



International Rules for Seed Testing 2024

Introduction to the ISTA Rules

**Including changes and editorial corrections adopted
at the Ordinary General Meeting 2023 in Verona, Italy**

Effective from 1 January 2024

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 2024 edition of the ISTA Rules

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

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:

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 Annexe to Chapter 7 of the ISTA Rules. They are now available as separate method sheets from the ISTA website at:

www.seedtest.org/seedhealthmethods.

Details of changes

The 2024 changes are editorial corrections or Rules changes adopted at the Ordinary General Meeting held in Verona, Italy in June 2023. 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 with yellow highlight boxes as a 'layer' over the English version 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 2023 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 2024

Introduction

- I-1:** New address for ISTA Secretariat updated.
- I-2.2:** Standard method provided to determine working weight of purity and other seed determination (OSD) for adding a new taxon [taxa] to Table 2C, including data rounding rules. Statistical methods applied and experimental design recommended in the Calculator developed by Statistics TCOM. Proposal submitted by Bulking & Sampling and Purity TCOMs, and approved by majority vote.
- Form 1:** Revision of form to improve procedures and guidance for proposing a new taxon to Table 2C. Section 2 updated with newly developed 'Calculator for adding working weights to Table 2C'. Section 3 revised to clarify purpose of providing morphological features for PSD (not for identification). New section 4 added to provide validated working weight for a new taxon or group of taxa. Proposal approved by Purity TCOM through vote.

Chapter 1

- 1.3:** Revision of maximum subplot size allowance for *Solanum lycopersicum* L. Proposal supported by experimental data, a summary report and analysis supplied by Statistics TCOM. Recommendation that a maximum of 20 sublots should be set for homogenous seed lots of tomato, accounting for a low level of other seed content. This would assure buyers and sellers that test results represent what is traded, and provides a practical solution for companies producing tomato seed lots. Proposal approved by Statistics TCOM and unanimous vote of Bulking & Sampling TCOM and ECOM-VSI WG.
- 1.4.1:** Revision to allow weight of original seed lot and weight of subplot to be recorded in same place on ISTA Certificate. Proposal approved by Statistics TCOM and unanimous vote of Bulking & Sampling TCOM and ECOM-VSI WG.
- 1.4.2:** Revision to allow weight of original seed lot and weight of subplot to be recorded in same place on ISTA Certificate. Statement '1' deleted and subsequent statements relabelled sequentially. Proposal approved by Statistics TCOM and unanimous vote of Bulking & Sampling TCOM and ECOM-VSI WG.
- 1.5.2.2:** Inconsistency with requirements in 3.6.1.3 identified by an ISTA member laboratory. Proposal submitted by Purity TCOM and approved by vote.

- 1.5.2.3:** Consequential change due to update to sections of Chapter 11, reflecting advancements in available seed treatments. Clarifications added on how and what to report when testing coated seeds. Proposal approved by majority vote of Purity TCOM.
- 1.5.2.7:** Consequential change due to improvements made to germination information in Chapter 11, rewording general principles, evaluation and reporting results. Proposal originates from and is supported by Germination TCOM.
- 1.5.2.12:** Clarification on reporting moisture tests for pelleted seeds and for coated seeds (mats and tapes), to align with Chapter 11. Proposal originates from and is supported by Moisture TCOM.
- 1.5.2.15:** Consequential change due to improvements made to germination information in Chapter 13, rewording calculation and expression of results, and reporting results. Proposal originates from and is supported by Germination TCOM, following consultation with Forest Tree and Shrub Seed TCOM.
- 1.5.2.16:** Consequential change due to revision and updating of Chapter 14 to improve clarity. Previous information obsolete due to new generations of X-ray machines and applications of digital technology. Significant changes are focused on equipment and its operation, as well as clarification on reporting. Proposal developed and approved by Advanced Technologies, Forest Tree & Shrub Seed and Purity TCOMs.
- 1.5.2.21:** Section updated to reflect changes on how to report results of GMO testing in 19.7.
- 1.5.3:** Change necessary to achieve consistency with 19.7 in reporting uncertainty of measurement when testing genetically modified organisms. Proposal approved by vote within GMO TCOM.

