



International Seed Testing Association

Secretariat, Zürichstrasse 50, CH-8303 Bassersdorf, Switzerland

Phone: +41 44 838 60 00 Fax: +41 44 838 60 01

Email: ista.office@ista.ch - <http://www.seedtest.org>

Document 05-2010-OM

Rules Proposals for the International Rules for Seed Testing 2011 Edition

This document was prepared by the Technical Committees and the Rules Committee of the Association and has been endorsed by the ISTA Executive Committee. The proposals are submitted to the ISTA Ordinary Meeting 2010 for voting by the nominated ISTA Designated Members on behalf of their respective Governments.

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

It contains proposed amendments and changes for all Chapters of the ISTA *International Rules for Seed Testing* and was discussed and accepted by vote at the Ordinary Meeting 2010, held on Tuesday, 22 June 2010 in Cologne, Germany, under Agenda point 11: Consideration and Adoption of the proposed Rules Changes.

Introduction to the ISTA Rules Proposals to become effective 1 January 2011

The current version of the ISTA International Rules for Seed Testing is the 2010 edition. Single copies of replacement pages and front covers for the 2010 edition have been sent free to all ISTA Member Laboratories. Extra copies are available for purchase from the ISTA Publications section. As the Rules are an evolving document, it is worth remembering that pages can be headed with different 'effective from' dates. The Preface for each edition includes details of changes and when replacement pages were issued. Previous Prefaces as a 'history of changes' are available on the ISTA website.

The ISTA Rules are the result of many years' worth of discussions and improvements from the various ISTA Technical Committees. Thanks to all the Technical Committee members and the ISTA Secretariat for all their help with this year's proposals.

The following Rules Proposals will be discussed at the ISTA Ordinary Meeting in Cologne, Germany, in June 2010. If the proposals are accepted by the membership, Amendments will be issued, and they will become the 2011 edition of the ISTA Rules.

Any subsequent editorial changes to any of the Rules remain the responsibility of the Chair of the Rules Committee.

Please let me know about any problems with these proposals.

Many thanks.

Steve Jones

Contact details for Chair of Rules Committee:

Dr. Steve Jones

Canadian Food Inspection Agency

Seed Science and Technology Section

301-421 Downey Road

Saskatoon, SK, S7N 4L8

Canada

Phone: +1 306 975 6505

Fax: +1 306 975 6450

E-mail: steve.jones@inspection.gc.ca

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PART A. INTRODUCTION OF EDITORIAL CHANGES

A.1. Editorial corrections

Shading of alternate rows in Rules tables

It is proposed that all tables in the Rules should have alternate rows shaded for better readability.

An example of this is shown in Appendix 1, following page 56.

Updating of cross-references

CURRENT VERSION	PROPOSED VERSION
<p>1.3 Conditions for issuance of ISTA Certificates</p> <p>...</p> <p>c) The tests must be carried out in accordance with the ISTA Rules. However, additionally and on request, results of tests not covered by these Rules may be reported on an ISTA Certificate (see 1.5.3.9).</p> <p>...</p>	<p>1.3 Conditions for issuance of ISTA Certificates</p> <p>...</p> <p>c) The tests must be carried out in accordance with the ISTA Rules. However, additionally and on request, results of tests not covered by these Rules may be reported on an ISTA Certificate (see 1.5.2.19).</p> <p>...</p>
<p><i>1.5.2.6 Germination</i></p> <p>...</p> <p>– the percentages, calculated to the nearest whole number (5.8.4), of normal seedlings, ...</p>	<p><i>1.5.2.6 Germination</i></p> <p>...</p> <p>– the percentages, calculated to the nearest whole number (5.8.2), of normal seedlings, ...</p>
<p><i>1.5.2.8 Tetrazolium test</i></p> <p>...</p> <p>At the discretion of the seed testing station, further information may be reported, e.g. percentage of seeds ...</p>	<p><i>1.5.2.8 Tetrazolium test</i></p> <p>...</p> <p>At the discretion of the seed testing laboratory, further information may be reported, e.g. percentage of seeds ...</p>
<p><i>1.5.2.13 Excised embryo</i></p> <p>...</p> <p>Further details may be given at the discretion of the seed testing station, e.g. percentages of seeds...</p>	<p><i>1.5.2.13 Excised embryo</i></p> <p>...</p> <p>Further details may be given at the discretion of the seed testing laboratory, e.g. percentages of seeds...</p>
<p><i>3.6.3.2 Test for variation between samples</i></p> <p>... If an ISTA International Seed Analysis Certificate has been issued already on the basis of the first test, refer to 17.6.</p>	<p><i>3.6.3.2 Test for variation between samples</i></p> <p>... If an ISTA International Seed Analysis Certificate has been issued already on the basis of the first test, refer to 1.6.</p>
<p>11.1.1 Definitions</p> <p>...</p> <p>Note: The numbering in this Chapter refers to the appropriate paragraphs of the other Chapters in the Rules, i.e. 11.2.4.1, cross references Chapter 11 to Chapter 2.4.1.</p>	<p>11.1.1 Definitions</p> <p>...</p> <p>Note: The numbering in this Chapter refers to the appropriate paragraphs of the other Chapters in the Rules, e.g. 11.3.2.1 cross references Chapter 11 to Chapter 3.2.1.</p>
<p>11.2.4.1 <i>Size of lot</i></p> <p>... subject to the seed number limitation prescribed in 11.2.4.1.A.</p>	<p>11.2.5.4.1 <i>Size of lot</i></p> <p>... subject to the seed number limitation prescribed in 11.2.5.4.1.A.</p>
<p>11.2.6.3 <i>Size of submitted sample</i></p>	<p>11.2.5.4.4 <i>Size of submitted sample</i></p>

CURRENT VERSION	PROPOSED VERSION
11.2.6.4-6.7 <i>Drawing and disposal of submitted sample</i>	<u>11.2.5.1.3-1.6</u> <i>Drawing and disposal of submitted sample</i>
11.2.7.1 <i>Size of working sample</i>	<u>11.2.5.2.1</u> <i>Size of working sample</i>
11.2.7.2 <i>Obtaining the working sample</i> For pelleted seeds use one of the dividers described in 2.7.2.A(i) . However the distance of fall must never exceed that indicated in 11.2.7.2.A . For seed tapes take pieces of tape at random, to provide sufficient seeds for the test.	<u>11.2.5.2</u> <i>Obtaining the working sample</i> For pelleted seeds use one of the dividers described in <u>2.5.2.2.1</u> . However the distance of fall must never exceed that indicated in <u>11.2.5.2.A</u> . For seed tapes take pieces of tape at random, to provide sufficient seeds for the test.
<i>11.3.5.1 Working sample</i> ... in accordance with 11.2.7.2 to one decimal place (see 3.5.1.A).	<i>11.3.5.1 Working sample</i> ... in accordance with <u>11.2.5.2</u> to one decimal place (see 3.5.1).
11.3.6 Calculation and expression of results ... the difference must not exceed the tolerance between duplicate analyses given in Chapter 16: Tolerances, Table 3.1 , column 3 or 4. ...	11.3.6 Calculation and expression of results ... the difference must not exceed the tolerance between duplicate analyses given in <u>Table 3C</u> , column 3 or 4. ...
11.4.6 Calculation and expression of results ... use Table 4.1 of Chapter 16: Tolerances	11.4.6 Calculation and expression of results ... use <u>Table 4A</u>
<i>11.5.6.4 Duration of test</i> ...	<i>11.5.6.4 Duration of <u>the</u> test</i> ...
11.10.2 Principles ... a sample of the size specified in Appendix A ...	11.10.2 Principles ... a sample of the size specified in <u>Chapter 16</u> ...
11.2.4.1.A <i>Size of lot</i> ...	<u>11.2.5.4.1.A</u> <i>Size of lot</i> ...
11.2.6.2.A <i>Sampling intensity</i> ... analogously following the prescriptions of 2.6.2 and 2.6.2.A , ...	<u>11.2.5.1.2.A</u> <i>Sampling intensity</i> ... analogously following the prescriptions of <u>2.5.1.2</u> , ...
11.2.7.2.A <i>Sampling in the laboratory</i> When using one of the dividers described in 2.7.2.A(i) for pelleted seed ...	<u>11.2.5.2.A</u> <i>Sampling in the laboratory</i> When using one of the dividers described in <u>2.5.2.2.1</u> for pelleted seed ...
11.5.4.A Materials ...	11.5.4.A <u>Growing media</u> ...
11.5.6.A Moisture and aeration ...	<u>11.5.6.2.2.A</u> <i>Moisture and aeration</i> ...
17.3 Principle ... and other information as designated in 6 below. ...	17.3 Principle ... and other information as designated in <u>17.6</u> below. ...

The following editorial changes are to simplify, clarify and synchronize Rules 1.5.2.1, 2.9.1.5 and 2.9.2.5. Since Chapter 2 has 2 rules for reporting results (2.9.1.5 for the H value test and 2.9.2.5 for the R value test), Rule 1.5.2.1. was correspondingly divided into 1.5.2.1.1 and 1.5.2.1.2, which are now identical to 2.9.1.5 and 2.9.2.5, respectively.

CURRENT VERSION	PROPOSED VERSION
<p><i>1.5.2.1 Sampling: heterogeneity testing for seed lots in multiple containers</i></p> <p>The result of a heterogeneity test for seed lots in multiple containers must be reported under ‘Other determinations’, as follows:</p> <p>a) The results of the H value test:</p> <ul style="list-style-type: none"> – X: test result of the adopted attribute in a container sample; – N: number of independent container samples; – No: number of containers in the lot; – the calculated H value. <p>b) The statement: ‘This H value does/does not indicate significant heterogeneity.’</p>	<p><i>1.5.2.1 Sampling: heterogeneity testing for seed lots in multiple containers</i></p> <p><u><i>1.5.2.1.1 The H value heterogeneity test</i></u></p> <p>The result of <u>the H value</u> heterogeneity test for seed lots in multiple containers must be reported under ‘Other determinations’, as follows:</p> <ul style="list-style-type: none"> – <u> </u>: <u>mean of all X values determined for the lot in respect of the adopted attribute</u>; – N: number of independent container samples; – No: number of containers in the lot; – the calculated H value; – <u>the</u> statement: ‘This H value does/does not indicate significant heterogeneity.’ <p><u>Note: the H value must not be calculated or reported if is outside the following limits:</u></p> <ul style="list-style-type: none"> – <u>purity components: above 99.8% or below 0.2%;</u> – <u>germination: above 99.0% or below 1.0%;</u> – <u>number of specified seeds: below two per sample.</u>
<p>c) The results of the R value test:</p> <ul style="list-style-type: none"> – X: test result of the adopted attribute in a container sample; – N: number of independent container samples; – No: number of containers in the lot; – the calculated <u>H</u> value. <p>d) The statement: “This R value does/does not indicate significant heterogeneity.”</p> <p>Note: the H value must not be calculated or reported if X is outside the following limits:</p> <ul style="list-style-type: none"> – Purity components: above 99.8% or below 0.2% – Germination: above 99.0% or below 1.0% – Number of specified seeds: below two per sample 	<p><u><i>1.5.2.1.2 The R value heterogeneity test</i></u></p> <p>The result of <u>the R value</u> heterogeneity test for seed lots in multiple containers must be reported under ‘Other determinations’, as follows:</p> <ul style="list-style-type: none"> – <u> </u>: <u>mean of all X-values determined for the lot in respect of the adopted attribute</u>; – N: number of independent container samples; – No: number of containers in the lot; – the calculated <u>R</u> value; – <u>the</u> statement: ‘This R value does/does not indicate significant heterogeneity.’

CURRENT VERSION	PROPOSED VERSION
<p>2.9.1 The H-value test</p> <p>...</p> <p>2.9.1.4 Use of Table 2D and reporting results</p> <p>...</p>	<p>2.9.1 The H value test</p> <p>...</p> <p>2.9.1.4 Use of Table 2D</p> <p>...</p>
<p>The results of the H-value test shall be reported as follows:</p> <p>X, N, No., calculated H-value and the statement that “This H-value does/does not indicate significant heterogeneity”. If X is outside of the following limits, the H-value shall not be calculated or reported:</p> <p>purity components: above 99.8% or below 0.2%</p> <p>germination: above 99.0% or below 1.0%</p> <p>number of specified seeds: below two per sample</p>	
<p>2.9.1.5 Heterogeneity testing for seed lots in multiple containers</p> <p>The result of a-heterogeneity test for seed lots in multiple containers must be reported under ‘Other determinations’, as follows:</p> <p>a) The results of the H-value test:</p> <p>— X: test result of the adopted attribute in a container sample;</p> <p>– N: number of independent container samples;</p> <p>– No: number of containers in the lot;</p> <p>– the calculated H-value.</p> <p>b) The statement: ‘This H-value does/does not indicate significant heterogeneity.’</p> <p>e) The results of the R-value test:</p> <p>— X: test result of the adopted attribute in a container sample;</p> <p>— N: number of independent container samples;</p> <p>— No: number of containers in the lot;</p> <p>— the calculated H-value.</p> <p>d) The statement: “This R-value does/does not indicate significant heterogeneity.”</p>	<p>2.9.1.5 Reporting results</p> <p>The result of <u>the H value</u> heterogeneity test for seed lots in multiple containers must be reported under ‘Other determinations’, as follows:</p> <p>– <u> : mean of all X values determined for the lot in respect of the adopted attribute;</u></p> <p>– N: number of independent container samples;</p> <p>– No: number of containers in the lot;</p> <p>– the calculated H value;</p> <p>– <u>the</u> statement: ‘This H value does/does not indicate significant heterogeneity.’</p>
<p>Note: the H-value must not be calculated or reported if X is outside the following limits:</p> <p>– Purity components: above 99.8% or below 0.2%</p> <p>– Germination: above 99.0% or below 1.0%</p>	<p>Note: the H value must not be calculated or reported if <u> </u> is outside the following limits:</p> <p>– purity components: above 99.8% or below 0.2%;</p> <p>– germination: above 99.0% or below 1.0%;</p>

CURRENT VERSION	PROPOSED VERSION
– Number of specified seeds: below two per sample	– number of specified seeds: below two per sample.
<p>2.9.2 The R-value test</p> <p>...</p> <p>2.9.2.4 Use of tables and reporting of results</p> <p>...</p> <p>The results of the R-value test must be reported as follows:</p> <p>X, N, No, calculated R-value and the statement that “This R-value does/does not indicate significant heterogeneity”.</p>	<p>2.9.2 The R value test</p> <p>...</p> <p>2.9.2.4 Use of tables</p> <p>...</p>
When using the tables, round averages to the next tabulated value (if in the middle, then downwards).	When using the tables, round averages to the next tabulated value (if in the middle, then downwards).
<p>2.9.2.5 Heterogeneity testing for seed lots in multiple containers</p> <p>The result of a heterogeneity test for seed lots in multiple containers must be reported under ‘Other determinations’, as follows:</p> <p>a) The results of the H-value test:</p> <p>— X: test result of the adopted attribute in a container sample;</p> <p>— N: number of independent container samples;</p> <p>— No: number of containers in the lot;</p> <p>— the calculated H-value.</p> <p>b) The statement: “This H-value does/does not indicate significant heterogeneity.”</p> <p>e) The results of the R-value test:</p> <p>— X: test result of the adopted attribute in a container sample;</p> <p>– N: number of independent container samples;</p> <p>– No: number of containers in the lot;</p> <p>– the calculated H-value.</p> <p>d) The statement: “This R-value does/does not indicate significant heterogeneity.”</p> <p>Note: the H-value must not be calculated or reported if X is outside the following limits:</p> <p>— Purity components: above 99.8% or below 0.2%</p> <p>— Germination: above 99.0% or below 1.0%</p> <p>— Number of specified seeds: below two per sample</p>	<p>2.9.2.5 Reporting results</p> <p>The result of <u>the R value</u> heterogeneity test for seed lots in multiple containers must be reported under ‘Other determinations’, as follows:</p> <p>– <u> </u>: <u>mean of all X values determined for the lot in respect of the adopted attribute;</u></p> <p>– N: number of independent container samples;</p> <p>– No: number of containers in the lot;</p> <p>– the calculated <u>R</u> value;</p> <p>– <u>the</u> statement: ‘This R value does/does not indicate significant heterogeneity.’</p>

Sections 1.5.2.18 and 1.5.2.19: numbering corrected.

