-\alpha,m-1}$$

where $\alpha$ is the significance level and $\chi^2_{1-\alpha,m-1}$ is the $1-\alpha$ quantile of a chi-square distribution with $m-1$ degrees of freedom.

# Support of TCOMs – Tool Development

(continued work under Special Project for comprehensive tool)



Heterogeneity test for conductivity

| | |
|---|---|
| PT number | 14-1 P.sat |
| Lot number | 2 |
| Species | *Pisum sativum* |
| Tolerated CV | 9.0% |

Change any value in a yellow cell

| | |
|---|---|
| Mean | 17.24 |
| Rep CV | 6.1% |
| Sample CV | 6.5% |

Chi-square test

| | |
|---|---|
| Significance level | 5% |
| Test Statistic | 4.76 |
| Critical value | 16.92 |

Accept homogeneity hypothesis

| Sample\Rep | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 | Number of reps | Mean | CV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample 1 | 17.0 | 17.0 | 18.2 | 18.4 | | | | | 4 | 17.65 | 4.3% |
| Sample 2 | 16.6 | 18.5 | 16.7 | 18.7 | | | | | 4 | 17.63 | 6.4% |
| Sample 3 | 15.4 | 16.8 | 17.0 | 16.1 | | | | | 4 | 16.33 | 4.5% |
| Sample 4 | 16.5 | 16.3 | 16.4 | 16.6 | | | | | 4 | 16.45 | 0.8% |
| Sample 5 | 17.0 | 14.9 | 14.8 | 17.2 | | | | | 4 | 15.98 | 8.2% |
| Sample 6 | 18.4 | 18.4 | 19.4 | 18.3 | | | | | 4 | 18.63 | 2.8% |
| Sample 7 | 14.9 | 16.1 | 14.2 | 18.2 | | | | | 4 | 15.85 | 11.1% |
| Sample 8 | 18.2 | 17.2 | 19.1 | 19.2 | | | | | 4 | 18.43 | 5.1% |
| Sample 9 | 16.7 | 15.0 | 16.9 | 17.9 | | | | | 4 | 16.63 | 7.2% |
| Sample 10 | 20.2 | 19.3 | 17.7 | 18.0 | | | | | 4 | 18.80 | 6.2% |
| Sample 11 | | | | | | | | | | | |
| Sample 12 | | | | | | | | | | | |
| Sample 13 | | | | | | | | | | | |
| Sample 14 | | | | | | | | | | | |
| Sample 15 | | | | | | | | | | | |

Moisture - PT 21-1 Z.may | Conductivity - PT 14-1 P.sat

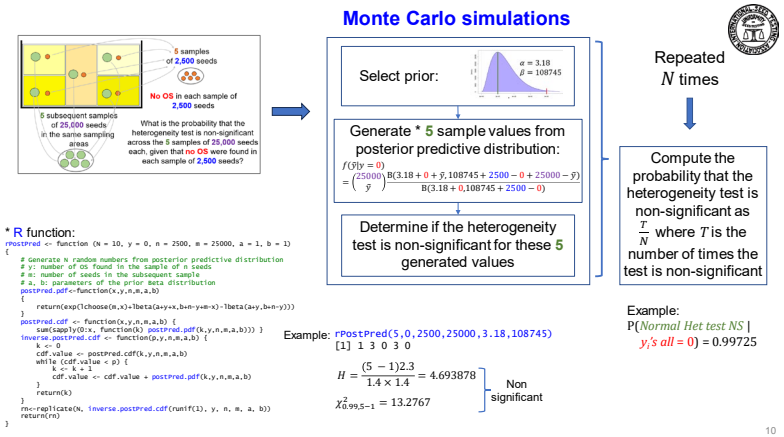
# Support of TCOMs – Workshops

- **ISTA GMO Testing Workshop** – Tavazzano, Italy
- ISTA supported **AOSA/SCST Method Validation Workshop** - Saskatoon, Canada

