International Rules for Seed Testing 2016

Chapter 7: Seed health testing

Including changes and editorial corrections adopted at the Ordinary General Meeting 2015, Montevideo, Uruguay

Effective from 1 January 2016
Note on the use of the translations

The electronic version of the International Rules for Seed Testing includes the English, French and German versions. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.
Contents

Preface to the 2016 Edition of the ISTA Rules .......... v

Chapter 7: Seed health testing ..................................... 7-1
  7.1 Object ................................................................... 7-1
  7.2 Definitions ............................................................ 7-1
    7.2.1 Seed health ...................................................... 7-1
    7.2.2 Pretreatment ..................................................... 7-1
    7.2.3 Seed treatment ................................................. 7-1
    7.2.4 ISTA Seed Health Method Validation
      Programme ............................................................ 7-1
  7.3 General principles .................................................. 7-1
  7.4 Procedures ............................................................. 7-1
    7.4.1 Working sample .............................................. 7-1
    7.4.2 Seed treatment ............................................... 7-2
    7.4.3 Sample storage ................................................ 7-2
    7.4.4 Specific directions ........................................... 7-2
  7.5 Calculation and expression of results .................... 7-2
  7.6 Reporting results .................................................... 7-2
    Table 7A. ISTA official seed health testing
    methods .............................................................. 7-3
Preface to the 2016 Edition of the ISTA Rules

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

http://www.ingentaconnect.com/content/ista/rules

If required, users of the ISTA Rules can print their own copies. For further information on the ISTA Rules, see:

http://www.seedtest.org/rules

The electronic version includes the English, French and German versions of the ISTA Rules. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.

Seed health testing methods

Previously, the seed health testing methods were published as a separate Annex to Chapter 7 of the ISTA Rules. They are now available as separate method sheets from the ISTA web site at:

http://www.seedtest.org/seedhealthmethods

Details of changes

The 2016 changes are editorial corrections or Rules changes adopted at the Ordinary General Meeting held at Montevideo, Uruguay, in June 2015.

The changes in the text content from the previous edition of the ISTA Rules are listed below. They can be displayed as yellow highlighted text as a ‘layer’ within the electronic copy with comments on what has changed.

For the previous history of amendments to the ISTA Rules, see the Prefaces for 2003 to 2015 on the ISTA web site.

Dr. Steve Jones, ISTA Rules Committee Chair
Craig McGill, ISTA Rules Committee Vice-Chair
ISTA Secretariat

General:
– Cross-references checked and updated
– ‘ISTA International Seed Analysis Certificate’ changed to ‘ISTA Certificate’

Title page: English version is official version
Preface: English version is official version

Chapter 1: 1.2, 1.3, 1.4: Clarification about issuance of certificates and other details related to duplicate and provisional ISTA Certificates
1.4.2: Clarification what should be entered under ‘Seal of lot’
1.5.2.19: Removal of ‘herbage’, where differentiation between ‘herbage’ and ‘amenity’ (turf) is no longer appropriate

Chapter 2: 2.5.4.2.1: Lolium ×boucheanum corrected to Lolium ×hybridum
2.8: Changes in Table 2A for lot size and sample sizes
2.5.4.1, 2.5.4.2, Fig. 2.1: Removal of ‘herbage’, where differentiation between ‘herbage’ and ‘amenity’ (turf) is no longer appropriate

Chapter 3: PSD 4: Revision re: Helianthus seeds with fused pericarps
3.5.2: Retention of separated components (other seeds only)

Chapter 4: 4.2, 4.5.1: Amendment of definitions for Other seeds determinations

Chapter 5: 5.6.1: Germination tests allowed with only 200 seeds, but only for Blue International Seed Sample
Certificates; clarifications on required actions when counting errors occur
5.6.3.4: Clarification on reporting germination when disinfection is applied
5.6.4: Omitting the first count when germination tests are carried out in Organic Growing Media
5.7, 5.9: Amendment to the process for retesting Table 5A: Corymbia corrected to Corymbia; Pinus pence entry changed from (20/>30) to 20/>30; dash added for Lomnas annua

Chapter 7: 7-007: Correction to text
7-022: Changes to 5. Examination; replacement of images
7-026: Additional grow-out method for existing seed health method
7-001a, b; 7-004; 7-010; 7-011; 7-012: Replacement and/or addition of photographs