CURRENT VERSION	PROPOSED VERSION
1.5.2.18 <i>Size and grading of seeds</i>	1.5.2.17 <i>Size and grading of seeds</i>
...	...
1.5.2.19 <i>Weighted average test for herbage and amenity seed lots transported loose in bulk containers</i>	1.5.2.18 <i>Weighted average test for herbage and amenity seed lots transported loose in bulk containers</i>
...	...
1.5.2.20 <i>Reporting of results of tests not covered by the Rules</i>	1.5.2.19 <i>Reporting of results of tests not covered by the Rules</i>
...	...

During preparation of the Revised PSD Handbook, Table 2A and Table 3B were cross-referenced, and it was found that following past nomenclature changes some entries in Table 3B are no longer listed in Table 2A. Therefore the following entries are to be deleted from Table 3B.

CURRENT VERSION	PROPOSED VERSION
10 <i>Caryophyllaceae</i> <i>Agrostemma</i>	(deleted)
11 <i>Fabaceae</i> <i>Cassia</i>	
<i>Cherianthus</i>	
10 <i>Onagraceae</i> <i>Godetia</i>	
<i>Lychnis</i>	
4 <i>Ranunculaceae</i> <i>Pulsatilla</i> <i>Chaffy</i>	
23 <i>Brassicaceae</i> <i>Rapistrum</i>	
11 <i>Fabaceae</i> <i>Senna</i>	
30 <i>Poaceae</i> <i>Taeniatherum</i> <i>Chaffy</i>	

The reference to ploidy testing was deleted in 8.2.2 of the 2010 edition. The following sentence should also have been deleted but was not.

CURRENT VERSION	PROPOSED VERSION
8.5.4 Examination of seedlings	8.5.4 Examination of seedlings
...	...
For a determination of ploidy level, a root tip or other tissue is excised and processed for ploidy analysis.	...
...	...

Section 11.12 Certificates was replaced by 11.3.7, 11.4.7 and 11.5.8.

CURRENT VERSION	PROPOSED VERSION
<p>11.12 Certificates</p> <p>11.12.6 Reporting results</p> <p>ISTA Certificates for coated seeds should be clearly marked in the space following the heading ANALYSIS RESULTS with the words ‘SEED PELLETS, ENCRUSTED SEEDS, SEED GRANULES, SEED TAPES or SEED MATS’. The name and number of seeds of each species found in the verification of species examination shall be reported under ‘Other Determinations’.</p>	<p>(deleted)</p>

Chapter 13: separate heading for Table 13C, for consistency with other chapters

CURRENT VERSION	PROPOSED VERSION
<p>Table 13C, based on the Poisson distribution, indicates the maximum range ...</p>	<p><u>13.9 Tolerance tables</u></p> <p>Table 13C, based on the Poisson distribution, indicates the maximum range ...</p>

[Reference in Chapter 3: 3.6.3.2 needs updating from 17.6 to 1.6](#)

PART B. NEW SPECIES AND CHANGES OF SPECIES NAMES**No new species for the 2011 Rules.****Changes to species names**

Species names for some of the pathogens detected by the ISTA Seed Health Testing Methods have had nomenclature changes. These changes therefore need to be made in the names used in the seed health methods.

Names will be updated in Methods 7-009 and 7-022 with an explanation about the changes added to the background of each method.

CURRENT NAME	PROPOSED NAME
7-009 <i>Fusarium moniliforme var. subglutinans</i>	<i>Fusarium <u>circinatum</u></i>
7-022 <i>Microdochium nivale</i>	<i>Microdochium nivale <u>and M. majus</u></i>

PROPOSED VERSION

7-009 Background

This method was originally published in the ISTA Handbook of Seed Health Testing in 1985 as Working Sheet No. 56 prepared by Robert L. Anderson, USDA Forest Service, Forest Pest Management, Region Asheville, North Carolina, USA. 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 & Koenraad, 2007) with the recommendation to accept for a further five years. [The pathogen *Fusarium circinatum*, with a teleomorph *Gibberella circinata*, was previously known as *Fusarium moniliforme* var. *subglutinans* \(Nirenberg and O'Donnell, 1998\).](#)

PROPOSED VERSION

7-022 Background

A method for *Microdochium nivale* (*Fusarium nivale*) was originally published in the *ISTA Handbook of Seed Health Testing* in November 1964 as S.3. No. 33 (Anon., 1964), and was never revised for inclusion in the second edition of the Handbook. The agar plate test proposed is based on the comparative test organized by the ISTA Seed Health Committee and experience of routine testing in a number of laboratories. Summary of changes to original working sheet: Either potato dextrose agar (PDA) or malt agar (MA) can be used; incubation temperature reduced to 20 °C; incubation in dark followed by 3-4 h in daylight or under near ultraviolet (NUV); changes to format and layout. [Within *Microdochium nivale* two varieties were recognised *M. nivale* var. *nivale* and *M. nivale* var. *majus*, in 2005 Glynn *et al.* provided molecular based phylogenetic evidence of two species when taken together with biological differences already reported, thus elevating the two varieties to species status, *M. nivale* and *M. majus*. Both species are detected on PDA and Malt Agar, however visually it is not possible to distinguish between the two and there is an overlap in spore morphology making it difficult to separate the two species on agar. Both species cause seedling blight in wheat although symptoms may be more severe with *M. majus*.](#)

5. Examination

CURRENT VERSION	PROPOSED VERSION
... conidia 10-30 × 2.5–5 µm; conidia <u>8-33</u> × 2.5–5 µm; ...

PART C. RULES CHANGES AND NEW METHODS REQUIRING A VOTE**Chapter 2: Sampling****C.2.1. Cargo sampler for seeds of the size of *Triticum aestivum* and larger**

The Bulking and Sampling Committee proposes that the cargo sampler be included in the Rules for seeds of the size of *Triticum aestivum* and larger. In the opinion of the Committee, a validation test is not necessary, for the following reasons:

- The cargo sampler has been successfully validated for chaffy and non-chaffy seeds of all sizes. Chaffy grass seeds are difficult seeds, when it is considered how the chamber of the cargo sampler fills. Since the cargo sampler functions with difficult seeds, it should also function with all seeds.
- The cargo sampler has been used for decades in national sampling without problems.
- there is currently no other manual sampling device for seed in bulk.

The following proposal was therefore developed by the Bulking and Sampling Committee, and approved by a vote:

CURRENT VERSION	PROPOSED VERSION
<p>2.5.1.3 Taking primary samples</p> <p>...</p> <p>e) <i>Cargo sampler (bulk sampler)</i>. The cargo sampler can be used for seeds smaller than the seeds of <i>Triticum aestivum</i>. The cargo sampler consists of a special type of chamber that is fixed to a shaft. ...</p>	<p>2.5.1.3 Taking primary samples</p> <p>...</p> <p>e) <i>Cargo sampler (bulk sampler)</i>. The cargo sampler consists of a special type of chamber that is fixed to a shaft. ...</p>

C.2.2. Hand halving method for *Gossypium* spp.

In some countries *Gossypium* seed is marketed without processing it with delinting methods. Fuzzy seed cannot be divided e.g. with soil divider and even modified halving method is seen to be useless. For these reasons the proposal is that hand halving method can be used for *Gossypium* if seed is not delinted.

CURRENT VERSION	PROPOSED VERSION
<p>2.5.2.2.4 The hand halving method</p> <p>This method is restricted to the following genera of chaffy seeds:</p> <p>...</p> <p><i>Elymus</i></p> <p><i>Eragrostis</i></p> <p><i>Gomphrena</i></p> <p><i>Melinis</i></p> <p><i>Oryza</i></p> <p>...</p>	<p>2.5.2.2.4 The hand halving method</p> <p>This method is restricted to the following genera of chaffy seeds:</p> <p>...</p> <p><i>Elymus</i></p> <p><i>Eragrostis</i></p> <p><i>Gomphrena</i></p> <p><u><i>Gossypium</i> (linted seed only)</u></p> <p><i>Melinis</i></p> <p><i>Oryza</i></p> <p>...</p>

C.2.3. Harmonization of sample sizes in Table 2A Part 1

The submitted sample sizes are proposed to be harmonized in *Table 2A Part 1. Agricultural and vegetable seeds*. According to the ISTA Rules *I-2.2 Proposals for new species* the submitted sample size should be 10 times the weight of the purity working sample to allow the determination of other species by number. However, submitted sample size is not proposed to exceed 1 000 g in any species and the minimum submitted sample size is proposed to be 5 g. Harmonization was requested at the ISTA Annual Meeting 2009.

The following proposal was developed by the Bulking and Sampling Committee and approved by a majority vote:

Species	CURRENT	PROPOSED
	VERSION	VERSION
	<u>Minimum</u>	<u>Minimum</u>
	Submitted	Submitted
	sample (g)	sample (g)
Achillea millefolium L.	25	5
Agrostis canina L.	25	5
Agrostis capillaris L.	25	5
Agrostis gigantea Roth	25	5
Agrostis stolonifera L.	25	5
Anthoxanthum odoratum L.	25	20
Apium graveolens L.	25	10
Axonopus compressus (Sw.) P. Beauv.	25	10
Axonopus fissifolius (Raddi) Kuhlm.	25	10
Beckmannia eruciformis (L.) Host	25	20
Bothriochloa insculpta (Hochst. ex A. Rich.) A. Camus	25	20
Bothriochloa pertusa (L.) A. Camus	25	10
Chloris gayana Kunth	25	10
Claytonia perfoliata Donn ex Willd.	25	20
Cynodon dactylon L. (Pers.)	25	10
Cynosurus cristatus L.	25	20
Deschampsia cespitosa (L.) P. Beauv.	25	10
Deschampsia flexuosa (L.) Trin.	25	10
Digitaria eriantha Steud. (includes Digitaria decumbens Stent)	25	12
Eragrostis curvula (Schrad.) Nees	25	10
Eragrostis tef (Zuccagni) Trotter	25	10
Fragaria spp.	25	10
Galega orientalis Lam.	250	200
Holcus lanatus L.	25	10
Koeleria macrantha (Ledeb.) Schult.	100	10
Lotononis bainesii Baker	25	10
Lotus uliginosus Schkuhr	25	20
Melinis minutiflora P. Beauv.	25	5
Nasturtium officinale R. Br.	25	5
Oenothera biennis L.	25	10
Origanum majorana L.	25	5
Origanum vulgare L.	25	5
Panicum antidotale Retz.	25	20
Panicum coloratum L.	25	20
Panicum maximum Jacq.	25	20
Papaver somniferum L.	25	10
Phleum nodosum L.	25	10
Phleum pratense L.	25	10
Physalis pubescens L.	25	20

	CURRENT VERSION	PROPOSED VERSION
Species	<u>Minimum</u> Submitted sample (g)	<u>Minimum</u> Submitted sample (g)
<i>Piptatherum miliaceum</i> (L.) Coss.	25	20
<i>Poa annua</i> L.	25	10
<i>Poa compressa</i> L.	25	5
<i>Poa nemoralis</i> L.	25	5
<i>Poa palustris</i> L.	25	5
<i>Poa pratensis</i> L.	25	5
<i>Poa secunda</i> J. Presl (includes <i>Poa ampla</i> Merr.)	25	15
<i>Poa trivialis</i> L.	25	5
<i>Portulaca oleracea</i> L.	25	5
<i>Pseudoroegneria spicata</i> (Pursh) Á. Löve	125	80
<i>Schizachyrium scoparium</i> (Mishx.) Nash	100	50
<i>Sorghum bicolor</i> (L.) Moench x <i>S. sudanense</i> (Piper) Stapf	500	300
<i>Thymus vulgaris</i> L.	25	5
<i>Trifolium campestre</i> L.	25	5
<i>Trifolium dubium</i> Sibth.	25	20
<i>Trifolium glomeratum</i> L.	25	10
<i>Trifolium hybridum</i> L.	25	20
<i>Trifolium lappaceum</i> L.	25	20
<i>Trifolium michelianum</i> Savi	25	20
<i>Trifolium repens</i> L.	25	20
<i>Trifolium resupinatum</i> L.	25	20
<i>Trifolium vesiculosum</i> Savi	100	30
<i>Trisetum flavescens</i> (L.) P. Beauv.	25	5
<i>Zoysia japonica</i> Steud.	25	10

Comments inserted

The Germination Committee have considered and discussed the debate and the points made at the Rules Committee meeting on Sunday.

In putting forward their proposals the Germination Committee consulted widely and all ISTA member laboratories were sent a questionnaire where they were asked to consider every paragraph of the Germination Chapter of the Rules and indicate where improvements could be made. ISTA auditors were consulted and provided a report which listed parts of the Germination Chapter that resulted in problems for accredited labs. Finally the committee considered questions it received from members over the last three years where these involved interpretation of the germination chapter of the rules.

Some of the issues raised at the Rules Committee meeting involved parts of the Germination Chapter where the Committee have not made any proposals. It is a pity that the committee’s efforts in information gathering failed to identify these issues. Unfortunately, the committee is unable to make proposals for changes to these parts at this meeting.

It would be pleased however, if those making such suggestions could send them to the committee. The suggestions will be given detailed consideration and could be included as part of the committee’s proposals to be considered at the 2011 Annual Meeting. Please send them to the committee as soon as possible, and no later than October, since the committee must submit its proposals by November, ready for consideration at the 2011 Annual Meeting.

Chapter 5: The Germination Test

Revision of Chapter 5

General comments to the Revision of Chapter 5:

The Germination Committee reviewed all occurrences of the words ‘must’ and ‘should’, and decided whether they were correct.

In the proposed version, the following changes have been made throughout without further indication in the text below:

- old family names have been removed;
- the term ‘ISTA International Seed Analysis Certificate’ has been replaced by the term ‘ISTA Certificate’. Further comments by the Committee regarding change proposals are inserted immediately above the relevant sections.

C.5.1. Revision of sections 5.1 Object, 5.2 Definitions: 5.2.1 Germination, 5.2.2 Germination percentage

CURRENT VERSION	PROPOSED VERSION
5.1 Object: We have removed the word ‘maximum’, since it could be regarded as superfluous and is not true in the case of seed lots with hard seed.	
<p>5.1 Object</p> <p>The object of the germination test is to determine the maximum germination potential of a seed lot, which can then in turn be used to compare the quality of different lots and also estimate the field planting value.</p>	<p>5.1 Object</p> <p>The object of the germination test is to determine the germination potential of a seed lot, which can then in turn be used to compare the quality of different lots and also estimate the field planting value.</p>

CURRENT VERSION	PROPOSED VERSION
...	...
5.2.1 Germination: It is an ISTA test rather than any laboratory test.	
<p>5.2 Definitions</p> <p>5.2.1 Germination</p> <p>Germination of a seed in a laboratory test is the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in soil.</p>	<p>5.2 Definitions</p> <p>5.2.1 Germination</p> <p>Germination of a seed in <u>an ISTA</u> test is the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in <u>the field</u>.</p>
5.2.2: Germination percentage: This is what germination is in an ISTA test.	
<p>5.2.2 Germination percentage</p> <p>The germination percentage reported on the ISTA International Seed Analysis Certificate indicates the proportion by number of seeds which have produced seedlings classified as normal under the conditions and within the period specified in Table 5A.</p>	<p><u>5.2.4</u> Germination percentage</p> <p>The germination percentage reported on the ISTA Certificate indicates the proportion by number of seeds which have produced seedlings classified as normal under the conditions and within the period specified in Table 5A, <u>i.e. the percentage of normal seedlings</u>.</p>
...	...

Section 5.2 has been restructured into 3 different votes. Procedure accepted

**Vote 1: Proposal 5.2a
5.5.2 QA for counting equipment**

**Vote 2: Proposal 5.2b
5.6.3.4 Disinfection of seed**

**Vote 3: Proposal 5.2c
The remaining part of 5.2**

Vote 1: Proposal 5.2a. 5.5.2 QA for counting equipment

<i>5.5.2 Counting equipment</i>	<i>5.5.2 Counting equipment</i>
	<p><u>Planting using counting boards or vacuum counters is permissible, as long as using these tools does not influence the germination result or cause replicate results to be biased. The laboratory must ensure that counting boards and vacuum counters are calibrated, and that the equipment does not influence the outcome of the germination test. With vacuum counters, some precautions should be observed to avoid biased replicates: the counting head must not be plunged into the working sample, and the vacuum must not be applied when seeds are poured onto the counting head, as both result in the selection of light seed.</u></p>

C.5.2.c Revision of sections 5.2 Definitions (remainder) to 5.6 Procedure

CURRENT VERSION	PROPOSED VERSION
New 5.2.2, 5.2.3: Definitions lacking in present rules.	
	<u>5.2.2 Double test</u> <u>A double test is where two tests are prescribed for certain tree and shrub seed species, and the results of both tests are reported.</u>
	<u>5.2.3 Parallel tests</u> <u>Parallel tests are where more than one test method from those prescribed is applied to a sample at the same time and the best result reported.</u>
	<u>(5.2.4 Germination percentage: see Voting Item C.5.1.)</u>
<u>5.2.3 Essential seedling structures</u> ... For further details see 5.2.8.	<u>5.2.5 Essential seedling structures</u> ... For further details see 5.2.11.
	<u>5.2.6 The 50% rule</u> <u>The 50% rule is used in the evaluation of cotyledons and primary leaves.</u> <u>Cotyledon tissue:</u> <ul style="list-style-type: none"> • <u>Seedlings are considered normal as long as half or more of the total cotyledon tissue is functional.</u> • <u>Seedlings are abnormal when more than half of the cotyledon tissue is missing, necrotic, decayed or discoloured.</u> <u>Primary leaves:</u> <ul style="list-style-type: none"> • <u>Primary leaves need to be evaluated in species such as <i>Phaseolus</i>.</u> • <u>Seedlings are considered normal as long as half or more of the primary leaf tissue is functional.</u> • <u>Seedlings are abnormal when more than half of the primary leaf tissue is missing, necrotic, decayed or discoloured.</u> <u>The 50% rule does not apply if the tissue around the terminal bud or the terminal bud itself is necrotic or decayed; such seedlings are abnormal irrespective of the condition of the cotyledons or primary leaves.</u> <u>Further details of how the 50% rule is applied can be found in the <i>ISTA Handbook on Seedling Evaluation</i>.</u>

CURRENT VERSION	PROPOSED VERSION
5.2.4 Normal seedlings	5.2.7 Normal seedlings
...	...
5.2.4.1 Intact seedlings	5.2.7.1 Intact seedlings
...	...
5.2.4.2 Slight defects: For greater clarity.	
5.2.4.2 Slight defects	5.2.7.2 Slight defects
The following defects are considered slight:	The following defects are considered slight <u>and therefore seedlings are classified as normal:</u>
– primary root with limited damage or slight growth retardation;	– primary root with limited damage (<u>e.g. not affecting the conductive tissue</u>) or slight growth retardation;
...	...
– hypocotyl, epicotyl or mesocotyl with limited damage;	– hypocotyl, epicotyl or mesocotyl with limited damage (<u>e.g. not affecting the conductive tissue</u>);
– cotyledons with limited damage (if half or more of the total tissue area is left functioning normally [50% rule] and if there is no evidence of damage or decay to the shoot apex or surrounding tissues);	– cotyledons with limited damage (if half or more of the total tissue area is left functioning normally [<u>i.e. the 50% rule; see 5.2.6</u>] and if there is no evidence of damage or decay to the shoot apex or surrounding tissues);
...	...
– three cotyledons instead of two (provided that they comply with the 50% rule);	– three <u>or more</u> cotyledons instead of two (provided that they comply with the 50% rule; <u>see 5.2.6</u>);
	– <u>fused cotyledons (provided that they comply with the 50% rule; see 5.2.6)</u> ;
– primary leaves with limited damage (if half or more of the total tissue area is left functioning normally [50% rule]);	– primary leaves with limited damage (if half or more of the total tissue area is left functioning normally [<u>the 50% rule; see 5.2.6</u>]);
...	...
– three primary leaves instead of two, e.g. in <i>Phaseolus</i> (provided that they comply with the 50% rule);	– three <u>or more</u> primary leaves instead of two, e.g. in <i>Phaseolus</i> (provided that they comply with the 50% rule; <u>see 5.2.6</u>);
...	...
5.2.5 Abnormal seedlings	5.2.8 Abnormal seedlings
...	...
decayed: seedlings with any of their essential structures so diseased or decayed as a result of primary infection (see definition in 5.2.8) that normal development is prevented.	decayed seedlings with any of their essential structures so diseased or decayed as a result of primary infection (see definition in 5.2.11) that normal development is prevented
5.2.5.1 Seedling abnormalities	5.2.8.1 Seedling abnormalities
One or more of the following defects in the seedling renders it abnormal.	One or more of the following defects in the seedling renders it abnormal:
0 Overall abnormalities	0 Overall abnormalities

CURRENT VERSION	PROPOSED VERSION
00 The seedling:	00 The seedling:
00/11: This was missed out in present rules by mistake.	
	00/11 is unbalanced
00/12: Common abnormality that should be included.	
	00/12 in Poaceae, detached endosperm
1 Abnormalities of the root system: Current text mixes up primary and secondary roots – amendment makes necessary correction.	
1 Abnormalities of the root system 11 The primary root: ...	1 Abnormalities of the root system 11 The primary root: ... Note: secondary roots showing one or more of the above defects are abnormal and cannot replace an abnormal primary root in cases where the presence of several secondary roots (e.g. Cucumis) determines the value of a seedling.
12 The seminal roots: 12/01 are stubby, weak or missing Note: secondary roots or seminal roots showing one or more of the above defects are abnormal and cannot replace an abnormal primary root in cases where the presence of several secondary roots (e.g. Cucumis), or at least one strong seminal root (e.g. Triticum), or two strong seminal roots (Cyclamen) determine the value of a seedling.	12 The seminal roots: 12/01 are stubby, weak or missing Note: At least one strong seminal root (e.g. Triticum), or two strong seminal roots (i.e. Cyclamen) are required for a normal seedling.
2 Abnormalities of the shoot system	2 Abnormalities of the shoot system
21 The hypocotyl, epicotyl or mesocotyl:	21 The hypocotyl, epicotyl or mesocotyl:
21/13: Additional type of abnormality that should be included.	
	21/13 shows negative phototropism
...	...
3 Abnormalities of the cotyledons and primary leaves	3 Abnormalities of the cotyledons and primary leaves
31 The cotyledons (apply the 50% rule):	31 The cotyledons (apply the 50% rule; see 5.2.6):
31/08: Although fused cotyledons can be considered as normal if the 50% Rule is complied with, they are considered abnormal if fused on both sides.	
	31/08 are fused on both sides
...	...
32: Order changed to match the Seedling Evaluation Handbook.	
32 In <i>Allium</i> spp., the cotyledon:	32 In <i>Allium</i> spp., the cotyledon:
32/01 is short and thick 32/02 is constricted 32/03 is bent over 32/04 forms a loop or spiral 32/05 is without a definite 'knee'	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	32/06 is spindly
33 The primary leaves (apply the 50%	33 The primary leaves (apply the 50%

CURRENT VERSION	PROPOSED VERSION
rule):	rule; see 5.2.6):
33/06: For clarity, as this only applies to <i>Phaseolus</i>	
33/06 are of normal shape, but less than one-quarter normal size	33/06 are of normal shape, but less than one-quarter normal size (only in <i>Phaseolus</i>)
...	...
4 Abnormalities of the coleoptile and first leaf:	4 Abnormalities of the coleoptile and first leaf:
41 The coleoptile:	41 The coleoptile:
41/11: To match what is given in the Seedling Evaluation Handbook	
41/11 is split at the base	41/11 is split other than from the tip
42: Primary leaf used throughout rather than first leaf to match handbook	
42 The first leaf:	42 The primary leaf:
...	...
5.2.6 Multigerm seed units: To provide greater clarity on how to deal with seedlings from multiple seed units.	
5.2.6 Multigerm seed units	5.2.9 Multigerm seed units
...	...
– fused embryos. Occasionally two seedlings which are fused together are produced from one seed.	– fused embryos. Occasionally two seedlings which are fused together are produced from one seed.
	When a unit produces more than one seedling, these are evaluated separately. One normal seedling is considered sufficient to classify the unit as normal. If a unit produces more than one normal seedling, only one is counted for determining the germination percentage.
5.2.7 Ungerminated seeds: Makes the link between dormancy and fresh seed clear.	
5.2.7 Ungerminated seeds	5.2.10 Ungerminated seeds
...	...
fresh seeds: seeds, other than hard seeds, which have failed to germinate under the conditions of the germination test, but which remain clean and firm and have the potential to develop into a normal seedling;	fresh seeds seeds, other than hard seeds, which because of dormancy have failed to germinate under the conditions of the germination test, but which remain clean and firm and have the potential to develop into a normal seedling
...	...
5.2.7.1 Hard seeds: Makes it clear that hard seeds are most often found in the <i>Fabaceae</i> , and should be looked for when testing this family.	
5.2.7.1 Hard seeds	5.2.10.1 Hard seeds
Hardseededness is a form of dormancy. It is common in many species of the <i>Fabaceae</i> (<i>Leguminosae</i>) but may also occur in other families. These seeds are not able to imbibe water under the conditions set out in Table 5A and remain hard.	Hardseededness is a form of dormancy. It is common in many species of the <i>Fabaceae</i> but may also occur in other families. These seeds are not able to imbibe water under the conditions set out in Table 5A and remain hard.
...	...

CURRENT VERSION	PROPOSED VERSION
5.2.7.3 Dead seeds: Makes clear the distinction between hard and dead.	
<i>5.2.7.3 Dead seeds</i>	5.2.10.3 <i>Dead seeds</i>
Dead seeds usually are soft, discoloured, frequently mouldy and show no sign of seedling development.	Dead seeds absorb water, are usually soft or discoloured or frequently mouldy , and show no sign of seedling development.
5.2.7.4 Other categories: Takes account of the possibility of finding hard seed in species other than those belonging to the Fabaceae	
<i>5.2.7.4 Other categories</i>	5.2.10.4 <i>Other categories</i>
On request, ungerminated seeds may be further subdivided into:	Ungerminated seeds may be further subdivided into:
...	...
insect-damaged seeds: ...	insect-damaged seeds
These categories may appear in all types of seeds, but are found more commonly in tree species.	These categories may appear in all species of seeds, but are found more commonly in tree species.
5.2.8 Additional definitions: definition of 'phototropism' added, since this is now included as a type of abnormality.	
<i>5.2.8 Additional definitions</i>	5.2.11 <i>Additional definitions</i>
...	...
	phototropism growth and response to a light stimulus. positive phototropism growth towards light negative phototropism growth away from light
...	...
5.3 General principles: To comply with rules for testing tapes and mats.	
5.3 General principles	5.3 General principles
§1 ...	§1 ...
§2 The pure seed definition for the species must be applied. The pure seed can be taken from either the pure seed fraction of a purity test carried out as prescribed in Chapter 3, or from a representative fraction of the submitted sample. When the seed lot has been coated, the pure pellet definition must be used.	§2 The pure seed definition for the species must be applied. The pure seed can be taken from either the pure seed fraction of a purity test carried out as prescribed in Chapter 3, or from a representative fraction of the submitted sample. When the seed lot has been coated, the pure pellet definition must be used except in the case of tapes or mats where the seeds are tested without removing the seed .
Changed for clarity.	
§3 The seeds must receive no pretreatments except those recommended in 5.6.3. Parallel or duplicate testing is permitted, the rules for reporting are as defined for retests in 5.7 . If additional tests are undertaken after any other pretreatment, the result and pretreatment must be	§3 Prescribed procedures for promoting germination are given in 5.6.3. Parallel testing is permitted. The rules for reporting parallel and double testing are defined in 5.9 . If additional tests are undertaken after any procedure other than those given in 5.6.3, the test is not

CURRENT VERSION	PROPOSED VERSION
reported under ‘Other Determinations’ on the ISTA International Seed Analysis Certificate.	covered by the Rules, and the result and procedure must be reported under ‘Other determinations’ on the ISTA Certificate (see 1.5.3.9).
§4 ...	§4 ...
5.4 Growing media	5.4 Growing media
5.4.1 Definition: ‘Anchorage’ would imply that the roots are growing into the media which is not permitted in paper media.	
5.4.1 Definition	5.4.1 Definition
Growing media used for germination tests are products which provide sufficient pore space for air and water, for the anchorage of the root system and for contact with solutions (water) needed for plant growth.	Growing media used for germination tests are products which provide sufficient pore space for air and water, for the growth of the root system and for contact with solutions (water) needed for plant growth.
5.4.2 Specifications: Gives a clear instruction. The Committee looked at every ‘should’ and ‘must’ and decided what was meant.	
5.4.2 Specifications	5.4.2 Specifications
The following general specifications apply for all growing media.	The following general specifications apply for all growing media and must be verified .
Composition: ...	Composition ...
Water retention: Changed for clarity.	
Water retention characteristics: ... A priori, the water content of the growing medium must be adjusted to the maximal water holding capacity. When necessary it can be adjusted to correspond to the needs of a particular species. The water retention must then be expressed as a percentage of the maximum retention.	Water retention characteristics ... The water content of the growing medium should be adjusted to correspond to the needs of the species being tested, based on the maximum water-holding capacity of the media . The water retention is then expressed as a percentage of the maximum retention.
pH: ...	pH ...
Conductivity: the salinity must be as low as possible and no more than 40 millisiemens per meter.	Conductivity The salinity must be as low as possible and no more than 40 millisiemens per meter.
pH and conductivity: Makes it clear that both pH and conductivity measurements can be replaced by biological tests in certain circumstances, and not just conductivity	
	Measurements of pH and conductivity can be replaced by biological tests (see 5.4.5)
...	...
5.4.3 Growing media characteristics	5.4.3 Growing media characteristics
5.4.3.1 Paper growing media	5.4.3.1 Paper growing media
The paper should be wood, cotton or other purified vegetable cellulose. ...	The paper must be wood, cotton or other purified vegetable cellulose. ...
...	...
5.4.4. Water: Makes it clear that all can be used	

CURRENT VERSION	PROPOSED VERSION
5.4.4 Water	5.4.4 Water
Demineralised water, de-ionized water, tap water and spring water are commonly used.	Demineralised water, de-ionized water, tap water and spring water are used and permitted .
<i>5.4.4.1 General specifications</i>	<i>5.4.4.1 General specifications</i>
Cleanness: ...	Cleanness ...
pH: Does not specify that statistical evidence is required, and makes it clear that when examining the effect of pH outside the range, a laboratory must provide evidence that a pH outside the range specified does not have a negative influence on germination.	
pH: the pH value should be within the range 6.0–7.5 when checked in the substrate, or there must be evidence, based on statistical data, that there is no influence of a pH outside this range of values on the germination test results.	pH: The pH value must be within the range 6.0–7.5 when checked in the substrate, unless there is evidence that the pH outside this range does not have a negative influence on the germination test results.
5.4.5 Quality control	5.4.5 Quality control
New deliveries of growing media must meet the requirements for the principal physical characteristics and be free of negative effects due to toxic substances or noxious micro-organisms.	New deliveries of growing media must meet the requirements for the principal physical characteristics and be free of negative effects due to toxic substances or noxious micro-organisms.
The characteristics composition, water retention, pH, cleanness and innocuousness (freedom from phytotoxic effects and negative effects due to micro-organisms) should be checked.	The characteristics composition, water retention, pH, cleanness and innocuity (freedom from phytotoxic effects and negative effects due to micro-organisms) must be checked.
Alternative measurements: Allows measurements of both pH and conductivity to be replaced by biological tests.	
	Alternative measurements: it may be difficult to check all the specifications or to get growing media from suppliers with the requested specifications. It is permissible to replace the measurements of pH and conductivity with biological tests, such as a test for phytotoxicity.
Adds clarity, and makes it clear that if an external lab carries out tests they do this on the basis of a subcontract.	
Examples of methods used to measure these characteristics are given in the <i>ISTA Handbook on Seedling Evaluation</i> . Quality control tests can be performed by the seed testing laboratory or by laboratories specializing in soil analyses or microbiology tests.	Examples of media quality control tests are given in the <i>ISTA Handbook on Seedling Evaluation</i> . Quality control tests can be performed by the seed-testing laboratory or subcontracted to laboratories specializing in soil analyses or microbiology tests.
5.5 Material and apparatus	5.5 Material and apparatus
5.5.1 Containers: For clarity.	
5.5.1 Containers	5.5.1 Containers
All kinds of plastic, glass, metal or pottery transparent or containers can be used, provided there are no toxic emanations and they are clean and free	All kinds of plastic, glass, metal or pottery containers can be used, provided that they have no toxic effects , and are clean and free from micro-organisms.

CURRENT VERSION	PROPOSED VERSION
from micro-organisms.	
5.5.2 Counting equipment: Details of the use of counting boards and vacuum counters should be in handbooks (or in Chapter 2: Sampling). Here we need only say for what species they are, or can be, used for, give generic warnings about their use, and state that they have to be shown to be fit for purpose through 'validation'	
5.5.2 Counting equipment	5.5.2 Counting equipment
	<u>Removed for separate vote.</u>
<i>5.5.2.1 Counting boards</i>	<i>5.5.2.1 Counting boards</i>
Counting boards are usually used for large seeds such as <i>Zea</i> , <i>Phaseolus</i> and <i>Pisum</i> . A counting board is approximately the size of the substrate on which the seeds are to be placed. The top consists of a board with 50 or 100 holes of the general size of the seeds to be counted, but large enough that the largest seeds of the sample fit in. Below this board is another board which serves as a bottom; it may be solid to be slid backward and forward or it may contain corresponding holes which can be closed or opened by moving top and bottom against each other. In operation the seeds are scattered over the board with the holes closed underneath. Excess seeds are removed after checking to see that all holes are filled and that there is only one seed in each hole. The holes are opened by sliding the movable board, and the seeds fall into place on the substrate.	Counting boards are <u>often</u> used for large seeds such as <i>Zea</i> , <i>Phaseolus</i> and <i>Pisum</i> .
<i>5.5.2.2 Vacuum counters</i>	<i>5.5.2.2 Vacuum counters</i>
Vacuum counters are mostly used for species with regular shaped and relatively smooth seeds such as cereals , <i>Brassica</i> and <i>Trifolium</i> species. A vacuum counter consists of three essential parts: a vacuum system including pipes which do not restrict air flow, a range of counting plates or heads to suit the range of seeds tested and the size of germination substrates, and a vacuum release valve. An ordinary household vacuum cleaner may be used as vacuum system. The heads, containing 50 or 100 holes, should be slightly smaller than the substrate and should have an edge to prevent the seeds from rolling off. The diameter of the holes should correspond with the seed size and the vacuum applied. In operation the seeds are poured evenly over the counting head with the vacuum off. The vacuum is then applied, surplus seeds removed and a check made that all	Vacuum counters <u>can in principle be used for all species, but</u> are mostly used for species with <u>regularly</u> shaped and relatively smooth seeds such as <u>cereals or species of Brassica or Trifolium.</u>

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<p>holes are filled and only one seed is on each hole. The head is then placed on the germination substrate and the vacuum released so that the seeds fall in place on the substrate.</p> <p>Care should be taken that there is no selection of seeds. With vacuum counters some precautions should be observed to avoid biased replicates: the counting head must not be plunged into the seed because this procedure selects light seeds. For the same reason the vacuum should not be applied when the seeds are being poured on to the counting head.</p>	
5.5.3 Germination apparatus	5.5.3 Germination apparatus
<i>5.5.3.1 The bell jar or Jacobsen apparatus (Copenhagen tank)</i>	<i>5.5.3.1 The bell jar or Jacobsen apparatus (Copenhagen tank)</i>
<p>This apparatus usually consists of a germination plate upon which filter paper substrates with seeds are placed. The substrate is kept continuously moist by means of a wick, which extends down through slits or holes in the germination plate into the underlying waterbath. To prevent drying out the substrate is covered with a bell jar provided with a hole allowing for ventilation without undue evaporation. The temperature is conditioned either indirectly by heating/cooling the water in the waterbath or directly by conditioning the germination plate and is usually automatically regulated. The apparatus may be used for all prescribed constant or alternating temperatures, although the range of temperatures it may be possible to achieve on a particular Jacobsen apparatus may be limited by its design.</p>	<p>This apparatus usually consists of a germination plate upon which filter paper substrates with seeds are placed. The substrate is kept continuously moist by means of a wick, which extends down through slits or holes in the germination plate into the underlying waterbath. To prevent drying out, the substrate is covered with a bell jar provided with a hole <u>which allows</u> for ventilation without undue evaporation. The temperature is conditioned either indirectly by heating or cooling the water in the waterbath, or directly by conditioning the germination plate, and is usually automatically regulated. The apparatus may be used for all prescribed constant or alternating temperatures.</p>
5.5.3.2: We have combined text regarding cabinets (incubators) and room germinators as they are the same thing only varying in terms of size/volume. This eliminates duplicate text.	
<i>5.5.3.2 The germination cabinet</i>	<i>5.5.3.2 The germination <u>incubator and the room germinator</u></i>
<p>Another type of apparatus is the closed cabinet for germinating seeds in darkness or light. Modern cabinets are well insulated and have both heating and cooling systems. Suitable models are available for constant and alternating temperatures covering the full range required. The temperature may be maintained by circulating water or air or both through the cabinet. If the equipment available is only capable of providing constant temperatures, the</p>	<p><u>The incubator is used for germinating seeds in darkness or light, or providing seeds with pretreatments to break dormancy, such as prechilling. The room germinator is a modification of the incubator but is large enough to permit workers to enter and place the tests within it. Germination incubators and room germinators are well insulated and are equipped with both heating and cooling systems to ensure the maintenance of required temperatures.</u></p>

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<p>transfer of tests from one cabinet to another running at different temperatures, will achieve the desired alternating cycle. Some cabinets have humidity control so that tests may be left uncovered in the cabinet without drying out ('wet' cabinets). However, not all so-called 'wet' cabinets maintain sufficient humidity and if there is any doubt about the humidity level, it is preferable to enclose tests in moisture-proof containers. Tests in 'dry' cabinets should always be enclosed.</p> <p>5.5.3.3 The room germinator</p> <p>The room germinator is a modification of the cabinet. It is constructed on the same principles, but is large enough to permit workers to enter and place the tests along either side of a central passageway. Alternatively tests can be placed on trolleys which are then wheeled into the room for the test period. Unless tests are enclosed in moisture-proof containers, room humidifiers should be installed to maintain a high level of humidity.</p>	<p><u>The temperature must be evenly distributed to ensure that all samples placed in the incubator/room have a temperature within the prescribed temperature limits for the test (± 2 °C) or pretreatment. If the incubator/room does not have a system capable of providing alternating temperatures, samples can be transferred from one incubator/room to another running at a different temperature to achieve the desired alternative temperature cycle. Tests must be supplied with sufficient water for germination and must not be allowed to dry out. This can be achieved through maintaining a high humidity by using 'wet' incubators or using humidifiers in germination rooms. Tests can also be enclosed in moisture-proof containers.</u></p>
5.6 Procedure	5.6 Procedure
5.6.1 Working sample	5.6.1 Working sample
5.6.1 Working sample: saprophytes added. It is not just seed-borne disease that causes problems – most often it is non-seed-borne pathogens and saprophytes such as <i>Pythium</i> and <i>Rhizopus</i> that cause the greatest problems.	
<p>Four hundred seeds are taken at random from the well-mixed pure seed (5.3) and spaced uniformly and adequately apart on the moist substrate. Care should be taken that there is no selection of seeds thus causing biased results. Replicates of 100 seeds are normally used, spaced sufficiently far apart on the seed bed to minimize the effect of adjacent seeds on seedling development. To ensure adequate spacing, split replicates of 50 or even 25 seeds may be necessary, particularly where there is seed-borne disease present. When the seeds are heavily infected it may also be necessary with a paper substrate to change the substrate at an intermediate count.</p>	<p>Four hundred seeds are taken at random from the well-mixed pure seed (5.3) and spaced uniformly and adequately apart on the moist substrate. Care <u>must</u> be taken <u>to ensure</u> that there is no selection of seeds, thus causing biased results. Replicates of 100 seeds are normally used, spaced sufficiently far apart on the seed bed to minimize the effect of adjacent seeds on seedling development. To ensure adequate spacing, split replicates of 50 or even 25 seeds may be necessary, particularly where there <u>are</u> seed-borne <u>pathogens or saprophytes</u> present. When seeds <u>grown on paper substrates</u> are heavily infected, it may be necessary at an intermediate count <u>to transfer remaining seeds and seedlings to fresh media.</u></p>
Multigerm seed units are not broken up for the germination test but are tested as though they were single seeds.	Multigerm seed units are not broken up for the germination test but are tested as though they were single seeds.
Testing fewer than 400 seeds: Chapter 2: Sampling gives this option, but no details have been provided in Chapter 5: Germination until now.	
	<u>The ISTA germination test is based on</u>

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	<p>400 seeds. In certain circumstances (see 2.5.4.4) it may be necessary to test fewer than 400 seeds. In such cases at least 100 seeds must be tested in replicates of 25 or 50.</p> <p>NOTE: If the submitted sample is smaller than prescribed, the sampler shall be notified accordingly and analysis withheld until sufficient seed is received in a single submitted sample; except that in the case of very expensive seed, the analysis may be completed to the extent possible and the following statement inserted on the certificate: “The sample submitted weighed only... g [or in the case of pelleted seeds ‘contained only ... pellets (seeds)’] and is not in accordance with the International Rules for Seed Testing.”</p>
5.6.2 Test conditions: For clarity.	
5.6.2 Test conditions	5.6.2 Test conditions
Permissible substrates, temperatures, duration and additional directions, including recommended special treatments for dormant samples , are indicated in Table 5A. Substrates, temperatures and duration of test indicated are prescriptive and no others may be used.	Permitted substrates, temperatures, duration of tests and additional directions, including recommended procedures for breaking dormancy , are indicated in Table 5A. Substrates, temperatures and duration of test indicated are prescriptive and no others may be used.
<i>5.6.2.1 Growing media</i>	<i>5.6.2.1 Growing media</i>
5.6.2.1.1 Paper substrates: “Cabinet” is replaced by “Incubator”. This makes it clear that the humidity is maintained at a level that prevents tests from drying out.	
<i>5.6.2.1.1 Paper substrates</i>	<i>5.6.2.1.1 Paper substrates</i>
Top of paper (TP): the seeds are germinated on top of one or more layers of paper which are placed:	Top of paper (TP) The seeds are germinated on top of one or more layers of paper which are placed:
...	...
§3 – directly on trays in cabinet germinators . The relative humidity in the cabinet must then be maintained as close to saturation as possible to prevent drying out. Moistened porous paper or absorbent cotton can be used as a base for the substrates.	§3 – directly on trays in germination incubators . The relative humidity in the incubators must then be maintained at a level that prevents tests drying out. Moistened porous paper or absorbent cotton can be used as a base for the substrates.
Between paper (BP): the seeds are germinated between two layers of paper. This may be achieved:	Between paper (BP) The seeds are germinated between two layers of paper. This may be achieved:
...	...
§3 – by placing the seeds in rolled towels (the rolls should be placed in an upright position).	§3 – by placing the seeds in rolled paper towels (the rolls must be placed in an upright position).

