ISTA
Seed Quality Assurance

ISTA Secretariat
Richtiarkade 18, 8304 Wallisellen, Switzerland
Phone: +41 44 838 60 00 | Fax: +41 44 838 60 01
Email: ista.office@ista.ch
www.seedtest.org

Seed Health Chapter Editorial Correction for the International Rules for Seed Testing 2024 Edition

This document was prepared by the Technical Committees (TCOMs) and the Rules Committee of the Association. The proposals are submitted to the ISTA Ordinary General Meeting 2023 for voting by the nominated ISTA Designated Members on behalf of their respective Governments.

It contains proposed amendments and changes for the ISTA *International Rules for Seed Testing* and will be discussed and voted on at the Ordinary General Meeting 2023 to be held on 01 June, 2023, in Verona, Italy.

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Introduction to the ISTA Rules Proposals to become effective 1 January 2024

The current version of the ISTA International Rules for Seed Testing (ISTA Rules) is the 2023 edition.

The ISTA Rules are only available electronically as a printable pdf file and are available for free download by ISTA members from the Ingenta website: http://www.ingentaconnect.com/content/ista/rules

The electronic version also includes the French, German, and Spanish versions of the ISTA Rules. If there are any questions on interpretation of the ISTA Rules the English version is the definitive version.

For further information on the ISTA Rules, see: http://www.seedtest.org/rules

The effective dates are changed annually. The changes from the previous edition of the ISTA Rules can be displayed as yellow highlighted text as a 'layer' within the electronic copy with comments on what has changed. Previous Prefaces as a 'history of changes' are available on the ISTA website.

The ISTA Rules are the result of the work of the ISTA Technical Committees (TCOMs) with input from many different sources. Thanks go to all the Technical Committee members and the ISTA Secretariat for their help with the annual proposals.

The following Rules Proposals will be discussed at the ISTA Ordinary General Meeting in Verona, Italy on 01 June, 2023, and may be amended without changing the intent of the proposal. If the proposals are accepted by the membership, amendments will be issued, and they will become the 2024 edition of the ISTA Rules.

Please let us know about any problems with these proposals.

Many thanks.

Ernest Allen and Sue Alvarez

Chair and Vice-Chair of ISTA Rules Committee

Contact details:

Ernest Allen

E-mail: ernest.allen@usda.gov

Sue Alvarez

E-mail: sue.alvarez@ransomseedlab.com

Key to text changes:

~~Deleted text~~

New text

New text in large blocks, not underlined for ease of reading

Any changes made after the proposals were published to the membership

PART A. INTRODUCTION OF EDITORIAL CHANGES

A.1. Chapter 7 Editorial corrections

General editorial corrections to Chapter 7

In order to harmonise "sample size" sentences throughout chapter 7, the SHC reviewed all chapter 7 methods and suggested improvements.

CURRENT VERSION	PROPOSED VERSION
Method 7-001a **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. …	**Method 7-001a** **Sample size** … The sample size (total number of seeds tested) 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. ….
Method 7-001b **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. …	**Method 7-001b** **Sample size** … The sample size (total number of seeds tested) 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. …
Method 7-002a **Sample size** … The sample (total number of seeds tested) size to be tested depends on ~~the desired tolerance~~	**Method 7-002a** **Sample size** … The sample size (total number of seeds tested) to be tested depends on intended use, the maximum acceptable infection level and the analytical

~~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. …	sensitivity of the method. The minimum sample size should be 400 seeds. …
Method 7-002b **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. …	**Method 7-002b** **Sample size** … The sample size (total number of seeds tested) 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. …
Method 7-003 **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. …	**Method 7-003** **Sample size** … The sample size (total number of seeds tested) 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. …
Method 7-004 **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. …	**Method 7-004** **Sample size** … The sample size (total number of seeds tested) 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. …

Method 7-005 **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. …	**Method 7-005** **Sample size** … The sample size (total number of seeds tested) 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. …
Method 7-006 **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. …	**Method 7-006** **Sample size** … The sample size (total number of seeds tested) 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. …
Method 7-007 **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. …	**Method 7-007** **Sample size** … The sample size (total number of seeds tested) 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. …
Method 7-008 **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~~	**Method 7-008** **Sample size** … The sample size (total number of seeds tested) to be tested depends on intended use, the maximum acceptable infection level and the analytical

