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Document OGM26-05

Rules Proposals for the International Rules for Seed Testing 2027 Edition

This document was prepared by the Technical Committees (TCOMs) and the Rules Committee of the Association and has been endorsed by the ISTA Executive Committee (ECOM). The proposals are submitted to the ISTA Ordinary General Meeting 2026 for voting by the nominated ISTA Designated Members on behalf of their respective Governments.

It is submitted to all ISTA Designated Authorities, ISTA Members and ISTA Observer Organizations for information two months prior to the ISTA Ordinary General Meeting 2026.

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 2026 to be held on 25 June 2026, in Calgary, Canada.

Introduction to the ISTA Rules Proposals to become effective 1 January 2027

The current version of the ISTA International Rules for Seed Testing (ISTA Rules) is the 2026 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.

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 Calgary, Canada on 25 June 2026, 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 2027 edition of the ISTA Rules. Please let us know about any problems with these proposals.

Many thanks.
Jodi Keeling and Janek Bartel
Chair and Vice-Chair of ISTA Rules Committee

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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

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Commented [JB1]: Corrected typographical error

PART A. INTRODUCTION OF EDITORIAL CHANGES

Form 1: Inclusion of new species in the ISTA Rules

Form 1 in the Introduction to the ISTA Rules is missing a place to record the minimum submitted sample for moisture testing for Table 2C. It is also missing a place to record the seedling evaluation group for Table 5A.

There is no corresponding AOSA Rule.

This editorial change proposal was submitted by the ISTA Rules Committee Chair and Vice-Chair.

RUL Committee Votes	Yes:	11	No:	0	Abstain/Absent:	8

CURRENT VERSION

Form 1: Proposal for inclusion of new species in the ISTA Rules

2. Lot and sample weights

(Information as it should appear in Table 2C)

Species	Maximum weight of lot (kg)	Minimum submitted sample (g)	Minimum working samples (g) Use 'Calculator for adding working weights to Table 2C'	
			Purity analysis (3.5.1)	Count of other species (4.5.1)

5. Validated germination test method(s)

(Information as it should appear in Table 5A)

Species	Prescriptions for:				Additional directions incl. recommendations for breaking dormancy
	Substrate	Temperature (°C)	First count (d)	Final count (d)	

PROPOSED VERSION

Form 1: Proposal for inclusion of new species in the ISTA Rules

2. Lot and sample weights

(Information as it should appear in Table 2C)

Species	Maximum weight of lot (kg)	Minimum submitted sample (g)	Minimum working samples (g) Use 'Calculator for adding working weights to Table 2C'		Minimum submitted sample for moisture testing (g)
			Purity analysis (3.5.1)	Count of other species (4.5.1)	

5. Validated germination test method(s)

(Information as it should appear in Table 5A)

Species	Prescriptions for:				Additional directions incl. recommendations for breaking dormancy	Seedling evaluation group
	Substrate	Temperature (°C)	First count (d)	Final count (d)		

Reporting Determination of Other Seeds by Number

4.7 was changed in 2024 to report to the number of decimal places indicated in Table 4A, instead of to the minimum number of decimals. The consequential change to 1.5.2.4 was missed.

This proposal aligns with the AOSA Rules.

This was noticed by an auditor and an editorial change proposed by the Rules Committee Chair and Vice-chair.

RUL Committee Votes	Yes:	10	No:	0	Abstain/Absent:	9

CURRENT VERSION	PROPOSED VERSION
1.5.2.4 Determination of other seeds by number …… The sample weight examined must be reported according to the ~~minimum~~ number ofdecimals indicated in ~~4.7~~.	**1.5.2.4 Determination of other seeds by number** …… The sample weight examined must be reported according to the number of decimal places indicated in Table 4A.

Minimum sampling intensity

BSC received questions regarding how to sample small containers. The ISTA Handbook on Seed Sampling covers this thoroughly and proposes a formula for determining sampling units. We propose to add this formula to the ISTA Rules as an example. This proposal also clarifies the statement that seed lots with up and including 15 containers must have the same number of primary samples from each container sampled.

There is no corresponding AOSA Rule.

This proposal was submitted by the Bulking and Sampling Committee

BSC Committee Votes	Yes:	14	No:	0	Abstain/Absent:	1

CURRENT VERSION	PROPOSED VERSION
2.5.1.2 Minimum sampling intensity For seed lots in containers holding up to and including 100 kg, …… b. For containers holding less than 15 kg of seed, containers must be combined into sampling units not exceeding 100 kg, e.g. 20 containers of 5 kg, 33 containers of 3 kg or 100 containers of 1 kg.	**2.5.1.2 Minimum sampling intensity** For seed lots in containers holding up to and including 100 kg, …… b. For containers holding less than 15 kg of seed, containers must be combined into sampling units not exceeding 100 kg, e.g. 20 containers of 5 kg, 33 containers of 3 kg or 100 containers of 1 kg. The following formula can be used to calculate the number of sampling units of a seed lot by always rounding up the calculated result: $$\text{Sampling units} = \frac{(\text{Number of containers*}) \times (\text{Container size in kg})}{100}$$ * smallest container the seed is packed in.

Commented [JB2]: After the open Rules discussion, the structure of the calculation was changed to harmonize with the rest of the rules

Commented [JB3R2]: Editorial Minimum Sampling intensity

The sampling units must be regarded as containers as described in Table 2A. …… When sampling a seed lot of up to 15 containers, regardless of their size, the same number of primary samples must be taken from each container.	The sampling units must be regarded as containers as described in Table 2A. See the ISTA Handbook on Seed Sampling for examples. …… When sampling a seed lot of up to and including 15 containers, regardless of their size, the same number of primary samples must be taken from each container.

Clarification of seed stream wording

This proposal is to make the wording of different Rules in Chapter 2 consistent with each other, and clarify the wording regarding sampling from a seed stream. Manual and automatic sampling from seed streams could take place at any point after all processing, conditioning and treatment of the seed has been done, not necessarily when it enters the containers, therefore it is proposed to clarify this and to delete this wording enters and entering.

There is no corresponding AOSA Rule.

This proposal was submitted by the Bulking and Sampling Committee

BSC Committee Votes	Yes:	14	No:	0	Abstain/Absent:	1

CURRENT VERSION	PROPOSED VERSION
2.5.1.1 Preparation of a seed lot and conditions for sampling …… Seed may be sampled in containers or from the seed stream~~, either before or when it enters containers~~.	**2.5.1.1 Preparation of a seed lot and conditions for sampling** …… Seed may be sampled in containers or from the seed stream. Sampling from the stream must be done after all processing and/or treatment of the seed has been completed as these actions may have an influence on the quality ofthe seed.
2.5.1.2 Minimum sampling intensity …… When sampling seed in containers holding more than 100 kg of seed, or from streams ~~of seed entering containers~~, the sampling intensity according to Table 2B must be regarded as the minimum requirement.	**2.5.1.2 Minimum sampling intensity** …… When sampling seed in containers holding more than 100 kg of seed, or from seed streams, the sampling intensity according to Table 2B must be regarded as the minimum requirement.
2.5.1.3 Taking primary samples …… When seed is to be packed in special types of containers (e.g. small, not penetrable, or moisture-proof containers), it should be sampled, if possible, ~~either before or during the filling of the containers~~.	**2.5.1.3 Taking primary samples** …… When seed is to be packed in special types of containers (e.g. small, not penetrable, or moisture-proof containers), it should be sampled, if possible, either from the containers of the seed lot the seed was originally packed in, or from the seed stream. See 2.5.1.1.

Table 2C: Minimum submitted sample for moisture testing

It was discovered that the minimum submitted sample size for moisture testing for *Pennisetum clandestinum* and *Pennisetum glaucum* are missing from Table 2C, despite there being methods in Chapter 9.

There is no corresponding AOSA Rule.

BSC Committee Votes	Yes:	13	No:	0	Abstain/Absent:	2

CURRENT VERSION

Table 2C: Lot sizes and sample sizes

Species	**Maximum weight of lot (kg) (except see 2.8 Note 2)**	**Minimum submitted sample (g)**	**Purity analysis (3.5.1)**	**Other seeds by number (4.5.1)**	**Minimum submitted sample size for moisture testing (g)**
1	**2**	**3**	4	**5**	**6**
Pennisetum clandestinum Hochst. ex Chiov.	10 000	70	7	70	~~N/A~~
Pennisetum glaucum (L.) R.Br.	10 000	150	15	150	~~N/A~~

PROPOSED VERSION

Table 2C: Lot sizes and sample sizes

Species	**Maximum weight of lot (kg) (except see 2.8 Note 2)**	**Minimum submitted sample (g)**	**Purity analysis (3.5.1)**	**Other seeds by number (4.5.1)**	**Minimum submitted sample size for moisture testing (g)**
1	**2**	**3**	4	**5**	**6**
Pennisetum clandestinum Hochst. ex Chiov.	10 000	70	7	70	50
Pennisetum glaucum (L.) R.Br.	10 000	150	15	150	50

Object of Other seed determination

This proposal is made for precision: In the other seed determination the number of seeds is not estimated but counted. There is not an Other seed determination test in the AOSA Rules.						
This proposal was submitted by the Purity Committee.						
PUR Committee Votes	Yes:	9	No:	0	Abstain/Absent:	1
CURRENT VERSION				PROPOSED VERSION		
4.1 Object The object ~~of the determination~~ is to ~~estimate~~ the number of seeds of other species stated by the applicant either generally (e.g. all other species) or by reference to one category of seeds (e.g. species scheduled as noxious in a certain country), or specifically (e.g. *Elymus repens*). In international trade this analysis is used mainly to determine the presence of seeds of noxious or undesirable species.				**4.1 Object** The object is to determine the number of seeds of other species stated by the applicant either generally (e.g. all other species) or by reference to one category of seeds (e.g. species scheduled as noxious in a certain country), or specifically (e.g. *Elymus repens*). In international trade this analysis is used mainly to determine the presence of seeds of noxious or undesirable species.		

Retesting section e clarification

Editorial change required at 5.7 e. to clarify the process for retesting and reporting test results when the range of replicates exceeds the maximum tolerated range.

This proposal is not in harmony with the AOSA Rules. The AOSA Rules Volume 1 section 6.7.b.(2) states that the replicates of retests, same as with the initial test, must be within tolerance for the retest results to be considered valid and usable.

This proposal was submitted by the Germination Committee.

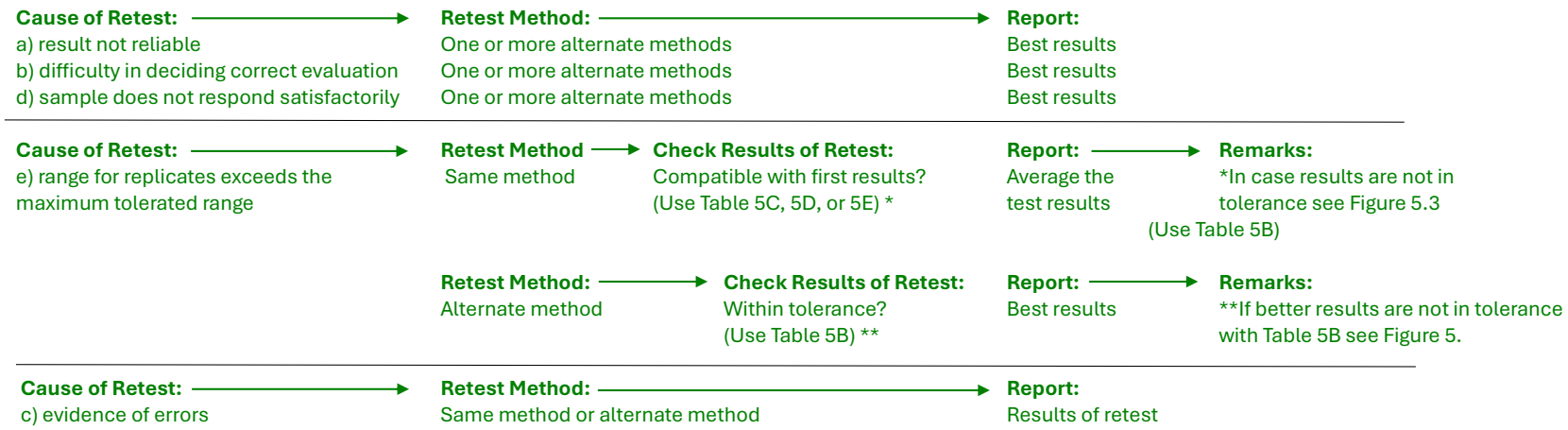
GER Committee Votes	Yes:	16	No:	0	Abstain/Absent:	0

CURRENT VERSION	PROPOSED VERSION
5.7 Retesting …. e. When the range for the replicates exceeds the maximum tolerated range in Table 5B, a retest must be carried out using the same test method or an alternative method. If the results of the retest using the same method are compatible with the first (i.e. the difference does not exceed the tolerance indicated in either Table 5C, 5D or 5E), the average of the test results must be reported on the ISTA Certificate (see 5.8.1 Tolerances). If an alternative method is used and if the results are better and within accepted tolerances, then these results must be reported on the ISTA Certificate (see 5.8.1 Tolerances) and must not be averaged with the previous test results. When retesting is carried out under the circumstances a., b. or d., the best results achieved must be indicated on the ISTA Certificate. The results of the other tests do not have to be reported on the ISTA Certificate, except on specific request by the applicant. ….	**5.7 Retesting** …. e. When the range for the replicates exceeds the maximum tolerated range in Table 5B, a retest must be carried out using the same test method and/or an alternative method. •If the results of the retest using the same method are compatible with the first (i.e. the difference does not exceed the tolerance indicated in either Table 5C, 5D or 5E), the average of the test results must be reported on the ISTA Certificate~~s~~ (see 5.8.1 Tolerances). If the results of the retest using the same method are not compatible with the first test see Figure 5.3 and conduct a third test. •If an alternative method is used and if the results are better and within accepted tolerances (according to Table 5B), then these better results must be reported on the ISTA Certificate (see 5.8.1 Tolerances) and must not be averaged with the previous test results. When retesting is carried out under the circumstances a., b., d., or e. (with an alternative method), the best results achieved must be indicated on the ISTA Certificate. The results of the other tests do not have to be reported on the ISTA Certificate, except on specific

Commented [JB4]: Question why "and was added"
And was included to add the possibility of having a parallel tests
Table changed by Germ Committee to a figure instead of a table to avoid relabelling of subsequent tables

Commented [JB5R4]: Editorial Retesting section e) clarification

ADD FIGURE:


Cause of Retest:
a) result not reliable
b) difficulty in deciding correct evaluation
d) sample does not respond satisfactorily
Retest Method:
One or more alternate methods
One or more alternate methods
One or more alternate methods
Report:
Best results
Best results
Best results
Cause of Retest:
e) range for replicates exceeds the maximum tolerated range
Retest Method
Same method
Check Results of Retest:
Compatible with first results?
(Use Table 5C, 5D, or 5E) *
Report:
Average the test results
Remarks:
*In case results are not in tolerance see Figure 5.3
(Use Table 5B)
Retest Method:
Alternate method
Check Results of Retest:
Within tolerance?
(Use Table 5B) **
Report:
Best results
Remarks:
**If better results are not in tolerance with Table 5B see Figure 5.
Cause of Retest:
c) evidence of errors
Retest Method:
Same method or alternate method
Report:
Results of retest

Beta vulgaris drying temperature

This editorial change is to change the drying temperature for prewashed *Beta vulgaris* in Table 5A Part 1 to match the instructions in 5.6.3.3.

This proposal does not harmonise with the AOSA Rules, which does not have a prewashing method for this species.

Commented [JB6]: This proposal was partially included in C.5.4. and was moved into Part C to not duplicate the vote, or have a partial vote.

This proposal was submitted by the Rule Chair and supported by the Germination Committee.

GER Committee Votes	Yes:	12	No:	2	Abstain/Absent:	3	

~~CURRENT VERSION~~

~~Species~~	~~Substrate~~	~~Temperature* (°C)~~	~~First count (d)~~	~~Final count (d)~~	~~Recommendations for breaking dormancy~~	~~Additional directions~~	~~Additional advice~~	~~Seedling Evaluation Group~~
~~1~~	~~2~~	~~3~~	~~4~~	~~5~~	~~6~~	~~7~~	~~8~~	~~9~~
~~*Beta vulgaris*~~	~~TP; BP; S~~	~~20<=>30; 15<=>25; 20~~	~~4~~	~~14~~	~~Presoak (2 h; 250 ml water per 100 seeds); prewash (multigerm: 2 h; genetic monogerm: 4 h); dry at max. 25 °C~~	~~–~~	~~–~~	~~A-2-1-1-1~~

~~PROPOSED VERSION~~

~~Species~~	~~Substrate~~	~~Temperature* (°C)~~	~~First count (d)~~	~~Final count (d)~~	~~Recommendations for breaking dormancy~~	~~Additional directions~~	~~Additional advice~~	~~Seedling Evaluation Group~~
~~1~~	~~2~~	~~3~~	~~4~~	~~5~~	~~6~~	~~7~~	~~8~~	~~9~~
~~*Beta vulgaris*~~	~~TP; BP; S~~	~~20<=>30; 15<=>25; 20~~	~~4~~	~~14~~	~~Presoak (2 h; 250 ml water per 100 seeds); prewash (multigerm: 2 h; genetic monogerm: 4 h); dry at 20-25 °C~~	~~–~~	~~–~~	~~A-2-1-1-1~~

Brassica perviridis

Brassica perviridis is now included in *Brassica rapa* and has not been included in Table 2C for many years. The validation study for *B. perviridis* did not include KNO_3 for a dormancy-breaking procedure, so it is not an exact separate entry that can be deleted from Table 5A. An editorial change to remove the separate entry for *B. perviridis* and add a note regarding KNO3 to the *B. rapa* entry is proposed by the Rules Committee and supported by the Germination Committee.

This proposal does not harmonise with the AOSA Rules, which does not have an equivalent of Table 2, and has *Brassica rapa* subsp. *napposinica* var. *perviridis* as a separate entry for germination.

This proposal was submitted by the Germination Committee.

GER Committee Votes	Yes:	15	No:	0	Abstain/Absent:	1	

CURRENT VERSION

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice	Seedling Evaluation Group
1	2	3	4	5	6	7	8	9
Brassica perviridis	~~BP; TP~~	~~20<=>30; 20~~	~~5~~	~~7~~	~~Pre-chill~~	~~–~~	~~–~~	~~A-2-1-1-1~~
Brassica rapa	BP; TP	20<=>30; 20	5	7	KNO_3; pre-chill	–	–	A-2-1-1-1

PROPOSED VERSION

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice	Seedling Evaluation Group
1	2	3	4	5	6	7	8	9
Brassica perviridis	~~BP; TP~~	~~20<=>30; 20~~	~~5~~	~~7~~	~~Pre-chill~~	~~–~~	~~–~~	~~A-2-1-1-1~~
Brassica rapa	BP; TP	20<=>30; 20	5	7	KNO_3*; pre--chill *KNO_3 not recommended for *Brassica rapa* var. *perviridis*)	–	–	A-2-1-1-1

Correct staining time for *Urochloa* spp. in Table 6A

The following proposal has been developed by ISTA Tetrazolium Committee with the intent to make a correction of a wrong transcription done in the past.

In 1996, 2 hours of staining seemed insufficient for transversal cut and, probably, when it was intended to make the correction, the error was made in 1999 by replacing it with 18 hours in staining time for both cuts. At that time the names of genus *Urochloa* was *Brachiaria* .

The AOSA Rules do not include detailed tetrazolium procedures.

This proposal was submitted by the Tetrazolium Committee.

TEZ	Yes:	9	No:	0	Abstain/Absent:	6	

CURRENT VERSION

Species	Pretreatment type/minimum time (h)	Preparation before staining	Staining solution (%)	Optimum staining time (h)	Preparation for evaluation	Permitted non-viable tissue	Remarks
1	2	3	4	5	6	7	8
Urochloa spp.	BP/18; W/6	Remove glumes, cut tranversely near embryo	1	18	Observe external embryo surface	1/3 radicle	-
	BP/18; W/6	Cut longitudinally through embryo and 3/4 of endosperm	1	18	Observe cut surface	1/3 radicle	-

PROPOSED VERSION

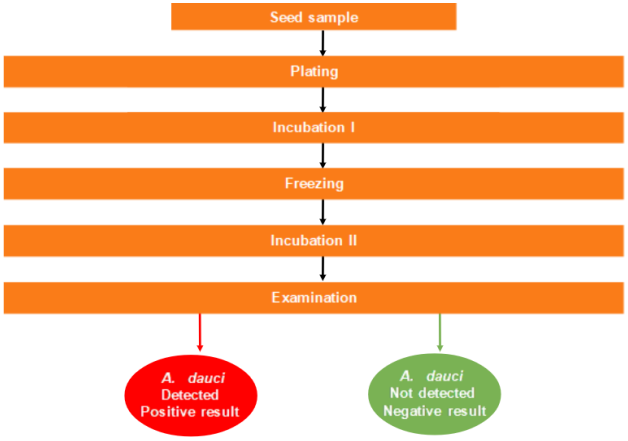
Species	Pretreatment type/minimum time (h)	Preparation before staining	Staining solution (%)	Optimum staining time (h)	Preparation for evaluation	Permitted non-viable tissue	Remarks
1	2	3	4	5	6	7	8
Urochloa spp.	BP/18; W/6	Remove glumes, cut tranversely near embryo	1	18	Observe external embryo surface	1/3 radicle	-
	BP/18; W/6	Cut longitudinally through embryo and 3/4 of endosperm	1	2	Observe cut surface	1/3 radicle	-

Add flow charts to seed health methods

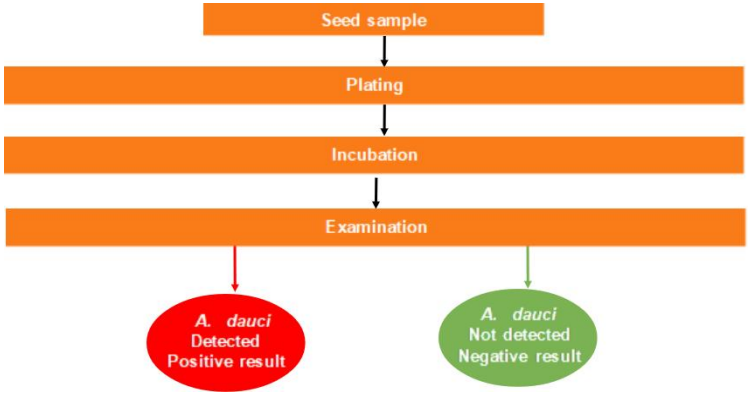
Over the course of the previous year, the SHCOM devoted considerable effort to the development of execution flow diagrams comparable to those used in ISTA Rule 7-019a, with the aim of supporting all rules within ISTA's International Rules for Seed Testing Chapter 7. The inclusion of these flow diagrams, designed in ISTA colours, is intended to assist laboratory technicians by visually outlining the sequence of procedural steps necessary to obtain valid test results which can be used for reporting on OICs. It is proposed that these flow diagrams be included in the Methods section, preceding the "1. Detection method" section. AOSA Rules do not have seed health methods and are therefore not affected by the proposed changes.					
This proposal was submitted by the Seed Health Committee.					
SHC Committee Votes	Yes:	14	No:	0	Abstain/Absent: 1
CURRENT VERSIONS of Seed Health methods 7-001a, 7-001b, 7-002a, 7-002b, 7-003, 7-004, 7-005, 7-006, 7-007, 7-008, 7-009, 7-010, 7-011, 7-012, 7-013a, 7-013b, 7-014, 7-015, 7-016, 7-019b, 7-020, 7-021, 7-022, 7-023, 7-024, 7-025, 7-026, 7-027, 7-028, 7-029, 7-030. 7-031, and 7-032 do not have flow charts.					

PROPOSED ADDITIONS:

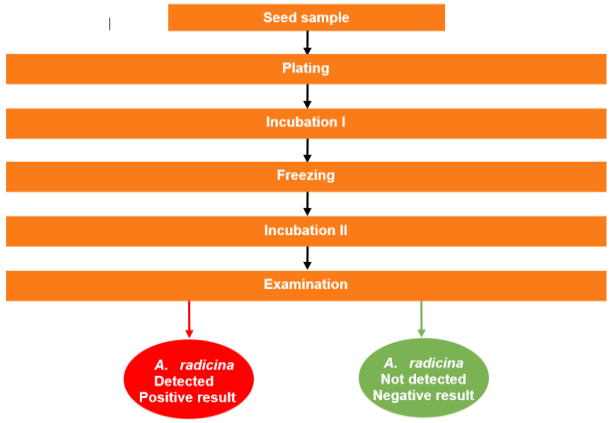
7-001a



7-001b



7-002a



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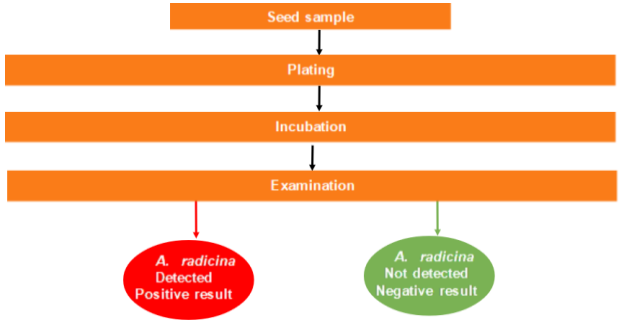
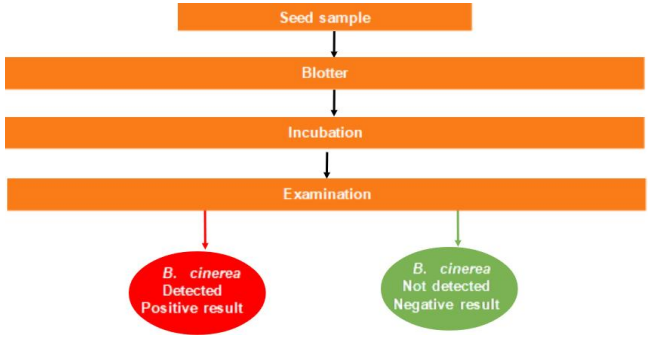
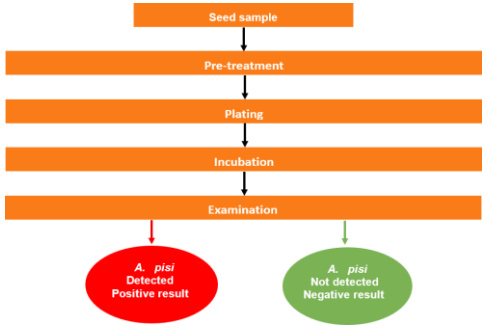


7-003

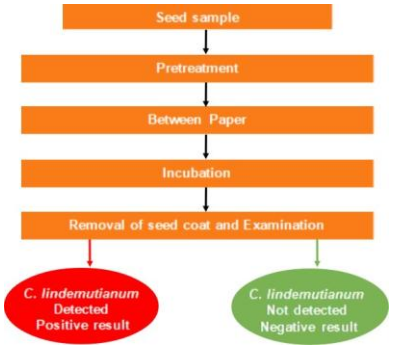


7-004

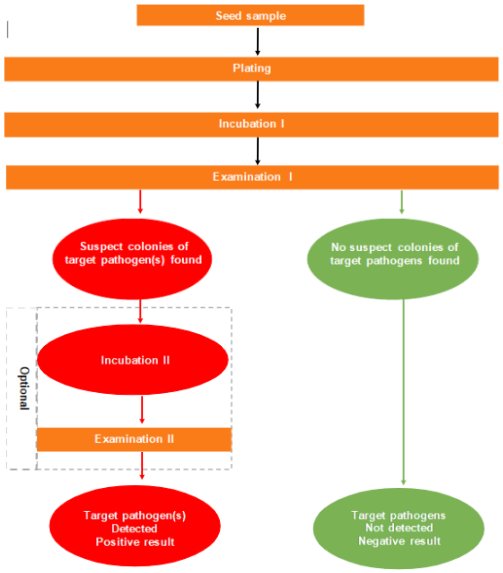
7-005



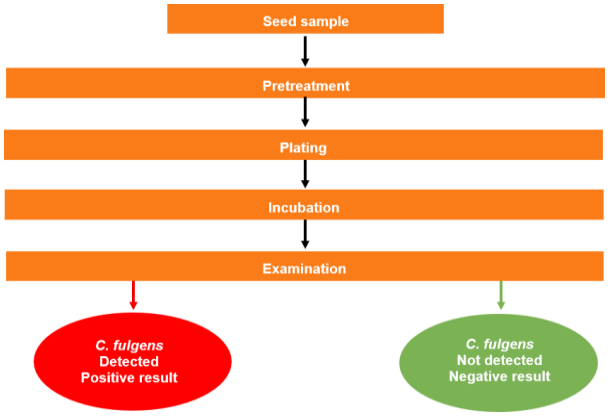
7-006



7-007



7-008



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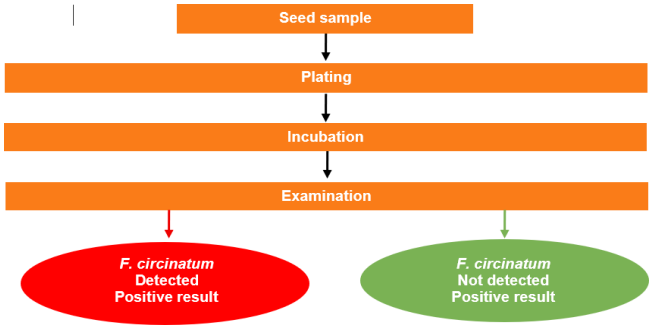

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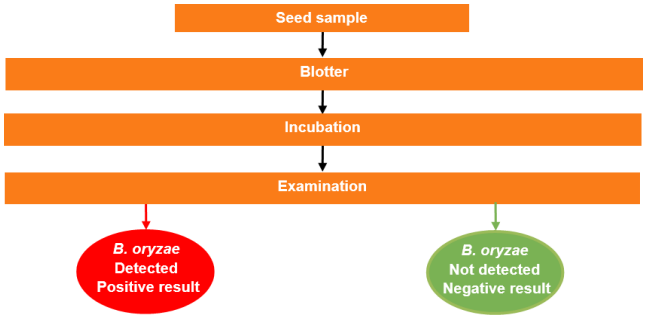

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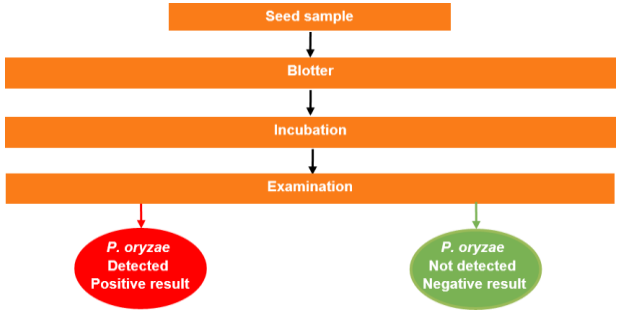

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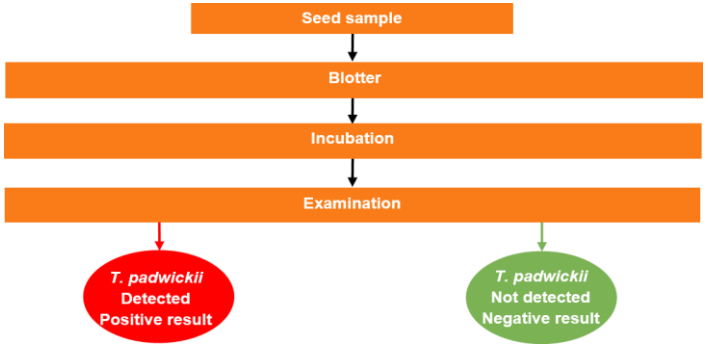
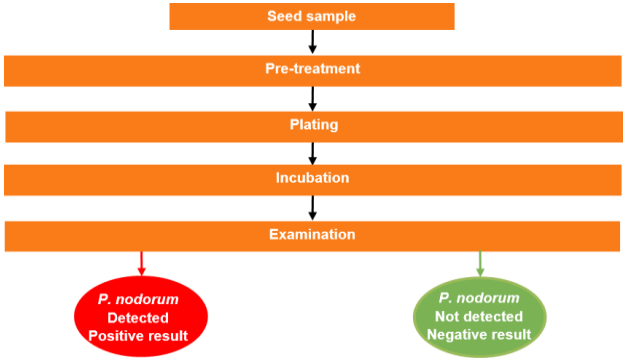


7-013a

7-014



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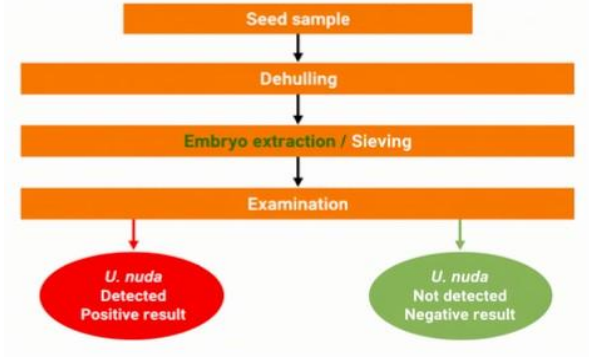

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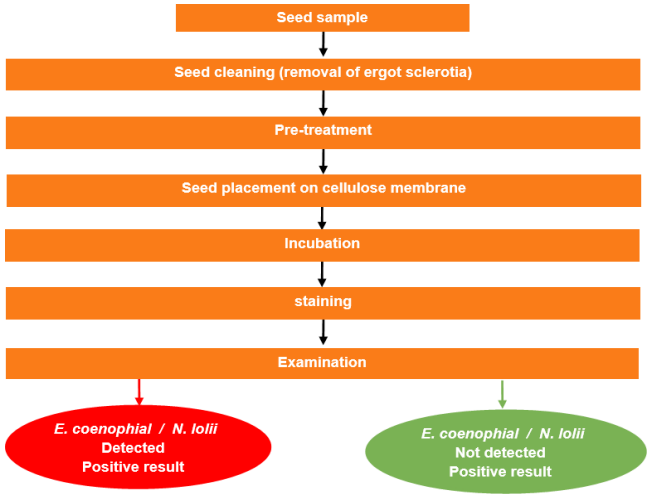
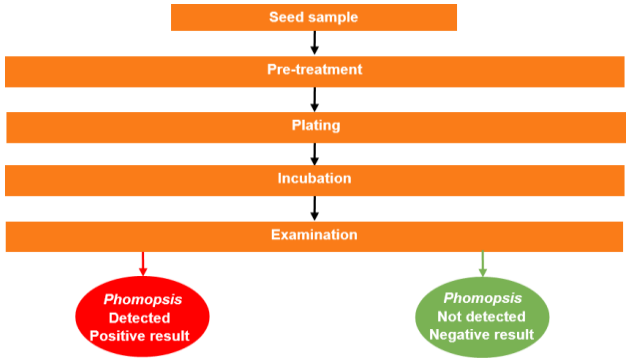


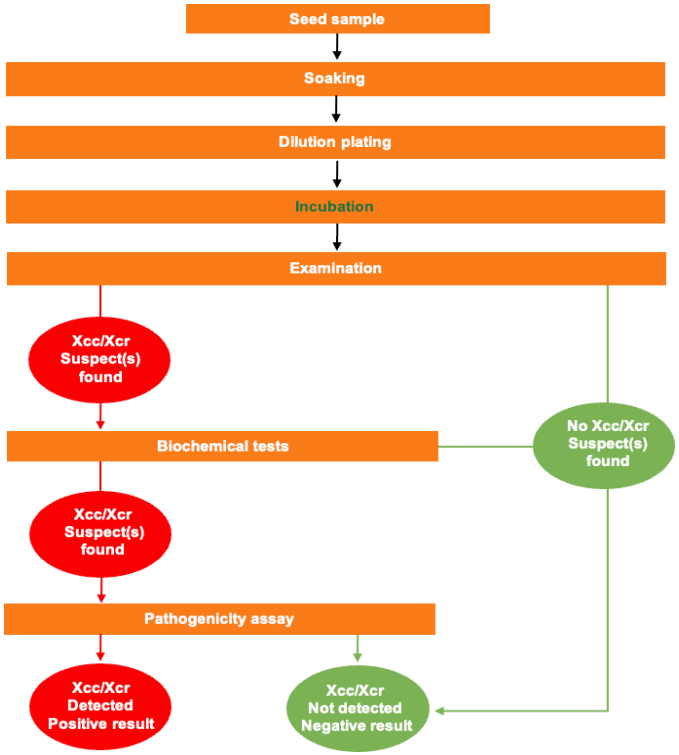
Commented [JB7]: Was updated to include Embryo extraction. This does not change the method and is still considered an editorial change.

Commented [JB8R7]: Same applies to 7-019b, 7-021, 7-023, 7-029

7-016



7-019b



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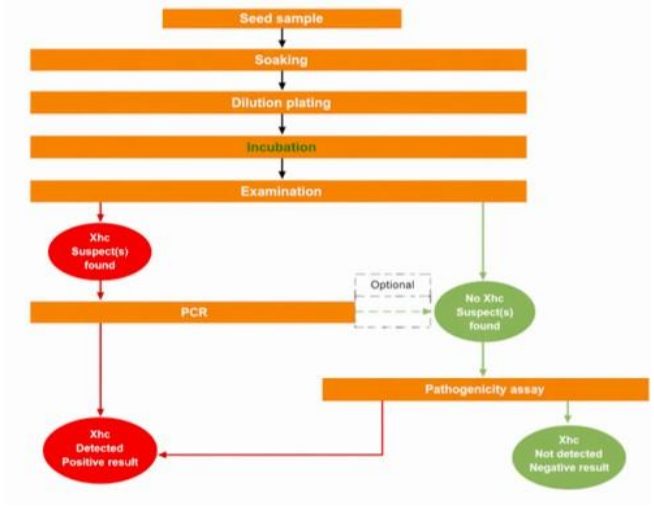
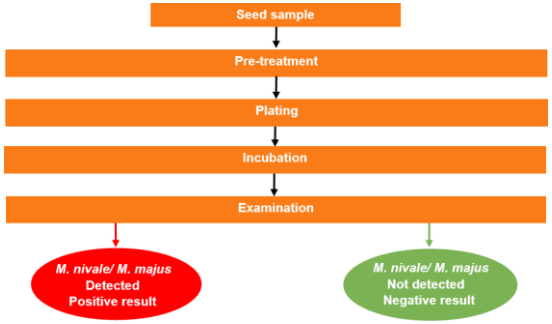


7-021

7-022



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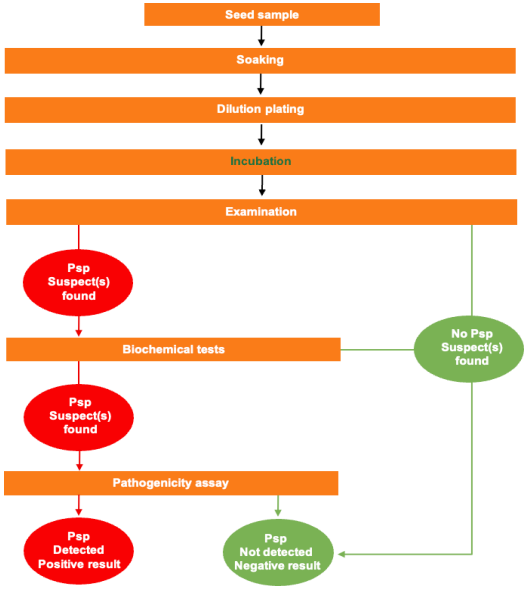

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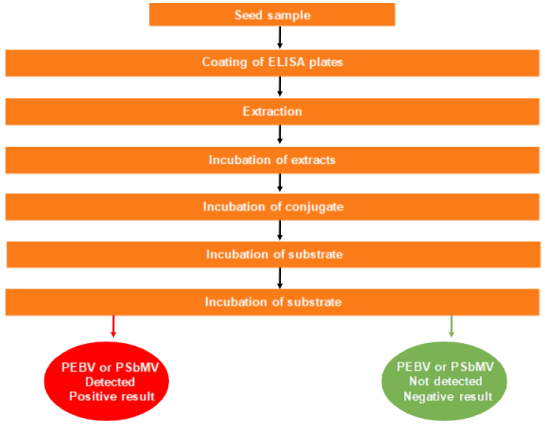

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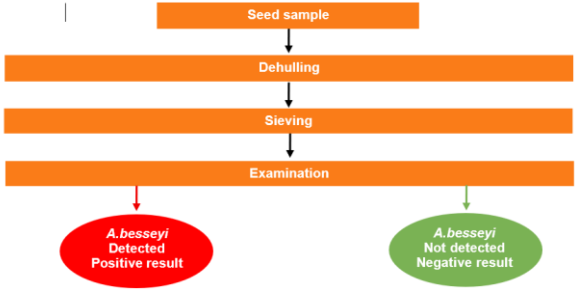
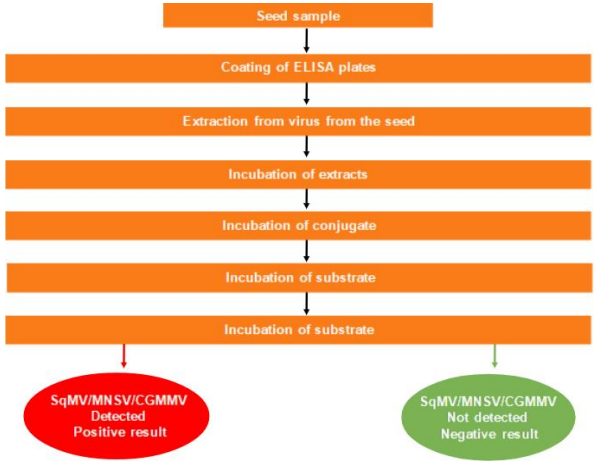
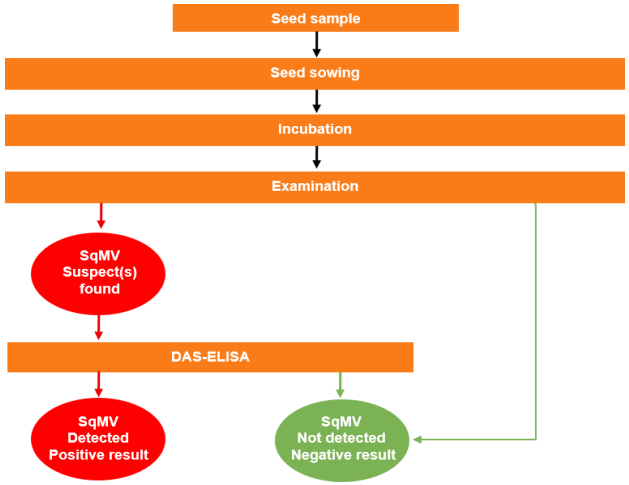


7-026
ELISA part



7-026 Grow-out part



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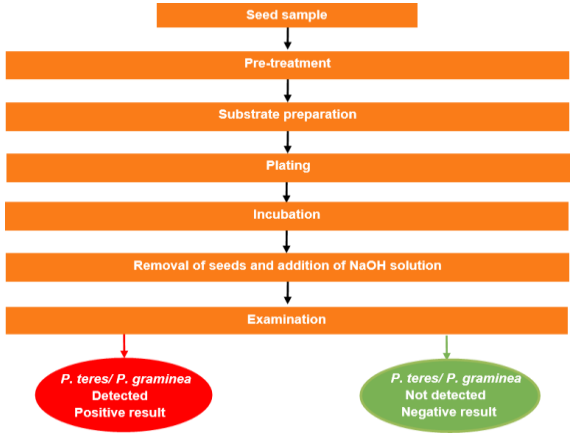

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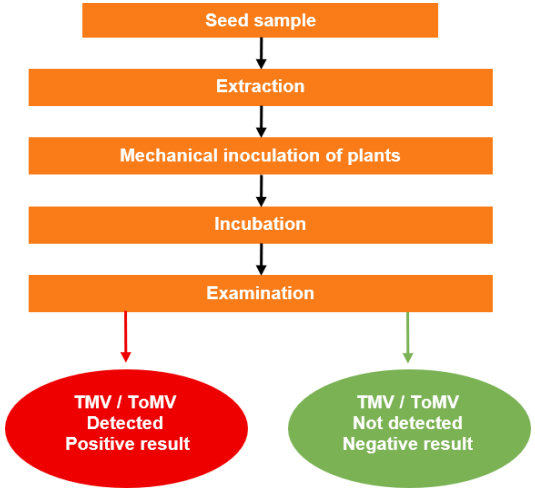

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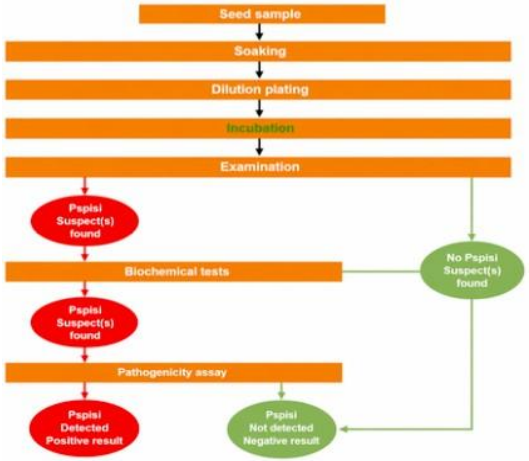

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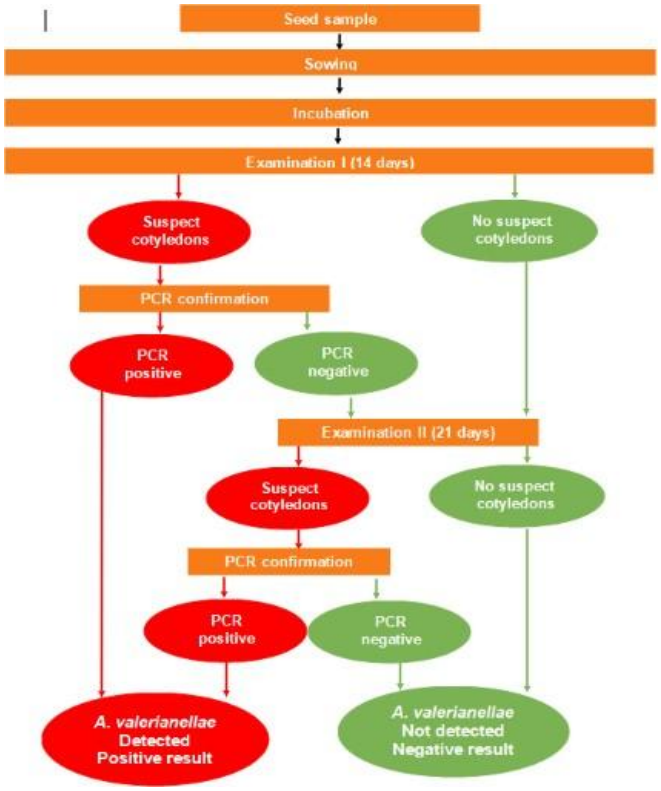

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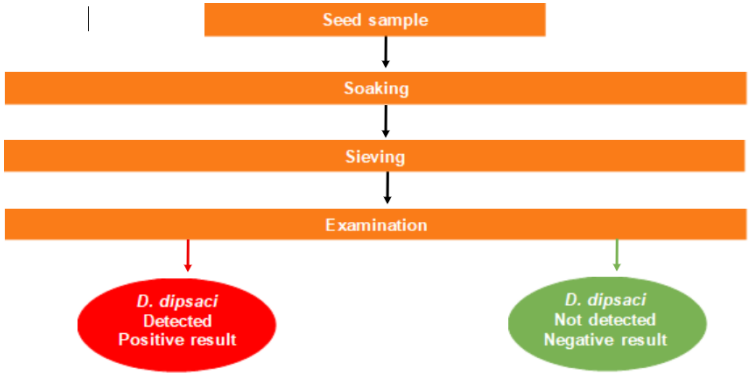

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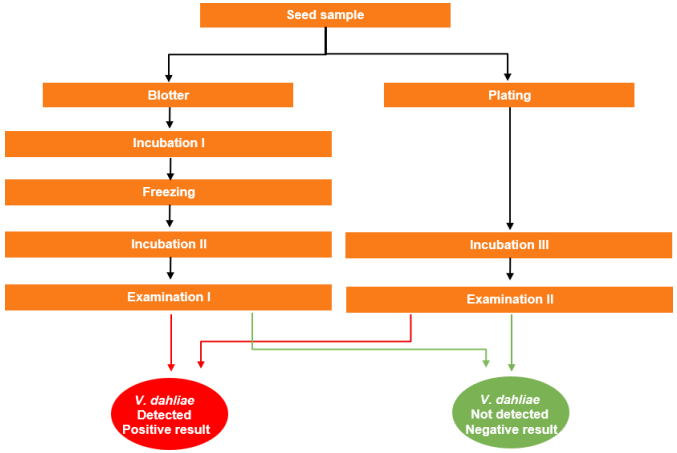
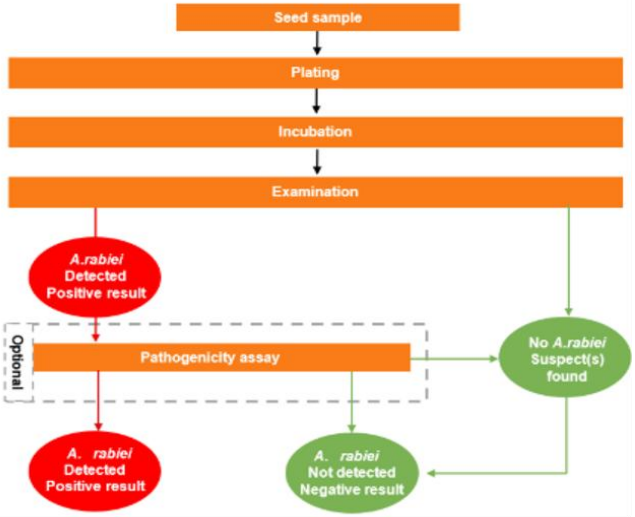


Seed health method synonym additions, change chart

This proposal adds full information on the pathogen naming and changes the flow chart. AOSA Rules do nothave seed health methods.							
This proposal was submitted by the Seed Health Committee.							
SHC Committee Votes	Yes:	14	No:	0	Abstain/Absent:	0	
CURRENT VERSION				PROPOSED VERSION			
7-033: Detection of *Ascochyta rabiei* in *Cicer arietinum* (chickpea) seed				**7-033: Detection of *Ascochyta rabiei* in *Cicer arietinum* (chickpea) seed**			
Pathogen(s): *Ascochyta rabiei* …. Remove:				**Pathogen(s):** *Ascochyta rabiei* (Passerini) Labrousse, syn. *Didymella rabiei* (Kovachevski) Arx, *Mycosphaerella rabiei* Kovachevski, *Phoma rabiei* (Passerini) Khune & J.N.Kapoor, *Phyllosticta rabiei* (Passerini) Trotter, *Zythia rabiei* Passerini …. Add: 			

Reporting of moisture test method

The words ‘oven method’ should be explicitly stated on the certificate to clearly identify the method used to measure moisture content. This aligns with existing requirements, under which the use of moisture meters must also be explicitly reported.

This proposal harmonises with the AOSA Rules with slight wording differences, which also require reporting the method used (‘air-oven method or electronic moisture balance method’).

Commented [JB9]: Corrected typographical error

This proposal was submitted by the Moisture Committee.

MOI Committee Votes	Yes:	13	No:	0	Abstain/Absent:	0

CURRENT VERSION	PROPOSED VERSION
9.2.7 Reporting of results The result of a moisture content test must be reported in the space provided to the nearest 0.1 %. The method must be reported (duration and temperature). The following additional information must also be reported under ‘Other determinations’: ….	**9.2.7 Reporting of results** The result of a moisture content test must be reported in the space provided to the nearest 0.1 %. The method must be reported (oven method: duration and temperature). The following additional information must also be reported under ‘Other determinations’: ….

CONSEQUENTIAL CHANGE TO CHAPTER 1

1.5.2.12 Moisture content	**1.5.2.12 Moisture content**
…. The following additional information must also be reported under ‘Other determinations’: • For the oven method (9.2.7), the method (~~i.e.~~ duration and temperature) must be reported. ….	…. The following additional information must also be reported under ‘Other determinations’: • For the oven method (9.2.7), the method (oven method: duration and temperature) must be reported. ….

Moisture test checking tolerances

The proposed wording makes it much clearer how to proceed, so the results are in agreement with the Moisture handbook (2007) and the moisture calculator.

This proposal harmonises with the AOSA Rules.

This proposal was submitted by the Moisture Committee.

MOI Committee Votes	Yes:	13	No:	0	Abstain/Absent:	0

CURRENT VERSION	PROPOSED VERSION
9.2.6.2 Tolerances The difference must be calculated to three decimal places and then rounded off to one decimal place. The maximum difference between the two replicates must not exceed 0.2 % ~~after rounding from three to one decimal place~~.	**9.2.6.2 Tolerances** The difference between two replicates must be calculated to three decimal places and then rounded off to one decimal place. The maximum difference between the two replicates must not exceed 0.2 %. ....

Definition of moisture content reported

The wording for reporting moisture content for pelleted seed for the oven method was changed some years ago. This proposal will harmonise the wording for the moisture meter method.

The AOSA Rules do not reference moisture testing of coated seed.

This proposal was submitted by the Moisture Committee.

MOI Committee Votes	Yes:	13	No:	0	Abstain/Absent:	0

CURRENT VERSION	PROPOSED VERSION
9.3.2.7 Reporting of moisture meter results …. In the case of pelleted seeds (see Chapter 11), the following statement must be entered: 'The seeds of the submitted moisture sample were pelleted, and the moisture content reported is ~~the average of~~ seed and pelleting materials'.	**9.3.2.7 Reporting of moisture meter results** …. In the case of pelleted seeds (see Chapter 11), the following statement must be entered: 'The seeds of the submitted moisture sample were pelleted, and the moisture content reported is that of the combined unit comprising seed and pelleting materials'. ....

Accelerated ageing temperature

Last year the temperature tolerance for the accelerated ageing test was changed to ± 0.5 °C in 15.8.2.5.1. It should have also been changed in 15.8.2.6.2, but this was missed.

This error was found by a member and the Chair of VIG confirmed that an amendment is needed.

CURRENT VERSION	PROPOSED VERSION
15.8.2.6.2 Ageing the seed … The 72 h ageing period starts with the placement of the shelves into the ageing chamber. The ageing chamber door should not be opened during the 72 h ageing period. After the temperature recovers to 41 ±0.~~3~~ °C, it should be monitored continuously during ageing to be certain that it remains at that level.	**15.8.2.6.2 Ageing the seed** … The 72 h ageing period starts with the placement of the shelves into the ageing chamber. The ageing chamber door should not be opened during the 72 h ageing period. After the temperature recovers to 41 ±0.5 °C, it should be monitored continuously during ageing to be certain that it remains at that level.

Commented [JB10]: This editorial change was submitted after the open Rules session.

Clarification for moistening paper in radicle emergence test

The proposed editorial change clarifies that the paper must only be moistened with water for the radicle emergence test. The AOSA Rules do nothave a radicle emergence test.						
This proposal was submitted by the Vigour Committee.						
VIG Committee Votes	Yes:	15	No:	0	Abstain/Absent:	1

CURRENT VERSION	PROPOSED VERSION
15.8.4.4.1 Setting up the radicle emergence test The test must be set up using the media and conditions described in Table 15B, following the normal procedure in your laboratory for a germination test using the prescribed medium.	**15.8.4.4.1 Setting up the radicle emergence test** The test must be set up using the media and conditions described in Table 15B, following the normal procedure in your laboratory for a germination test using the prescribed medium. Only water must be used to moisten the medium.

Vote to accept editorial changes	Yes votes	No votes	Result
	24	0	

Chapter 1: ISTA Certificates

C.1.1 Indicating Tests in Other Determinations

Without labels, it is not clear on ISTA certificates to what test or determination the information in Other Determinations refers. For example, "PP 20C" may not be clear to the user that it refers to the germination test.

The Accreditation Standard 8.1 states: " The results of each test or series of tests carried out by the accredited member must be reported accurately, clearly, unambiguously and objectively, and in accordance with any specific instructions in the ISTA Rules."

This rule proposal is to add a requirement that will ensure transparency and traceability, allowing users to accurately interpret the context and relevance of the reported data in accordance with the applicable test method.

There is no corresponding AOSA Rule.

The proposal was submitted by the ISTA Rules Committee Chair and Vice-Chair.

RUL Committee Votes	Yes:	10	No:	0	Abstain/Absent:	9

CURRENT VERSION	PROPOSED VERSION
1.5.2 Certificates …… Test results must be reported in accordance with the rules for calculating, expressing and reporting results in the appropriate chapter of the ISTA Rules. …… ‘N’ must not be used for components of a test such as hard or fresh seeds. For example, even if hard seeds do not usually occur in the species they must be looked for and if not found, then reported as ‘0’.	**1.5.2 Certificates** …… Test results must be reported in accordance with the rules for calculating, expressing and reporting results in the appropriate chapter of the ISTA Rules. …… ‘N’ must not be used for components of a test such as hard or fresh seeds. For example, even if hard seeds do not usually occur in the species they must be looked for and if not found, then reported as ‘0’. When reporting results in the “Other Determinations” section of an ISTA Certificate, the specific test or determination to which the result pertains must be clearly stated (e.g. Germination test: ...), followed by the specific reporting instructions for that test.

Vote to accept item	Yes votes	No votes	Result
C.1.1	23	1	

Chapter 2: Sampling

C.2.1 Definitions of containers, sealed & self-sealing containers

During audits the ISTA Accreditation Department has found that some laboratories regarded carton boxes, that are used to ship small packets in seed in, as the container for determining the sampling intensity and minimum number of primary samples. To clarify this, the BSC agreed that a definition of 'container' could be added to Chapter 2.

'Self-sealing containers' was moved to 'Sealing', and in order to get a more logical flow of the definitions, it was decided to put 'Containers' before 'Sealing'. This change would not necessitate any consequential changes as neither 2.2.9 or 2.2.10 is referenced at any place in the Rules.

In order to not change the numbering of the current Rules, we have amended the definition of 'Self-sealing containers'. Self-sealing containers are mentioned only once in Chapter 2, therefore a specific definition for self-sealing containers is not necessary, since it is already covered by 'are capable to be sealed'.

There is no corresponding AOSA Rule.

This proposal was submitted by the Bulking and Sampling Committee

BSC Committee Votes	Yes:	14	No:	0	Abstain/Absent:	1

CURRENT VERSION	PROPOSED VERSION
2.2.~~10 Self-sealing c~~ontainers	**2.2.9 Containers**
~~The 'valve-pack' bag is a specific type of self-sealing container. It is~~ filled through a sleeve-shaped valve which is automatically closed by ~~the completion of filling the bag.~~	Container means a vessel (bag, tin, hermetically sealed packet, bin, wooden box, silo, etc.), which is filled with seed. It must be firm enough to hold the seed and be either self-sealing or can be sealed. A shipping container, into which several smaller containers holding the seed are packed such as a bigger bag or carton box, is not considered as the container for the purpose of sampling.
2.2.~~9~~ Sealed	**2.2.10 Sealed**
Sealed means that a container in which seed is held is closed in such a way, that it cannot be opened to gain access to the seed…	Sealed means that a container, in which seed is held, is closed in such a way, that it cannot be opened to gain access to the seed... The specific type of self-sealing container, called 'valve-pack' bag, is accepted in seed lots as being sealed.

Vote to accept item	Yes votes	No votes	Result
C.2.1	24	0	

C.2.2 Marking/labelling and sealing ofcontainers

Following questions from the ISTA Accreditation Department, the BSC proposes to make a provision in Rule 2.5.4.3 that if hundreds or thousands of small packets, each containing for example 5 g of seed, are packed in carton boxes (shipping container), that only the carton boxes need to be marked with the lab-allocated seed lot identification number (where applicable) and sealed by the sampler. If the small packets are sealed according to the definition of sealing, it would not need further sealing. Traceability between the batch/lot number on the packets and the identification number allocated by the laboratory, must be maintained by the laboratory.

There is no corresponding AOSA Rule.

This proposal was submitted by the Bulking and Sampling Committee

BSC Committee Votes	Yes:	11	No:	1	Abstain/Absent:	3

CURRENT VERSION	PROPOSED VERSION
2.5.4.3 Marking/labelling and sealing of containers	**2.5.4.3 Marking/labelling and sealing of containers**
.... Where the seed lot is already marked/labelled and sealed before sampling, the seed sampler must verify the marking/labelling and sealing on the containers. Otherwise the sampler has to mark/label the containers and must seal every container before the seed lot leaves their control.	.... Where the seed lot is already marked/labelled and sealed before sampling, the seed sampler must verify the marking/labelling and sealing on the containers. Otherwise, the sampler has to mark/label the containers and must seal every container or shipping container, as appropriate, before the seed lot leaves their control. If sealed containers holding the seed are packed in larger shipping containers, and where the ~~laboratory~~ accredited member allocates a specific seed lot identification that is different than the marks of lot ~~numbers~~ indicated on the containers inside the shipping container, the shipping containers must be marked and sealed with the ~~laboratory~~ accredited member-allocated identification mark and seal. The ISTA Certificate must show the identification marks, seals, and number of containers of both the sealed small seed containers and of the larger shipping containers.

Commented [JB11]: During the rule session it was identified that the correct term should be accredited member instead of laboratory to include sampling entities

Commented [JB12R11]: Lot number was changed to marks of the lot to harmonize with the rest of the rules

Commented [JB13R11]: C2.2

Commented [JB14R11]: Due to the perspective of testing lab's scope of responsibility,
The inclusion of multiple lot numbers in the "Marks of lot" column and the inclusion of the seal status in the "Seal of lot" column should be exercised more cautionary.
Testing Lab assigns a sealing number to each container (carton box), and that of inner bags should be responsible for the client; Testing Lab is not involved.
Furthermore, we, Testing lab, do not allow the client to replace bags after sealing, and the lot identification is guaranteed by the outer seal.
Therefore, under this situation, including the client's lot number in the "Marks of Lot" field and the inner bag sealing condition in the "Seal of Lot" field
could lead to the misunderstanding that the Testing laboratory is also responsible for the sealing of the inner bags.
If this misunderstanding caused by the lack of information on the number of sealed containers in "Number of containers",
issue the number of sealed containers in this column primarily and the number of small packets supplementary, ex. 2 containers (2000 bags),
then the mandatory information can be appropriately conveyed. In addition, if necessary for the information ...

Commented [JB15R11]: It is **NOT a requirement of the ISTA Rules** that ISTA Laboratories should allocate their own identification mark, or to add a physical seal to the containers, should the containers already conform to the ISTA definition of sealing. In my opinion, it is completely unnecessary for laboratories to allocate their own Identification mark, as all seeds lots presented fo ...

Commented [JB16R11]: In many countries, accredited sampling entities and accredited laboratory do not add their own lot identification and use systematically the one allocated by the seed company (the numbering method has been defined in advance to ensure a unique batch number). In this case, only the identification mark on the small packets will be used ...

Consequential changes

1.4.2 Orange International Seed Lot Certificate It is stated on the Orange International Seed Lot Certificate: .... The completed Orange International Seed Lot Certificate must show the following information: .... c. seed lot identification (i.e. marks of lot); d. Under 'Seal of lot': the method of sealing (e.g. stitching, metal seal) and/or the authority (e.g. ISTA laboratory, Ministry); e. either the number of containers for which the certificate is issued; or 'N/A' for 'notapplicable';	1.4.2 Orange International Seed Lot Certificate It is stated on the Orange International Seed Lot Certificate: .... The completed Orange International Seed Lot Certificate must show the following information: .... c. seed lot identification (i.e. marks of lot); If larger shipping containers are marked with a different ~~laboratory~~ accredited member-allocated seed lot identification than the inner contents, both identification marks must be shown on the ISTA Certificate. d. Under 'Seal of lot': the method of sealing (e.g. stitching, metal seal) and/or the authority (e.g. ISTA laboratory, Ministry); If larger shipping containers are sealed with a different ~~laboratory~~ accredited member-allocated seal than the inner containers, both types of seals must be shown on the ISTA Certificate. e. either the number of containers for which the certificate is issued; or 'N/A' for 'not applicable'; If larger shipping containers are sealed and marked with a different ~~laboratory~~ accredited member-allocated seed lot identification and seal than the inner containers, both the number of shipping containers and the number of containers inside must be shown on the ISTA Certificate. ....

Vote to accept item	Yes votes	No votes	Result
C.2.2	23	2	

Chapter 3: The purity analysis

C.3.1 Pure seed definition for *Paspalum* spp.

Pure seed definition 36 mentions “no need to check for the presence of a caryopsis” only for *Megathyrsus, Panicum* and *Digitaria* . The genus *Paspalum* included in PSD 36 has the same characteristics: it is exceptionally difficult to differentiate between pure seed and florets without a caryopsis. Paspalum is also difficult to blow as the seeds tend to be tacky and stick together. To not have to check for the presence of a caryopsis would make the purity test easier for the analyst. As with *Megathyrsus, Panicum* and *Digitaria* , and knowing that the germination percentage would decrease due to the pure seed containing more seed without a caryopsis, the use of pure live seed (PLS) would be convenient. This proposal also puts the genera in alphabetical order.

It is not possible to harmonise this proposal with the AOSA Rules because species of the genus *Paspalum* are generally treated differently as in ISTA.

This proposal was submitted by the Purity Committee

PUR Committee Votes	Yes:	11	No:	0	Abstain/Absent:	2

CURRENT VERSION	PROPOSED VERSION
Table 3B Part 2. Numbered pure seed definitions 36. Spikelet, with or without pedicel, with glumes, lemma… ... *Megathyrsus, Panicum* and ~~*Digitaria*~~ : no need to check for the presence of a caryopsis.	**Table 3B Part 2. Numbered pure seed definitions** 36. Spikelet, with or without pedicel, with glumes, lemma… ... *Digitaria, Megathyrsus, Panicum* and *Paspalum* : no need to check for the presence of a caryopsis.

Vote to accept item	Yes votes	No votes	Result
C.3.1	19	5	

C.3.2 Reporting specified inert matter in Chapters 3 and 4

A proposal for reporting inert matter in the OSD has been received from the New Zealand ISTA Laboratory Members.

The New Zealand ISTA Laboratory Members are proposing the Rules change to enable the reporting of undesirable inert matter as individual components. This is also important for use of the ISTA Certificates in international trade.

The submission of this proposal follows the guidelines for Rules proposals from ISTA members as outlined in I-2 Guidelines for ISTA Rules Proposals in the ISTA Rules.

This proposal has been sent to the ISTA Purity Committee and has been considered by the ISTA Executive Committee with advice from the ISTA Accreditation and Technical Department.

The Executive Committee is proposing a modification of the New Zealand proposal as reporting by percentage should be in Chapter 3.

If accepted this Rules change proposal will result in consequent changes to 1.5.2.2 Purity and 1.5.2.4 Determination of other seeds by number.

PUR Committee Votes	Yes:	11	No:	2	Abstain/Absent:	2	

Chapter 3: The purity analysis

CURRENT VERSION	PROPOSED VERSION
3.5.2 Separation	**3.5.2 Separation**
… 4. …. until sample disposal (see 2.5.3 and 2.5.4.7). **3.5.2.1 All families except Poaceae** …	… 4. …. until sample disposal (see 2.5.3 and 2.5.4.7). 5. Upon request, and in addition to the purity analysis, specified inert matter (e.g., soil, sclerotia, ergot, smut balls, nematode galls) may be separated on the purity working weight, or on a weight as specified by the customer that is more than the minimum working weight of Table 2C column 4. For reporting the absence, presence or percentage by weight refer to 3.7. **3.5.2.1 All families except Poaceae** …

CURRENT VERSION	PROPOSED VERSION
3.7 Reporting results	**3.7 Reporting results**
... Upon request, the following information must be reported under 'Other determinations' as follows: • Other seeds may be divided into 'other crop seeds' and 'weed seeds'... ... • The percentage by weightof broken pure seed. The percentages may be reported to more than one decimal place if requested.	... Upon request, the following information must be reported under 'Other determinations' as follows: • Other seeds may be divided into 'other crop seeds' and 'weed seeds' ... • The percentage by weight of broken pure seed. • The percentage by weight of specified inert matter (e.g., soil, sclerotia, ergot, smut balls, nematode galls) on applicant specified weight (see 3.5.2.5). • Absence or presence of components of specified inert matter (e.g., soil, sclerotia, ergot, smut balls, nematode galls) on applicant specified weight (see 3.5.2.5). The percentages may be reported to more than one decimal place if requested. If the weight of the working sample tested for specified inert matter is more than 10 % higher than the weight specified in Table 2C, column 4 (Purity analysis), the actual weight examined must be reported according to the minimum number of decimal places indicated in 3.5.1. Upon request, the following information must be reported under 'Other determinations' as follows: The percentages may be reported to more than one decimal place if requested.

Chapter 4: Determination of other seeds and specified inert matter by number	
CURRENT VERSION	PROPOSED VERSION
4.1 Object	**4.1 Object**
The object of the determination is to estimate the number of seeds of other species stated by the applicant either generally (e.g. all other species) or by reference to one category of seeds (e.g. species scheduled as noxious in a certain country), or specifically (e.g. *Elymus repens*). In international trade this analysis is used mainly to determine the presence of seeds of noxious or undesirable species. ...	The object of the determination is to estimate the number of seeds of other species or specified inert matter stated by the applicant either generally (e.g. all other species) or by reference to one category of seeds (e.g. species scheduled as noxious in a certain country), or specifically (e.g. *Elymus repens*). In international trade this analysis is used mainly to determine the presence of seeds of noxious or undesirable species. …
CURRENT VERSION	PROPOSED VERSION
4.2 Definitions	**4.2 Definitions**
... **4.2.5 Reduced-limited test** ... **4.3 General principles**	... **4.2.5 Reduced-limited test** ... **4.2.6 Specified inert matter** Inert matter specified in 3.2.3 (e.g., sclerotia, ergot, smut balls, nematode galls) as stated by the applicant. **4.3 General principles**

4.5.2 Determination	**4.5.2 Determination**
The working sample is searched either for seeds of all other species or of certain stated species, as required by the applicant. The number of seeds found of each species sought is counted. If the search is limited to certain stated species, the examination may be stopped when one or more seeds of one or all of the stated species (as appropriate to the applicant's requirements) has been found. Seeds of the other species found must be retained and stored for reference until sample disposal (see 2.5.3 and 2.5.4.7).	The working sample is searched either for seeds of all other species or of certain stated species or specified inert matter as defined in 4.2.6, as required by the applicant. The number of seeds found of each species or specified inert matter sought is counted. If the search is limited to certain stated species, the examination may be stopped when one or more seeds of one or all of the stated species (as appropriate to the applicant's requirements) has been found. Seeds of the other species or specified inert matter found must be retained and stored for reference until sample disposal (see 2.5.3 and 2.5.4.7).
4.6 Calculation and expression of results	**4.6 Calculation and expression of results**
The result is expressed as the number of seeds belonging to each stated species or category found in the actual quantity examined. In addition the number per unit weight (e.g. per kilogram) may be calculated. If a second or more tests are carried out on the same sample, then the result must be expressed as the total number of seeds found in the total weight examined. …	The result is expressed as the number of seeds belonging to each stated species or category found in the actual quantity examined. In addition the number per unit weight (e.g. per kilogram) may be calculated. Upon request, the specified inert matter (e.g., sclerotia, ergot, smut balls, nematode galls) separated at the purity test (3.2.3) can also be separated and expressed as a number. When it is not possible to express the specified inert matter as a number (e.g., soil or dust), it must be expressed as a percentage by weight (see 3.5.2.5). If a second or more tests are carried out on the same sample, then the result must be expressed as the total number of seeds found in the total weight examined. …

4.7 Reporting results	**4.7 Reporting results**
The result of a determination of other seeds by number must be reported under 'Other determinations' as follows: … If no seeds were found it can be reported as: 'No seeds of ... species were found in … g of seed examined.' The sample weight examined must be reported according to the number of decimal places indicated in Table 4A.	The result of a determination of other seeds or specified inert matter by number must be reported under 'Other determinations' as follows: … If no seeds were found it can be reported as: 'No seeds of ... species were found in … g of seed examined.' The sample weight examined must be reported according to the number of decimal places indicated in Table 4A. The result of a determination of specified inert matter is expressed as the number of each requested specific inert matter found in the actual quantity examined. Upon request, the results may in addition be expressed in some other way, such as 'weight of specified inert matter found' or 'number of specified inert matter per kilogram'. Upon request, the absence of specified inert matter is reported as: 'No (name of specified inert matter) found in X g of seed examined.' The sample weight examined must be reported according to the number of decimal places indicated in Table 4A.

Consequential changes to Chapter 1

CURRENT VERSION	PROPOSED VERSION
1.5.2.2 Purity ... Upon request, the following information must be reported under 'Other determinations' as follows: • Other seeds may be divided into 'other crop seeds' and 'weed seeds'... ... The percentages may be reported to more than one decimal place if requested.	**1.5.2.2 Purity** ... Upon request, the following information must be reported under 'Other determinations' as follows: • Other seeds may be divided into 'other crop seeds' and 'weed seeds'... ... • The percentage by weight of specified inert matter (e.g., soil, sclerotia, ergot, smut balls, nematode galls) on applicant specified weight (see 3.5.2.5). • Absence or presence of components of specified inert matter (e.g., soil, sclerotia, ergot, smut balls, nematode galls) on applicant specified weight (see 3.5.2.5). The percentages may be reported to more than one decimal place if requested. If the weight of the working sample tested for specified inert matter is more than 10 % higher than the weight specified in Table 2C, column 4 (Purity analysis), the actual examined must be reported according to the minimum number of decimal places indicated in 3.5.1.

CURRENT VERSION	PROPOSED VERSION
1.5.2.4 Determination of other seeds by number	**1.5.2.4 Determination of other seeds or specified inert matter by number**
The result of a determination of other seeds by number must be reported under 'Other determinations' as follows: … If no seeds were found it can be reported as: 'No seeds of ... species were found in … g of seed examined.' The sample weight examined must be reported according to the number of decimal places indicated in Table 4A.	The result of a determination of other seeds or specified inert matter by number must be reported under 'Other determinations' as follows: … If no seeds were found it can be reported as: 'No seeds of ... species were found in … g of seed examined.' The sample weight examined must be reported according to the number of decimal places indicated in Table 4A. The result of a determination of specified inert matter is expressed as the number of each requested specific inert matter found in the actual quantity examined. Upon request, the results may in addition be expressed in some other way, such as 'weight of specified inert matter found' or 'number of specified inert matter per kilogram'. If no component of the inert matter specified by the applicant was found, it can be reported as: 'No (name of specified inert matter) found in X g of seed examined.' The sample weight examined must be reported according to the number of decimal places indicated in Table 4A.

Vote to accept item	Yes votes	No votes	Result
C.3.2	19	3	

Commented [JB17]: Renamed chapter, which was originally missed but identified and corrected in the open rules session

Commented [JB18R17]: Original comment received before open Rules session:
The proposal allows under 4.6 to express inert matter separated at the purity test (3.2.3), but it does not describe the expression of inert matter separated according to 4.5.2 in the same chapter.
Why shall chapter 4 describe what is possible in chapter 3 without describing the procedure in chapter 4?

Clarification of question:
The result is expressed as the number of seeds belonging to each
stated species or category found in the actual quantity examined. In
addition the number per unit weight (e.g. per kilogram) may be
calculated.

Upon request, the specified inert matter (e.g., sclerotia, ergot, smut
balls, nematode galls) separated at the purity test (3.2.3) can also be
separated and expressed as a number. When it is not possible to
express the specified inert matter as a number (e.g., soil or dust), it
must be expressed as a percentage by weight (see 3.5.2.5).
If a second or more tests are carried out on the same sample, then the
result must be expressed as the total number of seeds found in the
total weight examined.

The first paragraph describes the expression of seeds found as a result of the determination according to 4.5.2.
The second paragraph describes the expression of inter matter found as a result of the purity test according to 3.2.3.
The third paragraph describes the expression of seeds, if several tests are done on the same sample.

Where is described how to express the inert matter ...

Commented [JB19R17]: C3.2

Chapter 5: The germination test

C.5.1 Alternating temperature change

There is a change required at 5.6.2.3 regarding alternating temperatures. The word “should” has been removed and replaced with “must” to make it clearer that alternating temperatures are 16 hours at the low temperature and 8 hours at the high temperature (changeover period permitted). This aligns with the text in the footnote at the bottom of all the pages for Table 5A that states "...alternating temperature regimes: 1st temperature, 16 h; 2nd temperature 8 h."

This proposal harmonises with the AOSA Rules.

This proposal originates from the ISTA Accreditation department and is supported by the Germination Committee.

GER Committee Votes	Yes:	15	No:	0	Abstain/Absent:	1

CURRENT VERSION	PROPOSED VERSION
5.6.2.3 Temperature …. Where alternating temperatures are indicated, the lower temperature ~~should~~ be maintained for 16 h and the higher for 8 h. A gradual changeover lasting no more than 3 h may be satisfactory... ….	**5.6.2.3 Temperature** …. Where alternating temperatures are indicated, the lower temperature must be maintained for 16 h and the higher for 8 h. A gradual changeover lasting no more than 3 h may be satisfactory... ….

Vote to accept item	Yes votes	No votes	Result
C.5.1	24	1	

C.5.2 Add cautionary statement for potassium nitrate (KNO3) solution usage

A change for 5.6.3.1 by adding a seedling evaluation cautionary statement when using potassium nitrate solution to break physiological dormancy. The use of a potassium nitrate (KNO3) solution for germination testing has been known to cause shortened roots and root damage for some species. It is critical for laboratories to be informed of this test artifact possibility and to use caution when evaluating seedlings that have been exposed to a KNO3 solution. Retesting is recommended when root injury may have been caused by using the KNO3 solution.

The following supporting evidence has been provided to support this proposal:
AOSA Rules contain a cautionary statement regarding the possible negative effects of KNO3 solution on seedling roots; Proof of concept study conducted by Takayuki Okuda confirmed shortened roots can be a side effect of KNO3 use; ISTA Rules 5.6.3.1 Gibberellic acid (GA3) provides a cautionary statement when using GA3 for dormancy breaking, regarding the potential negative impact of GA3 on seedling development. See Appendix 1.

This proposal harmonises with the AOSA Rules Volume 1 Section 6.9.a. and AOSA Rules Volume 4 POACEAE, GRASS FAMILY V – OTHER kinds Notes 1. cautionary statements.

This proposal was submitted by the Germination Committee.

GER Committee Votes	Yes:	16	No:	0	Abstain/Absent:	0

CURRENT VERSION	PROPOSED VERSION
5.6.3.1 Procedures for breaking physiological dormancy …. Potassium nitrate (KNO3): Instead of water, up to 0.2 % KNO3 solution, prepared by dissolving up to 2 g KNO3 in 1 litre of water, is used to saturate the germination substrate at the beginning of the test. Water is used for moistening thereafter. ….	**5.6.3.1 Procedures for breaking physiological dormancy** …. Potassium nitrate (KNO3): Instead of water, up to 0.2 % KNO3 solution, prepared by dissolving up to 2 g KNO3 in 1 litre of water, is used to saturate the germination substrate at the beginning of the test. Water is used for moistening thereafter. The use of KNO3 may cause shortened or damaged roots and promote fungal growth for some species and for some germination tests. If these observed symptoms are suspected due to the use of KNO3, it is recommended to retest on substrate moistened with a lower concentration of KNO3. ….

Vote to accept item	Yes votes	No votes	Result
C.5.2	25		

Commented [JB20]: Water was replaced with KNO3 to address concern with limiting the use of KNO3 brought up in the open rules webinar

Commented [JB21R20]: C5.2

C.5.3 Add ethephon for breaking dormancy in *Helianthus annuus* ; add requirement to report concentration of dormancy breaking solutions

The purpose of this proposal is to add a dormancy breaking procedure using ethephon, to more efficiently overcome physiological dormancy of *Helianthus annuus* L. seeds and require the reporting of dormancy breaking solutions on certificates.

Immediately after harvesting sunflower, seed dormancy is sometimes very deep and ISTA methods recommended break dormancy of sunflower (i.e., prechilling and preheating) do not completely release the dormancy. It is in that case impossible to assess the full germination potential of the seeds with a germination test. Preliminary experiments and a validation study involving 7 laboratories on 10 sunflower seed samples have demonstrated that ethephon is particularly efficient when high levels of physiological dormancy occur.

It is therefore proposed to add this procedure to break sunflower seed dormancy. It requires adding the description of the procedure in 5.6.3.1 Procedures for breaking physiological dormancy, and to include the recommendation for sunflower in Table 5A. Part 1.

This proposal would also amend 1.5.2.6 and 5.9 requiring the reporting of the concentration of solutions used for promoting germination, such as ethephon.

The proposal is supported by a validation study that has been reviewed and approved by the STATS TCOM.

This proposal does not harmonise with the AOSA Rules. There are no dormancy breaking recommendations or requirements listed in AOSA Rules Volume 1 Table 6A for *Helianthus annuus* . The AOSA Rules do not require reporting the use of germination promoting solution concentrations.

This proposal was submitted by the Germination Committee

GER Committee Votes	Yes:	16	No:	0	Abstain/Absent:	0	

CURRENT VERSION	PROPOSED VERSION
5.6.3.1 Procedures for breaking physiological dormancy .... When a fuller germination assessment is required by the laboratory or upon the request of the customer, retesting utilising a procedure for removing dormancy is essential. The best result achieved must be reported and the procedure must be stated on the ISTA Certificate. [None - New section to be added before Prechilling] **Prechilling**: The replicates for germination are placed in...	**5.6.3.1 Procedures for breaking physiological dormancy** .... When a fuller germination assessment is required by the laboratory or upon the request of the customer, retesting utilising a procedure for removing dormancy is essential. The best result achieved must be reported and the procedure must be stated on the ISTA Certificate. **Ethephon**: *Helianthus annuus* seeds are soaked in a 0.3 g/l (300 ppm) active ingredient ethephon solution for 18 hours (± 1 hour) at room temperature. After soaking, the seeds are drained, poured onto absorbent paper and wiped dry. The germination test must start promptly after drying. **Prechilling**: The replicates for germination are placed in...

CURRENT VERSION

Table 5A Part 1.

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice	Seedling Evaluation Group
1	2	3	4	5	6	7	8	9
Helianthus annuus	BP; TPS; S; O	20<=>30; 25; 20	4	10	Preheat; prechill	–	–	A-2-1-1-2

PROPOSED VERSION

Table 5A Part 1.

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice	Seedling Evaluation Group
1	2	3	4	5	6	7	8	9
Helianthus annuus	BP; TPS; S; O	20<=>30; 25; 20	4	10	Preheat; prechill; ethephon	–	PP not advisable with ethephon	A-2-1-1-2

CURRENT VERSION	PROPOSED VERSION
5.9 Reporting results	**5.9 Reporting results**
…. The following additional information must be reported under 'Other determinations': …. • any special treatment or method used for promoting germination (5.6.3); ….	…. The following additional information must be reported under 'Other determinations': …. • any special treatment or method used for promoting germination (5.6.3), concentration of solutions used to promote germination (e.g., KNO3, GA3, Ethephon); ….

Consequential change to Chapter 1

CURRENT VERSION	PROPOSED VERSION
1.5.2.6 Germination	**1.5.2.6 Germination**
…. The following additional information must be reported under 'Other determinations': …. • any special treatment or method used for promoting germination (5.6.3); ….	…. The following additional information must be reported under 'Other determinations': …. • any special treatment or method used for promoting germination (5.6.3), concentration of solutions used to promote germination (e.g., KNO3, GA3, Ethephon); ….

Vote to accept item	Yes votes	No votes	Result
C.5.3	25	0	

C.5.4 Add drying instructions for presoaked *Beta vulgaris*

A change is required at 5.6.3.3 and Table 5A Part 1 to add the drying temperature after presoaking to harmonise with drying temperature after prewashing. An editorial change is also needed to the prewashing drying temperature to align with 5.6.3.3.

This proposal does not harmonise with the AOSA Rules.

This proposal was submitted by the Germination Committee.

GER Committee Votes	Yes:	12	No:	2	Abstain/Absent:	3

CURRENT VERSION	PROPOSED VERSION
5.6.3.3 Procedures for removing inhibitory substances …. Presoaking: Soak seeds in water for 2 h using 250 ml of water per 100 seeds. Rinse in running water and blot the surface dry. The temperature of the soaking and rinsing water must be 20–25 °C. Pelleted seed must not be presoaked. Prewashing: ... After washing, the seeds must be dried at a temperature of 20–25 °C (e.g. *Beta vulgaris*). Pelleted seed must not be prewashed.	**5.6.3.3 Procedures for removing inhibitory substances** …. Presoaking: Soak seeds in water for 2 h using 250 ml of water per 100 seeds. Rinse in running water and blot the surface dry. The temperature of the soaking and rinsing water must be 20–25 °C. After soaking, the seeds must be dried at a temperature of 20–25 °C (e.g. *Beta vulgaris*). Pelleted seed must not be presoaked. Prewashing: ... After washing, the seeds must be dried at a temperature of 20–25 °C (e.g. *Beta vulgaris*). Pelleted seed must not be prewashed.

CURRENT VERSION

Table 5A Part 1.

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice	Seedling Evaluation Group
1	2	3	4	5	6	7	8	9
Beta vulgaris	TP; BP; S	20<=>30; 15<=>25; 20	4	14	Presoak (2 h; 250 ml water per 100 seeds); prewash (multigerm: 2 h; genetic monogerm: 4 h); dry at ~~max.~~ 25 °C	–	–	A-2-1-1-1

PROPOSED VERSION

Table 5A Part 1.

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice	Seedling Evaluation Group
1	2	3	4	5	6	7	8	9
Beta vulgaris	TP; BP; S	20<=>30; 15<=>25; 20	4	14	Presoak (2 h; 250 ml water per 100 seeds); dry at 20-25 °C prewash (multigerm: 2 h; genetic monogerm: 4 h); dry at 20-25 °C	–	–	A-2-1-1-1

Vote to accept item	Yes votes	No votes	Result
C.5.4	24	1	

C.5.5 Add tolerance tables for replicates of 50 and 25 seeds

The purpose of this proposal is to add five additional replicate to replicate tolerance tables to section 5.11 Part B for replicates of 50 seeds and 25 seeds. The following tolerance tables would be added: Table 5B Part 4. (Four reps of 50 seeds); Table 5B Part 5. (eight reps of 50 seeds); Table 5B Part 6. (four reps of 25 seeds); Table 5B Part 7. (eight reps of 25 seeds); Table 5B Part 8. (sixteen reps of 25 seeds).

The proposal provides laboratories with tolerance tables for germination tests that consist of replicates of 50 seeds and 25 seeds. Laboratories would no longer be permitted to combine replicates of less than 100 seeds to artificially create replicates of 100 seeds. The new tolerance tables will reduce the frequency of required retests due to replicates being out of tolerance.

The STATS TCOM created the proposed additional tolerance tables and supports this proposal. If this proposal is adopted, the STATS TCOM will update the related tolerance calculation tools in the GER TCOM Toolbox.

This proposal does not harmonise with the AOSA Rules. AOSA Volume 1 section 6.6 states sub-replicates of 25 or 50 seeds are required to be combined to form replicates of 100 seeds, since section 14.5 Germination tolerances does not include tolerance tables for replicates of less than 100 seeds.

This proposal was submitted by the Germination Committee.

GER Committee Votes	Yes:	16	No:	0	Abstain/Absent:	0	

CURRENT VERSION	PROPOSED VERSION
5.11 Tolerance tables	**5.11 Tolerance tables**
Table 5B gives the maximum tolerated differences between the highest and lowest germination percentages of the replicates of a germination test, allowing only for random sampling variation at a probability of 0.025. ~~To determine whether a test is reliable, calculate the average germination percentage over all replicates, to the nearest whole number. If necessary, in tests of 400 or 200 seeds, four or two replicates, respectively, of 100 seeds each can be formed by combining the subreplicates of 50 or 25 seeds which were closest together in the germinator. In tests of 100 seeds, two replicates of 50 seeds each can be formed by combining the subreplicates of 25 seeds which were closest together in the germinator, and multiplying the results of each of the two replicates by 2 to obtain an average germination percentage.~~ ….	Table 5B gives the maximum tolerated differences between the highest and lowest germination percentages of the replicates of a germination test, allowing only for random sampling variation at a probability of 0.025. To determine whether a test is reliable, calculate the average germination percentage over all replicates, to the nearest whole number. ….

CURRENT VERSION	PROPOSED VERSION
Table 5B	**Table 5B**

....

Table 5B Part 3. Two replicates of 50 seeds

Average germination percentage of test		Tolerance
51–100 %	0–50 %	
99	2	5
98	3	7
97	4	8
96	5	9
95	6	10
94	7	11
92–93	8–9	12
90–91	10–11	13
89	12	14
86–88	13–15	15
84–85	16–17	16
81–83	18–20	17
78–80	21–23	18
74–77	24–27	19
70–73	28–31	20
63–69	32–38	21
51–62	39–50	22

Table 5C. Tolerances between results of two tests on the same or a different submitted sample when tests are made in the same laboratory (two-way test at the 2.5 % significance level)

Table 5C Part 1. Two tests of 400 seeds

....

....

Table 5B Part 3. Two replicates of 50 seeds

Average germination percentage of test		Tolerance
51–100 %	0–50 %	
99	2	5
98	3	7
97	4	8
96	5	9
95	6	10
94	7	11
92–93	8–9	12
90–91	10–11	13
89	12	14
86–88	13–15	15
84–85	16–17	16
81–83	18–20	17
78–80	21–23	18
74–77	24–27	19
70–73	28–31	20
63–69	32–38	21
51–62	39–50	22

INSERT THE FOLLOWING TABLES HERE

Table 5C. Tolerances between results of two tests on the same or a different submitted sample when tests are made in the same laboratory (two-way test at the 2.5 % significance level)

Table 5C Part 1. Two tests of 400 seeds

Table 5B Part 4. Four replicates of 50 seeds

Average germination percentage of test		Tolerance
51-100%	0-50%	
99	2	7
98	3	8
97	4	10
96	5	11
95	6	13
94	7	14
92-93	8-9	15
91	10	16
90	11	17
88-89	12-13	18
87	14	19
85-86	15-16	20
83-84	17-18	21
80-82	19-21	22
78-79	22-23	23
75-77	24-26	24
71-74	27-30	25
66-70	31-35	26
59-65	36-42	27
51-58	43-50	28

Table 5B Part 5. Eight replicates of 50 seeds

Average germination percentage of test		Tolerance
51-100%	0-50%	
99	2	8
98	3	10
97	4	12
96	5	13
95	6	15
94	7	16
93	8	17
92	9	18
91	10	19
89-90	11-12	20
88	13	21
87	14	22
85-86	15-16	23
83-84	17-18	24
81-82	19-20	25
79-80	21-22	26
77-78	23-24	27
74-76	25-27	28
71-73	28-30	29
67-70	31-34	30
62-66	35-39	31
51-61	40-50	32

Table 5B Part 6. Four replicates of 25 seeds

Average germination percentage of test		Tolerance
51-100%	0-50%	
99	2	9
98	3	12
97	4	14
96	5	16
95	6	18
94	7	19
93	8	21
92	9	22
91	10	23
90	11	24
89	12	25
88	13	26
87	14	27
86	15	28
84-85	16-17	29
83	18	30
81-82	19-20	31
79-80	21-22	32
77-78	23-24	33
75-76	25-26	34
73-74	27-28	35
70-72	29-31	36
67-69	32-34	37
62-66	35-39	38
52-61	40-49	39
51	50	40

Table 5B Part 7. Eight replicates of 25 seeds

Average germination percentage of test		Tolerance
51-100%	0-50%	
99	2	11
98	3	14
97	4	17
96	5	19
95	6	21
94	7	22
93	8	24
92	9	25
91	10	27
90	11	28
89	12	29
88	13	30
87	14	31
86	15	32
85	16	33
84	17	34
82-83	18-19	35
81	20	36
80	21	37
78-79	22-23	38
76-77	24-25	39
74-75	26-27	40
72-73	28-29	41
69-71	30-32	42
66-68	33-35	43
62-65	36-39	44
56-61	40-45	45
51-55	46-50	46

Table 5B Part 8. Sixteen replicates of 25 seeds

Average germination percentage of test		Tolerance
51-100%	0-50%	
99	2	12
98	3	16
97	4	19
96	5	21
95	6	23
94	7	25
93	8	27
92	9	28
91	10	30
90	11	31
89	12	33
88	13	34
87	14	35
86	15	36
85	16	37
84	17	38
83	18	39
81-82	19-20	40
80	21	41
79	22	42
77-78	23-24	43
76	25	44
74-75	26-27	45
72-73	28-29	46
69-71	30-32	47
67-68	33-34	48
63-66	35-38	49
59-62	39-42	50
51-58	43-50	51

Vote to accept item	Yes votes	No votes	Result
C.5.5	24	0	

Chapter 6: Biochemical test for viability: the topographical tetrazolium test

C.6.1 Add new tetrazolium method for *Glycine max*

The purpose of this proposal is to add a new tetrazolium method for *Glycine max* .

The AOSA Rules do not include detailed tetrazolium procedures.

This proposal was submitted by the Tetrazolium Committee and is supported by a validation study.

TEZ Committee Votes	Yes:	13	No:	0	Abstain/Absent:	0	

PROPOSED ADDITION

Table 6A Part 1.

Species	Pretreatment type/minimum time (h)	Preparation before staining	Staining solution (%)	Optimum staining time (h)	Preparation for evaluation	Permitted non-viable tissue	Remarks
1	2	3	4	5	6	7	8
Glycine max	BP/18	Leave seeds intact*	1	6	Remove seed coat to expose embryo and cut longitudinally through cotyledons and the radicle-hypocotyl axis. Observe both sides of the seed	⅓ radicle-hypocotyl axis measured from radicle tip, ½ of distal end of cotyledons if superficial, ⅓ of distal end of cotyledons if pervading	*If the viability of hard seeds is to be determined, the seed coat can be incised at distal end of cotyledons and soaked (W/4)

Vote to accept item	Yes votes	No votes	Result
C.6.1	25	0	

C.6.2 Checking the quality of tetrazolium solution

The following proposal has been developed by ISTA Tetrazolium Committee with the aim of introducing criteria for the quality checking of tetrazolium solution.

There is no equivalent in the AOSA Rules.

This proposal was submitted by the Tetrazolium Committee.

TEZ Committee Votes	Yes:	13	No:	0	Abstain/Absent:	0

CURRENT VERSION	PROPOSED VERSION
6.4.1 Tetrazolium solution An aqueous solution of 2,3,5-triphenyl tetrazolium... The tetrazolium solution can be stored in the dark at 5–10 °C for up to one year. **6.4.2** Buffer solution	**6.4.1 Tetrazolium solution** An aqueous solution of 2,3,5-triphenyl tetrazolium... The tetrazolium solution can be stored in the dark at 5–10 °C for up to one year. **6.4.1.1 Effectiveness verification of the Tetrazolium solution and salt** If the tetrazolium solution is prepared with salt of a new batch, a test of effectiveness must be done. For example, during routine testing, two replicates will be tested with the old solution and two replicates with the new one. The check tetrazolium test can also be carried out on a sample with a known quality (i.e. a sample whose quality has been ascertained either from a tetrazolium or a germination test). If the same batch of salt is used for preparing new tetrazolium solution, no additional test of effectiveness is necessary, provided that the reagent has been stored correctly (or in accordance with the instructions).

Vote to accept item	Yes votes	No votes	Result
C.6.2	25	0	

Chapter 7: Seed health testing

C.7.1 Change temperature ranges 7-019a

Changing the to specified temperature ranges into a more flexible temperature indication for 7 -019a: Detection of *Xanthomonas campestris* pv. *campestris* and *Xanthomonas campestris* pv. *raphani* in *Brassica* spp. seed. This is also now in harmony with other seed health rules in Chapter 7.

The AOSA Rules do nothave seed health methods.

This proposal was submitted by the Seed Health Committee.

SHC Committee Votes	Yes:	13	No:	0	Abstain/Absent:	2

CURRENT VERSION	PROPOSED VERSION
7 -019a: Detection of *Xanthomonas campestris* pv. *campestris* and *Xanthomonas campestris* pv. *raphani* in *Brassica* spp. seed	**7 -019a: Detection of *Xanthomonas campestris* pv. *campestris* and *Xanthomonas campestris* pv. *raphani* in *Brassica* spp. seed**
Incubator: operating at 28~~–30~~ °C	**Incubator:** operating at 28 ±2 °C
3.1.3 incubate for 48h at 28 ºC	3.1.3 incubate for 48h at 28 ±2 ºC
4.3 Incubate plates at 28~~–30~~ °C and examine after 3–4 d.	4.3 Incubate plates at 28 ±2 °C and examine after 3–4 d.
5.3 Incubate sectored plates for 25-48 h at 28~~–30 °C~~	5.3 Incubate sectored plates for 25-48 h at 28 ±2 ºC
6.1 Grow seedlings of a Brassica cultivar known to be susceptible to all races of Xcc/Xcr (e.g. cabbage 'Wirosa', see Vicente et al., 2001) in small pots or modules until at least 3~~–4~~ true leaf stage.	6.1 Grow seedlings of a Brassica cultivar known to be susceptible to all races of Xcc/Xcr (e.g. 'Wirosa'; see Vicente et al., 2001) at 20–30 °C ~~(±2 °C)~~ in small pots or modules until at least 2–3 true leaf stage.
6.6 Grow on plants at 20~~–25 °C~~.	6.6 Grow on plants at 20–30 °C ~~(±2 °C)~~.

Commented [JB22]: Erroneous tolerance, as this is a range same as 6.6

Commented [JB23R22]: C7.1

Vote to accept item	Yes votes	No votes	Result
C.7.1	24	0	

C.7.2 Change temperature ranges

Changing the to specified temperature ranges into a more flexible temperature indication. This is also now in harmony with other seed health rules in Chapter 7.

The AOSA Rules do nothave seed health methods.

This proposal was submitted by the Seed Health Committee.

SHC Committee Votes	Yes:	13	No:	0	Abstain/Absent:	2

CURRENT VERSION	PROPOSED VERSION
7-019b: Detection of *Xanthomonas campestris* pv. *campestris* in disinfested/disinfected *Brassica* spp. seed **Incubator:** operating at 28~~–30 °C)~~ 2.7 Incubate plates at 28~~–30~~ °C upside down and examine after 4–6 days (CCP). 6.3 Incubate sectored plates for 3–4 d at 28~~–30~~ °C. 7.6 Grow on plants at 20–30 °C	**7-019b: Detection of *Xanthomonas campestris* pv. *campestris* in disinfested/disinfected *Brassica* spp. seed** **Incubator:** operating at 28 ±2 °C 2.7 Incubate plates at 28 ±2 °C upside down and examine after 4–6 days (CCP). 6.3 Incubate sectored plates for 3–4 d at 28 ±2 °C. 7.6 Grow on plants at 20–30 °C. ~~(±2 °C)~~

Vote to accept item	Yes votes	No votes	Result
C.7.2	24	0	

Commented [JB24]: Erroneous tolerance, as this is a range

Commented [JB25R24]: C7.2

C.7.3 Seed health method for *Fusarium* on cereal seeds

The following proposal was submitted by the ISTA Seed Health Committee with the aim of introducing a new seed health method for the detection of *Fusarium* species on cereal seeds. The AOSA Rules do nothave seed health testing.						
This proposal was submitted by the Seed Health Committee and is supported by a validation study.						
SHC Committee Votes	Yes:	13	No:	0	Abstain/Absent:	2

PROPOSED VERSION

7-035: Detection of *Fusarium* species on cereal seeds Host:

Triticum aestivum L.; *Hordeum vulgare* L.; *Avena sativa* L.

Pathogen(s):

- *Fusarium avenaceum* (Fries) Saccardo
- *Fusarium graminearum* Schwabe
- *Fusarium culmorum* (W.G. Smith) Saccardo
- *Fusarium crookwellense* Burgess, Nelson & Toussoun
- *Fusarium langsethiae* Torp & Nirenberg
- *Fusarium poae* (Peck) Wollenweber
- *Fusarium tricinctum* (Corda) Saccardo
- *Fusarium sporotrichioides* Sherbakoff
- *Fusarium pseudograminearum* Aoki & O'Donnell

Prepared by: ISTA Seed Health Committee

Authors: Le Daré, L[1]., Sérandat, I.[1], McEwan, M[2], Brodal, G[3], Udnes Aamot, H[3], Isaksen, B[4], Alberti, I[5]

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Commented [JB26]: Numbering assigned after open rules session C7.3

Commented [JB27R26]: Address corrected after open rules session

Commented [JB28R26]: Corrected wording after comment in open rules session

Commented [JB29R26]: Incubation step added to flow chart, no change in method.

Revision history

Version 1.0

Background

Besides *Microdochium majus* and *Microdochium nivale*, *Fusarium* head blight (FHB) complex is also caused by several *Fusarium* species and is considered one of the most important diseases of cereals worldwide. The disease may result in significant yield losses and poor seed quality. Several of these *Fusarium* species can produce toxic secondary metabolites (mycotoxins) (Desjardins, 2006) that can reduce the use of the grain for human and/or animal consumption. For example, the species *Fusarium graminearum*, one of the most aggressive FHB pathogens and producer of the important mycotoxin deoxynivalenol (DON), was ranked number four in an international nomination of the top ten most economically important fungal pathogens (Dean *et al*., 2012).

At the ISTA seed health workshop in Angers, France in 2010, participants commented that the ISTA method 7-022 for detecting *Microdochium* spp. is also suitable for detection of *Fusarium* spp. It was stated in the validation report for 7-022 that the method was appropriate for the generic detection of *Fusarium* species, with the addition of further steps to aid species identification.

Potato dextrose agar (PDA) and malt agar (MA) are suitable media to detect *Fusarium* species on cereal seed (Leslie and Summerell, 2006) and were used for the validation. Incubation at 20 ±2 °C will either be with near-ultraviolet (NUV) light, or in darkness followed by exposure to daylight or NUV light. However, the transfer of colonies to special low-nutrient media, e.g. spezieller nährstoffarmer agar (SNA) or carnation leaf-piece agar (CLA), would facilitate species identification. The low-nutrient media induce sporulation and reliably produce uniform conidia that are more representative of the species than those produced on PDA or MA. If plated on different media, *Fusarium* species can present notoriously different phenotypes. For identification, it is thus important to standardise the media used within the laboratory and ensure it is of a consistent make-up.

The ISTA Seed Health Committee decided to carry out a validation study for the detection of *Fusarium* spp. on cereal seeds, based on the ISTA 7-022 method for detection of *Microdochium* spp. on wheat (ISTA, 2020).

Nine species of *Fusarium* were tested using this method, as well as *M. majus* and *M. nivale*: *F. graminearum, F. culmorum, F. poae*, *F. langsethiae*, *F. avenaceum*, *F. crookwellense*, *F. pseudograminearum*, *F. sporotrichioides* and *F. tricinctum*.

Hazard group

Fusarium species have been categorised in hazard group 2 as cereal pathogens, due to their capacity to produce mycotoxins, and in hazard group 2 as human pathogens which can cause disease, especially in immunosuppressed individuals. These pathogens should be handled at containment level 2 and any procedures likely to cause airborne spores should be conducted within a microbiological safety cabinet. Please ensure you have carried out a risk assessment for handling these pathogens and consult the Health and Safety Executive (HSE) guidance (Biosafety and microbiological containment; www.hse.gov.uk/biosafety/) for further information.

Treated seed

This method has not been validated for the detection of *Fusarium* on treated seed. (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) and subsample size depend on the desired tolerance standard (maximum acceptable number of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum recommended sample size should be 400 seeds.

Materials

Reference material: reference cultures or other appropriate material
Media: malt agar or potato dextrose agar with streptomycin sulphate (for detection); carnation leaf-piece agar or spezieller nährstoffarmer agar (for species confirmation)
Autoclave
1 % sodium hypochlorite solution (NaOCl): for seed disinfection
Deionised or sterile distilled water
Sterile or aseptic blotter paper: for blotting seed dry
Plates: 90 mm sterile Petri dishes (one for a maximum of ten seeds)
Steriliser: microbead steriliser or methylated spirit burner for sterilisation of microscope tools
Forceps
Dissecting needles
Microscope slides and coverslips
Incubator: operating at 20 ±2 °C darkness (incubation of samples)
Incubator: operating at 20 ±2 °C with 12 h near-ultraviolet light (NUV) 360 nm (after subculture on CLA and SNA)
Stereomicroscope: ×6.5–50 magnification)
Compound microscope (×100–400 magnification)

Methods

Note: All the steps described here are important and should be followed as written. Critical control points are indicated by CCP.

This method includes a procedure to detect *Fusarium** species, and descriptors to identify some *Fusarium* species (Fig. 1).

**Fusarium* reporting results can be expressed as genus, part of a complex or to species level (see 'Reporting results').

Overview of method

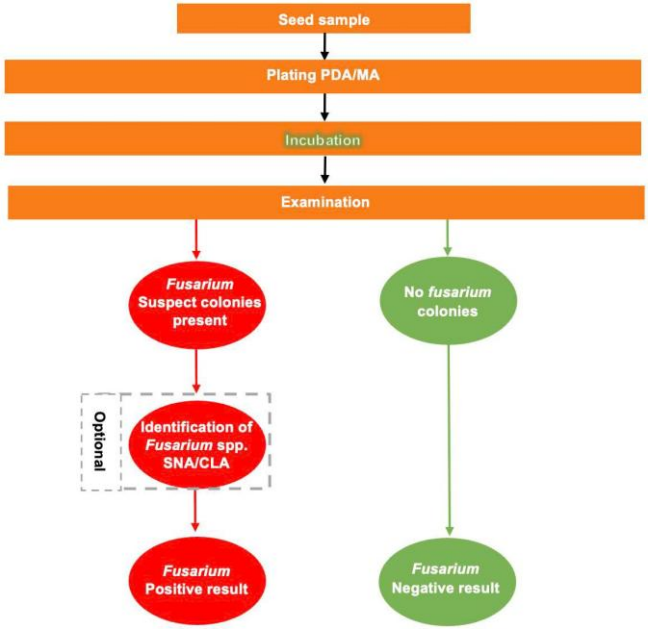

Figure 1. Workflow describing the steps and decisions for detection of *Fusarium* spp. by plating on media and morphological identification.

1. Detection method

1.1 Pretreatment: Immerse seeds in a 1% sodium hypochlorite (NaOCl) solution for 10 min, then drain, rinse well in deionised or sterile distilled water and drain again. Dry the seeds on clean blotting paper.

1.2 Plating: Aseptically place a maximum of ten seeds, evenly spaced around the perimeter of the plate, onto the agar surface of each PDA or MA plate (CCP).

1.3 Positive control (reference material): Aseptically plate a reference culture corresponding to at least one of the *Fusarium* species, on the same medium as that used for the detection.

1.4 Incubation: Incubate plates for 7 d at 20 ±2 ˚C in the dark.

1.5 Examination: Examine the plates after 7 d of incubation.

Examine the plates by eye, looking at each colony arising from the seeds for cultures with a morphology matching a *Fusarium* species. If required, subculturing can be done onto CLA or SNA plates for an accurate *Fusarium* species identification (optional). Some isolates may be easily identified without further subculturing. *Fusarium* species are very diverse in colour, shape and size; the main species found on cereals are described at the end of the method. The identity of colonies and conidia from sporodochia may be confirmed by compound microscope (×100–1000 magnification). Incubation can be extended under NUV at 360 nm for a few days to stimulate sporulation. When the identity of a colony remains uncertain, these colonies can be subcultured on CLA adjacent to the carnation leaf piece or on SNA + filter paper (see Fig. 2) medium with incubation under NUV.

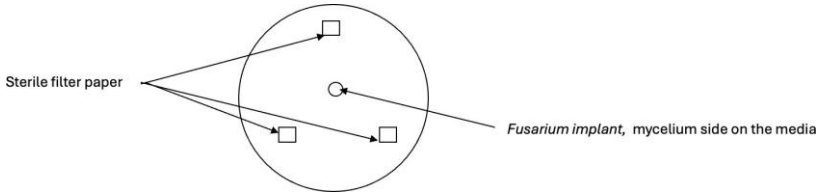

Figure 2. Subculture for identification of *Fusarium* on spezieller nährstoffarmer agar medium.

The main morphological criteria that must be considered for the identification are:

- Presence or absence of microconidia
- Microconidia shape
- Type of conidiophores
- Phialide (conidiophore) type
- Presence or absence of macroconidia
- Macroconidia shape
- Presence or absence of chlamydospore
- Other structures: coiled sterile hyphae, pseudochlamydospores, swollen hyphae

The morphological criteria are described at the end of the method.

General methods

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.

Depending on whether it has been possible to identify the species, the results reported could be:

- *Fusarium* spp.
- *Fusarium* in a named complex following Crous *et al*. (2021), O'Donnel *et al*. (2022) (percentage for each complex). The complexes concerning the *Fusarium* defined in this method are described in the table below.
- Individual *Fusarium* species (percentage for each identified species).
- A combination of these three propositions.

Morphological criteria of main *Fusarium* on cereals:

The morphological criteria listed below are based on descriptions from literature (see References) and from observations obtained during the performance study of the method in analytical specificity on a collection of target isolates. The media used, the components of the media and incubation conditions (darkness/NUV light) can lead to slight differences in morphological appearance. Main criteria are described below and are exemplified in the images of different species.

The species described here are based on Index Fungorum (www.speciesfungorum.org/Names/Names.asp). When other species names are described, the reference is given in the text.

***Fusarium* species included in the *Fusarium* complex**

Species	*Fusarium* complex
F. graminearum Schwabe	*F. sambucinum* complex
F. pseudograminearum Aoki & O'Donnell	
F. culmorum (W.G. Smith) Saccardo	
F. crookwellense Burgess, Nelson & Toussoun	
F. langsethiae Torp & Nirenberg	
F. poae (Peck) Wollenweber	
F. sporotrichioides Sherbakoff	
F. tricinctum (Corda) Saccardo	*F. tricinctum* complex
F. avenaceum (Fries) Saccardo	

Quality assurance

Critical control points (CCP)

- Method section 1.2: Many saprophytic fungi present on cereals are fast growing in comparison to the target pathogens; there is a risk of the plates being unreadable if the seeds are too close.
- Preparation of PDA or MA plates: The source of agar may influence the results. The level of available nutrients may vary between manufacturers. Both PDA and MA can be bought as a powdered medium, or MA can be made up following the recipe below. Whenever a new batch of agar is used, a check on the quality should be made, using a reference lot with a known infection level, or a reference isolate and sustainability of isolate measured. Pay particular attention to the growth characteristics of reference isolates.

Media and solutions

Potato dextrose agar + streptomycin (CCP)

Potato dextrose agar: make according to specification of supplier
Distilled/deionised water: 1000 ml
Streptomycin sulphate*: may be used between 50 mg and 130 mg, depending on the level of saprophytic bacterial contamination commonly encountered
*Added after autoclaving. Streptomycin sulphate can be dissolved in water. Filter sterilisation is required.

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 ml of distilled/deionised water.
3. Dissolve powdered PDA in the water by stirring.
4. Autoclave at 121 °C and 15 psi for 15 min.
5. Adjust to pH 5.6 ±0.2 pH units.
6. Allow agar to cool to approximately 50 °C and add streptomycin sulphate dissolved in water.
7. Pour 20–22 ml of molten agar into 90 mm Petri dishes and allow to solidify before use.

Storage

Prepared plates may be stored at 5 ±2 °C for up to 6 weeks.

Malt agar (MA) + streptomycin (CCP)

Agar: 20 g
Malt: 10 g

Distilled/deionised water: 1000 ml
Streptomycin sulphate*: may be used between 50 mg and 130 mg, depending on the level of saprophytic bacterial contamination commonly encountered
*Added after autoclaving. Streptomycin sulphate can be dissolved in water. Filter sterilisation is required.

Preparation

1. Weigh out the malt into a suitable container.
2. Add 1000 ml of distilled/deionised water.
3. Add the agar and the remainder of the water, and dissolve completely before autoclaving.
4. Autoclave at 121 °C and 15 psi for 15 min.
5. Allow agar to cool to approximately 50 °C and add streptomycin sulphate dissolved in water.
6. Pour 18–20 ml of molten agar into 90 mm Petri dishes and allow to solidify before use.

If using ready-to-use 1 % malt agar, prepare according to specifications of supplier.

Storage

Prepared plates may be stored at 5 ±2 °C for up to 6 weeks.

Spezieller nährstoffarmer agar (synthetic nutrient-poor agar) + filter paper

Agar: 15 g
K_2HPO_4: 1.0 g
KNO_3: 1.0 g
$MgSO_4 \cdot 7H_2O$: 0.50 g
KCl: 0.50 g
Glucose: 0.20 g
Saccharose: 0.20 g
Deionised or distilled water: 1000 ml
Sterile or aseptic filter paper (around 1 cm² each)

1. Weigh out agar and other compounds into a suitable autoclavable container.
2. Add 1000 ml of distilled/deionised water.
3. Completely dissolve ingredients in water by stirring.

4. Autoclave at 121 °C and 15 psi for 15 min.
5. Allow to cool to approximately 50 °C.
6. Pour 20 ml of molten agar into 90 mm Petri dishes and allow to solidify before use.
7. Aseptically place three squares of filter paper onto the surface of each media.

Storage
Prepared plates may be stored at 5 ±2 °C for up to 6 weeks.

Carnation leaf-piece agar
Agar: 20 g
Distilled/deionised water: 1000 ml
Carnation leaf pieces: 5–8 mm^2, dried at 70°C for 3–4 h, and gamma irradiated at 2.5 megarads (CCP); up to ten pieces are placed onto a 90 mm water agar plate

1. Weigh out agar into a suitable autoclavable container.
2. Add 1000 ml of distilled/deionised water.
3. Completely dissolve ingredients in water by stirring.
4. Autoclave at 121 °C and 15 psi for 15 min.
5. Allow to cool to approximately 50 °C.
6. Pour 20 ml of molten agar into 90 mm Petri dishes and allow to solidify before use.
7. Aseptically place 10 pieces (spaced apart) of carnation leaves onto the surface of each plate.

Storage
Prepared plates may be stored at 5 ±2 °C for up to 6 weeks.

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 a NaClO 1 % solution must be stored in the dark and used within 3 d of preparation. It is possible to check chlorine concentration with chlorine strip tests.

References

Crous, P.W., Lombard, L., Sandoval-Denis, M., Seifert, K.A., Schroers, H.-J., Chaverri, P. *et al.* (2021). *Fusarium*: more than a node or a foot cell. *Studies in Mycology*, **98**, 1–184.

Dean, R., Van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D. *et al.* (2012). The top ten fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, **13**, 414–430.

Desjardins, A.E. (2006). Fusarium *Mycotoxins: Chemistry, Genetics, and Biology*. American Phytopathological Society (APS Press), St. Paul, MN, USA. 260 pp.

ISTA (2020). 7-022: Detection of *Microdochium nivale* and *Microdochium majus* in *Triticum* spp. (wheat) seed. Validated Seed Health Testing Methods, *International Rules for Seed Testing*. International Seed Testing Association, Wallisellen, Switzerland.

Leslie, J.F. and Summerell, B.A. (2006). *The* Fusarium *Laboratory Manual*. Blackwell Publishing, Ames, IA, USA. 388 pp.

Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. (1983). Fusarium *Species: An Illustrated Manual for Identification*. Pennsylvania State University Press, USA. 183 pp.

O'Donnell, K., Whitaker, B.K., Laraba, I., Proctor, R.H., Brown, D.W., Broders, K. *et al.* (2022). DNA sequence-based identification of *Fusarium*: a work in progress. *Plant Disease*, **106**(6), 1597–1609.

Validation references

ISTA (2025). Method validation reports on Rules proposals for the *International Rules for Seed Testing* 2026 Edition. International Seed Testing Association, Wallisellen, Switzerland.

***Fusarium avenaceum* (Fr.) Sacc.**

(see Fig. 3)

On MA the mycelium is abundant and aerial, yellow to pink coloured, sometimes creamy white; sporodochia are orange, concentrated in the centre of the colony.

On PDA growth can vary from slow to somewhat rapid, the aerial mycelium is dense and white but frequently varies in colour from yellow/tan to reddish-brown; the undersurface colouration varies from tan to carmine red to dark brown; orange sporodochia may form on colonies originating from seed, depending on growth conditions; sporodochia quite often form in the centre of the colony on pure cultures.

Conidiophores: Unbranched and branched monophialides.

Macroconidia are very long, slender and thin walled with a foot-shaped basal cell and an elongated apical cell that may be bent.

Microconidia (cigar-shaped) are rare; mixed sizes of conidia (micro- and macro-) are observed from sporodochia on isolates on PDA, but on CLA only macroconidia true to type are observed.

Chlamydospores are absent.

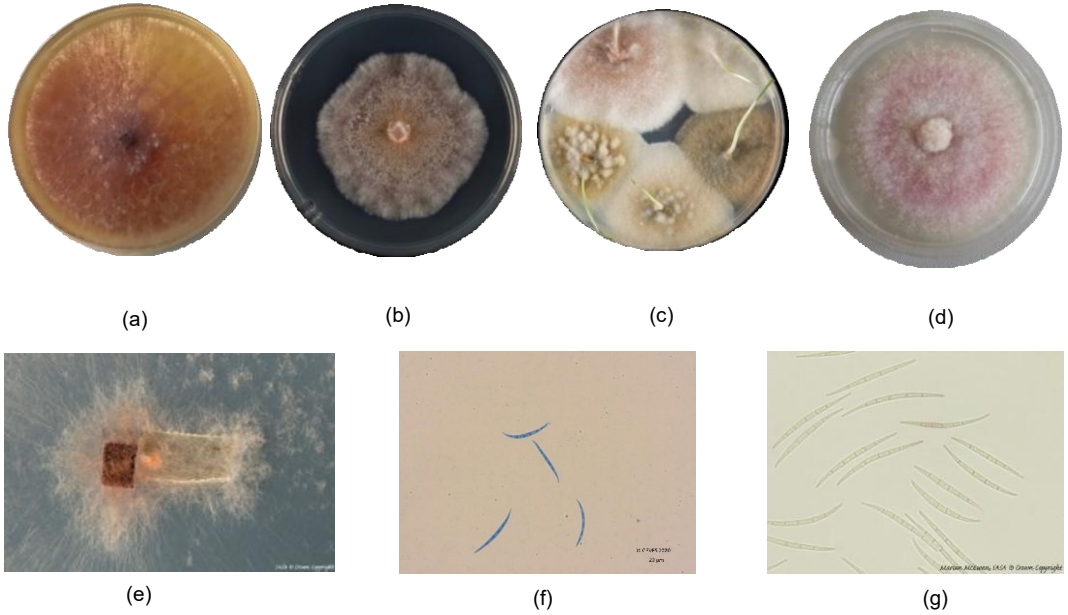

Figure 3. *Fusarium avenaceum*: **a** malt agar in darkness for 14 d; **b** potato dextrose agar (PDA) (Oxoid) with near-ultraviolet (NUV) light for 5 d; **c** PDA (Oxoid) after 7 d growth on seed (top left colony); **d** PDA (Difco) in darkness for 7 d; **e** formation of orange sporodochia containing macroconidia on carnation leaf-piece agar (CLA) after incubation under NUV light; **f** macroconidia stained with methyl blue (×200); **g** macroconidia unstained from CLA (×400).

***Fusarium graminearum* Schwabe**
***Fusarium pseudograminearum* Aoki & O'Donnell**
(see Fig. 4)
On MA the mycelium is abundant and aerial, pink coloured and white to yellow on the aerial part of the mycelium; sporodochia are orange and concentrated in the centre of the colony.
On PDA growth is usually rapid (e.g. Petri dishes filled within 4 d on pure culture) but can be variable, with dense aerial mycelium and frequently yellow to tan with the margins white to carmine red; the undersurface is usually carmine red; red-brown to orange sporodochia, if present, are sparse in the dark; sporodochia often appear in less than a week when under NUV light.
Conidiophores: Unbranched and branched monophialides.
Macroconidia are often present, distinctly septate, thick walled, straight to moderately sickle-shaped; the ventral surface is almost straight, and the dorsal surface smoothly arched; the basal cell is distinctly foot-shaped; the apical cell is curved, cone-shaped like a beak.
Microconidia are absent.
Chlamydospores are rare, formed in the macroconidia or the mycelium.
As *F. avenaceum* and *F. graminearum* share common morphological criteria, they cannot be distinguished visually on plates.

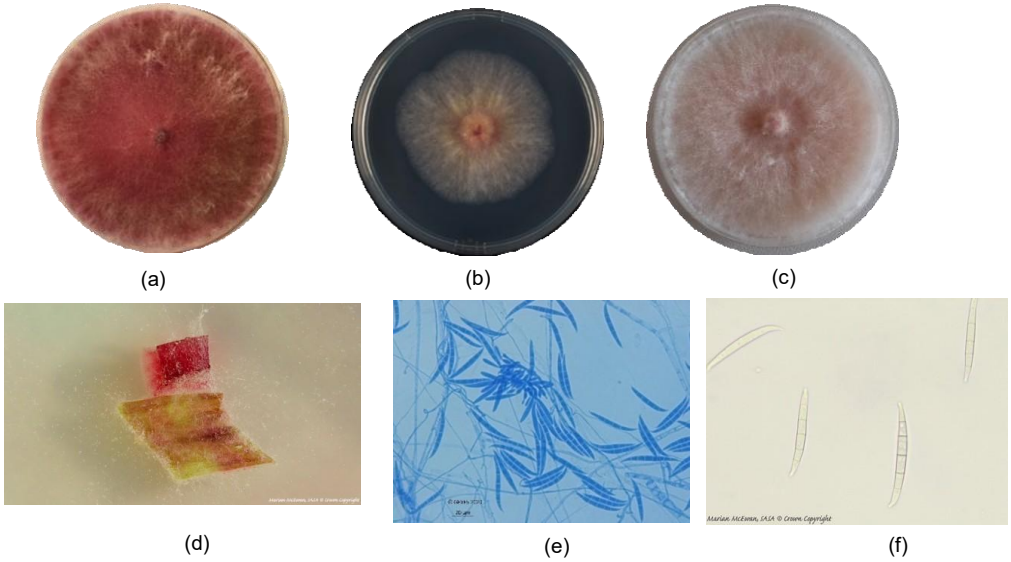

Figure 4. *Fusarium graminearum*:
a malt agar in darkness for 14 d;
b potato dextrose agar (PDA) (Oxoid) in darkness;
c PDA (Difco) in darkness for 7 d;
d *F. graminearum* on carnation leaf-piece agar (CLA);
e macroconidia with methyl blue stain (×200);
f macroconidia unstained from CLA (×400).

***Fusarium culmorum* (Wm.G. Sm.) Sacc.**
(see Fig. 5)
On MA the mycelium is short, pink to carmine red; sporodochia form on older cultures and are orange to red-brown in colour.
On PDA growth is rapid, with dense aerial mycelium, generally white but often yellow to tan on the base; the undersurface is carmine red; orange to red-brown sporodochia appear readily.
Conidiophores: Unbranched and branched monophialides.
Macroconidia are stout, distinctly septate, thick walled and have straight to curved ventral and dorsal surfaces; the basal cell is usually distinctly round and can occasionally be foot-shaped to slightly notched.
Microconidia are absent.
Chlamydospores generally form abundantly and quickly; they may occur singly, in chains or in clumps.

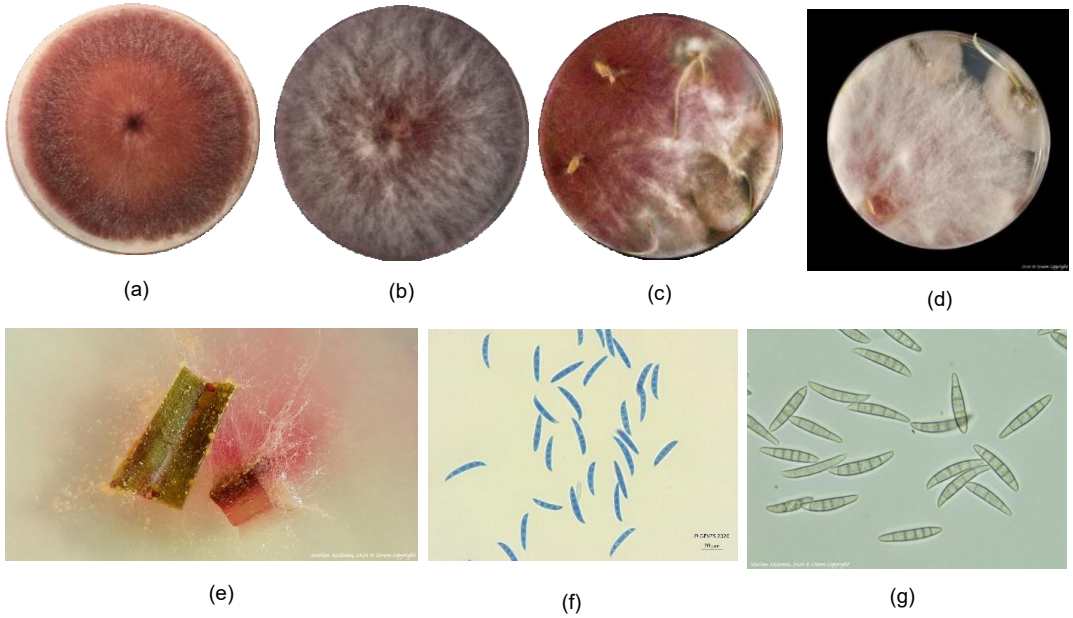

Figure 5. *Fusarium culmorum*:
a malt agar in darkness for 14 d;
b potato dextrose agar (PDA) (Oxoid) in darkness;
c high sporulating isolate PDA (Oxoid) (on left), 7 d in darkness;
d low sporulating isolate PDA (Oxoid) (on left), 7 d in darkness;
e *F. culmorum* on carnation leaf-piece agar (CLA) with orange sporodochia;
f macroconidia with methyl blue stain (×200);
g macroconidia unstained from CLA (×400).

***Fusarium crookwellense* Burgess, Nelson & Toussoun**
***Fusarium cerealis* (ref)**
(see Fig. 6)
On MA the mycelium is abundant and aerial, white to pink, sometimes yellow; sporodochia are orange to red-brown.
On PDA growth is rapid, with dense aerial mycelium, white in colour and then tan; the undersurface is carmine red; orange to red-brown sporodochia generally appear early in the centre of the culture and later in other areas of the culture.
Conidiophores: Unbranched and branched monophialides.
Macroconidia are strongly septate, thick walled and unequally curved, with the ventral surface less curved than the dorsal surface, which is strongly arched; the basal cell is distinctly foot-shaped; the apical cell is distinctly curved and tapers to a narrow tip.
Microconidia are absent.
Chlamydospores are present and are formed in the hyphae and the macroconidia.

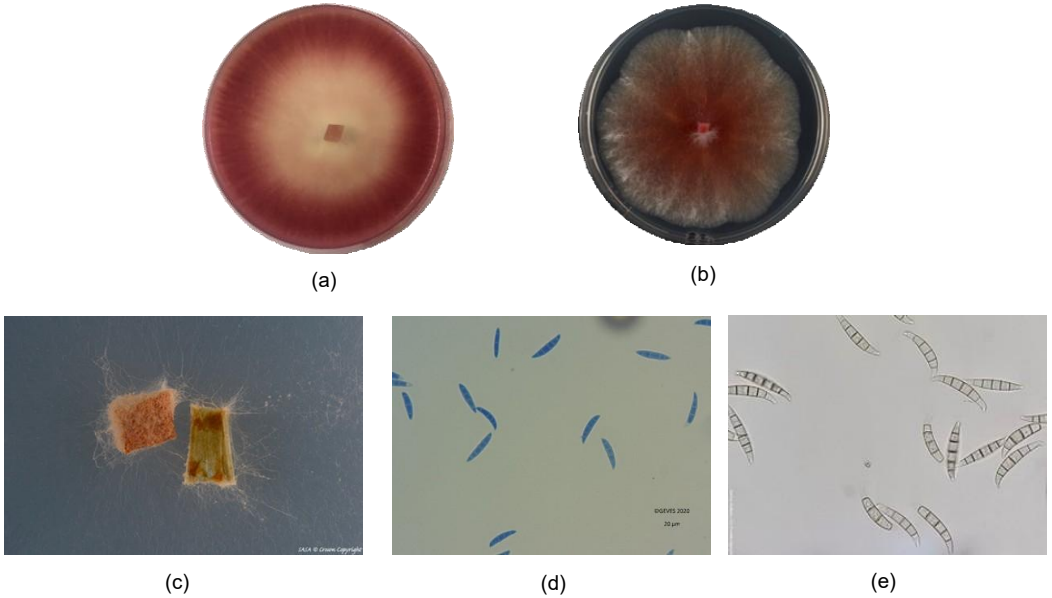

Figure 6. *Fusarium crookwellense*:
a malt agar in darkness for 14 d;
b potato dextrose agar (PDA) (Oxoid) in darkness for 7 d;
c *F. crookwellense* on carnation leaf-piece agar (CLA) with dark orange sporodochia containing macroconidia forming readily;
d macroconidia with methyl blue stain (×200);
e macroconidia unstained (×400) from sporodochia on CLA.

***Fusarium langsethiae* Torp & Nirenberg**
(see Fig. 7)
On MA the mycelium is short, white to yellow or pale orange; sporodochia are rare.
On PDA growth is not very rapid; usually white powdery mycelium; the undersurface is often white but can vary with several colours (yellow, pink, purple); very little aerial mycelium, variation can occur; globose microconidia are formed in abundance in the powdery mycelium.
Conidiophores: Unbranched and branched monophialides and polyphialides.
Macroconidia are absent.
Microconidia are abundant, globose, in clumps.
Chlamydospores are absent.

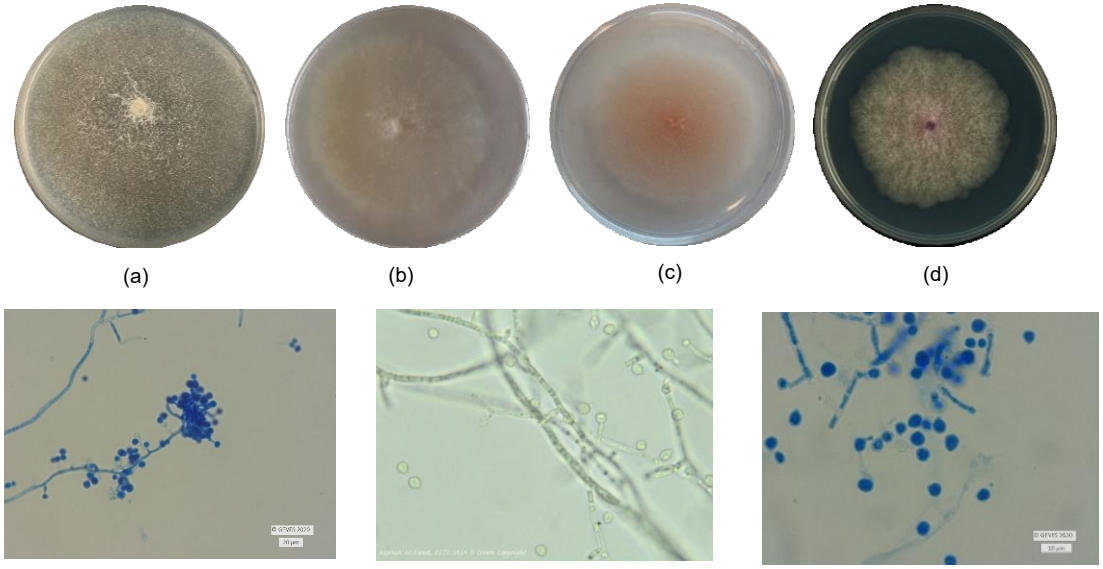

Figure 7. *Fusarium langsethiae*:
a malt agar in darkness for 17 d;
b potato dextrose agar (PDA) (Difco);
c PDA (Difco) in darkness for 7 d;
d PDA (Oxoid) darkness for 7 d;
e microconidia with methyl blue stain (×200);
f microconidia unstained (×400);
g microconidia with methyl blue stain (×1000).

***Fusarium poae* (Peck) Wollenw.**
(see Fig. 8)
Colonies have a distinct smell of apples.
On MA the mycelium is dense, aerial, white to pink; sporodochia are rare.
On PDA growth is rapid, with dense aerial, cotton-like mycelium that is white, yellow to pink in colour; spores are readily formed in the mycelium; the undersurface is usually carmine red; sporodochia are rare.
Conidiophores: Unbranched and branched monophialides; microconidiophores are short and fat, almost globose and quite distinctive.
Macroconidia are generally rare, typically sickle-shaped, 3–5 septate and have a foot-shaped basal cell.
Microconidia are abundant, globose or oval in shape (napiform), 0–1 septate, and often have distinct papilla.
Chlamydospores occur infrequently and may be in clumps or chains

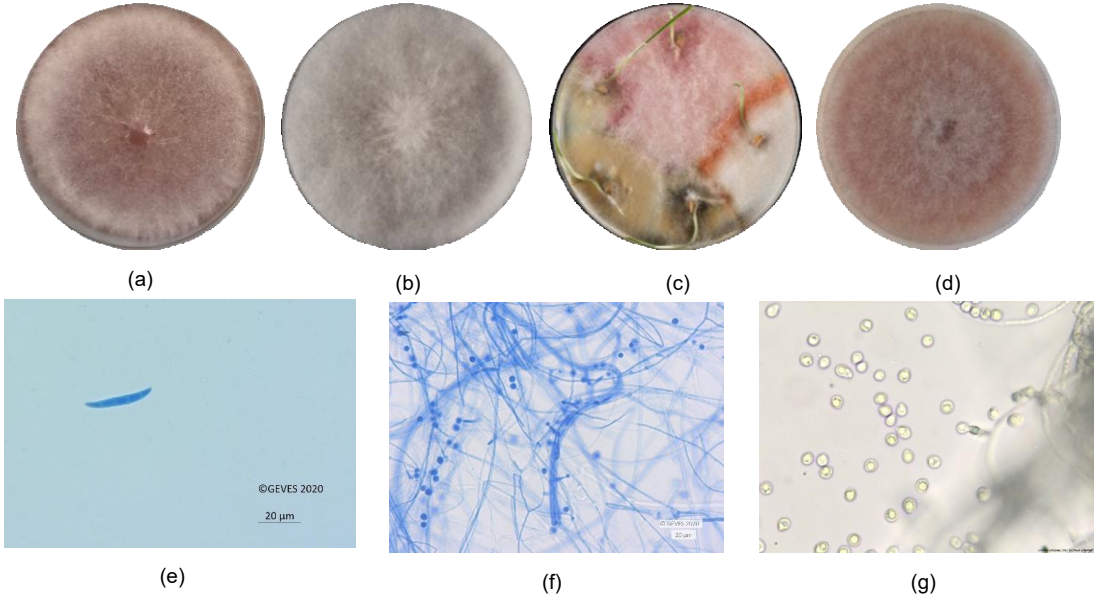

Figure 8. *Fusarium poae*:
a malt agar in darkness for 14 d;
b potato dextrose agar (PDA) (Oxoid) after 7 d in darkness;
c PDA (Oxoid) on seed sample after 7 d in darkness, colony at top;
d PDA (Difco) darkness for 7 d;
e macroconidia with methyl blue stain (×400);
f microconidia with methyl blue stain (×200);
g microconidia unstained (×400).

***Fusarium tricinctum* (Corda) Sacc.**
(see Fig. 9)
On MA the mycelium is short, white to dark pink, sometimes orange; sporodochia only appear on old cultures.
On PDA the aerial mycelium is dense and white to yellow; the undersurface is carmine red; sporodochia appear as the culture ages.
Conidiophores: Unbranched and branched monophialides.
Macroconidia are abundant and sickle-shaped; the basal cell is distinctly foot-shaped or notched.
Microconidia are abundant and are lemon- to pear-shaped or spindle-shaped, 0–1 septate, and often have papilla at the base; often appear in false heads like a cluster of grapes in the arial mycelium.
Chlamydospores can be formed by some isolates, but form very slowly; if present, they are formed singly or in chains.

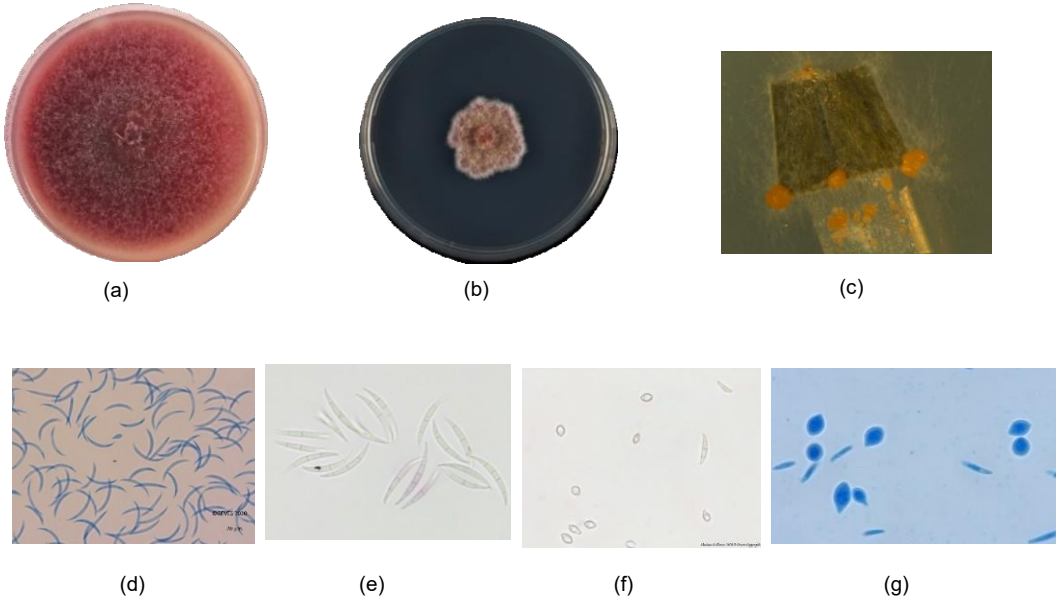

Figure 9. *Fusarium tricinctum*:
a malt agar in darkness for 17 d;
b potato dextrose agar (PDA) (Oxoid) darkness after 7 d;
c orange sporodochia on carnation leaf-piece agar (CLA);
d macroconidia with methyl blue stain (×400);
e macroconidia unstained from CLA (×200);
f juvenile macroconidia and microconidia (×400);
g juvenile macroconidia and microconidia with methyl blue stain (×1000).

***Fusarium sporotrichioides* Sherb.**
(see Fig. 10)
On MA the mycelium is aerial, white to pink, sometimes orange; sporodochia only appear on old cultures.
On PDA growth is rapid and the aerial mycelium is dense and white to yellow; the undersurface is carmine red; sporodochia appear as the culture ages.
Conidiophores: Unbranched and branched monophialides and polyphialides.
Macroconidia are abundant, sickle-shaped, 3–5 septate; the basal cell is not distinctly foot-shaped or notched.
Microconidia are abundant and are oval to pear-shaped or spindle-shaped, 0–1 septate, often with papilla at the base.
Chlamydospores are present and abundant, formed singly, in chains or in clumps.

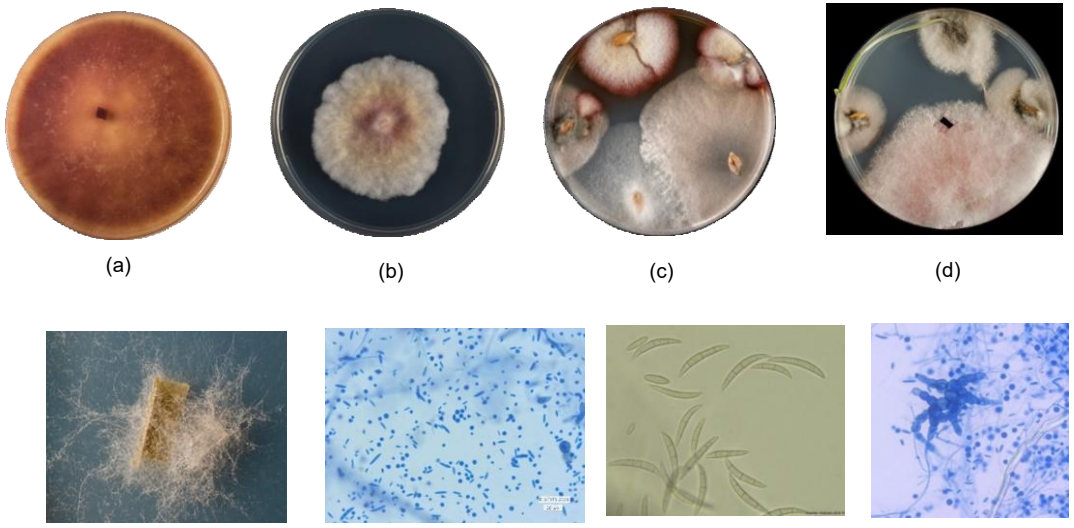

Figure 10. *Fusarium sporotrichioides*: **a** malt agar in darkness for 21 d; **b** potato dextrose agar (PDA) (Oxoid) after 7 d in darkness; **c** PDA (Oxoid) on seed sample (bottom left) at 7 d; **d** PDA (Oxoid) (bottom colony) on seed sample after 7 d; **e** isolate on carnation leaf-piece agar (CLA); **f** microconidia with methyl blue stain (x200); **g** macroconidia unstained from CLA (x400); **h** chlamydospores with methyl blue stain (x400).

References

- Champion, R. (1997). *Identifier les champignons transmis par les semences.* INRA, France.
- Gavrilova, O., Skritnika, A. and Gagkaeva, T. (2017). Identification and characterization of spontaneous auxotrophic mutants in *Fusarium langsethiae. Microorganisms*, **5**, 14.
- Leslie, J.F. and Summerell, B.A. (2006). *The* Fusarium *Laboratory Manual*. Blackwell Publishing, Ames, IA, USA. 388 pp.
- Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. (1983). Fusarium *Species: An Illustrated Manual for Identification*. Pennsylvania State University Press, USA. 183 pp.
- Summerell, B.A. (2019). Resolving *Fusarium*: current status of the genus. *Annu Rev Phytopathol.*, **57**, 323–339. pmid:31226019
- Valverde-Bogantes, E., Herr, J.R., Rose, D.J., Wegulo, S.N. and Hallen-Adams, H.E. (2019). Recent population changes of *Fusarium* head blight pathogens: drivers and implications. *Canadian Journal of Plant Pathology*, **42**(3), 315–329.
- Summerell, B.A. and Leslie, J.F. (2011). Fifty years of *Fusarium*: how could nine species have ever been enough? *Fungal Diversity*, **50**, 135–144.

Photograph credits

- Photographs on MA, courtesy of GEVES (France)
- Photographs on PDA Oxoid and CLA, courtesy of SASA (Scotland)
- Photographs on PDA Difco (Fig. 1d; Fig. 2c; Fig. 5b), courtesy of CREA (Italy)
- Photographs on PDA Difco (Fig. 5c; Fig. 6d), courtesy of Kimen Seed Laboratory (Norway)

Summary of morphological criteria

	Fusarium avenaceum (see Fig. 3)	*Fusarium graminearum* *F. pseudograminearum* (see Fig. 4)	*Fusarium culmorum* (see Fig. 5)	*Fusarium crookwellense* *F. cerealis* (see Fig. 6)	*Fusarium langsethiae* (see Fig. 7)	*Fusarium poae* (see Fig. 8)	*Fusarium tricinctum* (see Fig. 9)	*Fusarium sporotrichioides* (see Fig. 10)
Note		As *F. avenaceum* and *F. graminearum* share common morphological criteria they cannot be distinguished visually on plates				Colonies have a distinct smell of apples		
On malt agar	mycelium is abundant and aerial, yellow to pink coloured, sometimes creamy white; sporodochia are orange, concentrated in centre of colony	mycelium is abundant and aerial, pink coloured and white to yellow on aerial part of mycelium; sporodochia are orange and concentrated in centre of colony	mycelium is short, pink to carmine red; sporodochia form on older cultures and are orange to red-brown in colour	mycelium is abundant and aerial, white to pink, sometimes yellow; sporodochia are orange to red-brown	mycelium is short, white to yellow or pale orange; sporodochia are rare	mycelium is dense, aerial, white to pink; sporodochia are rare	mycelium is short, white to dark pink, sometimes orange; sporodochia only appear on old cultures	mycelium is aerial, white to pink, sometimes orange; sporodochia only appear on old cultures

On potato dextrose agar	growth can vary from slow to somewhat rapid, aerial mycelium is dense and white but frequently varies in colour from yellow/tan to reddish-brown; undersurface colouration varies from tan to carmine red to dark brown; orange sporodochia may form on colonies originating from seed, depending on growth conditions; sporodochia quite often form in centre of colony on pure cultures	growth is usually rapid (e.g. Petri dishes filled within 4 d on pure culture) but can be variable, with dense aerial mycelium and frequently yellow to tan with margins white to carmine red; undersurface usually carmine red; red-brown to orange sporodochia, if present, are sparse in dark; sporodochia often appear in less than a week when under near-ultraviolet light	growth is rapid, with dense aerial mycelium, generally white but often yellow to tan on base; undersurface is carmine red; orange to red-brown sporodochia appear readily	growth is rapid, with dense aerial mycelium, white in colour and then tan; undersurface is carmine red; orange to red-brown sporodochia generally appear early in centre of culture and later in other areas of culture	growth is not very rapid; usually white powdery mycelium; undersurface is often white but can vary with several colours (yellow, pink, purple); very little aerial mycelium, variation can occur; globose microconidia are formed in abundance in powdery mycelium	growth is rapid, with dense aerial, cotton-like mycelium that is white, yellow to pink in colour; spores are readily formed in mycelium; undersurface is usually carmine red; sporodochia are rare	aerial mycelium is dense and white to yellow; undersurface is carmine red; sporodochia appear as culture ages	growth is rapid and aerial mycelium is dense and white to yellow; undersurface is carmine red; sporodochia appear as culture ages

Conidiophores	unbranched and branched monophialides	unbranched and branched monophialides	unbranched and branched monophialides	unbranched and branched monophialides	unbranched and branched monophialides and polyphialides	unbranched and branched monophialides; microconidiophores are short and fat, almost globose and quite distinctive	unbranched and branched monophialides	unbranched and branched monophialides and polyphialides
Macroconidia	very long, slender and thin walled with foot-shaped basal cell and an elongated apical cell that may be bent	often present, distinctly septate, thick walled, straight to moderately sickle-shaped; ventral surface is almost straight and dorsal surface smoothly arched; basal cell is distinctly foot-shaped; apical cell curved, cone-shaped like a beak	stout, distinctly septate, thick walled and have straight to curved ventral and dorsal surfaces; basal cell is usually distinctly round and can occasionally be foot-shaped to slightly notched	strongly septate, thick walled and unequally curved, with ventral surface less curved than dorsal surface, which is strongly arched; basal cell is distinctly foot-shaped; apical cell is distinctly curved and tapers to a narrow tip	absent	generally rare, typically sickle-shaped, 3–5 septate and have a foot-shaped basal cell	abundant and sickle-shaped; basal cell is distinctly foot-shaped or notched	abundant, sickle-shaped, 3–5 septate; basal cell is not distinctly foot-shaped or notched

Microconidia	(cigar-shaped) are rare; mixed sizes of conidia (micro- and macro-) are observed from sporodochia on isolates on PDA, but on CLA only macroconidia true to type are observed	absent	absent	absent	abundant, globose, in clumps	abundant, globose or oval in shape (napiform), 0–1 septate, and often have distinct papilla	abundant and are lemon- to pear-shaped or spindle-shaped, 0–1 septate, and often have papilla at base; often appear in false heads like a cluster of grapes in arial mycelium	abundant and are oval to pear-shaped or spindle-shaped, 0–1 septate, often with papilla at base
Chlamydospores	absent	rare, formed in macroconidia or mycelium	generally form abundantly and quickly; may occur singly, in chains or in clumps	present and are formed in hyphae and macroconidia	absent	occur infrequently and may be in clumps or chains	can be formed by some isolates, but form very slowly; if present, formed singly or in chains	present and abundant, formed singly, in chains or in clumps

Vote to accept item	Yes votes	No votes	Result
C.7.3	22	0	

C.7.4 Reporting seed health tests

The ISTA Seed Health Committee proposes a modification to the rules governing the specification of results for seed health tests under the "Other Determinations" section of the OICs. The current two-option result scoring system, as defined in Chapter 1, Section 5.2.10, and Chapter 7, Section 6 (Reporting Results), is considered insufficiently descriptive. Information on the level of infection is regarded as highly valuable and should therefore be included as a mandatory element on ISTA certificates. This consideration has led to the following proposed changes.

The AOSA Rules do not have seed health tests specified so no there is no impact of the proposed rule changes.

This was noticed by an auditor and an editorial change proposed by the Rules Committee Chair and Vice-chair.

RUL Committee Votes	Yes:	14	No:	0	Abstain/Absent:	0

CURRENT VERSION	PROPOSED VERSION
7.6 Reporting results The results of a test for seed health must be reported under 'Other determinations' as follows: • either qualitative or quantitative results, as specified in the individual methods; • ~~negative and positive results~~, as specified in the individual methods; •the scientific name of the pathogen detected; •the percentage of infected seeds;	**7.6 Reporting results** The results of a test for seed health must be reported under 'Other determinations' as follows: • either qualitative or quantitative results, as specified in the individual methods; • not detected and detected with the percentage/number of infected seeds/subsamples out of the total tested between brackets, as specified in the individual methods; •the scientific name of the pathogen detected; •the percentage of infected seeds; ....

CURRENT VERSION	PROPOSED VERSION
1.5.2.10 Seed health test The results of a test for seed health must be reported under 'Other determinations' as follows: • either qualitative or quantitative results, as specified in the individual methods; • ~~negative and positive results~~, as specified in the individual methods; •the percentage of infected seeds;	**1.5.2.10 Seed health test** The results of a test for seed health must be reported under 'Other determinations' as follows: • either qualitative or quantitative results, as specified in the individual methods; • not detected and detected with the percentage/number of infected seeds/subsamples out of the total tested between brackets, as specified in the individual methods; ....

Vote to accept item	Yes votes	No votes	Result
C.7.4	21	0	

Chapter 8: Species and variety testing

C.8.1 DNA method for *Hordeum vulgare*

The following proposal was submitted by the ISTA Variety Committee with the aim of introducing a new DNA method for barley varietal identification. There is no equivalent rule test the AOSA Rules. ***Please note that if this rule proposal is accepted, the tables will be renumbered when the new method is incorporated into Chapter 8.***						
This proposal was submitted by the Variety Committee and is supported by a validation study.						
VAR Committee Votes	Yes:	14	No:	0	Abstain/Absent:	1

PROPOSED VERSION

8.10.6. Hordeum vulgare (Barley)

The DNA-based method for verifying varieties of Hordeum is by analysis of a minimum set of four microsatellite markers. Verification of the identity of a single-constituent seed lot may be achieved using pooled seed samples or analysis of a small number of individual seeds. Estimates of varietal purity will require analysis of larger numbers of individual seeds.

8.10.6.1 Microsatellite markers

Table 8R contains the prescribed microsatellite markers required for reports and issuance of ISTA Certificates.
Addition of 5′-tail sequences for labelling, using a universal primer approach or direct labelling through the addition of a fluorophore, are the only modifications permitted to PCR primers of the prescribed microsatellite markers. If these markers do not provide sufficient discrimination among the varieties for the purpose at hand, they may be supplemented with additional microsatellite markers of the laboratory's choosing.

Table 8R. Prescribed microsatellite markers and PCR primers for verification of barley varieties.

SSR	5' to 3'	Approximate allele size range (bp)
HvM36-F	TCCAGCCGACAATTTCTTG	120 - 160
HvM36-R	AGTACTCCGACACCACGTCC	
Bmag0009-F	AAGTGAAGCAAGCAAACAAACA	185 - 195
Bmag0009-R	ATCCTTCCATATTTTGATTAGGCA	
Bmac0032-F	CCATCAAAGTCCGGCTAG	225 - 260
Bmac0032-R	GTCGGGCCTCATACTGAC	
Bmag0120-F	ATTTCATCCCAAAGGAGAC	240 - 280
Bmag0120-R	GTCACATAGACAGTTGTCTTCC	
M13	CACGACGTTGTAAAACGAC	

8.10.6.2. Recommended DNA extraction protocol

DNA extraction protocol is up to each laboratory, as per ISTA semi-performance-based approach. DNA can be extracted using column-based kits; procedures such as those based on CTAB are also adequate for extraction of DNA from barley seeds. For any DNA extraction procedure chosen, it is recommended to use one seed as starting material. Following DNA extraction, DNA quality and quantity can be verified by means of a 1 % agarose gel or spectrophotometry. Once the extraction procedure has been validated for the matrix, verification of DNA quality and quantity may not be necessary for all samples.

8.10.6.3. Recommended PCR procedures

The microsatellite markers set was selected based on performance in two comparative tests. Each marker can be run individually or multiplexed. If multiplexed, it is recommended to use PCR master mix that is specially designed for multiplexing. However, optimization of the primer concentration within a multiplex may still be required. Table 8S shows recommended primer concentrations for each marker.

Table 8S. Recommended final primer concentrations for barley markers.

SSR	Final primer concentration in PCR mix
HvM36	0.04 µM (Forward and Reverse)
Bmag0009	0.025 µM (Forward and Reverse)
Bmac0032	0.03 µM (Forward and Reverse)
Bmag0120	0.08 µM (Forward and Reverse)

Fluorescent labelling can be accomplished using a universal primer approach (Oetting et al., 1995) in which the M13 sequence 5′-CACGACGTTCTAAAACGAC-3′ is added to the 5′ end of each forward primer and a single fluorescently labelled M13 primer having the identical sequence is included in the reaction mixture. During PCR, this universal fluorescent primer hybridizes with complementary sequences generated in early amplification cycles, resulting in the synthesis of fluorescent products for all the microsatellite markers in the reaction.
Fluorescent labelling can also be achieved through the addition of fluorophores.

8.10.6.3.1 Reaction components

For each marker, a master mix with all reaction components except the template DNA should be set up and aliquoted into reaction tubes or plate wells. Table 8T contains a list of reagents for a single 10 µL reaction with multiplexed primers.

Table 8T. Recommended reaction composition for PCR amplification of microsatellite markers for verification of barley variety.

Amount per reaction (µL)	Component	Final concentration
0.2	dNTP	0.2 mMol
0.1	Gold Taq	0.05 U
1	Buffer	1 x
0.04	HvM36-F	0.04 µM
0.04	HvM36-R	0.04 µM
0.025	Bmag0009-F	0.025 µM
0.025	Bmag0009-R	0.025 µM
0.03	Bmac0032-F	0.03 µM
0.03	Bmac0032-R	0.03 µM
0.08	Bmag0120-F	0.08 µM
0.08	Bmag0120-R	0.08 µM
0.05	M13	0.05 µM
0.8	$MgCl_2$	2 mM
6.5	H_2O	

Concentrations for all markers in multiplexed reactions may require adjustment depending upon the relative product intensities observed. For a given microsatellite marker, forward and reverse primers should be adjusted equally.

When preparing a master mix, component quantities are determined by multiplying the amounts indicated per reaction by the number of samples to be tested, plus one or two extras to accommodate for pipetting inaccuracies. The components should be combined in a microcentrifuge tube in the order listed. The mixture should be gently vortexed, briefly centrifuged to collect contents at the bottom of the tube and then distributed into reaction tubes or wells (9 µL each). Lastly, 1 µL (approximately 10 ng/µL) of template DNA is added to each reaction tube, resulting in a final reaction volume of 10 µL.

8.10.6.3.2 Thermal cycling profile

The thermal cycling protocol for the PCR reaction presented in Table 8U has been used successfully with the prescribed markers in comparative tests and is recommended.

Table 8U. Recommended thermal cycling protocol for PCR amplification of microsatellite markers for verification of barley varieties.

Number of cycles	Temperature	Duration
1 cycle	95 °C	10' (Initial denaturation conditions according to manufacturer of Taq polymerase used)
1 cycle	95 °C	45"
	60 °C	5'
	72 °C	1'
1 cycle	95 °C	45"
	59 °C	3'
	72 °C	1'
9 cycles	95 °C	45"
	58 °C /decrease 1 °C per cycle	2'
	72 °C	1'
33 cycles	95 °C	45"
	55 °C	2'
	72 °C	1'
1 cycle	72 °C	10'
Hold	4 °C	

8.10.6.3.3 Marker detection system

The amplified DNA fragments are separated according to size using electrophoresis; both conventional and capillary electrophoresis are suitable for separation. Detection and size determination is performed using an appropriate technique, either visually or through fluorometry, and by comparison to appropriate DNA ladders or control samples. Generally, electrophoresis and fragment detection are accomplished concurrently by the same instrument.

8.10.6.4 Evaluation of results

Alleles are called according to their sizes in base pairs. However, since detection systems and reagents used may differ between laboratories, differences in the migration of the PCR products is possible. To minimize this variation, it is recommended to use this method to verify varieties

in a comparative manner, i.e., to determine whether the allele profile of a sample is identical to that of an authentic reference variety.

It can be useful, particularly in gel-based analysis systems, to include samples of known varieties with known allele profiles to assist in the determination of sample allele sizes.

If analyses are performed on individual seeds, reference profiles should be determined using a sufficient number of individual authentic reference variety seeds to ensure that variation within a variety is adequately represented. If analyses are performed on pooled samples, it is recommended that the reference profiles used should also be based upon pooled seeds of authentic reference varieties.

Bibliography

Daniel J. Perry, Ursla Fernando, Sung- Jong Lee (2013) Simple sequence repeat- based identification of Canadian malting barley varieties

Oetting, W.S., Lee, H.K., Flanders, D.J., Wiesner, G.L., Sellers, T.A. and King, R.A. (1995). Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. Genomics 30(3), 450–458.

Vote to accept item	Yes votes	No votes	Result
C.8.1	21	0	

C.8.2 *Lolium* spp. fluorescence test duration

Some years ago, the germination duration of *Lolium* species were reduced from 14 to 10 days, and 10 days is now the common duration for most of the *Lolium* species in chapter 5. This proposed amendment is to harmonise the content of Chapter 8 on *Lolium* spp. with Chapter 5 of the ISTA Rules. *Lolium* spp. seedling root development is sufficient within the standard germination period of 10 days to perform the fluorescence test.

This proposal does not harmonise with the AOSA Rules, which still prescribes a 14 day final count for *Lolium* spp.

This proposal was submitted by the Variety Committee.

VAR Committee Votes	Yes:	12	No:	0	Abstain/Absent:	3

CURRENT VERSION	PROPOSED VERSION
8.11.4 *Lolium* spp. For other germination conditions (pre-chilling etc.) see Chapter 5. The examination should be made only when the roots are sufficiently well developed, which may not be until the ~~fourteenth~~, or in the case of dormant seed the ~~eighteenth~~ day.	**8.11.4 *Lolium* spp.** For other germination conditions (pre-chilling, etc.) see Chapter 5. The examination should be made only when the roots are sufficiently well developed, which may not be until the tenth, or in the case of dormant seed, the 14th day.

Vote to accept item	Yes votes	No votes	Result
C.8.2	23	1	

Chapter 11: Testing coated seed

C.11.1 Add moisture testing

While testing for moisture content of pelleted seeds is described in Chapter 9 (Moisture testing), it will also be helpful to find this reference in Chapter 11 (Testing coated seeds).

The AOSA Rules do not reference moisture testing of coated seed.

This proposal was submitted by the Moisture Committee.

MOI Committee Votes	Yes:	13	No:	0	Abstain/Absent:	0

CURRENT VERSION	PROPOSED VERSION
11.6.7 Reporting results ... percentage of viable seed`. 11.10 Thousand-seed weight determination and size grading of pelleted seeds	11.6.7 Reporting results ... percentage of viable seed`.  11.9 Determination of moisture content - Pelleted seeds have to be tested the same way as unpelleted seeds and the following statement must be entered: `The seeds of the submitted sample were pelleted, and the moisture content reported is that of the combined unit comprising seed and pelleting material.` - The moisture content of seed tapes and seed mats cannot be reported on an Orange International Seed Lot Certificate or a Blue International Seed Sample Certificate, since there is no ISTA method for this kind of sample. 11.10 Thousand-seed weight determination and size grading of pelleted seeds

Vote to accept item	Yes votes	No votes	Result
C.11.1	25	0	

Chapter 15: Seed vigour testing

C.15.1 Add new radicle emergence method for *Allium* spp.

The radicle emergence test has been shown to identify differences in the vigour of *Allium* spp. It is proposed to add *Allium cepa* as a species to which the radicle emergence test can be applied.

The AOSA Rules do nothave a radicle emergence test.

This proposal was submitted by the Vigour Committee and is supported by a validation study.

VIG Committee Votes	Yes:	15	No:	0	Abstain/Absent:	1	

CURRENT VERSION

Table 15B Specific conditions for the radicle emergence test procedures…

Species	Germination medium	Replication	Germination temperature	Criterion of radicle emergence	Timing of radicle emergence count
Brassica spp. **specifications for B. napus*	Top of paper or pleated paper	4 x 50 seeds or 2 x 100 with PP	20 ±1°C	Appearance of a radicle after breaking through the seed coat. Seeds in which the seed coat has split, but no radicle has emerged, must not be included.	48h ± 15 min *30h ±15 min for B.napus

PROPOSED VERSION

Table 15B Specific conditions for the radicle emergence test procedures…

Species	Germination medium	Replication	Germination temperature	Criterion of radicle emergence	Timing of radicle emergence count
Allium cepa	Top of paper	4 x 50 seeds	20 ± 1°C	Production of 2mm radicle	72h ±15 min
Brassica spp. **specifications for B. napus*	Top of paper or pleated paper	4 x 50 seeds or 2 x 100 with PP	20 ±1°C	Appearance of a radicle after breaking through the seed coat. Seeds in which the seed coat has split, but no radicle has emerged, must not be included.	48h ± 15 min *30h ±15 min for B.napus

CONSEQUENTIAL CHANGES	
CURRENT VERSION	PROPOSED VERSION
15.3 General principles	**15.3 General principles**
…. **Radicle emergence test**: *Brassica* spp. (species included in Table 2C), *Glycine max, Raphanus sativus, Triticum aestivum* subsp. *aestivum, Zea mays* ….	…. **Radicle emergence test**: *Allium cepa* , *Brassica* spp. (species included in Table 2C), *Glycine max, Raphanus sativus, Triticum aestivum* subsp. *aestivum, Zea mays* ….

Vote to accept item	Yes votes	No votes	Result
C.15.1	25	0	

C.15.2 New method: cold test for *Zea mays*

This method has been validated for maize. For details of the research supporting this proposal, see the validation report.

The ISTA cold test is similar in principle to the AOSA method, specifically the AOSA box method. However the ISTA method avoids using soil to eliminate sources of variation in results between laboratories. Unlike AOSA we do not include a control sample in ISTA vigour test methods.

This proposal was developed by a working group of the ISTA Vigour Committee, approved by the Vigour Committee, and is supported by a validation study.

VIG Committee Votes	Yes:	15	No:	0	Abstain/Absent:	1	

PROPOSED VERSION

15.8.6 Cold test

15.8.6.1 Principle

Early sowing dates usually produce higher grain yield but also subject seeds to unfavourable cold soil temperatures. The cold test is a vigour test created to reproduce in the laboratory the conditions encountered by spring cultivars following early sowings in the field i.e. cold and wet conditions. It has also been shown that a cold test can be more reliable than standard germination to predict field emergence for maize even when spring conditions are not so stressful.

15.8.6.2 Scope and field of application

The cold test provides a vigour test for *Zea mays* L. which relates to field emergence.

15.8.6.3 Apparatus

Sand or paper (BP method) growing media: As used in a germination test (Chapter 5.4.3.2)
Containers: to prevent drying out during the test.
Germination test facilities: Germination tests are conducted using the methods and test conditions described in Chapter 5 of the ISTA Rules.

15.8.6.4 Cold test procedure

Commented [JB30]: BP added by Vig TCOM C15.2

Commented [JB31R30]: Feedback received before and after the webinar and subsequent discussions within the VIG Committee for in the inclusion of BP led to revisions to incorporate BP into the method to allow for wider acceptance of the test.

Commented [JB32R30]: This proposed method is available for the Vigor test but is impossible to detect the poor germination that occurs during low temperature season in the field, caused by Pythium in soil mainly. Therefore, while we do not object to adding this new method, it should not be defined as an improved method of the conventional one, but as a new method with a different purpose.

15.8.6.4.1 Working samples

A test is carried out on four replicates of 50 pure seeds drawn at random from a representative sample of the submitted sample.

15.8.5.4.2 Growing medium
Substrate: sand moistened at 14% of its weight or BP moistened at 292% of blotter weight.

15.8.5.4.3 Temperature

10 ± 2°C for 7 days followed by 25 ± 2°C for 5 days.

15.8.5.4.4 Evaluation

Evaluation of normal seedlings must be reported after the 12 days test (7 days in cold conditions for germination followed by 5d in warm condition for seedling growth).

15.8.6.5 Calculation and expression of results

Record the number of seeds having developed a normal seedling for each replicate. Calculate the average normal seedling percentage. Use the Germination toolbox 'Tolerances Calculation Program' available on ISTA website (https://www.seedtest.org/en/services-header/tools/germination-committee/germination-toolbox.html) to calculate the repeatability of the test. When four 50-seed replicates differ by more than the maximum tolerance value shown in the tool, the seed lot must be re-tested. If the second test result is compatible with the first (i.e. the difference does not exceed the tolerance indicated in Table 5C part 2), the average of the two tests must be reported.

15.8.6.6 Reporting results

The result of a seed vigour test using the cold test must be reported under 'Other determinations' as follows:
Results are reported as a percentage of normal seedlings calculated to the nearest whole number (5.8.2). If the result is found to be nil, it must be entered as '0'.
The results must be accompanied by a statement of the specific variables used in the test: substrate with its moisture, time and temperatures used for the cold incubation and during the growth period.

CONSEQUENTIAL CHANGES	
CURRENT VERSION	PROPOSED VERSION
15.3 General principles	**15.3 General principles**
…. **Tetrazolium vigour test**: *Glycine max* Detailed methods are given in 15.8.	…. **Tetrazolium vigour test**: *Glycine max* **Cold test**: *Zea mays* Detailed methods are given in 15.8.
CURRENT VERSION	PROPOSED VERSION
	1.5.2.17.6 Cold test
1.5.2.17.5 Tetrazolium vigour test The result of a seed vigour test using the TZ method… **1.5.2.18 Size and grading of Beta seeds and pelleted seeds** The results of seed size grading… ….	**1.5.2.17.5 Tetrazolium vigour test** The result of a seed vigour test using the TZ method… **1.5.2.17.6 Cold test** The result of a seed vigour test using the cold test must be reported under 'Other determinations' as follows: Results are reported as a percentage of normal seedlings calculated to the nearest whole number (5.8.2). If the result is found to be nil, it must be entered as '0'. The results must be accompanied by a statement of the specific variables used in the test: substrate with the moisture, time and temperatures used for the cold incubation and during the growth period. **1.5.2.18 Size and grading of Beta seeds and pelleted seeds** The results of seed size grading… ….

Vote to accept item	Yes votes	No votes	Result
C.15.2	21	1	

C.15.3 Radicle emergence soybean evaluation in Table 15B.

Clarification of criterion of radicle emergence for soybean evaluation in Table 15B. Two accredited laboratories requested clarification from the VIG Committee regarding how to evaluate germinated soybean seeds in cases where the radicle tip exceeds 2 mm but remains enclosed within the seed coat. During the radicle emergence (RE) test, this type of seed occasionally occurs. Photographs of such cases were submitted to the working group leader responsible for method validation to determine whether these seedlings should be counted as germinated.

To clarify the evaluation criterion for the soybean radicle emergence test, it has been determined that seeds in which the radicle tip remains trapped within the seed coat will not be counted, even if the radicle length exceeds 2 mm. Further investigation will be conducted to determine whether paper moistening conditions may contribute to the occurrence of trapped radicles.

The AOSA Rules do not have a radicle emergence test.

This proposal was submitted by the Vigour Committee.

VIG Committee Votes	Yes:	15	No:	0	Abstain/Absent:	1	

CURRENT VERSION

Table 15B Specific conditions for the radicle emergence test procedures…

Species	Germination medium	Replication	Germination temperature	Criterion of radicle emergence	Timing of radicle emergence count
Glycine max	Between paper	4 x 50 seeds	20 ±1°C	Production of 2mm radicle	48h ±15 min

PROPOSED VERSION

Table 15B Specific conditions for the radicle emergence test procedures…

Species	Germination medium	Replication	Germination temperature	Criterion of radicle emergence	Timing of radicle emergence count
Glycine max	Between paper	4 x 50 seeds	20 ±1°C	Production of 2mm radicle Seeds in which the radicle has emerged, but the tip remains trapped within the seed coat are not included.	48h ±15 min

Vote to accept item	Yes votes	No votes	Result
C.15.3	24	0	