



# Method Validation Reports on Rules Proposals for the International Rules for Seed Testing 2024 Edition

- ISTA Statistics TCOM review to determine homogeneity in *Solanum lycopersicum* seed lots and the number of sub-lots for which an OIC established for the lot is still valid**  
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## Study to determine homogeneity in *Solanum lycopersicum* seed lots and the number of sub-lots for which an OIC established for the lot is still valid

Jean-Louis Laffont and Kirk Remund - ISTA Statistics Committee

### Summary of Experiment Design

- Six companies participated, providing samples from three tomato seed lots produced in 11 different countries.
- Five sub-samples from each seed lot were taken to cover spatially across the lot using an ISTA certified sampler (total of 90 samples).
- Purity tests and germination tests (1<sup>st</sup> count at day 6, final count at days 8 to 14) were performed on each sub-sample by one laboratory.

### Sub-samples homogeneity for purity

Given that purity percentages are all equal to 100%, no statistical analysis has been performed.

### Sub-samples homogeneity for germination

#### Checking homogeneity of the test replications

For each of the 90 sub-samples, the four reps of the normal seedlings (final count) are all within ISTA tolerance (see Excel file *Tomato homogeneity expt - Ger tol.xlsx*).

#### Background on the heterogeneity test

Let:

- $p_i$  be the average percentages of normal seedlings (1<sup>st</sup> count or final count), abnormal seedlings or dead seeds of each sub-sample  $i$  ( $i = 1, 2, \dots, m$ ).
- $\hat{p} = \frac{1}{m} \sum_{i=1}^m p_i$  .
- $V = \frac{1}{m-1} \sum_{i=1}^m (p_i - \hat{p})^2$  .

The random variable:

$$H = \frac{n(m-1)V}{\hat{p}(100 - \hat{p})}$$

(where  $n$  is the total number of seeds in the sub-sample) is then distributed according to a chi-squared distribution with  $m - 1$  degrees of freedom if the seed lot is homogeneous. For the tomato experiment,  $m = 5$  and  $n = 400$ .

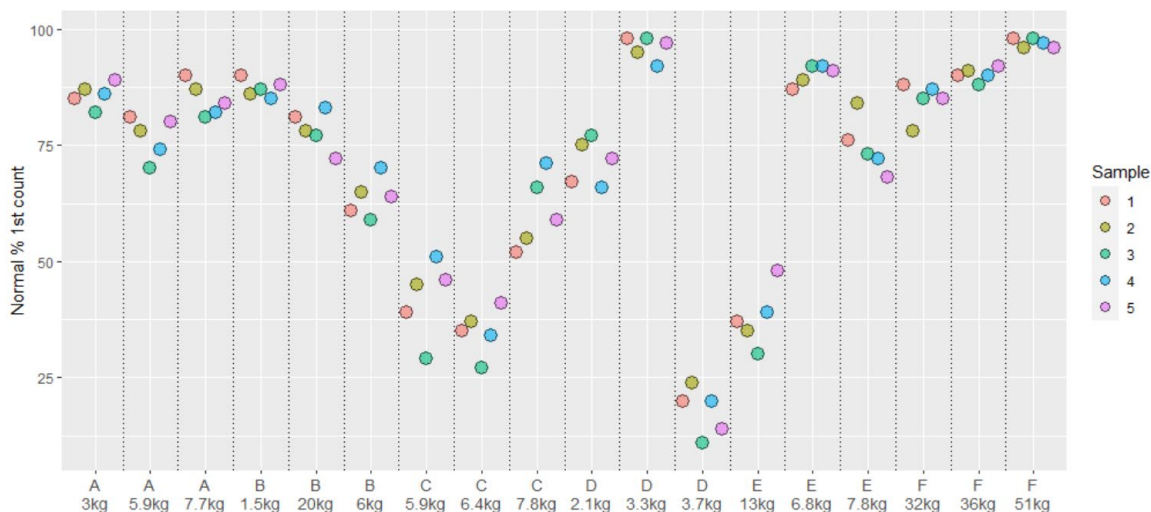
### Heterogeneity test results

The  $H$  statistic and the p-value of the one-sided upper tail test with the null hypothesis that all the sample values are equal (the lower the p-value, the greater the statistical evidence for heterogeneity) are reported in Table 1.

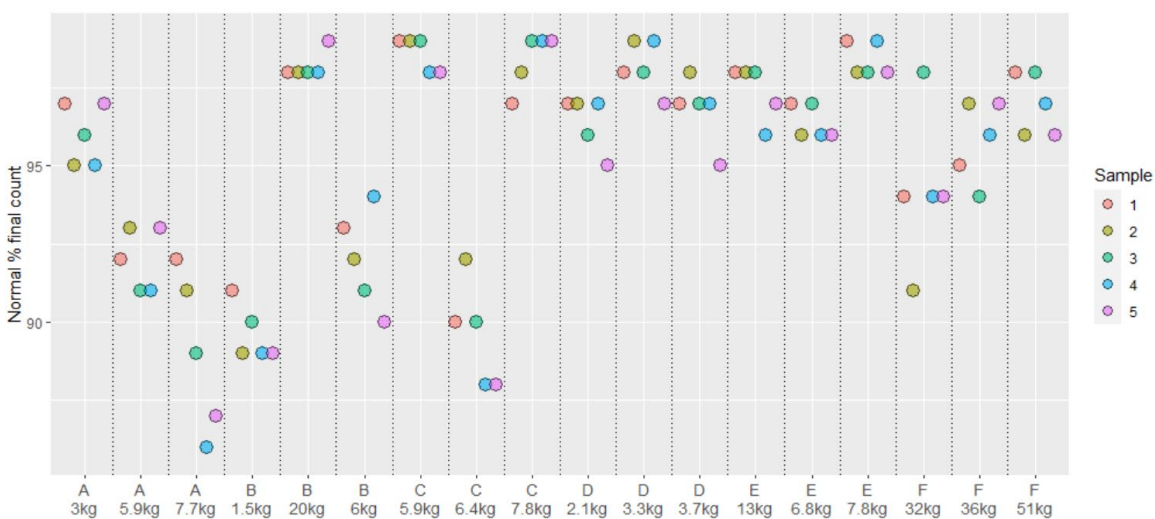
**Table 1.** Mean, *H* statistic and p-value for each sub-sample and for different germination attributes.  
p-values less than 0.05 are highlighted in yellow.