~~minimum number of pathogen propagules per seed which can be detected)~~. The minimum sample size should be 400 seeds. … **Method 7-009** **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. … **Method 7-010** **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. … **Method 7-011** **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. … **Method 7-012**	sensitivity of the method. The minimum sample size should be 400 seeds. … **Method 7-009** **Sample size** … The sample size (total number of seeds tested) 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. … **Method 7-010** **Sample size** … The sample size (total number of seeds tested) 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. … **Method 7-011** **Sample size** … The sample size (total number of seeds tested) 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. …

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. …	**Method 7-012** **Sample size** … The sample size (total number of seeds tested) 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. …
Method 7-013a **Sample size** … The sample (total number of seeds tested) and subsample size to be tested depend 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 work-ing sample consists of 100–120 g containing 2000–4000 seeds depending on TSW. A minimum of 1000 embryos are examined. …	**Method 7-013a** **Sample size** … The sample size (total number of seeds tested) and subsample size to be tested depend on intended use, the maximum acceptable infection level and the analytical sensitivity of the method. The working sample consists of 100–120 g containing 2000–4000 seeds depending on TSW. A minimum of 1000 embryos are examined. …
Method 7-013b **Sample size** … The sample (total number of seeds tested) and subsample size to be tested depend 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 work-ing sample consists of 100–120 g containing 2000–4000 seeds depending on TSW. A minimum of 1000 embryos are examined. …	**Method 7-013b** **Sample size** … The sample size (total number of seeds tested) and subsample size to be tested depend on intended use, the maximum acceptable infection level and the analytical sensitivity of the method. The working sample consists of 100–120 g containing 2000–4000 seeds depending on TSW. A minimum of 1000 embryos are examined. …
Method 7-014 **Sample size** …	**Method 7-014** **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.	The sample size (total number of seeds tested) 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.
…	…
Method 7-015	**Method 7-015**
Sample size	**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 100 seeds.	The sample size (total number of seeds tested) 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 100 seeds.
…	…
Method 7-016	**Method 7-016**
Sample size	**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.	The sample size (total number of seeds tested) 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.
…	…
Method 7-019a	**Method 7-019a**
Sample size	**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 30 000 seeds and the maximum subsample size should be 10 000 seeds.	The sample size (total number of seeds tested) 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 30 000 seeds and the maximum subsample size should be 10 000 seeds.
…	…
Method 7-019b	**Method 7-019b**
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 30 000 seeds and the maximum subsample size should be 10 000 seeds. … **Method 7-020** **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 10 000 seeds and the maximum subsample size should be 10 000 seeds. … **Method 7-021** **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 5 000 seeds and the maximum subsample size should be 1000 seeds. … **Method 7-022** **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. …	**Sample size** … The sample size (total number of seeds tested) 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 30 000 seeds and the maximum subsample size should be 10 000 seeds. … **Method 7-020** **Sample size** … The sample size (total number of seeds tested) 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 10 000 seeds and the maximum subsample size should be 10 000 seeds. … **Method 7-021** **Sample size** … The sample size (total number of seeds tested) 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 5 000 seeds and the maximum subsample size should be 1000 seeds. … **Method 7-022** **Sample size** … The sample size (total number of seeds tested) 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.

…	…
Method 7-023 **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 5 000 seeds and the maximum subsample size should be 1 000 seeds. …	**Method 7-023** **Sample size** … The sample size (total number of seeds tested) 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 5 000 seeds and the maximum subsample size should be 1000 seeds. …
Method 7-024 **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 2 000 seeds and the maximum subsample size should be 100 seeds. …	**Method 7-024** **Sample size** … The sample size (total number of seeds tested) 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 2 000 seeds and the maximum subsample size should be 100 seeds. …
Method 7-025 **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 1 000 seeds and the maximum subsample size should be 250 seeds. …	**Method 7-025** **Sample size** … The sample size (total number of seeds tested) 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 1000 seeds and the maximum subsample size should be 250 seeds. …
Method 7-026	**Method 7-026**

