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

7-018: Malt agar method for the detection of *Colletotrichum lini* on *Linum usitatissimum*

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Crop: *Linum usitatissimum* (Linseed, Flax)

Pathogen: *Colletotrichum lini*

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Background

Although an agar method has been used in routine seed health testing in Northern Ireland and the UK since 1939 (Muskett and Malone, 1941), the method was not studied in comparative tests until 1999. Twelve laboratories in 6 countries studied 3 methods: blotter; malt agar; and malt extract agar (Sheppard *et al*., 2003). The study concluded that in general the blotter method gave lower infection levels and more variability between laboratories than either malt agar or malt extract agar. There were no significant differences in results obtained using either malt agar or malt extract agar. However, this method sheet recommends the use of malt agar (Difco) to complement the test for *A. linicola*, as there is normally a requirement to test for both pathogens (see method sheet 7-017).

Validation studies

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

Please send comments, suggestions or reports of problems relating to this method to the leader of the ISTA-PDC Mycology Working Group, c/o 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 a 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, environmental and safety 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 petri dishes (one plate per ten seeds.)
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, evenly spaced, onto the agar surface of each malt agar plate.
2. Incubate plates for 7 days at 22 ± 2°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. *C. lini* is easily recognised by visual examination. Examine the plates for shell pink to salmon coloured colonies (Fig. 1). Colonies of *C. lini* are a fine wooly-gray at the centre to salmon pink at the outer edge. Dark globose fuiting bodies (acervuli) may be scattered throughout the agar adjacent to the seed. Characteristic long, black tapering hairs or setae 2-5 septate, 60-120 x 2-4 µm arise from the base of each acervulus. Bright orange conidial masses appear on the seed and agar adjacent to the seed. Conidia are hyaline, oblong to dumbell shaped, one celled, straight ends 9-15 x 3-4 µm (Malone and Muskett, 1997, Kulshrestha *et al.,* 1976).
5. Record the number of infected seeds on each plate.

General Methods (common to many test procedures)

1. *Checking Tolerances*

   Tolerances provide a means of assessing whether or not the variation in results within or between tests is sufficiently wide as to raise doubts about the accuracy of the result. 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.

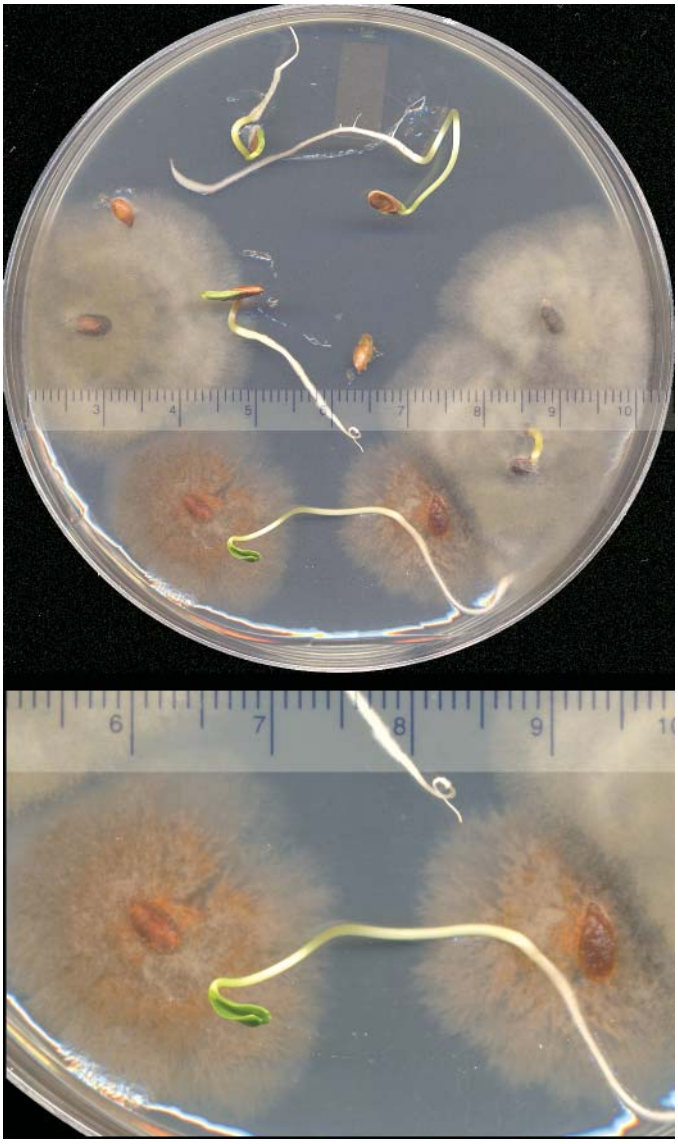

Fig. 1. Salmon coloured colonies of *C.lini* growing from flax seed on malt agar.

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/deionised 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

Kulshrestha, D.D, Mathur, S. B. and Neergaard, P. (1976) Identification of Seed-borne Species of *Colletotrichum*. *Friesa* **11**, 116-125.

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