

# **PROPOSED RULES CHANGES 2005**

**FOR CONSIDERATION AND DECISION AT THE  
ORDINARY MEETING 2005**





### **Introduction by the Chairman of the ISTA Rules Committee**

The loose-leaf edition of the ISTA rules has been updated. The current version is the 2005 edition. Single copies of replacement pages and front covers for the 2005 edition have been sent free to all ISTA member laboratories. Extra copies are available for purchase from the ISTA publications section. As the Rules are an evolving document it is worth remembering that pages could be headed as 'effective from' 2003, 2004 or 2005. The preface for each edition includes details of changes and when replacement pages have been issued. A complete reprint of the rules is not currently planned. As the amalgamation of rules and annexes into continuous text, without being split into brown and white pages, progresses it may become necessary. Until then replacement Chapters will be issued as they are revised and accepted by the membership.

The following proposals will be discussed at the voting part of the ISTA meeting in Bangkok, Thailand. If the proposals are accepted by the membership amendments will be issued and they will become the 2006 edition of the ISTA Rules. This year sees the inclusion of the first chapter (Chapter 2: Bulking and Sampling) to have the rules and annexes combined into a continuous text. This process has two stages: firstly the rules and annexes were combined section by section without any changes, they were then read through and editorial changes like renumbering sections and removal of redundant text was suggested. This version was sent to a two-person team of ISTA scrutineers, both ex-members of the ISTA Executive Committee. They have agreed that the editorial changes were indeed editorial so do not need to be voted on at the voting meeting. Thanks go to Doug Ashton and Simon Cooper for volunteering to be the scrutiny team. This editorially merged version has been e-mailed to all ISTA members and is available by contacting the ISTA Secretariat.

The second stage was that the Bulking and Sampling Committee worked on improving the merged version by proposing a set of changes. These changes are presented here and will be voted on in the normal way. Because the changes are extensive it has not been possible to show 'tracked changes' as we normally do. It was felt that it was better to read and consider the chapter as it would appear once it has been accepted. The vote will be to accept or not the proposals 2a to 2g for Chapter 2 as a whole. Proposal 2ah, which is the proposal for an increase in seed lot size for cereals, will be the subject of a separate vote.

The other major changes/amendments are to Chapter 8 (Verification of Species/Cultivar) to include testing for GM material. Like Chapter 2 Chapter 8 has been modified to fit in with the new style of amalgamated rules and annexes but has also had several sections added. It is planned that the amendments should be discussed in some detail during the technical committee sessions in Bangkok and then voting on the whole of this chapter would proceed as normal. Like Chapter 2 the changes are extensive and it has not been possible to show tracked changes as we normally do. Again it was felt that it was better to read and consider the chapter, as it would appear once it has been accepted.

Any subsequent editorial changes to any of the Rules remain the responsibility of the Chair of the Rules Committee.

Please let me know of any problems with these proposals.

Many thanks.

**Steve**

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~~Text proposed for deletion~~

Proposed new text

Text added or amended since printing

## **ITEM 1 GENERAL EDITING ISSUES**

### **Item 1 Corrections**

The following are editorial changes/errors that have been noticed in the 2005 edition of the Rules.

#### **Item 1a List of edits**

Preface to 2005 edition p4 has 'number' 'percentage' for 13.5. It should be 'percentage' changing to 'number' as is correctly printed in section 13.5 of the 2005 amended rules.

**ITEM 2 PROPOSALS FOR CHAPTER 2 AND ANNEXE, SAMPLING****Item 2a Amendments to Chapter 2 following amalgamation of the Rules and Annexes**

**NOTE: There will be one vote on items 2a to 2ag inclusive to accept or not all the changes. There will be another separate vote on item 2ah.**

The ISTA Bulking and Sampling Committee,

- in view of the discussions within the Committee during establishing the 2<sup>nd</sup> Edition of the ISTA Seed Sampling Handbook
- taking into consideration the discussions during the ISTA Congress 2004 about a new structure of the ISTA Rules
- having regard to the editorial changes being adopted for amalgamation of the Rules and the Annexe and for inclusion of Appendix D and the sampling part of chapter 11 into chapter 2
- in view of the frequent use of the ISTA Rules in national or international seed legislation by excluding the use of ISTA Certificates,
- respecting the usual regulation of topics like lot size, sealing or licensing of samplers in national and international seed certification schemes,
- contributing to the ongoing process of harmonisation of the ISTA Rules with national Rules concerning the technical methods,
- considering the increasing discussion about the title of the book now called “International Rules for Seed Testing”
- and finally realising that the object of chapter 2 is not to sample for issuing ISTA Certificates,

is proposing a restructuring of Chapter 2. The guiding principle of this restructuring is to separate the technical content describing how to take a representative sample according to the object from additional, regulative, non-technical measures that aim to improve the trustworthiness of the ISTA Certificate and that are linked to sampling but not part of the sampling methodology according to the object. This applies e.g. to conditions concerning sealing, recognition of samplers, sizes of submitted samples and seed lots. As an example we realise, that from a technical point of view, sampling can be done from a 100 t seed lot, there is no technical restriction, but a threshold in trustworthiness requires the definition of maximum lot size for ISTA Certificates or in seed certification systems.

In the following proposal, the non-technical, Certificate related measures are collected and listed in paragraph 2.5.4 which is partly identical to paragraphs in chapter 17. Topics that are regulated in the ISTA Laboratory Accreditation Standard are proposed to be deleted at all from the Rules. In the future, depending on the ongoing discussion about the Rules, paragraph 2.5.4 might even go completely to chapter 17 since there is no sampling methodology described in that paragraph but detailed conditions to the general condition in paragraph 17.3 (2).

The BSC hopes that this separation will document and strengthen the technical, methodological character of the ISTA Rules in total and of chapter 2 in particular, will support the process of technical harmonisation with other national or international methods for seeds testing and will allow a better assignment of Chapter 2 with other chapters of the ISTA Rules, other documents within ISTA and with standards outside ISTA.

The changes are shown as individual proposals to allow the reader to see what has changed and why. The vote on acceptance of proposals 2a-2ag will be one vote to accept or not, with a separate vote on proposal 2ah as this is a substantial change, i.e. the increase of seed lot sizes.



## Chapter 2: Sampling

### 2.1 Object

The object of sampling is to obtain a sample of a size suitable for tests, in which the probability of a constituent being present is determined only by its level of occurrence in the seed lot.

### 2.2 Definitions

#### Proposal 2a

It is proposed to change the definition of a seed lot to a purely technical one that is not linked to an ISTA certificate. This would be in line with the object of chapter 2

##### 2.2.1 Seed lot

A *seed lot* is a specified quantity of seed that is physically and uniquely identifiable.

#### Proposal 2b

It is proposed to delete “small” since it is not very precise and samples may be great compared to the seed lot.

##### 2.2.2 Primary sample

A *primary sample* is a portion taken from the seed lot during one single sampling action.

##### 2.2.3 Composite sample

The *composite sample* is formed by combining and mixing all the primary samples taken from the seed lot.

#### Proposal 2c Editorial only

2.2.4 moved from 2.2.6 to here to have the definition of sub-samples before the definition of submitted and working samples in which sub-sample is mentioned.

##### 2.2.4 Sub-sample

A *sub-sample* is a portion of a sample obtained by reducing a sample.



### Proposal 2d

It is proposed to define “submitted sample” technically and to clarify the terminology for sub-samples in different containers.

#### 2.2.5 Submitted sample

A *submitted sample* is a sample that is to be submitted to the testing laboratory and may comprise either the whole of the composite sample or a sub-sample thereof. The submitted sample may be divided into sub-samples packed in different material meeting conditions for specific tests (e.g. moisture or health).

### Proposal 2e

It is proposed to include a definition of “Duplicate sample” for clarification purposes.

#### 2.2.6 Duplicate sample

A duplicate sample is another sample obtained for submission from the same composite sample and marked “Duplicate sample”.

### Proposal 2f

It is proposed to change the definition of working sample by adding “the whole or” to include the other seed count – working sample and purity test working sample in case of 2.6.3 third bullet point. It is proposed to delete “in the laboratory” since technically, it is irrelevant where the working sample is drawn, as long as it is done properly. If the whole submitted sample is working sample, the obtaining of the working sample is done in the warehouse.

#### 2.2.7 Working sample

The *working sample* is the whole of the submitted sample or a sub-sample thereof, on which one of the quality tests described in these Rules is made and must be at least the weight prescribed by the Rules for the particular test.

### Editorial only, required as a result of Proposal 2c

2.2.6 moved to 2.2.4

### Proposal 2g

It is proposed to delete the separation into “the container” and “individual containers” as it is not relevant for the definition.

#### 2.2.8 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 and closed again, without either destroying the seal or



leaving evidence of tampering. This definition refers to the sealing of seed lots, as well as of seed samples.

### Proposal 2h

It is proposed to shorten the definition of self-sealed containers since it is too specific for a definition and in national or other regulations there are specifications laid down that are more relevant for the seed trade than the definition in ISTA.

#### 2.2.9 Self-sealing 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.

### Proposal 2i

It is proposed to delete the ISTA certificate from this paragraph since this is not relevant for the definition of marking/labelling. Marking of working samples is changed to marking of sub-samples as being more general.

#### 2.2.10 Marked/labelled

A container of a seed lot can be considered as marked or labelled when there is a unique identification mark on the container, which defines the seed lot to which the container belongs. All containers of a seed lot must be marked with the same unique seed lot designation (numbers, characters or combination of both). Marking of sub-samples must ensure that there is always an unambiguous link between the seed lot and the sub-samples.

### Proposal 2j

It is proposed to enter a general definition of coated seeds, to specify the kind of material that may be added in this definition and to delete these sentences from the individual definitions. Since in encrusted seed "greater or lesser extent" is very imprecise, it is proposed to be replaced by "measurable". The definition of granules appear now clearer. The definition of treated seeds is proposed to be shortened and moved after "encrusted seed" to be in the sequence of decreasing mass added.

#### 2.2.11 Coated seeds

Coated seeds are seeds covered with material that may contain pesticides, fungicides, dyes or other additives. The following types of coated seeds are defined:

*Seed pellets.* More or less spherical units, usually incorporating a single seed with the size and shape of the seed no longer readily evident.

