ISTA
Seed Quality Assurance

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Document OGM21-05

Rules Proposals for the International Rules for Seed Testing 2022 Edition

This document was prepared by the Technical Committees (TCOMs) and the Rules Committee of the Association and has been endorsed by the ISTA Executive Committee (ECOM). The proposals are submitted to the ISTA Ordinary General Meeting 2021 for voting by the nominated ISTA Designated Members on behalf of their respective Governments.

It is submitted to all ISTA Designated Authorities, ISTA Members and ISTA Observer Organizations for information two months prior to the ISTA Ordinary General Meeting 2021.

It contains proposed amendments and changes for the ISTA *International Rules for Seed Testing* and will be discussed and voted on at the Ordinary General Meeting 2021 to be held virtually on Thursday, June 03, 2021.

Introduction to the ISTA Rules Proposals to become effective 1 January 2022

The current version of the ISTA International Rules for Seed Testing (ISTA Rules) is the 2021 edition.

The ISTA Rules are only available electronically as a printable pdf file and are available for free download by ISTA members from the Ingenta website: http://www.ingentaconnect.com/content/ista/rules

The electronic version also includes the 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.

For further information on the ISTA Rules, see: http://www.seedtest.org/rules

The effective dates are changed annually. The changes from the previous edition of the ISTA Rules can be displayed as yellow highlighted text as a 'layer' within the electronic copy with comments on what has changed. Previous Prefaces as a 'history of changes' are available on the ISTA website.

The ISTA Rules are the result of the work of the ISTA Technical Committees (TCOMs) with input from many different sources. Thanks go to all the Technical Committee members and the ISTA Secretariat for their help with the annual proposals.

The following Rules Proposals will be discussed at the ISTA Ordinary General Meeting on June 03, 2021 and may be amended during the meeting. If the proposals are accepted by the membership, amendments will be issued, and they will become the 2022 edition of the ISTA Rules.

Please let us know about any problems with these proposals.

Many thanks.

Ernest Allen and Sue Alvarez

Chair and Vice-Chair of ISTA Rules Committee

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Key to text changes:

~~Deleted text~~

New text

New text in large blocks, not underlined for ease of reading

Any changes made after the proposals were published to the membership

Contents

PART A. INTRODUCTION OF EDITORIAL CHANGES

A.1. Editorial corrections

The proposal removes obsolete website information from 1.5.2.2. The same editorial correction will occur in 3.7.

Submitted by the Purity TCOM

CURRENT VERSION	PROPOSED VERSION
1.5.2.2 Purity The results of a purity test must be reported in the spaces provided as follows: • The scientific name of the species of pure seed, in accordance with Table 2C (e.g. *Triticum aestivum* subsp. *aestivum*). Where it is impossible to determine the species with certainty on the basis of seed characteristics, reporting must be done to the most precise taxon possible. • The percentage….. • The kind of inert… • The scientific name of every species of other seeds found, in accordance, where applicable, with the current *ISTA List of Stabilised Plant Names*~~,~~ ~~available at www.seedtest.org/stablist~~ (e.g. *Elymus repens)*.	**1.5.2.2 Purity** The results of a purity test must be reported in the spaces provided as follows: • The scientific name of the species of pure seed, in accordance with Table 2C (e.g. *Triticum aestivum* subsp. *aestivum*). Where it is impossible to determine the species with certainty on the basis of seed characteristics, reporting must be done to the most precise taxon possible. • The percentage….. • The kind of inert… • The scientific name of every species of other seeds found, in accordance, where applicable, with the current *ISTA List of Stabilised Plant Names* (e.g. *Elymus repens*).

The Accreditation Working Group proposed an improvement to the text from chapter 3 related to the proposal C.2.3.- Storage of the samples. The changes are intended to clarify the intent of sections 3.5.2 and harmonize it with the wording in section 4.5.2.

Submitted by the ISTA Accreditation Department.

CURRENT VERSION	PROPOSED VERSION
3.5.2 ..... 4. After separation, each component part (3.3) and any species of seed or kind of other matter for which a percentage is to be reported, must be weighed in grams to the minimum number of decimal places necessary to calculate the percentage to one decimal place (3.5.1). After weighing, the other seed~~s~~ component~~s~~ must be retained and stored for reference until sample disposal (see 2.5.3 and 2.5.4.7).	3.5.2 ..... 4. After separation, each component part (3.3) and any species of seed or kind of other matter for which a percentage is to be reported, must be weighed in grams to the minimum number of decimal places necessary to calculate the percentage to one decimal place (3.5.1). After weighing, the other seed component must be retained and stored for reference until sample disposal (see 2.5.3 and 2.5.4.7).

An incorrect reference is used in Rule **2.5.2.1 Minimum size of working sample** that needs to be corrected, 2.2.11 covers marking and labelling, treated seeds are defined in 2.2.12

CURRENT VERSION	PROPOSED VERSION
2.5.2.1 Minimum size of working sample ……. Working samples of all coated seeds except those defined as treated seed in ~~2.2.11~~ must contain at least the number of pellets, seeds or granules indicated in column 3 of Table 2D, Part 1 and Part 2. If a smaller sample is used, the actual number of pellets, seeds or granules in the sample must be reported.	**2.5.2.1 Minimum size of working sample** ……. Working samples of all coated seeds except those defined as treated seed in 2.2.12 must contain at least the number of pellets, seeds or granules indicated in column 3 of Table 2D, Part 1 and Part 2. If a smaller sample is used, the actual number of pellets, seeds or granules in the sample must be reported.

Corrections to the *Protocol for the Approval of Automatic Seed Samplers* required due to the 2019 update of the ISTA List of Stabilised Plant Names.

CURRENT VERSION	PROPOSED VERSION
6. Approval of the automatic seed sampler, its installation and operation …… • which of the four species groups will be sampled by the automatic seed sampler. It is up to the ISTA Seed Testing Laboratory/Sampling Entity to decide in which group a species belongs: A. Less sensitive bigger seeds (species with seeds equal to or bigger than *Triticum aestivum* seeds) B. Sensitive bigger seeds (e.g. Pulses) C. Small-seeded species (species with seeds smaller than *Triticum aestivum* seeds) that are non-chaffy species D. Small seeded chaffy species	**6. Approval of the automatic seed sampler, its installation and operation** …… • which of the four species groups will be sampled by the automatic seed sampler. It is up to the ISTA Seed Testing Laboratory/Sampling Entity to decide in which group a species belongs: A. Less sensitive bigger seeds (species with seeds equal to or bigger than *Triticum aestivum* ~~L.~~ subsp. *aestivum* seeds) B. Sensitive bigger seeds (e.g. Pulses) C. Small-seeded species (species with seeds smaller than *Triticum aestivum* ~~L.~~ subsp. *aestivum* seeds) that are non-chaffy species D. Small seeded chaffy species

Correction to the *Protocol for the Approval of Automatic Seed Samplers* required due to the renumbering of the ISTA Tables in the 2020 ISTA Rules.

CURRENT VERSION	PROPOSED VERSION
6. Approval of the automatic seed sampler, its installation and operation …… Thousand seed weight (TSW) TSW can be used for species for which the seed size can vary within the same seed lot to check, if the automatic seed sampler selects on weight. The submitted and working sample size must be in accordance with the sample sizes specified in Table 2~~A~~. ……..	**6. Approval of the automatic seed sampler, its installation and operation** …… Thousand seed weight (TSW) TSW can be used for species for which the seed size can vary within the same seed lot to check, if the automatic seed sampler selects on weight. The submitted and working sample size must be in accordance with the sample sizes specified in Table 2C. …….

Additions required due to the 2019 update of the ISTA List of Stabilised Plant Names.