# Special project – Vegetable Seed Industry Working group (VSI WG)

- **Guidance on extending veg sub-lotting increases to other veg species**

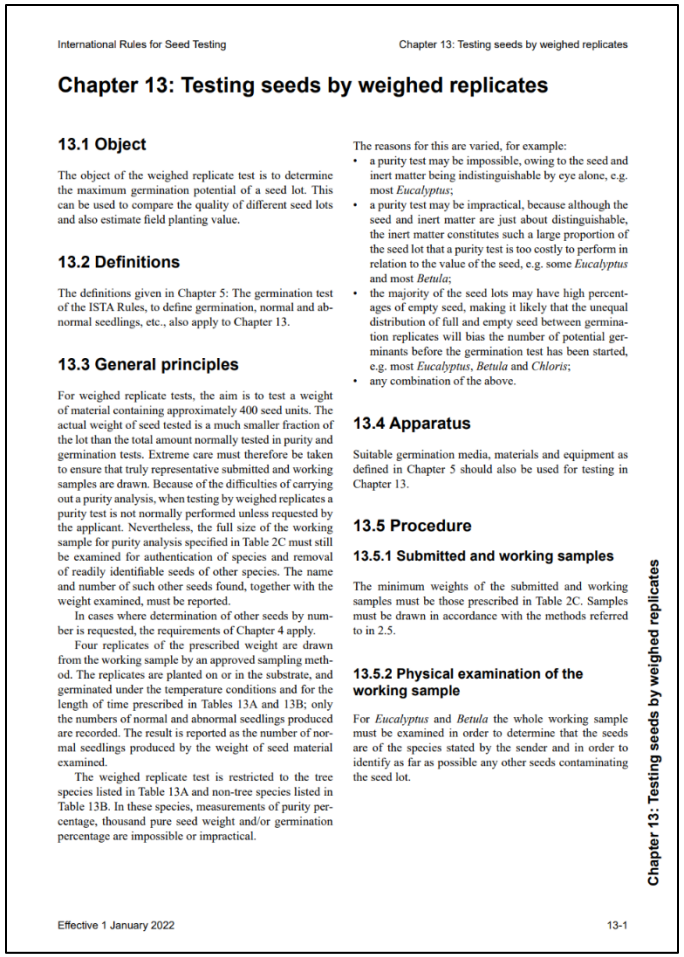
- **Guidance on OSD lot heterogeneity assessment using a light OSD test**
  **(Jean-Louis present Bayesian/simulation modeling in ATC open session)**



# Special project – Testing Seed by weighed replicates (Rules Chapter 13)

**For some species (e.g., some trees and shrubs), it is impractical to count out 100 seed replicates since seeds and inert matter are indistinguishable.**

**Therefore, weighed replicates using Table 13A and 13B are used which give approximate "100 seed replicates".**



International Rules for Seed Testing — Chapter 13: Testing seeds by weighed replicates

## Chapter 13: Testing seeds by weighed replicates

### 13.1 Object

The object of the weighed replicate test is to determine the maximum germination potential of a seed lot. This can be used to compare the quality of different seed lots and also estimate field planting value.

### 13.2 Definitions

The definitions given in Chapter 5: The germination test of the ISTA Rules, to define germination, normal and abnormal seedlings, etc., also apply to Chapter 13.

### 13.3 General principles

For weighed replicate tests, the aim is to test a weight of material containing approximately 400 seed units. The actual weight of seed tested is a much smaller fraction of the lot than the total amount normally tested in purity and germination tests. Extreme care must therefore be taken to ensure that truly representative submitted and working samples are drawn. Because of the difficulties of carrying out a purity analysis, when testing by weighed replicates a purity test is not normally performed unless requested by the applicant. Nevertheless, the full size of the working sample for purity analysis specified in Table 2C must still be examined for authentication of species and removal of readily identifiable seeds of other species. The name and number of such other seeds found, together with the weight examined, must be reported.

In cases where determination of other seeds by number is requested, the requirements of Chapter 4 apply.

Four replicates of the prescribed weight are drawn from the working sample by an approved sampling method. The replicates are planted on or in the substrate, and germinated under the temperature conditions and for the length of time prescribed in Tables 13A and 13B; only the numbers of normal and abnormal seedlings produced are recorded. The result is reported as the number of normal seedlings produced by the weight of seed material examined.

The weighed replicate test is restricted to the tree species listed in Table 13A and non-tree species listed in Table 13B. In these species, measurements of purity percentage, thousand pure seed weight and/or germination percentage are impossible or impractical.

The reasons for this are varied, for example:

- a purity test may be impossible, owing to the seed and inert matter being indistinguishable by eye alone, e.g. most *Eucalyptus*;
- a purity test may be impractical, because although the seed and inert matter are just about distinguishable, the inert matter constitutes such a large proportion of the seed lot that a purity test is too costly to perform in relation to the value of the seed, e.g. some *Eucalyptus* and most *Betula*;
- the majority of the seed lots may have high percentages of empty seed, making it likely that the unequal distribution of full and empty seed between germination replicates will bias the number of potential germinants before the germination test has been started, e.g. most *Eucalyptus*, *Betula* and *Chloris*;
- any combination of the above.

### 13.4 Apparatus

Suitable germination media, materials and equipment as defined in Chapter 5 should also be used for testing in Chapter 13.