Chapter 9: 9.1.5.2, 9.1.5.5: Changes to methods for cutting seeds
9.1.5.5: Changes to methods for cutting large tree seeds for moisture testing

Chapter 11: 11.5.6.3, 11.5.6.5, 11.5.7, 11.5.8: Clarification of procedures for seed pellets

Chapter 17: 17.6, 17.7: Removal of ‘herbage’, where differentiation between ‘herbage’ and ‘amenity’ (turf) is no longer appropriate

Effective 1 January 2016
Chapter 7: Seed health testing

7.1 Object

The object of a seed health test is to determine the health status of a seed sample, and by inference that of the seed lot. Health testing of seed is important for four reasons:

a) Seed-borne inoculum may give rise to progressive disease development in the field and reduce the commercial value of the crop.
b) Imported seed lots may introduce diseases into new regions. Tests to meet quarantine requirements may therefore be necessary.
c) Seed health testing may elucidate seedling evaluation and causes of poor germination or field establishment and thus supplement germination testing.
d) Seed health test results can/may indicate the necessity to carry out/perform seed lot treatment(s) in order to eradicate seed-borne pathogens or to reduce the risk of disease transmission.

7.2 Definitions

7.2.1 Seed health

Health of seed refers primarily to the presence or absence of disease-causing organisms, such as fungi, bacteria and viruses, and animal pests, including nematodes and insects, but physiological conditions such as trace element deficiency may be involved.

7.2.2 Pretreatment

Any physical or chemical laboratory treatment of the working sample preceding incubation, given solely to facilitate testing.

7.2.3 Seed treatment

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.

7.2.4 ISTA Seed Health Method Validation Programme

Before publication in the International Rules for Seed Testing, the ISTA seed health testing methods (new or equivalent) are validated. The principles and factors which should be considered in the validation of methods for the detection of seed-borne pathogens are described in the ISTA Handbook of Method Validation for the Detection of Seed-borne Pathogens.

7.3 General principles

Seed health testing should be performed using methods and equipment which have been tested to ensure they are fit for purpose. Different methods of testing are available, varying in sensitivity and reproducibility and in the amount of training and equipment required. The method used will depend on the pathogen or condition to be investigated, the species of the seed, and the purpose of the test. Selection of the method and evaluation of the results requires knowledge and experience of the methods available. The presence or absence of disease organisms, pests and deleterious physiological conditions specified by the sender is estimated as accurately as the method used permits.

7.4 Procedures

7.4.1 Working sample

The entire submitted sample, or a proportion of it, depending on the test method, may be used as a working sample. The sample should be packaged and submitted in a manner which will not alter its seed health status.

Exceptionally, a submitted sample larger than that prescribed in 2.8 may be required and in such cases the sampler must be instructed accordingly.

When a portion of the submitted sample is required as a working sample, the reduction must be carried out in accordance with 2.5.2, taking appropriate precautions to avoid cross-contamination.

Normally the working sample must not be less than that specified in the method description.

Replicates containing a specified number of seeds, if required, must be taken at random from a subsample after thorough mixing.
7.4.2 Seed treatment

Test results may be influenced by treatment applied to the seed lot. Seed health tests on treated seeds will generally deliver unreliable test results caused by masking or inhibition of the growth of the target organism. Individual Method Sheets will determine whether the testing of treated seeds is acceptable.

7.4.3 Sample storage

The microflora of seed, in the lot or the sample, may change considerably during storage in conditions in which seed viability is satisfactorily maintained. The selection of the appropriate storage conditions must take into account the optimal storage temperature and container in order to maintain sample integrity.

Abundant development of saprophytic moulds including ‘storage fungi’ in tests can be an indication that the seed is not of good quality due to unfavourable harvesting, processing or storage conditions, or to ageing. Some fungi (such as *Rhizopus* spp.) spread rapidly over tests on blotters and may rot originally healthy seedlings or may interfere with outgrowth of the pathogen from the plated infected seeds. Pretreatment as described in the specific method may be advisable.