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 2 000 seeds and the maximum subsample size should be 100 seeds. …	**Sample size** … The sample size (total number of seeds tested) 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 2 000 seeds and the maximum subsample size should be 100 seeds. …
Method 7-027 **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. …	**Method 7-027** **Sample size** … The sample size (total number of seeds tested) 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. …
Method 7-028 **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 3 000 seeds and the maximum subsample size should be 250 seeds. …	**Method 7-028** **Sample size** … The sample size (total number of seeds tested) 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 3 000 seeds and the maximum subsample size should be 250 seeds. …
Method 7-029 **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 5 000 seeds and the maximum subsample size should be 1000 seeds.	**Method 7-029** **Sample size** … The sample size (total number of seeds tested) 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 5 000 seeds and the maximum subsample size should be 1000 seeds. …
Method 7-030 **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 10 000 seeds and the maximum subsample size should be 5 000 seeds. …	**Method 7-030** **Sample size** … The sample size (total number of seeds tested) 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 10 000 seeds and the maximum subsample size should be 5 000 seeds. …
Method 7-031 **Sample size** … The sample (total number of seeds tested) and subsample size to be tested depend on ~~the desired tolerance standard (~~maximum acceptable ~~number of seeds infested) and detection limit (theoretical minimum number of nematodes per sample which can be detected). An example of a recommended minimum sample size for faba bean with a detection limit of 1.5 nematodes per 100 g in the seed lot and a zero tolerance, is 900 g, using ISTA sampling methodology calculation adapted to sieving method (Macarthur et al., TESTA Deliverable 2.4).~~ The minimum sample size should be 100 g of seeds for alfalfa and 300 g for faba bean (TESTA WP2-Sampling), and the maximum subsample size should be 100 g of seeds for alfalfa and 300 g for faba bean. ~~The whole sample is tested.~~	**Method 7-031** **Sample size** … The sample size (total number of seeds tested) and subsample size to be tested depend on intended use, the maximum acceptable infection level and the analytical sensitivity of the method. The minimum sample size should be 100 g of seeds for alfalfa and 300 g for faba bean, and the maximum subsample size should be 100 g of seeds for alfalfa and 300 g for faba bean.
Method 7-032 **Sample size** … The sample (total number of seeds tested) size to be tested depends on ~~the desired tolerance standard (~~maximum acceptable ~~percentage of~~	**Method 7-032** **Sample size** …

~~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. …	The sample size (total number of seeds tested) 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. …

General editorial changes: Seed Health Testing Method: 7-019a and 7-019b

Editorial changes harmonizing the description of dilutions and recording cfu for bacteria is necessary since is not compulsory to record colony count numbers. For these methods, recording the presence, "detected/not detected," is more important.

Chapter 7: Methods: Interpretation & decision – bio-PCR (7-019a-8;7-019a-10).

CURRENT VERSION	PROPOSED VERSION
4. Dilution and plating **4.1** Prepare two serial tenfold dilutions from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (~~10^{1}~~ dilution). Pipette 0.5 ml of the ~~10^{1}~~ dilution into another 4.5 ml of sterile saline and vortex to mix (~~10^{2}~~ dilution) (see General method).	**4. Dilution and plating** **4.1** Prepare two serial tenfold dilutions from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (10^{-1} dilution). Pipette 0.5 ml of the 10^{-1} dilution into another 4.5 ml of sterile saline and vortex to mix (10^{-2} dilution) (see General method).
4.4.2 Dilute sufficiently to obtain dilutions containing approx. ~~10^{2} to 10^{4}~~ cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.	**4.4.2** Dilute sufficiently to obtain dilutions containing approx. 10^{-2} to 10^{-4} cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.
4.6.6 Record the ~~number~~ of suspect ~~and other~~ colonies (see General methods).	**4.6.6** Record the presence of suspect colonies (see General methods). If necessary estimate the number of cfu of suspect and other colonies.
9. ~~Polymerase chain reaction (PCR)~~ option 3.	**9.** Quantitative polymerase chain reaction (qPCR) option 3.
9.6 Determine Ct-values; Ct-values of positive control should consistently be lower than 30. The cut-off ~~Ct -alue~~ of internal amplification control (IAC) should…	**9.6** Determine Ct-values; Ct-values of positive control should consistently be lower than 30. The cut-off Ct - value of internal amplification control (IAC) should…

Chapter 7: General Methods (7-019a-10;7-019a-11; 7-019b-7).

