International Seed Testing Association

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Rules Proposals for the International Rules for Seed Testing 2010 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 were submitted to the ISTA Ordinary Meeting 2009 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 approved by vote at the Ordinary Meeting 2009, held on Thursday, 18 June 2009 in Glattbrugg, Zurich, Switzerland, under Agenda point 9: Consideration and Adoption of the proposed Rules Changes 2010.

Introduction to the ISTA Rules Proposals to become effective 1 January 2010

The current version of the ISTA International Rules for Seed Testing is the 2009 edition. Single copies of replacement pages and front covers for the 2009 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 were discussed at the ISTA Ordinary Meeting in Glattbrugg, Zurich, Switzerland, in June 2009. For the proposals which were accepted by the voting delegates of the ISTA Ordinary Meeting 2009, Amendments will be issued, and they will become the 2010 edition of the ISTA Rules.

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

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CONTENTS

PART A. INTRODUCTION OF EDITORIAL CHANGES

A.1. Editorial corrections

CURRENT VERSION	PROPOSED VERSION
Table5A Part 3 **…** *Kochia scoparia*	**Table5A Part 3** **…** *Kochia scoparia*
Annexe to Chapter7: Seed Health Methods	**Annexe to Chapter7: Seed Health Methods**
p. 7-014-4: Preparation of Media and Solutions -- 2. Malt Agar … Streptomycin sulfate …	**p. 7-014-4: Preparation of Media and Solutions -- 2. Malt Agar** … Streptomycin sulfate* * Added after autoclaving …
p. 7-014-5: Potato Dextrose Agar … Streptomycin sulfate …	**p. 7-014-5: Potato Dextrose Agar** … Streptomycin sulfate* * Added after autoclaving …
p. 7-016-7: Preparation of Acidified Potato Dextrose Agar (APDA) **…** 4. Autoclave at 121°C, ~~1~~15 psi for 15 min. …	**p. 7-016-7: Preparation of Acidified Potato Dextrose Agar (APDA)** **…** 4. Autoclave at 121 °C, 15 psi for 15 min. …
p. 7-019-10: Preparation of sterile saline **…** 4. Autoclave at 121°C, ~~1~~15 psi for 15 min. …	**p. 7-019-10: Preparation of sterile saline** **…** 4. Autoclave at 121 °C, 15 psi for 15 min. …
p. 7-019-11: Preparation of mCS20ABN agar medium **…** 5. Autoclave at 121°C, ~~1~~15 psi for 15 min. 7. Allow medium to cool to approx. 50˚C and add antibiotic ~~and methionine~~ solutions …	**p. 7-019-11: Preparation of mCS20ABN agar medium** **…** 5. Autoclave at 121 °C, 15 psi for 15 min. 7. Allow medium to cool to approx. 50 °C and add antibiotic solutions …

CURRENT VERSION	PROPOSED VERSION
p. 7-020-12: Preparation of sterile saline **…** 5. Autoclave at 121°C, ~~1~~15 psi for 15 min. …	**p. 7-020-12: Preparation of sterile saline** **…** 5. Autoclave at 121 °C, 15 psi for 15 min. …
p. 7-020-13: Preparation of MKM agar medium **…** 4. Autoclave at 121°C, ~~1~~15 psi for 15 min. …	**p. 7-020-13: Preparation of MKM agar medium** **…** 4. Autoclave at 121 °C, 15 psi for 15 min. …
p. 7-021-9: Quality Assurance … The quality of milk powder is vital to develop the hydrolysis of ~~starch~~ in MT medium. …	**p. 7-021-9: Quality Assurance** … The quality of milk powder is vital to develop the hydrolysis of milk in MT medium.” …
15.1 Object The object of a seed vigour test is to provide information about the planting value in a wide range of environments and/or the storage potential of seed lots. The test provides additional information to the standard germination test (see Chapter 5) to assist in differentiation of seed lots of acceptable germination.	**15.1 Object** The object of a seed vigour test is to provide information about the planting value in a wide range of environments and/or the storage potential of seed lots. The test provides additional information to the standard germination test (see Chapter 5) to assist in the differentiation of seed lots of acceptable germination.
15.2.2 Seed vigour test A seed vigour test is either a direct or indirect analytical procedure to evaluate the vigour of a seed lot under standardised conditions. Direct tests ~~are those tests for which an~~ environmental stress or other conditions are reproduced in the laboratory and the percentage and/or rate of seedling emergence are recorded. Indirect tests ~~are those tests which~~ measure other characteristics of the seed that have proved to be associated with some aspect of seedling performance.	***15.2.2 Seed vigour test*** A seed vigour test is either a direct or an indirect analytical procedure to evaluate the vigour of a seed lot under standardized conditions. Direct tests reproduce environmental stresses or other conditions in the laboratory, and the percentage and/or rate of seedling emergence are recorded. Indirect tests measure other characteristics of the seed that have proved to be associated with some aspect of seedling performance.
15.2.3 ~~Seed lot of~~ ‘acceptable germination’	***15.2.3 Acceptable germination***

CURRENT VERSION	PROPOSED VERSION
15.5.4 Control samples All vigour tests require rigid control of test conditions and should include a control seed sample, to provide internal quality control of vigour test uniformity. Variability in control seed sample results provides an indication of slight fluctuations in test conditions (e.g. changes in temperature ~~and/~~or seed moisture) which can significantly affect reliability of results. Specific guidelines for ~~the~~ seed lot selection, storage and handling of control samples are described in the *ISTA Handbook of Vigour Test Methods*.	***15.5.4 Control samples*** All vigour tests require rigid control of test conditions and, where specified, should include a control seed sample, to provide internal quality control of vigour test uniformity. Variability in control seed sample results provides an indication of slight fluctuations in test conditions (e.g. changes in temperature or seed moisture) which can significantly affect the reliability of results. Specific guidelines for seed lot selection, storage and handling of control samples are described in the *ISTA Handbook of Vigour Test Methods*.
15.6 Calculation and expression of results Results are expressed in different formats for ~~various~~ vigour tests as shown in 15.8.	**15.6 Calculation and expression of results** Results are expressed in different formats for different vigour tests, as shown in 15.8.
15.8.1.1 Principle … Seed lots ~~that have~~ high electrolyte leakage, ~~that is, having~~ high leachate conductivity, are considered ~~as having~~ low vigour, whilst those with low leakage (low conductivity) are considered high vigour.	*15.8.1.1 Principle* … Seed lots with high electrolyte leakage, i.e. high leachate conductivity, are considered to have low vigour, whilst those with low leakage (low conductivity) are considered to have high vigour.
15.8.1.2 Scope and field of application Submitted seed lots may ~~be~~ fungicide ~~treated~~. Various ~~sources of~~ fungicide preparations with different purity levels are commercially available and some fungicides may possess additives that may significantly alter conductivity results. Thus, caution must be exercised when using the conductivity test for treated seeds.	*15.8.1.2 Scope and field of application* Submitted seed lots may have been treated with fungicide. Various fungicide preparations with different purity levels are commercially available, and some fungicides may possess additives that may significantly alter conductivity results. Thus, caution must be exercised when using the conductivity test for treated seeds.

CURRENT VERSION	PROPOSED VERSION
15.8.1.3 Apparatus Conductivity meter: ~~A suitable conductivity meter is~~ direct reading using AC or DC current, with a dip cell ~~having~~ a cell constant of 1.0. The meter specifications should include a range of 0-1999 µS cm^{-1}, a resolution of at least 0.1 µS cm^{-1} and an accuracy of ± 1%. ~~The~~ temperature range ~~for the meter should include~~ 20-25°C. Erlenmeyer flasks~~, conical flasks~~ or ~~glass~~ beakers: …	*15.8.1.3 Apparatus* Conductivity meter: a direct-reading meter using AC or DC current, with a dip cell that has a cell constant of 1.0, is suitable. The meter specifications should include a conductivity range of 0–1999 µS cm^{-1}, a resolution of at least 0.1 µS cm^{-1}, an accuracy of ±1% and a temperature range of 20–25 °C. Containers (Erlenmeyer flasks or beakers): …
Water: Deionised water or distilled water should be used. The conductivity of ~~either~~ the deionised or distilled water must be measured and must not exceed 5 µS cm^{-1} at 20°C. … Germinator, incubator or walk-in room: ~~A germinator, incubator or walk-in room at~~ a constant temperature of 20 ± 2°C is required. …	Water: deionized water or distilled water should be used. The conductivity of the deionized or distilled water must be measured and must not exceed 5 µS cm^{-1} at 20 °C. … Germinator, incubator or walk-in room: a constant temperature of 20 ± 2 °C is required. …
15.8.1.4 Preparation of the sample before measuring conductivity Determine the moisture content ~~(mc)~~ 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%~~ mc.~~ …	*15.8.1.4 Preparation of the sample before measuring conductivity* 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%. …
In the case of a moisture content below 10%, raise the moisture content by placing each weighed sub~~-~~sample between moist cloths (paper towels) until it reaches a weight equivalent to between 10 and 14%~~ mc.~~ …	In the case of a moisture content below 10%, raise the moisture content by placing each weighed subsample between moist cloths (paper towels) until it reaches a weight equivalent to a moisture content between 10 and 14%. …