Company	Lot weight	Normal seedlings %, 1 <sup>st</sup> count			Normal seedlings %, final count			Abnormal seedlings %			Dead seeds %		
		Mean	<i>H</i>	p-value	Mean	<i>H</i>	p-value	Mean	<i>H</i>	p-value	Mean	<i>H</i>	p-value
A	3kg	85.8	8.80	0.0663	96.0	4.17	0.3839	3.6	1.38	0.8471	0.4	12.05	0.0170
A	5.9kg	76.6	18.57	0.0010	92.0	2.17	0.7038	3.0	2.75	0.6006	5.0	1.68	0.7936
A	7.7kg	84.8	17.01	0.0019	89.0	10.62	0.0311	8.2	2.55	0.6356	2.8	15.87	0.0032
B	1.5kg	87.2	5.30	0.2575	89.6	1.37	0.8488	3.0	0.00	1.0000	7.4	1.87	0.7600
B	20kg	78.2	16.61	0.0023	98.2	1.81	0.7706	1.8	1.81	0.7706	0.0		
B	6kg	63.8	12.26	0.0155	92.0	5.43	0.2455	4.6	8.39	0.0784	3.4	3.90	0.4201
C	5.9kg	42.0	46.63	0.0000	98.6	3.48	0.4813	1.2	2.70	0.6094	0.2	16.03	0.0030
C	6.4kg	34.8	18.48	0.0010	89.6	4.81	0.3076	7.6	2.96	0.5642	2.8	4.12	0.3906
C	7.8kg	60.6	41.08	0.0000	98.4	8.13	0.0869	1.0	8.08	0.0887	0.6	8.05	0.0898
D	2.1kg	71.4	18.26	0.0011	96.4	3.69	0.4498	1.6	3.05	0.5497	2.0	4.08	0.3951
D	3.3kg	96.0	27.08	0.0000	98.2	6.34	0.1754	1.0	8.08	0.0887	0.8	4.03	0.4017
D	3.7kg	17.8	29.74	0.0000	96.8	6.20	0.1848	1.8	6.34	0.1754	1.4	3.48	0.4813
E	13kg	37.8	29.74	0.0000	97.4	5.05	0.2818	1.6	8.13	0.0869	1.0	8.08	0.0887
E	6.8kg	90.2	8.51	0.0747	96.4	1.38	0.8471	1.4	3.48	0.4813	2.2	5.21	0.2669
E	7.8kg	74.6	30.23	0.0000	98.4	3.05	0.5497	1.2	2.70	0.6094	0.4	12.05	0.0170
F	32kg	84.6	18.79	0.0009	94.2	18.16	0.0012	1.2	2.70	0.6094	3.4	23.38	0.0001
F	36kg	90.2	3.98	0.4084	95.8	6.76	0.1491	2.0	4.08	0.3951	2.2	5.21	0.2669
F	51kg	97.0	5.50	0.2399	97.0	5.50	0.2399	1.6	3.05	0.5497	1.4	3.48	0.4813

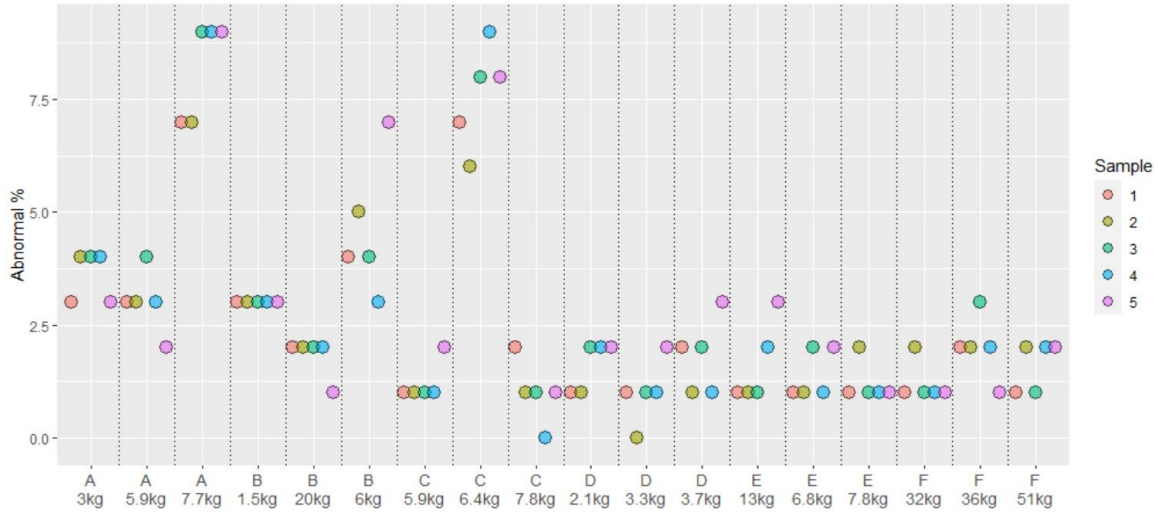
The results in each sub-sample for the different germination attributes are visualized with dot plots in Figures 1, 2, 3 and 4.



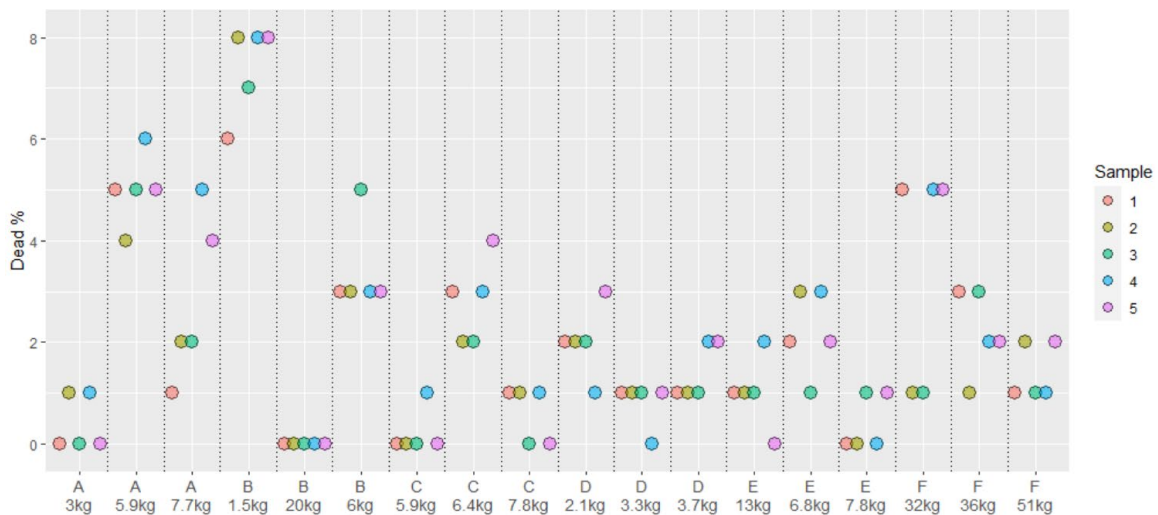
**Fig. 1.** Dot plot of the sub-samples normal seedlings percentages, 1<sup>st</sup> count.



**Fig. 2.** Dot plot of the sub-samples normal seedlings percentages, final count.



**Fig. 3.** Dot plot of the sub-samples abnormal seedlings percentages.



**Fig. 4.** Dot plot of the sub-samples dead seeds percentages.

## Discussion

There is evidence for heterogeneity of the sample results for normal seedlings percentages, 1<sup>st</sup> count (13 p-values less than 0.05 out of 18, i.e. 72%). This heterogeneity is less clear for dead seeds (five p-values less than 0.05 out of 18, i.e. 28%). There is clear evidence for homogeneity for abnormal seedlings percentages (all the p-values are above 0.05) and to a lesser degree for normal seedlings percentages, final count (two p-values less than 0.05 out of 18, i.e. 11%).

In conclusion and based on this study, there is strong evidence of heterogeneity in germination 1<sup>st</sup> count within tomato lots. However, there is evidence of tomato lot homogeneity in final germination counts particularly in counts of normal and abnormal seedlings.

## Follow-up Study Summary

The 2021 Tomato experiment has shown that different lots (different origins, different sizes) are homogeneous for the final count germination tests with regards to a heterogeneity test performed on five samples taken to cover spatially across the lot (Laffont and Remund, 2021). Assuming therefore that the lots are homogeneous, we generated the minimum value for the sub-lots for germination proportions between 0.5 and 0.99 using the multivariate hypergeometric distribution. Then, taking into account Miles' overdispersion factor (Miles, 1963) for tests performed in different laboratories, we generated values for these minimum sub-lot values using the Beta-binomial distribution.

Using Monte Carlo simulations, we finally computed the probability that the test value of the original lot is not different from the test value from a different laboratory performed on the minimum sub-lot value, the difference being assessed using Table 5F from the ISTA Rules (ISTA, 2022). These probabilities being very high (above 0.98) for various situations, we conclude that there is no limit regarding the number of sub-lots that can be elaborated from an homogeneous lot.

