



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



7-014: Detection of *Septoria nodorum* on *Triticum aestivum* (Wheat)

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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 damage etc., resulting from the use of this method.

- Crop:** *Triticum* spp. (Wheat)
- Pathogen:** *Stagonospora nodorum* Berk. = syn *Septoria nodorum* Berk., Perfect state: *Leptosphaeria nodorum* Mailer
- Prepared by:** ISTA-PDC Method Validation Sub-committee
- Revision History:** Version 1.0 November 20, 2001
Revised 20.11.2001 J. Sheppard
Reprinted with editorial changes 2003
Version 1.1 2008-01-01
“Treated seed” revised; “Reporting results” revised
Version 1.2. 2010-01-01
Footnote 2 added to malt agar ingredients
- Submitted by:** ISTA-PDC Method Validation Sub-committee

Method Abstract

This method was originally published in the ISTA Handbook of Seed Health Testing in November 1964 as S.3. No. 19 and revised in 1984 by M. Kietreiber, Bundesanstalt für Pflanzenbau, Wien, Austria. 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 & Koenraad, 2007) with the recommendation to accept for a further five years.

Summary of Validation Study:

Studied in International Comparative Testing: 1959, 1961, 1962, 1964 and 1979-81

Using potato dextrose in darkness, Hewett (1975) found a correlation coefficient of 0.95 between counts in the laboratory and the number of diseased seedlings in the field. Comparative tests organized by the ISTA Plant Disease Committee gave reasonable agreement between stations (Rennie, 1982).

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 safety regulations.

Treated Seed

This method has not been validated for the determination of *Septoria nodorum* 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	The use of reference cultures or other appropriate material is recommended when ever possible.
Media	Malt Agar or Potato Dextrose Agar containing 100 ppm streptomycin sulphate.
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 International Rules for Seed Testing.

Method

1. *Pretreatment*
10 minutes in 1% (available chlorine) sodium hypochlorite.
2. *Agar method*
Malt agar or Potato Dextrose Agar containing 100 ppm streptomycin sulphate.
3. *Incubation*
7 days at 20 °C in darkness.
4. *Examination.*
After 7 days examine each seed by naked eye for slow-growing circular colonies of dense white or cream mycelium that often covers infected seeds. The reverse of the colony is yellow/brown becoming darker with age.

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. Suitable tolerances, which can be applied to most direct seed health tests, can be found in Tables 5B of Chapter 5 of the ISTA Rules, or in the *Handbook of Tolerances and Measures of Precision for Seed Testing* by S.R. Miles (*Proceedings of the International Seed Testing Association* 28 (1963) No 3, p 644).

2. 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 International Seed Analysis Certificate*, results are entered under Other Determinations.

Preparation of Media and Solutions

1. 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 liter solution of sodium hypochlorite containing 1% chlorine from a stock of commercial bleach containing 12% available chlorine:

$$V_{\text{stock}} = V_{\text{final}} \times C_{\text{final}} / C_{\text{stock}} \quad V_{\text{stock}} = 1 \times 1/12 = 0.083$$

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

2. Malt Agar

Compound	g/L
Malt Agar ¹	According to manufacturer's instructions
Distilled/de-ionized water	1000 mL
Streptomycin sulfate ²	1 mg

CCP¹ Malt agar constituents should be equivalent to those of the following manufacturers Difco, USA or Oxoid, UK.

² Added after autoclaving

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 mL of distilled/de-ionized water.
3. Dissolve powdered Malt Agar in distilled/de-ionized 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.

Potato Dextrose Agar

Compound	g/L
Potato Dextrose Agar ¹	According to manufacturer's instructions
Distilled/de-ionized Water	1000 mL
Streptomycin sulfate ²	1 mg

CCP¹ PDA constituents should be equivalent to those of the following manufacturers Difco, USA or Oxoid, UK.

² Added after autoclaving

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 mL of distilled/de-ionized water.
3. Dissolve powdered PDA in distilled/de-ionized 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 cm Petri plates and allow to solidify before use.

Storage

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

Quality Assurance**Critical Control Points**

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

References

The following references are extracted from the ISTA Handbook on Seed Health Testing, Working Sheet No. 19, M. Kietreiber, 1984.

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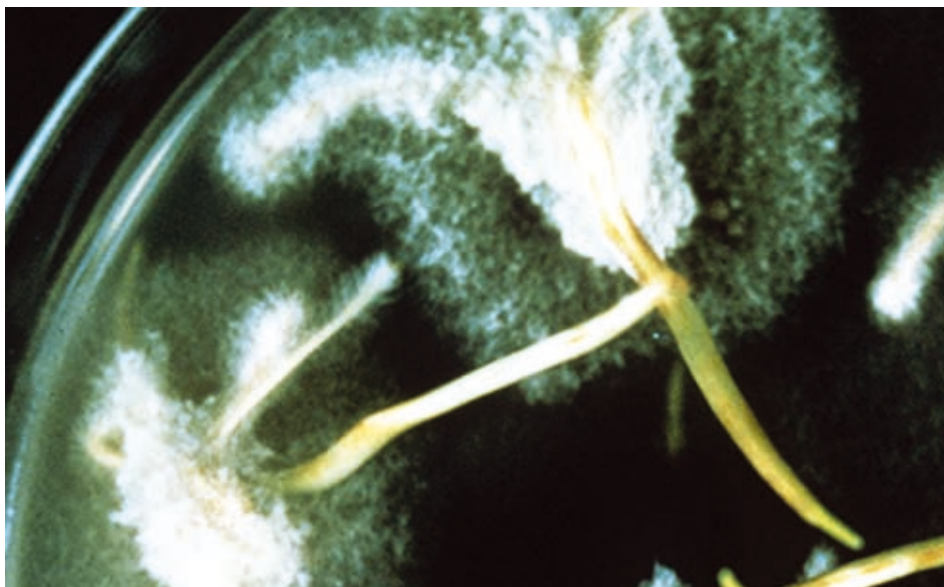


Fig. 1. Slow growing, finely tufted, white aerial mycelium of *Septoria nodorum* covering grain in an agar plate test.