*Encrusted seed.* Units more or less retaining the shape of the seed with the size and weight changed to a measurable extent.

*Seed granules.* Units more or less cylindrical, including types with more than one seed per granule.

*Seed tapes.* Narrow bands of material, such as paper or other degradable material, with seeds spaced randomly, in groups or in a single row.



*Seed mats.* Broad sheets of material, such as paper or other degradable material, with seeds placed in rows, groups or at random throughout the sheets.

*Treated seed.* Seeds with treatments, which have not resulted in a significant change in size, shape or addition to the weight of the original seed.

## 2.3 General principles

### Proposal 2k

It is proposed to delete the following paragraph since it is only explanatory and is not a rule.

### Proposal 2l

It is proposed to use the definitions given above in the following sentences. Since mixing is not obligatory in some sampling reduction methods, the third sentence is proposed for deletion.

Since the object of chapter 2 is not to obtain a sample for issuing ISTA certificates but to obtain a representative sample, the technical part of Chapter 2 is proposed to be free of specific, more regulative than technical requirements for issuing ISTA Certificates. These requirements are collected in paragraph 2.5.5, which might finally go to Chapter 17.

Reference is made to the new Handbook for obtaining further help and information.

A composite sample is obtained from the seed lot by taking primary samples from different positions in the whole seed lot and combining them. From this composite sample, sub-samples are obtained by sample reduction procedures at one or more stages forming the submitted sample and finally the working samples for testing. For issuing ISTA International Seed Analysis Certificates, specific requirements have to be fulfilled as given under 2.5.4. Further help in interpreting this chapter may be found in the current ISTA Handbook on Seed Sampling.

## 2.4 Apparatus

### Proposal 2m

It is proposed to change the following sentence for clarification.

Sampling and sample reduction must be performed using appropriate techniques and equipment that is clean and in good condition as described in 2.5.1.4 and 2.5.2.2.



## 2.5 Procedures

### Proposal 2n

It is proposed to delete the requirements concerning lot size from the technical part of Chapter 2 and to move this to 2.5.5 as it is technically possible to take samples from seed lots bigger than ISTA maximum lot size.

#### 2.5.1 Procedures for sampling a seed lot

##### 2.5.1.1 Preparation of a seed lot and conditions for sampling

### Proposal 2o

It is proposed to shorten the following paragraphs to the important sentence and to delete the explanatory parts.

At the time of sampling, the seed lot shall be as uniform as practicable, i.e. there is only tolerable variation among different parts of the seed lot. If there is documentary or other evidence of heterogeneity, or the seed lot is found to be obviously heterogeneous, sampling must be refused or stopped. In cases of doubt heterogeneity can be determined as described under 2.8.2.

Seed may be sampled in containers or when it enters containers. The containers must be strong enough, must not damage the seed, avoid cross contamination and can be breathable or not. The containers must be labelled or marked before or just after sampling is completed.

The seed lot shall be so arranged that each part of the seed lot is conveniently accessible.

##### 2.5.1.2 Sampling intensity

### Proposal 2p

It is proposed to include coated seeds into the sampling schemes by the following changes without changing of how samples have to be taken. Also, in the Tables 2.1 and 2.2 it shall be indicated that this is the minimum intensity.

For seed lots in containers of 15 kg to 100 kg capacity (inclusively), the sampling intensity according to Table 2.1 shall be regarded as the minimum requirement.

**Table 2.1:** Minimum sampling intensity for seed lots in multiple containers of 15 kg to 100 kg capacity (inclusively).



Number of containers	Minimum number of primary samples to be taken
1 - 4 containers:	3 primary samples from each container
5 - 8 containers:	2 primary samples from each container
9 - 15 containers:	1 primary sample from each container
16 - 30 containers:	15 primary samples from the seed lot
31 - 59 containers:	20 primary samples from the seed lot
60 or more containers:	30 primary samples from the seed lot

For seed lots in containers smaller than 15 kg capacity, containers shall 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. For seed mats and tapes, small packets or reels may be combined to sampling units of not exceeding 2,000,000 seeds. The sampling units shall be regarded as containers as described in Table 2.1.

When sampling seed in containers of more than 100 kg, or from streams of seed entering containers the sampling intensity according to Table 2.2 shall be regarded as the minimum requirement.

**Table 2.2:** Minimum sampling intensity for seed lots in containers of more than 100 kg, or from streams of seed entering containers.

Seed lot size	Number of primary samples to be taken
Up to 500 kg	At least five primary samples.
501-3,000 kg	One primary sample for each 300 kg, but not less than five.
3,001-20,000 kg	One primary sample for each 500 kg, but not less than 10.
20,001 kg and above	One primary sample for each 700 kg, but not less than 40.

When sampling a seed lot of up to 15 containers, regardless of their size, the same number of primary samples shall be taken from each container.

Sampling intensity for coated seeds is as described in Tables 2.1 and 2.2.

### Proposal 2r

It is proposed to move the definition of the size of the submitted sample to 2.5.5 since technically there must only be seed enough for performing the test. If there are additional requirements, then they are not technically and therefore are proposed to move to 2.5.4.



### 2.5.1.3 Taking primary samples

#### Proposal 2s

It is proposed to change the following text in order to obtain a logic sequence of the content and to move the conditions for taking primary samples from the big containers for an ISTA certificate valid for the small containers to 2.5.4.

When defining the number and/or the size of primary samples, the seed sampler needs to ensure (besides meeting the minimum sampling intensity) that the minimum amount of seed required for the requested test(s) is sent to the testing laboratory and enough seed remains available for obtaining duplicate samples if requested.

Primary samples of approximately equal size shall be taken from a seed lot, regardless from where in the lot or container it is taken.

When the seed lot is in containers, the containers to be sampled shall be selected at random or according to a systematic plan throughout the seed lot. Primary samples shall be drawn from top, middle and bottom of containers, but not necessarily from more than one position in any container, unless so specified in Tables 2.1 and 2.2.

When the seed is in bulk or in large containers, the primary samples shall be drawn from random positions.

Containers shall be opened or pierced for abstraction of primary samples. The sampled containers shall then be closed or the contents transferred to new containers.

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.

Sampling seed lots of seed tapes and seed mats should be done by taking packets or pieces of tape or mat.

The instruments being used must neither damage the seed nor select according to seed size, shape, density, chaffiness or any other quality trait. All sampling apparatus must be clean before use to prevent cross contaminations. Triers must be long enough so that the opening at the tip reaches at least half of the diameter of the container. When the container is not accessible from opposite sides, the trier must be long enough to reach the opposite side. Sampling seed lots may be done by one of the methods listed below.

#### Proposal 2t

The sampling from a seed stream automatically or by hand is included as a method and mentioned in the first place since it is the best method.

(a) *Automatic sampling from a seed stream.* Seed may be sampled by automatic sampling devices, provided that the instrument uniformly samples the cross section of the seed stream and the material entering the instrument does not bounce out again. It may be operated either under manual or automatic control. The intervals between taking primary samples should be constant but may also vary randomly.



(b) *Manual sampling from a seed stream.* Seed streams may also be sampled by using manual instruments when fulfilling the requirements listed under (a).

### Proposal 2u

The descriptions of the trier and the procedures how to use them are proposed to be shortened and to be of similar specificity. For further additional information reference is made to the ISTA Seed Sampling Handbook. Since the length of triers clearly depend on the dimensions of the containers, all values for the length are proposed to be deleted.

(c) *Sampling stick (synonym: stick trier, sleeve type trier).* The sampling stick consists of an inner tube which fits loosely inside an outer tube but tightly enough so that seed or impurities do not slip between them. The outer tube has a solid pointed end. Both tubes have slots cut into their walls so that the cavity of the inner tube can be opened and closed by twisting the tubes against each other. The sampling stick may be used horizontally, diagonally or vertically. However, when used vertically the sampling stick must have partitions dividing the instrument into a number of compartments. The minimum inside diameter should be about 25 mm for all species.

When using the sampling stick, insert it in the closed position into the container, gently push it so that the point reaches the required position, open the sampling stick, agitate it slightly to allow it to fill completely, gently close and withdraw it and empty the primary sample into a container. Care should be exercised in closing the sampling stick so that seeds are not damaged.

(d) *Nobbe trier.* The Nobbe trier (dynamic spear) is a pointed tube with an opening near the pointed end. Seed passes through the tube and is collected in a bucket or bag. The minimum internal diameter of the Nobbe trier should be about 10 mm for clovers and similar seeds, about 14 mm for cereals and about 20 mm for maize.

When using the Nobbe trier, insert it at an angle of about 30° to the horizontal plane with the opening facing down, push the trier until it reaches the required position and revolve it through 180°. Withdraw it with decreasing speed from the container, gently agitating the trier to help maintain an even flow of seed, and collect the seed sample coming from the trier in a suitable container.

(e) *Sampling by hand.* This method is sometimes the most satisfactory method in the following examples *Agropyron, Agrostis, Alopecurus, Anthoxanthum, Arrhenatherum, Axonopus, Bromus, Chloris, Cynodon, Cynosurus, Dactylis, Deschampsia, Digitalia, Elymus, Elytrigia, Festuca, Holcus, Lolium, Melinis, Panicum, Pascopyrum, Paspalum, Poa, Psathyrostachys, Pseudoroegneria, Trisetum, Zoysia.*

Sampling by hand is also the most suitable method for seed that may be damaged by the use of triers e.g. in seed lots of large seeded legumes, for seed with wings or seeds which have a low moisture content or for seed tapes and seed mats.

For hand sampling seed in containers, all positions inside the containers must be accessible. Containers with layers which are not accessible from the regular opening may have to be cut open, sampled and repackaged. Containers may also be partially or completely emptied during the sampling process to gain access to all positions in the containers. For sampling by hand, clean the hand and roll the sleeve up if necessary, insert the open hand into the container to the required position, close and withdraw the hand, taking great care that the



fingers remain tightly closed about the seeds so none may escape and empty the hand into a receiving pan.

#### *2.5.1.4 Obtaining the composite sample*

##### **Proposal 2v**

It is proposed to include the obtaining of the composite sample by collecting the primary samples in one container without inspection as usually done in the case of automatic seed sampler.