### Minimum sub-lot values

Let:

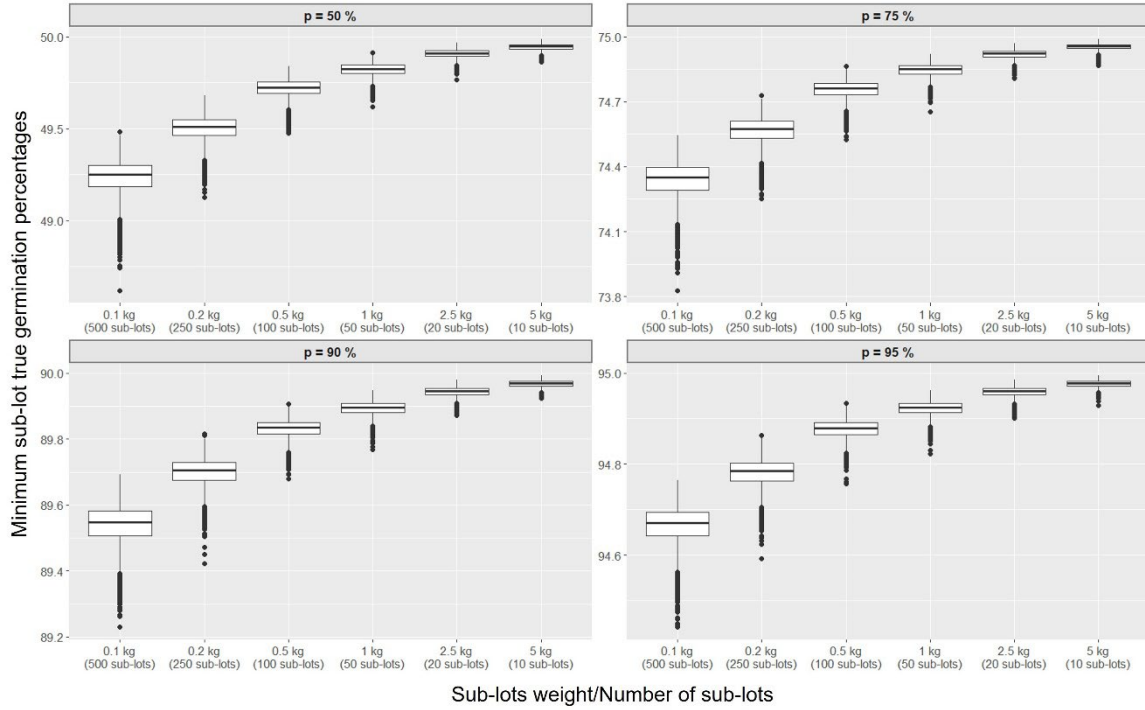
- .  $W_{Lot}$  be the lot weight;
- .  $W_{1000}$  be the 1000-seeds weight;
- .  $W_{Sub}$  be the weight of each sub-lot;
- .  $N = 1000 \times W_{Lot}/W_{1000}$  be the number of seeds in the lot;
- .  $n = 1000 \times W_{Sub}/W_{1000}$  be the number of seeds in each sub-lot;
- .  $M = N/n$  be the number of sub-lots (assuming that  $N$  is divisible by  $n$ );
- .  $\pi$  be the true germination proportion in the lot;
- .  $Y$  be the random variable "*tuple*<sup>1</sup> with 1<sup>st</sup> element the number of seeds to germinate in a random sample of  $n$  seeds out of  $N$  seeds, 2<sup>nd</sup> element the number of seeds to germinate in a random sample of  $n$  seeds out of the remaining seeds (i.e.,  $N - n$ ), ...,  $i$ <sup>th</sup> element the number of seeds to germinate in a random sample of  $n$  seeds out of the remaining seeds, ...,  $M$ <sup>th</sup> element the number of seeds to germinate in the remaining  $n$  seeds".

$Y$  has a multivariate hypergeometric distribution with parameters  $N, (n, n, \dots, n)$  and  $N\pi$  (Laffont et al., 2019). Assuming that the lot is homogeneous, any processes used to elaborate sub-lots lead to assume that the joint distribution of the number of seeds to germinate in each sub-lot is multivariate hypergeometric, and therefore, it is easy to generate the true germination proportion  $\pi_i$  in each sub-lot  $i$  ( $i = 1, 2, \dots, M$ )<sup>2</sup>. For example, 10,000  $\pi_i$ 's sets have been generated considering  $W_{Lot} = 50$  kg,  $W_{1000} = 2.5$  g and  $W_{Sub} = 0.1$  kg, 0.2 kg, 0.5 kg, 1 kg, 2.5 kg, 5 kg. Figure 1 shows the distributions of the minimum values of the 10,000 sets. We can see that the larger the number of sub-lots, the lower the minimum values. In Figure 2, 10,000  $\pi_i$ 's sets have been generated considering  $W_{Lot} = 1$  kg, 2 kg, 5 kg, 10 kg, 50 kg, 100 kg,  $W_{1000} = 2.5$  g and  $M = 20$  sub-lots. Interestingly, we can see that the lower the lot sizes, the lower the minimum values. We note also from these two Figures that the minimum sub-lot values are not very far from the lot true value (maximum difference of 1.64% for  $\pi = 50\%$  in Figure 2).

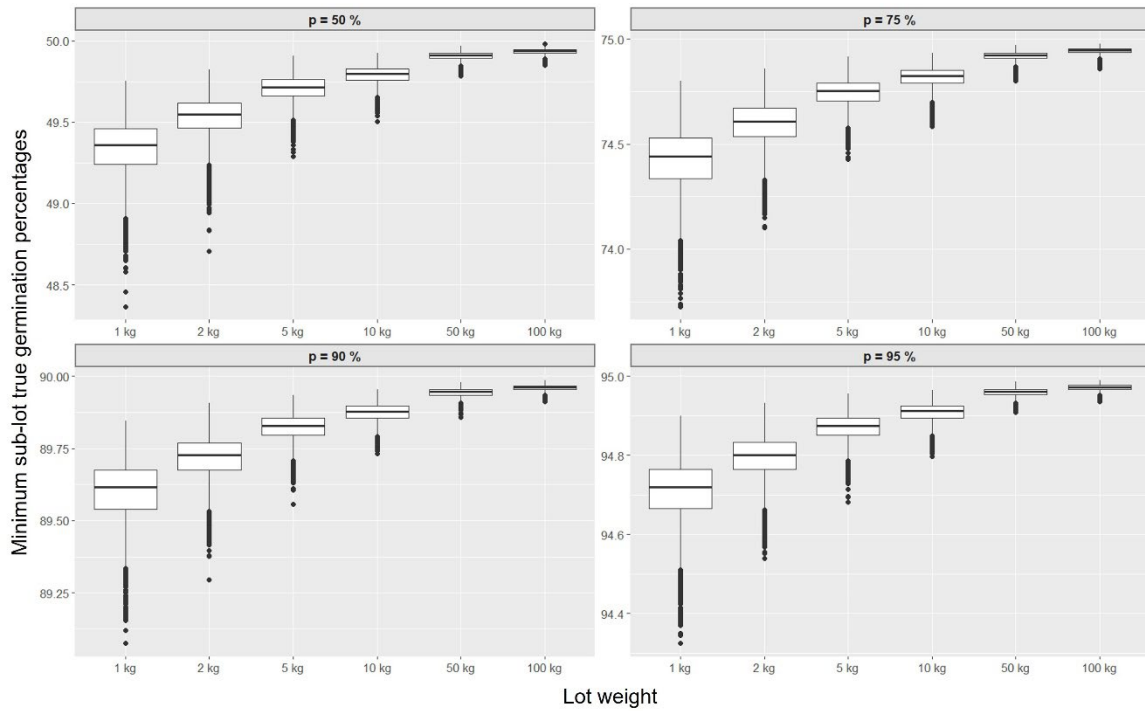
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<sup>1</sup> A tuple is an ordered set of elements. When there are two elements, the tuple is called an ordered pair and when there are three elements, it is called a triplet.