CURRENT VERSION	PROPOSED VERSION
Reporting results: The results… In the case of a positive result, the report must indicate ~~the mean number of pathogen propagules (cfu) per seed and~~ the number of positive subsamples out of the total number tested.	**Reporting results:** The results… In the case of a positive result, the report must indicate the number of positive subsamples out of the total number tested. The number of cfu can be indicated.

General editorial changes: Seed Health Testing Method: 7-019b

Chapter 7: Methods: (7-019b).

CURRENT VERSION	PROPOSED VERSION
3.2 Dilute the suspension sufficiently to obtain dilutions containing approx. ~~10^2-10^4~~ cfu/ml. This…	**3.2** Dilute the suspension sufficiently to obtain dilutions containing approx. 10^{-2} to 10^{-4} cfu/ml. This…

5.5 Record the ~~number of suspect and other colonies (CCP)~~(see General methods) **7.7** Examine plants for the appearance of typical progressive V-shaped, yellow/necrotic lesions with blackened veins after 10–14 days (Fig. 4). Symptoms may be visible earlier depending on temperature and the aggressiveness of the isolate. Compare with positive control (CCP). It is important to discriminate between the progressive lesions caused by the vascular pathogen Xcc and the limited dark necrotic lesions at the inoculation site caused by leaf spot Xanthomonas (classified as *X. c. pv. raphani* ~~*or X.c. pv. armoraciae*~~ (see Kamounv et al., 1992; Alvarez et al., 1994; Tamura et al., 1994; Vicente et al., 2001; Roberts et al., 2004)	**5.5** Record the presence of suspect colonies (see General methods). If necessary estimate the number of cfu of suspect and other colonies. **7.7** Examine plants for the appearance of typical progressive V-shaped, yellow/necrotic lesions with blackened veins after 10–14 days (Figure. 4). Symptoms may be visible earlier depending on temperature and the aggressiveness of the isolate. Compare with positive control (CCP). It is important to discriminate between the progressive lesions caused by the vascular pathogen Xcc and the limited dark necrotic lesions at the inoculation site caused by leaf spot Xanthomonas (classified as *X. c. pv. raphani* (see Kamounv et al., 1992; Alvarez et al., 1994; Tamura et al., 1994; Vicente et al., 2001; Roberts et al., 2004)

Chapter 7: General methods (7-019b-7).

CURRENT VERSION	PROPOSED VERSION
Reporting results: The results of a seed health (…) In the case of a positive result, the report must indicate ~~the mean number of pathogen propagules (cfu) per seed and~~ the number of positive subsamples out of the total number tested.	**Reporting results:** The results of a seed health (…) In the case of a positive result, the report must indicate the number of positive subsamples out of the total number tested. The number of cfu can be indicated.

General editorial changes: Seed Health Testing Method: 7-020

Chapter 7: Methods: (7-020-4; 7-020-5).

CURRENT VERSION	PROPOSED VERSION
2.2 Prepare a tenfold dilution series from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (~~10^{1} dilution~~). Pipette 0.5 ml of the ~~10^{1} dilution~~ into another 4.5 ml of sterile saline and vortex mix (~~10^{2} dilution~~). Pipette 0.5 ml of the ~~10^{2} dilution~~ into another 4.5 ml of sterile saline and vortex to mix (~~10^{3} dilution~~) (See General methods).	**2.2** Prepare a tenfold dilution series from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (10^{-1} dilution). Pipette 0.5 ml of the 10^{-1} dilution into another 4.5 ml of sterile saline and vortex mix (10^{-2} dilution). Pipette 0.5 ml of the 10^{-2} dilution into another 4.5 ml of sterile saline and vortex to mix (10^{-3} dilution) (See General methods).

3.2 Dilute sufficiently to obtain dilutions containing approximately ~~10^{2} to 10^{4}~~ cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.	**3.2** Dilute sufficiently to obtain dilutions containing approximately 10^{-2} to 10^{-4} cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.
5.7 Record the ~~number~~ of suspect ~~and other~~ colonies (see General methods).	5.7 Record the presence of suspect colonies (see General methods). If necessary, estimate the number of cfu of suspect and other colonies.

Chapter 7: General methods (7-020-8).