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...	...
<i>5.6.2.1.2 Methods using sand or organic growing medium</i>	<i>5.6.2.1.2 Methods using sand or organic growing <u>media</u></i>
Sand and organic growing medium are used as follows:	Sand and organic growing <u>media</u> are used as follows:
...	...
5.6.2.2 Moisture and aeration: For clarity.	
<i>5.6.2.2 Moisture and aeration</i>	<i>5.6.2.2 Moisture and aeration</i>
Precautions should be taken to ensure that the medium cannot dry out and that sufficient water is supplied continuously throughout the test period. Subsequent watering should be avoided wherever possible as it is likely to increase variability between replicates and between tests.	Precautions <u>must</u> be taken to ensure that the medium cannot dry out and <u>contains</u> sufficient water <u>for</u> the <u>whole</u> test period. Subsequent watering should be avoided wherever possible, as it is likely to increase variability between replicates and between tests. <u>However, it may be necessary to add water at intermediate counts.</u>
Special measures for aeration are not usually necessary for TP and PP tests enclosed in boxes or Petri dishes. For BP, however, care should be taken that envelopes and towel-rolls are loose enough to allow for sufficient air around the seeds. For the same reason, the material covering the seeds in sand and organic growing media tests should not be compressed.	Special measures for aeration are not necessary for TP and PP tests enclosed in boxes or Petri dishes. For BP, however, care <u>must</u> be taken that envelopes and <u>rolled paper towels</u> are loose enough to allow for sufficient air around the seeds. For the same reason, sand and organic growing media <u>must</u> not be compressed.
5.6.2.3 Temperature: This makes it clear that the maximum temperature variation is ± 2 °C, and that this is prescribed and is not a recommendation.	
<i>5.6.2.3 Temperature</i>	<i>5.6.2.3 Temperature</i>
Temperatures prescribed in Table 5A are those to which the seed is exposed on or inside the substrate. They should be as uniform as possible throughout the germination apparatus, cabinet or room germinator. It is recommended that for tests, either in darkness or under an artificial source of light or in indirect daylight, variation from the prescribed temperature due to the apparatus should not be more than ± 2 °C.	<u>The</u> temperatures prescribed in Table 5A <u>for the germination of a species</u> are those to which the seed is exposed on or inside the substrate. They should be as uniform as possible throughout the germination apparatus, <u>incubator</u> or room germinator. <u>For any</u> test, <u>whether</u> in darkness or under artificial light or in indirect daylight, variation from the prescribed temperature <u>must</u> not be more than ± 2 °C.
Changeover of alternating temperatures: How this is achieved is up to the laboratory. Details need not be given in the Rules.	
Where alternating temperatures are indicated, the lower temperature should usually be maintained for 16 hours and the higher for eight hours. A gradual changeover lasting three hours may be satisfactory, but a sharp changeover lasting one hour or less, or transference of the tests to another germinator at a lower temperature, may be necessary for seeds which are likely to be dormant.	Where alternating temperatures are indicated, the lower temperature should be maintained for 16 hours and the higher for eight hours. A gradual changeover lasting <u>no more than</u> three hours may be satisfactory, but a sharp changeover lasting one hour or less <u>may be necessary for breaking dormancy.</u>
The following change makes it clear that, when a range of temperatures is given, no	

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additional tolerances may be applied to the	upper or lower limits of the range.
	<u>Where a temperature range is given, no tolerances may be applied to the upper or lower temperatures. For example, when a prechilling temperature of 5 to-10 C is prescribed, this means that the allowed temperature range is 5 to-10°C, and not 5 ± 2°C to 10 ± 2°C.</u>
5.6.2.4 Light: This is a repeat of the information in 5.6.3.1, but the committee thinks it is necessary here also.	
<i>5.6.2.4 Light</i>	<i>5.6.2.4 Light</i>
...	...
In certain cases (e.g. some tropical and subtropical grasses), light may promote germination of dormant samples (5.6.3.1-4), but on the other hand there are also a few species (e.g. <i>Phacelia tanacetifolia</i>) which should be germinated in darkness, as light may be inhibitory. Specific recommendations for light or darkness, respectively , are given in the last column of Table 5A.	In certain cases (e.g. some tropical and subtropical grasses), light may promote germination of dormant samples (5.6.3.1). <u>In such cases the light should be between 750–1250 lux from cool white lamps.</u> There are also a few species (e.g. <i>Phacelia tanacetifolia</i>) which <u>must</u> be germinated in darkness, as light may be inhibitory. Specific recommendations for light or darkness are given in the last column of Table 5A.
5.6.2.5 Choice of method: the information deleted here is given later when retesting is described.	
<i>5.6.2.5 Choice of method</i>	<i>5.6.2.5 Choice of method</i>
When alternate methods are indicated in Table 5A one of them (any combination of substrate and temperature) must be used. The choice of method will depend largely on the facilities and experience of the testing station and to some extent on the provenance and condition of the sample. If occasionally a sample does not respond satisfactorily to the method selected, it will be necessary to re-test it by one or more of the alternative methods.	When <u>alternative</u> methods are indicated in Table 5A, one of them (any combination of substrate and temperature) must be used. The choice of method will depend largely on the facilities and experience of the testing <u>laboratory</u> and to some extent on the provenance and condition of the sample.
5.6.3 Treatments for promoting germination: this proposed change makes it clear that as well as the recommended treatments for breaking dormancy given in Table 5A for a species, any of the treatments listed in 5.6.3.1, 5.6.3.2 and 5.6.3.3 can be used, but that precise details of the treatment used must be given on the ISTA Certificate.	
<i>5.6.3 Treatments for promoting germination</i>	<i>5.6.3 <u>Procedures</u> for promoting germination <u>of dormant seed</u></i>
For various reasons (e.g. physiological dormancy, hardseededness, inhibitory substances) a considerable number of hard or fresh seeds may remain at the end of the germination test. More complete germination may be obtained by retesting after one or a combination of the treatments given below . These may be applied to the original test, if dormancy is suspected. Recommended	For various reasons (e.g. physiological dormancy, hardseededness, inhibitory substances) a considerable number of hard or fresh seeds may remain at the end of the germination test. More complete germination may be obtained by retesting after one or a combination of the <u>procedures listed in 5.6.3.1, 5.6.3.2 and 5.6.3.3</u> . These <u>procedures</u> may be applied to the original test, if

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<p>treatments are indicated in the last column of Table 5A. The period of pretreatment is not included in the germination test period. Pretreatment and duration of pretreatment must be reported on the ISTA International Seed Analysis Certificate.</p>	<p>dormancy is suspected. Recommended <u>procedures</u> are indicated in <u>column 6</u> of Table 5A, <u>but this does not prevent the use of other procedures listed in 5.6.3.1, 5.6.3.2 and 5.6.3.3</u>. The period of <u>treatment</u> is not included in the germination test period. <u>Precise details</u> and duration of <u>the dormancy-breaking procedure</u> must be reported on the ISTA Certificate.</p>
<p>This paragraph describes a double test, so this should be specifically mentioned.</p>	
<p>For some tree and shrub seeds, where it is known from experience that a proportion of the seeds will not germinate due to dormancy, a second test incorporating a special treatment is prescribed which preferably should run concurrently with the normal test.</p>	<p>For some tree and shrub seeds, where it is known from experience that a proportion of the seeds will not germinate <u>because of</u> dormancy, a second test incorporating a special <u>dormancy-breaking procedure</u> is prescribed which preferably should run concurrently with the normal test (<u>double test</u>).</p>
<p>Disinfection: the committee want this option to be available for all species, not just <i>Beta</i> and <i>Arachis</i>.</p>	
<p>For certain species mentioned in 5.6.3.1 to 5.6.3.3, disinfection of the seed prior to the test is permitted and described in 5.6.3.4.</p>	<p>Disinfection of the seed prior to the test is permitted and described in 5.6.3.4.</p>
<p>'Dry storage' renamed 'prestorage' and moved down.</p>	
<p>5.6.3.1 Methods for breaking physiological dormancy</p>	<p>5.6.3.1 <u>Procedures</u> for breaking physiological dormancy</p>
<p>Dry storage:—for species where dormancy is naturally of short duration, it is often sufficient to store the sample in a dry place for a short period.</p>	
<p>Prechilling: the replicates for germination are placed in contact with the moist substrate and kept at a low temperature for an initial period before they are moved to the temperature indicated in Table 5A column 3. Agricultural, vegetable, and flower, spice, herb and medicinal seeds are usually kept at a temperature of between 5 °C and 10 °C for an initial period of up to 7 days. In some cases it may be necessary to extend the prechilling period or to re chill.</p> <p>Tree and shrub seeds are usually prechilled at a temperature of between 1 °C and 5 °C for a period, ranging with the species, from 2 weeks to 12 months prior to the germination test, but care must be taken to avoid freezing them. For seeds where a long period of prechilling is required and a germination test cannot be completed within two months, quick viability tests are</p>	<p>Prechilling The replicates for germination are placed in contact with the moist substrate and kept at a low temperature for an initial period before they are moved to the temperature indicated in Table 5A column 3. Agricultural, vegetable, flower, spice, herb and medicinal seeds are usually kept at a temperature of 5 to- 10°C for an initial period of up to 7 days. In some cases it may be necessary to extend the prechilling period or to re chill.</p> <p>Tree and shrub seeds are usually prechilled at a temperature of 1 to- 5°C for a period, ranging with the species, from 2 weeks to 12 months prior to the germination test, but care must be taken to avoid freezing. For seeds where a long period of prechilling is required and a germination test cannot be completed within two months, quick viability</p>

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recommended (e.g. tetrazolium test: see Chapter 6; excised embryo test: see Chapter 12). For some tree and shrub species with a varying degree of dormancy, duplicate tests with and without prechilling are prescribed ('double tests'), as indicated in Table 5A Part 2, which should if possible be set to germinate at the same time.	tests are recommended (e.g. tetrazolium test: see Chapter 6; excised embryo test: see Chapter 12). For some tree and shrub species with a varying degree of dormancy, duplicate tests with and without prechilling are prescribed ('double tests'), as indicated in Table 5A Part 2, which should if possible be set to germinate at the same time.
Preheating: This is a temperature range. "Not exceeding" would refer to a particular temperature.	
Preheating: the replicates for germination are heated at a temperature not exceeding 30–35 °C with free air circulation for a period of up to 7 days before they are placed under the prescribed germination conditions. ...	Preheating The <u>non-imbibed seeds of the</u> replicates for germination are heated at a temperature <u>of</u> 30–35 °C with free air circulation for a period of up to 7 days before they are placed under the prescribed germination conditions. ...
Prestorage: renamed from "dry storage" and details given	
	<p>Prestorage <u>For some temperate herbage grass species, the seed submitted for testing is stored at a temperature of 15 to- 25°C for a period of 14 to 98 days with free air circulation before they are tested.</u></p> <p><u>A prestorage period of up to one year can be used.</u></p> <p>NOTE: the reporting requirement under proposal C. 5.4 has been amended to state what must be stated under 'Other determinations', i.e.</p> <p>Any special treatment or method used for promoting germination (5.6.3). <u>The duration in days of any special treatment or method used for promoting germination, except in the case of prestorage.</u></p>
Light: the tests should be illuminated during at least 8 hours in every 24-hour cycle and during the high temperature period when the seeds are germinated at alternating temperatures. The light intensity should be approximately 750–1250 lux from cool white lamps. ...	Light The tests should be illuminated during at least 8 hours in every 24-hour cycle and during the high temperature period when the seeds are germinated at alternating temperatures. <u>The quality and intensity of light may be important.</u> The light intensity should be <u>between</u> 750–1250 lux from cool white lamps. ...
Potassium nitrate (KNO₃): ...	Potassium nitrate (KNO₃) ...

