International Rules for Seed Testing
2017

Validated Seed Health Testing Methods

7-005: Detection of Ascochyta pisi in Pisum sativum (pea) seed

Including changes and editorial corrections adopted at the Ordinary General Meeting 2016, Tallinn, Estonia

Effective from 1 January 2017
Validation reports

See References. Copies are available by e-mail from the ISTA Secretariat at ista.office@ista.ch.

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

Disclaimer

Whilst ISTA has taken care to ensure the accuracy of the methods and information described in this method description, ISTA shall not be liable for any loss or damage, etc. resulting from the use of this method.

Safety precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during weighing out of ingredients. It is assumed that persons carrying out this test are in a laboratory suitable for carrying out microbiological procedures and familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic techniques. Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.

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.

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7-005-2 Effective 1 January 2017
7-005: Detection of *Ascochyta pisi* in *Pisum sativum* (pea) seed

**Host:** *Pisum sativum* L.s.l.
**Pathogen(s):** *Ascochyta pisi* Lib.

**Prepared by:** ISTA-PDC Method Validation Sub-committee
**Authors:** ISTA-PDC Method Validation Sub-committee

**Revision history**
Version 1.0, 2000-07-13
Revised 2001-19-11: J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.1, 2008-01-01: Treated seed revised; Reporting results revised
Version 1.2, 2017-01-01: Reporting results revised

**Background**
This method was originally published in the ISTA Handbook of Seed Health Testing in November 1964 as S.3. No. 16 revised 1987 by P.D. Hewett, Official Seed Testing Station for England and Wales, Cambridge, United Kingdom. The method was incorporated into the newly revised Annexe to Chapter 7 in 2002 from the 1999 edition of the ISTA Rules. The method was reviewed by the ISTA-Seed Health Committee in 2006 (Cockerell & Koenraadt, 2007) with the recommendation to accept for a further five years.

**Treated seed**
This method has not been validated for the determination of *Ascochyta pisi* on treated seed. Seed treatments may affect the performance of the method.
(Definition of treatment: any process, physical, biological or chemical, to which a seed lot is subjected, including seed coatings. See 7.2.3)

**Materials**
**Reference material:** reference cultures or other appropriate material
**Media:** malt agar or potato dextrose agar
**Sodium hypochlorite solution (1 % available chlorine):** for seed disinfection
**Petri dishes:** When sowing density is given by a number of seeds per Petri dish, a diameter of 90 mm is assumed.
**Incubator:** capable of operating in the range 20 ±2 °C.

**Sample preparation**
The test is carried out on a working sample of 400 seeds as described in Section 7.4.1 of the ISTA Rules as appropriate.

**Method**
1. **Pretreatment:** 10 min. in a 1 % (available chlorine) sodium hypochlorite solution followed by draining.
2. **Agar method:** Malt or potato dextrose agar. Place 10 seeds on the agar surface in each Petri dish.
3. **Incubation:** 7 days at 20 °C in darkness.
4. **Examination:** After 7 days examine each seed by naked eye for abundant white mycelium which often covers infected seeds (Fig. 1). Doubtful colonies may be confirmed by the presence of wavy hyphae at the edge of the colony when examined at ×25 magnification. Colony diameter typically 20–30 mm, occasionally smaller or incompletely surrounding the seed. Reverse of colonies medium to dark orange-brown centrally, opaque and even, becoming lighter in colour towards the edge of the colony. Gelatinous-looking orange-brown pycnidia often present (although only sometimes clearly visible), particularly where seed touches agar. Under STM at ×20–25 magnification, using both transmitted and incident light, hyphae are curled, often
several running together, typically with moisture drops (although these evaporate easily)(Fig. 2). Very limited growths from some seeds may only be seen if dishes tilted to get lighting at best angle, or under STM examination or after extended incubation. Pycnidia are up to 250 µm in diameter. Spores, hyaline, cylindric, of slightly curved with rounded ends, 1-septate, slightly constricted at septum, mostly 12 × 4.5 µm (Punithalingam, & Holliday, 1972).

Notes: Samples frequently bear A. pinodes (Mycosphaerella pinodes (Berk. & Blox.) Westerg.) and, occasionally, A. pinodella (Phoma medicaginis Malbr. & Roum. var. pinodella (Jones) Boerema). These pathogens differ markedly from A. pisi in their colony and mycelial characters and in spore morphology (see CMI descriptions Nos. 340 and 518, respectively).

General methods

Checking tolerances: Tolerances provide a means of assessing whether or not the variation in results within or between tests are wide as to raise doubts about the accuracy of the results. Suitable tolerances, which can be applied to most direct seed health tests, can be found in Table 5B Part 1 of Chapter 5 of the ISTA Rules, or Table G1 in Miles (1963).

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

The report must indicate the number of seeds tested. In the case of a negative result (pathogen not detected), the results must be reported as “not detected”. In the case of a positive result, the report should indicate the percentage of infected seeds.

Quality assurance

Critical control points (CCP)

Where the wording of the original Working Sheet suggests that an action is critical this has been marked with CCP.

Media and solutions

Sodium hypochlorite solution

Sodium hypochlorite for pretreatment of seed can be prepared from commercial bleach diluted to 1 % available chlorine. The concentration of chlorine in commercial bleach varies considerably. Use the formula:

\[ V_{\text{stock}} = V_{\text{final}} \times C_{\text{final}} / C_{\text{stock}} \]

(where \( V \) = volume and \( C \) = % available chlorine) to calculate the volume of commercial bleach stock solution required to prepare sodium hypochlorite solutions for use in seed pretreatment.

To prepare a 1 L solution of sodium hypochlorite containing 1 % chlorine from a stock of commercial bleach containing 12 % available chlorine:

\[ V_{\text{stock}} = 1 \times 1/12 = 0.083 \]

Thus add 83 mL of the 12 % stock to 917 mL water.

Malt agar

Malt agar: According to manufacturer’s instructions

Distilled/deionized water: 1000 mL

¹ Malt agar constituents should be equivalent to those of the manufacturers BD, USA or Oxoid, UK (CCP)

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 mL of distilled/deionized water.
3. Dissolve powdered Malt agar in distilled/deionized water by stirring.
4. Autoclave at 15 p.s.i. and 121 °C for 15 min.
5. Allow agar to cool to approx. 50 °C.
6. Pour 15–22 mL of molten agar into 90 mm Petri plates and allow to solidify before use.

Storage

Prepared plates may be stored at 4 °C for up to 6 weeks.
Figure 1. Colonies of *A. pisi*, face (left) and reverse (right), from test on PDA, following hypochlorite pretreatment. Incubation for 7 days at 21 °C in darkness.

Figure 2. Typical appearance of hyphae of *A. pisi*. Test conditions as for Figure 1.
Potato dextrose agar

**Potato dextrose agar**: according to manufacturer's instructions

**Distilled/deionized water**: 1000 mL

1 Potato dextrose agar constituents should be equivalent to those of the following manufacturers BD, USA or Oxoid, UK (CCP)

**Preparation**

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 (or 500) mL of distilled/deionized water.
3. Dissolve powdered PDA in distilled/deionized water by stirring.
4. Autoclave at 15 p.s.i. and 121 °C for 15 min.
5. Allow agar to cool to approx. 50 °C.
6. Pour 15–22 mL of molten agar into 90 mm Petri plates and allow to solidify before use.

**Storage**

Prepared plates may be stored at 4 °C for up to 6 weeks.

**References**

The following references are extracted from the ISTA Handbook on Seed Health Testing, Working Sheet No. 16, P. D. Hewett, 1987.


**Validation reports**


**Summary of validation studies**

Agar tests detect approximately 50 % more infection by *Ascochyta* spp. than blotter tests (Anselme & Champion, 1962; Tempe, 1968).

International comparative tests (Hewett, 1987) showed that of over 350 results obtained by experienced stations, 95 % fell within tolerance limits used for germination tests.