



International Rules for Seed Testing 2020

Introduction to the ISTA Rules

**Including changes and editorial corrections adopted at the
Ordinary General Meeting 2019, Hyderabad, India**

Effective from 1 January 2020

Note on the use of the translations

The electronic version of the International Rules for Seed Testing includes the English, French, German and Spanish versions. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.

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Preface to the 2020 Edition of the ISTA Rules

Since 2014, the *International Rules for Seed Testing* (ISTA Rules) are primarily available in electronic form only. The ISTA Rules can be downloaded as a complete PDF file or as individual chapters from:

<http://www.ingentaconnect.com/content/ista/rules>

If required, users of the ISTA Rules can print their own copies. For further information on the ISTA Rules, see:

<http://www.seedtest.org/rules>

The electronic version includes the English, French, German and Spanish versions of the ISTA Rules. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.

Seed health testing methods

Previously, the seed health testing methods were published as a separate Annex to Chapter 7 of the ISTA Rules. They are now available as separate method sheets from the ISTA web site at:

<http://www.seedtest.org/seedhealthmethods>

Details of changes

The 2020 changes are editorial corrections or Rules changes adopted at the Ordinary General Meeting held at Hyderabad, India, in June 2019. Edits were made in Adobe InDesign by Vanessa Sutcliffe of HeartWood Editorial (www.heartwoodeditorial.co.uk).

The changes in the text content from the previous edition of the ISTA Rules are listed below. They can be displayed as yellow highlighted text as a 'layer' within the electronic copy with comments on what has changed.

For the previous history of amendments to the ISTA Rules, see the Prefaces for 2003 to 2019 on the ISTA website.

Ernest Allen, ISTA Rules Committee Chair

Susan Alvarez, ISTA Rules Committee Vice-Chair

ISTA Secretariat

Changes to the ISTA Rules for 2020

General editorial

Re-labelling of Tables throughout the Rules for consistency, with Chapter number and a capital letter; subsequent cross-referencing updated:

- Table 2.1 becomes Table 2A;
- Table 2.2 becomes Table 2B;
- Table 2A becomes Table 2C;
- Table 2B becomes Table 2D;
- Table 2C becomes Table 2E;
- Table 2D becomes Table 2F;
- Table 2E becomes Table 2G;
- Table 2F becomes Table 2H;
- Table 2G becomes Table 2I;
- Table 4.1 no longer has a title, consistent with the similar table under section 3.5.1;
- Table 8.5 becomes Table 8B;
- Table 8.6 becomes Table 8C;
- Table 8.7 becomes Table 8D;
- Table 8.8 becomes Table 8E;
- Table 8.9 becomes Table 8F;
- Table 8.10 becomes Table 8G;
- Table 8.11 becomes Table 8H;
- Table 8B becomes Table 8I;
- [changed for 2019 Rules] Table 17.1 to Table 17A;
- [changed for 2019 Rules] Table 17.2 to Table 17B;
- [changed for 2019 Rules] Table 17.3 to Table 17C;
- [changed for 2019 Rules] Table 17.4 to Table 17D.

Changes to nomenclature throughout the Rules, according to the 7th edition of the *ISTA List of Stabilised Plant Names*, prepared by the Nomenclature Committee.

Introduction

I-1: Sentences added in response to a Motion concerning 'ISTA's position on integrating advanced technologies in classical seed testing methods' discussed at 2018 OGM.

Chapter 1

- 1.3:** Editorial change requested by BSC to correct erroneous reference; approved by BSC by vote.
- 1.5.2.2:** Editorial change required to clarify placement of specified 'species' or 'inert matter' in the Purity section on the OIC.
- 1.5.2.4:** *Phelipanche* added where *Orobanche* is mentioned, with the assumption that the *Orobanche/Phelipanche* species concerned are parasitic plants with small dust-like seeds.
- 1.5.2.6:** It was no longer obvious why the % of normal seedlings obtained at the end of the test period, when the test is extended, must be reported. This prescription has been removed from the Rules.

1.5.2.20: Editorial change to 'Seed mixtures' under 'Certificates'. As a general principle, any statement of the applicant may be reported only in the space reserved for applicant statements/declarations. This space is reserved at the top of the ISTA certificate under 'Stated by applicant'. Therefore, the components of seed mixtures as reported by the applicant cannot be reported under 'Analysis results' as this space is reserved for laboratory results obtained by an ISTA laboratory through seed testing.

1.5.2.20.1: Clarification on how to report purity content of seed mixtures (i.e. the percentage by weight of pure seed, inert matter, and other seeds).