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Gibberellic acid: a note to labs to ensure that use of concentrations greater than 0.1% do not have an adverse effect on germination.	
<p>Gibberellic acid (GA₃): the GA₃ method is recommended mainly for ...</p> <p>When dormancy is weaker, 0.02% may be enough; when it is stronger, up to 0.1% may be used. When a concentration higher than 0.08% is required, ...</p>	<p>Gibberellic acid (GA₃) The GA₃ <u>treatment</u> is recommended mainly for ...</p> <p>When dormancy is weaker, 0.02% may be enough; when it is stronger, up to 0.1% may be used <u>routinely</u>. <u>If it is necessary to use concentrations higher than 0.1%, care must be taken to ensure that the development of seedlings is not adversely affected.</u></p> <p>When a concentration higher than 0.08% is required, ...</p>
Sealed polyethylene envelopes: ...	Sealed polyethylene envelopes ...
Mechanical and acid scarification: this text was moved to here from 5.6.3.2, since these treatments are also used for breaking dormancy in seeds other than hard seeds. The treatment for <i>Oryza sativa</i> was corrected from KNO ₃ to HNO ₃ .	
	<p>Acid scarification The seeds are <u>soaked in concentrated sulphuric acid (H₂SO₄) until the seed coat becomes pitted. Digestion may be rapid, or take more than one hour, but the seeds should be examined every few minutes. After digestion, seeds must be thoroughly washed in running water before the germination test is commenced (e.g. <i>Brachiaria</i> spp.). In the case of <i>Oryza sativa</i>, scarification may be performed by soaking the seed in 1 M nitric acid (HNO₃) for 24 hours (after preheating at 50 ± 2 °C).</u></p>
	<p>Mechanical scarification The seed is <u>cut, pierced, filed or sandpapered to improve permeability to moisture and gasses. Care must be taken to scarify the seed coat at a suitable place in order to avoid damaging the embryo and the resulting seedling. The best places are either immediately above the tips of the cotyledons or to the sides of the cotyledons.</u></p>
5.6.3.2 Treatments for removing hardseededness: this proposed change makes it clear that this is only necessary on request of the customer, and need not be done routinely.	
5.6.3.2 Methods for removing hardseededness	5.6.3.2 <u>Procedures</u> for removing hardseededness
For many species where hard seeds occur, no attempt is made to germinate them and the percentage found is reported. Where a fuller assessment is required, some special treatment is essential. This treatment may be applied prior to the commencement of the germination test or, if it is suspected that the treatment may adversely affect non-	For many species where hard seeds occur, no attempt is made to germinate them and the percentage found is reported. Where a fuller assessment is required <u>on the request of the customer, some special procedure for removing hardseededness</u> is essential. This <u>procedure</u> may be applied prior to the commencement of the germination test,

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hard seeds, it should be carried out on the hard seeds remaining after the prescribed test period.	or, if it is suspected that the procedure may adversely affect non-hard seeds, it should be carried out on the hard seeds remaining after the prescribed test period.
Soaking: ...	Soaking ...
Mechanical and acid scarification: text deleted here was moved to 5.6.3.1.	
Mechanical scarification: careful piercing, chipping, filing or sandpapering of the seed coat may be sufficient to break the dormancy condition. Care must be taken to scarify the seed coat at a suitable part in order to avoid damaging the embryo and the resulting seedling. The best site for mechanical scarification is that part of the seed coat immediately above the tips of the cotyledons.	Mechanical scarification Careful piercing, chipping, filing or sandpapering of the seed coat may be sufficient (see 5.6.3.1).
Acid scarification: digestion in concentrated sulphuric acid (H₂SO₄) is effective with some species (e.g. <i>Macroptilium</i> spp., <i>Brachiaria</i> spp.). The seeds are soaked in the acid until the seed coat becomes pitted. Digestion may be rapid, or take more than one hour, but the seeds should be examined every few minutes. After digestion, seeds must be thoroughly washed in running water before the germination test is commenced. In the case of <i>Oryza sativa</i>, scarification may be performed by soaking the seed in 1 N nitric acid (HNO₃) for 24 hours (after preheating at 50 °C).	Acid scarification This procedure is effective with some species (e.g. <i>Desmodium</i> spp., <i>Macroptilium</i> spp., <i>Stylosanthes guianensis</i>) (see 5.6.3.1).
5.6.3.3 Methods for removing inhibitory substances	5.6.3.3 Procedures for removing inhibitory substances
Prewashing: temperature limits are given, and it is made clear that pelleted seed should not be washed.	
Prewashing: naturally occurring substances in the pericarp or seed coat which act as inhibitors of germination may be removed by washing the seeds in running water at a temperature of 25 °C before the germination test is made. After washing, the seeds should be dried at a maximum temperature of 25 °C (e.g. <i>Beta vulgaris</i>).	Prewashing Naturally occurring substances in the pericarp or seed coat which act as inhibitors of germination may be removed by washing the seeds in running water at a temperature of 25 ± 2 °C before the germination test is made. After washing, the seeds must be dried at a temperature of 20–25 °C (e.g. <i>Beta vulgaris</i>). Pelleted seed must not be prewashed.
Removal of outer structures: ...	Removal of outer structures ...
Disinfection: the committee want this option to be available for all species, not just <i>Beta</i> and <i>Arachis</i> .	
5.6.3.4 <i>Disinfection of the seed</i>	5.6.3.4 <i>Disinfection of the seed</i>
	Removed for separate vote.

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5.6.4 Duration of the test: the procedure added to the end of paragraph 3 is used by many laboratories, and should be in the Rules with a warning regarding the integrity of replicates and avoidance of damage.	
5.6.4 Duration of the test	5.6.4 Duration of the test
§1 ...	§1 ...
§2 ...	§2 ...
§3 ... Number and date of intermediate counts may be left to the discretion of the analyst, but should be kept at a minimum to reduce the risk of damaging any seedlings which are not sufficiently developed.	§3 ... Number and date of intermediate counts may be left to the discretion of the analyst, but should be kept at a minimum to reduce the risk of damaging any seedlings which are not sufficiently developed. When samples are tested on paper, ungerminated seed and seedlings requiring additional time to reach the stage of development that allows for accurate evaluation, can be transferred to fresh substrate at intermediate counts. In doing so, care must be taken to ensure the integrity of replicates and to avoid any damage to the transferred seeds and seedlings.
5.6.5 Evaluation: deleted information moved to 5.7 Retesting	
5.6.5 Evaluation	5.6.5 Evaluation
Every seedling must be evaluated in accordance with the general principles laid down in 5.2.3 and 5.2.4 . For evaluation, the essential structures must be sufficiently developed to permit detection of any abnormality. When samples tested on paper produce seedlings which cannot readily be evaluated, a retest should be made in sand or organic growing media at the temperature indicated in Table 5A and under favourable conditions of moisture and light.	Every seedling must be evaluated in accordance with the general principles laid down in 5.2.5–5.2.7 . For evaluation, the essential structures must be sufficiently developed to permit detection of any abnormality.
Information moved down.	
At the end of the germination test, the classification of ungerminated seeds as fresh must be determined as prescribed in 5.6.5.3. In the case of tree seeds, the evaluation of empty and insect damaged seeds may be made, on request only, prior to the germination test. The classes and methods to be used are described in 5.2.7 and 5.6.5.3.	At the end of the germination test, the classification of ungerminated seeds must be determined as prescribed in 5.6.5.3.
5.6.5.1 Seedlings: it should be allowed to remove obviously abnormal seedlings at intermediate counts.	
5.6.5.1 Seedlings	5.6.5.1 Seedlings
Seedlings which have reached a stage when all essential structures can be accurately assessed must be removed	Seedlings which have reached a stage when all essential structures can be accurately assessed must be removed

CURRENT VERSION	PROPOSED VERSION
from the test at the first and any other intermediate counts. Badly decayed seedlings should be removed in order to reduce the risk of secondary infection, but abnormal seedlings with other defects should be left on the substrate until the final count.	from the test at the first and any other intermediate counts. Badly decayed seedlings should be removed in order to reduce the risk of secondary infection, but <u>doubtful</u> seedlings with other defects <u>must</u> be left on the substrate until the final count, <u>unless it is obvious that they will never develop into normal seedlings, e.g. broken seedlings and white seedlings.</u>
Information moved down.	
Seedlings usually can be readily evaluated on primary substrates. However, when seedlings occur which cannot be easily evaluated or show phytotoxic symptoms, a retest should be made in sand, organic growing media, or soil at the temperature prescribed in Table 5A. Planting another sample of the same cultivar, known to germinate satisfactorily, alongside, may provide a useful guide to evaluation of this retest.	
5.6.5.2 <i>Multigerm seed units</i> ...	5.6.5.2 <i>Multigerm seed units</i> ...
5.6.5.3 Ungerminated seeds: information already given.	
5.6.5.3 <i>Ungerminated seeds</i>	5.6.5.3 <i>Ungerminated seeds</i>
Hard seeds: at the end of a germination test hard seeds are counted and reported as such on the ISTA International Seed Analysis Certificate. However, where it is necessary to remove hardseededness prior to the germination test, measures as described in 5.6.3 must be taken to promote germination.	Hard seeds At the end of a germination test hard seeds are counted and reported as such on the ISTA Certificate.
Fresh seeds: ...	Fresh seeds ...
Dead seeds: ...	Dead seeds ...
Other categories: upon request of the sender the number of empty, embryoless or insect-damaged seeds may be determined and reported under 'Other Determinations' on the ISTA International Seed Analysis Certificate.	Other categories Upon request of the <u>customer</u> , the number of empty, embryoless or insect-damaged seeds may be determined and reported under 'Other determinations' on the ISTA Certificate.
To detect these categories of seeds, the following methods may be used:	To detect these <u>other</u> categories of seeds, the following methods may be used:
a) Before the germination test: ...	a) Before the germination test: ...
• cutting test, which is performed on four separate replicates of 100 seeds, soaked for up to 24 hours at room temperature. Each seed is cut along its longitudinal axis and the content examined and classified;	• cutting test, which is performed on four separate replicates of 100 seeds, soaked for up to 24 hours at room temperature. Each seed is cut along its longitudinal axis and the content examined and classified <u>as full, empty,</u>

CURRENT VERSION	PROPOSED VERSION
	embryoless or insect-damaged ;
X-ray can also be done after the germination test on ungerminated seed	
b) After the germination test:	b) After the germination test:
<ul style="list-style-type: none"> cutting test of fresh ungerminated seeds. 	<ul style="list-style-type: none"> cutting test or X-ray test of apparently fresh ungerminated seeds.
...	...

Proposal 5.3c
Reporting the number on the ISTA Certificate.

When the germination percentage is reported on the ISTA Certificate, the method used must be given. The ISTA germination test is based on 400 seeds. In cases where less than 400 seeds are tested, the number tested must be reported.

Proposal 5.3d
Remainder of section 5.3

C.5.3.d. Revision of sections 5.7 Retesting and 5.8 Calculation and expression of results

5.7 Retesting	
...	5.7 Retesting
...	...
Paragraph a): this clarifies that it is a treatment for breaking dormancy.	
a) When dormancy is suspected (fresh ungerminated seeds), any method indicated in column 6 of Table 5A or in 5.6.3. † to break dormancy may be applied in one or more additional tests. The best result achieved must be reported and the method must be indicated on the ISTA International Seed Analysis Certificate.	a) When dormancy is suspected (fresh ungerminated seeds), any procedure to break dormancy indicated in column 6 of Table 5A or in 5.6.3 may be applied in one or more additional tests. The best result achieved must be reported and the procedure must be indicated on the ISTA Certificate.
b) ...	b) ...
c) ...	c) ...
Paragraph d): There is no reason why an alternative treatment for breaking dormancy cannot be used.	
d) When there is evidence of errors in test conditions, seedling evaluation or counting, a retest must be made using the same method and the result of the retest must be reported on the ISTA International Seed Analysis Certificate.	d) When there is evidence of errors in test conditions, seedling evaluation or counting, a retest must be made using the same method or an alternative method as prescribed in Table 5A , and the result of the retest must be reported on the ISTA Certificate.
Paragraph e): this is the principle of parallel testing, which is permitted.	
	e) If a sample does not respond satisfactorily to the method selected, it will be necessary to re-test it by one or more of the alternative methods. When seedlings occur which cannot be easily evaluated or show phytotoxic symptoms, a retest should be made in sand, organic growing media, or soil at the temperature prescribed in Table 5A. Planting another sample of the same

	<p><u>cultivar, known to germinate satisfactorily, alongside, may provide a useful guide to evaluation of this retest. The best result achieved must be reported and the method used must be indicated on the ISTA Certificate.</u></p>
<p>Section on tolerances and tolerance checking: amended to allow third and fourth tests to be carried out and to provide details of how to calculate and report results – something lacking in the present Rules.</p>	
<p>e) When the range for the 100 seed replicates exceeds the maximum tolerated range in Table 5B, a retest must be made using the same method. If the second result is compatible with the first (i.e. the difference does not exceed the tolerance indicated in Table 5C) the average of the two tests must be reported on the ISTA International Seed Analysis Certificate. If the second result is not compatible with the first and the difference exceeds the tolerance indicated in Table 5C, a third test using the same method must be made. The average of compatible results must be reported.</p>	<p>f) When the range for the replicates exceeds the maximum tolerated range in Table 5B, a retest must be <u>carried out</u> using the same <u>test</u> method. <u>If the results of the repeat test</u> are compatible with the first (i.e. the difference does not exceed the tolerance indicated in <u>either</u> Table 5C, <u>5D or 5E</u>), the average of the <u>test results</u> must be reported <u>on the ISTA Certificate (see 5.8.1 Tolerances).</u></p>
<p>5.8 Calculation and expression of results</p>	<p>5.8 Calculation and expression of results</p>
<p>Results are expressed as percentage by number calculated to the nearest whole number. When four 100 seed replicates of a test are within the maximum tolerated range (Table 5B) the average represents the percentage germination to be reported on the ISTA International Seed Analysis Certificate. The average percentage is calculated to the nearest whole number.</p>	
<p>Paragraph on multigerm seed units moved from 5.8.1</p>	
<p>The result of the germination test is calculated as the average of four 100 seed replicates (sub-replicates of 50 or 25 seeds are combined into 100 seed replicates). It is expressed as a percentage by number of normal seedlings. The percentage is calculated to the nearest whole number (0.5 is taken to the higher figure). The percentage of abnormal seedlings, hard, fresh and dead seeds is calculated in the same way. The sum of the percentage of normal and abnormal seedlings and ungerminated seeds must be 100.</p>	<p>The result of the germination test is <u>expressed as percentages by number of normal and abnormal seedlings and hard, fresh and dead seeds. The percentages are rounded</u> to the nearest whole number. The sum of the percentages <u>of normal and abnormal seedlings and hard, fresh and dead seeds</u> must be 100 (<u>see 5.8.2 Rounding results</u>).</p> <p><u>For multigerm seed units, only one normal seedling per unit is counted to calculate the result of the germination test. On request, the number of units producing one, two or more than two normal seedlings may also be reported, expressing the results as a percentage of</u></p>

	the total number of units which have produced at least one normal seedling, or alternatively the total number of seedlings produced by a given number of seed units.
5.8.2 Tolerances	5.8.1 Tolerances
<p>Section ‘Tolerances’ moved to before section ‘Calculation’ (renamed ‘Rounding results’).</p> <p>Complete revision of the tolerance section and new tables replacing present table which contain errors. Instruction given on how to carry out tolerance checks and flow chart given to add interpretation of text and guide the user through the procedure</p>	
<p>The result of a germination test can be relied upon only if the difference between the highest and the lowest replicates is within accepted tolerances. To check the reliability of a test result, the average percentage of the replicates is calculated and compared with Table 5B. The result is considered reliable, if the difference between the highest and the lowest replicate does not exceed the tolerance indicated. Tolerances are applied to at least the category of normal seedlings.</p>	<p>The result of a germination test can be relied upon only if the difference between the highest and the lowest replicates is within accepted tolerances. To check the reliability of a test result, the average percentage of the replicates is rounded to the nearest whole number and compared with Table 5B. The result is considered reliable, if the difference between the highest and the lowest replicate does not exceed the tolerance indicated. Tolerances are applied to at least the category of normal seedlings.</p>
<p>To decide whether two test results of the same sample are compatible, Table 5C is used. The average percentage germination of the two tests is calculated and compared with Table 5C. The tests are compatible if the difference between the germination percentage of the two tests does not exceed the tolerance indicated.</p>	<p>If the range of the replicates exceeds the maximum tolerated range in Table 5B, a retest must be made. If the second result, using the same method, is in tolerance with the first (i.e. the difference between the two test results does not exceed the tolerance indicated in Table 5C), the average of the two test results must be reported on the ISTA Certificate.</p>
	<p>If the second result is not in tolerance with the first (i.e. the difference between the two test results exceeds the tolerance indicated in Table 5C), a third test must be made. If the three test results are in tolerance (i.e. the difference between the three test results does not exceed the tolerance indicated in Table 5D), the average of the three test results, using the same method, must be reported. If the three test results are not in tolerance (i.e. the difference between the three test results exceeds the tolerance indicated in Table 5D), the highest compatible result obtained from comparison of the three test pairs of the two tests is reported (i.e. comparison of tests 1 and 3 and tests 2 and 3). If after carrying out the second retest no compatible result is obtained, a third retest is carried out.</p>
	<p>The average of the four test results, using the same method, must be reported if the four test results are in tolerance (i.e. the difference between the four test</p>

	<p><u>results does not exceed the tolerance indicated in Table 5E). If the four test results are not in tolerance (i.e. the difference between the four test results exceeds the tolerance indicated in Table 5E), the highest compatible result obtained from comparison of the three test trios of the four tests is reported (i.e. comparison of tests 1, 2 and 3; tests 1, 2 and 4; and tests 2, 3 and 4). If after carrying out the comparison of trios of tests no compatible result is obtained, the highest compatible result obtained from comparison of the six pairs of the four tests is reported (i.e. comparison of tests 1 and 2; tests 1 and 3; tests 1 and 4; tests 2 and 3; tests 2 and 4; and tests 3 and 4). If after carrying out the comparison of six pairs of tests no compatible result is obtained, no test result is reported, and the customer is informed that the sample appears to have unacceptable variation in germination.</u></p>
	<p><u>Figure 5.2 illustrates, in the form of a flow chart, the retesting procedure to obtain compatible results within tolerance.</u></p>
	<p><u>Note: section removed for a separate vote.</u></p>
<p><u>5.8.1 Calculation</u></p>	<p><u>5.8.2 Rounding results</u></p>
<p>The percentage of normal seedlings is rounded to the nearest whole number, 0.5 is taken to the higher figure (xx.0 and xx.25 are rounded to xx; xx.50 and xx.75 are rounded to xx + 1). Calculate the integer part of the remaining percentages, sum the values obtained.</p>	<p><u>First, round</u> the percentage of normal seedlings <u>up or down</u> to the nearest whole number (xx.0 and xx.25 are rounded <u>down</u> to xx; xx.50 and xx.75 are rounded <u>up</u> to xx + 1). <u>Add up</u> the integer parts of the remaining percentages.</p>
<p>If the sum is 100, the procedure ends; otherwise, continue with the following steps. For the percentage of abnormal seedlings, hard seeds, fresh seeds and dead seeds:</p>	<p>If the sum is 100, the procedure ends; otherwise, continue with the following steps:</p>
<p>1. Find the value with the greatest decimal part among the remaining percentages and round this percentage to the upper whole number; keep this value as a final result, and calculate the integer part of the remaining percentages.</p>	<p>1. Find the value with the greatest decimal part among the remaining percentages (<u>abnormal seedlings, hard seeds, fresh seeds and dead seeds</u>) and round this percentage to the upper whole number; keep this value as a final result.</p>

<p>2. Sum the values obtained.</p> <p>3. If the sum is 100, the procedure ends, else continue with further steps 1. and 2.</p>	<p>2. <u>Add up the integer parts of the remaining percentages.</u></p> <p>3. If the sum is 100, the procedure ends; <u>otherwise</u> continue with further steps 1. and 2.</p>
<p>In the case of equal decimal parts, the priority order is abnormal seedlings — hard seeds — fresh seeds — dead seeds.</p>	<p>In the case of equal decimal parts, the priority order is abnormal seedlings — hard seeds — fresh seeds — dead seeds.</p>
<p>For multigerm seed units, only one normal seedling per unit is counted to calculate the result of the germination test. On request, the number of units producing one, two or more than two normal seedlings may also be reported, expressing the results as a percentage of the total number of units which have produced at least one normal seedling, or alternatively the total number of seedlings produced by a given number of seed units.</p>	

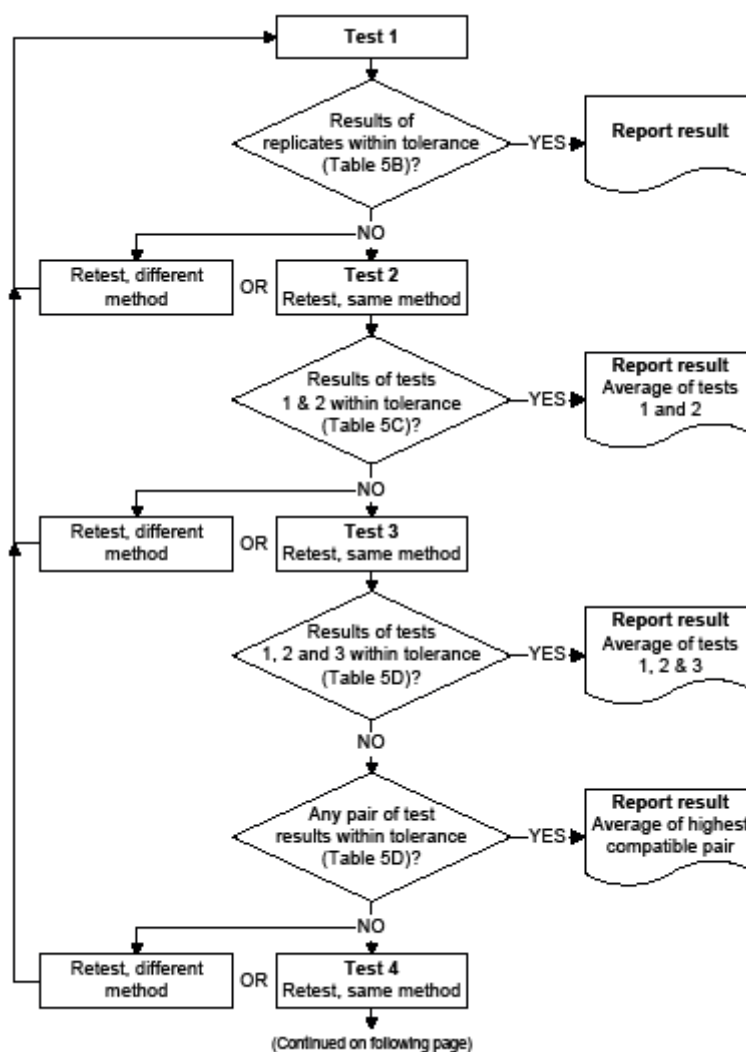


Figure 5.2. Part 1. Flow chart to illustrate the retesting procedure when test replicates and repeat tests are out of tolerance.

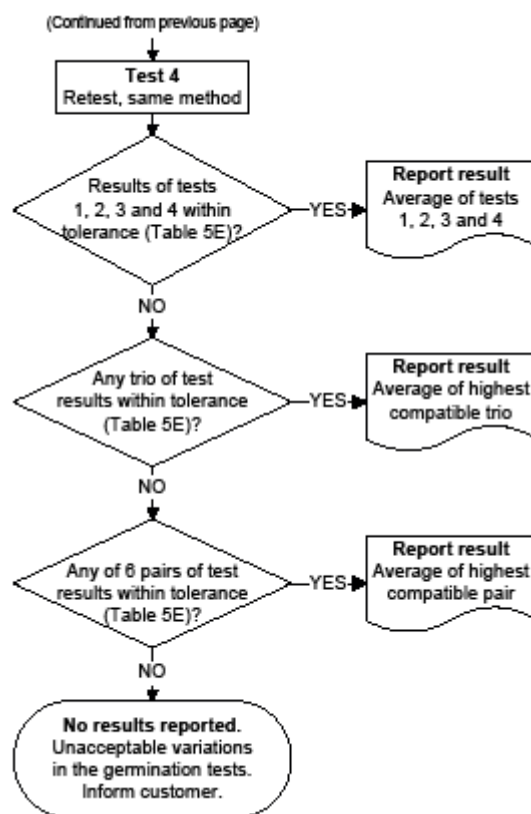


Figure 5.2. Part 2. Flow chart to illustrate the retesting procedure when test replicates and repeat tests are out of tolerance.

C.5.4. Revision of 5.9 Reporting results

The same changes will also be applied to Chapter 1: Certificates, 1.5.2.6 Reporting results.	
5.9 Reporting results	5.9 Reporting results
The result of a germination test must be reported in the spaces provided as follows:	The result of a germination test must be reported in the spaces provided as follows:
The following change proposal clarifies that the duration of the test does not include the periods of treatments for breaking dormancy, such as prechilling or predrying.	
– the duration of the test;	– the actual duration of the test (in days), excluding the period of special treatment or method used for promoting germination).
– the percentages, calculated to the nearest whole number (5.8. 1), of ...	– the percentages, calculated to the nearest whole number (5.8. 2), of ...
In all cases, the total number of seeds used to calculate the result must be given. It could be 100 (in a 100-seed test), 800, if a 400-seed test is retested and the 1st and 2nd tests are in tolerance, or even 1600, if 4 x 400-seed tests are required to obtain a result with the required tolerance that can be reported.	
	– The number of seeds used to calculate the percentages Note: This will need to be amended depending on the Vote of parts C.5.3.1a to c.
The following additional information must also be reported under ‘Other determinations’: – ... – Any special treatment or method used for promoting germination (5.6.3).	The following additional information must also be reported under ‘Other determinations’: – ... – Any special treatment or method used for promoting germination (5.6.3). The duration in days of any special treatment or method used for promoting germination, except in the case of prestorage.
Instructions as to how double tests are reported.	
– The germination percentage obtained within the prescribed time, if the germination period was extended beyond the period indicated in Table 5A. The statement must be entered as follows: ‘After the prescribed period of ... days, there were ...% normal seedlings.’ — The second result obtained, if duplicate tests were indicated in Table 5A.	– when double tests are prescribed in Table 5A Part 2, the result of the first test, with treatment for breaking dormancy, is reported in the appropriate space on the ISTA Certificate, and the result of the second test, without treatment for breaking dormancy, is reported under ‘Other determinations’.
Upon request, the following information may be reported as follows: – the result of any additional test; – ...	Upon request, the following information may be reported as follows: – the result of parallel tests or any additional test; – ...

C.5.5. Revision of 5.10 Germination methods/Table 5A

Changes made to this are to aid understanding – the last column is split into new columns – one for recommended dormancy breaking treatments, one for additional recommendations and one for additional prescriptions. For the forest tree seed germination methods, the current table gives the impression that for some species methods are a mix of TZ, excised embryo and germination; this has been clarified.

The complete revised Table 5A is shown in Appendix 2.

The symbols <=> will now be used for alternating temperatures.

The two way arrow symbol (⇔) will be replaced throughout the document and details of the alternating temperature symbol added to each page of Table 5A, e.g.

[Alternating temperature regimes are indicated by symbols between the temperatures, e.g., 20<=>30 is an alternating temperature regime of 20 °C for 16 hours and 30 °C for 8 hours.](#)

CURRENT VERSION	PROPOSED VERSION
<p>Table 5A. Germination methods</p> <p>This table indicates the permissible substrates, the duration of the test and recommended additional treatments for dormant samples. Where methods are prescribed for a group of species, only those species specifically listed in Table 2A shall be considered to be covered.</p>	<p><u>5.10 Germination methods</u></p> <p><u>Table 5A indicates the prescribed substrates, temperatures and test durations, recommended procedures for breaking dormancy, additional directions and additional advice.</u> Where methods are prescribed for a group of species, only those species specifically listed in Table 2A shall be considered to be covered.</p> <p><u>For certain species in Table 5A Part 2, ‘double tests’ (with and without pre-chilling) are mandatory, as indicated in column 6. Less desirable methods are placed in brackets, e.g. TTZ (or EET).</u></p>
<p>Substrates: ...</p>	<p>Substrates ...</p>
<p>Temperatures: the sequence of alternative temperatures is the same throughout and does not indicate any preference: alternating temperatures, highest first; constant temperatures, highest first.</p>	<p>Temperatures The sequence of alternative temperatures is the same throughout and does not indicate any preference: alternating temperatures, highest first; constant temperatures, highest first. <u>Alternating temperature regimes are indicated by the symbols between, i.e. 20<=>30 is an alternating temperature regime of 20 °C for 16 hours and 30 °C for 8 hours.</u></p>
<p>First count: ...</p>	<p>First count ...</p>
<p>Light: illumination of the tests is generally recommended for the sake of better developed seedlings. If in certain cases light is needed to promote germination of dormant samples or if, on the other hand, light may be inhibitory to germination and the substrates should be kept in darkness, this is indicated in the</p>	<p>Light Illumination of the tests is generally recommended for better developed seedlings. If in certain cases light is <u>required</u> to promote germination of dormant samples, <u>this is indicated in column 6.</u> If light is inhibitory to germination and the substrates should be kept in darkness, this is indicated in the</p>

CURRENT VERSION	PROPOSED VERSION
last column.	last -column <u>7</u> .
	<p><u>Where more than one dormancy breaking method is indicated, the sequence of alternative methods does not indicate any preference, and any method or combination of methods can be used. However, if predrying or H₂SO₄ is used in combination with any other method, they must be used prior to the other methods.</u></p> <p><u>If tests are illuminated during an alternating temperature regime, it is usually, at a minimum, for the duration of the higher of the two temperatures, i.e. for 8 hours in a 20↔30 alternating temperature regime.</u></p>
<p>Abbreviations</p> <p>...</p> <p>TT tetrazolium test</p>	<p>Abbreviations</p> <p>...</p> <p>TTZ tetrazolium test</p>

Table 5A. Detailed methods for germination tests (Parts 1–3)

See Appendix 1

C.5.6. Revision of 5.11 Tolerance tables

The new tolerance tables address problems with those currently in the Rules.

- They allow checking of 200- and 100-seed tests as well as 400-seed tests – something that is needed for testing expensive hybrid seed where fewer seed might be required to be tested (something alluded to in Chapter 2) – and these will be necessary when rules for testing mixtures are introduced (regardless of whether it will be possible to issue Orange or only Blue Certificates for mixtures).
- They permit the checking of tolerances when up to (and including) 3 retests have had to be carried out (using the same method). The current rules only give tables for checking 1 retest.
- They correct errors in the present tables – the table for checking repeat tests is only for checking repeat tests carried out in the same laboratory.