CURRENT VERSION	PROPOSED VERSION
In the case of a moisture content above 14%, reduce the moisture content by placing the weighed sub-sample in an oven at 30°C until it reaches a weight equivalent to between 10 and 14% ~~mc.~~ Experience indicates that seeds ~~having~~ an initial ~~mc~~ of around 15% take 1h to reach 14% ~~mc~~ and 5-6 h to reach 10% when dried in this way. When the initial seed moisture content is approximately 16%, it takes 1-2 h drying to reach 14% ~~mc~~ and 8-10 h to reach 10% ~~mc~~. …	In the case of a moisture content above 14%, reduce the moisture content by placing the weighed subsample in an oven at 30 °C until it reaches a weight equivalent to a moisture content between 10 and 14%. Experience indicates that seeds with an initial moisture content of around 15% take 1 h to reach 14%, and 5–6 h to reach 10%, when dried in this way. When the initial seed moisture content is approximately 16%, it takes 1–2 h drying to reach 14%, and 8–10 h to reach 10%. …
Weight of subsample at 10 or 14% mc = (initial weight) • $\frac{(100 - \text{initial mc})}{(100 - \text{desired seed mc*})}$ mc = moisture content	
*The desired ~~mc~~ will be either 10 or 14%. When the sub-sample has reached a weight equivalent to between 10 and 14% ~~mc~~, it should be sealed in a moisture-proof container such as an aluminium foil packet or polythene bag and held for 12-18 h at 5-10°C ~~for~~ the moisture content to equilibrate throughout the seed.	*The desired moisture content will be either 10 or 14%. When the subsample has reached a weight equivalent to a moisture content between 10 and 14%, it should be sealed in a moisture-proof container, such as an aluminium foil packet or polythene bag, and held for 12–18 h at 5–10 °C to allow the moisture content to equilibrate throughout the seed.
15.8.1.5.1 Calibrating the dip cell … Note that calibration of the meter using these solutions is carried out at 25 °C, which is possible when using a meter with the specifications described ~~above~~. …	*15.8.1.5.1 Calibrating the dip cell* … Note that calibration of the meter using these solutions is carried out at 25 °C, which is possible when using a meter with the specifications described in 15.8.1.3. …
15.8.1.5.2 Checking the cleanliness of equipment Each testing day, select at random 2 out of every 10 ~~flasks~~ to be used, … (etc.)	*15.8.1.5.2 Checking the cleanliness of equipment* Each testing day, select at random 2 out of every 10 containers to be used, … (etc.)
15.8.1.6.2 Preparing the ~~flasks/beakers~~ … (etc.)	*15.8.1.6.2 Preparing the containers* … (etc.)

CURRENT VERSION	PROPOSED VERSION
15.8.1.6.5 Measuring the conductivity of the solution … Once the leachate has been mixed, take several measurements of the conductivity until a stable value is obtained. If hard seeds are observed during testing, they should be removed after the conductivity ~~has been measured, the number recorded, surface dried, weighed and the~~ weight subtracted from the initial weight of the 50 seed replicate.	*15.8.1.6.5 Measuring the conductivity of the solution* … Once the leachate has been mixed, take several measurements of the conductivity until a stable value is obtained. If hard seeds are observed during testing, they should be removed after the conductivity test, and their number recorded. They should then be surface dried and weighed, and their weight subtracted from the initial weight of the 50-seed replicate.
15.8.1.6.6 Accounting for the conductivity of the original water source Measure the conductivity of one control ~~flask/beaker~~. Any increase in ~~the reading~~ above 5 µS cm^{-1} indicates a potential problem with the cleanliness of the dip cell. Re-wash the dip cell and ~~retest~~ the conductivity of the other control ~~flask/beaker~~. If this also indicates an increase in ~~reading~~, there is a problem with the dip cell and conductivity measurements cannot be made until this has been satisfactorily cleaned. Most conductivity meters provide instructions for cleaning the dip cell. Where the conductivity of the second control ~~flask/beaker~~ does not show an increase above 5 µS cm^{-1}, this ~~conductivity reading~~, or the mean of the two controls if neither has increased, represents the background ~~reading~~ and should be subtracted from the ~~conductivity reading~~ already recorded for each replicate flask/beaker.	*15.8.1.6.6 Accounting for the conductivity of the original water source* Measure the conductivity of one control container. Any increase in conductivity above 5 µS cm^{-1} indicates a potential problem with the cleanness of the dip cell. Rewash the dip cell and measure the conductivity of the other control container. If this also indicates an increase in conductivity, there is a problem with the dip cell, and conductivity measurements cannot be made until this has been satisfactorily cleaned. Most conductivity meters provide instructions for cleaning the dip cell. Where the conductivity of the second control container does not show an increase above 5 µS cm^{-1}, this value, or the mean of the two controls if neither has increased, represents the background conductivity, which should be subtracted from the values already recorded for each replicate container.
15.8.1.7 Calculation and expression of results …, are shown in Table 15D. ~~When reported on an *ISTA International Seed Analysis Certificate*, results are entered under 'Other Determinations'.~~	*15.8.1.7 Calculation and expression of results* …, are shown in Table 15D. *15.8.1.8 Reporting results* The result of a seed vigour test using the conductivity test method must be reported under 'Other determinations' as follows:…(see 1.5.2.16.1)
15.8.2.3 Apparatus Balance: Analytical balance capable of weighing to 0.001 g. …	*15.8.2.3 Apparatus* Balance: analytical balance capable of weighing to the nearest 0.001 g. …

CURRENT VERSION	PROPOSED VERSION
15.8.2.4 Preparation of the sample Determine the moisture content ~~(mc)~~ 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% although it is not necessary for the ~~mc~~ of all samples to be the same within this range. …	*15.8.2.4 Preparation of the sample* 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%, although it is not necessary for the moisture content of all samples to be the same within this range. …
In the case of a moisture content below 10%, raise the moisture content by placing each weighed sub-sample between moist cloths (paper towels) or in a high humidity environment until it reaches a weight equivalent to between 10 and 14% ~~mc~~. In the case of a moisture content above 14%, reduce the moisture content by placing the weighed sub-sample in an oven at 30°C until it reaches a weight equivalent to between 10 and 14% ~~moisture content~~. …	In the case of a moisture content below 10%, raise the moisture content by placing each weighed subsample between moist cloths (paper towels) or in a high-humidity environment until it reaches a weight equivalent to a moisture content between 10 and 14%. In the case of a moisture content above 14%, reduce the moisture content by placing the weighed subsample in an oven at 30 °C until it reaches a weight equivalent to a moisture content between 10 and 14%. …
Weight of subsample at 10 or 14% mc = (initial weight) • $\frac{(100 - \text{initial mc})}{(100 - \text{desired seed mc*})}$ mc = moisture content	
*The desired ~~mc~~ will be either 10 or 14%. When the sub-sample has reached a weight equivalent to between 10 and 14% ~~mc~~, it should be sealed in a moisture-proof container such as an aluminium foil packet or polythene bag and held for 12-18 h at 5-10°C ~~for~~ the moisture content to equilibrate throughout the seed.	*The desired moisture content will be either 10 or 14%. When the subsample has reached a weight equivalent to a moisture content between 10 and 14%, it should be sealed in a moisture-proof container, such as an aluminium foil packet or polythene bag, and held for 12–18 h at 5–10 °C to allow the moisture content to equilibrate throughout the seed.
15.8.2.6.1 Preparing the plastic AA boxes and seed sample Place 40 ml (± 1.0 ml) of distilled or deionized water in each plastic AA box and insert a dry screen tray, being ~~certain~~ not to splash water onto the screen. …	*15.8.2.6.1 Preparing the plastic AA boxes and seed sample* Place 40 ml (± 1.0 ml) of distilled or deionized water in each plastic AA box and insert a dry screen tray, being careful not to splash water onto the screen. …

CURRENT VERSION	PROPOSED VERSION
15.8.2.7 Calculation and expression of results …, are shown in Table 15G. ~~When reported on an *ISTA International Seed Analysis Certificate*, results are entered under 'Other Determinations'.~~	*15.8.2.7 Calculation and expression of results* …, are shown in Table 15G. *15.8.2.8 Reporting results* The result of a seed vigour test using the accelerated ageing test method must be reported under 'Other determinations' as follows:…(see 1.5.2.16.2)
~~**Chapter 16: Tolerances**~~	…
All tolerance tables from Chapter 16 have been transferred to their respective chapters.	
~~**Appendix A:**~~ **Rules for size and grading of seeds**	**Chapter 16: Rules for size and grading of seeds**
~~**Appendix B:**~~ **Rules for the Issue of…**	**Chapter 17: Rules for the issue of…**

Section 8.2.2

The text mentions as an example of a test included in Chapter 8 ploidy testing. This is not true so the words ploidy testing will be deleted.

PART B. NEW SPECIES AND CHANGES OF SPECIES NAMES

B.1. Addition of *Brachiaria brizantha*

This Rules proposal could be withdrawn if it has not been approved by the Germination Committee or the validation report is not available for discussion at the Zurich meeting.

New entries: Table 2A Part 1 Agricultural and vegetable species

Species	Maximum weight of lot	Minimum sample weights		
		Submitted sample	Working sample for purity analysis	Working sample for count of other species
	kg	g	g	g
Brachiaria brizantha (Hochst. ex A. Rich.) Stapf	10 000	100	10	100

New entries: Table 5A Part 1 Agricultural and vegetable seeds

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature (°C)	First count (d)	Final count (d)	
1	2	3	4	5	6
Brachiaria brizantha	TP	20–35	7	21	~~H_2SO_4 followed by~~ Preheat; KNO_3

PART C. RULES CHANGES AND NEW METHODS REQUIRING A VOTE

Chapters 2, 7 and 11

C.0.1. Revised definition of seed treatment applicable to several Rules chapters

At present, the ISTA Rules contain three different definitions of "Seed Treatment". At the 2007 Congress, the ISTA members requested that these inconsistencies be removed, and in response to this, the Secretariat prepared a document for discussion within the ECOM at the 2008 Ordinary Meeting. The ECOM duly considered the document, and decided that a Working Group be established to define "Seed Treatment" for the ISTA Rules. The following proposal has been developed by a working group of the ISTA Executive Committee and approved by a majority vote of the Rules Committee.

The current proposal does not go as far as to review all occurrences of the word "treatment" in the ISTA Rules. Such changes will coincide with on-going reviews by technical committees of individual Chapters of the Rules, in particular for the Germination Chapter where the terms treatment and pre-treatment are used.

ISTA considers that "Seed Treatment" is a generic term and that the following definition is proposed.

If this generic term is accepted changes will be required in Chapters 7 and 11 as detailed below.

CURRENT VERSION	PROPOSED VERSION
2.2.~~11~~ Coated seeds Coated seeds…….. ~~Treated seed. Seeds with treatments, which have not resulted in a significant change in size, shape or addition to the weight of the original seed.~~	**2.2.11 Treated seed** "Seed treatment" is a generic term which indicates that a seed lot has been subjected to: a) the application of a compound including chemicals, nutrients or hormones b) the application of a biological product including micro-organisms c) a process including wetting and drying d) an energy form including heat, radiation, electricity or magnetism; but does not specify the application method. Seed treatment does not significantly change the size, shape or add to the weight of the seeds in the lot. **2.2.12 Coated seeds** Coated seeds……..

CURRENT VERSION	PROPOSED VERSION
7.2.3 ~~T~~reatment ~~Any process, physical, biological or chemical, to which a seed lot is subjected, including seed coatings.~~	**7.2.3** Seed t**reatment** See 2.2.11. For seed health testing, a seed lot may be treated for the purpose of controlling plant pathogens or insect pests, or correcting trace element deficiencies.
CURRENT VERSION	PROPOSED VERSION
11.1 Objects … However, ~~seed~~ treated ~~in traditional ways with pesticides alone is~~ not covered and …	**11.1 Objects** …However, treated seeds are not covered and …
CURRENT VERSION	PROPOSED VERSION
11.1.1 Definitions … ~~Treated seed. Seed to which only pesticides, dyes or other additives have been applied which have not resulted in a significant change in size, shape or addition to weight of the original seed and which can still be tested according to the methods prescribed in the other chapters.~~	**11.1.1 Definitions** … *Seed treatment.* See 2.2.11. Seeds which have received seed treatment must still be tested according to the methods prescribed in other chapters.

Chapter 1: ISTA Certificates

C.1.1. Revision of 1.5 Reporting results, and synchronization with corresponding paragraphs in other Chapters

Although these are considered as editorial-only changes, they affect how tests results are presented on the ISTA Certificates; therefore, the ECOM would like the membership to vote on the principle of including the expanded Reporting section in Chapter 1 and including the same text in each Chapter, rather than voting on the wording, since the changes are editorial.

For many years, the technical Chapters and Chapter 1 (previously Chapter 17) have had a paragraph on how to "report the results" on an ISTA Certificate.

These paragraphs contain either complementary information or redundant information.

The Executive Committee decided to introduce more consistency into reporting results in the Rules. Therefore, it was decided:

– to merge the paragraphs from Chapter 1 and the related technical chapters;

– to adopt a common structure for each chapter;

– to duplicate the related paragraphs from Chapter 1 into the technical chapters.

The new structure is largely inspired from the current structure in Chapters 2, 3 and 5.

The new structure is intended to give guidance on:

– mandatory information;

– data;

– format;

– places to report additional information;

– mandatory upon request information;

– conditional information.

The following text would completely replace the existing sections 1.5.1 to 1.5.3.8; as this is a complete replacement, the existing text has not been shown here.

If this proposal is accepted, the existing sections concerned with reporting results would be deleted from all Chapters, and editorially replaced with the text from the relevant section from Chapter 1; for example, the section 1.5.2.2 below would also be included as section 3.7 in the Purity Chapter. This is indicated in brackets for each section of the proposal for information only, and would not be printed in the final version of the Rules.

Some of the proposed text in Chapter 1 will be affected by other proposals being proposed this year. The Chapter 1 text will need to be updated to be in line with the new proposals, for example proposal C.3.1.

PROPOSED VERSION 1.5.

(Note: All existing text in 1.5 Reporting results would be deleted and replaced with the proposed version. For ease of reading the text is not underlined)

1.5 Reporting results

1.5.1 Sampling and testing

From one sampling operation, only one sample may be submitted for testing. The sample may be subjected to one or more of the tests described in the ISTA Rules as requested by the applicant. However, in certain situations (see 2.5.1.6) the submission of separate moisture-proof-packed subsample(s) from the same sampling operation attached to the submitted sample is required.

1.5.2 Certificates

The results of tests may be reported on one or more ISTA Certificates, separately or combined.

Test results must be reported in accordance with the rules for calculating, expressing and reporting results in the appropriate chapter of the ISTA Rules. If there is a space on the certificate for certain determinations which are not made or applicable, 'N' for 'not tested' must be placed in the space.

1.5.2.1 Sampling: heterogeneity testing for seed lots in multiple containers (also 2.9.1.5 (new), 2.9.2.5(new))

The result of a heterogeneity test for seed lots in multiple containers must be reported under 'Other determinations', as follows:

a) The results of the H value test:

– 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.

b) The statement: 'This H value does/does not indicate significant heterogeneity.'

c) The results of the R value test:

– 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.

d) The statement: "This R value does/does not indicate significant heterogeneity."

Note: the H value must not be calculated or reported if X is outside the following limits:

– 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

1.5.2.2 Purity (also 3.7)

The results of a purity test must be reported in the spaces provided as follows:

– The scientific name of the species of pure seed, in accordance with Table 2A. Where it is impossible to determine the species with certainty on the basis of seed

characteristics, the genus name only must be reported (e.g. *Malus* sp.).

– The percentage by weight of pure seed, inert matter and other seeds, given to one decimal place. The percentage of all components must total 100%. Components amounting to less than 0.05% must be reported as 'Trace' or 'TR' (for 'Trace'). If no inert matter or other seeds are found, this must be reported as '0.0'.

– The kind of inert matter.

– The scientific name of every species of other seeds found, in accordance, where applicable, with the current *ISTA List of Stabilized Plant Names*, available at www.seedtest.org.

– The actual weight of the working sample tested for purity, if it deviates from the weight prescribed in Table 2A, column 4.

Note: the paragraph above will be amended and expanded if Proposal C.3.1. is accepted.

– The percentage of winged seed (as defined in Pure Seed Definitions 47 and 51), if winged seeds are found.

Upon request, the following information ~~may~~ must be reported under 'Other determinations' as follows:

– The percentage by weight of a specified species, entered immediately after the name of the species to the nearest 0.1%. Species for which the percentage by weight has been requested are listed first.

– Other seeds may be divided into 'other crop seeds' and 'weed seeds'. In this case, the words 'Other crop seeds' must be entered, followed by the percentage by weight of other crop seeds and the name(s) of the species found. This procedure must also be used for 'Weed seeds'.

– Multiple seed units must be reported as '% MSU'.

– Seeds with appendages attached must be reported as '% seeds with appendages attached'.

– The kinds of inert matter, together with the percentage by weight of any particular kind (to one decimal place).

The percentages may be reported to more than one decimal place if requested.

1.5.2.3 Purity tests on coated seeds (also 11.3.7)

The result of a purity test on coated seeds must be reported as follows:

– Following the species name, the words 'seed pellets', 'encrusted seeds', 'seed granules', 'seed tapes' or 'seed mats', as applicable, must be clearly entered.

– The results must be reported to one decimal place, and the percentage of all components must total 100%. Components amounting to less than 0.05% must be reported as 'Trace' or 'TR' (for 'Trace').

– In the case of pelleted seeds only, the percentages of pure pelleted seeds, inert matter and unpelleted seeds must be reported in the spaces provided for 'Pure seeds', 'Inert matter', and 'Other seeds', respectively.

– The name and number of the seeds of each species found in the examination of the 100 seeds removed from the pellets or tapes must be reported under 'Other determinations'.

Upon request, the following information may be reported under 'Other determinations' as follows:

– Purity test on depelleted seeds. The component parts (pure seed, other seeds and

inert matter) may be reported as percentages of their total weight, ignoring the pelleting material.

The percentage of pelleting material must be reported separately only on request. The result of this test is to be reported: 'weight of … material excluded".

– Purity of seeds removed from tapes. The component parts (pure seed, other seeds, and inert matter) may be reported as percentages of their total weight, ignoring the tape material. The result of this test is to be reported: 'weight of … material excluded'.

1.5.2.4 Determination of other seeds by number (also 4.7)

Note: 1.5.2.4 will be amended and expanded if Proposal C.4.1 is accepted.

The result of a determination of other seeds by number must be reported under 'Other determinations' as follows:

– The actual weight of seed examined.

– The scientific name and number of seeds of each species sought and found in this weight.

– If the full weight prescribed in Table 2A was examined for all other species present, then the words 'Complete test' must be entered, alongside the weight of seed examined.

– If the examination was for only a limited range of other species, then the words 'Limited test' must be entered.

– If the weight examined for all other species was less than the prescribed weight, then the words 'Reduced test' must be entered.

– If the weight examined was less than the prescribed weight in Table 2A, and only a limited range of other species was examined, then the words 'Reduced-limited test' must be entered.

– If a sample of at least 25 000 seeds was examined, and this sample was below the weight prescribed in Table 2A, then the weight of seed examined and the statement 'Test based on at least 25 000 seeds' must be entered.