<sup>2</sup> Using for example the function `rmvhyper()` from the R package `extraDistr` (Wolodzko, 2020).



**Fig. 1.** Distributions of the minimum sub-lot true germination percentages for  $W_{Lot} = 50$  kg,  $W_{1000} = 2.5$  g,  $\pi = 50\%, 75\%, 90\%, 95\%$  and  $W_{Sub} = 0.1$  kg,  $0.2$  kg,  $0.5$  kg,  $1$  kg,  $2.5$  kg,  $5$  kg.



**Fig. 2.** Distributions of the minimum sub-lot true germination percentages for  $W_{Lot} = 1$  kg,  $2$  kg,  $5$  kg,  $10$  kg,  $50$  kg,  $100$  kg,  $W_{1000} = 2.5$  g,  $\pi = 50\%, 75\%, 90\%, 95\%$  and  $M = 20$ .

### Test values from a different laboratory of the minimum sub-lot values

Suppose that the test of the sub-lot with the minimum true value  $\pi_m$  is performed by a different laboratory than the one which established the value  $p$  for the original lot. Generating the test value for this new laboratory requires us to take into account over-dispersion related to the inter-laboratory variation. Let  $\sigma$  be the standard-deviation of a test on  $k$  seeds of a lot performed by any laboratories.  $\sigma$  is related to the binomial standard-deviation  $\sigma_B = k\pi_m(1 - \pi_m)$  by a dispersion factor  $f$  as follows (Miles, 1963):

$$\sigma = f\sigma_B$$

with

$$f = 2.38 - 0.8321\pi_m .$$

A model for generating over-dispersed binomial data is the Beta-binomial model with parameters  $k$ ,  $\alpha$  and  $\beta$ . The mean of the Beta-binomial distribution is the same as the mean of the binomial distribution with parameters  $k$  and  $\pi_m$ :

$$\mu = \frac{k\alpha}{\alpha + \beta} = k\pi_m$$

and its variance is:

$$\sigma^2 = k\pi_m(1 - \pi_m) \left( 1 + \frac{k - 1}{\alpha + \beta + 1} \right) .$$

Then:

$$f^2 = 1 + \frac{k - 1}{\alpha + \beta + 1}$$

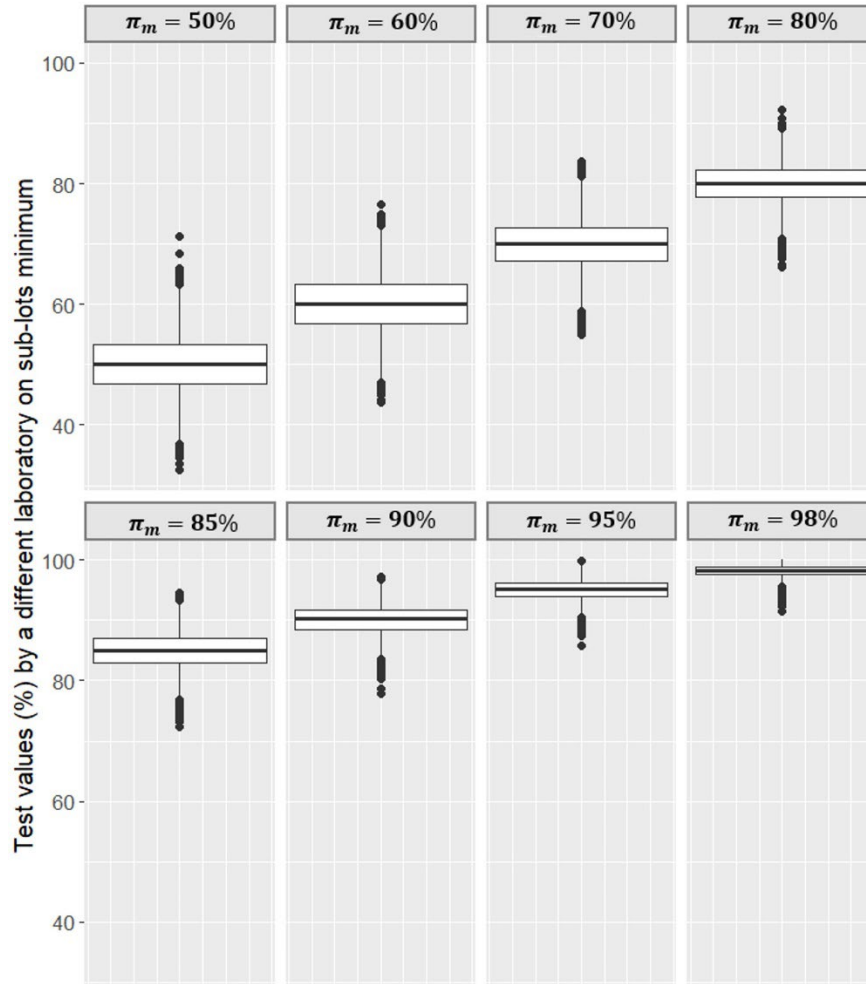
and:

$$\alpha = \pi_m \left( \frac{k - 1}{f^2 - 1} - 1 \right) ,$$

$$\beta = \alpha \left( \frac{1}{\pi_m} - 1 \right) .$$

For a given number of sub-lots  $M$ , 10,000 test values from a different laboratory of the minimum sub-lot value  $\pi_m$  have been generated using the Beta-binomial distribution defined above. For illustration purposes, Figure 3 shows the distribution of these test values from a different laboratory for different minimum sub-lot values  $\pi_m$ ,  $M = 20$  and  $k = 400$ .





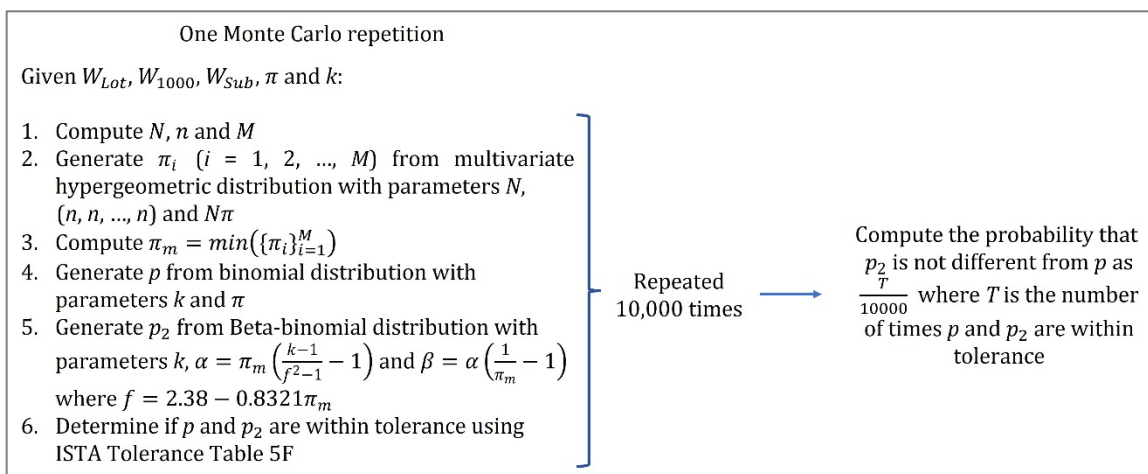
**Fig. 3.** Distributions of the test results from a different laboratory of the minimum sub-lot values  $\pi_m = 50\%$ ,  $60\%$ ,  $70\%$ ,  $80\%$ ,  $85\%$ ,  $90\%$ ,  $95\%$ ,  $98\%$  for  $M = 20$  and  $k = 400$ .

### Acceptable number of sub-lots

Let:

- .  $p$  be the test value of the original lot established from a sample of  $k$  seeds;
- .  $p_2$  be the test value of the sub-lot with minimum true value  $\pi_m$  established from a sample of  $k$  seeds by a different laboratory than the one which established  $p$ .