Chapter 2

2.5.1.1: Revision discussed by the BSC and approved by vote, to specify that seed may also be sampled from the seed stream, including before it enters containers, as for automatic sampling. This revision is consistent with wording in Rule 2.5.1.3 'Taking primary samples'.

2.5.1.5: Revision to clarify procedures for obtaining submitted samples for moisture testing; proposal discussed by BSC and approved by vote.

2.5.1.6: 'Packing' added to heading. Many seed companies have ISTA accredited laboratories, where the warehouse in which the samples are taken and the testing laboratory are on the same premises, making it superfluous to seal the sample if the ISTA Sampler delivers it personally to the laboratory. The former wording was not consistent with 2.5.4.3, which already makes provision for this. Proposal discussed by BSC and approved by vote.

2.5.2.2, 2.5.2.2.1d and e: For variable and rotary dividers it is not necessary to mix the composite sample before dividing, as mixing of the seed takes place during the dividing process (stated in the *ISTA Sampling Handbook*). In the Rules it is required that 'the seed sample must first be thoroughly mixed' and the auditors apply this strictly, also for variable and rotary dividers. The exclusion of the pre-mixing requirement of these two dividers is now included in the Rules. Proposal discussed by the BSC and approved by vote.

2.5.2.2: Two paragraphs deleted. The first paragraph has been moved to 2.5.1.5 as it is more applicable to obtaining a submitted sample (for moisture) than it is for sample reduction methods. The deletion of the second paragraph is for consistency. Obtaining working samples for specific tests is generally specified in the applicable Chapters. This paragraph was a duplication of what is already stated in 9.2.5.2. Proposal was discussed by BSC and approved by vote.

2.5.2.2.3: Revision requested by BSC for readability and to remove the part that the spoon method is the recommended method for seed health testing. Proposal discussed by BSC and approved by vote.

2.5.4.2.1: *Festuca trachyphylla* was erroneously omitted from the Species group 2 list some years ago. It was part of the experiment and should be included.

2.5.4.4: Editorial change requested by BSC to correct erroneous reference; approved by BSC by vote.

Table 2C (formerly 2A): Updates to nomenclature as a result of changes to the *ISTA List of Stabilised Plant Names*.

Table 2C (formerly 2A) Part 3: Lot sizes and sample sizes moved from Table 2C (formerly 2A) Part 2 to Part 3 for *Malva sylvestris*, as this is not a woody species.

Table 2C (formerly 2A) Part 3: Inclusion of *Salvia hispanica* L. to the ISTA Rules; proposed sample and maximum seed lot size submitted to BSC by Purity Committee based on thousand-seed weight determinations. Discussed by the members of BSC and approved by vote.

Chapter 3

3.2.3: Editorial change required due to species in Taxodiaceae being absorbed into Cupressaceae. Corrections approved by Nomenclature Committee.

Table 3B Parts 1 and 2: Updates to nomenclature as a result of changes to the *ISTA List of Stabilised Plant Names*.

Chapter 4

Throughout Chapter, *Phelipanche* added where *Orobanche* is mentioned, with the assumption that the *Orobanche/Phelipanche* species concerned are parasitic plants with small dust-like seeds.

Chapter 5

5.6.4: Precision added relating to the extension of the duration of the germination test, and adapting the date of the final count when it ends on a non-working day.

5.9: It was no longer obvious why the % of normal seedlings obtained at the end of the test period, when the test is extended, must be reported. This prescription has been removed from the Rules.

Table 5A: Updates to nomenclature as a result of changes to the *ISTA List of Stabilised Plant Names*.

Table 5A Part 1: GA₃ is indicated as a dormancy breaking treatment for *Avena sativa* in section 5.6.3.1, and now added to Table 5A for this species, to achieve greater concordance and understanding.

Table 5A Part 1: Organic growing media added as a primary media for the germination of *Glycine max*.

Proposal supported by a validation study done within the Germination Committee.

Table 5A Part 1: Organic growing media added as a primary media for the germination of *Phaseolus vulgaris*. Proposal supported by a validation study done within the Germination Committee.

Table 5A Part 1: The Germination Committee has conducted a validation study on *Zea mays* to compare the results obtained with TP method using CCP, to the results obtained with the other ISTA approved substrates.

Table 5A Part 3: Germination method moved from Table 5A Part 2 to Part 3 for *Malva sylvestris*, as this is not a woody species.

Table 5A Part 3: Germination methods for *Salvia hispanica* included in the Rules, following validation studies carried out within the Germination Committee.

Chapter 6

Table 6A: Updates to nomenclature as a result of changes to the *ISTA List of Stabilised Plant Names*.