If the primary samples appear uniform they are combined to form the composite sample, if not, the sampling procedure must be stopped. When primary samples are collected directly into one container, the content of this container shall be regarded as the composite sample only if it appears uniform, if not, it must not be used for obtaining a submitted sample.

#### *2.5.1.5 Obtaining the submitted sample*

##### **Proposal 2w**

It is proposed to change the following text for better and more efficient wording.

The submitted sample shall be obtained by reducing the composite sample to an appropriate size by one of the methods referred to in 2.5.2.2. Obtaining sub-samples such as for moisture testing must be carried out in such a way that changes in moisture content are minimal.

The composite sample can be submitted to the seed testing laboratory if it is of appropriate size or if it is difficult to mix and reduce the composite sample properly under warehouse conditions.

Duplicate samples, which were requested not later than at the time of sampling shall be prepared in the same way as the submitted sample.

#### *2.5.1.6 Dispatch of the submitted sample*

The submitted sample must be marked with the same identification as the seed lot. For an ISTA International Seed Lot Certificate, the sample must be sealed. The additional information required according to 17.4.2 and 17.4.3, as well as the name of any chemical treatment applied must be provided.

Submitted samples shall be packed so as to prevent damage during transit. Submitted samples should be packed in breathable containers.

Sub-samples for moisture testing, and samples from seed lots which have been dried to low moisture content, shall be packed in moisture proof containers which contain as little air as possible. Submitted samples for germination tests, viability tests and health tests may only be packed in moisture proof containers if suitable storage conditions can be assured.



Submitted samples shall be dispatched by the sampler to the seed testing laboratory without delay.

#### *2.5.1.7. Storage of submitted samples before testing*

Every effort must be made to start testing a submitted sample on the day of receipt. Storage of orthodox seeds, when necessary, should be in a cool, well-ventilated room.

Non-orthodox (i.e. recalcitrant or intermediate) seeds should be tested as soon as possible after obtaining the submitted sample from the composite sample without any storage. Handling of the submitted sample and, if necessary, storage should be done under species specific optimum conditions.

#### *2.5.2 Procedure for obtaining the working sample*

##### *2.5.2.1 Minimum size of working sample*

Minimum sizes of working samples are prescribed in the appropriate chapter for each test. The working sample weights for purity analyses given in Table 2A are calculated to contain at least 2500 seeds. These weights are recommended for normal use in purity tests, see 3.5.1.

The sample weights in column 5 of Table 2A, Part 1, for counts of other species are 10 times the weights in column 4, subject to a maximum of 1000 g.

Working samples of coated seeds shall contain at least the number of pellets or seeds indicated in column 3 of Table 2B, Part 1 and Part 2. If a smaller sample of pelleted seed is used, the actual number of pellets or seeds in the sample shall be reported.

##### *2.5.2.2 Sample reduction methods*

If the seed sample needs to be reduced to a size equal to or greater than the size prescribed, the seed sample shall first be thoroughly mixed. The submitted/working sample shall then be obtained either by repeated halving or by abstracting and subsequently combining small random portions. The apparatus and methods for sample reduction are described in 2.5.2.2.1. to 2.5.2.2.4. One, two or more of these methods may be used in one sample reduction procedure. When using one of the dividers described for seed pellets the distance of fall must not exceed 250 mm.

Except in the case of seed health, the method of hand halving shall be restricted to certain genera listed in 2.5.2.2.4. Only the spoon method and the hand halving method may be used in the laboratory to obtain working samples for seed health testing where other samples or equipment may be contaminated by spores or other propagating material.

For seed tapes and mats take pieces of tape or mat at random, to provide sufficient seeds for the test.

After obtaining a working sample or half-working sample the remainder shall be re-mixed before a second working sample or half-working sample is obtained.



### Proposal 2x

It is proposed to add the following paragraph for taking moisture samples which is the procedure as described in 9.1.5.3. For moisture samples the need to produce a sample without exposing it too the air for more than 30 seconds is important.

Sub-samples for moisture content determination may be taken in the following way: Before taking the sub-sample, mix the sample by either stirring the sample in its container with a spoon or place the opening of the original container against the opening of a similar container and pour the seed back and forth between the two containers. Take at minimum three sub-samples with a spoon from different positions and combine them to the sub-sample of the required size. The seed may not be exposed to the air during sample reduction for more than 30 seconds.

#### 2.5.2.2.1 Mechanical divider method

This method is suitable for all kinds of seeds except some very chaffy seeds. The apparatus divides a sample passed through it into two or more approximately equal parts. The submitted sample can be mixed by passing it through the divider, recombining the parts and passing the whole sample through a second time, and similarly, a third time if necessary. The sample is reduced by passing the seed through repeatedly and removing parts on each occasion. This process of reduction is continued until a working sample of approximately, but not less than, the required size is obtained.

The dividers described below are examples of suitable equipment.

(a) *Conical divider*. The conical divider (Boerner type) consists of a hopper, cone, and series of baffles directing the seed into two spouts. The baffles form alternate channels and spaces of equal width. They are arranged in a circle and are directed inward and downward, the channels leading to one spout and the spaces to an opposite spout. A valve or gate at the base of the hopper retains the seed. When the valve is opened the seed falls by gravity over the cone where it is evenly distributed to the channels and spaces, then passes through the spouts into the seed pans.

The following dimensions have been found suitable: About 19 channels and 19 spaces, each about 25 mm wide for large seeds and about 22 channels and 22 spaces, each about 8 mm wide for small free-flowing seeds.

(b) *Soil divider*. The soil divider consists of a hopper with about 18 attached channels or ducts alternately leading to opposite sides.

A channel width of about 13 mm was found to be suitable.

In using the divider the seed is placed evenly into a pouring pan and then poured in the hopper at approximately equal rates along the entire length. The seed passes through the channels and is collected in two receiving pans.

(c) *Centrifugal divider*. In the centrifugal divider (Gamet type) the seed flows downward through a hopper onto a shallow cup or spinner. Upon rotation of the spinner by an electric motor the seeds are thrown out by centrifugal force and fall downward. The circle or area where the seeds fall is equally divided into two parts by a stationary baffle so that approximately half the seeds fall in one spout and half in the other spout.



The centrifugal divider tends to give variable results unless the spinner is operated after having poured the seed centrally into the hopper.

(d) *Rotary divider*. The rotary divider comprises a rotating crown unit with 6 to 10 attached sub-sample containers, a vibration chute and a hopper. In using the divider the seed is poured into the hopper and the rotary divider is switched on so that the crown unit with the containers rotates with approx. 100 rpm and the vibration chute starts to feed the seed into the inlet cylinder of the rotating crown. The feeding rate and therefore the duration of the dividing operation can be adjusted by the distance between the funnel of the hopper and the chute and the vibration intensity of the chute. There are two principles: (i) The inlet cylinder feeds the seed centrally onto a distributor within the rotating crown distributing the seed to all containers simultaneously. (ii) The inlet cylinder feeds the seed de-centrally into the inlets of the containers rotating underneath the inlet cylinder so that the seed stream is subdivided into a lot of sub-samples.

(e) *Variable sample divider*. The variable sample divider consists of a pouring hopper and a tube underneath that rotates with about 40 revolutions per minute. The tube distributes the seed stream from the pouring hopper onto the inner surface of a further hopper, which is well fitted into a third hopper all being concentric. In the second and the third hopper there are slots that comprise 50% of the perimeter of the hoppers. 50% of the seed will pass through the two hoppers into a collecting pan. The other 50% will stay within the hoppers and will then go into a second collecting pan. The two hoppers can be twisted against each other resulting in more narrow slots. The effect is that a smaller percentage will pass the slots. Either the smaller sample outside the hoppers or the bigger sample inside the hoppers can be used as the required sample. The position of the two hoppers in relation to each other can be adjusted accurately, resulting in pre-determined sub-sample sizes.

#### 2.5.2.2.2 Modified halving method

The apparatus comprises a tray into which fits a grid of equal-sized cubical cells, open at the top and every alternate one having no bottom. After preliminary mixing, the seed is poured evenly over the grid. When the grid is lifted, approximately half the sample remains on the tray. The submitted sample is successively halved in this way until a working sample, of approximately but not less than the required size, is obtained.

#### 2.5.2.2.3 Spoon method

##### Proposal 2y

It is proposed to delete the restriction to “single seeded species” since there is doubt about the meaning and background of this definition. The assumption that species containing MSU would not fall under this definition is often found, but there was no reference available to the BSC, why they are excluded. Therefore, it is proposed to delete “single” and to specify the maximum size of seed by adding “*Triticum* sp.” as the often used threshold in seed size. The Moisture Committee also suggests the method as described should **not** be allowed for moisture content determinations.

The spoon method is recommended for sample reduction for seed health testing (7.4.1). For other tests it is restricted to species with seeds smaller than *Triticum* sp.. A tray, a spatula and a spoon with a straight edge are required. After preliminary mixing, pour the seed evenly over the tray; do not shake the tray thereafter. With the spoon in one hand, the spatula in the other,



and using both, remove small portions of seed from not less than five random places. Sufficient portions of seed are taken to constitute a sub-sample of the required size.

#### 2.5.2.2.4 *The hand halving method*

This method is restricted to the following genera of chaffy seeds:

<i>Agrimonia</i>	<i>Cenchrus</i>	<i>Melinis</i>
<i>Andropogon</i>	<i>Chloris</i>	<i>Oryza</i>
<i>Anthoxanthum</i>	<i>Dichanthium</i>	<i>Pennisetum (non glaucum)</i>
<i>Arrhenatherum</i>	<i>Digitaria</i>	<i>Psathyrostachys</i>
<i>Astrebla</i>	<i>Echinochloa</i>	<i>Scabiosa</i>
<i>Beckmannia</i>	<i>Ehrharta</i>	<i>Sorghastrum</i>
<i>Bouteloua</i>	<i>Elymus</i>	<i>Stylosanthes (non guianensis)</i>
<i>Brachiaria</i>	<i>Eragrostis</i>	<i>Taeniatherum</i>
<i>Briza</i>	<i>Gomphrena</i>	<i>Trisetum</i>

and to the following genera of tree and shrub seeds:

<i>Acer</i>	<i>Corylus</i>	<i>Populus</i>
<i>Aesculus</i>	<i>Fraxinus</i>	<i>Quercus</i>
<i>Ailanthus</i>	<i>Juglans</i>	<i>Salix</i>
<i>Castanea</i>	<i>Liriodendron</i>	<i>Tectona</i>
<i>Cedrela</i>	<i>Platanus</i>	<i>Ulmus</i>

For all other species it can be used only to obtain working samples in the laboratory for seed health tests (7.4.1).

#### **Proposal 2z**

It is proposed to re-arrange the following paragraph to be formally in line with the descriptions of how to use the other sampling tools.

For applying the hand halving method, pour the sample evenly onto a smooth clean surface, thoroughly mix the seed into a mound with a flat-edged spatula, divide the mound into half and halve each half again - giving four portions - and halve each portions again - giving eight portions -, arrange the portions in two rows of four, combine and retain alternate portions: e.g. combine the first and third portions in the first row with the second and fourth in the second row, remove the remaining four portions. Repeat the procedure using the retained portions until obtaining the required sample size.



### Proposal 2aa

It is proposed to move the heterogeneity test to the end of chapter 2 into the Annex 2.8 behind tables 2A and 2B.

### 2.5.3 Storage of samples after testing

#### Proposal 2ab

It is proposed to describe here the technical requirements for sample on a general level and to move the time period to 2.5.4. From the technical point of view, a differentiation between orthodox and recalcitrant/intermediate seeds is necessary.

The primary aim of storage of samples after testing is to be able to repeat the original tests carried out on the submitted sample. Therefore, storage conditions should be such that changes in the seed quality traits tested are minimal. E.g. in the case of purity test or other seed count, the sample should be stored in such a way that the physical identity is kept. In the case of germination, viability or health test in orthodox seeds the sample should be stored under cool and dry conditions. For such tests in recalcitrant and intermediate seeds of tropical and sub-tropical species, long term storage is not possible. For such seed of temperate species storability is depending on the fungal status and to some extent whether the seed is dormant or not. All factors pertaining to storage need to be determined on a species basis. Protection against insects and rodents may be necessary.

When a re-test in a different testing laboratory is required, a portion shall be drawn from the stored sample in accordance with 2.5.2.2, submitted to the designated testing laboratory. The remainder shall be retained in store.

#### Proposal 2ac

The following paragraph contains those conditions that were deleted in the paragraphs below as being not related to the technical method of sampling and sampling reduction.

### 2.5.4. Conditions for issuing ISTA International Seed Lot Certificates

The sampling methods laid down in the ISTA Rules shall be followed when seed samples are drawn for the issue of ISTA International Seed Lot Certificates. Further conditions have to be fulfilled as listed below.

#### 2.5.4.1. Seed lot size

The seed lot shall not exceed the quantity indicated in column 2 of Table 2A, subject to a tolerance of 5% with the exception of

(i) herbage and amenity seed being transported loose in bulk containers. The conditions under which this exception may be permitted are laid down in Appendix B.



(ii) seed pellets, seed granules, seed tapes or seed mats. The maximum number of seeds that a seed lot of seed pellets, seed granules, seed tapes or seed mats may contain is 1,000,000,000 (10,000 units of 100,000) except that the weight of the seed lot, including the coating material may not exceed 40,000 kg subject to a tolerance of 5% (42,000 kg). When seed lot size is expressed in units the total weight of the seed lot must be given on the ISTA International Seed Lot Certificate.

Maximum lot size for treated and encrusted seeds is defined by applying the quantities indicated in Table 2.A to the seeds without coating material.

A seed lot in excess of the prescribed quantity shall be subdivided into seed lots not larger than the prescribed quantity, each of which shall be labelled or marked with a separate seed lot identification.

#### *2.5.4.2 Marking/labelling and sealing of containers*

The seed lot shall be in marked/labelled containers which are self-sealing, sealed (or capable of being sealed) or under the control of the seed sampler.

Where the seed lot is already marked/labelled and sealed before sampling, the seed sampler must verify marking/labelling and sealing on every container. Otherwise the sampler has to mark/label the containers and must seal every container before the seed lot leaves his/her control.

#### **Proposal 2ad Editorial change**

Correction: “unsealed” before submitted sample is unnecessary as ‘unless they are sealed’ qualify it.

The samplers are personally responsible for the seals, labels and bags supplied to them and it is their duty to ensure that primary, composite or submitted samples shall never be left in the hands of persons not authorised by the seed testing laboratory unless they are sealed in such a way that they cannot be tampered with.

#### *2.5.4.3 Sampling from the seed lot*

For sampling from the seed lot methods listed under 2.5.1.4.1 must be used. Automatic seed samplers must be approved by the ISTA seed testing laboratory.

#### **Proposal 2ae**

It is proposed to allow the so-called “re-sealing” that is allowed in several national and international seed certification systems. This is an extension of the former rule for small moisture proof containers (2.6.4, fifth paragraph).

An ISTA International Seed Lot Certificate issued on a seed lot (2.2.1) is still valid after re-packaging the seed lot in new containers provided that:

- The identity of the seed in the initial seed lot is preserved.
- The identification code of the seed lot is not changed.



- The moving of the seed into the new containers is done under the control of an ISTA seed sampler.
  - There is no processing of the seed during filling of the new containers.

### Proposal 2af

It is proposed to delete the following paragraph since the topic is covered by the ISTA Laboratory Accreditation Standard (see definition of seed sampler, 3.5, 3.6, 3.7, 6.1.2, 6.1.4).

### Proposal 2ag

It is proposed to add a reference to moisture meters to be in line with 9.2.2.5.2.

#### 2.5.4.4 Submitted sample

Minimum size of submitted samples are as follows:

- For moisture determination - 100 g for species that have to be ground (see Table 9A) and 50 g for all other species. When moisture meters are to be used for testing, a larger sample size may be necessary. Contact the ISTA seed testing laboratory for specific instructions.

- For verification of species and cultivar - As prescribed in Chapter 8.
- For all other tests - At least the weight prescribed in column 3 of Table 2A. As long as a determination of other seeds by number is not requested, the submitted sample shall weigh at least the amount indicated for the working sample for purity analysis in column 4 of Table 2A. In the case of coated seeds, the submitted samples shall contain not less than the number of pellets or seeds indicated in column 2 of Table 2B, Part 1 and Part 2.

In case the submitted sample is smaller than prescribed, the sampler shall be notified accordingly and analysis withheld until sufficient seed is received in a single submitted sample; except that in the case of very expensive seed, the analysis may be completed to the extent possible and the following statement inserted on the certificate: "The sample submitted weighed only ..... g [or in the case of pelleted seeds 'contained only .... pellets (seeds)'] and is not in accordance with the International Rules for Seed Testing."

The submitted sample must be sealed and labelled or marked.

#### 2.5.4.5 Sample reduction

For sample reduction methods listed under 2.5.2.2 must be used.



#### *2.5.4.6 Storage of submitted samples after testing*

To provide for re-testing by the original or by another seed testing laboratory, submitted samples on which ISTA International Seed Analysis Certificates have been issued shall be stored for one year from the date of issue of the certificate. Only in the case of very expensive seed, the remainder of the submitted sample, except 25 seeds for assurance of identity, may be sent back to the applicant. The seed testing laboratory cannot be held responsible for any deterioration of the sample during storage.

### **2.6. Calculation and expression of results**

See under 2.8.2 for calculating the results of heterogeneity tests.

### **2.7 Reporting of results**

See under 2.8.2 for reporting the results of heterogeneity tests.

## **2.8 Annexe**

### *2.8.1 Tables for lot size and sample sizes*





**Table 2A**

Note: Table 2A is not included here as it remains unchanged from the 2005 edition except for those suggested in Proposal 2ah which follows.

**Table 2B Part 1 Sample sizes of pelleted seeds in number of pellets**

Determinations	Submitted samples not less than	Working samples not less than
1	2	3
Purity analysis (including verification of species)	7500	2500
Weight determination	7500	Pure pellet fraction
Germination	7500	400
Determination of other seeds	10000	7500
Determination of other seeds (encrusted seeds and seed granules)	25000	25000
Size grading	10000	2000

**Table 2B Part 2 Sample sizes of seed tapes**

Determinations	Submitted samples not less than	Working samples not less than
1	2	3
Verification of species	2500 seeds	100 seeds
Germination	2500 seeds	400 seeds
Purity analysis (if required)	2500 seeds	2500 seeds
Determination of other seeds	10000 seeds	7500 seeds





### *2.8.2 Heterogeneity testing for seed lots in multiple containers*

The object of heterogeneity testing is to detect the presence of heterogeneity which makes the seed lot technically unacceptable for sampling according to the object as defined in 2.1.

#### *2.8.2.1 The H-value test*

##### *2.8.2.1.1 Definitions of terms and symbols*

**Note: Numbering only changed no textual changes so editorial only and not included. Table headings follow for reference only.**

#### **Table 2C Sampling intensity and critical H-values**

**Table 2D Part 1 Maximum tolerated ranges for the R-value test at a significance level of 1% probability using components of purity analyses as indicating attribute in non-chaffy seeds.**

**Table 2D Part 2 Maximum tolerated ranges for the R-value test at a significance level of 1% probability using components of purity analyses as indicating attribute in chaffy seeds.**

**Table 2E Part 1 Maximum tolerated ranges for the R-value test at a significance level of 1% probability using components of germination tests as indicating attribute in non-chaffy seeds.**

**Table 2E Part 2 Maximum tolerated ranges for the R-value test at a significance level of 1% probability using components of germination tests as indicating attribute in chaffy seeds.**

**Table 2F Part 1 Maximum tolerated ranges for the R-value test at a significance level of 1% probability using components of other seed count analyses as indicating attribute in non-chaffy seeds.**

**Table 2F Part 2 Maximum tolerated ranges for the R-value test at a significance level of 1% probability using components of other seed count analyses as indicating attribute in chaffy seeds.**

**Note: this ends the proposals to the text of Chapter 2 following amalgamation of the Rules and Annexes and the subsequent review of the text.**

**Separate vote required on Proposal 2ah**

Following the presentations and discussion during the Meeting in Budapest (2004), the BSC was proposing to increase seed lot size for cereals from 25 to 30 tonne.

Table 2A Part 1:

1	2	3	4	5
<i>Avena sativa</i> L.	30 000	1000	120	1000
<i>Avena strigosa</i> Schreb.	30 000	500	50	500
<i>Hordeum vulgare</i> L.	30 000	1000	120	1000
<i>Oryza sativa</i> L.	30 000	700	70	700
<i>Secale cereale</i> L.	30 000	1000	120	1000
x <i>Triticosecale</i> Wittm. ex A. Camus	30 000	1000	120	1000
<i>Triticum aestivum</i> L.	30 000	1000	120	1000
<i>Triticum durum</i> Desf.	30 000	1000	120	1000
<i>Triticum spelta</i> L.	30 000	1000	270	1000



### ITEM 3 PROPOSALS FOR CHAPTER 3, PURITY

#### Item 3a *Cuscuta* spp. seeds as inert matter

For harmonisation with statement in 3.2.3 *Inert matter*: “6. Seeds of *Cuscuta* spp. which are fragile or ashen grey to creamy white in colour”

##### 3.2.2 *Other seeds*

...

3. *Cuscuta* spp. seed units which are fragile ~~and~~ or ashen grey to creamy white in colour are classified as inert matter.

#### Item 3b Sieving reference

For harmonisation with rule change for beet adopted in: no sieving is now obligatory.

##### 3.5.2 *Separation*

1. The working sample (or sub-sample) after weighing, shall be separated into its component parts as defined in 3.2. In general, the separation shall be based on an examination of each particle in the sample, but in certain cases special procedures are obligatory, such as uniform blowing ~~or sieving~~.

#### Item 3c *Hordeum* no longer considered as chaffy species

*Hordeum* are no longer considered as chaffy

##### 3.2.1.A.1 Part 1 Pure seed definition numbers by genus and family.

...

<i>Hordeum</i>	Poaceae (Gramineae)	62	€
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##### Changes to PSDs 3.2.1.A.2 Part 2 Numbered Pure Seed Definitions

#### Item 3d PSD 1

Some samples present bracts attached to the seed and removing bracts is very time consuming. *Cannabis* is the only *Cannabaceae* genus taken into account in the PSD and no other *Cannabaceae* are included in other PSD. Other genera taken into account in PSD 1 are not concerned by bracts.

1. Achene, with or without bracts, unless it is obvious that no seed is present.  
Piece of achene larger than one-half the original size, unless it is obvious that no seed is present.



Seed, with the pericarp/testa partially or entirely removed.

Piece of seed larger than one-half the original size, with the pericarp/testa partially or entirely removed.

#### Item 3e PSD 4

Some samples of *Cichorium* present bracts attached to the achene and removing bracts is very time consuming.

Bracts can be attached to the achene, but PSD 4 mentions only the pappus and the beak as appendages to be found on the achene. Other PSDs mention appendages for genera of *Asteraceae* family. In these cases, both the presence and the absence of appendage (bract, wing, pappus, bristle, and calyx) are accepted.

4. Achene, with or without beak, ~~or with or without~~ pappus or bracts, unless it is obvious that no seed is present.  
Piece of achene larger than one-half the original size, unless it is obvious that no seed is present.  
Seed, with the pericarp/testa partially or entirely removed.  
Piece of seed larger than one-half the original size, with the pericarp/testa partially or entirely removed.

#### Item 3f PSD 10

*Pinaceae* can be removed as there are no members of the family listed in Table 3.2.1.A.1 that refer to this PSD.

10. Seed, with or without testa.  
Piece of seed larger than one-half the original size, with or without testa.  
*Fabaceae* (*Leguminosae*), *Brassicaceae* (*Cruciferae*), *Cupressaceae*, *Pinaceae*, *Taxaceae*, *Taxodiaceae*: seeds and pieces of seed without testa are regarded as inert matter. For *Fabaceae* (*Leguminosae*): separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.

#### Item 3g PSD 21

- In some samples of *Onobrychis viciifolia* seeds with the calyx attached to the fruit can be found. The addition of “with or without calyx” could save very time consuming work during the analysis of this kind of samples. In other PSD when calyx is mentioned (6, 7 and 22) presence or absence is accepted on pure seed.  
- Considering that all genera covered by PSD 21 (*Coronilla*, *Melilotus*, *Onobrychis*) belong to the *Fabaceae* family, “only” is not necessary.

21. Pod, with or without calyx, with seed(s).  
Seed, provided a portion of the testa is attached.



Piece of seed larger than one-half the original size, provided a portion of the testa is attached.

For *Fabaceae* (*Leguminosae*) ~~only~~: Seeds and pieces of seed without testa are regarded as inert matter. Separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.

### Item 3h PSD 23

- Considering the difficulty of evaluating the presence of the seed without opening the fruit, it would be useful for routine analysis to avoid this work by adding “unless it is obvious that no seed is present” to the definition.

23. One-seeded segment of pod or siliqua, with or without stalk or terminal beak, unless it is obvious that no seed is present.  
Seed, provided a portion of the testa is attached.  
Piece of seed larger than one-half the original size, provided a portion of the testa is attached.  
For *Ornithopus compressus* only, one-seeded pod segment, with or without attached empty pod segments or partial segments.  
*Fabaceae* (*Leguminosae*) and *Brassicaceae* (*Cruciferae*): Seeds and pieces of seed without testa are regarded as inert matter. For *Fabaceae* (*Leguminosae*) only: Separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.

### Item 3i PSD 25

Sometime, seeds with pedicels and stalk fragments are present on the samples of *Valerianella* genus. To detach these appendages is a very time consuming work for purity analyst. Another genus of *Valeranaceae* family is taken into account on PSD 7, where the presence of the calyx attached to the achene is accepted.

25. Dry, indehiscent fruit with one-three loculi, with or without calyx or pedicel or stalk fragment, unless it is obvious that no seed is present.  
Seed, with or without testa.  
Piece of seed larger than one-half the original size, with or without testa.



### Item 3j Weighing

Harmonisation with the paragraph 3.5.1 of the Rules (see below) where is specified that the number of decimal places indicated is a minimum. It will result in more uniform interpretation of the Rule.

#### 3.5.1 Working sample

...

The working sample (or each sub-sample) shall be weighed in grams to the minimum numbers of decimal places necessary to calculate the percentage of its component parts to one decimal place (3.5.1.A).

#### 3.5.1.A Weighing

The minimum number of decimal places necessary for weighing, in order to calculate percentages to one decimal place, is indicated below.

*Weight of working sample or half working sample in grams*      *Weigh the working sample or half working sample and its components to the following number of decimal places*

### Item 3k Magnification

Remove the reference to magnification: use of magnification is current in purity analysis. The previous statement does not correspond to the actual situation of laboratories.

#### 3.5.2.A.1 All families except Poaceae (Gramineae)

Achenes, schizocarps and mericarps, other fruits and seeds are to be examined superficially only, without the use of pressure, **magnification**, a diaphanoscope or other special equipment. If it is obvious on such an examination that there is no seed in the structure, it is to be regarded as inert matter.



## ITEM 4 PROPOSALS FOR CHAPTER 5, GERMINATION, ANNEXE

### Item 4a Clarification of how to report ungerminated seeds

Proposal from the Germination Committee.

If more than 5% ungerminated seeds are found at the end of the germination test a method is required to check whether they are fresh or dead. It needs to be made clear in the rules that whatever method is used the subsequent classification should be into fresh or dead. Also x-ray tests will only determine if seeds are full or empty, they will not determine if the seed is viable, hence this term is deleted as an appropriate method.

#### 5.6.5.A.3. Ungerminated seeds:

.....

2. Fresh seeds: when more than 5% of fresh seeds are believed present, the potential for these alleged fresh seeds to germinate must be determined by dissection, tetrazolium, or excised embryo. Those determined to have the potential to germinate are reported as fresh. Those determined to not have the potential to germinate are reported as dead. ~~measures as described in Annexe 5.6.3.A. must be taken to induce germination, especially if large numbers are found. If fresh seeds are to be reported at a rate of 5% or more, it must be verified that these seeds have the potential to produce a normal seedling. This may be done with a tetrazolium test (Chapter 6) or other appropriate method (such as dissection, embryo excision or x-ray).~~ If there is any doubt as to whether the seed is fresh or dead, then it must be classified as dead. If not already applied, measures described in Annexe 5.6.3.A. must be taken to induce germination if more than 5% of fresh ungerminated seeds are found.

.....



#### **Item 4b Addition of Organic Growing Medium as a primary substrate for sunflower germination**

Proposal from the Germination Committee.

To include organic growing media as a primary substrate for sunflower germination.

In order to decide if an Organic Growing Media (O) can be used as primary substrate for sunflower germination, a comparative test involving 16 ISTA and AOSA laboratories was organised to compare germination of sunflower with paper, sand and growing media.

The ISTA Method Validation Program was used for the planning and the elaboration of the comparative test protocol.

Statistical analysis of the results demonstrated that the level of normal seedlings is higher when Organic Growing Media is used for sunflower germination. When Organic Growing Media was used as the substrate for sunflower germination repeatability (within laboratories) and reproducibility (between laboratories) of the results was also better than that obtained using paper or sand media. Results of the comparative trial were presented at the 2004 ISTA Congress in Budapest and the validation report is available from the ISTA Secretariat.

The results obtained in this experiment allow the recommendation of Organic Growing Media as primary substrate for sunflower germination tests. A definition of a Growing Media is proposed to be included in ISTA Rules.

Background to changes in terminology relating to media used in germination test:

According to standards (EN standards) on substrates used for the sowing and culture of plantlets, the generic term for substrates is "Growing Media". The proposal is then to use Growing Media as a generic term for all substrates – paper, sand and other substrates such as the organic mixtures of peat, sand, perlite, etc.

General definitions are given for the main parameters to be taken into account for all the media. The idea is that general parameters should be the same for all Growing Media: i.e. paper and pure sand, as well as, Organic Growing Media (referred to as compost in the present rules).

According to the soil scientists, the same parameters can be applied to all media and it should be easier to fulfil these requirements for the Organic Growing Media than it is for the pure sand.



## 5.4 Materials

~~Paper or sand are commonly used substrates according to Table 5A. Soil or artificial compost are not recommended primary testing substrates. They are, however, permitted in special cases only as indicated in 5.6.2.A.1. Specifications and instructions as to permissible substrates, their preparation and use are given in 5.4.A and 5.6.A. Specifications as to water quality and substrate moisture content are given in 5.4.A and 5.6.A.~~

### 5.4 Growing Media: substrates and water

Growing Media used for germination tests are commonly Paper, Sand and Organic Growing Media according to Table 5A.

Soil is not permissible.

Water: demineralised, de-ionised, tap water and spring water are commonly used, specifications are given in 5.4.5.

#### 5.4.1 Definition

Product used as Growing Media for plants. Its use results in the development of media with sufficient pore space for air and water, for the anchorage of the root system and for contact with solutions (water) needed for plant growth.

#### 5.4.2 Specifications

The following general specifications apply for all Growing Media: Paper, Sand or Organic Growing Media.

*Composition:* the Growing Media can be paper, pure sand or mixtures of organic compounds with added mineral particles

*Water retention characteristics:* when the appropriate amount of water is added, the particles of the Growing Media should have the capacity to hold sufficient water to provide continuous movement of water to the seeds and seedlings, but also provide sufficient pore space for aeration required for optimal germination and root growth. *A priori*, the water content of the Growing Media shall be adjusted to the maximal water holding capacity. When necessary, for certain species, it can be adjusted to correspond to the particular species. The water retention shall then be expressed as a percentage of the maximum retention.

*pH:* The Growing Media must have a pH value within the range 6.0-7.5 when checked in the substrate.

*Conductivity:* The salinity must be as low as possible and no more than 40 milliSiemens per meter.

*Cleanliness and inocuity:* The Growing Media must be free from seeds, fungi, bacteria or toxic substances, which may interfere with the germination of seeds, the growth of seedlings or their evaluation.

*Re-use of substrates:* It is strongly recommended that the Growing Media is only used once

*Alternative.* It may be difficult to check all the specifications or to get Growing Media from suppliers with the requested specifications. It is permissible to replace the measure of



conductivity with biological tests such as phytotoxicity and innocuousness. If not, all the characteristics described in 5.4.2 must be verified.

### 5.4.3. Growing Media Characteristics

#### (1) Paper Growing Media

The paper should be wood, cotton or other purified vegetable cellulose. The paper may take the form of filter papers, blotters or towels. The paper should be such that:

- the roots of the seedlings will grow on and not into the paper.
- it possesses sufficient strength to enable it to resist tearing when handled during the test.

#### (2) Pure Sand Growing Media

The sand should be reasonably uniform and free from very small and large particles. Round particles are preferable and it is recommended that sand with sharp particles, which may impair seedling development, is avoided. It is recommended that 90% of the particles should pass through a sieve having holes or meshes of 0.8mm width and be retained on a sieve having holes or meshes of 0.05 mm width.

#### (3) Organic Growing Media

The mixtures are defined as containing the following elements in known proportions and fitting the requirements of 5.4.2:

- **Organic compounds:** Fibres such as peat, coconut fibres and wood fibres, with a size less than 5 mm.
- **Mineral particles:** for example sand, perlite and vermiculite. The proportion should be around 20% in volume. It is recommended that 90% of the particles should pass through a sieve having holes or meshes of 2 mm width and be retained on a sieve having holes or meshes of 0.05 mm width.

### 5.4.4 Water

#### (1) General specifications

*Cleanliness:* The water used to moisten the substrate should be reasonably free from organic or inorganic impurities.

*pH:* The pH value should be within the range 6.0-7.5 when checked in the substrate, or there must be evidence based on statistical data there is no influence of a pH outwith this range of values on the germination test results.

### 5.4.5. Quality control

*Objective:* to ensure that new deliveries of Growing Media meet the requirements for the principal physical characteristics and are free of negative effects due to toxic substances or noxious microorganisms.

The following characteristics: composition, water retention characteristic, pH, cleanliness and innocuousness (freedom from phytotoxic effects and negative effects



due to microorganisms) should be controlled<sup>1</sup>. Quality control tests can be performed by the seed testing laboratory or by laboratories specialising in soil analyses or microbiology tests.

<sup>1</sup> Examples of methods used to measure these quality attributes are given in the ISTA Handbook on Seedling Evaluation

## 5.5 Material, Apparatus

Containers: all kinds of plastic, glass (transparent or translucent), metal or pottery transparent or containers can be used provided there are no toxic emanations and they are clean and free from microorganisms.

Types of counting equipment and germination apparatus are described in 5.5.A.

## 5.6 Procedure

.....

### 5.6.2 Test conditions

Permissible substrates, temperatures, duration and additional directions, including recommended special treatments for dormant samples, are indicated in Table 5A. Substrates, temperatures and duration of test indicated are prescriptive and no others may be used. ~~if an ISTA International Seed Analysis Certificate is issued.~~

.....

### 5.6.5 Evaluation

Every seedling must be evaluated in accordance with the general principles laid down in 5.2.3 and 5.2.4. For evaluation, the essential structures must be sufficiently developed to permit detection of any abnormality. For further details see 5.6.5.A.

When samples tested on paper produce seedlings which cannot readily be evaluated, a retest should be made in sand or Organic Growing Media at the temperature indicated in Table 5A and under favourable conditions of moisture and light.

...

**NOTE: All sections of 5.4.A must be deleted as the information is now in 5.4**

....

### 5.6.2.A Test conditions

#### 5.6.2.A.1 Growing Media

*Methods using paper*

.....

*Methods using Sand or Organic Growing Media*

Sand and Organic Growing Media are used as follows:



1. TS (Top of Ssand) or TO (Top of Organic Growing Media)

The seeds are pressed into the surface of the Sand or the Organic Growing Media.

2. S (in Ssand) or O (Organic Growing Media)

The seeds are planted on a level layer of moist Sand or Organic Growing Media and covered with 10-20 mm of uncompressed ~~sand~~ substrate depending on the size of the seed. To ensure good aeration it is recommended that the bottom layer ~~of sand~~ be loosened by raking before sowing.

Sand or Organic Growing Media may be used instead of paper, even if not prescribed in Table 5A,:

- when the evaluation of a diseased sample proves impracticable because of ~~contamination of the spread of infection between seeds and seedlings on~~ paper substrate;
- ~~Sand is sometimes used~~ for investigative purposes and to confirm evaluation of seedlings in cases of doubt; ~~though for these objectives soil is the preferred medium.~~
- when seedlings show phytotoxic symptoms

*Methods using soil, compost*

~~Soil and compost are generally not recommended as a primary testing substrate. However, it may be necessary to use them, for example when seedlings show phytotoxic symptoms or if evaluation of seedlings is in doubt on paper or sand. Soil or compost is commonly used for comparative or investigative purposes.~~

5.6.2.A.2 Moisture and aeration

~~The substrate must at all times contain sufficient moisture to meet the requirements for germination. However, moisture content must not be excessive, or aeration may be limited.~~

~~The initial quantity of water to be added will depend on the nature and dimensions of the substrate and also on the size and species of the seed to be tested. The optimum amount should be determined by experiment.~~

Subsequent watering should be avoided wherever possible as it is likely to increase variability between replicates and between tests. Therefore, precautions should be taken to ensure that the substrate cannot dry out and that sufficient water is supplied continuously during the test period.

Special measures for aeration are not usually necessary for TP and PP tests enclosed in boxes or petri dishes. For BP however, care should be taken that envelopes and towel rolls are loose enough to allow for sufficient air around the seeds. For the same reason the material covering the seeds in sand and ~~soil~~ Organic Growing Media tests should not be compressed.

.....



**Table 5A. Germination methods**

....  
Substrates     The sequence.....  
                  TP; BP; S; [O](#)

....

The abbreviations have the following meanings:

- TP    top of paper
- BP    between paper
- PP    pleated paper
- S     sand
- TS    top of sand
- [O](#)    organic growing media
- [TO](#)   top of organic growing media

....

**Table 5A, amend entry for *Helianthus annuus***

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature °C	First count (days)	Final count (days)	
1	2	3	4	5	6
<i>Helianthus annuus</i>	BP; S; <a href="#">O</a>	20-30; 25; 20	4	10	Preheat, prechill

**Item 4c To include details of the tolerances checks to be made on replicate results.**

Proposal from the Germination Committee.

ISTA auditors have stated that the Rules 5.8.A “Calculation and expression of results – *Tolerances*” are not clear. The Germination Committee discussed this in Budapest and decided that the statement “Tolerances are to be applied at least on the category of normal seedlings“ should be added to make it clear that only the number of normally germinated seedlings in the replicates need to be checked using tolerances.

**5.8.A Calculation and expression of results**

The result .....

*Tolerances*

The result of a germination test can be relied upon only if the difference between the highest and the lowest replicates is within accepted tolerances. To check the reliability of a test result, the average percentage of the replicates is calculated and compared with Table 5.1 of Chapter 16: Tolerances. The result is considered reliable, if the difference between the highest and the lowest replicate does not exceed the tolerance indicated. **Tolerances are to be applied at least on the category of normal seedlings.**

To decide.....

**Item 5 Proposals for Annexe to Chapter 7, Seed Health testing methods****Item 5a: Modifications and additions to the validated seed health testing methods.**

The following method sponsored by ISHI-Veg has been validated by the Seed Health Committee, and is submitted for acceptance by the membership to be included in Annexe to Chapter 7 of the International Rules for Seed Testing as Method 7-020. Any problems or errors should be directed to the Chairpersons of the Seed Health and Rules Committees.

**New Method:****7-020 Detection of *Xanthomonas hortorum* pv. *carotae* (bacterial leaf blight) on carrot (*Daucus carota*)**

**Crop:** *Daucus carota*

**Pathogen:** *Xanthomonas hortorum* pv. *carotae*

**Background:**

There is no validated method for *Xanthomonas hortorum* pv. *carotae* on carrot seed. The most commonly used method in seed health testing laboratories is based on a seed wash dilution-plating assay. This method involves washing seeds in buffer and plating serial dilutions of the extract on a semi-selective medium. Various semi-selective media are currently used as described or adapted from the following papers: Cubeta and Kuan, (1986); Williford and Schaad, (1984); Kim *et al.*, (1982); and McGuire *et al.*, (1982). These media have been tested by ISHI-Veg and ISHI-Veg/ISTA in a number of comparative studies (Asma, 1999, Asma, 2000a and Asma, 2000b). In addition to comparing selective media the latter comparative study (Asma, 2000b) concluded that the confirmation method chosen had an affect on test results, with ELISA and IF giving false positive confirmations due to poor specificity of antisera. The 2000 study (Asma, 2000b) also looked at the effect of antibiotics and agar source on the performance of the test. Further work by Asma *et al.*, (2002) has shown PCR to be a reliable and quick confirmation method when compared to pathogenicity tests.

This method is derived from the previous comparative tests and the validation studies carried out by ISHI-Veg in 2003 (Asma, 2005). For routine testing of carrot seed a combination of two semi-selective media, MKM/MD5A or MKM/mTBM is recommended. If nystatin at a concentration of 35 mg/l is not enough to completely inhibit fungal growth, cycloheximide should be used. Either a pathogenicity test or a PCR test is used to confirm suspect isolates.

**Method Abstract:**

Seeds are suspended in saline plus Tween 20 in a conical flask, soaked overnight at 4-7°C. The flask is then shaken at room temperature for 5 minutes and the extract diluted. The diluted extracts are plated on either MKM and MD5A or MKM and mTBM media. Plates are incubated at 28°C for 4-8 days and then examined for the presence of suspect colonies of *Xanthomonas hortorum* pv. *carotae*. Suspect colonies are sub-cultured to plates of YDC medium and their identity confirmed by a pathogenicity test on susceptible carrot seedlings or using PCR with *X. hortorum* pv. *carotae* specific primers.



## ITEM 8 PROPOSALS FOR CHAPTER 8, VERIFICATION OF SPECIES AND CULTIVAR

### Item 8a

As laid down in the ISTA Position paper and presented during the EOM 2003 in Zurich, the GMOTF Rules chapter working group developed a proposal to include GMO testing of conventional seed lots into the ISTA. As also presented during that meeting and during the ISTA Congress 2004, a performance based approach is included for these tests.

The proposal below is now submitted by the Strategy Working Group of the GMOTF and the Variety Committee.

The proposal comprises a complete new text of Chapter 8 since there are comprehensive new paragraphs and movements of existing paragraphs. Documentation of the individual changes would cause too much confusion. Instead, a new text is submitted without differentiating the old text and the changes.

In addition, the new general headings for chapter of the ISTA Rules were used in this proposal as defined in Budapest 2004. In the former Annexe to Chapter 8 (now paragraph 8.8) only the numbering of the paragraphs was changed and no text.

The following comments are given for explanation and support of the proposal:

The testing for GM impurities in conventional seed lots was seen as a testing for the existence of a specified trait in a submitted sample. Since the object of the present Chapter 8 is to verify the trueness of a sample claimed by the applicant, the GMO testing issue is not covered by the present object of Chapter 8. Therefore, a second object was added.

Since for some GMOs no authentic standard sample may be available but a standard reference (like a base sequence of characteristic DNA regions), this kind of reference was added.

**Performance based approach:** The ability of a laboratory to perform bio-molecular tests and bioassays accurately and precisely depends on a number of factors including method validation, familiarisation and training. Numerous suitable methods for testing bio-molecular traits and performing bioassays are available. These methods require highly sophisticated equipment, skilled operators and specialized laboratory environments. Since these methods are under a rapid development and improvement and may require specific laboratory optimisation and updating, they are not included as standardised methods in the ISTA Rules. Instead, a performance based approach is established in order to assure the highest achievable quality standard for reporting uniform results.

Performance approved methods are evaluated and approved according to the principles of the performance based approach as it will be laid down in the relevant ISTA accreditation document. This document will be established by the Executive Committee (ECOM) and will be presented together with this Rules Change Proposal at the Ordinary Meeting 2005.

The initial idea of the performance based approach was that the laboratories organise “mini proficiency tests” to evaluate their performance in applying a new method. However, the group realised that reference material may be available for this purpose so that the strategy of



in-house validation by using reference material was developed. Consequently, the Rules Chapter Working group made the following proposal to the ECOM for the key-points of the performance based approach:

- The laboratory can freely choose a method. It is recommended to choose methods that are already validated by an inter-laboratory study according to ISO 5725 or other internationally or nationally recognised standards. But the laboratory is free to develop a method, use a commercially available test kit or adopt an unvalidated method from either the scientific literature or from national and international standards. In this case, at minimum a single laboratory validation according to IUPAC (Pure Appl. Chem. 2002, 74(5), 835-855) or other internationally or nationally recognised standards must be performed, to determine its fitness for the purpose. For further information refer to the ISTA Method Validation Handbook.
- The laboratory is documenting its performance in applying the method in terms of:
  - Accuracy: Closeness of the agreement between the result of a measurement and a true value of the measurand (VIM 3.5),
  - Repeatability: Closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement (VIM 3.6), and
  - Reproducibility: Closeness of the agreement between the results of measurement of the same measurand carried out under changed conditions of measurement (VIM 3.7).

This is done in-house with samples produced from reference material (but of unknown identity for the analyst) when starting a new method (if not already done during the single laboratory validation) and on a routine basis as long as using the method.
- The whole performance approval of methods is done under the responsibility of the laboratories.

## Chapter 8: Species and Cultivar Testing

### 8.1 Objects

#### *8.1.1 Verification of species and cultivar*

The object is to determine the extent that the submitted sample conforms to the species or cultivar as requested by the applicant using methods not permissible in a purity test according to Chapter 3.

#### *8.1.2 Testing for the presence of specified traits*

The object is to test for the presence of traits in the submitted sample as specified by the applicant (for examples see 8.2.2) using methods not permissible in a purity test according to Chapter 3.



## 8.2 Definitions

### 8.2.1 *Authentic standard sample*

An authentic standard sample is a valid seed sample of cultivar or species identity or a valid sample with presence of the specified traits.

### 8.2.2 *Standard reference*

A standard reference is a valid descriptive attribute of a cultivar or species, e.g. ploidy level, zygosity; isozyme, protein or DNA banding pattern produced by gel electrophoresis or similar techniques; allelic profile or nucleotide sequence.

### 8.2.3 *Performance approved methods*

Performance approved methods are evaluated and approved according to the principles of the performance based approach as laid down in the relevant ISTA accreditation document under the responsibility of the laboratory. They are restricted to bio-molecular tests and bioassays for the object of testing for the presence of specified traits. The laboratory is responsible for approving these methods. Performance approved methods can only be applied when no standardised method is included in this chapter for the test required.

## 8.3 General principles

### 8.3.1 *Field of application*

#### 8.3.1.1 *Verification of species and cultivar*

The determination is valid only if the species or cultivar is stated by the applicant and an authentic standard sample of the species or cultivar is available for comparison to ensure the certainty of the determination. The traits compared may be morphological, physiological, cytological or chemical.

#### 8.3.1.2 *Testing for the presence of specified traits*

The determination is valid only if the trait is specified by the applicant and either an authentic standard sample or a standard reference for that specified trait is available for comparison to ensure the certainty of the determination. The trait specified by the applicant may be morphological, physiological, cytological or chemical including bio-molecular.

### 8.3.2 *Testing principles*

The determination is carried out, depending on the species or cultivar or specified trait in question on seeds, seedlings or more mature plants grown in a laboratory, a glasshouse, a growth chamber or a field plot.

When an authentic standard sample is available, the working sample is compared with the authentic standard sample. Whenever possible, the working sample and the authentic standard sample shall be handled in the same way, e.g. in field plots they shall be grown contemporaneously, near-by and in identical environmental conditions and the evaluation shall be done at the same stage of development.



When a standard reference is available, the test is done by comparing the traits of the seeds, seedlings or plants of the working sample with the standard reference.

#### *8.3.2.1 Principles for verification of species and cultivars*

In the case of species or cultivars that are sufficiently uniform in one or more traits (e.g. in self-pollinated species), the conformity of the working sample with an authentic standard sample can be determined and if possible, the degree of conformity may be quantified. If the species or cultivar is not sufficiently uniform (e.g. in cross-pollinated species), the proportion of any obvious off-types is calculated and the conformity of the working sample is expressed.

#### *8.3.2.2 Principles for testing for the presence of specified traits*

A test is performed to determine either the proportion of that specified trait in the working sample based on number or mass of seeds or on extracted components, and/or the confidence probability by which the seed lot meets a specification. The test can be either a qualitative test, in which the presence or absence of the specified trait in the working sample is determined or a quantitative test in which the proportion of the specified trait is determined. The laboratory must ensure that the selected methods meet the request of the applicant. Appropriate controls must be included.

### **8.4 Personnel and Equipment**

The determination shall be made by a specialist familiar with the morphological, physiological, bio-molecular or other trait of seeds. The specialist must possess specific knowledge of procedures, apparatus and equipment required for determining species and cultivar. It may be necessary to consult the international scientific literature, official government documents, other laboratories or other resources for guidance.

Appropriate facilities and equipment must be available as specified in detail in 8.8 for testing the specified trait, and in general as follows:

- (a) In the laboratory - apparatus and reagents for morphological, physiological, cytological or bio-molecular examinations, chemical tests and germination of seeds as appropriate.
- (b) In glasshouses and growth chambers - provision of controlled environmental conditions adequate to induce the development of the trait.
- (c) In field plots - climatic, soil and cultural conditions to permit normal development of the trait and sufficient protection against pests and diseases.



## 8.5 Procedures

### 8.5.1 Submitted sample

The testing laboratory shall ensure that the size of the submitted sample is sufficient to perform the tests as requested by the applicant.

Guiding values for the size of the submitted sample for tests covered by this chapter are as follows:

	Laboratory only (g)	Field plot and laboratory (g)
<i>Glycine, Lupinus, Phaseolus, Pisum, Vicia, Zea</i> and species of other genera with seeds of similar size	1000	2000
<i>Avena, Hordeum, Secale, Triticum</i> and species of other genera with seeds of similar size	500	1000
<i>Beta</i> and species of other genera with seeds of similar size	250	500
All other genera	100	250

Depending on the method and the degree of precision required, more seeds or less seeds than the amount listed above may be necessary.

### 8.5.2 Working sample

The size of the working sample and the number and size of replicates will depend on the object, the method to be used and the degree of precision as requested by the applicant. If technically possible and justified, replicates should be tested to improve the reliability of the test result. Preparation of the working sample and the replicates shall be done according to procedures described under 2.7.2.

#### 8.5.2.1 Working samples for testing for the presence of specified traits

Tests can be performed with seeds from the pure seed fraction (according to Chapter 3) or the whole working sample with the exclusion of the inert matter (according to Chapter 3). The working sample shall be washed to remove dust, treatments or in the case of coated seeds, pelleting material or tapes if these materials may affect the test result. The kind of preparation of the working sample must be reported according to 8.7.

### 8.5.3 Examination of seeds

There may be different procedures for examining seeds:

For testing morphological traits, the seeds shall be examined with the aid of a suitable magnifying apparatus when necessary. For testing colour traits, the seeds may be examined under full daylight or light of limited spectrum, e.g. ultra-violet. For testing chemical traits, the seeds shall be treated with the appropriate reagent and the reaction of each seed noted. For



a determination of ploidy level, tissue is excised and processed for analysis. For testing bio-molecular traits, DNA, RNA, protein or other specific metabolic products are extracted from the seeds and the traits may be detected, elucidated and quantified.

Standardised methods for examining seeds listed under 8.8.1 are applicable to both objects according to 8.1. For the application of performance approved methods see 8.2.3.

#### *8.5.4 Examination of seedlings*

The seeds shall be germinated on an appropriate medium. When the seedlings have reached a suitable stage of development, they are examined in whole or in part, with or without further treatment. For a determination of ploidy level, a root tip or other tissue is excised and processed for ploidy analysis. For testing bio-molecular traits, DNA, RNA, protein or other specific metabolic products are extracted from the seedlings and the traits may be detected, elucidated and quantified. In bioassays, seeds may be treated before germination or the seedlings may be treated to induce the expression of the traits if present.

Standardised methods for examining seedlings listed under 8.8.2 are applicable to both objects according to 8.1. For the application of performance approved methods see 8.2.3.

#### *8.5.5 Examination of plants in glasshouse or growth chamber*

The seeds shall be sown in suitable containers and maintained in environmental conditions necessary for the development of the traits. When the plants have reached a suitable stage of development, the traits shall be observed on each plant and noted. For testing bio-molecular traits, DNA, RNA, protein or other specific metabolic products are extracted from the plants and the traits may be detected, elucidated and quantified. In bioassays, seeds may be treated before germination or the seedlings or plants may be treated directly to induce the expression of the traits if present.

Standardised methods for examining plants listed under 8.8.2 are applicable to both objects according to 8.1. For the application of performance approved methods see 8.2.3.

#### *8.5.6 Examination of plants in field plots*

Each working sample shall be sown in at least two replicate plots. As insurance against failure the replicates should be situated in different fields or different parts of the same field. The plots may be of any convenient size that will provide enough plants for the determination to be of the accuracy required. If the seed is sown *in situ*, it shall be sown in rows, mechanically if possible. Spacing between rows and between plants shall be sufficient to allow development of the traits. Both transplanting and thinning are possible sources of error and the sowing rate shall be adjusted to produce approximately the same number of plants in the plots produced from the working sample and the authentic standard sample. When absolutely necessary, thinning or transplanting of seedlings from elsewhere into the plot is permitted.

Observations shall be made during the whole growing period, but particularly at times indicated in 8.8.3. Plants showing the traits shall be counted and recorded.

When practical, either an actual count or an estimate of the number of plants in the plot shall be made, preferably at the time the plants are examined.

Standardised methods for examining plants listed under 8.8.3 are applicable to both objects according to 8.1. For the application of performance approved methods see 8.2.3.



## 8.6 Calculation and expression of results

The calculation and expression of results depends on the object, the method used, the testing plan and whether a qualitative or quantitative result or a confidence probability for meeting a threshold is requested by the applicant. The mean and other statistics may be calculated and reported when results of replicates are within the range of expected variability. Methods for determining tolerances may be found in the ISTA Handbook of Variety Testing (Electrophoresis Testing) as well as in the ISTA Handbook on Statistics in Seed Testing (Appendix II). In the case of verification of species and cultivar, the determined proportion of other species, other cultivars or aberrant (e.g. fatuoid oats, speltoid wheat) is calculated and expressed.

In the case of testing for the presence of specified traits the result shall be expressed as agreed with the applicant by either

- reporting whether the trait is present or not,
- calculating and expressing the proportion of the trait or
- calculating and expressing the confidence probability that the true proportion of the trait meets or exceeds a specification on the basis of the test result.

### *8.6.1 Examination of individual seeds, seedlings or plants*

Whenever possible, the number of divergent seeds, seedlings or plants or those with the trait under test shall be calculated as a percentage of the number of seeds, seedlings or plants examined.

When testing seedlings, the result is expressed as the proportion of the number of normal seedlings (as defined in Chapter 5). If the applicant requested reporting in a different way, it shall be given in addition.

When testing plants in field plots in rows without wide spacing, it may be difficult to estimate the total number of plants examined per plot. The result may be expressed as the number of divergent plants or plants with the trait under test produced by the mass of seed sown.

### *8.6.2 Tests for traits of bulk samples*

Tests may be done by measuring traits of a bulk sample that do not allow a reference to individual seeds, seedlings or plants. There are various different principles for calculation and expression of test results of such measurements. The result shall be expressed as agreed with the applicant.

### *8.6.3. Calculation of the confidence probability that the seed lot meets or exceeds a specification*

For the calculation of the confidence probability the software “SeedCalc” in the latest version available on the ISTA homepage or other means with appropriate algorithms must be used.

## 8.7 Reporting results

The results shall be reported under ‘Other Determinations’ on an ISTA International Seed Analysis Certificate and in addition the following information shall be given:



- The request of the applicant.
- The trait(s) and the method(s) used.
- The kind of preparation of the working sample (e.g. the whole working sample excluding the inert matter or only the pure seed fraction, washing).
- Whether an authentic standard sample or a standard reference was used. In case of the use of a standard reference, its origin must be indicated.
- The number of seeds, seedlings or plants examined. When it is difficult to determine the total number of plants examined in field plots, the mass of seed sown must be reported.

### *8.7.1 Reporting results of verification of species and cultivar*

#### *8.7.1.1 Results of examination of individual seeds or seedlings*

Suggested phrases for reporting divergent seeds or seedlings depending upon the result are as follows:

- If none was found: "The test performed revealed nothing indicating that the species (and/or cultivar) stated by the applicant is incorrect."
- If non-conforming seeds were found: "Out of .... seeds examined .... seeds do not conform to the authentic standard sample of the species (and/or cultivar) stated by the applicant."
- If non-conforming seedlings were found: "Out of .... seeds producing normal seedlings, ....% do not conform to the authentic standard sample of the species (and/or cultivar) stated by the applicant."
- If the total working sample was found to be of a species and/or cultivar other than that stated by the applicant: "The sample does not conform to the authentic standard sample of the species (and/or cultivar) stated by the applicant."

#### *8.7.1.2 Results of a field plot examination*

The results shall, whenever possible, be reported as a percentage of each other species, other cultivars or aberrant found. When the expression of the result as a percentage is not possible, appropriate comments regarding the conformity of the sample may be reported.

If nothing worthy of special comment was found the following statement is suggested: "The results of a field plot examination of this sample revealed nothing indicating that the species (and/or cultivar) stated by the sender is (are) incorrect."

#### *8.7.1.3 Reporting probabilities of meeting specifications*

The result may be reported as: "On the basis of the traits tested, the seed lot meets the specification of ...% minimum species (or cultivar) purity with ...% confidence."

### *8.7.2 Reporting test results of presence of specified traits*

#### *8.7.2.1 Qualitative test results*

Suggested phrases for reporting depending upon the result are as follows:

- If the specified trait was not found: "The test performed revealed nothing indicating the presence of the trait specified by the applicant."
- If the specified trait was found: "The presence of the trait specified by the applicant was detected."



The limit of detection of the method used should be provided.

#### *8.7.2.2 Quantitative results obtained by multiple qualitative tests of individual or bulks of seeds or seedlings*

Results may be reported as the percentage of seeds or seedlings showing the trait specified by the applicant. Suggested phrases for reporting depending upon the result are as follows:

- If none was found: “The test performed revealed nothing indicating the presence of the trait specified by the applicant.”
- If seeds showing the trait were found: “Out of .... seeds examined .... seeds showed the trait specified by the applicant.”
- If seedlings showing the trait were found: “Out of .... seeds producing normal seedlings, .... % showed the trait specified by the applicant.”

#### *8.7.2.3. Quantitative measurements of traits in bulk samples*

Units may be percent seeds by number, percent seeds by mass, percent by protein, percent by number of DNA copies, or any other determinant by percent. The limit of detection (LOD) and the limit of quantification (LOQ) of the method used, the testing plan (e.g. number of replicate seed samples, number of replicate flour samples per seed sample, number of extracts per flour sample, number of replicate measurements per extract) and the standard deviation of the test result should be provided.

Suggested phrases for reporting depending upon the result are as follows:

- If the specified trait was not found: “The test performed revealed nothing indicating the presence of the trait specified by the applicant at a level above LOD.”
- If the specified trait was found at a level above LOD and below LOQ: “The trait specified by the applicant was present at a level below the LOQ of the method used.”
- If the specified trait was found at a level above LOQ: “The trait specified by the applicant was found at a percentage of ..... % by number of DNA copies.”

#### *8.7.2.4 Reporting probabilities of meeting or exceeding specifications*

Independent of the type of method used, a suggested phrase for reporting the results is: “For the trait specified by the applicant, the seed lot meets the specification of ...% (maximum or minimum) with ...% confidence.”

## **8.8 Annexes**

### **8.8.1 Standardised methods for examination of seeds**

**Note: Numbering only changed in this section no textual changes so editorial changes only.**