Rules changes for Table 3B Part 1 and 2: Result of changes to the ISTA List of Stabilised Plant Names used in Table 2C for:

Megathyrsus maximus (Jacq.) B.K.Simon & S.W.L.Jacobs (synonym *Panicum maximum* Jacq.)
Thinopyrum elongatum (Host) D.R.Dewey (synonym *Elytrigia elongata* (Host) Nevs)

The following proposal has been developed by the Purity TCOM (approved by majority vote) and the Rules committee.

~~Four~~ Five nomenclature changes approved in the 2019 LSPN update were not included in Table 3B Part 1 and Table 5A Part 1. These editorial corrections will be made in the 2022 edition.

CURRENT VERSION

Table 3B Part 1. Pure seed definition numbers and chaffiness of seeds, listed by genus

Genus	Family	PSD no.	Chaffiness
…..			
Medicago	Fabaceae	11	
Melilotus	Fabaceae	21	
Melinis	Poaceae	36	C
….			
Tetragonia	Aizoaceae	19	
Thuja	Cupressaceae	49	C
Thunbergia	Acanthaceae	10	

Updates: Table 3B Part 1. Pure seed definition numbers and chaffiness of seeds, listed by genus

Genus	Family	PSD no.	Chaffiness
1	2	3	4
Allium	~~Alliaceae~~	10	
Asclepias	~~Asclepiadaceae~~	10	C
Liquidambar	~~Hamamelidaceae~~	48	C
Tectona	~~Verbenaceae~~	54	

Table 3B Part 2. Numbered pure seed definitions

…….

PROPOSED VERSION

Table 3B Part 1. Pure seed definition numbers and chaffiness of seeds, listed by genus

Genus	Family	PSD no.	Chaffiness
…..			
Medicago	Fabaceae	11	
Megathyrsus	Poaceae	36	C
Melilotus	Fabaceae	21	
Melinis	Poaceae	36	C
….			
Tetragonia	Aizoaceae	19	
Thinopyrum	Poaceae	28	C
Thuja	Cupressaceae	49	C
Thunbergia	Acanthaceae	10	

Updates: Table 3B Part 1. Pure seed definition numbers and chaffiness of seeds, listed by genus

Genus	Family	PSD no.	Chaffiness
1	2	3	4
Allium	Amaryllidaceae	10	
Asclepias	Apocynaceae	10	C
Liquidambar	Altingiaceae	48	C
Tectona	Lamiaceae	54	

Table 3B Part 2. Numbered pure seed definitions

…….

36. Spikelet, with or without pedicel, with glumes, lemma and palea enclosing a caryopsis, plus attached sterile lemma. Floret, with lemma and palea enclosing a caryopsis. Caryopsis. Piece of caryopsis larger than one-half the original size. *Axonopus:* spikelet, with single glume, lemma and palea enclosing a caryopsis, plus attached sterile lemma. *Echinochloa* and *Melinis:* attached sterile lemma with or without awn. *Panicum* and *Digitaria*: no need to check for the presence of a caryopsis.	36. Spikelet, with or without pedicel, with glumes, lemma and palea enclosing a caryopsis, plus attached sterile lemma. Floret, with lemma and palea enclosing a caryopsis. Caryopsis. Piece of caryopsis larger than one-half the original size. *Axonopus*: spikelet, with single glume, lemma and palea enclosing a caryopsis, plus attached sterile lemma. *Echinochloa* and *Melinis*: attached sterile lemma with or without awn. *Megathyrsus*, *Panicum* and *Digitaria*: no need to check for the presence of a caryopsis.

Current Version: Table 5A Part 1. Detailed methods for germination tests

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Hordeum vulgare	BP; S	20	4	7	Preheat at....		

Proposed Version: Table 5A Part 1. Detailed methods for germination tests

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Hordeum vulgare subsp. *vulgare*	BP; S	20	4	7	Preheat at....		

The Germination Committee received a request to consider the symbols used to denote division for 5.6.1 of the ISTA Rules (proportional calculation). The request was to change the colon ':' symbol to a forward slash '/'.

This change is supported by the Germination Committee.

CURRENT VERSION	PROPOSED VERSION
5.6.1 Working sample **…..** If there are up to 5 seeds lost or found as extra in the test, then each replicate must be adjusted to 100 by calculation. For example, if one replicate had 80 normal seedlings, 10 abnormal seedlings and 9 dead seeds, with one seed missing, then the result must be adjusted to 100 with the following proportional calculation: 80 × 100 ~~÷~~ 99 normal seedlings, 10 × 100 ~~÷~~ 99 abnormal seedlings and 9 × 100 ~~÷~~ 99 dead seeds. Rounding follows the principles described in 5.8.2…..	**5.6.1 Working sample** **…..** If there are up to 5 seeds lost or found as extra in the test, then each replicate must be adjusted to 100 by calculation. For example, if one replicate had 80 normal seedlings, 10 abnormal seedlings and 9 dead seeds, with one seed missing, then the result must be adjusted to 100 with the following proportional calculation: 80 × 100 / 99 normal seedlings, 10 × 100 / 99 abnormal seedlings and 9 × 100 / 99 dead seeds. Rounding follows the principles described in 5.8.2…..

The temperature for DNA extraction indicated in the validation report was reported wrongly in the method.

CURRENT VERSION	PROPOSED VERSION
Method 7.019a 7.1 Make a slightly turbid cell suspension (OD600 nm approximately 0.05) in 1.0 ml sterile saline from the suspected cultures on YDC medium and the positive control. In addition a non-suspect isolate should be used as a negative control (CCP). Centrifuge bacterial suspensions for 5 min at 8000 rpm. Discard the supernatant and resuspend the pellet with 500 µl of 0.5 M NaOH. Incubate for 10 min at ~~65 °C~~ by shaking at 1000 rpm.	**Method 7.019a** 7.1 Make a slightly turbid cell suspension (OD600 nm approximately 0.05) in 1.0 ml sterile saline from the suspected cultures on YDC medium and the positive control. In addition a non-suspect isolate should be used as a negative control (CCP). Centrifuge bacterial suspensions for 5 min at 8000 rpm. Discard the supernatant and resuspend the pellet with 500 µl of 0.5 M NaOH. Incubate for 10 min at 100 °C by shaking at 1000 rpm

18.8.1 Clarification on how to report purity content of seed mixtures. This proposal was approved for the 2020 edition. The change, however, was placed in the wrong place within 18.8.1. The editorial change corrects the error.

CURRENT VERSION	PROPOSED VERSION
18.8.1 Purity and component analysis	**18.8.1 Purity and component analysis**
The results of the purity analysis are reported according to Chapter 3.	The results of the purity analysis are reported according to Chapter 3.
	The percentage by weight of pure seed (if applicable, including declared inert material); inert matter and other seeds must be given to one decimal place and entered in the spaces for purity. The pure seed percentage is calculated using the total weight of the pure seed of all mixture components.
The actual weight of sample examined to the minimum number of decimal places indicated in 4.7 must be reported under 'Other determinations', i.e. 'Purity and composition analysis: … g of seed examined.'	The actual weight of sample examined to the minimum number of decimal places indicated in 4.7 must be reported under 'Other determinations', i.e. 'Purity and composition analysis: … g of seed examined.'
The mixture composition is reported under 'Other determinations' in one of the following formats, as requested by the applicant:	The mixture composition is reported under 'Other determinations' in one of the following formats, as requested by the applicant:
~~1. The percentage by weight of pure seed (if applicable, including declared inert material); inert matter and other seeds must be given to one decimal place~~ **~~and entered in the spaces for purity~~**~~. The pure seed percentage is calculated using the total weight of the pure seed of all mixture components.~~	
~~2~~. The percentage by weight of the pure seeds of the mixture components using the total weight of the pure seed fraction. In addition, if applicable, the percentage by weight of the 'inert material according to declaration' referred to the sum of the	1. The percentage by weight of the pure seeds of the mixture components using the total weight of the pure seed fraction. In addition, if applicable, the percentage by weight of the 'inert material according to declaration' referred to the sum of the

weights of all mixture components (pure seeds and inert material according to declaration) must be given to one decimal place under 'Other determinations'. ~~3.~~ The percentage by weight of mixture components, pure seeds or inert material according to declaration using the sum of the weights of the pure seed fraction and the declared inert material. ~~4.~~ The percentage by number of the pure seeds of the mixture components using the total number of seeds of the pure seed fraction.	weights of all mixture components (pure seeds and inert material according to declaration) must be given to one decimal place under 'Other determinations'. 2. The percentage by weight of mixture components, pure seeds or inert material according to declaration using the sum of the weights of the pure seed fraction and the declared inert material. 3. The percentage by number of the pure seeds of the mixture components using the total number of seeds of the pure seed fraction."

To Address ISTA member's inquiry: Is it redundant to write… ergot, sclerotia…? Could only sclerotia be used?" The purity committee consulted with the Seed Health committee and provided the re-wording.

The following editorial clarification was submitted by PUR Committee and approved by a majority vote of the Committee.

CURRENT VERSION	PROPOSED VERSION
3.2.1 Pure seed The pure seed must refer to the species stated by the applicant, or found to predominate in the test, and must include all botanical varieties and cultivars of that species including: 1. The following structures (even if immature, undersized, shrivelled, diseased or germinated, providing they can be definitely identified as of that species) unless transformed into partially or fully ~~ergotised~~ visible ~~fungal~~ sclerotia, smut balls or nematode galls…or nematode galls (see 3.5.2.5.1 for exceptions when the uniform blowing method is used):	**3.2.1 Pure seed** The pure seed must refer to the species stated by the applicant, or found to predominate in the test, and must include all botanical varieties and cultivars of that species including: 1. The following structures (even if immature, undersized, shrivelled, diseased or germinated, providing they can be definitely identified as of that species) unless transformed into partially or fully visible sclerotia (of ergot or other fungi), smut balls or nematode galls (see 3.5.2.5.1 for exceptions when the uniform blowing method is used):

ACCEPTED BY VOTE	RESULT
YES	47/47

PART B. NEW SPECIES AND CHANGES TO SPECIES NAMES

B.1.1 Addition of new species to Table 2C

An ISTA method validation study conducted on *Chenopodium quinoa* provides evidence showing the best germination method for repeatability and reproducibility is 20C, TP or BP and first count after 4 days and final count 7 days. Evaluations are carried out according to Seedling Type E – Seedling Group A-2-1-1-1. Sample and maximum seed lot size, for this species, is based on thousand seed weight determinations and was approved by the Bulking and Sampling and Purity Committees. The Purity Committee proposed the PSD is PSD 2 and non-chaffy.

This proposal is supported by the Germination Committee and a method validation study.

New entries: Table 5A Part 1. Detailed methods for germination tests

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Chenopodium quinoa	TP; BP	20	4	7	-		

New entries: Table 2C Part 1. Lot sizes and sample sizes: agricultural and vegetable species

Species	Maximum weight of lot (kg) except see 2.8 Note 2	Minimum submitted sample (g)	Minimum working samples (g)	
			Purity analysis (3.5.1)	Other seeds by number (4.5.1)
1	2	3	4	5
Chenopodium quinoa Willd.	10000	100	10	100

New entries: Table 3B Part 1. Pure seed definition numbers and chaffiness of seeds, listed by genus

Genus	Family	PSD no.	Chaffiness
1	2	3	4
Chenopodium	Chenopodiaceae	2	

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
B.1.1	**47**	**0**	**YES**

B.1.2 Changes to the ISTA Stabilised List

None this year.

The next revision of the ISTA Stabilised List will be considered at the 2025 ISTA Meeting.

PART C. RULES CHANGES AND NEW METHODS REQUIRING A VOTE

Chapter 1: ISTA Certificates

C.1.1 Clarifying primary nomenclature reporting source in ISTA

The proposal is to add the ISTA List of Stabilised Plant Names as the primary source for reporting other seeds.

The following proposal has been developed by PUR Committee and approved by a majority vote of the Committee.

If approved, this change will also apply in the reporting results section in Chapter 4 (4.7).

CURRENT VERSION	PROPOSED VERSION
1.5.2.4 Determination of other seeds by number The result of a determination of other seeds by number must be reported under 'Other determinations' as follows: • The actual weight of seed examined to the minimum number of decimal places indicated in 4.7. • The scientific name and number of seeds of each species ~~sought and~~ found in this weight. If no other seeds are found, this must be indicated on the certificate.	**1.5.2.4 Determination of other seeds by number** The result of a determination of other seeds by number must be reported under 'Other determinations' as follows: • The actual weight of seed examined to the minimum number of decimal places indicated in 4.7. • The scientific name in accordance, where applicable, with the current *ISTA List of Stabilised Plant Names* (e.g. *Elymus repens*), and number of seeds of each species found in this weight. If no other seeds are found, this must be indicated on the certificate.

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.1.1	**47**	**0**	**YES**

Chapter 2: Sampling

C.2.1. Revision of 2.2.12 Treated seed & 2.2.13 Coated seeds

This Rules proposal has been submitted by the Bulking and Sampling Committee. These changes are requested to clarify that additives that are used to either treat or to coat seed may contain more than one of the listed ingredients. In the current wording, it could be construed that only one additive could be used as either Treated seed or Coated seed.

The proposals were discussed and unanimously approved by the Bulking and Sampling Committee.

CURRENT VERSION	PROPOSED VERSION
2.2.12 Treated seed 'Seed treatment' is a generic term which indicates that a seed lot has been subjected to: a. the application of a compound including film coatings, polymers, pesticides, fungicides, biologicals, identifying colourants, dyes and other additives; b. …….. **2.2.12 Coated seeds** Coated seeds are seeds covered with material in such a way that in most cases the seeds cannot be identified without removing the covering material. The material may contain pesticides, fungicides, biologicals, identifying colourants, dyes or other additives. The following types of coated seeds are defined: ……	**2.2.12 Treated seed** 'Seed treatment' is a generic term which indicates that a seed lot has been subjected to: a. the application of a compound including film coatings, polymers, pesticides, fungicides, biologicals, identifying colourants, dyes and/or other additives; b. …….. **2.2.12 Coated seeds** Coated seeds are seeds covered with material in such a way that in most cases the seeds cannot be identified without removing the covering material. The material may contain pesticides, fungicides, biologicals, identifying colourants, dyes and/or other additives. The following types of coated seeds are defined: ……

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.2.1	47	0	YES

C.2.2. Revision of 2.5.2.2.1 Mechanical divider method

This Rules proposal has been submitted by the Bulking and Sampling Committee. These changes are requested to enable a more accurate description of the various types of dividers that may be used for sample reduction.
The proposals were discussed and unanimously approved by the Bulking and Sampling Committee through voting.

