International Rules for Seed Testing 2027

Validated Seed Health Testing Methods

7-001a: Detection of *Alternaria dauci* in *Daucus carota* (carrot) seed by blotter method

Including changes and editorial corrections adopted at the Ordinary General Meeting 2026 in Calgary, Canada

Effective from 1 January 2027

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

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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

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Revision history

Version 1.0, 2003-01-01
Version 1.1, 2013-01-01: Definition of sample size
Version 1.2, 2014-01-01: Addition of positive control; common name of host added
Version 1.3, 2016-01-01: New Figures 1a, 1b
Version 1.4, 2017-01-01: Reporting results revised
Version 1.5, 2021-01-01: Sample size added and Methods revised
Version 1.6, 2024-01-01: Sample size and Figures revised
Version 1.7, 2025-01-01: Critical control points and figure captions revised
Version 1.8, 2027-01-01: Flowchart added in Methods

Background

This method was originally published in the *ISTA Handbook of Seed Health Testing* in November 1964 as S.3. No. 4 and revised in 1987 (Gambogi, 1987). It was incorporated into the *Annexe to Chapter 7: Seed Health Testing Methods* as method 7-001 (Sheppard & Cockerell, 2002). It has been renumbered (7-001a) and slightly modified following studies conducted by the International Seed Health Initiative for Vegetable Crops, ISF (ISHI-Veg) in 1999 and 2001 (Van Bilsen, 2003). The studies compared blotter and malt agar methods and concluded that the two were equivalent. Note that seeds can be simultaneously tested for the presence of *Alternaria radicina* using the same method (see method 7-002a).

Treated seed

Seed treatments may affect the performance of this test. It should only be performed on untreated seed.

Sample size

The sample size (total number of seeds to be tested) depends on intended use, the maximum acceptable infection level and the analytical sensitivity of the method. The minimum sample size should be 400 seeds.

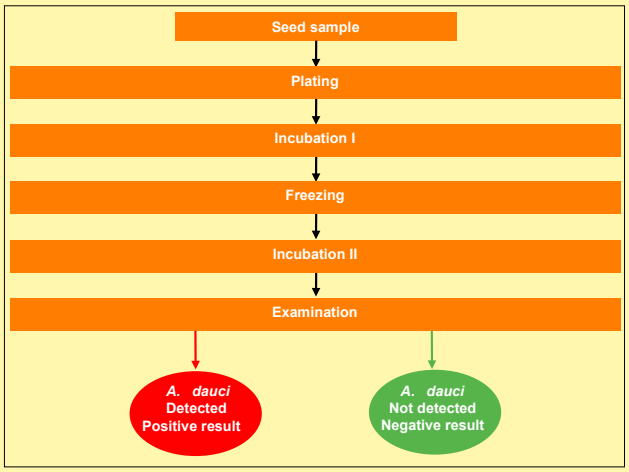
Materials

Reference material: reference cultures or other appropriate material
Substrate: blotters or filter papers, 90 mm, circular (e.g. Whatman No. 1 or equivalent), free from micro-organisms and inhibitors (3 per plate)
Plates: 90 mm sterile Petri dishes (one per ten seeds)
Incubator: operating at 20 ±2 °C, equipped with timer-controlled near-ultraviolet lights (NUV, peak at 360 nm, e.g. colour number 08, Philips; BLB, Sylvania)
Freezer: operating at –20 ±2 °C

Sample preparation

It is vital to exclude any possibility of cross-contamination between seed samples. This can be achieved by swabbing/spraying equipment and gloved hands with 70 % ethanol.

Methods



Critical control points are indicated by CCP.

1. Pretreatment: None.
2. Place three 90 mm filter papers in each plate and soak with sterile distilled/deionised water. Drain away excess water.
3. Plating

3.1 Aseptically place 10 seeds, evenly spaced (CCP), on the surface of the filter paper in each plate.

3.2 Positive control (reference material): Aseptically place seeds evenly spaced (CCP) onto the surface of the filter paper in an appropriate number of plates to obtain the reference culture, or plate a reference culture on media. The number of plates required will depend on the level of contamination of the positive-control seed lot.

4. Incubate for 3 d at 20 ±2 °C in the dark.
5. Transfer plates to freezer and maintain at –20 ±2 °C for 24 h.
6. After freezing, incubate for 6 d at 20 ±2 °C with alternating 12 h periods of darkness and NUV light (ISTA,1984; Tempe, 1968). Plates should be approx. 25 cm below the lights and should not be stacked.
7. Examine seeds under a stereomicroscope at ×30 magnification for fungal growth and up to ×80 for identification of conidia. Conidiophores are simple or slightly branched (Fig. 1), arising singly or in small groups from the surface of the seed or on aerial mycelium. Conidia are usually solitary, obclavate, up to 450 µm long (including beak), pale olivaceous brown at first, becoming brown with age, with a long pale beak up to 3 times the length of the body (Ellis, 1971). Groups of sunken conidia are sometimes visible by the emerging clusters of their bright long beaks (Fig. 1c). Compare with positive control. Record the number of infected seeds in each plate (CCP).

General methods

Checking tolerances: Tolerances provide a means of assessing whether or not the variation in results within or between tests are sufficiently 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 must indicate the percentage of infected seeds.

Quality assurance

Specific training

This test should only be performed by persons who have been trained in fungal identification or under their direct supervision.

Critical control points (CCP)

- Spreading hyphae may lead to contamination of other seeds. Seeds must therefore be spaced at least 20 mm from each other with not more than 10 seeds per 90 mm Petri dish (Step 3).
- Samples may be difficult to examine due to the growth of contaminants, especially *Alternaria tenuis/alternata*, and/or *A. radicina*. Experience and great care is required for the detection of all occurrences (ISTA, 1984) (Step 7).
- Supplementary examination may be required 13–14 d after plating of seeds to allow hampered or deeper inoculum to exceed contaminants by its fructification (Hewett, 1964) (Step 7).

References

Ellis, M. B. (1971). *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. 609 pp.



Figure 1. a Conidia of *Alternaria dauci* on simple or slightly branched conidiophores. **b** Conidia of *A. dauci*. **c** Conidia of *A. dauci* on simple or slightly branched conidiophores borne on a single rootlet initial. **d** Conidiophores and conidia of *A. dauci* and groups of the saprophyte *Alternaria alternata* on carrot seed.

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Validation references

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