CURRENT VERSION	PROPOSED VERSION
Reporting results: The results of a seed health test should (…) In the case of a positive result, the report must indicate ~~the mean number of pathogen propagules (cfu) per seed and~~ the number of positive subsamples out of the total number tested.	**Reporting results:** The results of a seed health test should (…) In the case of a positive result, the report must indicate the number of positive subsamples out of the total number tested. The number of cfu can be indicated.

General editorial changes: Seed Health Testing Method: 7-021

Chapter 7: Methods: (7-021-5;7-021-9).

CURRENT VERSION	PROPOSED VERSION
2.2 Prepare a tenfold dilution series from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (~~10^{1} dilution~~). Pipette 0.5 ml of the ~~10^{1} dilution~~ into another 4.5 ml of sterile saline and vortex mix (~~10^{2} dilution~~) (See General methods).	**2.2** Prepare a tenfold dilution series from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (10^{-1} dilution). Pipette 0.5 ml of the 10^{-1} dilution into another 4.5 ml of sterile saline and vortex mix (10^{-2} dilution) (See General methods).

3.2 Dilute sufficiently to obtain dilutions containing approximately ~~10^{2} to 10^{4}~~ cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.	**3.2** Dilute sufficiently to obtain dilutions containing approximately 10^{-2} to 10^{-4} cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.
5.6 Record the ~~number~~ of suspect ~~and other~~ colonies (see General methods).	5.6 Record the presence of suspect colonies (see General methods). If necessary, estimate the number of cfu of suspect and other colonies.

7.5 Inoculate plants with one positive X. ~~apisolate~~, and 2 negative controls: *X. vesicatoria* and distilled/deionised water.	**7.5** Inoculate plants with one positive *Xap* isolate, and 2 negative controls: *X. vesicatoria* and distilled/deionised water.

Chapter 7: General methods (7-021-10).

CURRENT VERSION	PROPOSED VERSION
Reporting results: The results of a seed health test should (…) In the case of a positive result, the report must indicate ~~the mean number of pathogen propagules (cfu) per seed and~~ the number of positive subsamples out of the total number tested.	**Reporting results:** The results of a seed health test should (…) In the case of a positive result, the report must indicate the number of positive subsamples out of the total number tested. The number of cfu can be indicated.

General editorial changes: Seed Health Testing Method: 7-023

Chapter 7: Methods: (7-023-4;7-023-5).

CURRENT VERSION	PROPOSED VERSION
2.2 Prepare a tenfold dilution series from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (~~10^{1} dilution~~). Pipette 0.5 ml of the ~~10^{1} dilution~~ into another 4.5 ml of sterile saline and vortex mix (~~10^{2} dilution~~) (See General methods).	**2.2** Prepare a tenfold dilution series from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (10^{-1} dilution). Pipette 0.5 ml of the 10^{-1} dilution into another 4.5 ml of sterile saline and vortex mix (10^{-2} dilution) (See General methods).

3.2 Dilute sufficiently to obtain dilutions containing approximately ~~10^{2} to 10^{4}~~ cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.	**3.2** Dilute sufficiently to obtain dilutions containing approximately 10^{-2} to 10^{-4} cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.
5.6 Record the ~~number~~ of suspect ~~and other~~ colonies (see General methods).	5.6 Record the presence of suspect colonies (see General methods). If necessary, estimate the number of cfu of suspect and other colonies.

Chapter 7: General methods (7-023-7).

CURRENT VERSION	PROPOSED VERSION
Reporting results: The results of a seed health test should (…) In the case of a positive result, the report must indicate ~~the mean number of pathogen propagules (cfu) per seed and~~ the number of positive subsamples out of the total number tested.	**Reporting results:** The results of a seed health test should (…) In the case of a positive result, the report must indicate the number of positive subsamples out of the total number tested. The number of cfu can be indicated.

General editorial changes: Seed Health Testing Method: 7-029

Chapter 7: Methods: (7-029-4;7-029-6).

CURRENT VERSION	PROPOSED VERSION
2.2 Prepare a tenfold dilution series from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (~~10^{1} dilution~~). Pipette 0.5 ml of the ~~10^{1} dilution~~ into another 4.5 ml of sterile saline and vortex mix (~~10^{2} dilution~~) (See General methods).	**2.2** Prepare a tenfold dilution series from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (10^{-1} dilution). Pipette 0.5 ml of the 10^{-1} dilution into another 4.5 ml of sterile saline and vortex mix (10^{-2} dilution) (See General methods).

3.2 Dilute sufficiently to obtain dilutions containing approximately ~~10^{2} to 10^{4}~~ cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.	**3.2** Dilute sufficiently to obtain dilutions containing approximately 10^{-2} to 10^{-4} cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.

SUBSECTION PROPOSED
5.2 : Examine the samples for the presence of typical *P syringae* pv. *pisi* colonies by comparison with the positive control plates. If necessary, estimate the number of cfu.

Chapter 7: General methods (7-029-7).

CURRENT VERSION	PROPOSED VERSION
Reporting results: The results of a seed health test should (…) In the case of a positive result, the report must indicate ~~the mean number of pathogen propagules (cfu) per seed and~~ the number of positive subsamples out of the total number tested.	**Reporting results:** The results of a seed health test should (…) In the case of a positive result, the report must indicate the number of positive subsamples out of the total number tested. The number of cfu can be indicated.

Modification of pictures

With the higher quality of pictures equipments, the SHC now suggest improved pictures for the Chap 7 methods

CURRENT VERSION	PROPOSED VERSION
Method 7-001a and 7-001b Fig 1a, b, c ~~b Single conidia~~ d ~~Conidium and simple conidiophores. ×350.~~	**Method 7-001a and 7-001b** See file attached b. Conidia of *Alternaria dauci*. d. Conidiophores and conidia of *Alternaria dauci* and chains of the saprophyte *Alternaria alternata* on carrot seed.
Method 7-002a and 7-002b Figure 1. ~~a Conidiophores and conidia of Alternaria radicina and chains of conidia of the saprophyte A. tenuis on a rootlet initial. ×80~~. ~~b Spreading hyphae and fructifications of the pathogen on the blotter. ×80. c Abundant growth and fructification of the pathogen on a rootlet initial. ×50. d Conidia of Alternaria radicina. ×350.~~	**Method 7-002a and 7-002b** See file attached a. Conidiophores and conidia of *Alternaria radicina* and chains of the saprophyte *Alternaria alternata* on a rootlet initial. b. Conidia and conidiophores of *Alternaria radicina* on carrot rootlet initial. c. Abundant growth and sporulation of *Alternaria radicina* on a rootlet initial. d. Conidia of *Alternaria radicina*
Method 7-003 Fig 1 left Fig 4 and 5	**Method 7-003** See file attached
Method 7-005 ~~Figure 2. Typical appearance of hyphae of A. pisi. Test conditions as for Figure 1~~	**Method 7-005** See file attached Figure 2. Colony of *Ascochyta pisi* on Malt Agar Figure 3. Conidia of *Ascochyta pisi* Figure 4. Pycnidia of *Ascochyta pisi*
Method 7-007 Figure 1. Olive-grey colonies of *A. linicola* and darker colonies of saprophytic *A. alternata* on malt agar ~~Figure 2. Conidia of Alternaria linicola. ×600~~	**Method 7-007** Figure 1. Olive-grey colonies of A. linicola and darker colonies of saprophytic *A. alternata* on malt agar (left) and close up of a colony of-*Alternaria linicola* (right) Figure 2. Conidia of *Alternaria linicola* x 400
Method 7-013a ~~Figure 1. Infected embryo, smut mycelium at S in scutellum~~ ~~Figure 2. Smut mycelium in scutellum~~	**Method 7-013a** Figure 1a. *U. nuda* in an embryo, 1b *U. nuda* in an embryo with stain 1c infected and healthy embryos with methyl blue stain

Method 7-013b	**Method 7-013b**
	Figure 1. *U. nuda* in an embryo
Method 7-014	**Method 7-014**
~~Figure 1. Slow growing, finely tufted, white aerial mycelium of Parastagonospora nodorum covering grain in an agar plate test.~~	Figure 1. *Parastagonospora nodorum* on PDA
Method 7-016	**Method 7-016**
~~Figure 1. Infected seeds overgrown by a dense, white, floccose mycelium (left) which often contains black, globose fruiting bodies (pycnidia) and/or black stromatic bodies (right)~~	Figure 1. Colonies of *Phomopsis complex* on APDA