CURRENT VERSION	PROPOSED VERSION
2.5.2.2.1 Mechanical divider method ……. **a. Conical divider.** The conical divider (Boerner type) consists of a hopper, cone, and series of baffles directing the seed into two spouts. The baffles form alternate channels and spaces of equal width. They are arranged in a circle and are directed inward and downward, the channels leading to one spout and the spaces to an opposite spout. A valve or gate at the base of the hopper retains the seed. When the valve is opened the seed falls by gravity over the cone where it is evenly distributed to the channels and spaces, then passes through the spouts into the seed pans. ~~Dividers with more than 18 channels have been found to be suitable.~~ Channels must be wide enough to allow the smooth free flow of seed and contaminants. b. Soil divider. The soil divider (riffle divider) consists of a hopper with ~~about~~ ~~18~~ attached channels or ducts alternately leading to opposite sides. Channels must be wide enough to allow the smooth free flow of seed and contaminants.	**2.5.2.2.1 Mechanical divider method** …… **a. Conical divider.** The conical divider (Boerner type) consists of a hopper, cone, and series of baffles directing the seed into two spouts. The baffles form alternate channels and spaces of equal width. They are arranged in a circle and are directed inward and downward, the channels leading to one spout and the spaces to an opposite spout. A valve or gate at the base of the hopper retains the seed. When the valve is opened the seed falls by gravity over the cone where it is evenly distributed to the channels and spaces, then passes through the spouts into the seed pans. Channels and spaces must be wide enough to allow the smooth free flow of seed and contaminants. The more channels and spaces, the better the accuracy. Typical commercial conical dividers have about 15 channels and 15 spaces. b. Soil divider. The soil divider (riffle divider) consists of a hopper with attached channels or ducts alternately leading to opposite sides. Channels must be wide enough to allow the smooth free flow of seed and contaminants. The more channels, the

	better the accuracy. ~~Ten channels must be regarded as the minimum~~. A minimum of ten channels is required.
In using the divider the seed is placed evenly into a pouring pan and then poured in the hopper at approximately equal rates along the entire length. The seed passes through the channels and is collected in two receiving pans.	In using the divider the seed is placed evenly into a pouring pan and then poured in the hopper at approximately equal rates along the entire length. The seed passes through the channels and is collected in two receiving pans.
d. Rotary divider. The rotary divider comprises a rotating crown unit with 6 to ~~10~~ attached subsample containers, a vibration chute and a hopper. In using the divider the seed is poured into the hopper and the rotary divider is switched on so that the crown unit with the containers rotates with ~~approx. 100 rpm~~ and the vibration chute starts to feed the seed into the inlet cylinder of the rotating crown.	**d. Rotary divider.** The rotary divider comprises a rotating crown or base unit usually with 6 to 32 attached subsample containers, a vibration chute and a hopper. In using the divider the seed is poured into the hopper and the rotary divider is switched on so that the crown or base unit with the containers rotates with high speed and the vibration chute starts to feed the seed into the inlet cylinder of the rotating crown/base unit. ~~The higher the speed and~~ The longer the duration of the dividing operation, the better the accuracy.
The feeding rate and therefore the duration of the dividing operation can be adjusted by the distance between the funnel of the hopper and the chute and the vibration intensity of the chute. There are two principles: (i) The inlet cylinder feeds the seed centrally onto a distributor within the rotating crown distributing the seed to all containers simultaneously. (ii) The inlet cylinder feeds the seed de-centrally into the inlets of the containers rotating underneath the inlet cylinder so that the seed stream is subdivided into a lot of subsamples. For this type of divider, mixing and dividing takes place in one operation.	The feeding rate and therefore the duration of the dividing operation can be adjusted by the distance between the funnel of the hopper and the chute and the vibration intensity of the chute. There are two principles: (i) The inlet cylinder feeds the seed centrally onto a distributor within the rotating crown or base unit distributing the seed to all containers simultaneously. (ii) The inlet cylinder feeds the seed de-centrally into the inlets of the containers rotating underneath the inlet cylinder so that the seed stream is subdivided into a lot of subsamples. For this type of divider, mixing and dividing takes place in one operation.
e. Variable sample divider. The variable sample divider consists of a pouring hopper and a tube underneath ~~that rotates with about 40 rpm~~. The	**e. Variable sample divider**. The variable sample divider consists of a

tube distributes the seed stream from the pouring hopper onto the inner surface of a further hopper, which is well fitted into a third hopper all being concentric. ~~In the second and the third hopper there are slots that comprise 50 % of the perimeter of the hoppers. 50 % of the seed will pass through the two hoppers into a collecting pan. The other 50 % will stay within the hoppers and will then go into a second collecting pan.~~ The ~~two~~ hoppers can be twisted against each other resulting in more narrow slots. The effect is that ~~a smaller percentage~~ will pass through the slots. ~~Either the smaller sample outside the hoppers or the bigger sample inside the hoppers can be used as the required sample.~~ The position of the ~~two~~ hoppers in relation to each other can be adjusted accurately, resulting in pre-determined ~~sub~~sample sizes. For this type of divider, mixing and dividing takes place in one operation.	pouring hopper and a rotating tube underneath. The tube distributes the seed stream from the pouring hopper onto the inner surface of a further hopper, which is well fitted into a third hopper, all being concentric. In the second and the third hopper there are slots that can be twisted against each other resulting in wider or narrower slots. The effect is that variable proportions will pass through the slots. The position of the hoppers in relation to each other can be adjusted accurately, resulting in pre-determined sample sizes. Depending on the design, the sample poured into the hopper can be divided into one or up to eight subsamples. The operation of these types of dividers can be controlled with computer software, which enables it to provide in one operation two or more subsamples with different predetermined sizes. For this type of divider, mixing and dividing takes place in one operation.

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.2.2	**45**	**2**	**YES**

C.2.3 Revision of 2.5.3 Storage of samples

This Rules proposal has been submitted by the Bulking and Sampling Committee This change is requested to refer to Chapters 3 and 4 respectively for clarification on the way which the different fractions of working samples should be stored after testing.

The proposal was drawn up in consultation with the Purity Committee and unanimously approved by the Bulking and Sampling Committee through voting.

CURRENT VERSION	PROPOSED VERSION
2.5.3 Storage of samples after testing The primary aim of storage of samples after testing is to be able to repeat the original tests carried out on the submitted sample. Therefore, storage conditions should be such that changes in the seed quality traits tested are minimal. For example, in the case of the purity test or other seed count, the sample should be stored in such a way that the physical identity is kept. In the case of germination, viability or health test of orthodox seeds the sample should be stored under cool and dry conditions. For such tests in recalcitrant and intermediate seeds of tropical and subtropical species, long term storage is not possible. For such seed of temperate species storability depends on the fungal status and to some extent whether the seed is dormant or not. All factors pertaining to storage need to be determined on a species basis. Protection against insects and rodents may be necessary. ……..	**2.5.3 Storage of samples after testing** The primary aim of storage of samples after testing is to be able to repeat the original tests carried out on the submitted sample. Therefore, storage conditions should be such that changes in the seed quality traits tested are minimal. For example, in the case of the purity test or other seed count, the sample should be stored in such a way that the physical identity is kept (see 3.5.2 and 4.5.2). In the case of germination, viability or health test of orthodox seeds the sample should be stored under cool and dry conditions. For such tests in recalcitrant and intermediate seeds of tropical and subtropical species, long term storage is not possible. For such seed of temperate species storability depends on the fungal status and to some extent whether the seed is dormant or not. All factors pertaining to storage need to be determined on a species basis. Protection against insects and rodents may be necessary. …….. .

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.2.3	47	0	YES

C.2.4 Revision of 2.8, Table 2C Parts 1 and 3

This Rules proposal has been submitted by the Bulking and Sampling Committee. When *Salvia hispanica* was added to the ISTA Rules in 2020, it was listed under Table 2C Part 3. The BSC has since then received requests from ISTA accredited laboratories to move it from Table 2C Part 3 to Table 2C Part 1, as it is used as a food and large quantities of the commodity, as well as seed lots, are produced. Therefore, it should be regarded as an Agricultural Crop. Consequently, the maximum seed lot size will also be adjusted to the maximum for small seeded agricultural species, i.e. 10 000 kg. From data supplied by the Purity Committee in 2019, the average TSW is 0.13g, therefore the minimum submitted sample size and other seed by number working sample are increased to 35 grams so that it would contain at least 25,000 seeds, and also be consistent with other species in Part 1 where the OSD is 10 times the PUR.

