



International Rules for Seed Testing 2021

Chapter 1: ISTA Certificates

**Including changes and editorial corrections adopted
at the online Ordinary General Meeting 2020**

Effective from 1 January 2021

Note on the use of the translations

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

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Preface to the 2021 Edition of the ISTA Rules

Since 2014, the *International Rules for Seed Testing* (ISTA Rules) are primarily available in electronic format only. The ISTA Rules can be downloaded as a complete PDF file or as individual chapters from:

<http://www.ingentaconnect.com/content/ista/rules>.

If required, users of the ISTA Rules can print their own copies. For further information on the ISTA Rules, see:

<http://www.seedtest.org/rules>.

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

Seed health testing methods

Previously, the seed health testing methods were published as a separate Annexe to Chapter 7 of the ISTA Rules. They are now available as separate method sheets from the ISTA web site at:

<http://www.seedtest.org/seedhealthmethods>.

Details of changes

The 2021 changes are editorial corrections or Rules changes adopted at the online Ordinary General Meeting held in June 2020. Edits were made in Adobe InDesign by Vanessa Sutcliffe of HeartWood Editorial (www.heartwoodeditorial.co.uk).

The changes in the text content from the previous edition of the ISTA Rules are listed below. They can be displayed with yellow highlight boxes as a 'layer' over the English version within the electronic copy, with comments on what has changed.

For the previous history of amendments to the ISTA Rules, see the Prefaces for 2003 to 2020 on the ISTA website.

Ernest Allen, ISTA Rules Committee Chair

Susan Alvarez, ISTA Rules Committee Vice-Chair

ISTA Secretariat

Changes to the ISTA Rules for 2021

Introduction

I-1: Health and Safety statement added. Statement reviewed and approved by ECOM and Rules Committee.

Chapter 1

1.5.2.2: Result of changes approved in Chapter 2 during OGM16.

1.5.2.2: Clarification that authorities are not required for reporting.

1.5.2.6: Result of proposal submitted to Germination Committee by ISTA Accreditation Department. Clarity in section 5.9 that only percentage germination is reported when germination test is terminated due to predetermined germination level.

1.5.2.17.2, 1.5.2.17.3 and 1.5.2.17.4: Reference for 5.8.2 replaces the current erroneous reference of 5.8.1.

1.5.2.21, 1.5.2.21.2 and 1.5.2.21.3: Result of changes approved in Chapter 19 during OGM18.

Chapter 2

2.8: Result of nomenclature changes to the *ISTA List of Stabilised Plant Names* that have been incorporated into Table 2C. Previous cross references were not removed prior to the printing of the 2020 Rules. Previous cross references will be removed in the 2021 Rules and the last sentence of the current version will be replaced.

Table 2C Part 1: Result of approved nomenclature changes to the *ISTA List of Stabilised Plant Names*.

Chapter 3

3.7: Result of changes approved in Chapter 2 during OGM16.

3.7: Clarification that authorities are not required for reporting.

Table 3A: Deletion of *Poa pratensis* varieties that are no longer being traded. Proposal approved by majority vote in Purity Committee.

Chapter 4

4.2.1: Result of changes approved in Chapter 4 during OGM18.

4.2.2: ‘Orobanchaceae’ replaced with ‘dust-like seeds’ to harmonise with other changes related to such seeds. Addition of ‘indistinguishable species’ to distinguish those seeds that are costly to or not reliably retrieved

in the complete test quantity. Proposal approved by majority vote in Purity Committee.

4.2.4: ‘Orobanchaceae’ replaced with ‘dust-like seeds’ and ‘very expensive seed’ replaced with ‘small seed lots’ to harmonise with other changes related to such seeds. A reduced test is allowed in the Rules for similar species searches. Proposal approved by majority vote in Purity Committee.

Chapter 5

5.2.7.2: Result of validation study carried out in Germination Committee to change evaluation of roots for *Helianthus annuus*, allowing for secondary roots when the primary root is defective. Proposal approved by Germination Committee.

5.6.2.4 and 5.6.3.1: Clarity that LED lights can be used for germination. Proposal supported by Germination Committee.

5.9: Proposal submitted to Germination Committee by ISTA Accreditation Department. Clarity that only percentage germination is reported when germination test is terminated due to predetermined germination level.

Table 5A Part 1: Result of validation study conducted on *Brassica napus* to determine which temperature requirements for germination promoted higher normal seedling development and enhanced testing reproducibility among labs. Proposal supported by Germination Committee.

Chapter 7

Method 7-001a, -001b, -002a, -002b, -003 -005, -006, -008, -010, -011, -012, -013a, -013b, -014, -015 and -016: Increasing harmonisation for sample and subsample size throughout Seed Health Methods. Proposal approved and supported by Seed Health Committee.

Methods 7-001a, -001b, -002a, -002b, -007 and -009: Clarifying instructions for preparing hypochlorite solutions for pretreatment as prescribed in Methods.

Method 7-004, -007, -019a, -019b, -020, -021, -022, -023, -024, -025, -026, -027, -028, -029, -030, -031 and -032: Increasing harmonisation for sample size with other methods to detect fungi. Proposal approved and supported by Seed Health Committee.

Methods 7-005, -006, -008, -014, -016, -022 and -032: Clarifying instructions for preparing hypochlorite solutions for pretreatment as prescribed in Methods. Adding use of hypochlorite solutions for methods

similar to those already approved in Rules. Proposal supported by Seed Health Committee.

Method 7-007: Revision requested by Seed Health Committee to make description of *Alternaria linicola* more informative.

Method 7-019a, -019b and -020: Addition of safety considerations when using ethidium bromide. Proposal supported by Seed Health Committee.

Method 7-019a and -020: Clarity that alternative chemicals other than ethidium bromide may be used for this method. Proposal supported by Seed Health Committee.

Method 7-021, -030 and -031: Clarity that alternative chemicals other than ethidium bromide may be used for this method. Addition of safety considerations when using ethidium bromide. Proposal supported by Seed Health Committee.

Chapter 9

9.2.4.2, 9.2.5.4, 9.2.5.6, 9.3.1.4, 9.3.1.5.1, 9.3.1.5.2, 9.3.1.5.3, 9.3.1.5.4, 9.3.1.5.5, 9.3.2.4.3, 9.3.2.8 and 9.3.2.9: Review of Chapter 9 with recommendation that some wording changed to improve clarity on when a laboratory *must* perform an action and when it is suggested that the laboratory *should* perform the action (but would not be found in violation during an ISTA audit if they used a different but acceptable alternative process). Proposal approved by Moisture Committee.

9.2.4.3: Clarification of definition of appropriate containers for use in oven moisture testing. Review of Chapter 9 by Moisture Committee.

9.2.4.6 and 9.2.5.4: Use of singular 'mesh' instead of misleading 'meshes'. Proposal approved by Moisture Committee.

9.2.5.2: Section re-ordered and modified to improve clarity and to avoid confusion about differences in how many working samples are drawn depending on whether seeds are whole or ground.

Table 9A Part 2: Moisture method for *Carica papaya* included in table. Proposal made following validation studies carried out within Moisture Committee.

Chapter 15

15.8.2.8, 15.8.3.6 and 15.8.4.6: Reference for 5.8.2 replaces the current erroneous reference of 5.8.1.

Table 15B: Clarification of assessment of 2 mm radicle; consistency in description of germination media for radicle emergence test for *Zea mays* and *Triticum aestivum* subsp. *aestivum*; all Latin names expressed according to *ISTA List of Stabilised Plant Names*. Additional text clarifies measurement of 2mm in radicle emergence test for these species and specifies that assessments should be made by eye. All validation for radicle emergence has been done without magnification and has achieved repeatability and reproducibility. Proposal supported by Vigour Committee.

Chapter 1: ISTA Certificates

1.1 Object

The object is to prescribe rules for the issue of ISTA Certificates for seed analysis. The completed certificates are available only from accredited member laboratories of ISTA and must only be issued in accordance with the ISTA Rules currently in force.

1.2 Definitions

Blank ISTA Certificates for seed analysis are produced by ISTA, and only provided to accredited laboratories (see 1.2.6) for reporting the results of tests. These completed certificates are the property of ISTA and may only be issued under the authority of ISTA.

1.2.1 Orange International Seed Lot Certificate

An Orange International Seed Lot Certificate is issued when both sampling from the lot and testing of the sample are carried out under the responsibility of an accredited laboratory, or when sampling from the lot and testing of the sample are carried out under the authority of different accredited laboratories. Where the accredited laboratory carrying out the sampling is different to the one carrying out the testing, this must be stated (see 1.4.2). The procedure followed links the Orange International Seed Lot Certificate with the seed lot. The certificate is coloured orange.

An Orange International Seed Lot Certificate may also be issued, without further sampling or testing, for a portion (sublot) of a seed lot which has been sampled and tested and for which an Orange International Seed Lot Certificate has been issued. The certificate for the sublot must carry the same test results as reported for the original seed lot.

The results reported on an Orange International Seed Lot Certificate refer strictly to the lot as a whole at the time of sampling.

1.2.2 Blue International Seed Sample Certificate

A Blue International Seed Sample Certificate is issued when sampling from the lot is not under the responsibility of an accredited laboratory. The accredited laboratory is responsible only for testing the sample as submitted. It is not responsible for the relationship between the sample and any seed lot from which it may have been derived. The certificate is coloured blue.

The results reported on a Blue International Seed Sample Certificate refer strictly to the sample at the time of receipt.

1.2.3 Original certificate

An original certificate is an ISTA Certificate issued after the completion of a test or tests. It is marked ORIGINAL.

1.2.4 Duplicate certificate

A duplicate certificate is an exact printed copy, not a photocopy, of a completed original ISTA Certificate, marked DUPLICATE.

1.2.5 Provisional certificate

A provisional certificate is an ISTA Certificate issued before the completion of a test or tests. It is marked PROVISIONAL, and must include a statement under 'Other determinations' that a final original certificate will be issued upon completion.

1.2.6 Accredited laboratory

An accredited laboratory is an ISTA-accredited member laboratory authorised by the ISTA Executive Committee under Article 4(i) of the Articles of ISTA to sample and test seeds and to issue ISTA Certificates.

1.3 Conditions for issuance of ISTA Certificates

ISTA Certificates must be issued only on forms obtained from the ISTA Secretariat and approved by the ISTA Executive Committee. There are two kinds of certificates: Orange International Seed Lot Certificates and Blue International Seed Sample Certificates, as defined in 1.2.

On request of the applicant, duplicate and provisional certificates as defined in 1.2 may be issued.

A duplicate certificate may be issued for an original certificate. More than one duplicate certificate may be issued.

A provisional certificate may be issued for any ISTA test result(s) that are later combined onto an original certificate. More than one provisional certificate may be issued.

If an applicant cancels testing, an ISTA Certificate does not need to be issued.

An ISTA Certificate may be issued only by the seed testing laboratory which either carried out all the tests to be reported, or subcontracted sampling and/or some of the tests to be reported (see 1.4.2 and 1.4.3), and under the conditions listed below:

- a. The issuing laboratory must be currently authorised to do so by the Executive Committee.
- b. The seed tested must be of a species listed in Table 2C (Lot and sample weights) of the ISTA Rules. Where in other tables, such as Table 5A and Table 6A, methods are prescribed for a group of species, only those species specifically listed in Table 2C may be considered to be covered.

Consequently, no certificates may be issued for species not listed in Table 2C of the current ISTA Rules, except in the case of seed mixtures, where for the species tested it is shown as 'Seed mixture'.

- c. The tests must be carried out in accordance with the ISTA Rules. However, additionally and on request, results of tests not covered by these Rules may be reported on an ISTA Certificate (see 1.5.2.22). Results of analyses not covered by the current ISTA Rules may be included on a certificate only if results of at least one test covered by the ISTA Rules are also being reported.
- d. For the result of a determination of moisture content to be reported on an ISTA Certificate, the sample must be submitted in an intact, moisture-proof container from which as much air as possible has been excluded (see 9.2.5.1).

- e. To report results of tests which are in the ISTA Rules, the laboratory must be accredited for these tests, either directly or through subcontracting to another laboratory accredited for these tests.
- f. The assessment of any attribute reported on a certificate must be calculated from tests carried out on one submitted sample.
- g. In the case of Orange International Seed Lot Certificates:
 - the seed lot must comply with the requirements prescribed in 2.5.4;
 - the submitted sample must be drawn and dealt with in accordance with 2.5.4.
- h. For an Orange International Seed Lot Certificate, each container in the lot must be marked, labelled and sealed in accordance with 2.5.4.3.
- i. For an Orange International Seed Lot Certificate to be issued for a subplot, the subplot must represent a minimum size of 20 % of the weight of the original seed lot. A maximum of five Orange International Seed Lot Certificates may be issued for sublots of any one original seed lot.
- j. For an Orange International Seed Lot Certificate, the submitted sample must be tested by an accredited laboratory. The issuing laboratory must ensure that sampling, sealing, identification, testing and issuance of the certificate is in accordance with the ISTA Rules, although subcontracting of sampling and/or testing to another accredited laboratory is permissible. The laboratory which carries out sampling must provide all the information that is necessary to complete the Orange International Seed Lot Certificate. The seed lot identification ('Marks of the lot'; see 2.2.11) may take the form of a sequential series of characters or a single reference character. Each container within the lot or subplot must be identified in such a way that the containers can be readily recognised by the information provided on the certificate issued. Each container of a subplot must be marked with the identification of the original seed lot. A subplot-specific identification is not necessary. When the seed lot is located in a different country to the sampling laboratory, the country where the seed lot has been sampled must be reported either under 'Sampling by' or under 'Additional observations'.

1.4 Completing ISTA Certificates

1.4.1 General

ISTA Certificates must be completed using a typewriter or machine-printer and can be completed in any language. No certificate may be issued that shows signs of amendment, alteration or erasure.

A completed certificate must show the following information:

- The name and address of the issuing laboratory; the laboratory must be on the ISTA list of accredited member laboratories.
- Dates, written in the ISO 8601 format: year in full – month – day, with two figures for both month and day (e.g. 2007-07-25).
- The scientific name of the species tested, as listed in the current ISTA Rules and (in most cases) also the *ISTA List of Stabilised Plant Names*. Where it is impossible to determine the species with certainty on the basis of seed characters, only the genus name must be stated (example: *Malus* sp.). In the case of seed mixtures, for the species tested ‘Seed mixture’ must be entered.
- The name and address of the applicant. Other information stated by the applicant, such as country of origin, species, cultivar, weight of lot or subplot, certification category and applicant’s lot reference must be entered as stated by the applicant.
Note: at the request of the applicant the name and address of the applicant may be omitted.
- The signature of the Head of the issuing laboratory or their assignee. It may be either a physical or an electronic signature, the use of which is authorised by the Head of the issuing laboratory.
- Under ‘Status of certificate’, the word ‘ORIGINAL’, ‘PROVISIONAL’ or ‘DUPLICATE’, as appropriate.

1.4.2 Orange International Seed Lot Certificate

It is stated on the back of the Orange International Seed Lot Certificate:

‘I certify that sampling, sealing and testing have been carried out in accordance with the *ISTA International Rules for Seed Testing* and that the tests have been made at a laboratory accredited by the International Seed Testing Association to issue International Seed Analysis Certificates.’

The completed Orange International Seed Lot Certificate must show the following information:

- name, address, ISTA member code and stamp (seal) of issuing laboratory;
- name and ISTA member code of laboratory responsible for sampling;
- seed lot identification (i.e. marks of lot);
- Under ‘Seal of lot’: the method of sealing (e.g. stitching, metal seal) and/or the authority (e.g. ISTA laboratory, Ministry).
- either the number of containers for which the certificate is issued; or ‘N/A’ for ‘not applicable’;
- date of sampling;
- date that the sample was received by the testing laboratory;
- date test was concluded;
- place, country and date of issue of the certificate;
- test or sample number of the testing laboratory;
- analysis results;
- In the case of certificates for sublots, under ‘Other determinations’: ‘The results reported represent the sample drawn from the original seed lot of ... kg’;
- country where the seed lot was sampled, when the seed lot is located in a different country to the sampling laboratory, reported under either ‘Sampling by’ or ‘Additional observations’;
- the signature of the Head of the issuing laboratory or their assignee which confirms the statement on the back of the certificate as true;
- under the signature it must state at least, the job position of the person signing or ‘Authorised signatory’.

1.4.3 Blue International Seed Sample Certificate

The Blue International Seed Sample Certificate refers only to the sample submitted for testing.

It is stated on the back of the Blue International Seed Sample Certificate:

‘I certify that testing has been carried out in accordance with the *ISTA International Rules for Seed Testing* and that the tests have been made at a laboratory accredited by the International Seed Testing Association to issue International Seed Analysis Certificates.’

The completed certificate must show the following information:

- name, address, ISTA member code and stamp (seal) of issuing laboratory;
- date that the sample was received by the testing laboratory;
- date test was concluded;
- place, country and date of issue of the certificate;
- test or sample number of the testing laboratory;

- f. results of tests;
- g. the signature of the Head of the issuing laboratory, or their assignee which confirms the statement on the back of the certificate as true;
- h. under the signature it must state at least, the job position of the person signing or 'Authorised signatory'.

1.4.4 Duplicate certificate

A duplicate ISTA Certificate may be issued on request of the applicant.

1.4.5 Provisional certificate

A provisional ISTA Certificate may be issued on request of the applicant. There is no limit to the number of copies of an ISTA certificate marked 'Provisional'.

If the sample is being tested for more than one test, but at least one test has been concluded, the 'date that the test was concluded' is entered.

1.5 Reporting results

1.5.1 Sampling and testing

From one sampling operation, only one sample may be submitted for testing. The sample may be subjected to one or more of the tests described in the ISTA Rules as requested by the applicant. However, in certain situations (see 2.5.1.6) the submission of separate moisture-proof-packed subsample(s) from the same sampling operation attached to the submitted sample is required.

1.5.2 Certificates

The results of tests may be reported on one or more ISTA Certificates, separately or combined.

Test results must be reported in accordance with the rules for calculating, expressing and reporting results in the appropriate chapter of the ISTA Rules. If there is a space on the certificate for a certain test which is not made, or where information is not provided by the ap-

plicant: 'N' for 'not tested' or 'NA' for 'not applicable', must be placed in the space.

'N' must not be used for components of a test such as hard or fresh seeds. For example, even if hard seeds do not usually occur in the species they must be looked for and if not found, then reported as '0'.

1.5.2.1 Sampling: heterogeneity testing for seed lots in multiple containers

1.5.2.1.1 The H value heterogeneity test

The result of the H value heterogeneity test for seed lots in multiple containers must be reported under 'Other determinations', as follows:

- \bar{X} : mean of all X values determined for the lot in respect of the adopted attribute;
- N: number of independent container samples;
- No: number of containers in the lot;
- the calculated H value;
- the statement: 'This H value does/does not indicate significant heterogeneity.'

Note: the H value must not be calculated or reported if \bar{X} is outside the following limits:

- purity components: above 99.8 % or below 0.2 %;
- germination: above 99.0 % or below 1.0 %;
- number of specified seeds: below two per sample.

1.5.2.1.2 The R value heterogeneity test

The result of the R value heterogeneity test for seed lots in multiple containers must be reported under 'Other determinations', as follows:

- \bar{X} : mean of all X values determined for the lot in respect of the adopted attribute;
- N: number of independent container samples;
- No: number of containers in the lot;
- the calculated R value;
- the statement: 'This R value does/does not indicate significant heterogeneity.'