The table for checking tolerances of germination tests carried out in different laboratories was deleted, as this is not required in the Rules.

5.11 Tolerance tables

Table 5B gives the maximum tolerated differences between the highest and lowest germination percentages of the replicates of a germination test, allowing only for random sampling variation at a probability of 0.025.

To determine whether a test is reliable, calculate the average germination percentage over all replicates, to the nearest whole number. If necessary, in tests of 400 or 200 seeds, four or two replicates, respectively, of 100 seeds each can be formed by combining the subreplicates of 50 or 25 seeds which were closest together in the germinator. In tests of 100 seeds, two replicates of 50 seeds each can be formed by combining the subreplicates of 25 seeds which were closest together in the germinator, and multiplying the results of each of the two replicates by 2 to obtain an average germination percentage.

Locate the average germination percentage in the appropriate part of the table for the number of seeds tested, and read off the tolerance in the adjoining column. If the difference between the highest and lowest replicates does not exceed this tolerance, the test is reliable.

The tolerances for tests with 400 and 200 seeds are extracted from Table GI, columns D and L respectively, in Miles (1963). The tolerances for tests with 100 seeds are calculated in accordance to Miles (1963).

Table 5B. Tolerances between highest and lowest germination percentages of replicates in one germination test (two-way test at the 2.5% significance level)

Table 5B Part 1. 4 replicates of 100 seeds			Table 5B Part 2. 2 replicates of 100 seeds			Table 5B Part 3. 2 replicates of 50 seeds		
Average germination percentage of test		Tolerance	Average germination percentage of test		Tolerance	Average germination percentage of test		Tolerance
51–100%	0–50%		51–100%	0–50%		51–100%	0–50%	
99	2	5	99	2	4	99	2	5
98	3	6	98	3	5	98	3	7
97	4	7	96–97	4–5	6	97	4	8
96	5	8	95	6	7	96	5	9
95	6	9	93–94	7–8	8	95	6	10
93–94	7–8	10	90–92	9–11	9	94	7	11
91–92	9–10	11	88–89	12–13	10	92–93	8–9	12
89–90	11–12	12	84–87	14–17	11	90–91	10–11	13
87–88	13–14	13	81–83	18–20	12	89	12	14
84–86	15–17	14	76–80	21–25	13	86–88	13–15	15
81–83	18–20	15	69–75	26–32	14	84–85	16–17	16
78–80	21–23	16	55–68	33–46	15	81–83	18–20	17
73–77	24–28	17	51–54	47–50	16	78–80	21–23	18
67–72	29–34	18				74–77	24–27	19
56–66	35–45	19				70–73	28–31	20
51–55	46–50	20				63–69	32–38	21
						51–62	39–50	22

Tables 5C–5E give the tolerances for percentages of normal seedlings, abnormal seedlings, dead seeds, hard seeds or any combination of these when tests are made on the same or a different submitted sample in the same laboratory. For two tests, use Table 5C, for three, Table 5D, and for four, Table 5E.

To determine whether tests are compatible, calculate the average of the test results to the nearest whole number. Locate this in the appropriate part of the table for the number of seeds tested, and read off the tolerance in the adjoining column. If the difference between the highest and lowest results of the tests does not exceed the tolerance, the tests are compatible.

The sources for tolerances are as follows:

- tests with 2 x 400 seeds: extracted from Table G2, column L, in Miles (1963);
- tests with 2 x 200 and 2 x 100 seeds: derived from Miles (1963);
- tests with 3 x 400 seeds: extracted from Table G2, column H, in Miles (1963);
- tests with 3 x 200 and 3 x 100 seeds: derived from Miles (1963);
- tests with 4 x 400 seeds: extracted from Table G2, column D, in Miles (1963);
- tests with 4 x 200 and 4 x 100 seeds: derived from Miles (1963).

Table 5C. Tolerances between results of two tests on the same or a different submitted sample when tests are made in the same laboratory (two-way test at the 2.5% significance level)

Table 5C Part 1. 2 tests of 400 seeds			Table 5C Part 2. 2 tests of 200 seeds			Table 5C Part 3. 2 tests of 100 seeds		
Average germination percentage of two tests		Tolerance	Average germination percentage of two tests		Tolerance	Average germination percentage of two tests		Tolerance
51–100%	0–50%		51–100%	0–50%		51–100%	0–50%	
98–99	2–3	2	99	2	2	99	2	4
95–97	4–6	3	98	3	3	98	3	5
91–94	7–10	4	96–97	4–5	4	96–97	4–5	6
85–90	11–16	5	94–95	6–7	5	95	6	7
77–84	17–24	6	91–93	8–10	6	93–94	7–8	8
60–76	25–41	7	87–90	11–14	7	90–92	9–11	9
51–59	42–50	8	82–86	15–19	8	88–89	12–13	10
			75–81	20–26	9	84–87	14–17	11
			64–74	27–37	10	81–83	18–20	12
			51–63	38–50	11	76–80	21–25	13
						69–75	26–32	14
						55–68	33–46	15
						51–54	47–50	16

Table 5D. Tolerances between results of three tests on the same or a different submitted sample when tests are made in the same laboratory (two-way test at the 2.5% significance level)

Table 5D Part 1. 3 tests of 400 seeds			Table 5D Part 2. 3 tests of 200 seeds			Table 5D Part 3. 3 tests of 100 seeds		
Average germination percentage of three tests		Tolerance	Average germination percentage of three tests		Tolerance	Average germination percentage of three tests		Tolerance
51–100%	0–50%		51–100%	0–50%		51–100%	0–50%	
99	2	2	99	2	3	99	2	4
97–98	3–4	3	97–98	3–4	4	98	3	5
94–96	5–7	4	96	5	5	97	4	6
90–93	8–11	5	94–95	6–7	6	96	5	7
85–89	12–16	6	91–93	8–10	7	95	6	8
78–84	17–23	7	88–90	11–13	8	93–94	7–8	9
66–77	24–35	8	84–87	14–17	9	91–92	9–10	10
51–65	36–50	9	79–83	18–22	10	89–90	11–12	11
			72–78	23–29	11	87–88	13–14	12
			60–71	30–41	12	84–86	15–17	13
			51–59	42–50	13	81–83	18–20	14
						77–80	21–24	15
						71–76	25–30	16
						64–70	31–37	17
						51–63	38–50	18

Table 5E. Tolerances between results of four tests on the same or a different submitted sample when tests are made in the same laboratory (two-way test at the 2.5% significance level)

Table 5E Part 1. 4 tests of 400 seeds			Table 5E Part 2. 4 tests of 200 seeds			Table 5E Part 3. 4 tests of 100 seeds		
Average germination percentage of four tests		Tolerance	Average germination percentage of four tests		Tolerance	Average germination percentage of four tests		Tolerance
51–100%	0–50%		51–100%	0–50%		51–100%	0–50%	
99	2	2	99	2	3	99	2	5
97–98	3–4	3	98	3	4	98	3	6
95–96	5–6	4	97	4	5	97	4	7
92–94	7–9	5	95–96	5–6	6	96	5	8
88–91	10–13	6	93–94	7–8	7	95	6	9
82–87	14–19	7	90–92	9–11	8	93–94	7–8	10
74–81	20–27	8	87–89	12–14	9	91–92	9–10	11
60–73	28–41	9	83–86	15–18	10	89–90	11–12	12
51–59	42–50	10	78–82	19–23	11	87–88	13–14	13
			72–77	24–29	12	84–86	15–17	14
			61–71	30–40	13	81–83	18–20	15
			51–60	41–50	14	78–80	21–23	16
						73–77	24–28	17
						68–72	29–33	18
						56–67	34–45	19
						51–55	46–50	20

Chapter 6: The Tetrazolium Test

C.6.1. Tetrazolium test for *Chloris gayana*

This method has been validated for this species. For details of the research supporting this proposal, see the validation report.

This proposal originates from and is supported by the Tetrazolium Committee.

Table 6A Part 1. Agricultural and horticultural seeds

Species	Pretreatment: type/min. time (h)	Preparation before staining	Staining solution (%)	Optimum staining time (h)	Preparation for evaluation	Permitted non-viable tissue	Remarks
1	2	3	4	5	6	7	8
<i>Chloris gayana</i>	Remove glumes before premoistening BP/16 at 10 °C; W/3	Cut transversely near embryo	1	6	Observe surface of embryo and scutellum	1/3 radicle, measured from radicle tip; in total 1/3 of extremities of scutellum	Empty seeds are reported as non-viable

Chapter 9: Determination of Moisture Content

C.9.1. Resolution of inconsistency between 9.1.5.5 and 9.1.5.2

This proposal was originally included in the Rules change proposals for 2010, to correct the inconsistency in the use of the words working sample, subsample and replicate, but disappeared when these Rules change proposals were withdrawn. However, it also had the purpose of correcting the inconsistency between 9.1.5.5 and 9.1.5.2, where the 7th paragraph states: “In the case of cutting or grinding, one working sample shall be drawn for cutting or grinding and from the cut/ground material two replicates shall be obtained.” Therefore, in 9.1.5.5, “subsamples” needs to be changed to “a working sample” to be consistent with the wording in paragraph in 9.1.5.2 above.

CURRENT VERSION	PROPOSED VERSION
<p><i>9.1.5.5 Cutting</i></p> <p>... The cutting shall be carried out on two subsamples each of a weight approximately equal to the weight of five intact seeds from the submitted sample.</p>	<p><i>9.1.5.5 Cutting</i></p> <p>... The cutting shall be carried out on <u>a working sample</u> of a weight approximately equal to the weight of <u>ten</u> intact seeds from the submitted sample.</p>

C.9.2. Increase in moisture test duration for *Lolium* spp.

The PT round 08-1 *Lolium multiflorum* included moisture determination. The results of this round indicated a difference in the moisture determined, depending on which of the two methods permitted in the ISTA Rules (103 °C for 17 h vs 130 °C for 1 h) was used. Comparative testing at four ISTA laboratories indicates that the duration of the moisture test for *Lolium* spp. should be increased from 1 h to 2 h. A validation report to support this Rules change proposal, based on the comparative testing undertaken by the four ISTA laboratories, data from PT round 08-1 for *Lolium multiflorum* and the literature, has been prepared and approved by the Moisture Committee.

Table 9A Part 1. Details of methods for moisture determination: agricultural and vegetable seeds

Species	Grinding/cutting (9.1.5.4, 9.1.5.5)	High temperature	Drying at high temperature (h)	Predrying requirement (9.1.5.6)
1	2	3	4	5
CURRENT VERSION				
<i>Lolium</i> spp.	No	Yes	1	–
PROPOSED VERSION				
<i>Lolium</i> spp.	No	Yes	<u>2</u>	–

Chapter 15: Seed Vigour Testing

C.15.1. Conductivity test for *Glycine max*

The conductivity test is currently validated for *Pisum sativum* and *Phaseolus vulgaris*. This test also identifies differences in seed vigour in *Glycine max*, and comparative tests have shown that the method is repeatable and reproducible (see Method Validation Report). *Glycine max* is therefore proposed as a third species to which the conductivity test can be applied. This change would also necessitate a corresponding change to Table 15A.

CURRENT VERSION	PROPOSED VERSION
<p><i>15.8.1.2 Scope and field of application</i></p> <p>The conductivity test offers a vigour test for <i>Pisum sativum</i> (garden peas only) and <i>Phaseolus vulgaris</i> which relates to the field emergence of seed lots. The test does not apply to field peas or the so-called 'petit pois' varieties of peas.</p> <p>...</p>	<p><i>15.8.1.2 Scope and field of application</i></p> <p>The conductivity test offers a vigour test for <i>Pisum sativum</i> (garden peas only), <i>Phaseolus vulgaris</i> and <i>Glycine max</i> which relates to the field emergence of seed lots. The test does not apply to field peas or the so-called 'petit pois' varieties of peas.</p> <p>...</p>

C.15.2. Change to list of validated tests; modification of Table 15A

Table 15A. Vigour tests that have completed validation

CURRENT VERSION

Vigour test	Species
Conductivity	<i>Pisum sativum</i> (garden pea only, excluding petit-pois varieties) <i>Phaseolus vulgaris</i>
Accelerated ageing	<i>Glycine max</i>
Controlled deterioration	<i>Brassica</i> spp.

PROPOSED VERSION

Vigour test	Species
Conductivity	<i>Pisum sativum</i> (garden pea only, excluding petit-pois varieties) <i>Phaseolus vulgaris</i> <i>Glycine max</i>
Accelerated ageing	<i>Glycine max</i>
Controlled deterioration	<i>Brassica</i> spp.

C.15.3. Amendment of 15.8.1.4 for consistency with 15.8.2.4

CURRENT VERSION	PROPOSED VERSION
<p><i>15.8.1.4 Preparation of the sample before measuring conductivity</i></p> <p>Determine the moisture content of the submitted sample according to Chapter 9. If the moisture content is below 10% or above 14%, it must be adjusted to between 10 and 14%.</p> <p>...</p>	<p><i>15.8.1.4 Preparation of the sample</i></p> <p>Determine the moisture content of the submitted sample according to Chapter 9. If the moisture content is below 10% or above 14%, it must be adjusted to between 10 and 14%, <u>although it is not necessary for the moisture content of all samples to be the same within this range.</u></p> <p>...</p>

Chapter 17: Bulk containers**C.17.1 Amendment of Table in 17.5: Calculation and expression of results**

Although this change could be considered to be editorial, the existing text has remained unchanged since its inclusion in the Rules in 1993. Therefore, the changes are being presented for a vote. The values amended in the Table have been amended to follow the rounding procedures detailed in the text of 17.5.

CURRENT VERSION

	Lot 1	Lot 2	Lot 3	Totals	Weighted average
Lot sizes (kg)	10 000	7 000	2 500	19 500	
Multiplication factor	0.513	0.359	0.129		
Germination (%)	91	87	92		
Proportional value	46.683	31.233	11.868	89.784	90%
Purity (%)	99.4	99.1	99.3		
Proportional value	50.9922	35.5769	12.8097	99.3788	99.4%
Other seed count	5	2	7		
Proportional value	2.565	0.718	0.903	4.136	4

PROPOSED VERSION

	Lot 1	Lot 2	Lot 3	Totals	Weighted average
Lot sizes (kg)	10 000	7 000	2 500	19 500	
Multiplication factor	0.513	0.359	<u>0.128</u>		
Germination (%)	91	87	92		
Proportional value	46.683	31.233	<u>11.776</u>	<u>89.692</u>	90%
Purity (%)	99.4	99.1	99.3		
Proportional value	50.9922	35.5769	<u>12.7104</u>	<u>99.2795</u>	<u>99.3%</u>
Other seed count	5	2	7		
Proportional value	2.565	0.718	<u>0.896</u>	<u>4.179</u>	4