The proposal was drawn up in consultation with the Purity Committee and unanimously approved by the Bulking and Sampling Committee.

If this proposal is approved, a consequencial change will occur for this species in Chapter 5. The *Salvia hispanica* will be moved from Table 5A Part 3 to Table 5A Part 1 (agricultural and vegetable seeds).

CURRENT VERSION: Table 2C Part ~~3~~

Species	Maximum weight of lot (kg) (except see 2.8 Note 2)	Minimum submitted sample (g)	Minimum working sample for purity analysis (3.5.1) (g)
1	2	3	4
~~*Salvia hispanica* L.~~	~~5 000~~	~~20~~	~~3.5~~

PROPOSED VERSION: Table 2C Part 1

Species	Maximum weight of lot (kg) (except see 2.8 Note 2)	Minimum submitted sample (g)	Minimum working sample for purity analysis (3.5.1) (g)	
			Purity Analysis (3.5.1)	Other Seeds by number (4.5.1)
1	2	3	4	5
Salvia hispanica L.	10 000	35	3.5	35

Consequential change if C.2.4 is approved

CURRENT VERSION: Table 5A Part 3. Detailed methods for germination tests: flower, spice,…

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy
1	2	3	4	5	6
~~*Salvia hispanica*~~	~~TP~~	~~20<=>30; 20~~	~~4-7~~	~~14~~	~~–~~

PROPOSED VERSION: Table 5A Part 1. Detailed methods for germination tests**:** agricultural and veg…

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Salvia hispanica	TP	20<=>30; 20	4 - 7	14	-	-	-

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.2.4	**47**	**0**	**YES**

Chapter 3: The purity analysis

C.3.1 Revised definition of inert matter (Withdrawn)

A question to the Purity TCOM noted that it was not obvious how to categorize Fern spores if they were found in purity test or other seed determination. After discussion, a majority of Purity TCOM members agreed that fern spores can be defined as inert matter.

The following proposal has been developed by the Purity TCOM and approved by a majority vote of the Committee.

CURRENT VERSION	PROPOSED VERSION
3.2.3 Inert matter …… 7. Unattached sterile florets, empty glumes, lemmas, paleas, chaff, stems, leaves, cone scales, wings, bark, flowers, nematode galls, fungus bodies such as ergot, sclerotia and smut balls, soil, sand, stones, and all other non-seed matter.…	**3.2.3 Inert matter** …… 7. Unattached sterile florets, empty glumes, lemmas, paleas, chaff, stems, leaves, cone scales, wings, bark, flowers, nematode galls, fungus bodies such as ergot, sclerotia and smut balls, soil, sand, stones, fern spores, and all other non-seed matter.

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.3.1 (Withdrawn)			

C.3.2. Revised Pure Seed Definition for *Ornithopus sativus*

Ornithopus compressus and *Ornithopus sativus* are in the ISTA RULES. *Ornithopus sativus* is currently not assigned a PSD. This proposal assigns a PSD to *Ornithopus sativus.*

PSD 23 is valid for *Ornithopus* in general. A specific mention is given for *O. compressus* only. In *O. sativus,* the morphological situation is comparable, to that in *O. compressus* but is not mentioned in PSD 23.

The following proposal has been developed by PUR Committee to correct this omission and approved by a majority vote of the Committee.

CURRENT VERSION	PROPOSED VERSION
Table 3B Part 2. Numbered pure seed definitions (continued)	**Table 3B Part 2.** Numbered pure seed definitions (continued)
…….	…….
23. One-seeded segment of pod or siliqua, with or without stalk or terminal beak, unless it is obvious that no seed is present.	23. One-seeded segment of pod or siliqua, with or without stalk or terminal beak, unless it is obvious that no seed is present.
Seed, provided a portion of the testa is attached.	Seed, provided a portion of the testa is attached.
Piece of seed larger than one-half the original size, provided a portion of the testa is attached.	Piece of seed larger than one-half the original size, provided a portion of the testa is attached.
Ornithopus compressus: one-seeded pod segment, with or without attached empty pod segments or partial segments.	*Ornithopus compressus and O. sativus*: one-seeded pod segment, with or without attached empty pod segments or partial segments.

Vote to accept item	Yes votes	No votes	Result
C.3.2	**47**	**0**	**YES**

Chapter 5: The germination test

C.5.1 Alternate germination method for *Eustoma exaltatum*

A validation study was carried out in 2016 to support the introduction of *Eustoma exaltatum* to the ISTA Rules Table 5A Part 3. Six laboratories participated. Three germination methods differing only in temperature (20°C, 25°C and 20°C<=>30°C) were evaluated in the study (Zecchinelli R. and Grim A., 2018). Statistical analysis showed only one method 20°C<=>30°C had acceptable repeatability and reproducibility. This temperature is now the prescribed ISTA method for seed germination of *Eustoma exaltatum*.

In 2019, a new study re-evaluated the 20°C temperature given its importance to laboratories testing seed for cut flowers of *Eustoma exaltatum* in Japan and the inclusion of this temperature in the AOSA Rules for Testing Seed. The proposed temperature was compared to the current prescribed ISTA temperature, 20°C <=>30°C.

The 2019 study has successfully shown that the 20°C method produces results comparable to those produced with the standard method. Statistical analysis shows the average percentage of normal seedlings, repeatability and reproducibility are acceptable.

This proposal is supported by the FSC, Germination Committee, and a method validation study.

CURRENT VERSION: Table 5A Part 3. Detailed methods for germination tests: Flower, spice, herb and medicinal species

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy
1	2	3	4	5	6
Eustoma exaltatum	TP	20<=>30	4-7	21	Light

PROPOSED VERSION: Table 5A Part 3. Detailed methods for germination tests: Flower, spice, herb and medicinal species

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy
1	2	3	4	5	6
Eustoma exaltatum	TP	20<=>30; 20	4-7	21	Light

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.5.1.	**47**	**0**	**Yes**

C.5.2 Germination method for *Glycine max* – addition of growing media

An ISTA method validation study was conducted to determine the suitability of utilizing crepe cellulose paper (CCP) as a primary media for the top of paper (TP) method for *Glycine max* and allow this this media to be added to the ISTA Rules Table 5A Part 1.

The current ISTA Rules germination media options for *Glycine max* include Sand (S), Between Paper (BP), Top of Paper with a Sand covering (TPS) with the paper being CCP and Organic Growing Media. The purpose of this study was to harmonize the ISTA Rules with the Association of Official Seed Analysts (AOSA) Rules. The use of CCP for the TP method was adopted into the AOSA Rules in 1980. Since then, this highly efficient test method has been successfully utilized for accurately assessing the germination of seed lots by both governmental and non-governmental laboratories across multiple decades.

Note: An abbreviation for crepe cellulose paper is not required as it is a type of paper used for the TP method and was added as method for *Zea mays* under “Additional Advice” in 2019. A comment from the ECOM suggested the statement “Use CCP for TP Method” was a direction and not additional advice. This proposal moves the statement to the Additional directions column for *Zea mays* and adds the statement to the same column for *Glycine max*.

This proposal is supported by the Germination Committee and a method validation study.