7.4.4 Specific directions

Specific seed health testing methods are published online on the ISTA website at:

www.seedtest.org/seedhealthmethods

Seed health methods are normally based on one host, and one pathogen, but multi-pathogen methods may be included. Before publication, all seed health test methods must be validated through the ISTA Seed Health Method Validation Programme. Methods validated in this way at the time of printing are listed in Table 7A. Additions, updates and deletions to this list can be found on the ISTA website (www.seedtest.org/seedhealthmethods). The definitive list is held by the ISTA Secretariat. It is the responsibility of the laboratory using the method to consult this list.

7.5 Calculation and expression of results

Results are expressed either qualitatively or quantitatively as specified in the individual prescribed methods.

7.6 Reporting results

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.
Table 7A. ISTA official seed health testing methods

7-001a: Detection of Alternaria dauci in Daucus carota (Carrot) seed by blotter method
Host: Daucus carota L.
Pathogen(s): Alternaria dauci (J.G.Kühn) J.J.Groves & Skolko, syn. A. porri f.sp. dauci (J.G.Kühn) Neerg., syn. A. carotae (Ellis & Langlois) Stevenson & Wellman
Date approved: 2012
Review due: 2017

7-001b: Detection of Alternaria dauci in Daucus carota (Carrot) seed by malt agar method
Host: Daucus carota L.
Pathogen(s): Alternaria dauci (J.G.Kühn) J.J.Groves & Skolko, syn. A. porri f.sp. dauci (J.G.Kühn) Neerg., syn. A. carotae (Ellis & Langlois) Stevenson & Wellman
Date approved: 2012
Review due: 2017

7-002a: Detection of Alternaria radicina in Daucus carota (Carrot) seed by blotter method
Host: Daucus carota L.
Pathogen(s): Alternaria radicina Meier, Drechsler & E.D.Eddy, syn. Stemphylium radicinum (Meier, Drechsler & E.D.Eddy) Neergaard
Date approved: 2012
Review due: 2017

7-002b: Detection of Alternaria radicina in Daucus carota (Carrot) seed by malt agar method
Host: Daucus carota L.
Pathogen(s): Alternaria radicina Meier, Drechsler & E.D.Eddy, syn. Stemphylium radicinum (Meier, Drechsler & E.D.Eddy) Neergaard
Date approved: 2012
Review due: 2017

7-003: Detection of Botrytis cinerea in Helianthus annuus (Sunflower) seed
Host: Helianthus annuus L.
Pathogen(s): Botrytis cinerea Pers. ex Pers. (Perfect state Botryotinia fuckeliana (de Bary) Whetzel, syn. Sclerotinia fuckeliana (de Bary) Fuckel.)
Date approved: 2011
Review due: 2016

7-004: Detection of Phoma lingam in Brassica spp. seed
Host: Brassicaceae
Pathogen(s): Phoma lingam (Tode ex Fr.) Desm., syn. Plenodomus lingam (Tode ex Fr.) Hohn (Perfect state Leptosphaeria maculans (Tode ex Fr.) Ces. & de Not.). Imperfect state Phoma lingam
Date approved: 2011
Review due: 2016

7-005: Detection of Ascochyta pisi in Pisum sativum (Pea) seed
Host: Pisum sativum L.s.l.
Pathogen(s): Ascochyta pisi Lib.
Date approved: 2011
Review due: 2016

7-006: Detection of Colletotrichum lindemuthianum in Phaseolus vulgaris (Bean) seed
Host: Phaseolus vulgaris L.
Pathogen(s): Colletotrichum lindemuthianum (Sacc. & Magn.) Briosi & Cav.
Date approved: 2011
Review due: 2016

7-007: Detection of Alternaria lini, Botrytis cinerea and Colletotrichum lini in Linum usitatissimum (Flax) seed
Host: Linum usitatissimum L.
Pathogen(s): Alternaria lini, J.W.Groves & Skolko; Botrytis cinerea Pers. ex Pers. (Perfect state Botryotinia fuckeliana (de Bary) Whetzel, syn. Sclerotinia fuckeliana (de Bary) Fuckel.); Colletotrichum lini (Westerd.) Tochinai, syn. C. lini in Lin. usitatissimum
Date approved: 2012
Review due: 2017

7-008: Detection of Caloscypha fulgens in Picea engelmannii and P. glauca (Spruce) seed
Host: Picea engelmannii Parry ex Engelm.; Picea glauca (Moench) Voss
Pathogen(s): Caloscypha fulgens (Pers.) Boud. (Imperfect state Geniculodontendor pyriiforme Salt)
Date approved: 2011
Review due: 2016
### Table 7A. ISTA official seed health testing methods (cont.)

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>7-010</td>
<td>Detection of Drechslera oryzae in <em>Oryza sativa</em> (Rice) seed&lt;br&gt;Host: <em>Oryza sativa</em> L.&lt;br&gt;Pathogen(s): Drechslera oryzae (Breda de Haan) Subram. &amp; Jain, syn. Bipolaris oryzae (Breda de Haan) Shoem., syn. Helminthosporium oryzae Breda de Haan (Perfect state <em>Cochliobolus miyabeanus</em> Ito &amp; Kurib., Drechsler ex Dastur, syn. Ophiobolus miyabeanus Ito &amp; Kuribayashi)&lt;br&gt;Date approved: 2011&lt;br&gt;Review due: 2016</td>
</tr>
<tr>
<td>7-011</td>
<td>Detection of Pyricularia oryzae in <em>Oryza sativa</em> (Rice) seed&lt;br&gt;Host: <em>Oryza sativa</em> L.&lt;br&gt;Pathogen(s): Magnaporthe grisea (Hebert) Barr (Imperfect state <em>Pyricularia oryzae</em> Cavara, syn. <em>P. grisea</em>)&lt;br&gt;Date approved: 2011&lt;br&gt;Review due: 2016</td>
</tr>
<tr>
<td>7-012</td>
<td>Detection of Alternaria padwickii in <em>Oryza sativa</em> (Rice) seed&lt;br&gt;Host: <em>Oryza sativa</em> L.&lt;br&gt;Pathogen(s): Alternaria padwickii (Ganguly) M.B.Ellis, syn. Trichoconis padwickii Ganguly, syn. Trichoconiella padwickii (Ganguly) Jain&lt;br&gt;Date approved: 2011&lt;br&gt;Review due: 2016</td>
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<tr>
<td>7-013a</td>
<td>Detection of <em>Ustilago nuda</em> in <em>Hordeum vulgare</em> (Barley) seed by embryo extraction&lt;br&gt;Host: <em>Hordeum vulgare</em> L.&lt;br&gt;Pathogen(s): <em>Ustilago nuda</em> (Jens.) Rostr.&lt;br&gt;Date approved: 2011&lt;br&gt;Review due: 2016</td>
</tr>
<tr>
<td>7-013b</td>
<td>Detection of <em>Ustilago nuda</em> in <em>Hordeum vulgare</em> (Barley) seed by dehulling and embryo extraction&lt;br&gt;Host: <em>Hordeum vulgare</em> L.&lt;br&gt;Pathogen(s): <em>Ustilago nuda</em> (Jens.) Rostr.&lt;br&gt;Date approved: 2011&lt;br&gt;Review due: 2016</td>
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<tr>
<td>7-014</td>
<td>Detection of <em>Stagonospora nodorum</em> in <em>Triticum aestivum</em> (Wheat) seed&lt;br&gt;Host: <em>Triticum aestivum</em> L.&lt;br&gt;Pathogen(s): Stagonospora nodorum Berk., syn. Septoria nodorum Berk. (Perfect state <em>Leptosphaeria nodorum</em> Maier)&lt;br&gt;Date approved: 2011&lt;br&gt;Review due: 2016</td>
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<td>7-015</td>
<td>Detection of Neotyphodium spp. in <em>Festuca</em> spp. (Fescue) and <em>Lolium</em> spp. (Rye grass) seed&lt;br&gt;Host: <em>Festuca</em> spp., <em>Lolium</em> spp.&lt;br&gt;Pathogen(s): Neotyphodium coenophialum (Morgan-Jones &amp; W.Gams) Glenn, C.W.Bacon &amp; Hanlin; Neotyphodium loli (Latch, M.J.Chr. &amp; Samuels) Glenn, C.W.Bacon &amp; Hanlin&lt;br&gt;Date approved: 2012&lt;br&gt;Review due: 2017</td>
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<td>7-016</td>
<td>Detection of <em>Phomopsis</em> complex in <em>Glycine max</em> (Soybean, Soya bean) seed&lt;br&gt;Host: <em>Glycine max</em> (L.) Merr.&lt;br&gt;Pathogen(s): Phomopsis longicolla Hobbs, Diaporthe phaseolorum var. <em>sojae</em> (Lehm.) Wehm. (Imperfect state <em>P. phaseoli</em> (Desm.) Sacc., syn. <em>P. sojae</em> Lehmann); Diaporthe phaseolorum (Cke. &amp; Ell.) Sacc. f. sp. <em>caulivora</em> (DPC), syn. <em>D. phaseolorum</em> var. <em>caulivora</em> Athow &amp; Caldwell&lt;br&gt;Date approved: 2012&lt;br&gt;Review due: 2017</td>
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<tr>
<td>7-017</td>
<td>(Replaced by 7-007)</td>
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<tr>
<td>7-018</td>
<td>(Replaced by 7-007)</td>
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<tr>
<td>7-019a</td>
<td>Detection of <em>Xanthomonas campestris</em> pv. <em>campestris</em> on <em>Brassica</em> spp. seed&lt;br&gt;Host: <em>Brassica</em> spp.&lt;br&gt;Pathogen(s): <em>Xanthomonas campestris</em> pv. <em>campestris</em> (Pammel) Dowson&lt;br&gt;Date approved: 2014&lt;br&gt;Review due: 2019</td>
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### Table 7A. ISTA official seed health testing methods (cont.)