1.5.2.2 Purity

The results of a purity test must be reported in the spaces provided as follows:

- The scientific name of the species of pure seed, in accordance with Table 2C (e.g. *Triticum aestivum* subsp. *aestivum*). Where it is impossible to determine the species with certainty on the basis of seed characteristics, reporting must be done to the most precise taxon possible.
- The percentage by weight of pure seed, inert matter and other seeds, given to one decimal place. The percentage of all components must total 100 %. Components amounting to less than 0.05 % must be reported as 'Trace' or 'TR' (for 'Trace'). If no inert matter or other seeds are found, this must be reported as '0.0'.
- The kind of inert matter.
- The scientific name of every species of other seeds found, in accordance, where applicable, with the current *ISTA List of Stabilised Plant Names*, available at www.seedtest.org/stablist (e.g. *Elymus repens*).
- When the weight of the working sample tested for purity equals or is no more than 10 % higher than the weight specified in Table 2C, column 4 (Purity analysis), no statement regarding the weight of the working sample is required on the ISTA Certificate.
- When the weight of the working sample tested for purity deviates from that specified in Table 2C, column 4, the actual weight of the working sample weighed according to 3.5.1 must be reported on the ISTA Certificate using one of the following, as applicable:
 - a. When testing a weight that exceeds by 10 % the weight specified in Table 2C, column 4, report under 'Other determinations' as:
'Purity: ... g'
 - b. When testing a weight estimated to contain 2500 seed units, report under 'Other determinations' as:
'Purity: ...g (approx. 2500 seeds)'
 - c. When the submitted sample received for purity testing weighs less than the weight in Table 2C, column 4, report under 'Other determinations' and use the statement:
'The submitted sample weighed only ... g and is not in accordance with the *International Rules for Seed Testing*.'
- The percentage of winged seed (as defined in Pure Seed Definitions 47 and 51), if winged seeds are found.

Upon request, the following information can be reported to one decimal place in the spaces provided for the 'Kind of inert matter' and 'Other seeds' results:

- The percentage by weight of a specified species, entered immediately after the name of the species to the nearest 0.1 %. Species for which the percentage by weight has been requested are listed first.
- The percentage by weight of any kind of inert matter.

Upon request, the percentages can be reported to more than one decimal place but only under 'Other determinations'.

Upon request, the following information must be reported under 'Other determinations' as follows:

- Other seeds may be divided into 'other crop seeds' and 'weed seeds'. In this case, the words 'Other crop seeds' must be entered, followed by the percentage by weight of other crop seeds and the name(s) of the species found. This procedure must also be used for 'Weed seeds'.
- Multiple seed units must be reported as '% MSU'.
- Seeds with appendages attached must be reported as '% seeds with appendages attached'.
- The percentage by weight of broken pure seed.

The percentages may be reported to more than one decimal place if requested.

1.5.2.3 Purity tests on coated seeds

The result of a purity test on coated seeds must be reported as follows:

- Following the species name, the words 'seed pellets', 'encrusted seeds', 'seed granules', 'seed tapes' or 'seed mats', as applicable, must be clearly entered.
- The results must be reported to one decimal place, and the percentage of all components must total 100 %. Components amounting to less than 0.05 % must be reported as 'Trace' or 'TR' (for 'Trace'). If no inert matter or other seeds are found, this must be reported as '0.0'.
- In the case of pelleted seeds only, the percentages of pure pelleted seeds, inert matter and unpelleted seeds must be reported in the spaces provided for 'Pure seeds', 'Inert matter', and 'Other seeds', respectively.
- The name and number of the seeds of each species found in the examination of the 100 seeds removed from the pellets or tapes must be reported under 'Other determinations'.

Upon request, the following information may be reported under ‘Other determinations’ as follows:

- Purity test on depelleted seeds. The component parts (pure seed, other seeds and inert matter) may be reported as percentages of their total weight, ignoring the pelleting material. The percentage of pelleting material must be reported separately only on request. The result of this test is to be reported: ‘weight of ... material excluded’.
- Purity of seeds removed from tapes. The component parts (pure seed, other seeds, and inert matter) may be reported as percentages of their total weight, ignoring the tape material. The result of this test is to be reported: ‘weight of ... material excluded’.