CURRENT VERSION: Table 5A Part 1. Detailed methods for germination tests

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Glycine max	BP; TPS; S; O	20<=>30; 25	5	8	-	-	-

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Zea mays	TP; BP; TPS; S	20<=>30; 25; 20	4	7	-	-	~~Use CCP for TP Method~~

PROPOSED VERSION: Table 5A Part 1. Detailed methods for germination tests

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Glycine max	TP; BP; TPS; S; O	20<=>30; 25	5	8	-	Use CCP for TP method	-

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Zea mays	TP; BP; TPS; S	20<=>30; 25; 20	4	7	-	Use CCP for TP Method	-

Consequential changes if C.5.2 is accepted…

CURRENT VERSION	PROPOSED VERSION
5.10 Germination methods	**5.10 Germination methods**
…..	…..
BP between paper	BP between paper
PP pleated paper	PP pleated paper
TP top of paper	TP top of paper
TPS top of paper covered with sand	CCP Crepe Cellulose Paper
	TPS top of paper covered with sand
S sand	
TS top of sand	S sand
	TS top of sand
O organic growing media	
TO top of organic growing media ….	O organic growing media
	TO top of organic growing media…

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.5.2.	**45**	**1**	**YES**

C.5.3. Germination method for *Pinus sylvestris* – addition of growing media

An ISTA method validation study was conducted to determine the suitability of utilizing agar (A) as a primary media for *Pinus sylvestris* and adding this media to the ISTA Rules Table 5A Part 2. Different combinations of media and temperatures were studied. The temperatures of 20° C and 20< =>30° C were compared and were used in combination with the following methods of top of paper (TP) and agar (A). Results showed good repeatability and reproducibility for both methods; therefore, it is proposed to add agar (A) to the ISTA Rules for *Pinus sylvestris*.

This proposal is supported by the Germination Committee, the Forest Tree and Shrub Committee, and a method validation study.

CURRENT VERSION: Table 5A Part 2. Detailed methods for germination tests

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Pinus sylvestris	TP	20 <=>30; (20)	7	21	Eastern and Mediterranean provenances may require prechill 21 d	-	-

PROPOSED VERSION: Table 5A Part 2. Detailed methods for germination tests

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Pinus sylvestris	A; TP	20 <=>30; (20)	7	21	Eastern and Mediterranean provenances may require prechill 21 d	-	-

Consequential changes if ~~C.5.2~~ C.5.3 is accepted…

CURRENT VERSION	PROPOSED VERSION
5.4 Growing Media ….. 5.4.2 Specifications The following general specifications apply for all growing media and must be verified: Composition: the growing medium can be paper, pure sand or mixtures of organic compounds with added mineral particles. **Water retention characteristics:** …. The water retention is then expressed as a percentage of the maximum retention.	**5.4 Growing Media** ….. 5.4.2 Specifications The following general specifications apply for all growing media and must be verified: Composition: the growing medium can be agar, paper, pure sand or mixtures of organic compounds with added mineral particles. **Water retention characteristics:** …. The water retention is then expressed as a percentage of the maximum retention. For agar, the depth of the medium must be sufficient to supply adequate moisture to the seeds and seedlings throughout the testing period and the sample container must be sealed to avoid excessive medium moisture loss. **5.4.3.4 Agar**

	Agar (CAS-9002-18-0) is a polysaccharide solidifier composed of agarose and agaropectin derived from the *Rhodophyta* phylum of red algae. Agar powder should be approximately 99% pure and must be free of extra salts that may inhibit seed germination or plant growth. Agar used must not contain any additional nutrients, vitamins, hormones, or antimicrobials additives unless permitted by methods stated in the ISTA Rules.
5.6.2.1 Growing media ….	**5.6.2.1 Growing media** ….. 5.6.2.1.4 Agar (A) the seeds are germinated on top of the agar of the appropriate concentration and adequate thickness for the species being tested. Agar concentration used typically ranges from 0.7 g/L to 1.0 g/L, when test containers are oriented horizontally. If test containers are oriented at a slant, then is recommended that the agar concentration be from 0.9 g/L to 1.0 g/L so that the medium remains in place in the container. To allow for adequate moisture for the seeds and seedlings, it is suggested that the agar thickness be no less than 3 mm for small seeded species and ~~much thicker~~ greater than 3 mm thick for large seeded species.
5.6.2.1.~~4~~ Soil Soil is generally not recommended as a primary growing medium. However, it may be used as an alternative to organic growing media when seedlings show phytotoxic symptoms or if evaluation of seedlings is in doubt on paper or sand. If soil is used it must meet the specifications given in 5.4.2.	**5.6.2.1.5 Soil** Soil is generally not recommended as a primary growing medium. However, it may be used as an alternative to organic growing media when seedlings show phytotoxic symptoms or if evaluation of seedlings is in doubt on paper or sand. If soil is used it must meet the specifications given in 5.4.2.
5.6.2.2 Moisture and aeration ….. Special measures for aeration are not necessary for	5.6.2.2 Moisture and aeration ….. Special measures for aeration are not necessary for A, TP and PP tests enclosed in boxes or Petri dishes…..

TP and PP tests enclosed in boxes or Petri dishes….. 5.10 Germination methods….. Substrates: The sequence of alternative substrates does not indicate any preference: TP; BP; TPS; S; O. BP and TP may be replaced by PP (pleated paper). …..Abbreviations For further details see 5.6.2 and 5.6.3. BP between paper PP pleated paper TP top of paper TPS top of paper covered with sand S sand TS top of sand O organic growing media TO top of organic growing media ….	5.10 Germination methods….. Substrates: The sequence of alternative substrates does not indicate any preference: A; TP; BP; TPS; S; O. BP and TP may be replaced by PP (pleated paper). …..Abbreviations For further details see 5.6.2 and 5.6.3. A agar BP between paper PP pleated paper TP top of paper TPS top of paper covered with sand S sand TS top of sand O organic growing media TO top of organic growing media…

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.5.3	**47**	**0**	**YES**

C.5.4. Revision of wording regarding germination using Top of Paper

The Germination Committee proposes to remove the wording “upright” regarding the Top of Paper (TP) method. This wording suggests that if seeds were planted in an upright position, they may fall off the TP. This wording is more appropriate for the Between Paper (BP) method.

This proposal is supported by the Germination Committee.

CURRENT VERSION	PROPOSED VERSION
5.6.2.1.1 Methods using paper Top of paper (TP): the seeds are germinated on top of one or more layers of paper which are placed: • on the Jacobsen apparatus (5.5.3.1); • into transparent boxes or Petri dishes which may be placed in a flat, inclined ~~or upright~~ position. The appropriate quantity of water is added at the beginning of the test and evaporation may be minimised by a tightly fitting lid or by enclosing the dishes in plastic bags; • directly on trays in germination incubators which may be placed in a flat, inclined ~~or upright~~ position….	**5.6.2.1.1 Methods using paper** Top of paper (TP): the seeds are germinated on top of one or more layers of paper which are placed: • on the Jacobsen apparatus (5.5.3.1); • into transparent boxes or Petri dishes which may be placed in a flat or inclined position. The appropriate quantity of water is added at the beginning of the test and evaporation may be minimised by a tightly fitting lid or by enclosing the dishes in plastic bags; • directly on trays in germination incubators which may be placed in a flat or inclined position….

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.5.4	**47**	**0**	**YES**

C.5.5. Changes in abnormal seedling evaluation

The Germination Committee proposes to add the seedling abnormality criterion 32/07 to *Allium* spp. regarding primary infection.

This proposal is supported by the Germination Committee.

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CURRENT VERSION	PROPOSED VERSION
5.2.8.1 Seedling abnormalities …….32 In *Allium* spp., the cotyledon: 32/01 is short and thick 32/02 is bent over or forms a loop 32/03 forms a spiral 32/04 does not show a definite 'knee' 32/05 is constricted 32/06 is spindly	**5.2.8.1 Seedling abnormalities** …….32 In *Allium* spp., the cotyledon: 32/01 is short and thick 32/02 is bent over or forms a loop 32/03 forms a spiral 32/04 does not show a definite 'knee' 32/05 is constricted 32/06 is spindly 32/07 is decayed as a result of primary infection

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.5.5.	**47**	**0**	**YES**

Chapter 7: Seed health testing

C.7.1 Revision of validated seed health methods 7-013a and 7-013b

This proposed change will allow the optional use of Methyl Blue stain to aid visualisation of the fungal hyphae in methods 7-013a and 7-013b. This option is based on a method validation carried out by SASA for the SHC coupled to a PT. The report 'Using methyl blue to stain *Ustilago nuda* hyphae in ISTA Methods 7-013a and 7-013b' found that the inclusion of methyl blue stain in the test method of *U. nuda* in barley could help laboratories that have difficulty when attempting to identify hyphae in the scutellum without staining.

This proposal was approved and supported by the Seed Health Committee.

CURRENT VERSION	PROPOSED VERSION
7-013a **Background** This method was originally published in the *ISTA Handbook of Seed Health Testing* in November 1964 as S.3. No. 25 and revised in 1988 by W J Rennie, Agricultural Scientific Services, East Craigs, Edinburgh, Scotland. The method was incorporated into the newly revised *Annexe to Chapter 7* in 2002 from the 1999 edition of the ISTA Rules. The method was reviewed by the ISTA-Seed Health Committee in 2006 (Cockerell & Koenraadt, 2007) with the recommendation to accept for a further five years.	**7-013a** **Background** This method was originally published in the *ISTA Handbook of Seed Health Testing* in November 1964 as S.3. No. 25 and revised in 1988 by W J Rennie, Agricultural Scientific Services, East Craigs, Edinburgh, Scotland. The method was incorporated into the newly revised *Annexe to Chapter 7* in 2002 from the 1999 edition of the ISTA Rules. The method was reviewed by the ISTA-Seed Health Committee in 2006 (Cockerell & Koenraadt, 2007) with the recommendation to accept for a further five years. Based on a method validation carried out by Science and Advice for Scottish Agriculture for the SHC coupled to a PT and a report 'Using methyl blue to stain *Ustilago nuda* hyphae in ISTA Methods 7-013a and 7-013b' an option of methyl blue stain was added and could help laboratories that have difficulty identifying hyphae in the scutellum
Materials **Reference material:** seed known to be infected or other appropriate material **Incubator:** operating at 20 ±2 °C **Brass sieves:** 1 mm mesh (2 additional sieves of larger mesh size can be useful; see point 2.3) **Microscope:** with substage illumination, ×25 and ×50 magnification **5 % sodium hydroxide:** see below **Lactic acid solution:** see below **Fume cupboard**	**Materials** **Reference material:** seed known to be infected or other appropriate material **Incubator:** operating at 20 ±2 °C **Brass sieves:** 1 mm mesh (2 additional sieves of larger mesh size can be useful; see point 2.3) **Microscope:** with substage illumination, ×25 and ×50 magnification **5 % sodium hydroxide:** see below **Lactic acid solution:** see below **Optional Methyl blue:** see below

Glycerol	**Fume cupboard** **Glycerol**
Methods 2.4 Transfer the embryos to a mixture of equal quantities of glycerol and water in which further separation of the embryos and chaff can be made.	**Methods** 2.4 Transfer the embryos to a mixture of equal quantities of glycerol and water in which further separation of the embryos and chaff can be made.
2.5 Transfer the embryos to a beaker containing 50 ml of lactic acid solution and clear them by maintaining the lactic acid solution at boiling point for approximately 5 min. in a fume cupboard.	2.5 a. Transfer the embryos to a beaker containing 50 ml of lactic acid solution and clear them by maintaining the lactic acid solution at boiling point for up to 5 min. in a fume cupboard, or b. Transfer the embryos to a beaker containing 50 ml of lactic acid solution with Methyl Blue and clear them by maintaining the lactic acid solution at boiling point for up to 5 min. in a fume cupboard.
2.6 Transfer the embryos to fresh glycerol for examination. The scutellum becomes more transparent when embryos are left in glycerol for 1–2 h, making examination easier.	2.6 Transfer the embryos to fresh glycerol for examination. The scutellum becomes more transparent when embryos are left in glycerol for 1–2 h, making examination easier.
Section Media and solutions **Lactic acid solution** **Glycerine:** 333.3 ml **Lactic acid (90 % pure, minimum assay 88 % purity):** 333.3 ml **Water:** 333.3 ml	**Section Media and solutions** **Lactic acid solution** **Glycerine:** 333.3 ml **Lactic acid (90 % pure, minimum assay 88 % purity):** 333.3 ml **Water:** 333.3 ml
Preparation 1. Add equal parts of glycerine, lactic acid and water. Mix thoroughly. 2. Final solution should be clear and almost colourless. The solution will turn yellow with age and exposure to light. Store in amber bottle and avoid exposure to light.	**Preparation** 1. Add equal parts of glycerine, lactic acid and water. Mix thoroughly. 2. Final solution should be clear and almost colourless. The solution will turn yellow with age and exposure to light. Store in amber bottle and avoid exposure to light. **Option: Lactic acid solution with Methyl blue** Add 0.16g/L of Methyl blue to lactic acid solution.
Validation references **Studied in international comparative testing:** 1960, 1963, 1964, 1979. Comparative tests organised by the ISTA Plant Diseases Committee gave reasonable agreement between stations when samples with more than 1.0 % infection were tested by stations experienced in the test procedure (Rennie, 1978; Tempe, 1976).	**Validation references** **Studied in international comparative testing:** 1960, 1963, 1964, 1979. Comparative tests organised by the ISTA Plant Diseases Committee (now Seed Health Committee) gave reasonable agreement between stations when samples with more than 1.0 % infection were tested by

	stations experienced in the test procedure (Rennie, 1978; Tempe, 1976). ISTA (2021). Using methyl blue to stain *Ustilago nuda* hyphae in ISTA Methods 7-013a and 7-013b. Method Validation Reports. International Seed Testing Association, Bassersdorf, Switzerland.
Method 7-013b **Background** Since the 1970s, the Nordic laboratories have used a modification of ISTA method 7-013a described by Joelson (1968). The method described here differs from 7-013a in the embryo extraction technique and the procedure used to clear embryos for examination of the *Ustilago* mycelium. A validation study comparing the two methods was carried out. Three seed lots with infection levels between 1 % and 4 % were tested by three laboratories using both the current method 7-013a and the 'Nordic' Method (7-013b). The validation study shows that the two methods produce equivalent results (Sperlingsson, 2011). The Nordic method offers an alternative method for laboratories that do not have access to plentiful warm water, nor a fume hood. The alternative embryo-clearing process adds a day to the duration of the test, so may not be suitable where a quicker turnaround is required. It does, however, offer an alternative clearing procedure which could be used in combination with the existing method to provide flexibility of resources within laboratories during busy periods.	**Method 7-013b** **Background** Since the 1970s, the Nordic laboratories have used a modification of ISTA method 7-013a described by Joelson (1968). The method described here differs from 7-013a in the embryo extraction technique and the procedure used to clear embryos for examination of the *Ustilago* mycelium. A validation study comparing the two methods was carried out. Three seed lots with infection levels between 1 % and 4 % were tested by three laboratories using both the current method 7-013a and the 'Nordic' Method (7-013b). The validation study shows that the two methods produce equivalent results (Sperlingsson, 2011). The Nordic method offers an alternative method for laboratories that do not have access to plentiful warm water, nor a fume hood. The alternative embryo-clearing process adds a day to the duration of the test, so may not be suitable where a quicker turnaround is required. It does, however, offer an alternative clearing procedure which could be used in combination with the existing method to provide flexibility of resources within laboratories during busy periods. Based on a method validation carried out by SASA for the SHC coupled to a PT and a report 'Using methyl blue to stain *Ustilago nuda* hyphae in ISTA Methods 7-013a and 7-013b' an option of methyl blue stain in the was added and could help laboratories that have difficulty identifying hyphae in the scutellum.
Materials **Reference material:** seed known to be infected or other appropriate material **Oven:** capable of operating at 75 ±5 °C **Sulphuric acid (H2SO4):** concentration 25–37 % by weight **Electric hand mixer:** at low speed	**Materials** **Reference material:** seed known to be infected or other appropriate material **Oven:** capable of operating at 75 ±5 °C **Sulphuric acid (H2SO4):** concentration 25–37 % by weight **Electric hand mixer:** at low speed

