International Rules for Seed Testing 2027

Validated Seed Health Testing Methods

7-006: Detection of *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* (bean) seed

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.

Published by
The International Seed Testing Association (ISTA)
Richtiarkade 18, CH-8304 Wallisellen, Switzerland

© 2027 International Seed Testing Association (ISTA)

Online ISSN 2310-3655

All rights reserved. No part of this publication may be reproduced, stored in any retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior permission in writing from ISTA.

7-006: Detection of *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* (bean) seed

Host: *Phaseolus vulgaris* L.
Pathogen(s): *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav.

Prepared by: ISTA-PDC Method Validation Subcommittee

Authors: ISTA-PDC Method Validation Subcommittee

Revision history

Version 1.0, November 19, 2001
Revised 19.11.2001 J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.1, 2008-01-01: Treated seed revised; Reporting results revised
Version 1.2, 2014-01-01: Addition to Background and caption of Figure 1
Version 1.3, 2017-01-01: Reporting results revised
Version 1.4, 2021-01-01: Sample preparation changed to Sample size and paragraph revised; Media and solutions revised
Version 1.5, 2022-01-01: Methods (pretreatment) revised
Version 1.6, 2024-01-01: Sample size revised
Version 1.7, 2027-01-01: Flowchart added in Methods

Background

This method was originally published in the *ISTA Handbook of Seed Health Testing* in 1981 as Working Sheet No. 45 prepared by C. Anselme & R. Champion, La Minière, France. 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. Lesions on severely infected seeds may be either brown with whitish centres surrounded by a pale brown to dark brown area or reddish lesions of variable size (Fig. 1). Direct inspection is not considered as a dependable method, as not all infected seeds bear symptoms and on dark-skinned varieties symptoms are more difficult to see.

Treated seed

This method has not been validated for the determination of *Colletotrichum lindemuthianum* 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)

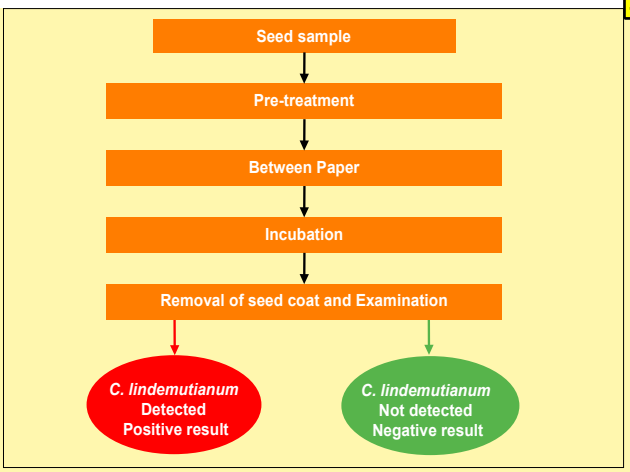
Sample size

The sample 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
Media: paper towelling
Sodium hypochlorite solution (1 % available chlorine): for seed disinfection
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. Between paper (BP): Spread the seeds in replicates of 50 on double sheets of paper towelling 350 × 450 mm which have been soaked in water. Cover seeds with one sheet of paper towelling soaked in water. Fold the paper twice lengthways and cover it with a sheet of polythene to maintain the moisture during incubation.
3. Incubation: 7 days at 20 °C in darkness
4. Examination: After 7 days remove the seed coats and examine the cotyledons by naked eye for black depressed areas with well delimited outlines (Fig. 2). Check each spot for the presence of acervuli with or without dark brown setae using ×25 magnification (Fig. 3). The septate setae measure approx. 6 µm × 100 µm. The pale orange acervuli contain cylindric, hyaline conidia with rounded ends containing one or two guttulaes. Conidia measure 2.5–5.5 µm × 11–20 µm (Mordue, 1971; Kulshrestha, Mathur, & Neergaard, 1976). The use of a high-power microscope (magnification ×200) is sometimes necessary (Fig. 3). Small spots may require longer incubation for development of acervuli.

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)

None listed.

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.



Figure 1. Dry, white bean seeds showing symptoms. Lesions on severely infected seeds may be either brown with whitish centres surrounded by a pale brown to dark brown area, or reddish lesions of variable size. Direct inspection is not considered as a dependable method, as not all infected seeds bear symptoms, and on dark-skinned varieties, symptoms are more difficult to see.

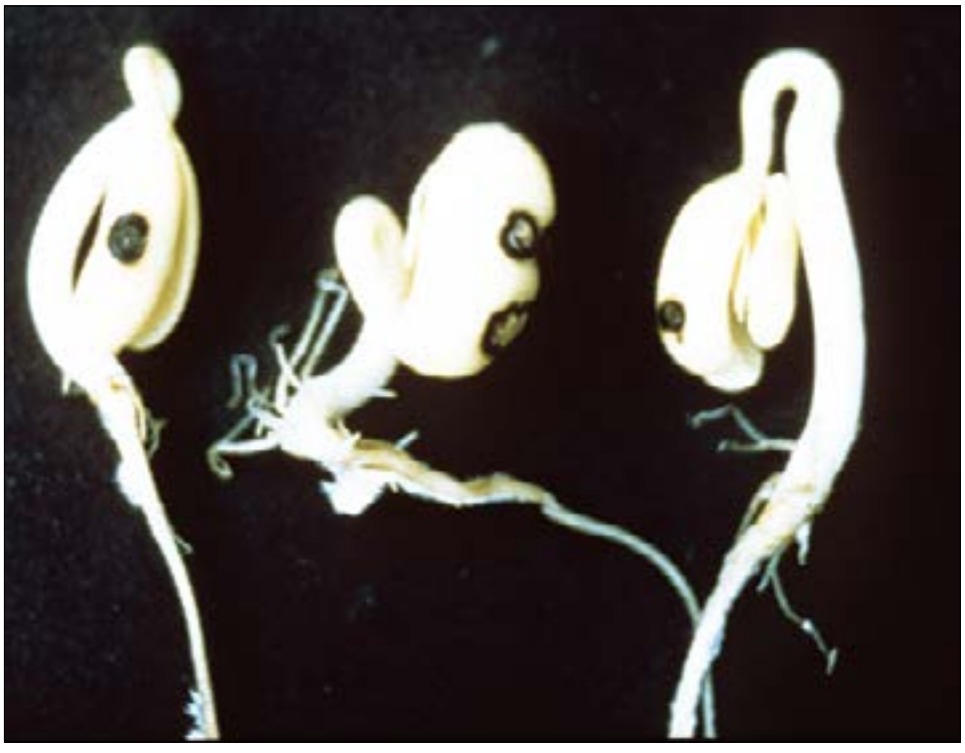


Figure 2. Cotyledons of seedlings after 7 days incubation with seed coats removed, showing black areas with well defined outline.

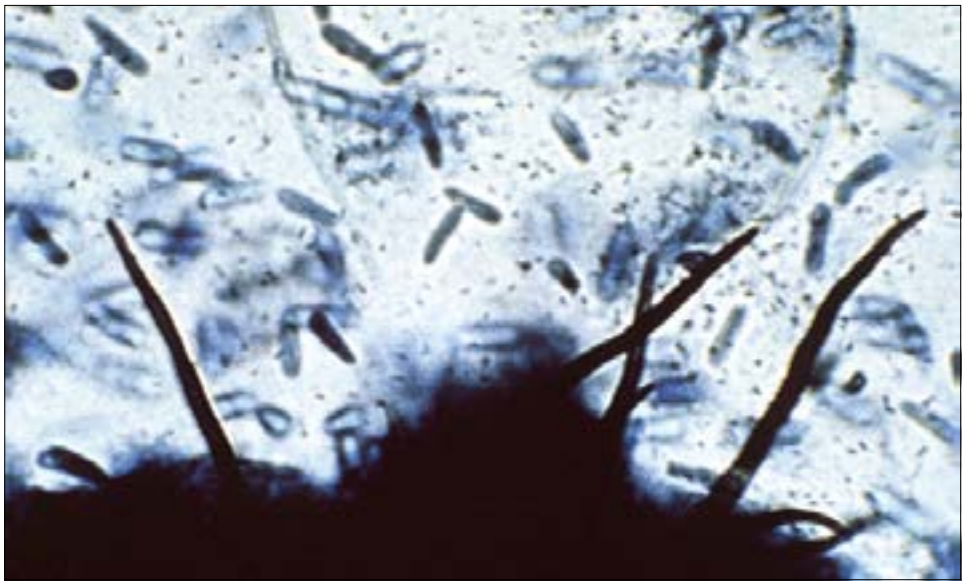


Figure 3. Acervuli with cylindric, hyaline conidia and dark brown septate setae. ×150.

References

The following references are extracted from the *ISTA Handbook of Seed Health Testing*, Working Sheet No. 45, C. Anselme & R. Champion, 1981.

Champion, R. (1969). Quelques parasites importants transmis par les semences. Identification au laboratoire, *Agriculture*, **322**, 3–8.

Kulshrestha, D. D., Mathur, S. B. & Neergaard, P. (1976). Identification of seed borne species of *Colletotrichum*. *Friesia* **11**, 116–125.

Miles, S. R. (1963). Handbook of tolerances and of measures of precision for seed testing. *Proceedings of the International Seed Testing Association*, **28** (3), 525–686.

Mordue, J. E. M. (1971). *C.M.I. Description of pathogenic fungi and bacteria No. 316.* Commonwealth Mycological Institute, Kew.

International Seed Testing Association, Committee on Plant Diseases (1963). *Report on the Fifth International Conference (workshop) on seed pathology sponsored by the International Seed Testing Association convened by the ISTA Committee on Plant Diseases.* Bundesanstalt fur Pflanzenbau and Samenprüfung in Wien 17th to 23rd September, 1962, p. 23. Copenhagen. Mimeographed.

Validation references

Studied in ISTA comparative tests: 1962