



*ISTA
Method
Validation
Reports*



International Seed Testing Association (ISTA)

ISTA Method Validation Reports

Published by:
The International Seed Testing Association
P.O. Box 308
8303 Bassersdorf, CH-Switzerland

ISBN: 3-906549-
Volume 2003/1: 500 copies

Copyright © 2003 by the International Seed Testing Association

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

Preface

ISTA Method Validation Reports is a new ISTA publication initiated by the Plant Disease Committee. It will contain reports of method validation studies which support proposals for new or modified methods to be included in the International Rules for Seed Testing. Publication will coincide with announcements of rules proposals to be voted on by the ISTA membership at the next Extraordinary meeting.

As other Technical Committees adopt method validation procedures, the scope of this publication will be expanded to include the reports of their work.

Contents

Preface	
Revised method for detection of <i>Xanthomonas campestris</i> pv. <i>campestris</i> in Brassica seed	1
Procedures, instructions to authors and reviewers, and evaluation criteria for validation of seed health test methods	10

Roberts, S.J and Koenraad, H. (2003) *ISTA Method Validation Reports* 1, 1-9.

ISTA-PDC Technical report: Revised method for detection of *Xanthomonas campestris* pv. *campestris* in Brassica seed

S.J. ROBERTS¹ AND H. KOENRAADT²

¹Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK
(E-mail: steve.roberts@hri.ac.uk).

²The Naktuinbouw, P.O.Box 40, 2370 AA Roelofarendsveen, The Netherlands.

Submitted 01 Jan 2003 and accepted 15 April 2003

Summary

A number of changes are proposed to the previous ISTA working sheet for the detection of *Xanthomonas campestris* pv. *campestris* on Brassica seed, based on the results of an inter-laboratory comparative test and a study done in a single laboratory. Other changes are proposed to improve the clarity of the method and its utility for routine seed testing. The changes proposed include: no fungicides used in extraction buffer; the use of mCS20ABN instead of NSCAA; centrifugation step done in micro-centrifuge tubes; only one plate of each medium per dilution; minor changes to media preparation; simplified pathogenicity test method; removal of IF and direct plating assays; changes to format and layout. In the recommended method seeds are suspended in saline plus Tween 20 in a conical flask, which is then shaken for 5 min. Two 1 ml samples are removed and centrifuged. The flask is then shaken for a further 2.5 h and the extract diluted. Both centrifuged and diluted extracts are plated on FS and mCS20ABN media. Plates are incubated at 28-30°C for 3-4 d and then examined for the presence of suspected colonies of *Xanthomonas campestris* pv. *campestris*. Suspected colonies are sub-cultured to plates of YDC medium and their identity confirmed by a pathogenicity test on susceptible Brassica seedlings. It should be noted that the absence of the centrifugation step would result in a greater detection limit and an increased likelihood of false negative results (Roberts *et al.* 2003).

Introduction

Working sheet No 50 (2nd Edn) in the ISTA handbook on seed health testing (Schaad and Franken, 1996) provides a seed washing liquid plating assay *and* a direct plating assay for the detection of *Xanthomonas campestris* pv. *campestris* (*Xcc*) in Brassica seed. In the liquid plating assay, bacteria are first extracted from the seed by shaking in saline plus Tween 20, an aliquot of the extract is centrifuged after 5 min and the remaining extract is then diluted after 2.5 h stationary incubation; centrifuged and diluted extracts are plated on FS and NSCAA semi-selective media; the identity of suspected *Xcc* colonies is confirmed by a pathogenicity test. Although this method has formed the basis of the method used in most laboratories around the world, many laboratories were not following it precisely: using variations of the extraction medium and procedure, semi-selective media and pathogenicity test method. Consequently, there has been much debate about the comparability of results obtained and the relative merits or otherwise of the various modifications.

A comparative inter-laboratory test, organised in the framework of the International Seed Health Initiative (ISHI), evaluated the selective media and pathogenicity test methods in common use (Koenraad, 1997; Koenraad *et al.* 2003). A further investigation

(Roberts *et al.* 2003), sponsored by ISHI, was conducted in a single laboratory and compared the extraction procedure commonly used in many laboratories and the method in the ISTA working sheet No 50 (2nd Edn) (Schaad and Franken, 1996)

In this report we summarise the results of these studies, propose a number of changes to be included in a revised method description, and provide justification for the proposed changes.

Inter-laboratory comparative test

A complete report and analysis of the inter-laboratory comparative test is given by Koenraadt *et al.* (2003). The primary aim of the inter-laboratory comparative test was to evaluate four semi-selective media and two pathogenicity assays which were in common use for the detection of *Xcc* in seeds in a number of laboratories, but also compared extraction for 5 min and 2.5 h.

Three sub-samples of 10,000 seeds of eight seedlots were tested in thirteen laboratories in France, the Netherlands and the USA. Seeds were extracted by shaking for 5 min or 2.5 h and then plated on mCS20ABN, mFS, NSCAA, and NSCA media. The extraction procedure differed from the previous working sheet (Schaad and Franken, 1996) in that there was no centrifugation step and the seed was shaken continuously for 2.5 h.

Preliminary analysis of the data indicated that two of the laboratories had not performed the test correctly; data from these two laboratories were therefore excluded from subsequent analyses. Analysis of the data from the remaining eleven laboratories clearly indicated that mCS20ABN and mFS media were better than NSCAA and NSCA media, and that 2.5 h shaking was better than 5 min for 7 out of the 8 seedlots. Results obtained with the 'cabbage plant' pathogenicity assay were more consistent and easier to interpret than those obtained with the excised cotyledon assay.

The report concluded that two media (mCS20ABN and mFS) should be used as it was known that some strains of *Xcc* may be neomycin sensitive (i.e. inhibited on mCS20ABN) (Schaad, unpublished) or cephalixin sensitive (i.e. inhibited on mFS) (van Bilsen and Asma, unpublished)

A preliminary report of this comparative test (Koenraadt, 1997) suggested that two out of three media (mCS20ABN, mFS, NSCAA) should be used. A re-analysis of the data, however, indicated that the proportion of positive sub-samples is maximised, and the reproducibility dispersion, d_R (analogous to the reproducibility variance, s_R^2 in ISO-6725-2, Anon., 1994) is minimised by the use of mCS20ABN with mFS; therefore this combination is to be preferred.

Comparison of extraction methods in a single laboratory

A complete report and detailed analysis of these studies is given by Roberts *et al.* (2003). Two series of seed tests were carried out for the detection of *Xcc* in 107 Brassica seedlots. Sub-samples of 5,000 or 10,000 seeds were extracted in saline with a centrifugation step after 5 min shaken incubation followed by static incubation for 2.5 h or shaken

incubation for 2.5 h. Extracts were plated on semi-selective media (FS and NSCAA or mCS20ABN).

The inclusion of a centrifugation step after 5 min incubation of extracts increased the proportion of *Xcc* positive sub-samples and seedlots. Omission of the centrifugation step was estimated to give a 25% chance of false-negative results. Numbers of *Xcc* were similar on FS and NSCAA media, but saprophytes were greater on NSCAA medium. Numbers of *Xcc* were greater on FS medium than on mCS20ABN. Shaking increased the numbers of both *Xcc* and saprophytes detected, but the increase in *Xcc* was greater than the increase in saprophytes; shaking also resulted in a higher proportion of positive sub-samples/lots.

The report concluded that both centrifugation after 5 min and dilution after 2.5 h incubation should be used to minimise the likelihood of false negative results, that two media should be used to minimise the likelihood of false negative results and that continuous shaking represents an improved extraction procedure compared to static incubation of extracts.

This conclusion about centrifugation was in contrast to that of Franken *et al.* (1991), however, their results were based on only two seedlots and their interpretation may have been flawed.

Proposed changes and justifications

Removal of direct plating assay from the method

The direct plating assay represents a quite separate method from liquid plating assay, this method is not used routinely and has not been subjected to inter-laboratory comparative test, this method is therefore removed.

Removal of identification by immunofluorescence

It is recognised that this step or another serological test may provide a useful pre-screen to minimise the number of isolates tested in pathogenicity. However, the performance of IF and other serological tests is dependent on the particular antiserum used and was not evaluated in the comparative test. As this step is not essential for the performance and reliability of the test method, it is removed.

Media

The use of the semi-selective media mCS20ABN and FS instead of FS and NSCAA is proposed, as it is clear from the comparative test results (see above) that the combined results of mCS20ABN and FS give improved detection and greater reproducibility than FS and NSCAA. It is also clear from the comparison of extraction methods (Roberts *et al.* 2003) that these two media are superior to NSCAA in terms of selectivity. mCS20ABN was not recommended in the previous working sheet (Schaad and Franken, 1996), because of concerns about the toxicity of neomycin, however, it has become apparent (Woudt, pers. comm.) that this toxicity is related to the pH of the medium, which should be adjusted to below 6.6 to avoid problems. In addition, as two media are used, detection

failure is unlikely unless the *Xcc* strain(s) present in a particular seedlot is/are sensitive to the antibiotics in both media. We believe that the benefits for *routine* testing of increased selectivity of mCS20ABN compared to NSCAA outweigh the potential problems associated with sensitivity of a small number of strains to neomycin.