Chapter 2

- 2.2.2:** Revision of maximum subplot size allowance for *Solanum lycopersicum* L. Proposal supported by experimental data, a summary report and analysis supplied by Statistics TCOM. Recommendation that a maximum of 20 sublots should be set for homogenous seed lots of tomato, accounting for a low level of other seed content. This would assure buyers and sellers that test results represent what is traded, and provides a practical solution for companies producing tomato seed lots. Proposal approved by Statistics TCOM and unanimous vote of Bulking & Sampling TCOM and ECOM-VSI WG.

2.5.2.1: Revision to provide standard method to determine working weight of purity and other seed determination (OSD) for adding new weights to Table 2C, including data rounding rules. Working weight determinations developed based on statistically recognised methods for estimating variables such as lots, variety and testing laboratories, and removing data outliers. Proposal provided by Bulking & Sampling and Purity TCOMs, and approved by majority vote. Statistical methods applied and experimental design recommended in the Calculator were developed by Statistics TCOM.

Table 2C Part 1: Revision following survey of ISF members producing tomato seed lots, concluding that 200 kg is a maximum lot size for international trade in tomato seed. Proposal discussed in depth within Bulking & Sampling TCOM, approved by Statistics TCOM, and approved by close majority vote of Bulking & Sampling TCOM and ECOM-VSI WG.

Chapter 3

3.5.2.4: Clarification regarding identification of indistinguishable species. Procedures only apply when seed is deemed by laboratories as ‘indistinguishable’, with discretion. Proposal supported by majority vote of Purity TCOM.

3.7: Correction of inconsistency with requirements in 3.6.1.3, as identified by an ISTA member laboratory. Proposal submitted by Purity TCOM and approved by vote.

Table 3B Part 2: Definition of PSD 15 made more inclusive for species where schizocarp could be broken and present in a sample. Schizocarp more than one-half original size is added. Proposal developed and approved by Purity TCOM.

Table 3B Part 2: Revision to correct discrepancy between PSD 33 (Fig. 3.1) and the *ISTA Handbook on Pure Seed Definitions* (Fig. 33.1), including multiple seed units with both fertile and sterile florets. Proposal developed and approved by Purity TCOM.

Chapter 4

4.5.3.2, 4.6, 4.7, 4.8, Table 4B [newly named], Table 4B [newly named]: Clarification on reporting sample weight of determination of other seeds to a fixed decimal place. Misleading use of ‘minimum’ deleted. Table given caption, subsequent tables renamed and cross references updated. Proposal developed and approved by Purity TCOM.

Chapter 5

5.6.3.1, 5.6.5.3, 5.7: Revision of rules on retesting when fresh seed present. Purpose of proposal is to address issue of considering test results of initial germination test ‘unsatisfactory’ and instruction to not report these test results and to require a retest when dormancy is suspected. Laboratories should have option to either report % germination and % fresh seeds determined by the initial test, or to not report results of initial test and to conduct additional testing using dormancy-breaking procedures listed in Table 5A. When fuller assessment is requested by customer or desired by laboratory, test results are not reported and a retest would be conducted. ISTA Rules should allow for same testing and reporting option for ‘fresh seeds’ as for ‘hard seeds’ (5.6.3.2). At end of test period, if ungerminated seeds are determined to be ‘fresh’, % fresh seeds is reported. Proposal originates from and is supported by Germination TCOM.

5.10: Editorial change clarifying column to be checked in Table 5A Part 2 for ‘double tests’.

Table 5A Part 1: Editorial change to correct alphabetical order of two *Centrosema* species.

Chapter 6

Table 6A Part 2: Addition of method to test *Ulmus* spp. seeds with tetrazolium salts. Proposal approved by Tetrazolium TCOM and supported by method validation study.

Chapter 7

All seed health methods: Sample size description revised for consistency across methods.

Methods 7-001a, 7001b, 7-002a, 7-002b, 7-003, 7-005, 7-007, 7-013a, 7-014, 7-016: Figure images updated and captions revised.

Method 7-013b: Figure and caption added.

Methods 7-019a, 7-019b, 7-020, 7-021, 7-023: Editorial changes to harmonise description of dilutions under Methods.

Methods 7-019a, 7-019b, 7-020, 7-021, 7-023, 7-029: Editorial changes to harmonise description of recording colony-forming units (cfu).

Method 7-019b: Editorial change to delete *X. c. pv. amoraciae* from classification of *Xanthomonas*.

Chapter 9

9.2.4.7, 9.2.5.1, 9.2.5.2, 9.2.5.3, 9.2.5.4, 9.2.5.5, 9.2.5.6, 9.2.5.7, 9.2.6.2: Updating of cross reference to Table 9A. Parts 1 and 2 of Table 9A merged due to changes in crop groups, to allow easier inclusions and modifications in future. Some species renamed according to Table 2C and additional information included for species new to taxonomy. Proposal originates from and is supported by Moisture TCOM.

9.2.5.7: Prescribed drying temperatures explicitly given, as in 9.1.2 and 9.1.3. Proposed tolerated range for high temperature method (127–133 °C) aligns with that prescribed by AOSA. Comparison conducted in two laboratories; Statistics TCOM analysed data and supports proposed change. Proposal originates from and is supported by Moisture TCOM.

9.2.6.2, Table 9B: Deletion of word ‘initial’ before ‘moisture content’. Proposal originates from and is supported by Moisture TCOM.

9.2.7: Clarification on reporting moisture tests for pelleted seeds and for coated seeds (mats and tapes), to align with Chapter 11. Proposal originates from and is supported by Moisture TCOM.

Table 9A: Parts 1 and 2 of Table 9A merged due to changes in crop groups (e.g. *Malva* transferred from tree & shrub to flower crop group), to allow easier inclusions and modifications in future. Some species renamed according to Table 2C and additional information included for species new to taxonomy. Proposal originates from and is supported by Moisture TCOM.

Chapter 11

11.1.2 [newly numbered], 11.3.2.2, 11.3.7, 11.10: Update to sections of Chapter 11 to reflect advancements in available seed treatments. Cross references to recent changes in Chapter 10 included. Clarifications added on how and what to report when testing coated seeds. Proposal approved by majority vote of Purity TCOM.

11.4.6: Cross reference to Table 4A updated due to re-belling of tables in Chapter 4.

11.5.3, 11.5.6.5, 11.5.8: Improvements made to germination information in Chapter 11 by rewording general principles, evaluation and reporting results. Proposal originates from and is supported by Germination TCOM.

Chapter 13

13.7: Improvements made to germination information in Chapter 13 by rewording calculation and expression of results, and reporting results. Proposal originates from and is supported by Germination TCOM, following consultation with Forest Tree and Shrub Seed TCOM.

Chapter 14

Revision and updating of chapter to improve clarity. Previous information obsolete due to new generations of X-ray machines and applications of digital technology. Significant changes are focused on equipment and its operation, as well as clarification on reporting. Proposal developed and approved by Advanced Technologies, Forest Tree & Shrub Seed and Purity TCOMs.

Chapter 18

18.8: Addition of section regarding reporting moisture content of seed mixtures. Subsequent sections in chapter renumbered and cross references updated. Proposal originates from and is supported by Moisture TCOM.

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 testing 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 at-

tributes 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.

Users of the ISTA Rules are responsible to comply with the Health and Safety Requirements for the jurisdiction in which they operate. ISTA does not audit or accept any responsibility for compliance to Health and Safety Regulations. Any statements relating to Health and Safety in the ISTA Rules are for guidance only.



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

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CH-8304 Wallisellen
Switzerland

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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. To determine the weight of the purity and other seed determination (OSD) working samples for a new taxon (or a group of taxa) to be added to Table 2C, conduct and analyse an experiment for assessing multiple sources of variation of 100-seed unit weights. Guidelines for the experimental design and data analysis for deriving the minimum 2500 or 25 000 seed weight are provided in the 'Calculator for adding working weights to Table 2C', available on the ISTA website.
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3. **Pure Seed Definition.** The ISTA Rules and the *ISTA Handbook on 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 website (www.seedtest.org/mv-prog)

1. Scientific name of proposed species

(Family)	Genus	Species	(Nominated Authority)

Genus and species names appear in *ISTA 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)
Use 'Calculator for adding working weights to Table 2C'				

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:	
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Characteristics to support the PSD proposal:

(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 working weight determinations

(Provided according to the guidelines and the experimental design of the 'Calculator for adding working weights to Table 2C')

YES	
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5. 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)	

6. 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)

7. 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)	

8. Thousand-seed weight = _____ g

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

Supporting evidence for proposal

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

11. Other countries or laboratories testing the proposed species:

Submitted by:

Signature:

Date: