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 2.2.12. 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 Technical Guidelines for Organising and Analysing Results of Proficiency Tests (PT) and Interlaboratory Tests for Validation of Methods (CT)*.

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 web site 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 web site (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 *Leptosphaeria maculans* and *Plenodomus biglobosus* in *Brassica* spp. seed
Host: *Brassica* spp.
Pathogen(s): *Leptosphaeria maculans* (Tode ex Fr.) Ces. & de Not (previously *Phoma lingam*) or *Plenodomus biglobosus* (Shoemaker & H. Brun) (previously *Leptosphaeria biglobosa*)
Date approved: 2017
Review due: 2022

7-005: Detection of *Ascochyta pisi* in *Pisum sativum* (pea) seed
Host: *Pisum sativum* L.s.l.
Pathogen(s): *Ascochyta pisi* Lib.
Date approved: 2022
Review due: 2027

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: 2022
Review due: 2027

7-007: Detection of *Alternaria linicola*, *Botrytis cinerea* and *Colletotrichum lini* in *Linum usitatissimum* (flax, linseed) seed
Host: *Linum usitatissimum* L.
Pathogen(s): *Alternaria linicola* 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. linicola* Pethybr. & Laff.
Date approved: 2012
Review due: 2017

7-008: Detection of *Caloscypha fulgens* in *Picea engelmannii* and *P. glauca* (spruce) seed
Host: *Picea engelmannii* Engelm.; *Picea glauca* (Moench) Voss
Pathogen(s): *Caloscypha fulgens* (Pers.) Boud. (Imperfect state *Geniculodendron pyriforme* Salt)
Date approved: 2011
Review due: 2016

Table 7A. ISTA official seed health testing methods (cont.)

7-009: Detection of *Fusarium circinatum* 1n *Pinus* spp. (pine) and *Pseudotsuga menziesii* (Douglas fir) seed
Host: *Pinus* spp.; *Pseudotsuga menziesii* (Mirb.) Franco
Pathogen(s): *Fusarium circinatum* Nirenberg & O'Donnell (syn. *Fusarium subglutinans* f. sp. *pini* Hepting, syn. *Fusarium lateritium* f. sp. *pini* Hepting, syn. *Gibberella circinata*)
Date approved: 2018
Review due: 2023

7-010: Detection of *Bipolaris oryzae* in *Oryza sativa* (rice) seed
Host: *Oryza sativa* L.
Pathogen(s): *Bipolaris oryzae* (Breda de Haan) Shoem., syn. *Drechslera oryzae*, syn. *Helminthosporium oryzae* Breda de Haan (Perfect state *Cochliobolus miyabeanus* (Ito & Kurib.) Drechsler ex Dastur, syn. *Ophiobolus miyabeanus* Ito & Kuribayashi)
Date approved: 2018
Review due: 2023

7-011: Detection of *Pyricularia oryzae* in *Oryza sativa* (rice) seed
Host: *Oryza sativa* L.
Pathogen(s): *Magnaporthe grisea* (Hebert) Barr (Imperfect state *Pyricularia oryzae* Cavara, syn. *P. grisea*)
Date approved: 2011
Review due: 2016

7-012: Detection of *Trichoconiella padwickii* in *Oryza sativa* (rice) seed
Host: *Oryza sativa* L.
Pathogen(s): *Trichoconiella padwickii* Ganguly, syn. *Alternaria padwickii* (Ganguly) Jain
Date approved: 2018
Review due: 2023

7-013a: Detection of *Ustilago nuda* in *Hordeum vulgare* subsp. *vulgare* (barley) seed by embryo extraction
Host: *Hordeum vulgare* L. subsp. *vulgare*
Pathogen(s): *Ustilago nuda* (Jens.) Rostr.
Date approved: 2022
Review due: 2027

7-013b: Detection of *Ustilago nuda* in *Hordeum vulgare* subsp. *vulgare* (barley) seed by dehulling and embryo extraction
Host: *Hordeum vulgare* L. subsp. *vulgare*
Pathogen(s): *Ustilago nuda* (Jens.) Rostr.
Date approved: 2022
Review due: 2027

7-014: Detection of *Parastagonospora nodorum* in *Triticum aestivum* subsp. *aestivum* (wheat) seed
Host: *Triticum aestivum* L. subsp. *aestivum*
Pathogen(s): *Parastagonospora nodorum* (Berk.) Quaedvl., Verkley & Crous 2013, syn. *Stagonospora nodorum*, syn. *Septoria nodorum* Berk. (Perfect state *Leptosphaeria nodorum* Mailer)
Date approved: 2022
Review due: 2027

7-015: Detection of *Epichloë coenophiala* in *Festuca* spp. (fescue) and of *Neotyphodium lolii* in *Lolium* spp. (ryegrass) seed
Host: *Festuca* spp., *Lolium* spp.
Pathogen(s): *Epichloë coenophiala* (Morgan-Jones & W. Gams) C.W. Bacon & Schardl; *Neotyphodium lolii* (Latch, M.J.Chr. & Samuels) Glenn, C.W.Bacon & Hanlin
Date approved: 2017
Review due: 2022

7-016: Detection of *Phomopsis* complex in *Glycine max* (soybean, soya bean) seed
Host: *Glycine max* (L.) Merr.
Pathogen(s): *Phomopsis longicolla* Hobbs, *Diaporthe phaseolorum* var. *sojae* (Lehm.) Wehm. (Imperfect state *P. phaseoli* (Desm.) Sacc., syn. *P. sojae* Lehmann); *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. f. sp. *caulivora* (DPC), syn. *D. phaseolorum* var. *caulivora* Athow & Caldwell
Date approved: 2022
Review due: 2027

7-017: (Replaced by 7-007)

7-018: (Replaced by 7-007)

7-019a: Detection of *Xanthomonas campestris* pv. *campestris* and *Xanthomonas campestris* pv. *raphani* in *Brassica* spp. seed
Host: *Brassica* spp.
Pathogen(s): *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson and *Xanthomonas campestris* pv. *raphani*
Date approved: 2022
Review due: 2027

7-019b: Detection of *Xanthomonas campestris* pv. *campestris* in disinfested/disinfected *Brassica* spp. seed
Host: *Brassica* spp.
Pathogen(s): *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson
Date approved: 2018
Review due: 2023

Table 7A. ISTA official seed health testing methods (cont.)

7-020: Detection of *Xanthomonas hortorum* pv. *carotae* in *Daucus carota* (carrot) seed
Host: *Daucus carota* L.
Pathogen(s): *Xanthomonas hortorum* pv. *carotae* (Kendrick) Vauterin, Hoste, Kersters & Swings, syn. *X. campestris* pv. *carotae* (Kend) Dye
Date approved: 2010
Review due: 2015

7-021: Detection of *Xanthomonas axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans* in *Phaseolus vulgaris* (bean) seed
Host: *Phaseolus vulgaris* L.
Pathogen(s): *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin, Hoste, Kersters & Swings, syn. *X. campestris* pv. *phaseoli* (Smith) Dye; *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* Vauterin, Hoste, Kersters & Swings, syn. *X. campestris* pv. *phaseoli* var. *fuscans* (Burkholder) Starr & Burkholder
Date approved: 2011
Review due: 2016

7-022: Detection of *Microdochium nivale* and *M. majus* in *Triticum* spp. (wheat) seed
Host: *Triticum* spp.
Pathogen(s): *Microdochium nivale* Samuels & Hallett, syn. *Fusarium nivale* (Fr.) Rabenh. (Perfect state *Monographella nivalis* (Schaff.) Müller); *M. majus* (Wollenw.) Glynn & S.G.Edwards, syn. *M. nivale* var. *majus* (Wollenw.) Samuels & I.C.Hallett
Date approved: 2022
Review due: 2027

7-023: Detection of *Pseudomonas savastanoi* pv. *phaseolicola* in *Phaseolus vulgaris* (bean) seed
Host: *Phaseolus vulgaris* L.
Pathogen(s): *Pseudomonas savastanoi* pv. *phaseolicola* (Burkh.) Gardan, Bollet, Abu, Ghorrah, Grimont & Grimont, syn. *P. syringae* pv. *phaseolicola* (Burkh.) Young, Dye & Wilkie
Date approved: 2012
Review due: 2017

7-024: Detection of *pea early browning virus* and *pea seed-borne mosaic virus* in *Pisum sativum* (pea) seed
Host: *Pisum sativum* L.s.l.
Pathogen(s): *Pea early browning virus* (PEBV) and *pea seed-borne mosaic virus* (PSbMV)
Date approved: 2012
Review due: 2017

7-025: Detection of *Aphelenchoides besseyi* in *Oryza sativa* (rice) seed
Host: *Oryza sativa* L.
Pathogen(s): *Aphelenchoides besseyi* Christie
Date approved: 2019
Review due: 2024

7-026: Detection of *squash mosaic virus*, *cucumber green mottle mosaic virus* and *melon necrotic spot virus* in cucurbit seed
Host: Cucurbits
Pathogen(s): *Squash mosaic virus* (SqMV); *cucumber green mottle mosaic virus* (CGMMV); *melon necrotic spot virus* (MNSV)
Date approved: 2014
Review due: 2019

7-027: Detection of *Pyrenophora teres* and *P. graminea* in *Hordeum vulgare* subsp. *vulgare* (barley) seed
Host: *Hordeum vulgare* L. subsp. *vulgare*
Pathogen(s): *Pyrenophora teres* Drechsler (Imperfect state *Drechslera teres* (Sacc.) Shoem.); *Pyrenophora graminea* Ito & Kurib. (Imperfect state *D. graminea* (Rabenh. Ex Schlecht.) Shoem.)
Date approved: 2011
Review due: 2016

7-028: Detection of infectious *tobacco mosaic virus* and *tomato mosaic virus* in *Solanum lycopersicum* (tomato) seed by the local lesion assay (indexing) in *Nicotiana tabacum* plants
Host: *Solanum lycopersicum* L.
Pathogen(s): *Tobacco mosaic virus* (TMV); *tomato mosaic virus* (ToMV)
Date approved: 2012
Review due: 2017

7-029: Detection of *Pseudomonas syringae* pv. *pisi* in *Pisum sativum* (pea) seed
Host: *Pisum sativum* L.s.l.
Pathogen(s): *Pseudomonas syringae* pv. *pisi* (Sack.) Young, Dye & Wilkie
Date approved: 2012
Review due: 2017

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

Table 7A. ISTA official seed health testing methods (cont.)

7-031: Filtration method for detection of *Ditylenchus dipsaci* in *Medicago sativa*; *D. dipsaci* and *D. gigas* in *Vicia faba*
Host: *Medicago sativa* L. and *Vicia faba* L.
Pathogen(s): *Ditylenchus dipsaci* Kuhn, 1857; *Ditylenchus gigas* n. sp.
Date approved: 2017
Review due: 2022

7-032: Detection of *Verticillium dahliae* in *Spinacia oleracea* (spinach) seed
Host: *Spinacia oleracea* L.
Pathogen(s): *Verticillium dahliae* Kleb.
Date approved: 2017
Review due: 2022