### 13.5 Procedure

#### 13.5.1 Submitted and working samples

The minimum weights of the submitted and working samples must be those prescribed in Table 2C. Samples must be drawn in accordance with the methods referred to in 2.5.

#### 13.5.2 Physical examination of the working sample

For *Eucalyptus* and *Betula* the whole working sample must be examined in order to determine that the seeds are of the species stated by the sender and in order to identify as far as possible any other seeds contaminating the seed lot.

Chapter 13: Testing seeds by weighed replicates

Effective 1 January 2022 — 13-1

# Special project – Testing Seed by weighed replicates (Rules Chapter 13)

- **Consider a germination test of four replicates of 100 seeds with normal seedlings 60, 60, 70 and 90**
- **Average germination is 70% and a tolerated range according to table 5B Part 1 of 18. Observed range is 30 and we conclude the results are out of tolerance.**
- **For testing seeds by weight, the sum of the number of normal seedings is 280 leading yielding a tolerance of 35 in Table 13C and we conclude the results are within tolerance.**
- **Large discrepancy in conclusions needs to be addressed and Poisson distribution basis in Table 13C needs to be corrected.**

# Special project – Testing Seed by weighed replicates (Rules Chapter 13)

**Y is the random variable "*number of seeds in one replicate*".**

**Proposed solution is a Binomial distribution-based approach with a mean and acceptable variance in the number of seeds per replicate (e.g., CV=4%) for parameter *y* versus the inferior Poisson approach of Table 13C.**

**Working with Forest Tree & Shrub Committee on practical CV values across species.**

We start with some notations. Let:

- $\pi$ be the true unknown percentage of seedlings in the lot.
- $Y$ be the random variable "*number of seeds in one replicate*". The mean and the variance of this distribution are $\mu_Y$ and $\sigma_Y^2$ respectively.
- $X$ be the random variable "*number of seedlings in one replicate*" out of $y$ seeds (realization of random variable Y).

The conditional variable $X|Y = y$ is binomial$(y, \pi)$ and we have:
$\mathrm{E}[X|Y = y] = y\pi$ and $\mathrm{Var}[X|Y = y] = y\pi(1 - \pi)$.
We are interested in the expectation and the variance of $X$ unconditionally on $Y$. We have:

$$\mathrm{E}[X] = \mathrm{E}\big[\mathrm{E}[X|Y]\big] = \mathrm{E}[y\pi] = \pi\mathrm{E}[y] = \pi\mu_Y$$

and

$$\begin{aligned}\mathrm{Var}[X] &= \mathrm{Var}\big[\mathrm{E}[X|Y]\big] + \mathrm{E}\big[\mathrm{Var}[X|Y]\big] \\ &= \mathrm{Var}[y\pi] + \mathrm{E}[y\pi(1-\pi)] \\ &= \pi^2\mathrm{Var}[y] + \pi(1-\pi)\mathrm{E}[y] \\ &= \pi^2\sigma_Y^2 + \pi(1-\pi)\mu_Y \\ &= \sigma_X^2.\end{aligned}$$

# Special project – Testing Seed by weighed replicates (Rules Chapter 13)

**Interim tool has been developed for tolerances for seeds tested by weighed replicates**

**Following example given with average germination of 70% a revised tolerance of 21 is calculated using a CV=4% and compared to the replicates range of 30 the results are out of tolerance. [CV=10% yields tolerance of 33]**

**Experiments can be conducted to calculate practical CVs for different species tested by weighing rather than counting replicates**

**Germination Tolerances for seeds tested by weighed replicates**

2-way test equivalent at 2.5% significance level

| | | |
|---|---|---|
| Number of replicates | 4 | |
| Average number of seeds per rep ($\mu_Y$) | 100 | |
| CV of the number of seeds per rep | 4% | |
| Average germination | 70 | |
| Reported germination | 70 | |
| | | Table 5B |
| Maximum range | 21 | 18 |

Change any value in a yellow cell

# 2024-2025 upcoming activities

Webinars

- ISF/ISTA Seed Testing Statistical Tools
- Potential SANSOR Seed Analyst Webinar

Young@ISTA STA Committee Member Workshop

Other ISTA Statistical Theory Special Project Deliverables

- Method Validation
- Sampling

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Young@ISTA STA Committee Member Workshop

Other ISTA Statistical Theory Special Project Deliverables

- Method Validation
- Sampling

# Acknowledgements


- **STA Committee members**
- **ECOM Liaison Officer, Vanessa Sosa**
- **ISTA Secretariat and ISTA ECOM**
- **TCOM members**
- **Users of the tools developed by the STA Committee**

ISTA ANNUAL MEETING 2024
01-04 JULY
CAMBRIDGE, UNITED KINGDOM

# Thank you