

Technical protocol for the ISTA Experiment on Sampling and Testing Seed Mixtures

Background

The present ISTA Rules only cover the issuance of ISTA Certificates for seed lots of one variety or species. The following experiment shall provide data to establish the necessary information that may allow a Rules change proposal for the testing of seed mixtures.

1. Scope of the experiment

This experiment is directed at checking the sampling and testing of seed mixtures. The experiment does not include either the certification of the mixture lot or the issuance of ISTA Certificates for the lot.

All accredited ISTA member laboratories can participate in this experiment.

2. Course of the experiment

1. ISTA laboratories should send a short informal notice about their willingness to participate in this experiment to the ISTA Secretariat.
2. Participants are expected to sample and test mixture seed lots during the course of the experiment. However, there is no minimum number of seed lots that have to be sampled and tested by participants.
3. For sampling and testing of seed mixture lots, the methods used shall be as described under 3. of this protocol.
4. Participants are expected to report the test results regarding purity, composition and thousand seed mass as well as the declared composition of the mixture lot together with some additional information on the attached form to the ISTA Secretariat.
5. Participants cannot get a financial refund from ISTA for sampling and testing expenses.
6. The ISTA Secretariat will forward submitted forms to Michael Kruse, member of the Bulking and Sampling Committee, for statistical analysis. The goal of this analysis is to quantify the distribution of deviations between the declared composition and the test results obtained by the proposed method. This distribution will be the basis used to establish tolerance tables as part of a Rules Change Proposal.
7. The Experiment will run up to the end of 2009. The results of the experiment shall be reported during the ISTA Congress 2010. The identity of the mixtures, companies, countries and ISTA laboratories will not be evaluated and published.

3. Methods for sampling and testing seed mixtures

Sampling

The ISTA Rules Chapter 2 shall be followed for sampling mixture seed lots and for obtaining working samples for testing by taking into account the following additional regulations, restrictions and changes.

Definitions

(The ISTA Rules 2.2)

2.2. Seed Mixture Lot

A seed mixture lot is a specified quantity of seed that is physically and uniquely identifiable and includes by declaration seed of two or more species.

2.2 Mixture Components

A mixture component is seed of a species or variety that is given in the declaration of the seed mixture lot. Inert Matter, e.g. additives to improve sowability, are also regarded as mixture components if given as such in the declaration.

The Seed Mixture Lot

2.5.4.1 Size of the lot

(iii) For mixtures where all the components have the same maximum seed lot size, the mixture will have this maximum seed lot size. Where the mixture contains components that have different maximum seed lot sizes the components are grouped according to the maximum seed lot size and the maximum seed lot size of the mixture is equal to that of the most abundant group in terms of percentage by weight of the lot. If a seed mixture lot includes coated seeds, the mass of the coated seeds is used for the determination of the lot size and not the mass of the seed without coating material. Inert additives, if given in the declaration, are included in the lot size.

Appendix B of the ISTA Rules as well as the "ISTA/ISF Herbage Seed Lot Size Experiment" are not extended to seed mixtures.

2.5.4.1.1 Declaration of the seed mixture lot

The applicant must provide information about the

- identity of the mixture components, i.e. the genus and, if applicable, the species within the genus and/or the varieties within the species (or the identity of the additives) and
- the mass proportions of the mixture components.

2.5.1.2 Sampling Intensity

Sampling intensity for seed mixtures is as described in The ISTA Rules, Tables 2.1 and 2.2, however it is strongly recommended to take more samples than the minimum number and when sampling containers to take care that all positions in the containers (i.e. center, top, bottom, corners, edges) are represented when primary samples are taken.

2.5.1.3 Taking primary samples

Seed mixtures. Containers or positions in the seed mixture lots to be sampled should be selected according to a systematic plan. If the sequence of the containers is not detectable, the containers to be sampled should be selected with equal distances from each other.

For taking primary samples from seed mixture lots in containers, methods listed in 2.5.1.3 must be used. It is also possible to obtain the primary sample from the container by one of the sample reduction method listed below.

For taking primary samples from the stream of seeds, automatic or hand sampling methods may be used according to the ISTA Rules.

2.5.4.4 Submitted sample

The size of the submitted sample shall be equal to the sample size of the component with the greatest sample size according to the ISTA-Rules Table 2A.

2.5.4.5 Sample reduction

For reduction of samples taken from seed mixture lots, the methods listed in 2.5.2.2 of the ISTA Rules may be used.

The variable divider is not recommended for seed mixtures containing species with multiple seed units since they may cause cross-contaminations.

For seed mixtures, the hand halving method or the spoon method may be used when hand halving or spoon method is allowed for at least one of the components in the mixture seed lot.

Chapter 3. Purity

3.2.4 Component species

In a seed mixture, the pure seed of each species stated by the applicant to be part of that mixture shall constitute a component species. The pure seed assessment shall follow the appropriate PSD of each species.

3.5.1 Working sample

The purity analysis shall be made on a working sample taken from the submitted sample in accordance with 2.5.2, the submitted sample having been received in accordance with 2.5.1. Except for species of *Poaceae* (*Gramineae*) for which the uniform blowing method is to be used, the size of the working sample shall be:

either a weight estimated to contain at least 2500 seed units,
or not less than the weight indicated in column 4 of Table 2A.

For seed mixtures, a calculation is required to define the right sample size on the basis of the weights of table 2.A assumed as weights of 2500 seeds of each species. The following formula can be used to obtain a mixture sample of 2500 seeds:

Working sample weight for mixture = $100 / (\%C_1/PWC_1 + \%C_2/PWC_2 + \%C_3/PWC_3 + \%C_4/PWC_4 + \dots)$

Where:

$\%C_i$ = percentage of the component "i" in the mixture

PWC_i = purity sample weight of component "i" (table 2.A)

To illustrate the use of this equation, see the following example:

Species in the mixture	Component percentage	Weight of purity working sample Table 2.A	Weight of each species in the sample of calculated weight	Number of each species in the sample of calculated weight
C1 <i>Festuca arundinacea</i>	30	5	0,97	484
C2 <i>Festuca rubra</i>	30	3	0,97	806
C3 <i>Lolium perenne</i>	30	6	0,97	403
C4 <i>Poa pratensis</i>	10	1	0,32	806
Total			3,23	2500

Working sample weight for mixture

$$= 100 / (\%C_1/PWC_1 + \%C_2/PWC_2 + \%C_3/PWC_3 + \%C_4/PWC_4).$$

$$= 100 / (30/5 + 30/3 + 30/6 + 10/1)$$

$$= 100 / (6+10+5+10)$$

$$= 100/31$$

$$= 3.23$$

3.2.1 Pure seed

The pure seed shall refer to the species stated by applicant, or found to predominate in the test, and shall include all botanical varieties (varietas) and cultivars of that species (or more than one species, in the case of seed mixtures) including:

3.5.3 Seed Mixtures

The pure seed shall be separated into the component species and each component species shall be weighed in grams to the minimum number of decimal places necessary to calculate the percentage to one decimal place (3.5.1.A).

3.5.2.A Separation

3.5.2.A.5 Uniform blowing method

This method is obligatory for *Poa pratensis*, *Poa trivialis* and *Dactylis glomerata*, except when they form components of a seed mixture. The uniform blowing method shall not be used for species components of seed mixtures.

Chapter 4. Other seeds by number

4.5.1 Working sample

a) The size of the working sample shall be either a weight estimated to contain at least 25000 seed units or not less than the weight indicated in Table 2A, Part 1, column 5. For seed mixtures the working sample size shall be ten times the purity sample working size determined according to the calculation in 3.5.1.

17.5.A Reporting results

17.5.A.23.1 Purity

The percentage by weight of pure seed, inert matter, and other seeds shall be given to one decimal place. In the case of a seed mixture, the pure seed percentage shall be calculated using the total weight of the pure seed of all component species. The percentage by weight of the pure seed of each component species shall be included under 'Other determinations'. If the percentage by weight is below 0.05, it shall be stated as 'TR' (for 'Trace'). If no 'Inert matter' or 'Other seeds' are found, this should be entered as '0.0'. The scientific name shall be used for every species of 'Other species' found in the purity test.

Chapter 5. Germination

5.6.1 Working sample

Four hundred seeds in replicates of 100 are taken at random from the pure seed (5.3) and spaced uniformly and adequately apart on the moist substrate. Replicates may be divided into sub-replicates of 50 or 25 seeds depending on the size of seeds and the amount of space needed between them. For seed mixtures, four hundred seeds are planted from the pure seed of each component species, except that for species representing less than 15% of the mixture, 2 x 100 seeds are planted. For species representing less than 5% of the mixture germination testing is not carried out except on the specific request of the customer. When such a request is made the germination test is carried out on 2 x 100 seed replicates of seed. If sufficient seed are not available from the pure seed fraction the test will be carried out on the all of the pure seed of the species in the pure seed fraction.

5.9 Reporting results

The following items shall be entered in the report when reporting the result of a germination test:

- duration of test.
- percentage of normal, abnormal seedlings, hard seeds, fresh seeds and dead seeds. If the result for any of these categories is found to be nil, it shall be entered as '0'.

In the case of a seed mixture, the results of the germination test are reported to the nearest whole number under the categories normal seedlings, hard seed, fresh seed, abnormal seedlings, and dead seeds. No germination results are reported in the 'Germination' section of a report. The germination results for each component species shall be included under 'Other determinations' under the categories normal seedlings, hard seed, fresh seed, abnormal seedlings, and dead seeds. When 400 or 200 seeds have been tested the results are reported as a percentage and the number of seeds tested is also reported. Where less than 200 seeds have been tested the actual number of seeds in each category are reported together with the total number of seeds tested.

Chapter 10: Weight Determination

10.5.4 Seed Mixtures

For seed mixtures the 1000 seed weight is determined by counting the number and weight of each seed component in the purity sample rather than counting replicates as in 10.5.3.

10 .6 Calculation and expression of results

If counting the number of seed of each component of a mixture in a purity sample the 1000 seed weight is calculated according to the weight and number of seeds of each component

e.g.

Species in the mixture	Number of seed in purity sample	Weight of seed	Thousand Seed Weight
<i>Festuca arundinacea</i>	484	0.9677	= (0.9677/484)*1000 = 2.00
<i>Festuca rubra</i>	806	0.9677	1.20
<i>Lolium perenne</i>	403	0.9677	2.40
<i>Poa pratensis</i>	806	0.3226	0.40

Chapter 17. Certificates

As ISTA certificates are not allowed to be issued to a for a seed lot of a mixture, chapter 17 should be referred to as 'Not applicable'.

2.5% TOLERANCE RANGES TO BE USED TO COMPARE REPLICATE RESULTS OF GERMINATION TESTS¹

Average Number of Category [*] in Sample		Seed Tested: 2x100 Tolerance	Seed Tested: 4x100 Tolerance
A	B	C	D
99	2	4	5
98	3	5	6
97	4	6	7
96	5	6	8
95	6	7	9
94	7	8	10
93	8	8	10
92	9	9	11
91	10	9	11
90	11	9	12
89	12	10	12
88	13	10	13
87	14	11	13
86	15	11	14
85	16	11	14
84	17	11	14
83	18	12	15
82	19	12	15
81	20	12	15
80	21	13	16
79	22	13	16
78	23	13	16
77	24	13	17
76	25	13	17
75	26	14	17
74	27	14	17
73	28	14	17
72	29	14	18
71	30	14	18
70	31	14	18
69	32	14	18
68	33	15	18
67	34	15	18
66	35	15	19
65	36	15	19
64	37	15	19
63	38	15	19
62	39	15	19
61	40	15	19
60	41	15	19
59	42	15	19
58	43	15	19
57	44	15	19
56	45	15	19
55	46	15	20
54	47	16	20
53	48	16	20
52	49	16	20
51	50	16	20

¹ Derived from

Miles S. R. (1963) *Handbook of tolerances and measures of precision for seed testing* Proc.Int. Seed Test. Ass. **28 pg 644**

* Category can be number of: normal or abnormal seedlings; hard, fresh, dormant or dead seed; or seed with a particular disease