<table>
<thead>
<tr>
<th>Code</th>
<th>Detection</th>
<th>Host</th>
<th>Pathogen(s)</th>
<th>Date approved</th>
<th>Review due</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-024</td>
<td>Detection of <em>Pea early browning virus</em> and <em>Pea seed-borne mosaic virus</em> in <em>Pisum sativum</em> (Pea) seed</td>
<td><em>Pisum sativum</em> L.s.l.</td>
<td><em>Pea early browning virus</em> (PEBV) and <em>Pea seed-borne mosaic virus</em> (PSbMV)</td>
<td>2012</td>
<td>2017</td>
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<tr>
<td>7-025</td>
<td>Detection of <em>Aphelenchoides besseyi</em> in <em>Oryza sativa</em> (Rice) seed</td>
<td><em>Oryza sativa</em> L.</td>
<td><em>Aphelenchoides besseyi</em> Christie</td>
<td>2013</td>
<td>2018</td>
</tr>
<tr>
<td>7-026</td>
<td>Detection of <em>Squash mosaic virus</em>, <em>Cucumber green mottle mosaic virus</em> and <em>Melon necrotic spot virus</em> in <em>Cucurbita pepo</em> seed</td>
<td><em>Cucurbita pepo</em></td>
<td><em>Cucumber green mottle mosaic virus</em> (CGMMV); <em>Melon necrotic spot virus</em> (MNSV)</td>
<td>2014</td>
<td>2019</td>
</tr>
<tr>
<td>7-027</td>
<td>Detection of <em>Pyrenophora teres</em> and <em>P. graminea</em> on <em>Hordeum vulgare</em> (Barley) seed</td>
<td><em>Hordeum vulgare</em> L.</td>
<td><em>Pyrenophora teres</em> Drechsler (Imperfect state <em>Drechslera teres</em> (Sacc.) Shoem.; <em>Pyrenophora graminea</em> Ito &amp; Kurib. (Imperfect state <em>D. graminea</em> (Rabenh. Ex Schlecht.) Shoem.)</td>
<td>2011</td>
<td>2016</td>
</tr>
<tr>
<td>7-028</td>
<td>Detection of infectious <em>Tobacco mosaic virus</em> and <em>Tomato mosaic virus</em> in <em>Solanum lycopersicum</em> (Tomato) seed by the local lesion assay (indexing) on <em>Nicotiana tabacum</em> plants</td>
<td><em>Solanum lycopersicum</em> L.</td>
<td><em>Tobacco mosaic virus</em> (TMV); <em>Tomato mosaic virus</em> (ToMV)</td>
<td>2012</td>
<td>2017</td>
</tr>
</tbody>
</table>
Table 7A. ISTA official seed health testing methods (cont.)

7-030: Detection of Acidovorax valerianellae in Valerianella locusta (corn salad) seed

Host: Valerianella locusta (L.) Laterr.
Pathogen(s): Acidovorax valerianellae sp. nov.
Date approved: 2014
Review due: 2019