Sodium hydroxide + sodium chloride: 10–15 % NaOH plus 110–175 g salt per litre of solution **Brass sieves:** 1 mm mesh, with one additional sieve of larger mesh (approx. 2.4 mm) and an additional fine sieve with mesh smaller than 1 mm **Glycerol-ethanol solution:** one part glycerol to two parts ethanol **Lactic acid:** more than 70 % **95 % ethanol** **Glycerol (glycerine)** **Microscope:** with substage illumination	**Sodium hydroxide + sodium chloride:** 10–15 % NaOH plus 110–175 g salt per litre of solution **Brass sieves:** 1 mm mesh, with one additional sieve of larger mesh (approx. 2.4 mm) and an additional fine sieve with mesh smaller than 1 mm **Glycerol-ethanol solution:** one part glycerol to two parts ethanol **Lactic acid:** more than 70 % **95 % ethanol** **Glycerol (glycerine)** **Optional Methyl blue:** see below **Microscope:** with substage illumination
Embryo extraction 11. Drain the embryos, place in a beaker and cover with lactic acid.	**Embryo extraction** 11. a. Drain the embryos, place in a beaker and cover with lactic acid or b. Drain the embryos, place in a beaker and cover with lactic acid/Methyl blue solution.
Section Media and solutions **Sodium hydroxide** For safety reasons, ready-made NaOH solution 10–15 % or ready-made NaOH-NaCl is preferable. If these are not available, dissolve 130–175 g sodium hydroxide pellets and 110–150 g sodium chloride in 1 l of cold tap water.	**Section Media and solutions** **Sodium hydroxide** For safety reasons, ready-made NaOH solution 10–15 % or ready-made NaOH-NaCl is preferable. If these are not available, dissolve 130–175 g sodium hydroxide pellets and 110–150 g sodium chloride in 1 l of cold tap water. **Lactic acid and Methyl Blue Solution**: Lactic acid 1000ml Methyl Blue 0.16g Mix thoroughly
Validation references ISTA (2011). Alternative embryo extraction procedure to 7-013b *Ustilago nuda/Hordeum vulgare*. *Method Validation Reports*. International Seed Testing Association, Bassersdorf, Switzerland.	**Validation references** ISTA (2011). Alternative embryo extraction procedure to 7-013b *Ustilago nuda/Hordeum vulgare*. *Method Validation Reports*. International Seed Testing Association, Bassersdorf, Switzerland. ISTA (2021). Using methyl blue to stain *Ustilago nuda* hyphae in ISTA Methods 7-013a and 7-013b. Method Validation Reports. International Seed Testing Association, Bassersdorf, Switzerland.

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.7.1.	**45**	**0**	**YES**

C.7.2 Harmonization of pretreatments for Methods 7-005, 7-006, 7014, 7-016, and 7-022

This proposal harmonizes pre-treatments and updates the description for several similar methods within Chapter 7. The harmonized pre-treatments are supported by validation studies carried out by the SHC.

This proposal was approved by majority vote of the Seed Health Committee.

CURRENT VERSION	PROPOSED VERSION
Method 7-005 **Methods** ~~1. Pretreatment: 10 min. in a 1 % (available chlorine) sodium hypochlorite solution followed by draining.~~	**Method 7-005** **Methods** 1. Pretreatment: Immerse seeds in a solution of sodium hypochlorite (NaOCl) (1% available chlorine) for 10 mins, then drain and rinse well in sterile water and drain.
Method 7-006 **Methods** ~~1. Pretreatment: Seeds are submerged in a solution of 1 % (available chlorine) sodium hypochlorite for 10 min and allowed to~~ drain.	**Method 7-006** **Methods** 1. Pretreatment: Immerse seeds in a solution of sodium hypochlorite (NaOCl) (1% available chlorine) for 10 mins, then drain and rinse well in sterile water and drain.
Method 7-014 **Methods** ~~1. Pretreatment: 10 min in 1 % (available chlorine) so-dium hypochlorite.~~	**Method 7-014** **Methods** 1. Pretreatment: Immerse seeds in a solution of sodium hypochlorite (NaOCl) (1% available chlorine) for 10 mins, then drain and rinse well in sterile water and drain.
Method 7-022 **Methods** Critical control points are indicated by CCP. ~~1. Pretreatment: Immerse seeds in NaOCl solution (1 % available chlorine) for 10 min, then drain.~~	**Method 7-022** **Methods** Critical control points are indicated by CCP. 1. Pretreatment: Immerse seeds in a solution of sodium hypochlorite (NaOCl) (1% available chlorine) for 10 mins, then drain and rinse well in sterile water and drain.
Method 7-016 **Methods** Critical control points are indicated by CCP.	**Method 7-016** **Methods** Critical control points are indicated by CCP.

~~1. Pretreatment: Gently rinse seeds in NaOCl solution (1 % available chlorine) for 30 s, then rinse for 30 s in sterile water. Blot the seed dry on sterile paper towels.~~	1. Pretreatment: Gently rinse seeds in a solution of sodium hypochlorite (NaOCl) (1% available chlorine) for 30 to 60 s, then rinse for 30 s in sterile water. Blot the seed dry on sterile paper towel.

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.7.2.	45	0	YES

Chapter 9: Determination of moisture content

C.9.1 Guidance for species not listed in Table 9A but is included in Table 2C

There have been questions how to report moisture results for species listed in Table 2C but not listed in Table 9A. The proposed cross reference in the reporting section of Chapter 9 gives clear advice.

The following proposal has been developed and approved by a majority vote of the Moisture Committee.

CURRENT VERSION	PROPOSED VERSION
9.2.7 Reporting of results … • In the case of pelleted seeds (see Chapter 11), the following statement must be entered: 'The seeds of the submitted moisture sample were pelleted, and the moisture content reported is the average of seed and pelleting materials.'	**9.2.7 Reporting of results** … • In the case of pelleted seeds (see Chapter 11), the following statement must be entered: 'The seeds of the submitted moisture sample were pelleted, and the moisture content reported is the average of seed and pelleting materials.' If the species being tested is not listed in Table 9A but is listed in Table 2C, the result must be reported according to 1.5.2.22. "NA" must be entered in the moisture test result space on the ISTA Certificate.
9.3.2.7 Reporting of moisture meter results … • In the case of pelleted seeds (see Chapter 11), the following statement must be entered: 'The seeds of the submitted moisture sample were pelleted, and the moisture content reported is the average of seed and pelleting materials.' ~~(shelled seeds).~~	**9.3.2.7 Reporting of moisture meter results** … • In the case of pelleted seeds (see Chapter 11), the following statement must be entered: 'The seeds of the submitted moisture sample were pelleted, and the moisture content reported is the average of seed and pelleting materials.' ~~(shelled seeds).~~ If the species being tested is not listed in Table 9A but is listed in Table 2C, the result must be reported according to 1.5.2.22. "NA" must be entered in the moisture test result space on the ISTA Certificate.

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.9.1	47	0	YES