



International Rules for Seed Testing 2023

Introduction to the ISTA Rules

**Including changes and editorial corrections adopted
at the Ordinary General Meeting 2022, Cairo, Egypt**

Effective from 1 January 2023

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.

Published by
The International Seed Testing Association (ISTA)
Richtiarkade 18, CH-8304 Wallisellen, Switzerland

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Online ISSN 2310-3655

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Contents

Preface to the 2023 edition of the ISTA Rules..... v

Introduction to the ISTA Rules.. I-1

I-1 General information..... I-1

I-2 Guidelines for ISTA Rules proposals..... I-2

 I-2.1 Proposals concerning test methods..... I-2

 I-2.2 Proposals for new species I-2

 I-2.3 Other proposals I-3

Form 1: Proposal for inclusion of new species in
the ISTA Rules.. I-4

Preface to the 2023 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 Annex 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 2023 changes are editorial corrections or Rules changes adopted at the Ordinary General Meeting held at Cairo, Egypt in May 2022. 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 2022 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 2023

Chapter 1

1.1, 1.2, 1.2.4, 1.3, 1.4.1, 1.4.2, 1.4.3, 1.6: Edits made following offer of optional electronic seed analysis certificates from 2023, for use by accredited member laboratories of ISTA. Proposal developed by ISTA Rules Chair with input from Secretariat and ECOM.

Chapter 3

3.7: Paragraph moved for clarification of reporting authorities when determining scientific names for pure and other seeds reported on ISTA Certificates. Harmonisation of reporting between 3.7 and 4.7. Proposal developed by Purity TCOM and approved by unanimous vote.

3.7: Proposal developed and approved by Purity TCOM to indicate how other seeds and inert matter found in the second whole sample test shall be reported, providing clear guidance to the second whole sample test.

Chapter 4

4.7: Wording altered for clarification of reporting authorities when determining scientific names for pure and other seeds reported on ISTA Certificates. Harmonisation of reporting between 3.7 and 4.7. Proposal developed by Purity TCOM and approved by unanimous vote.

Chapter 5

5.6.2.3, 5.6.3.1: Proposal gives clarification that the temperature prescribed for prechilling should be measured from on or in the substrate during prechilling. Proposal developed and approved by Germination TCOM.

5.6.5.3: Proposal gives clarification that a tetrazolium test can be conducted to determine viability of fresh

ungerminated seeds, at the end of a germination test, for species listed in Table 5A. Proposal developed by Germination TCOM and approved by Germination and Tetrazolium TCOMs.

5.10: Proposal to add Seedling Evaluation Groups to Table 5A (Parts 1, 2 and 3), giving benefit to seed analysts and ensuring that correct group is used. Proposal developed and approved by Germination TCOM.

Table 5A Part 1: Addition of new temperature regime (20 °C) for germination of *Anethum graveolens* following an ISTA peer method validation study. Proposal developed and approved by Germination TCOM.

Table 5A Parts 1, 2 and 3: Proposal to add Seedling Evaluation Groups to Table 5A, giving benefit to seed analysts and ensuring that correct group is used. Proposal developed and approved by Germination TCOM.

Chapter 7

Method 7-031: Addition of option to use a sieve with filter paper or an equivalent nematode-permeable container (such as a non-woven plant growth bag) for filtration. Proposal developed and approved by Seed Health TCOM, and supported by method validation study.

Chapter 8

8.10.4 (new section): Inclusion of DNA-based test for testing *Pisum* varieties. Proposal developed by working group within Variety TCOM and supported by validation study. Proposal approved by Variety TCOM.

8.10.5 (new section): Inclusion of DNA-based test for testing *Avena sativa*. Proposal supported by validation study. Proposal developed and approved by Variety TCOM.

Table 8I: Table relabelled as 8R due to addition of new methods in Chapter 8.

Chapter 9

9.2.7: Paragraph moved and bullet point added to improve clarity. Proposal developed and approved by Moisture TCOM.

9.3.2.6: Editorial correction to improve sentence clarity.

9.3.2.7: Bullet point added to improve clarity. Proposal developed and approved by Moisture TCOM.

Table 9A Part 1: Editorial corrections of nomenclature changes approved in 2019 *ISTA List of Stabilised Plant Names*. *Elytrigia* spp. updated to *Elymus* spp.

Chapter 19

19.1: Introduction of acronym TP for ‘trait purity’. Small risk of ambiguity with TP used in Chapter 5 for ‘top of paper’. In cases where ambiguity might exist, ‘trait purity’ should be used in full. Proposal developed and approved by GMO TCOM.

19.2: Editorial corrections made that do not modify intent, including renumbering of paragraphs and word substitutions.

19.2: New definitions added and numbering of definitions throughout section modified as a result.

19.2.17: Improvement of definition of term ‘analyte’, relating it to term ‘target’ widely used in Chapter 19.

19.2.20: Improved definition of ‘performance-based approach’ as a mechanism to ensure uniformity in testing.

19.2.21: Improved definition of ‘proficiency test’ to clarify object of assessing ability of subject (laboratory, operator) to carry out a test, not a specific method.

19.3: Section revised, including precise definition of pieces of evidence a laboratory must provide to apply for accreditation under performance-based approach. Requirement of method validation or verification is introduced. Precise specification of requirement of production of performance data on seed samples is given. Use by laboratories of results obtained in non-ISTA proficiency tests is accepted as evidence.

19.4: Section retitled ‘Objectives and approaches’ consistent with revised terminology. Testing objectives and approaches defined in relation to each other, with reference to Fig. 19.1. Two paragraphs describing ‘technical aspects’ moved to end of section. Sentence indicating need for reference material in quantitative

PCR deleted since digital PCR does not necessarily require it. Several editorial changes made.

19.4.1: Redundant sentence deleted regarding size of working sample compared to submitted sample. Specification that working sample can be analysed in single or in multiple units of observation. Clarification of concept of limit of group/bulk size (not of working sample size) in relation to limit of quantification.

19.5: Section retitled ‘Testing technologies’ consistent with revised terminology.

19.5.1.1: Minor changes and specifications in bullet points.

19.5.1.3: Addition of need to verify identity of PCR products in real-time PCR when using intercalating dyes.

19.5.2.2: Simplification of text on lateral flow strip test.

19.5.2.3: Simplification of text on enzyme-linked immunosorbent assay.

19.5.3.1: Replacement of ‘resistance’ with ‘tolerance’ as a more appropriate term when referring to herbicides.

19.6.1: Specification added that testing results for both assessment of presence or estimation of level can be referred either to seed sample or seed lot. Deletion of unnecessary sentence, and other minor changes and specifications.

19.6.2: Modification of section title and text for consistency with revised terminology and to improve clarity.

19.6.3: New reference (Remund *et al.*, 2020) added.

19.7: Specification of details to be provided when reporting results on an ISTA Certificate, including conditions for reporting results of GMO testing on Orange or Blue International Certificates.

19.7.1: Section retitled ‘Assessment of presence of GMO’ consistent with revised terminology, and editorial changes made. Clarification of how to express compliance to a given standard when a working sample tests negative to a qualitative assay for a given target.

19.7.2: Section retitled ‘Estimation of the level of GMO by multiple qualitative tests of individuals or groups’ consistent with revised terminology.

19.7.3: Section retitled ‘Estimation of the level of GMO by quantitative measurements on groups or bulks’ consistent with revised terminology. Specification of conditions for reporting results of GMO testing on Orange or Blue International Certificates. Other editorial changes made to improve clarity.

19.8: New reference (Remund *et al.*, 2020) added and link for SeedCalc download on ISTA website updated.

Table 19A (new table): Table to summarise types of analytical output that can be attained using different approaches in GMO testing.

Figure 19.1: Figure redrawn according to revised terminology. More information provided including new concepts (unit of observation, testing approach) and relating workflow to different analytical outputs.

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 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.

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:

ISTA Secretariat
Zürichstrasse 50
CH-8303 Bassersdorf
Switzerland
Phone +41 44 838 6000
Fax +41 44 838 6001

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 *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)

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: