International Rules for Seed Testing
Annexe to Chapter 7: Seed Health Testing Methods

7-017: Malt agar method for the detection of *Alternaria linicola* on *Linum usitatissimum*

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Crop: *Linum usitatissimum* (Flax, Linseed)

Pathogen: *Alternaria linicola*

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Background

This method was originally published in the *ISTA Handbook of Seed Health Testing* in 1982 as Working Sheet No 46 (Malone,1982). Though the method had been used in routine testing in the UK and Northern Ireland since 1939 (Muskett, and Malone, 1941), it had not been studied in comparative tests. The method has since been studied in 12 laboratories in six countries (Sheppard *et al.,* 2003). Although there were no statistically significant differences between results obtained using either malt agar or malt extract agar, malt agar was preferred due to increased sporulation of *A. linicola,* enabling easier assessment of fungal colonies. This method is also suitable for evaluation and determination of the percentage of *Colletotrichum lini* on *Linum usitatissimum* (Linseed, Flax), see method sheet 7-018 (2003).

Validation studies

Sheppard *et al*. (2003)
Copies are available: by e-mail from ista.office@ista.ch; by mail from the ISTA Secretariat.

Safety Precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during preparation of media, autoclaving and weighing out of ingredients. It is assumed that this procedure is being carried out in microbiological laboratory by persons 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, safety and environmental regulations.

Treated Seed

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

Materials

Reference material	-	The use of reference cultures or other appropriate material is recommended.
Malt Agar plates	-	See page 6. 9.0 cm plates (petri dishes, one plate per ten seeds).
Petri dishes	-	9.0 cm.
Incubator	-	Operating at 22 ± 2°C, equipped with timer-controlled near-ultraviolet lights (NUV, peak at 360 nm).

Sample Preparation

The test is carried out on a working sample as described in Section 7.4.1 of the International Rules for Seed Testing.

Method

[Critical control points are indicated by **CCP**]

1. Aseptically place a maximum of 10 seeds per plate, evenly spaced, onto the agar surface of each malt agar plate.
2. Incubate plates for 7 d at 22°C with alternating 12 h periods of darkness and NUV.
3. Sub-culture a reference culture to a malt agar plate at the same time the seeds are plated and incubate with the test plates.
4. Examine plates for dense olive grey colonies, 1.5-3 cm diameter. Some colonies of saprophytic *Alternaria* spp. can resemble those of *A. linicola* but the conidia of *A. linicola* are diagnostic. Colonies should therefore be examined under x50 – x100 magnification. Conidiophores are simple, occurring singly or in bundles, pale olive-brown, septate, and variable in length 5-8 µm. Conidia form singly, are smooth walled, olive-brown, obclavate with long, tapering occasionally branched beaks muriform 4-16 µm with transverse septa and occasionally 1-4 longitudinal septa, sometimes slightly constricted at the septa (Corlett and Corlett,1999; David, 1991; Malone and Muskett, 1997). Record the number of infected seeds in each plate.

   Short red streaks and water soaked areas may be visible on the hypocotyls and cotyledons of some infected seedlings.

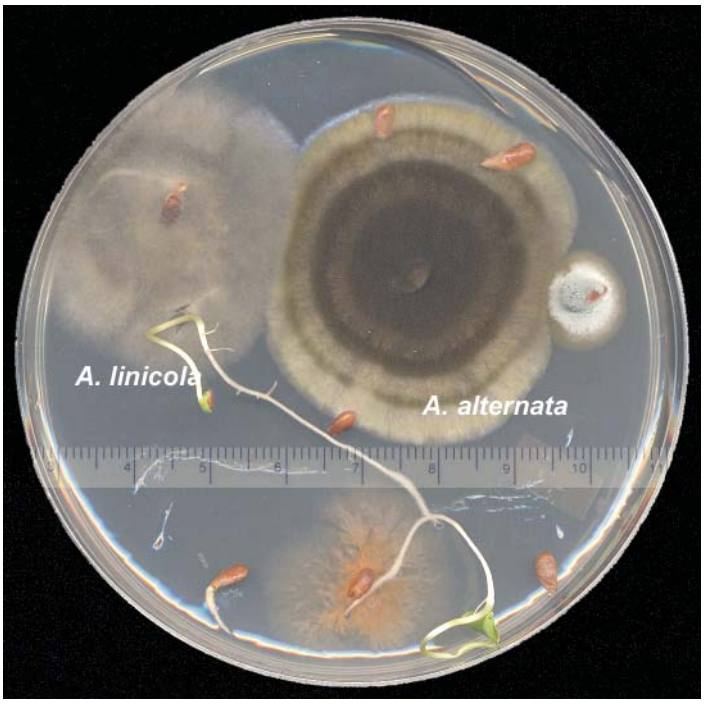

Fig. 1. Olive-grey colonies of *A. linicola* and darker colonies of saprophytic *A. alternata* on malt agar.

Fig. 2. Reddish streaks on cotolyedons and hypocotyls casued by *A. linicola.*

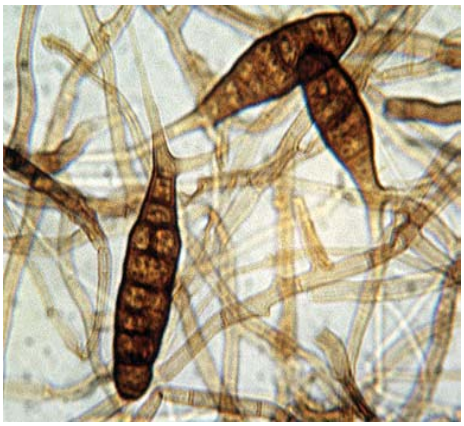

Fig. 3. Conidia of *Alternaria linicola x600*

General Methods (common to many test procedures)

1. *Checking Tolerances*

   Tolerances provide a means of assessing whether or not the variation in result within or between tests is sufficiently wide as to raise doubts about the accuracy of the results. A tolerance table, which can be applied to most direct seed health tests, can be found in Table 5.1 of Annexe 16 of the International Rules for Seed Testing or in Table G1 of the *Handbook of Tolerances and Measures of Precision for Seed Testing* (Miles, 1963).

2. *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), the results should be reported in terms of the tolerance standard (e.g. infection level less than 1% with 95% probability). The tolerance standard depends on the total number of seeds tested, n, and is approximately 3/n (P=0.95) (see Roberts *et al.,* 1993).

   In the case of a positive result the report should indicate 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

[identified by **CCP** in the methods].

The malt agar used must have equivalent constituents to DIFCO, USA. The malt agar source can influence the results. Whenever a new batch is used a check on the quality should be made using a reference lot with known infection or a reference culture.

Preparation of Malt Agar

Compound	g/l	g/500 ml
Malt Agar (BD DifcoTM, Cat No. 224200, BD, USA) (**CCP**)[1]	30 g	15 g
De-ionised/Distilled Water	1000 ml	500 ml

[1]Quantities of malt agar powder may differ between manufacturers: check the label carefully.

Preparation

1. Weigh out all ingredients into a suitable autoclavable container.
2. Add 1000 ml (or 500 ml) of distilled/de-ionised water.
3. Steam or boil to dissolve.
4. Autoclave at 121°C, and 15 psi for 15 min.
5. Allow agar to cool to approx 50°C.
6. Pour 22 ml of molten agar into 9.0 cm Petri plates and allow to solidify at room temperature (20-25°C) before use.

Storage

Plates may be stored at room temperature or 4ºC for up to four weeks before use.

References

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