Upon request, the results may in addition be expressed in some other way, such as 'weight of seeds found' or 'number of seeds per kilogram'.

1.5.2.5 Determination of other seeds by number on coated seeds (also 11.4.7)

The result of a determination of other seeds by number on ~~other~~ coated seeds must be reported as follows:

– Following the species name, the words 'seed pellets', 'encrusted seeds', 'seed granules', 'seed tapes' or 'seed mats', as applicable, must be clearly entered.

– Under 'Other determinations', the actual weight (or length of tape, or area of mat) and approximate number of pelleted seeds examined must be entered, together with the scientific name and number of seeds of each species sought and found in this weight, length or area.

Upon request, the result may in addition be expressed in some other way, such as number of seeds per kilogram, per metre or per square metre.

1.5.2.6 Germination (also 5.9)

The result of a germination test must be reported in the spaces provided as follows:

– the duration of the test;

– the percentages, calculated to the nearest whole number (5.8.1), of normal seedlings, hard seeds, fresh seeds, abnormal seedlings and dead seeds. If the result for any of these categories is found to be zero, it must be reported as '0'.

The following additional information must also be reported under 'Other determinations':

– The germination method using the abbreviations used in Table 5A, including at least substrate and temperature.

– Any special treatment or method used for promoting germination (5.6.3).

– 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.

Upon request, the following information may be reported as follows:

– the result of any additional test;

– the viability of ungerminated seeds and the method used to determine it;

– the categories of ungerminated seeds (as listed in 5.6.5.3) and the method used to determine them;

– in the case of multigerm seed units: the number of normal seedlings produced by 100 units, and the proportion of units producing one, two or more than two normal seedlings.

1.5.2.7 Germination of coated seeds (also 11.5.8)

The result of a germination test on coated seeds must be reported as follows:

– Following the species name, the words 'seed pellets', 'encrusted seeds', 'seed granules', 'seed tapes' or 'seed mats', as applicable, must be clearly entered in the space provided.

– The percentage of pellets or seed in tapes with normal seedlings, with abnormal seedlings and without seedlings.

– The duration of the test.

The following additional information must also be reported under 'Other determinations':

– The method used for the germination test.

– For seed tapes or mats: the number of normal seedlings per metre of tape or square metre of mat.

Seedlings that are obviously not of the species stated by the applicant, even if otherwise normal, must not be included in the germination result, but their number must be reported separately.

1.5.2.8 Tetrazolium test (also 6.7)

The result of a tetrazolium test must be reported under 'Other determinations' as follows:

– The statement 'Tetrazolium test: …% of seeds were viable' must be entered.

– In cases where the testing procedure (premoistening time, tetrazolium concentration, staining temperature, staining time) deviates from that prescribed in Table 6A, the corresponding deviating procedures must also be reported.

– If individual seeds are tested at the end of the germination test, the result must be reported in accordance with 5.9.

In addition, in the case of species of *Fabaceae*, one of the following, and only one, must be reported:

Either the percentage of hard seeds found in the test

or the percentage of hard seeds included in the reported percentage of viable seeds.

At the discretion of the seed testing station, further information may be reported, e.g. percentage of seeds that were empty, with larvae, broken or decayed.

1.5.2.9 Seed health test (also 7.6)

The results of a test for seed health must be reported under 'Other determinations' as follows:

– either qualitative or quantitative results, as specified in the individual methods;

– negative and positive results, as specified in the individual methods;

– the scientific name of the pathogen detected;

– the percentage of infected seeds;

– the method used, including any pretreatment (7.2.2);

– the size of the sample or fraction examined;

– any additional permitted procedure used.

The absence of a statement concerning the health condition of the seed does not necessarily imply that the health condition is satisfactory.

1.5.2.10 Species and variety testing (also 8.7)

The results must be reported under 'Other determinations' and in addition the following information must be given:

a) the request of the applicant;

b) the trait(s) and the method(s) used;

c) the kind of preparation of the working sample (e.g. the whole working sample excluding the inert matter or only the pure seed fraction, washing);

d) whether an authentic standard sample or a standard reference was used; if a standard reference was used, its origin must be indicated;

e) the number of seeds, seedlings or plants examined. When it is difficult to determine the total number of plants examined in field plots, the mass of seed sown must be reported.

1.5.2.10.1 Reporting results of verification of species and variety

1.5.2.10.1.1 Results of examination of individual seeds or seedlings

Suggested phrases for reporting divergent seeds or seedlings depending upon the result are as follows:

a) if none was found: "The test performed revealed nothing to indicate that the species (and/or variety) stated by the applicant is incorrect."

b) if non-conforming seeds were found: "Out of … seeds examined, … seeds do not conform to the authentic standard sample of the species (and/or variety) stated by the applicant."

c) if non-conforming seedlings were found: "Out of .... seeds producing normal seedlings, …% do not conform to the authentic standard sample of the species (and/or variety) stated by the applicant."

d) if the total working sample was found to be of a species and/or variety other than that stated by the applicant: "The sample does not conform to the authentic standard sample of the species (and/or variety) stated by the applicant."

1.5.2.10.1.2 Results of a field plot examination

The results must, whenever possible, be reported as a percentage of each other species, other variety or aberrant found. When the expression of the result as a percentage is not possible, appropriate comments regarding the conformity of the sample may be reported.

If nothing worthy of special comment was found the following statement is suggested: "The results of a field plot examination of this sample revealed nothing to indicate that the species (and/or variety) stated by the sender is (are) incorrect."

1.5.2.10.1.3 Reporting probabilities of meeting specifications

The result may be reported as: "On the basis of the traits tested, the seed lot meets the specification of …% minimum species (or variety) purity with …% confidence."

1.5.2.10.2 Reporting test results of presence of specified traits

1.5.2.10.2.1 Qualitative test results

Suggested phrases for reporting depending upon the result are as follows:

a) if the specified trait was not found: "The test performed revealed nothing to indicate the presence of the trait specified by the applicant."

b) if the specified trait was found: "The presence of the trait specified by the applicant was detected."

The limit of detection of the method used should be provided.

1.5.2.10.2.2 Quantitative results obtained by multiple qualitative tests of individual or bulks of seeds or seedlings

Results may be reported as the percentage of seeds or seedlings showing the trait specified by the applicant. Suggested phrases for reporting depending upon the result are as follows:

a) if none was found: "The test performed revealed nothing to indicate the presence of the trait specified by the applicant."

b) if seeds showing the trait were found: "Out of .... seeds examined .... seeds showed the trait specified by the applicant."

c) if seedlings showing the trait were found: "Out of .... seeds producing normal seedlings, …. % showed the trait specified by the applicant."

1.5.2.10.2.3 Quantitative measurements of traits in bulk samples

Units may be percent seeds by number, percent seeds by mass, percent by protein, percent by number of DNA copies, or any other determinant by percent. The limit of detection (LOD) and the limit of quantification (LOQ) of the method used, the testing plan (e.g. number of replicate seed samples, number of replicate flour samples per seed sample, number of extracts per flour sample, number of replicate measurements per extract) and the standard deviation of the test result should be provided.

Suggested phrases for reporting depending upon the result are as follows:

a) if the specified trait was not found: "The test performed revealed nothing to indicate the presence of the trait specified by the applicant at a level above LOD."

b) if the specified trait was found at a level above LOD and below LOQ: "The trait specified by the applicant was present at a level below the LOQ of the method used."

c) if the specified trait was found at a level above LOQ: "The trait specified by the applicant was found at a percentage of … % [units]." (where [units] are the units of measurement of the test used)

1.5.2.10.2.4 Reporting probabilities of meeting or exceeding specifications

Independent of the type of method used, a suggested phrase for reporting the results is: "For the trait specified by the applicant, the seed lot meets the specification of …% (maximum or minimum) with …% confidence."

1.5.2.11 Moisture content (also 9.1.7, 9.2.2.7)

This Rule is applicable to both the oven method (9.1.7) and the moisture meter method (9.2.2.7).

The result of a moisture content test must be reported in the space provided to the nearest 0.1%.

The method must be reported (duration and temperature).

The following additional information must also be reported under 'Other Determinations':

– If germinating seeds were present in the sample, the following statement must be entered: 'Germinating seeds were found in the submitted moisture sample.'

– If mouldy seeds were present in the sample, the following statement must be entered: 'Mouldy seeds were found in the submitted moisture sample.'

– In the case of pelleted seeds (see Chapter 11), the following statement must be entered: 'The seeds of the submitted moisture sample were pelleted, and the moisture content reported is the average of seed and pelleting materials.'

Note: 1.5.2.12 will be amended and expanded if Proposal C.10.1 is accepted.

1.5.2.12 Weight determination (also 10.7)

The result of a weight determination test must be reported under 'Other determinations' to the number of decimal places used in the determination (10.5.3).

1.5.2.13 Excised embryo (also 12.7)

The result of an excised embryo test must be reported under 'Other determinations' as follows:

'Excised embryo test: ……% of seeds had viable embryos'

Further details may be given at the discretion of the seed testing station, e.g. percentages of seeds that were empty, insect-damaged or physically damaged.

1.5.2.14 Weighed replicates (also 13.7)

The result of a weighed replicates test must be reported in the space provided as follows:

– The result of the purity test (if requested), in the spaces provided for purity tests.

– 'N' must be entered in all the spaces provided for reporting the percentages of the components of the germination tests.

The following additional information must also be reported under 'Other determinations':

– average weight of four replicates;

– average number of normal seedlings in four replicates;

– number of normal seedlings per kilogram;

– other information as specified in 1.5.2.6 Germination;

Upon request, other seeds found to be present in the weighed replicates may be reported, giving the scientific name(s) and number(s) of seeds found.

1.5.2.15 X-ray test (also 14.7)

The results of an X-ray test must be reported under 'Other determinations' as

percentages of filled, empty, insect-damaged or physically damaged seeds, as follows:

'X-ray test results:

……% filled

……% empty

……% insect-damaged

……% physically damaged'.

1.5.2.16 Seed vigour test

1.5.2.16.1 Conductivity test (also 15.8.1.8)

The result of a seed vigour test using the conductivity test method must be reported under 'Other determinations' as follows:

– The result must be expressed in µS cm^{-1} g^{-1} to the nearest 0.1 µS cm^{-1} g^{-1}.

– The seed moisture content before the test must be reported. Where the moisture content has been adjusted before the test, both the initial moisture content and the calculated moisture content after adjustment must be reported.

– The results must be accompanied by a statement of the specific variables used in the test (soaking time and temperature)

1.5.2.16.2 Accelerated ageing test (also 15.8.2.8)

The result of a seed vigour test using the accelerated aging (AA) method must be reported under 'Other determinations' as follows:

– Results are expressed as a percentage, calculated to the nearest whole number (5.8.1) of normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds. If the result for any of these categories is found to be zero, it must be reported as '0'.

– The seed moisture content before the test must be reported. Where the moisture content has been adjusted before the test, both the initial moisture content and the calculated moisture content after adjustment must be reported.

– The results must be accompanied by a statement of the specific variables used in the test (seed weight per AA box both before and after ageing, ageing time and temperature)

1.5.2.16.3 Controlled deterioration test (15.8.3.6)

The result of a seed vigour test using the controlled deterioration test method must be reported under 'Other determinations' as follows:

– Results are expressed as a percentage, calculated to the nearest whole number (5.8.1), of total germination (normal plus abnormal seedlings) and normal germination. If the result for either of these is found to be zero, it must be reported as '0'.

The results must be accompanied by a statement of the specific variables used in the test (raised seed moisture content, deterioration period and temperature)

1.5.2.18 Size and grading of seeds (also Chapter 16)

The result of a screening analysis test for size and grading of seeds must be reported under 'Other determinations' as the average of two screening analyses falling within the permitted tolerance limits.

1.5.2.19 Weighted average test for herbage and amenity seed lots transported loose in bulk containers (also 17.6)

The result of a weighted average test performed on herbage and amenity seeds lots, as described in Chapter 17, must be reported in the normal way, except that:

a) across the date of sampling, date sample received, date test concluded and test number boxes insert the statement:

'Seed loose in bulk container(s) – see under Other determinations.'

b) Under 'Other determinations', list the test number, date of sampling and date test concluded of all constituent lots together with the statement:

'The test results reported represent the weighted average of the results reported on these certificates which were not significantly different from each other.'

1.5.2.20 Reporting of results of tests not covered by the Rules

Results must be reported under 'Other determinations'. The test method must be reported and followed by:

"(this method is not covered by the *International Rules for Seed Testing*)."

1.5.3 Reporting of uncertainty of measurement on ISTA Certificates

…

1.5.4 Statement referring to compliance with legislative requirements

…

C.1.2. Requirement for moisture-proof containers for moisture testing

The view of the Moisture Committee is that it does not make practical sense to submit a sample for moisture testing in a container that is not moisture proof. If a sample is received for a moisture test in a container that is not moisture proof, the result of the moisture test would generally not represent the moisture of the seed lot, and could be misleading to the customer**.** This issue has also been raised by an ISTA laboratory as a question to the Moisture Committee.

The proposal is that a new condition d) be inserted into 1.3 to require that samples submitted for moisture determination for both an Orange International Seed Lot Certificate and a Blue International Seed Sample Certificate be in a moisture-proof container. "Moisture-proof" being estimated by the receiving laboratory based on own experiences or on evidence submitted by the sender.

CURRENT VERSION	PROPOSED VERSION
1.3 Conditions for issuance of ISTA Certificates … d) To report results of tests …	**1.3 Conditions for issuance of ISTA Certificates** … d) For the result of a determination of moisture content to be ~~carried out and the result~~ reported on an ISTA Certificate, the sample must be submitted in an intact, moisture-proof container from which as much air as possible has been excluded (see 9.1.5.1). e) To report results of tests …

C.1.4. Requirement for reporting the name of the sampling laboratory

It has been reported via the ISTA auditors that there is some confusion about when to report the sampling laboratory details on the ISTA Orange International Seed Lot Certificates. This proposal is aimed at correcting that by simply always requiring the name and member code of the sampling laboratory even if it is the same as the issuing laboratory.

CURRENT VERSION	PROPOSED VERSION
1.4.2 Orange International Seed Lot Certificate … b) name and ISTA member code of laboratory responsible for sampling~~, if different from the issuing laboratory~~; …	**1.4.2 Orange International Seed Lot Certificate** … b) name and ISTA member code of laboratory responsible for sampling; …

Chapter 2: Sampling

C.2.1. Size of submitted sample for *Nicotiana tabacum*

The submitted sample size for *Nicotiana tabacum* is currently 25 g and it is suggested that it should be 5 g. Seeds of *Nicotiana tabacum* are very small. There are more than 15 600 seeds per gram. The size of the working sample for purity analysis is 0.5 g and the size of the working sample for count of other species is 5 g, ten times the size of the working sample for the purity weight. For agricultural species, the submitted sample size needs to be large enough to allow for determination of other species by number based on 10 times the purity weight. Accordingly, the Bulking and Sampling Committee proposes that the submitted sample size is decreased to 5 g.

CURRENT VERSION

Species	Maximum weight of lot	Minimum sample weights		
		Submitted sample	Working sample for purity analysis	Working sample for count of other species
	kg	g	g	g
Nicotiana tabacum L.	10 000	~~25~~	0.5	5

PROPOSED VERSION

Species	Maximum weight of lot	Minimum sample weights		
		Submitted sample	Working sample for purity analysis	Working sample for count of other species
	kg	g	g	g
Nicotiana tabacum L.	10 000	5	0.5	5

C.2.2. Addition of spiral-slot sampling stick without compartments and cargo sampler

There is a need to validate other sampling equipment not currently included as approved in the ISTA Rules. A spiral-slot sampling stick and a cargo sampler were evaluated by members of the Bulking and Sampling Committee.

This Rules proposal could be withdrawn if it has not been approved by the Bulking and Sampling Committee or the validation report is not available for discussion at the Zurich meeting.

Note: the validations have only been completed for seed smaller than cereals, i.e. grasses and clovers.

CURRENT VERSION	PROPOSED VERSION
2.5.1.3 Taking primary samples … *c) Sampling stick (synonym: stick trier, sleeve type trier).* The sampling stick consists of an inner tube which fits loosely inside an outer tube but tightly enough so that seed or impurities do not slip between them. The outer tube has a solid pointed end. Both tubes have slots cut into their walls so that the cavity of the inner tube can be opened and closed by twisting the tubes against each other. The sampling stick may be used horizontally, diagonally or vertically. However, when used vertically the sampling stick must have partitions dividing the instrument into a number of compartments**.** The minimum …	*2.5.1.3 Taking primary samples* … *c) Sampling stick (~~synonym~~: e.g. stick trier, sleeve type trie, spiral trier).* The sampling stick consists of an inner tube which fits loosely inside an outer tube, but tightly enough so that seed or impurities do not slip between them. The outer tube has a solid pointed end. Both tubes have slots cut into their walls so that the cavity of the inner tube can be opened and closed by twisting the tubes against each other. The sampling stick may be used horizontally, diagonally or vertically. However, when used vertically the sampling stick must have partitions dividing the instrument into a number of compartments. For seeds of a size smaller than *Triticum aestivum*, a sampling stick with a spiral arrangement of slots and no compartments may also be used vertically. The minimum … **NEW editorially changed version** The spiral trier has slots in a spiral arrangement for their subsequent opening from the tip to the handle and may only be used for seeds of a size smaller than *Triticum aestivum* However, when used vertically the sampling stick must either have partitions dividing the instrument into a number of compartments or have slots in a spiral arrangement. The minimum …

d) Nobbe trier. …	*d) Nobbe trier.* …
	e) Cargo sampler (bulk sampler). The cargo sampler can be used for seeds smaller than seeds of *Triticum aestivum*. The cargo sampler consists of a special type of chamber that is fixed to a shaft. The lower part of the chamber is cone-shaped with a pointed end. To reach a greater depth, the shaft may be lengthened by screwing on successive extensions. There is a closing system in the chamber that may be a collar on the outside of the instrument, a wing connected to a door or a valve with a spring. Some cargo samplers can be closed before they are drawn back from the sampling position; others cannot be closed, so that the filled chamber is open during withdrawal. For all species, the minimum inside diameter can be about 35 mm and the depth 75 mm. When using the cargo sampler, insert it in the closed position into the container, gently push it vertically into the seed so that the point reaches the required position, pull the cargo sampler back about 10 cm or turn it (depending on the closing system), agitate it slightly to allow it to fill completely, gently close if possible and withdraw it and empty the primary sample into a container. Care should be exercised in closing the cargo sampler, so that the seeds are not damaged.
e) Sampling by hand. This method…	*f) Sampling by hand.* This method …

Chapter 3: The Purity Analysis

C.3.1. Reporting purity sample weight

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

CURRENT VERSION	PROPOSED VERSION
3.7 Reporting results	**3.7 Reporting results** When the weight of the working sample tested for purity equals or is no more than 10% higher than the weight specified in Table 2A, column 4 (Purity analysis), no statement regarding the weight of the working sample is required on the ISTA Certificate.
When the weight of the working sample tested for purity deviates from that ~~prescribed~~ in Table 2A, column 4, the actual weight ~~examined~~ must be reported on the certificate. …	When the weight of the working sample tested for purity deviates from that specified in Table 2A, column 4, the actual weight of the working sample weighed according to 3.5.1 must be reported on the ISTA Certificate using one of the following, as applicable: a) When testing a weight that exceeds by 10% the weight specified in Table 2A, column 4, report under other determinations as: ~~'Weight of purity working sample examined = ……g'~~ 'Purity: ……g' b) When testing a weight estimated to contain 2500 seed units, report under other determinations as: ~~'Weight of purity working sample examined = ……g (estimated to contain 2500 seeds)'~~ 'Purity: ……g (approx. 2500 seeds)' c) When the submitted sample received for purity testing weighs less than the weight in Table 2A, column 4, report under other determinations and use the current statement, according to 2.5.4.4: 'The submitted sample weighed only … g and is not in accordance with the *International Rules for Seed Testing.'* …

Chapter 5: The Germination Test

C.5.1. Top of paper covered with sand (TPS) method for *Glycine max, Helianthus annuus, Phaseolus vulgaris* and *Zea mays*

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

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

Table 5A Part 1 Agricultural and vegetable seeds: modified entries for *Glycine max, Helianthus annuus, Phaseolus vulgaris* and *Zea mays*

PROPOSED VERSION

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature (°C)	First count (d)	Final count (d)	
1	2	3	4	5	6
Glycine max	BP; TPS; S	20–30; 25	5	8	–
Helianthus annuus	BP; TPS; S; O	20–30; 25; 20	4	10	Preheat; prechill
Phaseolus vulgaris	BP; TPS; S	20–30; 25; 20	5	9	–
Zea mays	BP; TPS; S	20–30; 25; 20	4	7	–

C.5.2. Between Paper (BP) method for *Brassica* spp. and *Sinapis alba*

The comparative tests have been completed and a validation report submitted. This report has been approved, with minor revisions recommended by the Technical Reviewers. The proposal is supported by the Germination Committee.

In Table 5A Part 1 under column 2 ‘substrate’, ‘BP’ should be added in addition to the substrates already listed there for the following species:

Brassica napus; Brassica napus var *napobrassica; Brassica nigra; Brassica oleracea; Brassica perviridis; Brassica rapa* and *Sinapis alba.*

Table 5A Part 1 Agricultural and vegetable seeds: modified entries for *Brassica* spp. and *Sinapis alba*

PROPOSED VERSION

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature (°C)	First count (d)	Final count (d)	
1	2	3	4	5	6
Brassica napus	BP; TP	20–30; 20	5	7	Prechill; KNO_3
Brassica napus var. *napobrassica*	BP; TP	20–30; 20	5	14	Prechill
Brassica nigra	BP; TP	20–30; 20	5	10	Prechill; KNO_3
Brassica oleracea	BP; TP	20–30; 20	5	10	Prechill; KNO_3
Brassica perviridis	BP; TP	20–30; 20	5	7	Prechill
Brassica rapa	BP; TP	20–30; 20	5	7	Prechill; KNO_3
Sinapis alba	BP; TP	20–30; 20	3	7	Prechill

C.5.3. Organic Growing Media (O) method for *Vicia faba*

The comparative tests have been completed, and a preliminary analysis of the results supports the use of organic growing media for *Vicia faba* germinations. A validation report has been completed and approved. The proposal originates from and is supported by the Germination Committee.

In Table 5A Part 1, under column 2 'substrate', '**O'** should be added in addition to the substrates already listed there for *Vicia faba*.

Table 5A Part 1 Agricultural and vegetable seeds: modified entries for *Vicia faba*

PROPOSED VERSION

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature (°C)	First count (d)	Final count (d)	
1	2	3	4	5	6
Vicia faba	BP; S; O	20	4	14	Prechill

Chapter 7: Seed Health Testing

C.7.1. Addition of new method. 7-026: Detection of Squash Mosaic Virus, Cucumber Green Mottle Mosaic Virus and Melon Necrotic Spot Virus in Cucurbits

Crop: Cucurbits.

Pathogen: Squash Mosaic Virus (SqMV), Cucumber Green Mottle Mosaic Virus (CGMMV) and Melon Necrotic Spot Virus (MNSV)

Prepared by: Koenraadt, H.M.S. and Remeeus, P.M.

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Revision History: Version 1.0, 1 September 2007.

Background

SqMV, CGMMV and MNSV are seed-transmissible viruses of cucurbits, and therefore the detection of these viruses in seeds of cucurbits is an important tool in control strategies. Enzyme-linked immunosorbent assay (ELISA) is widely used for the detection of plant viruses (Clark and Adams, 1977). ELISA methods have also been described for the detection of PEBV and PSbMV (Hamilton and Nichols, 1978; Van Vuurde and Maat, 1985, Maury *et al.*, 1987).

The method, using ground seed and a DAS-ELISA, can be used to simultaneously detect SqMV, CGMMV and MNSV in a single extract. Note that the extract is tested in three microtiter plates, one each for SqMV, CGMMV and MNSV. The theoretical detection limit is one infested seed in 100 seeds. To ensure a 95% probability that infestations of 0.15% or higher are detected it is necessary to test 20 subsamples of 100 seeds each. ELISA positive seed lots will not necessarily lead to seed transmission. Seed transmission of these viruses can be monitored in a grow-out, but this technique is time consuming and rather laborious.

Validation studies

Koenraadt, H.M.S. and Remeeus, P.M. (2007)

Copies are available by E-mail from ista.office@ista.ch; by mail from the ISTA Secretariat.

Please send comments, suggestions or reports of problems relating to this method to the ISTA Seed Health Committee, c/o ISTA Secretariat.

International Seed Testing Association (ISTA)

Zürichstr.50

CH-8303 Bassersdorf

Switzerland

Safety precautions

Make sure you are familiar with the hazardous nature of the materials being used and take appropriate safety precautions, especially during preparation of buffers, grinding, autoclaving, and weighing out of ingredients. It is assumed that persons carrying out this procedure are in a microbiological laboratory and are familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic technique. Dispose of all waste materials in an appropriate way (e.g. autoclave, disinfect) and in accordance with local health, environmental and safety regulations.

Treated seed

Dry heat is often used for the control of CGMMV in contaminated seed lots. ELISA does not discriminate between infectious and non-infectious CGMMV, and a positive reaction in this test may cause a non CGMMV-infected seed lot to be unnecessarily discarded.

This method has not been validated for the determination of SqMV, CGMMV or MNSV in seed treated with crop protection products or with heat. Although ELISA is compatible with some seed treatment chemicals (Pataky *et al.*, 2004), seed treatments may affect the performance of this test. This method must only be performed on untreated seed.

Sample and subsample size

The sample (total number of seeds tested) and subsample size to be tested depend on the desired tolerance standard (maximum acceptable number of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). In any case the subsample size should not exceed 100 seeds.

Materials

Reference material: SqMV-, CGMMV- and MNSV-infested seeds or standardized reference material (flour of seeds containing SqMV, CGMMV and MNSV)

Microtiter plates: 96-well plates suitable for ELISA (CCP)

Antisera: suitable for detection of SqMV-, CGMMV- and MNSV-infested seeds (e.g. PRI, Wageningen, the Netherlands)

Balance: capable of weighing to the nearest 0.01 g

pH meter: capable of being read to the nearest 0.1 pH unit

Automatic pipettes: capable of pipetting to the nearest 0.001 mL

Grinder: capable of grinding seeds to fine flour (e.g. Retsch Grindomix GM 200)

Incubator: capable of maintaining a temperature of 4 ± 2 °C

Incubator: capable of maintaining a temperature of 37 ± 2 °C

ELISA plate reader

Tubes: 10 ml (LDPE)

Vortex: suitable for vortexing 10 ml tubes

Sample preparation

This can be done in advance of the assay.

It is vital to exclude any possibility of cross-contamination between seed samples. It is therefore essential to clean all equipment, surfaces, containers, hands, etc. both before and after handling each sample.

Count the number of seeds in a known weight. Calculate the estimated thousand-seed weight as:

$$\textit{Estimated thousand-seed weight} = \frac{\textit{Wt of seed}}{\textit{No of seed}} \times 1000$$

Based on the estimated thousand-seed weight, weigh out sub-samples of the required size into new and clean bags or containers.

Method

[Critical control points are indicated by CCP]

1. Coating of ELISA plates

1.1. Add appropriate (as defined by supplier) dilution of SqMV, CGMMV and MNSV-coating serum to coating buffer to obtain coating solution. Be sure that the antisera are

not only suitable for diagnostics but also for the detection of viruses in seed extracts (CCP).

1.2. Coat one plate with 100 µl of SqMV-coating solution per well. Coat a second plate with 100 µL of CGMMV-coating solution per well. Coat a third plate with 100 µL of MNSV-coating solution per well.

1.3. Cover ELISA plates with lid or wrap with plastic to minimize evaporation.

1.4. Incubate plates overnight at 4 ± 2 °C or as defined by the supplier.

2. Extraction of virus from the seed and incubation of extracts

2.1. Count or weigh 20 x 100 seeds per subsample.

2.2. Grind each subsample to fine flour in a grinder (CCP).

2.3. From each subsample, weigh out 0.5 g of flour and transfer to a 10 mL tube.

2.4. Add 5 mL of extraction buffer to each tube.

2.5. Vortex each tube for 15 s. Allow extract to settle for at least 5 min on the bench to facilitate pipetting.

2.6. Remove coating solution from ELISA plates and immediately rinse plates thoroughly, three times, using PBS/Tween 20 to remove residues (CCP).

2.7. Immediately after rinsing, pipette 100 µL of each seed extract into a well. Use two wells per subsample.

2.8. Add positive and negative controls to each ELISA plate. Use at least two dilutions for the positive control: one “low” dilution that gives a high extinction and a “high” dilution that gives an extinction just above the detection threshold (CCP). Negative controls must include a healthy seeds extract.

2.9. Cover plates with lid or wrap with plastic to minimize evaporation and incubate overnight at 4 ± 2 °C or as defined by the supplier.

3. Incubation of conjugate

3.1. Prepare appropriate dilution of SqMV-, CGMMV- and MNSV-conjugated antiserum using conjugate buffer as defined by the supplier.

3.2. Remove seed extracts from ELISA plates and rinse plates three times with washing buffer PBS/Tween 20 to remove residues of seed extract (CCP).

3.3. Immediately after rinsing, add 100 µL of diluted conjugate to each well of the ELISA plate.

3.4. Cover plates with lid or wrap with plastic to minimize evaporation and incubate for 3 h at 37 ± 2 °C or as defined by the supplier.

4. Addition of substrate to ELISA plates

4.1. Prepare substrate solution (10 mg para-nitrophenyl phosphate in 20 mL of substrate buffer).

4.2. Remove conjugate from ELISA plates and rinse thoroughly 3 times by hand using washing buffer PBS/Tween 20. Alternatively use a reliable washing device (CCP).

4.3. Add 100 µL of substrate solution to each well.

4.4. Incubate in the dark for 2 h at 20 ± 2 °C or as defined by the supplier.

4.5. Measure extinction value (A_{405}) with ELISA plate reader. (See General Methods, point 2.)

General methods (common to many test procedures)

1. Grinding seeds

Grind each sub sample of 100 seeds to give a fine flour. Be sure to use a grinder that can be cleaned thoroughly since cross-contamination is likely during the grinding step.

2. Recording of ELISA extinction

Record the results for all wells in the microtiter plate. Check first whether the positive and negative controls meet the expectations since otherwise the results of the test are invalid and the test must be repeated.

It is recommended to use a negative-positive threshold of 2.5 times the background of healthy samples.

3. Reporting results

The result of a seed health test should indicate the scientific name of the pathogen and the test method used. When reported on an ISTA Certificate, results are entered under *Other Determinations*.

In the case of a negative result (pathogen not detected in any of the subsamples), the results should be reported in terms of the tolerance standard and detection limit. The tolerance standard depends on the total number of seeds tested, n, and is approximately 3/n (P=0.95) (Roberts *et al.,* 1993);

In the case of a positive result, the report should indicate the number of positive subsamples out of the total number tested and the sample size or the maximum likelihood estimate of the proportion of infested seeds.

Quality assurance

Critical Control Points [Identified by CCP in the methods]

Using different types of microtiter plates can influence sensitivity.

The quality of antisera from different sources is known to be variable. Therefore, be sure that the antisera are not only suitable for diagnostics but also for the detection of viruses in seed extracts. Step 1.1.

The use of fine flour will improve the efficacy of extraction. Therefore, grind seeds for 20 s at 10 000 rpm to get a fine flour. Note that some blades easily get blunt and therefore grind less efficiently in time. Step 2.2.

Coated microtiter plates will lose activity rapidly when they are left to dry on the bench for some time. Therefore, limit the time as much as possible that empty microtiter plates are left on the bench. Step 2.6.

The use of appropriate positive and negative controls is very important to validate the result. Be sure that, apart from a “high” positive control, there is always a “low” positive control in each plate. Step 2.8.

Poorly washed microtiter plates between the different incubation steps often cause high backgrounds in ELISA. Washing can be done by hand using PBS/Tween 20 or with a washing device. Thoroughly washing microtiter plates is very critical in several steps (2.6, 3.2 and 4.2) in the ELISA, particularly after the incubation with the conjugated antiserum. Step 4.2.

Coating buffer (pH 9.6)

Na_2CO_3: 1.59 g

$NaHCO_3$: 2.93 g

Extraction buffer (0.05 M, pH 7.4)

NaCl: 8.0 g

KH_2PO_4: 1.0 g

$Na_2HPO_4.12H_2O$: 14.5 g

Ovalbumine (Grade II): 2.0 g

Tween 20: 10.0 mL

PVP (ELISA grade, mol. wt. 10 000): 20.0 g

Conjugate buffer (0.05 M, pH 7.4)

NaCl: 8.0 g

KH_2PO_4: 1.0 g

$Na_2HPO_4.12H_2O$: 14.5 g

Tween 20: 0.5 mL

PVP (ELISA grade, mol. wt. 10 000): 20.0 g

BSA (ELISA grade, e.g. BSA fraction 5): 5.0 g

Substrate buffer (pH 9.6)

Diethanolamine: 97 mL

HCl (32%): 15 mL

Washing buffer PBS/Tween 20 (0.05 M, pH 7.4)

NaCl: 8.0 g

KH_2PO_4: 1.0 g

$Na_2HPO_4.12H_2O$: 14.5 g

Tween 20: 1.5 mL

Preparation of individual buffers

1. Weigh or measure out all ingredients into a suitable container.

2. Dissolve/mix ingredients and adjust volume to 1000 mL with distilled/de-ionized water.

3. Check the pH with a pH meter and adjust if necessary.

Storage of buffers

Store buffers as mentioned above at 4 ± 2 °C. Use them within a month after preparation.

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Chapter 9: Determination of Moisture Content

C.9.1. Change to 9.1.6 Calculation and expression of results

If the rounding is done to early samples that should have been retested are not and some samples that do not require retesting are retested.

This proposal is submitted and supported by the Moisture Committee.

CURRENT VERSION	PROPOSED VERSION
9.1.6.1 Constant-temperature oven methods The moisture content as a percentage by weight must be calculated to ~~one~~ decimal ~~place~~ by means of the following formula: …	*9.1.6.1 Constant-temperature oven methods* The moisture content as a percentage by weight must be calculated to three decimal places for each replicate by means of the following formula: …
9.1.6.2 Tolerances ~~Report the result as the arithmetic mean of the duplicate determinations carried out on a sample if the difference between the two determinations does not exceed the 0.2% tolerance.~~ For tree and shrub…	*9.1.6.2 Tolerances* The difference must be calculated to three decimal places and then rounded off to one decimal place. The maximum difference between the two replicates must not exceed 0.2% after rounding from three to one decimal place. Otherwise, repeat the determination in duplicate. The reported result is the arithmetic mean of the results for two working samples (see 9.1.7). For tree and shrub….

C.9.2. Change to 9.1.4.3 Containers

The lid is present only to prevent contamination of the sample from other material and the time taken to fit a tight lid on a large number of containers may introduce errors into the moisture determination.

This proposal is submitted and supported by the Moisture Committee.

CURRENT VERSION	PROPOSED VERSION
9.1.4.3 Containers ….this, glass dishes, with ~~sufficiently tight fitting~~ lids, and an …	*9.1.4.3 Containers* ….this, glass dishes, with lids, and an …

C.9.3. Addition to 9.1.5.1 General Directions and Precautions

Some laboratories are not retaining moisture samples after moisture determination. This does not allow for re-testing in cases where errors are detected, in response to complaints or for other purposes. This has been recorded as a non-conformity for laboratories during audits and the requirement in ISTA Accreditation Standard that samples be stored for one year is not appropriate for moisture samples.

The Moisture Committee has discussed this problem and recommends a Rule change proposal.

The Rule change proposal is the following paragraph be added to the end as a fifth paragraph of 9.1.5.1.

This proposal is submitted and supported by the Moisture Committee.

PROPOSED VERSION	PROPOSED VERSION
9.1.5.1 General directions and precautions …	*9.1.5.1 General directions and precautions* … The remaining submitted sample after determination of moisture must be stored under controlled conditions in a moisture proof container for a period defined by the laboratory, but long enough to ensure the possibility for re-testing in case of errors.

C.9.4. Clarify 9.1.5.6 Predrying

After predrying it is not clear how the working sample is drawn for the moisture determination.

CURRENT VERSION	PROPOSED VERSION
9.1.5.6 Predrying …in a warm place. After predrying, the subsamples are reweighed in their containers to determine the loss in weight. Immediately thereafter the two partly dried subsamples are separately ground ~~and t~~he moisture determined as prescribed in 9.1.5.3.	*9.1.5.6 Predrying* …in a warm place. ((New paragraph)) After predrying, the subsamples are reweighed in their containers to determine the loss in weight. Immediately thereafter the two partly dried subsamples are separately ground. One working sample is drawn from each subsample. Drawing of the working sample should be in accordance with 9.1.5.2. The moisture is determined as prescribed in 9.1.5.3.

Chapter 10: Weight Determination

C.10.1. Reporting method used for weight determination

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

CURRENT VERSION	PROPOSED VERSION
10.7 Reporting results The result must be reported under 'Other Determinations' on an *ISTA International Seed Analysis Certificate* ~~as calculated according to 10.6~~.	**10.7 Reporting results** The method used ('Counting the entire working sample' or 'Counting replicates') and the result as calculated according to 10.6 must be reported under 'Other Determinations'.

Chapter 15: Seed Vigour Testing

C.15.1. *Phaseolus vulgaris* validated for conductivity test

The conductivity test is currently validated for *Pisum sativum*. This test also identifies differences in seed vigour in *Phaseolus vulgaris* and comparative tests have shown that the method is repeatable and reproducible (see Method Validation Report). *Phaseolus vulgaris* is therefore proposed as a second species to which the conductivity test can be applied

CURRENT VERSION	PROPOSED VERSION
15.8.1 Conductivity test ~~for Pisum sativum~~	***15.8.1 Conductivity test***
15.8.1.1 Principle ... Conductivity measurement of the soak water in which a bulk sample of ~~pea~~ seeds has been steeped…	*15.8.1.1 Principle* … Conductivity measurement of the soak water in which a bulk sample of seeds has been steeped…
15.8.1.2 Scope and field of application The conductivity test offers a vigour test for *Pisum sativum* which relates to the field emergence of seed lots. The test does not apply to the so-called 'petit pois' varieties.	*15.8.1.2 Scope and field of application* The conductivity test offers a vigour test for *Pisum sativum* (Garden peas only) and *Phaseolus vulgaris* 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 ~~*Pisum sativum*~~. Garden peas.
15.8.1.4 Preparation of the sample before measuring conductivity … Experience indicates that to raise the moisture content of ~~pea~~ seeds ~~having~~ an initial moisture content…..	*15.8.1.4 Preparation of the sample before measuring conductivity* … Experience indicates that to raise the moisture content of seeds with an initial moisture content…..

C.15.2. Calculation and expression of results in Sections 15.8.1.7 and 15.8.2.7

These sections do not currently provide guidelines on how to report results following a re-test when the data for the original replicates of the test were out of tolerance. This proposal to report the average of the two tests follows the same approach as that used when germination has been re-tested.

CURRENT VERSION	PROPOSED VERSION
15.8.1.7 Calculation and expression of results … If the mean conductivity of the four replicates differs by more than the tolerance value (see Table 15B) for that conductivity, the lot ~~should~~ be re-tested. …	*15.8.1.7 Calculation and expression of results* … If the mean conductivity of the four replicates differs by more than the tolerance value (see Table 15B) for that conductivity, the lot must be re-tested. If the second result is compatible with the first (i.e. the difference does not exceed the tolerance indicated in Table 15C), the average of the two tests must be reported. …
15.8.2.7 Calculation and expression of results … If the two 100 seed replicates differ by more than the maximum tolerance value for AA germination shown in Table 15E, the seed lot ~~should~~ be re-tested. …	*15.8.2.7 Calculation and expression of results* … If the two 100-seed replicates differ by more than the maximum tolerance value for AA germination shown in Table 15E, the seed lot must be re-tested. If the second result is compatible with the first (i.e. the difference does not exceed the tolerance indicated in Table 15F), the average of the two tests must be reported. …

C.15.3. New validated vigour test: controlled deterioration (CD) test for *Brassica* spp.

There is currently no vigour test in the ISTA Rules for any small seeded vegetable species. The results of the controlled deterioration (CD) test have been related to the emergence (in the field and glasshouse) and storage potential of a number of vegetable species. A series of comparative tests (see Method Validation report) has established the repeatability and reproducibility of the CD test as applied to *Brassica* species. The controlled-deterioration test is therefore proposed as an addition to Chapter 15: Seed Vigour Testing.

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

PROPOSED VERSION

15.8.3 Controlled deterioration test for* Brassica *spp.

15.8.3.1 Principle

The controlled deterioration (CD) test exposes seeds to a high temperature while at a specified and constant raised seed moisture content. These conditions cause seeds to deteriorate, or age, rapidly. The moisture content of a seed sample is raised before the seeds are placed at the raised temperature, thus ensuring that all samples tested are exposed to a predetermined degree of deterioration during the test. High vigour seeds retain a high germination after deterioration, while the germination of low vigour seeds is reduced.

15.8.3.2 Scope

The CD test provides a vigour test for *Brassica* species which relates to both field emergence and storage potential. This test has not been validated on treated seed. Seed treatments may affect the performance of the method.

15.8.3.3 Apparatus

Water bath: this must have a temperature range to include 45 °C and be accurate to ±0.5 °C. Alternatively, an incubator giving the same degree of accuracy could be used. A water bath maintains the required temperature more uniformly when a number of tests are being conducted. If an incubator is used, care must be taken to ensure that there are no differences in temperature within it, especially when many tests are being conducted.

Analytical balance: capable of weighing to the nearsest 0.0001g.

Aluminium foil packets: suitable for holding 100 seeds in a single layer, with at least 3 cm space above the seeds after the packet is sealed. Packets approximately 5–6 cm deep and 7–10 cm wide are suitable. Packets must be impermeable to moisture once sealed. A range of packets are available, but example specifications are: paper (white kraft 60 g) covered by aluminium foil of 8 µm and polyethylene film of 40 µm.

Packet sealer: any instrument capable of producing a watertight seal to the foil packets is suitable.

Filter paper or germination paper: e.g. as used in the germination test.

Containers: to hold seeds and filter and germination papers during the procedure of raising the seed moisture content. A range of dishes or containers may be suitable, e.g. 9 cm Petri dishes, germination boxes.

Refrigerator or cooled incubator: capable of maintaining 7 ± 2 °C.

Germination test facilities: germination tests are conducted using the methods and test conditions described in Chapter 5 of the ISTA Rules.

Moisture content test facilities: moisture content tests are conducted according to

Chapter 9 of the ISTA Rules.

15.8.3.4 Controlled deterioration procedure

15.8.3.4.1 Raising and equilibration of seed moisture content

Determine the initial moisture content of the submitted sample according to Chapter 9 of the ISTA Rules. This is subsequently referred to as the initial seed moisture content. To adjust the seed moisture content, mix the fraction of pure seed thoroughly and draw randomly four replicates of at least 100 seeds. Weigh each replicate to four decimal places. Raise the seed moisture content of each replicate to 20%. The weight of seed at this moisture content is calculated as:

$$\text{Weight of replicate at 20\% mc} = (\text{initial seed weight}) \times \frac{(100 - \text{initial seed mc})}{(100 - \text{desired mc})^*}$$

*i.e. 80
mc = moisture content

Calculate the required weight to four decimal places. The acceptable required weight is then correct to three decimal places.

Place each of the four replicates to imbibe on a moist germination/filter paper, placed in a suitable container. There should be no free water on the surface of the paper. If 9 cm germination papers are used, 3–4 ml water per paper usually gives a moist but not wet paper. Use the same volume of water for a standard amount of paper on each test occasion.

Weigh seeds regularly to determine when they reach the required moisture content. Weighing must be accurate and correct to three decimal places. Seeds may begin to reach the required moisture content after 1.25–1.5 h depending on the seed lot, laboratory temperature and relative humidity.

Once seeds have reached the required weight, place each replicate immediately into an aluminium foil packet. The seeds can lose moisture rapidly at this stage, so speed is essential. Flatten the packets with the edge of the hand to remove air, and heat-seal the packets approximately 3 cm above the level of the seeds.

Place the sealed packets at 7 ± 2 °C for 24 h.

15.8.3.4.2 Deterioration of the seed

Place the four replicate packets of each seed lot into a water bath at 45 °C for 24 h ± 15 min. When the packets have been removed from the water bath, cool the seeds within the packets by placing the packets under cold running water for 5 min.

15.8.3.4.3 Testing for germination

A CD germination test should be set up using the deteriorated seed within 30 min of removing the seeds from the water bath. Set up the CD germination test using 100 seeds from each replicate packet. The seeds may be divided into subreplicates for the germination test. The germination conditions for a CD germination test are the same as those outlined for the standard germination test for *Brassica* spp. in Chapter 5 of the ISTA Rules.

15.8.3.5 Calculation and expression of results

The total germinated~~tion~~ percentage (normal plus abnormal seedlings) and percentage of normal seedlings are noted in each replicate. The result of the CD test is calculated as the average of the four 100-seed replicates, as described for the standard germination test in Chapter 5. Both the total germinated~~tion~~ percentage and the percentage of

normal seedlings are reported.

15.8.3.6 Reporting results

The result of a seed vigour test using the controlled deterioration test method must be reported under 'Other determinations' as follows:

– Results are expressed as a percentage, calculated to the nearest whole number (5.8.1), and stated as ~~of~~ 'Total germinated~~tion~~ (normal plus abnormal seedlings) ….%' and '~~n~~Normal ~~germination~~ seedlings….%'. If the result for either of these is found to be zero, it must be reported as '0'.

The results must be accompanied by a statement of the specific variables used in the test (raised seed moisture content, deterioration period and temperature)

Controlled deterioration vigour test

Total germinated seeds (normal plus abnormal seedlings) …….%

Normal seedlings …….%

C.15.4. Addition of a definition to clarify the unit of assessment used at the end of a controlled deterioration test

This proposal is required if proposal C.15.3 is accepted.

CURRENT VERSION	PROPOSED VERSION
15.2.4 Additional definitions **Seedling emergence:** … **Seedling performance:** …	*15.2.4 Additional definitions* **Seedling emergence:** … **Seedling performance:** … **Total germinated seeds ~~tion~~** **~~tion~~ ~~percentage~~:** the sum of the proportion of seedlings classified as normal and abnormal at the end of a controlled deterioration (CD) germination test conducted under the conditions and within the period specified in Chapter 5, Table 5A.

C.15.5. Change to list of validated tests; modification of Table 15A

CURRENT VERSION

Vigour test	~~**Crop**~~	**Species**
Conductivity	~~Garden pea~~	*Pisum sativum*
Accelerated ageing	~~Soya bean, soybean~~	*Glycine max*

PROPOSED VERSION

Vigour test	Species
Conductivity	*Pisum sativum* (Garden pea only, excluding petit-pois varieties) *Phaseolus vulgaris*
Accelerated ageing	*Glycine max*
Controlled deterioration	*Brassica* spp.