Chapter 7

Table 7A: Updates to nomenclature as a result of changes to the *ISTA List of Stabilised Plant Names*.

Method 7-019a: Addition of *Xanthomas campestris* pv. *raphani* in the plating assay as the pathovars are undistinguishable on the semi-selective media used. Inclusion of a process flow diagram in Background section to reflect optional and mandatory steps in the method. Proposal approved by a vote of Seed Health TCOM and supported by the Committee.

Method 7-025: Improvements to the description of the method and detailed descriptions of nematode identification. Figure added to assist in nematode identification. Proposal approved by vote and supported by Seed Health Committee.

Chapter 8

8.5.3: Editorial change required due to an incorrect cross reference to performance approved methods; corrections approved by Variety Committee.

Chapter 9

9.2.4.2: Changes to wording for oven requirements, to avoid specifying ‘ventilation’ and ‘capacity’ of the oven. The need to check whether the oven is fit for purpose or not is highlighted and text has been revised for readability. Proposal approved by Moisture Committee.

9.2.4.4: Adding additional possibilities to desiccator, such as perforated porcelain or other material instead of metal. Proposal approved by Moisture Committee.

9.2.5.1: References corrected and optimised where needed; an additional reference is added, and it is stressed that the correct sample size shall be used and where to find it. Proposal approved by Moisture Committee.

9.2.5.1, 9.2.5.2, 9.2.5.4 and 9.2.5.5: Changes to time limits for drawing working samples; time limits are given for mixing, grinding, cutting and drawing the working sample separately. Proposal approved by Moisture Committee.

Table 9A: Updates to nomenclature as a result of changes to the *ISTA List of Stabilised Plant Names*.

9.3.2.4.1: Addition of requirement for storing the remainder of the submitted sample for moisture using moisture meters; addition of the same requirement to storing time as for the submitted sample using the oven method. Proposal approved by Moisture Committee.

Chapter 15

15.3 and Table 15B: Addition of *Triticum aestivum* to the radicle emergence (RE) test, following a method validation study illustrating that the test identifies differences in vigour (field emergence) of seed lots of this species and is repeatable and reproducible.

15.8.4.4.1: Removal of requirement for a control seed lot for the radicle emergence (RE) test. Following their experience with the test, the Vigour Committee believes that when the test is completed following the prescribed ISTA Rules protocol, the use of a control seed lot is not necessary.

Chapter 18

18.8: Editorial change to 'Reporting results'. As a general principle, any statement of the applicant may be reported only in the space reserved for applicant statements/declarations. This space is reserved at the top of the ISTA certificate under 'Stated by applicant'. Therefore, the components of seed mixtures as reported by the applicant cannot be reported under 'Analysis results' as this space is reserved for laboratory results obtained by an ISTA laboratory through seed testing.

18.8.1: Clarification on how to report purity content of seed mixtures (i.e. the percentage by weight of pure seed, inert matter, and other seeds).

Introduction to the ISTA Rules

I-1 General information

The International Seed Testing Association (ISTA) was established in 1924 to work towards a vision of uniformity in seed testing internationally. ISTA's current mission is to develop, adapt and publish standard procedures for sampling and testing seeds, and to promote uniform application of these procedures for evaluation of seeds moving in international trade. The need for seed testing methods that are reliable and reproducible among its accredited member laboratories is therefore a basic need for ISTA. This is achieved through the publication of the International Rules for Seed Testing (hereafter 'ISTA Rules'). The primary aim of the ISTA Rules is to provide testing methods for seeds designated for growing of crops or production of plants. In addition, most of the testing methods can also be applied for evaluation of the quality of seeds used as food or for technical purposes.

ISTA's seed sampling and testing methods have been developed by its members since its formation in 1924. Methods have gone through appropriate validation studies to ensure that test procedures give reliable and reproducible results. Following agreement between ISTA's member countries, the validated methods have been included in the ISTA Rules.

Seed quality testing therefore requires test methods and equipment that have been tested to ensure they are fit for purpose, i.e. validated. The ISTA Method Validation Programme (see section I-2) provides the mechanism for the inclusion of test methods in the ISTA Rules. New methods and modifications to existing methods need to be validated through the ISTA Method Validation Programme. Equipment needs to be fit for the purpose described in each chapter, and not influence the accuracy or reliability of results. Rules proposals can include the use of technologies new to the ISTA Rules, whether these are the basis of new methods or new tools within existing methods, provided they meet these requirements.

Seed is a living biological product, and its behaviour cannot be predicted with the certainty that characterises the testing of inert or non-biological material. The test methods used must be based on scientific knowledge and the accumulated experience of those working in seed test-

ing and quality control. This expertise is provided largely by the members of ISTA's Technical Committees.

The ISTA Rules contain 19 chapters, 17 of which provide internationally accepted test methods for various attributes of seed quality. Chapter 2 (Sampling) provides the required methods for sampling of seed lots, because for ISTA, a direct connection between the seed lot from which the sample was drawn and the results of quality tests conducted on that seed lot must always be evident. The 'end product' for an accredited ISTA laboratory following quality tests on a seed lot is an ISTA Certificate. Information on how to use ISTA Certificates is presented in Chapter 1.

Each of the 17 chapters on test methods includes sections on the Object (of the test), Definitions (of terms used in the chapter), General Principles (for the test), Apparatus (required for the test), Procedure (how to conduct the test), Calculation and Expression of Results (specific to each test), Reporting Results (how to report results correctly on an ISTA Certificate), and Tolerances (statistical tables for use in determining whether test results are acceptable or not acceptable). Note that where, to provide adequate guidance, it has been necessary in the Apparatus section to refer to a particular manufacturer's piece of equipment, this should not be construed that ISTA endorses that piece of equipment in preference to, or to the exclusion of, equivalent products from other manufacturers.

The ISTA Rules are designed for the principal crop species of the world. Species are broadly classified as agricultural and vegetable, tree and shrub, and flower, spice, herb and medicinal. ISTA encourages proposals for the addition of new species to the ISTA Rules.

ISTA Certificates can only be issued by ISTA accredited laboratories. For seed quality test results to be reported on an ISTA Certificate, it is mandatory that all the requirements of the ISTA Rules are strictly followed.

ISTA also recommends that the ISTA Rules be used by all seed testing laboratories (including non-ISTA member laboratories) when testing seed for trade transactions which do not require the use of an ISTA Certificate (e.g. within a country), and for the enforcement of national laws for the control of seed quality.

For further information on the ISTA Rules and their use, please contact:

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or visit the ISTA website: www.seedtest.org

I-2 Guidelines for ISTA Rules proposals

Proposals to amend the ISTA Rules or to introduce new species are welcomed from any source. ISTA operates an open system, and proposals are not restricted to ISTA members only. Any external proposal needs to have been submitted to the ISTA Secretariat by 1 November.

Following receipt, the ISTA Secretariat may send the proposal to the relevant ISTA Technical Committee or directly to the ISTA Rules Committee, which will review all the proposals received. The ISTA Executive Committee will then either approve a proposal for consideration by the ISTA membership or request further work on the proposal. All approved Rules proposals are then sent to the ISTA membership two months before the Ordinary Meeting. At the Ordinary Meeting, the ISTA voting delegates may vote to accept a proposal (which will then be implemented in the ISTA Rules, effective 1 January of the following year), to withdraw a proposal (for further consideration), or to reject a proposal.

I-2.1 Proposals concerning test methods

All seed quality test methods proposed for inclusion in the ISTA Rules must have gone through the ISTA Method Validation Programme. This is required for both new test methods (i.e. not currently in the ISTA Rules) and modifications to existing methods already included in the ISTA Rules. A four-step process is involved:

1. method selection and development;
2. validation through comparative testing;
3. review of comparative test results and preparation of a Method Validation Report;
4. approval of validation status by the relevant ISTA Technical Committee and preparation and of an ISTA Rules proposal for the method.

Final acceptance of the proposal by vote of the ISTA membership at an Ordinary Meeting will allow publication of the validated method in the ISTA Rules.

Further information on the ISTA Method Validation Programme can be obtained from the ISTA Secretariat.

I-2.2 Proposals for new species

For a proposal to introduce a new species, Form 1 on pages I-4 to I-6 may be used. The following information must be supplied by the applicant:

1. **Names of species.** The scientific name (including author) plus common names and synonyms must be given. The common names will be used by the ISTA Nomenclature Committee to update the Multilingual Glossary of Common Plant Names. The ISTA Nomenclature Committee will stabilise the scientific name for at least six years so that laws and trade agreements do not have to be altered frequently. For assistance in determining the correct scientific name and its author, the ISTA Nomenclature Committee may be contacted.
2. **Maximum lot size and sample sizes.** Proposals for maximum lot size should take into account the general principles that have been applied to species already in the ISTA Rules and to the feasibility of achieving reasonably homogenous seed lots. Seed size is generally the significant factor in determining maximum lot size, but this is also influenced by whether the species is for agriculture or horticulture use, a tree or shrub species, or a flower, spice, herb or medicinal species. This, in turn, will determine whether the species should be placed in Part 1, 2 or 3, respectively, of Table 2C. Proposals for maximum lot size and submitted sample size should then be based on those already to be found in the corresponding part of Table 2C. For agricultural and horticultural species, the submitted sample is larger in relation to the purity working sample, based on the weight of 2500 seeds, than for the other species, to allow for determination of other species by number based on 10 times the purity weight.
3. **Pure Seed Definition.** The ISTA Rules and the *Handbook of Pure Seed Definitions* already list many pure seed definitions. The appropriate one should be given. If none of them apply, a proposal for a new definition should be submitted.