We are now interested in the probability that  $p_2$  is not different from  $p$  using ISTA Tolerance Table 5F. For this, we have used Monte Carlo simulations described in Figure 4. Table 1 provides probabilities for two extreme lot sizes (1.5 kg and 50 kg) and different number of sub-lots (15, 50 and 500). All the probabilities are very high (above 0.98) giving evidence that given that the original lot is homogeneous, there is no limit in the number of sub-lots that can be elaborated from it.



**Fig. 4.** Monte Carlo simulation details for the computation of the probability that  $p_2$  is not different from  $p$ .

**Table 1.** Probability that  $p_2$  is not different from  $p$  for  $k = 400$  and  $W_{1000} = 2.5$  g.

	$W_{Lot}= 1.5$ kg, $W_{Sub}= 0.1$ kg ( $M=15$ )	$W_{Lot}= 50$ kg, $W_{Sub}= 1$ kg ( $M= 50$ )	$W_{Lot}= 50$ kg, $W_{Sub}= 0.1$ kg ( $M= 500$ )
$p$ (%)	Prob( $p$ and $p_2$ are within Tol)	Prob( $p$ and $p_2$ are within Tol)	Prob( $p$ and $p_2$ are within Tol)
50	0.9865	0.9857	0.9887
55	0.9858	0.9854	0.9836
60	0.9870	0.9871	0.9867
65	0.9852	0.9866	0.9854
70	0.9850	0.9853	0.9860
75	0.9840	0.9845	0.9842
80	0.9848	0.9858	0.9832
85	0.9861	0.9851	0.9849
90	0.9861	0.9892	0.9887
95	0.9893	0.9894	0.9885
99	0.9951	0.9959	0.9958

## References

ISTA (2022). International Rules for Seed Testing, Chapter 5: The Germination Test. *International Seed Testing Association*, Bassersdorf, Switzerland.

Laffont, J-L., Hong, B., Kuo, B-J. and K.M. Remund (2019). Exact theoretical distributions around the replicate results of a germination test. *Seed Science Research* **29**, 64-72.

Miles S.R. (1963) Handbook of Tolerances and Measures of Precision for Seed Testing. *Proceedings of the International Seed Testing Association* 28/3.

Wolodzko, T. (2020). extraDistr: additional univariate and multivariate Distributions. R package version 1.9.1.

<https://CRAN.R-project.org/package=extraDistr>.

## ISTA Peer Method Validation for Tetrazolium test in *Ulmus* spp. L.

*Sergio Pasquini. Centro Nazionale Carabinieri Biodiversità - Peri (VR), Italy.*

### Summary

The scope of the present document is introducing into Chapter 6 of the ISTA Rules a method to test *Ulmus* spp. seeds with Tetrazolium salts.

The comparative testing has been conducted with three labs (two ISTA accredited, one not accredited) experts on daily testing of tree seeds, using 3 different commercial seed lots. The statistical analysis has been conducted by Jean Louis Laffont of the STA Committee.

The method validation test plan was reviewed and approved by two technical ISTA reviewers appointed by the ISTA Tetrazolium Committee:

Rita Zecchinelli  
José Franca Neta

### Introduction

*Ulmus* is an important genus of trees with a broad diffusion in Europe. Usually, the test used for determining the quality of seed lots is Germination test, as the species is easy to germinate. Sometimes it could be useful determine the seed quality using Tetrazolium test, a more rapid test than germination one.

The scope of this test plan is introducing into Chapter 6 of the ISTA Rules an official method to test *Ulmus* spp. with Tetrazolium salts, following the method already described in ISTA Working Sheets on Tetrazolium Testing Volume II, published by ISTA in 2003 (ISBN 978-3-906549-41-5).

### Materials and Methods

#### Seed material

Three lots (lot#1, lot#2 and lot#3) with 2 different levels of quality (1 medium, 2 high) of *Ulmus glabra* L. seeds will be taken from commercial production of this species. From the seed lots, 18 blind samples with 200 seeds each will be prepared and dispatched to participating labs according to the scheme below reported, plus 10 samples for each seed lot for heterogeneity test.

Lot#1	1 sample x 2 replicates x 3 labs + 10 samples for heterogeneity test
Lot#2	1 sample x 2 replicates x 3 labs + 10 samples for heterogeneity test
Lot#3	1 sample x 2 replicates x 3 labs + 10 samples for heterogeneity test

The homogeneity of the seed lots was assessed by the organizer determining the germination of 10 randomly selected samples for each seed lot.

#### Participant laboratories

Samples have been sent to the 3 laboratories listed in Table 1 at the end of October 2021 and the results have been sent to organizer the next month.

Table 1. Participants laboratories to the peer method validation

Participant	Country
Stefanie Kraemer (DEDL04)	Germany
Ralph Jenner (no ISTA lab)	Germany
Sergio Pasquini (ITML06)	Italy

### Germination and Tetrazolium methods

The tests conducted on seeds were Germination test, according to ISTA Rules Chapter 5, Table 5A part 3 for species *Ulmus* spp. and Tetrazolium test, according to how described in ISTA Working Sheets on Tetrazolium Testing Volume II.

Germination test (already existing in ISTA Rules, Chapter 5) - Table 1

Species	Prescription for				
	Substrate	Temp. (°C)	First count (d)	Final count (d)	Additional direction incl. recommendation from breaking dormancy
<i>Ulmus americana</i> <i>Ulmus parviflora</i> <i>Ulmus pumila</i>	TP	20⇔30; (20)	7	14	pericarp may be removed

**Tetrazolium test (to add to ISTA Rules, Chapter 6) - Table 2**

Species	Pretreatment						
	type/minimum time (h)	Preparation before staining	Staining solution (%)	Optimum staining time (h)	Preparation for evaluation	Permitted non-viable tissue	Remarks
<i>Ulmus</i> spp.	Soak 18 hours in water at 20°C	Cut transversely 1/3 from stalk base	1	18 hours, 30°C	Extract embryo	none	none

### Statistical analyses

The comparative test results have been reviewed by Jean-Louis Laffont in March 2022 and a complete Method Validation report has been prepared by the organiser in August 2022.

### Results and Discussion

The comparative test germination/tetrazolium conducted on seeds of *Ulmus* spp. didn't show any difference between the two way of analyse the commercial seed lots tested (Appendix 2).

This peer-validation study supports the introduction of the Tetrazolium test for *Ulmus* spp.: the test can differentiate different seed lots, there are no repeatability issues and results are consistent across laboratories (reproducibility).

### Acknowledgements

Thanks to Jean-Louis Laffont, Statistical Committee chair

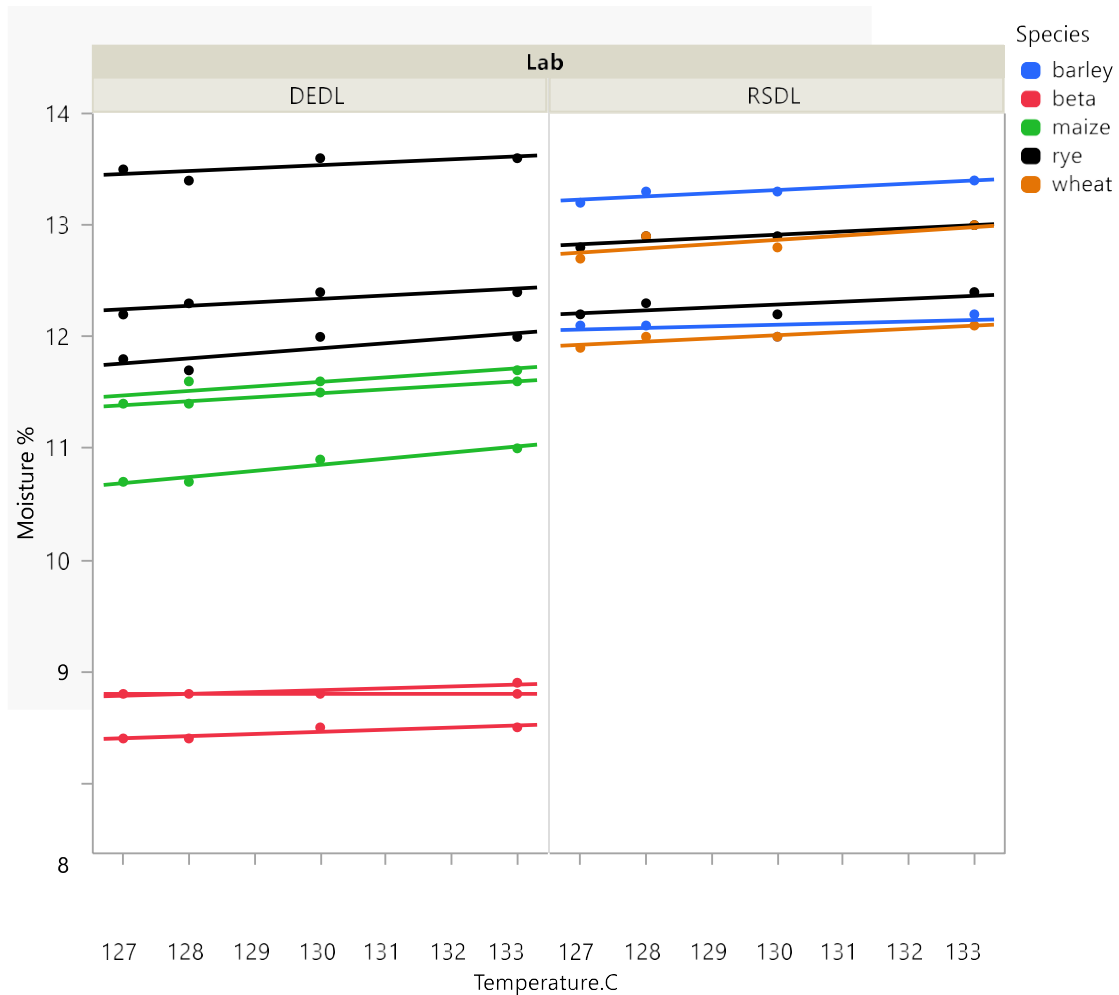
## Statistical analysis of high oven temperature tolerance for seed moisture – peer method validation

Kirk Remund & Jean-Louis Laffont – ISTA Statistics Committee

### 1. Overview of the data analyzed

- Trait analyzed: seed moisture
- Data structure: 2 laboratories, 5 species, 15 seed lots across species and labs
- Oven temperatures: 127°C, 128°C, 130°C, 133°C

### 2. Data exploration with scatter plots



### 3. Assess oven temperature trend in seed moisture

We consider the following linear model with a temperature covariate:

$$y_{ij} = \mu + \alpha_i + L_j + (\alpha L)_{ij} + e_{ij} \quad (1)$$

in which:

- .  $y_{ij}$  is the moisture % for temperature  $i$  and lot  $j$ .
- .  $\mu$  is the intercept.
- .  $\alpha_i$  is the covariate at temperature  $i$  to assess trend (slope).
- .  $L_j$  is the fixed effect of seed lot  $j$ .
- .  $(\alpha L)_{ij}$  is the fixed interaction effect between temperature  $i$  and lot  $j$  (assess heterogeneous slopes).
- .  $e_{ij}$  are the residuals about the linear fit for temperature  $i$  and lot  $j$ .  $e_{ij} \sim$  i.i.d.  $N(0, \sigma^2)$ .

Labs and species combinations are represented in the model in the lots fixed factor in the model. Lots are considered fixed so that lot-to-lot variance is not included in the margin of error around seed moisture across oven temperatures within a specific batch.  $(\alpha L)_{ij}$  was not significant ( $p=0.558$ ) indicating a homogeneous slope model is adequate. The oven temperature covariate ( $\alpha_i$ ) was significant ( $p<0.001$ ) with slope estimate 0.029% ( $\pm 0.007$ ) increase in moisture with each degree increase in oven temperature. While statistically significant this is only a nominal increase as illustrated in the example table below of predicted values for one of the maize seed lots for DEDL lab:

Oven Temp. (C)	Pred. Seed Moisture (95% Pred. Int.)
127	11.40% ([11.26% ; 11.55%])
<b>128</b>	<b>11.43%</b> ([11.29% ; 11.58%])
<b>130</b>	<b>11.49%</b> ([11.35% ; 11.63%])
<b>132</b>	<b>11.55%</b> ([11.40% ; 11.69%])
133	11.58% ([11.43% ; 11.72%])

There is a 0.12% nominal difference in seed moisture predictions in oven temperature from 128°C to 132°C. This moisture difference is below the 0.2% tolerance from the ISTA Rules (paragraph 9.2.6.2) and therefore the high oven temperature tolerance can be expanded to 130°C  $\pm$  2°C for seed moisture in the ISTA Rules. Note that even for the high oven temperature range of 127°C to 133°C the seed moisture prediction range is only 0.17% moisture.

#### 4. Conclusion

The high oven temperature tolerance can be expanded to 130°C  $\pm$  2°C for seed moisture in the ISTA Rules.

#### Reference

Statistical analysis was conducted using JMP version 16 software (a SAS product) and R.



## Appendix 1

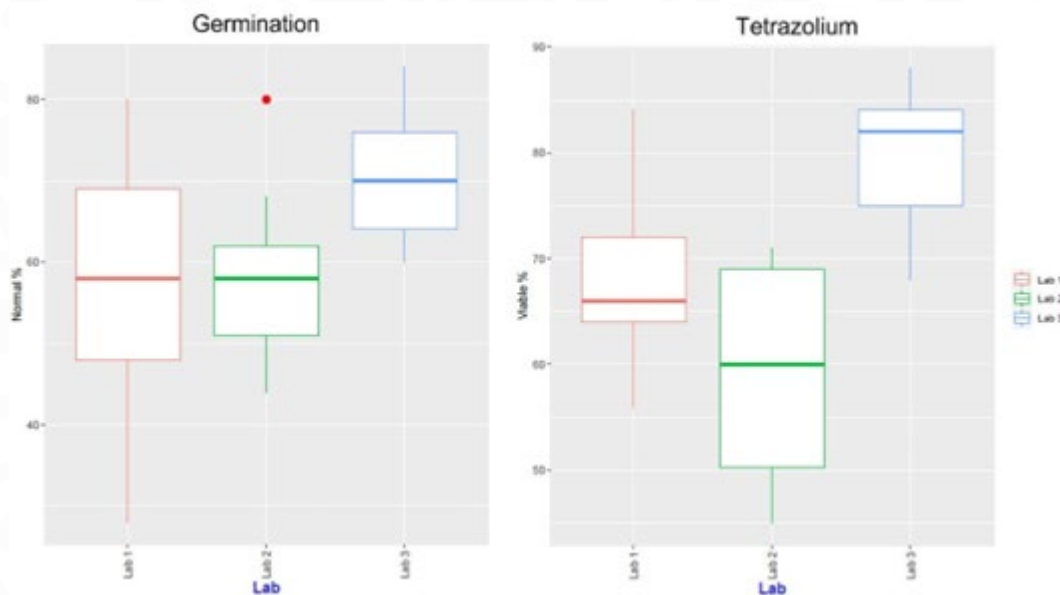
### Tetrazolium testing in *Ulmus* spp. L.: Statistical analysis of peer-validation data

Jean-Louis Laffont – ISTA Statistics Committee

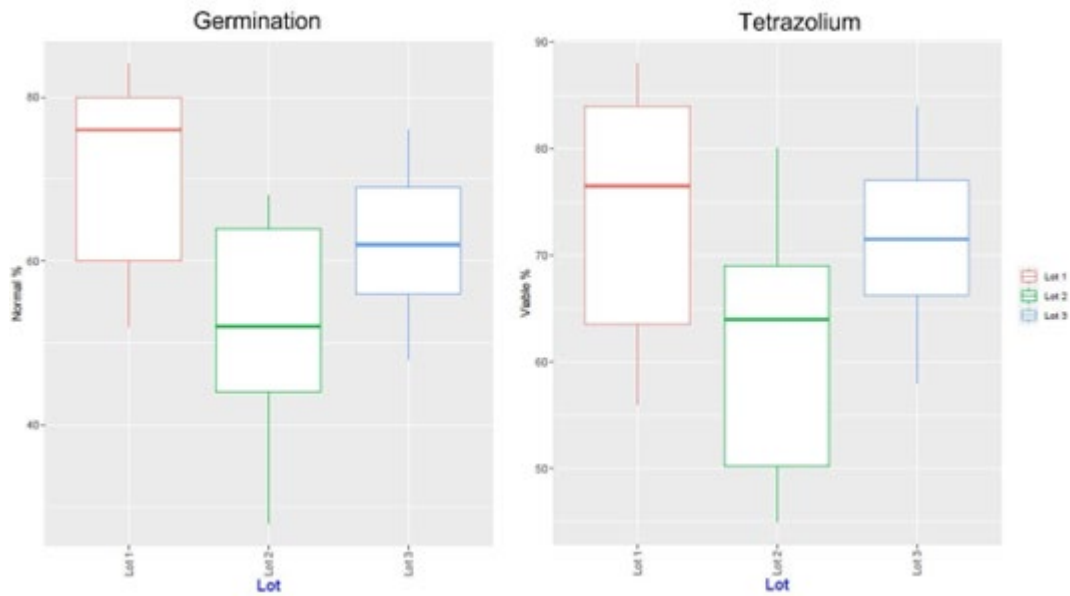
Possible outliers have been assessed using boxplots and using the ISTA Tolerance tables.

The success of the method will be evaluated by estimating the repeatability and reproducibility parameters.

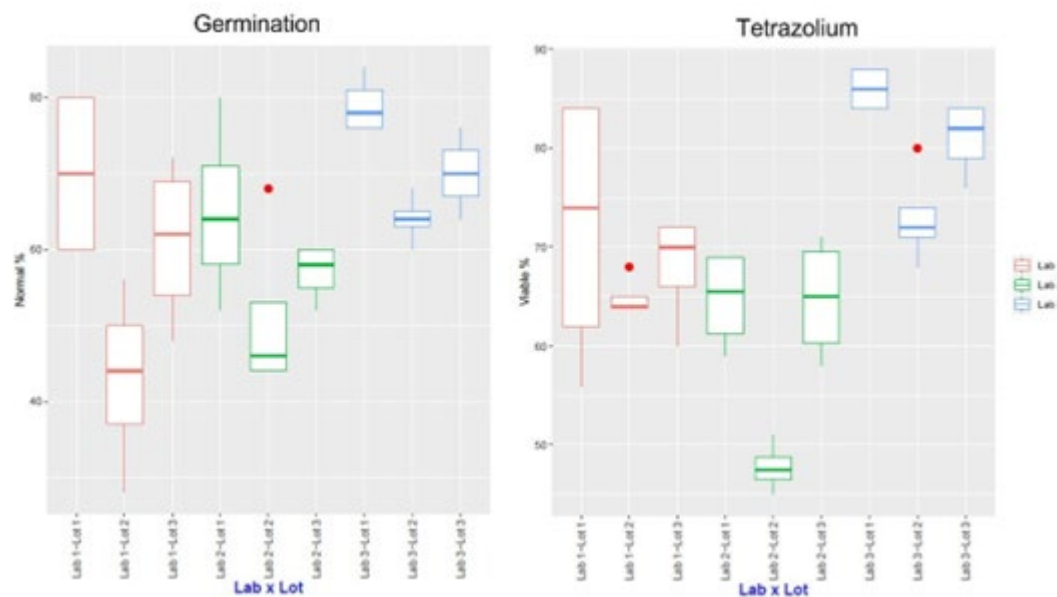
#### 1. Data exploration with side-by-side boxplots



There are differences between laboratories.



There are differences between seed lots.



## 2. Data checking

Data checking has been performed according to ISTA rules by computing tolerances for germination test replicates.

No results are out of tolerance.



**Germination:**

Lot	Lab	Mean	# Reps	# seeds/rep	Range	Tol	Out of Tol
Lot 1	Lab 1	70	4	25 20 36		No	
Lot 1	Lab 2	65	4	25 28 38		No	
Lot 1	Lab 3	79	4	25 8 32		No	
Lot 2	Lab 1	43	4	25 28 39		No	
Lot 2	Lab 2	51	4	25 24 40		No	
Lot 2	Lab 3	64	4	25 8 38		No	
Lot 3	Lab 1	61	4	25 24 39		No	
Lot 3	Lab 2	57	4	25 8 39		No	
Lot 3	Lab 3	70	4	25 12 36		No	

**Tetrazolium:**

Lot	Lab	Mean	# Reps	# seeds/rep	Range	Tol	Out of Tol
Lot 1	Lab 1	72	4	25 28 36		No	
Lot 1	Lab 2	65	4	100 10 19		No	
Lot 1	Lab 3	86	4	25 4 28		No	
Lot 2	Lab 1	65	4	25 4 38		No	
Lot 2	Lab 2	48	4	100 6 20		No	
Lot 2	Lab 3	73	4	25 12 35		No	
Lot 3	Lab 1	68	4	25 12 37		No	
Lot 3	Lab 2	65	4	100 13 19		No	
Lot 3	Lab 3	81	4	25 8 31		No	

**3. Repeatability/Reproducibility**

**Germination:**

Mean	s_repeatability	disp	s_Reproducibility	s_Lab	s_LotxLab
62	8.95	0.92	11.46	7.15	0

The dispersion factor is below 1 (0.92) indicating no over-dispersion of the results within repeatability conditions.

The Lot by Lab interaction standard deviation is very low (zero) indicating consistent results across laboratories.

**Tetrazolium:**

Mean s\_repeatability disp s\_Reproducibility s\_Lab s\_LotxLab  
69 6.28 0.96 12.19 10.26 1.98

The dispersion factor is below 1 (0.96) indicating no over-dispersion of the results within repeatability conditions.

The Lot by Lab interaction standard deviation is very low (1.98) indicating consistent results across laboratories.

**4. Mixed model for comparing Lot means**

**Germination:**

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	2094.222	1047.111	2	31	13.06547	7.666548e-05

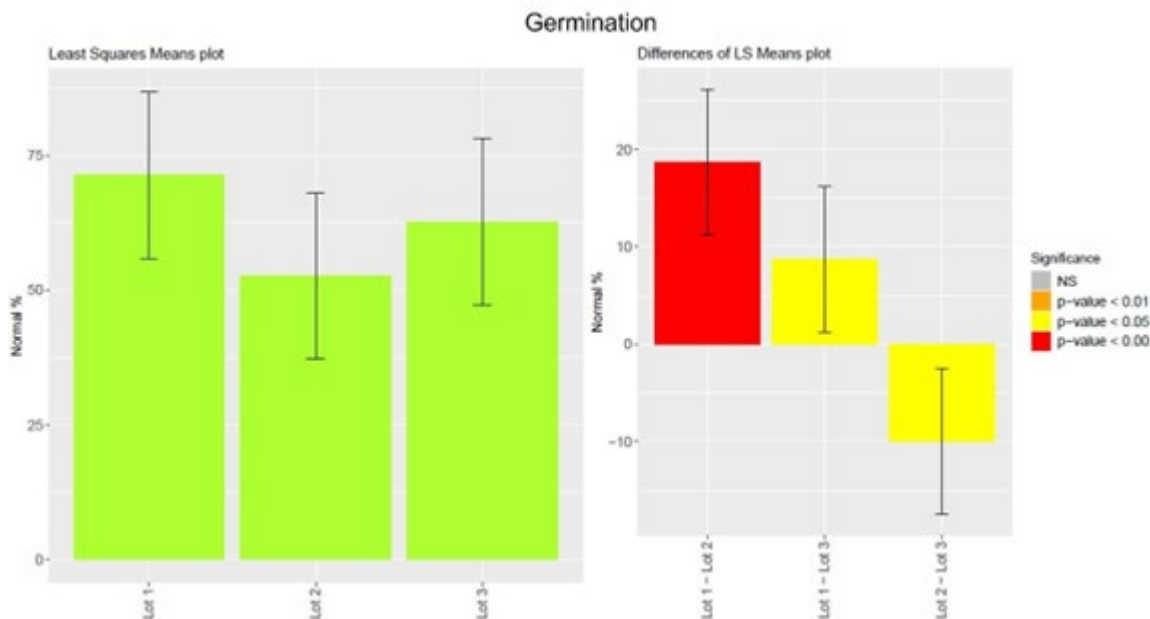
Least Squares Means Table:

	Estimate	Std. Error	lower	upper
Lot 1	71.33333	4.870724	55.89189	86.77478
Lot 2	52.66667	4.870724	37.22522	68.10811
Lot 3	62.66667	4.870724	47.22522	78.10811

Differences of Least Squares Means Table:

	Estimate	Std. Error	lower	upper
Lot 1 - Lot 2	18.66667	3.654755	11.212746	26.120588
Lot 1 - Lot 3	8.66667	3.654755	1.212746	16.120588
Lot 2 - Lot 3	-10.00000	3.654755	-17.453921	-2.546079

The Lot effect is highly significant (p-value <0.01). The Least Squares Means are displayed in the following bar plot with error bars added corresponding to their standard errors:



**Tetrazolium:**

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

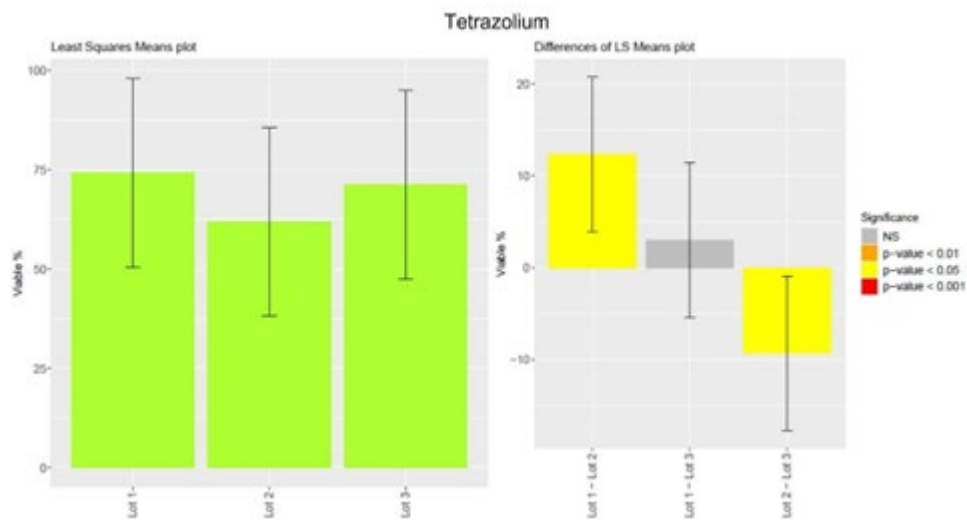
Differences of Least Squares Means Table:

---

	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

The Lot effect is significant at the 5% level. The Least Squares Means are displayed in the following bar plot with error bars added corresponding to their standard errors:

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## Appendix 2

R function for producing Table 1 (requires R package extraDistr):

```
MC.sublots <- function (W=50,w=0.1,w1000=2.5,k=400,nrep=10000)
{
  # Probability that the test value from a different laboratory for the minimum sub-lot
  # is different from the test value of the original lot using ISTA Tolerance Table 5F
  # W: lot weight (kg)
  # w: sub-lot weight (kg)
  # w1000: weight of 1000 seeds (g)
  # k: number of seeds tested
  # nrep: number of Monte Carlo simulations
  # Package extraDistr required

  # Function called:
  germTol2Labs <- function(n = 400, avg)
  {
    # Germination Tolerance for tests in 2 laboratories
    # Miles (1963) Table G5, columns C, F and I, 400 seed tests
    #-----
    #
    # Studentized range quantile, two-way test at 5% significance level, df=infinity
    sr.q <- 2.77
    # Reported germination
    avg.rep <- round(avg, 0)
    # Tolerances
    Tol <- vector("numeric", 0)
    for(i in 1:length(avg)) {
      if(avg[i] > 50)
      {
        f<-round(2.38-0.008321*avg.rep[i],2)
        Tol <- c(Tol, floor(round(sr.q * f * sqrt(((avg.rep[i] - 0.5) *
          (100 - avg.rep[i] + 0.5))/n) + 0.2, 2)))
      }
      else
      {
        f<-round(2.38-0.008321*(101-avg.rep[i]),2)

```

```
      if (avg.rep[i]==0) avg.rep[i]<-1
      Tol <- c(Tol, floor(round(sr.q * f * sqrt(((101 - avg.rep[i] -
          0.5) * (avg.rep[i] - 0.5))/n) + 0.2, 2)))
    }
  }
  Tol
}

W<-W*1000 # Weights in grams
w<-w*1000
N1<-round(W*1000/w1000)
n<-round(w*1000/w1000) # Sub-lots size
M<-round(N1/n) # Number of sub-lots
N<-n*M # Adjusted lot size

p<-vector("numeric",0)
minSubLot2<-vector("numeric",0)
P<-c(seq(.5,.95,.05),.99) # True lot values
for (i in 1:length(P))
{
  p<-c(p,rbinom(nrep,k,P[i])/k) # Original lot test value
  N.g<-round(P[i]*N,0) # True number of seeds to germinate in the lot
  minSubLot<-vector("numeric",0)
  for (j in 1:nrep)
  {
    # Determining min sub-lot assuming the lot is homogeneous
    pm.ij<-min(rmvhyper(1, rep(n,M), N.g))/n
    # Simulating test results in a different laboratory for the min sub-lot
    # Overdispersion factors (Miles, 1963)
    if(pm.ij > .5) f<-round(2.38-0.8321*pm.ij,2)
    else f<-round(2.38-0.8321*(1.01-1*pm.ij),2)
    # Parameters of the Beta-binomial distribution
    alpha<-pm.ij*(k-1)/(f^2-1)-pm.ij
    beta<-alpha*(1/pm.ij-1)
    minSubLot<-c(minSubLot,rbinom(1,k,alpha,beta)/k)
  }
  minSubLot2<-c(minSubLot2,minSubLot)
}
}
```

```
# Output
P<-rep(P*100,each=nrep)
p<-round(p*100)
minSubLot2<-round(minSubLot2*100)
tol<-germTol2Labs(k,round((p+minSubLot2)/2))
withinTol<-rep(0,length(p))
withinTol[round(abs(p-round(minSubLot2)))<=tol]<-1
proba<-as.vector(sapply(split(withinTol,P), function(x){sum(x)/length(x)}))
P<-as.vector(sapply(split(P,P), function(x){x[1]}))
cat(paste("M=",M),sep="\n")
return(data.frame(pi=P,proba))
}
```