Media recipes

Some changes have been made to the recipes and specifications of the media to facilitate routine preparation and reduce costs. Wherever possible, stock solutions of antibiotics, and other components added after autoclaving, are prepared in alcoholic solutions to avoid the need for, and cost of, filter sterilisation. Wherever possible, the concentrations of stock solutions have been adjusted to minimise the number of different volumes of components which are added, thus reducing the likelihood of errors.

There are a number of slightly different published recipes for FS medium (Schaad, 1989; Yuen *et al.* 1987; Schaad and Franken, 1996; Schaad *et al.* 2002). The prefix 'm' is used to denote one of these variations in Koenraadt *et al.* (2003). The basis for some differences is not clear and it is possible they result from typing errors. The most important difference, however, is the inclusion or omission of gentamycin, which is reported to be inhibitory to some Xanthomonads (Schaad *et al.* 2002). It should be noted that the concentration of gentamycin (0.4 mg/l) is well below the minimum inhibitory concentration of 80 mg/l reported by Chang *et al.* (Chang *et al.* 1991). The recipe given by Schaad (1989) which is the reference given in the previous working sheet (Schaad and Franken, 1996) and which contains gentamycin is used as the basis for the recipe for FS in the proposed working sheet. As two media are used, detection failure is unlikely unless the *Xcc* strain(s) present in a particular seedlot are sensitive to the antibiotics in both media.

Extraction medium

The extraction medium (0.85% saline) in the previous working sheet (Schaad and Franken, 1996) contains Tween 20 and suggests the inclusion of the fungicides benomyl and chlorothalonil, if fungi are expected to be a common contaminant. Franken *et al.* (1991) suggested that chlorothalonil (as Daconil) was inhibitory to *Xcc* and that benomyl was not necessary because cycloheximide in the selective media gave adequate suppression of fungi. We cannot see any benefit from the inclusion of these fungicides and therefore they are omitted.

Centrifugation step

The previous working sheet (Schaad and Franken, 1996) includes a centrifugation step after 5 min extraction. The centrifugation step makes a 10× concentrate of the seed extract. Theoretically, therefore, it provides a tenfold reduction in the detection limit of the test in terms of the number of *Xcc* that can be detected in an individual sub-sample. The results of the study to compare extraction methods (see above) clearly showed a benefit in terms of the proportion of *Xcc*-positive sub-samples/lots detected from inclusion of a centrifugation step.

Detection of *Xanthomonas campestris* pv. *campestris*

The principle of the test method is not affected by the inclusion/exclusion of the centrifugation step, but clearly the detection limit is affected and therefore, indirectly, the tolerance standard. It has been agreed by the ISTA-PDC that it is not the role of ISTA to set tolerance standards for seedborne plant pathogens, as these may vary according to the purpose of the test and the environment in which the crop is grown. Following a lengthy debate, the ISTA-PDC Bacteriology Working Group (Wageningen, The Netherlands, 01 May 2002) decided that it would be acceptable to make the centrifugation step optional. However, following further consideration by the ISTA-PDC Method Validation sub-committee (Conference Call, October 2002), it was concluded that optional steps could not be permitted in *Official ISTA Methods* and therefore the centrifugation step will be included as an obligatory part of the method.

Centrifugation method

In the previous working sheet (Schaad and Franken, 1996), the centrifugation step is done on a 20 ml sample of extract which is then pelleted and re-suspended in 2 ml. In order to facilitate routine application, and avoid the need for an expensive centrifuge, centrifugation will now be done on 1 ml samples in micro-centrifuge tubes, with re-suspension of the pellet in 0.1 ml.

Test for antagonists

The previous working sheet (Schaad and Franken, 1996) contains a test for the presence of antagonists, but no indication how this information should be used or reported. The presence of antagonists alone does not necessarily imply that un-detected *Xcc* are present in a sub-sample and therefore would appear to be uninformative with respect to the health status of a seedlot. We consider that this test is not appropriate for routine testing and may present quality assurance problems due to the production of aerosols of *Xcc* in the laboratory. We are also not aware of any laboratories that implement this test on a routine basis. Although the potential for detection failures due to the presence of antagonists can never be ruled out, and will vary for different seed lots, we believe that the use of the two selective media containing different selective agents obviates this problem as far as possible. The test for antagonists is removed.

Number of plates per dilution

In the previous working sheet (Schaad and Franken, 1996), each dilution is plated on four replicate plates. One of these is used in the test for antagonists, leaving three replicate plates at each dilution for evaluation. This level of replication greatly increases the number of plates required for the test, the preparation time, and the amount of labour required to prepare, plate and evaluate the test. Examination of data from several comparative tests for bacterial pathogens has indicated that there is little information to be gained from even duplicate plates, as the variance is less than or equal to the expected Poisson variance for counts. Replication of plates therefore seems to achieve little other than a check that the operator can use a pipette correctly. As the operator should have received appropriate training, and as this check can be partly achieved by comparison of counts of dilutions of

a reference (control) strain on the two semi-selective media, there seems to be no practical value, for routine testing, in preparing even duplicate plates. Therefore each dilution is plated onto one plate of each of two selective media.

Controls

The inclusion of positive control strains was only implied in the previous working sheet. The new method makes explicit reference to the use of positive control strains or reference materials and a sterility check.

Pathogenicity test

The pathogenicity test in the previous working sheet required preparation of a suspension from each isolate. The pathogenicity test is simplified by inoculation of plants using inoculum scraped directly from an agar plate with a pin or toothpick. This reduces the labour and consumables required. The method gave consistent results in all laboratories that took part in the comparative test (see above). In addition, during an ISTA teaching workshop on detection of bacteria in seeds at the University of Lavras, Brazil, 1999, inexperienced participants obtained reproducible and unambiguous results using the method.

Format and layout

Changes have been made to the format and layout of the method to improve readability and reduce ambiguity.

Estimation of reproducibility and repeatability

International standard ISO 5725-2 (Anon, 1994) presents guidelines for the determination of the precision (i.e. repeatability and reproducibility) of a test method, under the assumption that the data follow a normal distribution, in which the variance is constant. The data from the comparative test follow a binomial distribution, in which the variance is a function of the mean. For binomial data, the statistic which is analogous to the variance is the mean deviance or dispersion parameter, d , where the variance of a proportion, p , = (variance function) \times (dispersion parameter) = $p \times (1 - p) \times d$. Data from the comparative test for the recommended combination of media (FS and mCS20ABN) and an extraction time of 2.5 h, were used to estimate the reproducibility dispersion at 0.72, repeatability dispersion at 0.63, and between laboratory dispersion at 0.09. As these values are less than the theoretical minimum of 1 for binomial data, it is suggested that a dispersion parameter of 1 is used to estimate the precision of test results.

Conclusions and recommendations

We believe that the results of the inter-laboratory comparative tests, the comparison of extraction methods in a single laboratory, and the arguments above present sufficient justification for the changes proposed to the previous working sheet. We recommend that

the revised method, as briefly described below, should be accepted as an ISTA Validated Method.

Seeds are suspended in saline plus Tween 20 in a conical flask, which is then shaken for 5 min. Two 1 ml samples are removed and centrifuged. The flask is then shaken for a further 2.5 h and the extract diluted. Both centrifuged and diluted extracts are plated on FS and mCS20ABN media. Plates are incubated at 28-30°C for 3-4 d and then examined for the presence of suspected colonies of *Xanthomonas campestris* pv. *campestris*. Suspected colonies are sub-cultured to plates of YDC medium and their identity confirmed by a pathogenicity test on susceptible Brassica seedlings.

References

- Anon. (1994) International Standard ISO 5725-2: Basic method for the determination of repeatability and reproducibility of a standard measurement method. Geneva, Switzerland: International Standards Organisation.
- Chang, C.J., Donaldson, R., Crowley, M. and Pinnow, D. (1991) A new semi-selective medium for the isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seed. *Phytopathology* **81**, 449-453.
- Franken, A.A.J.M., van Zeijl, C., van Bilsen, J.G.P.M., Neuvel, A., de Vogel, R., van Windgerden, Y., Birnbaum, Y.E., van Hateren, J. and van der Zouwen, P.S. (1991) Evaluation of a plating assay for *Xanthomonas campestris* pv. *campestris*. *Seed Science and Technology* **19**, 215-226.
- Koenraad, H. (1997) Comparative test for the detection of *Xanthomonas campestris* pv. *campestris* in crucifer seeds. In: Hutchins, J.D. and Reeves, J.C., (Eds.) *Seed Health Testing. Progress towards the 21st Century*, pp. 205-209. CAB International, Wallingford, Oxon, UK.
- Koenraad, H., van Bilsen, J.G.P.M. and Roberts, S.J. (submitted) Comparative test for the detection of *Xanthomonas campestris* pv. *campestris* in Brassica seeds. *Seed Science and Technology*.
- Roberts, S.J., Brough, J., Everett, B. and Redstone, S. (submitted) Extraction methods for *Xanthomonas campestris* pv. *campestris* from brassica seed. *Seed Science and Technology*.
- Schaad, N.W. (1989) Detection of *Xanthomonas campestris* pv. *campestris* in Crucifers. In: Saettler, A.W., Schaad, N.W. and Roth, D.A., (Eds.) *Detection of bacteria in seeds and other planting material*, pp. 68-75. American Phytopathological Society, St. Paul, USA.
- Schaad, N.W. and Franken, A.A.J.M. (1996) ISTA Handbook on Seed Health Testing Working Sheet No 50 (2nd Ed): *Xanthomonas campestris* pv. *campestris*. ISTA, Zurich.
- Schaad, N.W., Jones, J.B., Lacy, G.H. (2002) *Xanthomonas*. In: Schaad, N.W., Jones, J.B. and Chun, W. (Eds) *Laboratory guide for the identification of plant pathogenic bacteria*. 3rd edn. pp. 175-200. American Phytopathological Society, St Paul, USA.
- Yuen, G.Y., Alvarez, A.M., Benedict, A.A. and Trotter, K.J. (1987) Use of monoclonal antibodies to monitor the dissemination of *Xanthomonas campestris* pv. *campestris*. *Phytopathology* **77**, 366-370.

Addendum

This addendum is provided as a summary of the main results contained in manuscripts which have been submitted to, but not yet published in, *Seed Science and Technology*.

The following two tables have been extracted from Koenraad *et al.* (submitted). Eight seed lots were distributed among thirteen laboratories in the USA, France, and the Netherlands. Bacteria from the seed lots were extracted by shaking for 5 minutes or 2.5 hours, followed by dilution plating onto mCS20ABN, mFS, NSCA, and NSCAA semi-selective media. Suspected *Xcc* colonies were sub-cultured to YDC medium and a selection were tested in ‘cabbage plant’ and ‘excised cotyledon’ pathogenicity assays.

Table 1 presents the mean proportions of positive sub-samples for each medium and extraction time. Although mCS20ABN gave slightly more positive samples than mFS this difference was not significant; both mCS20ABN and FS were significantly better than NSCAA; and mCS20ABN, FS and NSCAA were all better than NSCA. Increasing extraction time generally gave significantly more positive sub-samples on the most selective media (mCS20ABN and mFS) but not on the least selective (NSCAA, NSCA).

Table 1. Proportion of positive sub-samples of 10,000 seeds for each medium and extraction time. Values were obtained as predictions from the appropriate model (from Koenraad *et al.*, submitted).

Medium	Extraction time				Mean \pm s.e. ¹	
	5 min \pm s.e. ¹		2.5 h \pm s.e. ¹			
mCS20ABN	0.62	0.03	0.72	0.03	0.67	0.02
mFS	0.58	0.03	0.67	0.03	0.62	0.02
NSCAA	0.55	0.03	0.57	0.03	0.56	0.02
NSCA	0.39	0.03	0.40	0.03	0.39	0.02

¹ Approximate standard errors, based on a dispersion parameter of 1.

Table 2 presents the mean proportion of positive sub-samples and estimates of the reproducibility and repeatability dispersion for an extraction time of 2.5 h and different media combinations. The proportion of positive sub-samples was maximised and the reproducibility and repeatability were minimised when using a combination of mCS20ABN and mFS media.

Table 2. Effect of different media combinations (2.5 h extraction time) on the proportion of positive sub-samples of 10,000 seeds, p , and dispersion values for reproducibility, d_R , repeatability, d_r , between laboratories d_l (from Koenraad *et al.*, submitted).

Media	p \pm s.e. ¹		d_R	d_r	d_l
mCS20ABN + mFS	0.78	0.03	0.722	0.630	0.092
mCS20ABN + NSCAA	0.72	0.03	1.077	1.133	0.000
mFS + NSCAA	0.70	0.03	1.407	1.308	0.099

¹ Approximate standard errors, based on a dispersion parameter of 1.

The following two tables have been extracted from Roberts *et al.* (submitted). Sub-samples of 5,000 (*Series 1*) or 10,000 seeds (*Series 2*) were extracted in saline with a centrifugation step after 5 min shaking incubation followed by stationary incubation or shaking incubation and plating on semi-selective media (FS and NSCAA or mCS20ABN).

Table 3 presents a summary of results for the first series of tests. The number of positive sub-samples and lots was maximised by a combination of centrifugation after 5 min and dilution after 2.5 h, and by a combination of both FS and NSCAA media.

Table 3. Numbers of sub-samples of 5,000 seeds and seedlots for *Series 1* (stationary incubation) in which *Xanthomonas campestris* pv. *campestris* was detected, summarised by centrifugation (media combined) or medium (methods combined) (from Roberts *et al.*, submitted).

	Sub-samples		Lots	
	Pos.	Neg.	Pos.	Neg.
<i>Centrifugation</i>				
Diluted	16	637	9	48
Centrifuged	17	636	10	47
Both ¹	6	647	5	52
Combined ²	27	626	14	43
<i>Medium</i>				
FS	18	635	9	48
NSCAA	22	631	12	45
Both ¹	13	640	7	50
Combined ²	27	626	14	43

¹ Sub-samples/lots in which *Xcc* detected in *both* diluted and centrifuged extracts or on *both* media

² Sub-samples/lots in which *Xcc* detected in *either* diluted or centrifuged extracts or on *either* medium.

Table 4 presents a summary of results for the second series of tests. Again the number of positive sub-samples and lots was maximised by a combination of centrifugation after 5 min and dilution after 2.5 h, and by a combination of both mCS20ABN and FS media. Shaking for 2.5 h gave a greater number of positive sub-samples and lots than stationary incubation for 2.5 h.

Table 4. Numbers of sub-samples of 10,000 seeds and seedlots for *Series 2* with suspected/confirmed colonies of *Xanthomonas campestris* pv. *campestris*, summarised by centrifugation, medium or extraction method (from Roberts *et al.*, submitted).

	Suspected				Confirmed			
	Sub-samples		Lots		Sub-samples		Lots	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
<i>Centrifugation</i>								
Diluted	57	243	20	30	21	279	9	41
Centrifuged	59	241	18	32	21	279	9	41
Both ¹	49	251	15	35	18	282	8	42
Combined ²	67	233	23	27	24	276	10	40
<i>Medium</i>								
mCS20ABN	44	256	16	34	17	283	8	42
FS	59	241	21	29	21	279	10	40
Both ¹	36	264	14	36	14	286	8	42
Combined ²	67	233	23	27	24	276	10	40
<i>Method (excluding centrifugation)</i>								
Stationary	24	126	12	38	9	141	5	45
Shaking	33	117	16	34	12	138	8	42

¹ Sub-samples/lots in which *Xcc* detected simultaneously in *both* diluted and centrifuged extracts or on *both* media

² Sub-samples/lots in which *Xcc* detected in *either* diluted or centrifuged extracts or on *either* medium.

Procedures, instructions to authors and reviewers, and evaluation criteria for validation of seed health test methods

ISTA-PDC METHOD VALIDATION SUB-COMMITTEE

Chairperson: J.W. Sheppard, *Canadian Food Inspection Agency, Canada*

Quality Assurance: V. Cockerell, *Scottish Agricultural Science Agency, United Kingdom*

Bacteriology: S.J. Roberts, *Horticulture Research International, United Kingdom*

Mycology: G. Brodal, *Norwegian Agricultural Inspection Service, Norway*

Virology: H. Koenraad, *Naktuinbouw, The Netherlands*

Nematology: P. Remeus, *Naktuinbouw, The Netherlands*

Statistics: S. Grégoire, *Geves, France*

12 February 2003

Introduction

The following pages set out the procedures to be followed for review and approval of seed health test methods, together with instructions to authors for the preparation of method descriptions and supporting method validation reports. The instructions and evaluation criteria for reviewers and the proposal form are also provided to assist authors.

Procedure for review and approval of Seed health test methods

The ISTA Secretariat maintains a record of the review process and endeavours to ensure that it proceeds in a timely fashion.

1. The proposed method description, validation report and other supporting documents are submitted to the ISTA Secretariat together with an application form.
2. Where validation reports are not presented according to the instructions to authors, these will be returned to the author, without review.
3. The Method Validation Sub-committee (MVSC) identifies an individual member of the MVSC ('editor') who will, acting on behalf of the MVSC, take primary editorial responsibility for the method and report, the review process, and for correspondence with the author and reviewers. The MVSC identifies two independent technical reviewers with acknowledged expertise in the field and a statistical reviewer. Method authors/proposers are welcome to nominate reviewers, who will be accepted at the discretion of the MVSC.
4. The proposed method description, validation report and other supporting documents are transmitted to reviewers, together with instructions and evaluation forms.
5. Technical reviewers evaluate both the proposed method description and the validation report, and any other supporting documents, the statistical reviewer evaluates the validation report and other supporting documents. If a reviewer does not respond or is not able to respond within a reasonable time, alternative reviewers will be sought.
6. Following review, and based on the advice of the reviewers, the editor will send copies and make a recommendation to the MVSC who will then decide to:
 - a) approve the method description and validation report without revision
 - b) approve the method description and validation report following minor revisions to the method description and/or validation report
 - c) defer a decision pending major revisions to the method description and/or validation report
 - d) reject the method
7. Where reviews are considered inadequate or reviewers do not agree, the MVSC will, at its discretion, make a final decision or seek the advice of additional reviewers.
8. The MVSC will notify the authors/proposer and reviewers of its decision with reasons and, if appropriate, request revisions.
9. Methods in category (b) will only be considered approved following completion of the required revisions to the satisfaction of the MVSC.
10. Methods in category (c) will be re-considered by the MVSC following completion of the required revisions and may be subjected to further independent review. Completion of revisions will not guarantee approval.
11. Following approval of the method by the MVSC, the method receives Validated status and is recommended to the Rules committee for Official status.
12. Methods recommended for Official status by the MVSC are listed on the *ISTA Online website* prior to a vote by the membership. The notice will include a citation for the validation report. Validation reports, if not previously published, will be formally

published on an annual basis in *ISTA Method Validation Reports*. Copies of the method description and validation report will be available from the ISTA Secretariat. Any interested party may submit comments and data in writing to ISTA for or against its adoption as an Official method. The ISTA Secretariat will forward copies of all comments to the MVSC for resolution.

13. Methods recommended by the MVSC, plus any comments received, will be sent to the Rules Committee for inclusion in a member ballot.
14. Methods will be adopted as Official ISTA Seed Health Testing Methods if accepted by a majority of voting members and will be published in the *International Rules for Seed Testing, Annexe to Chapter 7: Seed Health Testing Methods*.

Instructions to authors for the preparation of seed health test method descriptions

The method should be technically sound and must be practical for use in routine seed health testing.

The method description should be written in English (UK).

The method description should follow the same style and format as method descriptions in the current *International Rules for Seed Testing, Annexe to Chapter 7: Seed Health Testing Methods*, with sections appropriate to the particular method.

A Microsoft Word™ template is available from the MVSC to assist with preparation.

The method description should be supported by a validation report which clearly states the basis for the method and wherever possible gives estimates of the accuracy (bias and precision) of the method.

The method description must be clear and unambiguous, remembering that the method may be followed by persons whose first language is not English.

The method description should include appropriate safety and environmental precautions.

All critical steps must be identified.

Critical control points must be identified and adequate to ensure reliable test results.

All reagents and instruments must be described in performance terms with system suitability tests where necessary.

Sources of critical materials and reagents should be given.

Estimates of the detection limits, reproducibility and repeatability should be given wherever possible.

All tables, figures and terms must be adequately explained.

Instructions to authors for the preparation of validation reports proposing new methods or revisions

The report should be a self-contained document suitable for publication in a scientific journal.

It should be written as a scientific paper following the general style of Seed Science and Technology (see instructions to authors in a recent edition of SST or on the ISTA Online web site).

The proposed new method or revisions should be clearly stated.

The report should present justifications for the new method or revisions based on: (a) the results of scientific studies contained in the report itself; or (b) by reference to scientific studies published elsewhere in a peer-reviewed journal; or (c) by reasoned argument; or any combination of the three.

The methods validation sub-committee would prefer to receive documents in electronic form whenever possible.

The report should normally contain the follow sections:

In all cases:

Title – which should begin: *A revised/new method for.....*

Authors – names and addresses of authors

Summary – a short summary of the study and the proposed method

Introduction – stating the problem, reasons for the study, the purpose of the method, pertinent background information and history of the method with reference to previously published information and if appropriate the objective(s) of the collaborative study(ies).

If reporting the results of scientific studies directly:

Materials and methods – full details of the materials and methods used and design of the study, including the method(s) of statistical analysis.

Results – of the study, statistical analysis and summaries of the data in the form of tables and/or figures, presented in sufficient detail and with appropriate measures of variation to allow the reader to draw independent conclusions. If appropriate, justifications for exclusion of raw data.

Discussion – discussion of the method performance including comments from collaborators and how they were addressed.

If referring to scientific studies published elsewhere:

Summaries and discussion of the external studies – results of external studies which have been published elsewhere should be summarised/reviewed and discussed in terms of method performance.

In all cases:

Proposed changes and justification – if appropriate clearly identify proposed changes to an existing method with justification.

Procedures, Instructions, Criteria

Estimates of repeatability and reproducibility – give estimates of the repeatability and reproducibility of the test method and how these are calculated.

Conclusions and recommendations – a clear statement of the conclusions of the report and recommendations for actions.

Acknowledgements – of collaborators if not co-authors, funding bodies, etc. as appropriate.

References – details of all cited references.

Reports which are not presented in the correct format and/or which do not fulfil these requirements will be rejected.

Raw data

A hard copy and an unformatted computer text file of the raw data should be deposited with the ISTA Secretariat. To maintain confidentiality the identity of individual participating laboratories should be indicated only by a coded identifier.

Copyright

Submission of a manuscript implies that it has not been published elsewhere and that all co-authors have approved the manuscript. The International Seed Testing Association will retain the copyright of the method description and the technical report.

Procedures, Instructions, Criteria

Recommendation (delete as applicable):

- a) approve the method without revision
- b) approve the method following minor revisions to the method/report
- c) defer a decision pending major revisions to the method/report
- d) reject the method

How confident are you with your assessment ?

Are you happy for your name to be revealed to the author(s) ?

Instructions and evaluation criteria for reviewers: Validation report

Please review the enclosed validation report with reference to the evaluation criteria below and making comments on additional sheets as appropriate. Please indicate any aspects on which you do not feel qualified to comment.

Method: Reviewer No:
 Author: Review request date:
 Submission date: Review returned date:

The method should be considered as:

New host/pathogen combination	
Additional method for an existing host/pathogen	
Replacement for method	
Method modification	

Evaluation Criteria (not all aspects will necessarily apply):

	Yes	No	See comments
Is the title appropriate ?			
Is the summary clear/adequate ?			
Is the reason for the study clearly stated ? (i.e. objective(s), aim, questions, hypothesis that test organiser wishes to address)			
Has previous literature/data been reviewed adequately ?			
Is the cited literature appropriate, are there any omissions ?			
In the case of inter-laboratory comparative test – is there evidence that the guidelines have been followed as far as possible ?			
Have technical difficulties/problems been highlighted ?			
Have the comments of participants been reported/addressed ?			
Has the practical application of the method been considered/discussed in terms of routine use: e.g. cost and time schedule, training/staff requirements, availability of reagents (esp. serological), licensing of PCR or other patented methods ?			
Has account been taken of other relevant international standards ?			
Was the design of the study appropriate ?			
Were the controls adequate to ensure reliability of the data reported ?			
Were reference materials/cultures included and are their results reported ?			

Procedures, Instructions, Criteria

Were steps taken to ensure the integrity of the data, i.e. blind testing/coding of samples ?			
What checks were done to ensure that each participant followed the protocol ?			
Has a statistical analysis been performed ?			
Is the statistical analysis appropriate to the data, has the approach been justified ?			
Has sufficient data been presented to allow independent assessment ?			
Is the exclusion of particular data/laboratories from the analysis justified ?			
Has the accuracy, reproducibility and repeatability of the method(s) been estimated and clearly stated ?			
Are the conclusions justified by the data and statistical analysis ?			
Are all tables, figures, equations, and terms sufficiently explained ?			
Are the summaries (graphs/tables) of the data appropriate ?			
Could any figures or tables be explained by a simple statement ?			
Are the proposed changes clearly stated and justified ?			
Have the conclusions and recommendations been clearly stated ?			
Are the references correct ?			
Are all the cited reports/data publicly available ?			
Have steps been taken to archive the raw data, ensure availability for re-analysis/future studies ?			

Please make comments on an additional sheet.

Recommendation (delete as applicable):

- a) approve the report without revision
- b) approve the report following minor revisions
- c) defer a decision pending major revisions to the report
- d) reject the report

How confident are you with your assessment ?

Are you happy for your name to be revealed to the author(s) ?