4. **Validated germination test methods.** The methods proposed must have been validated, either by multi-laboratory collaborative testing or peer validation (see ISTA Method Validation Programme). Advice as to requirements can be obtained from the ISTA Germination Committee. Please specify the data as required for insertion in Table 5A.
5. **Validated tetrazolium test procedures.** Procedures for tetrazolium testing should be given if known. A proposal to amend Chapter 6 may be submitted following the appropriate method validation.
6. **Validated moisture content determination methods.** A validated method for moisture determination must be provided if the method is different to the reference (i.e. low-constant-temperature) method.
7. **Thousand-seed weight**
8. **Varietal identification.** Using current techniques, it is possible to verify a descriptor to check varietal purity in some species. Please indicate validated techniques.
9. **Seed health tests.** The methods proposed must have been validated, either by multi-laboratory collaborative testing or peer validation (see ISTA Method Validation Programme). Advice as to requirements can be obtained from the ISTA Seed Health Committee.

I-2.3 Other proposals

Within a chapter of the ISTA Rules, a change to the existing text (e.g. amendment of a definition) or introduction of new text (e.g. introduction of a new definition) may be proposed. Providing the proposal does not directly involve a test method or new species, it should be sent directly to the ISTA Secretariat.

Thousand-seed weight of small-seeded varieties of *Poa pratensis*

Before a small-seeded variety can be included in Table 3A, a determination of the thousand-seed weight must be performed on at least 20 samples from different seed lots, representing seeds grown either in two different harvest years or in two different countries.

The determination of the thousand-seed weight must be carried out on pure seeds, obtained by blowing a 1 g sample of *Poa pratensis* using the standard blower setting (factor 1.00). Only seed remaining in the heavy fraction may be used for the thousand-seed weight. See Chapter 10 of the ISTA Rules for the weight determination procedure.

Results should be submitted to the ISTA Purity Committee with a request to change the ISTA Rules.

Form 1: Proposal for inclusion of new species in the ISTA Rules

Note: this form is also available on the ISTA web site (www.seedtest.org/mv-prog)

1. Scientific name of proposed species

(Family)	Genus	Species	(Nominated Authority)

Genus and species names appear in List of Stabilised Plant Names: Yes/No

Known synonyms: _____

Common plant name: _____ in _____ (Member country)
(required for Multilingual Glossary)

2. Lot and sample weights

(Information as it should appear in Table 2C)

Species	Maximum weight of lot (kg)	Minimum submitted sample (g)	Minimum working samples (g)	
			Purity analysis (3.5.1)	Count of other species (4.5.1)

3. Pure Seed Definition

(Table 3B Part 1)

The following Pure Seed Definition (PSD) covers the proposed species:

Genus	Family	PSD number	Chaffiness

No existing definition covers this species:

Distinguishing characteristics of this species:

(List distinguishing characteristics. Attach drawings, if available, and be prepared to send to the Secretariat five seed samples from well-processed, as well as from incompletely cleaned, seed.)

4. Validated germination test method(s)

(Information as it should appear in Table 5A)

Species	Prescriptions for:				Additional directions incl. recommendations for breaking dormancy
	Substrate	Temperature (°C)	First count (d)	Final count (d)	

5. Validated tetrazolium test procedure

(Information as it should appear in Table 6A)

Species	Pretreatment: type/minimum time (h)	Preparation before staining	Staining solution (%)	Optimum staining time (h)	Preparation for evaluation	Permitted non-viable tissue	Remarks

(If no existing drawings apply, attach if available)

6. Validated moisture test methods

Specify appropriate methods or details for inclusion in Table 9A Part 1 or 2:

Species	Grinding/cutting (9.2.5.4, 9.2.5.5)	High temperature	Drying at high temperature (h)	Predrying requirement (9.2.5.6)	Remarks
(Part 1)					(Not applicable)
(Part 2)		(Not applicable)	(Not applicable)	(Not applicable)	

7. Thousand-seed weight = _____ g

8. Validated varietal identification method (attach separate sheet, if necessary)

Supporting evidence for proposal

9. Number of national seed analysis certificates issued per year:

10. Other countries or laboratories testing the proposed species:

Submitted by:

Signature:

Date: