International Rules for Seed Testing 2023

Validated Seed Health Testing Methods

7-014: Detection of *Parastagonospora nodorum* in *Triticum aestivum* subsp. *aestivum* (wheat) seed

Including changes and editorial corrections adopted at the Ordinary General Meeting 2022, Cairo, Egypt

Effective from 1 January 2023

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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7-014: Detection of *Parastagonospora nodorum* in *Triticum aestivum* subsp. *aestivum* (wheat) seed

Host: *Triticum aestivum* L. subsp. *aestivum*
Pathogen(s): *Parastagonospora nodorum* (Berk.) Quaedvl., Verkley & Crous 2013, syn. *Stagonospora nodorum*, syn. *Septoria nodorum* Berk. (Perfect state *Leptosphaeria nodorum* Mailer)

Prepared by: ISTA-PDC Method Validation Sub-committee

Authors: ISTA-PDC Method Validation Sub-committee

Revision history

Version 1.0, 2001-11-20
Revised 2001-11-20, 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
Version 1.3, 2014-01-01: Streptomycin sulphate added to malt agar; addition of positive control; pathogen renamed *Stagonospora*; reference culture mandatory
Version 1.4, 2017-01-01: Reporting results revised
Version 1.5, 2018-01-01: Changes to the taxonomic names of fungi
Version 1.6, 2021-01-01: Sample preparation changed to Sample size and paragraph revised; Media and solutions revised
Version 1.7, 2022-01-01: Methods (pretreatment) revised

Background

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 & Koenraadt, 2007) with the recommendation to accept for a further five years.

Treated seed

This method has not been validated for the determination of *Parastagonospora 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.)

Sample size

The sample (total number of seeds tested) size to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum sample size should be 400 seeds.

Materials

Reference material: reference cultures or other appropriate material
Media: malt agar or potato dextrose agar with 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

Methods

1. Pretreatment: Immerse seeds in a solution of sodium hypochlorite (NaOCl) (1 % available chlorine) for 10 min, then drain, rinse well in sterile water and drain again.
2 Plating:
2.1 Aseptically place a maximum of 10 seeds, evenly spaced, onto the agar surface of each malt agar or potato dextrose agar plate.
2.2 Positive control (reference material): Aseptically place seeds 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.
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. Compare with positive control.

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

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 and declines with storage. Use the formula:

$$V_{stock} = V_{final} \times C_{final} / C_{stock}$$

(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:

$$V_{stock} = 1 \times 1/12 = 0.083$$

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

The percentage of active chlorine decreases rapidly in solution so, NaClO 1 % solution must be stored in the dark and used within 3 days of preparation. It is possible to check chlorine concentration with chlorine strip tests.

Malt agar

Malt agar[1]: according to manufacturer's instructions
Distilled/deionised water: 1000 ml
Streptomycin sulphate[2]: may be used between 50 and 100 ppm, depending on the level of saprophytic bacterial contamination commonly encountered

[1] CCP: Malt agar constituents should be equivalent to those of the following manufacturers: BD, USA or Oxoid, UK.
[2] Added after autoclaving

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 ml of distilled/deionised water.
3. Dissolve powdered malt agar in distilled/deionised water by stirring.
4. Autoclave at 121 °C and 15 psi for 15 min.
5. Allow agar to cool to approx. 50 °C, and add streptomycin sulphate dissolved in water.
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.

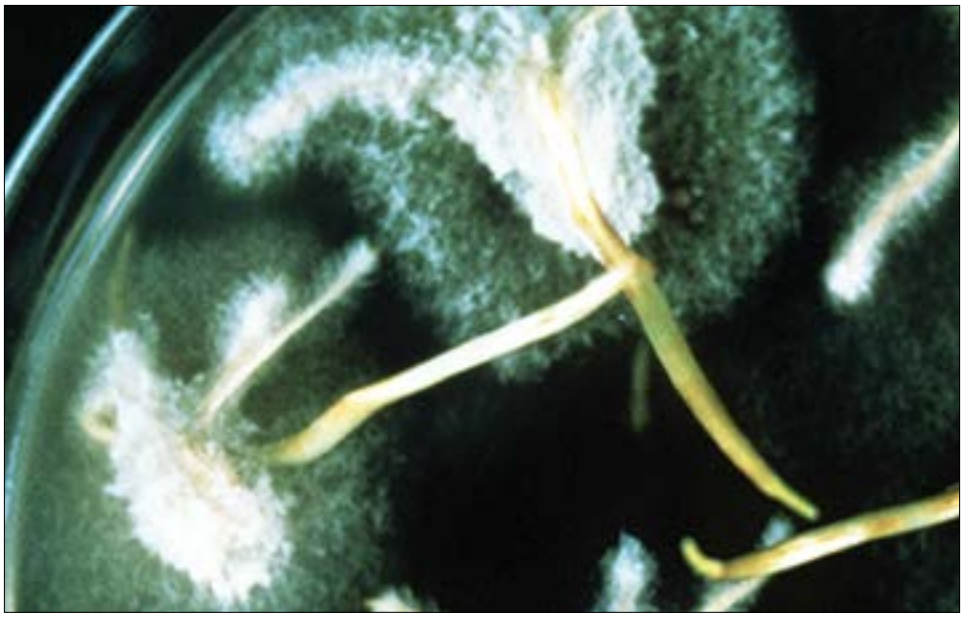

Figure 1. Slow growing, finely tufted, white aerial mycelium of *Parastagonospora nodorum* covering grain in an agar plate test.

Potato dextrose agar (PDA)

Potato dextrose agar[1]: according to manufacturer's instructions
Distilled/deionised water: 1000 ml
Streptomycin sulphate[2]: may be used between 50 and 100 ppm, depending on the level of saprophytic bacterial contamination commonly encountered

[1] CCP: PDA constituents should be equivalent to those of the following manufacturers. BD, USA or Oxoid, UK.
[2] Added after autoclaving

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 ml of distilled/deionised water.
3. Dissolve powdered PDA in distilled/deionised water by stirring.
4. Autoclave at 15 psi and 121 °C for 15 min.
5. Allow agar to cool to approx. 50 °C, and add streptomycin sulphate dissolved in water.
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.

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

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Validation references

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).