1.5.2.4 Determination of other seeds by number

The result of a determination of other seeds by number must be reported under ‘Other determinations’ as follows:

- The actual weight of seed examined to the minimum number of decimal places indicated in 4.7.
- The scientific name and number of seeds of each species sought and found in this weight. If no other seeds are found, this must be indicated on the certificate.
- Where it is impossible to determine with certainty on the basis of seed characteristics, reporting must be done to the most precise taxon possible.
- If the full weight prescribed in Table 2C was examined for all other species present, then the words ‘Complete test’ must be entered, alongside the weight of seed examined.
- If the examination was for only a limited range of other species, then the words ‘Limited test’ must be entered.
- If the weight examined for all other species was less than the prescribed weight, then the words ‘Reduced test’ must be entered.
- If the weight examined was less than the weight prescribed in Table 2C, and only a limited range of other species was examined, then the words ‘Reduced-limited test’ must be entered.
- If a sample of at least 25 000 seeds was examined, and this sample was below the weight prescribed in Table 2C, then the weight of seed examined and the statement ‘Test based on at least 25 000 seeds’ must be entered.

Upon request, the results may in addition be expressed in some other way, such as ‘weight of seeds found’ or ‘number of seeds per kilogram’.

Upon request, the presence of one or more of the following genera: *Aeginetia*, *Alectra*, *Orobanche*, *Phelipanche* and *Striga*, can be reported only on a Blue International Seed Sample Certificate (see 1.2.2) and must be reported as: ‘Test for presence of ... species – ... seeds of ... species were found in ... g of seed examined.’

If no seeds were found it can be reported as: ‘No seeds of ... species were found in ... g of seed examined.’

The sample weight examined must be reported according to the minimum number of decimals indicated in 4.7.

1.5.2.5 Determination of other seeds by number on coated seeds

The result of a determination of other seeds by number on coated seeds must be reported as follows:

- Following the species name, the words ‘seed pellets’, ‘encrusted seeds’, ‘seed granules’, ‘seed tapes’ or ‘seed mats’, as applicable, must be clearly entered.
- Under ‘Other determinations’, the actual weight (or length of tape, or area of mat) and approximate number of pelleted seeds examined must be entered, together with the scientific name and number of seeds of each species sought and found in this weight, length or area.

Upon request, the result may in addition be expressed in some other way, such as number of seeds per kilogram, per metre or per square metre.

1.5.2.6 Germination

The result of a germination test must be reported in the spaces provided as follows:

- The actual duration of the test (in days, excluding the period of special treatment or method used for promoting germination).
- The percentages, calculated to the nearest whole number (5.8.2), of normal seedlings, hard seeds, fresh seeds, abnormal seedlings and dead seeds. If the result for any of these categories is found to be zero, it must be reported as ‘0’.
- If an applicant requests that the test be terminated when the sample reaches a predetermined germination percentage, before the final count, then only the percentage of normal seedlings is reported. The results of the other categories (abnormal seedlings, hard seeds, fresh seeds and dead seeds) must be reported as ‘N’.



The following additional information must be reported under ‘Other determinations’:

- the number of seeds tested, if fewer than 400 seeds;
- the germination method using the abbreviations used in Table 5A, including at least substrate and temperature;
- any special treatment or method used for promoting germination (5.6.3);
- the duration in days of any special treatment or method used for promoting germination, except in the case of prestorage;
- the method for evaluating fresh seeds (dissection, tetrazolium or excised embryo – see paragraph 5.6.5.3) when 5 % or more of fresh seeds are believed to be present;
- If an applicant requests that the germination test be terminated when the sample reaches a predetermined germination percentage, the following statement: ‘Upon request of the applicant, the germination test was terminated after ... days. The prescribed test period is ... days.’

When double tests are prescribed in Table 5A Part 2, the result of the first test, with treatment for breaking dormancy, is reported in the appropriate space on the ISTA Certificate, and the result of the second test, without treatment for breaking dormancy, is reported under ‘Other determinations’.

Upon request, the following information may be reported as follows:

- the germination percentage obtained within the prescribed time, if the germination period was extended beyond the period indicated in Table 5A. The statement must be entered as follows: ‘After the prescribed period of ... days, there were ... % normal seedlings’;
- the result of parallel tests or any additional test;
- the results of other tests made when retesting is necessary;
- the viability of ungerminated seeds and the method used to determine it;
- the categories of ungerminated seeds (as listed in 5.6.5.3) and the method used to determine them;
- in the case of multigerm seed units: the number of normal seedlings produced by 100 units, the number of units which have produced one, two or more than two normal seedlings, or the proportion of units producing one, two or more than two normal seedlings.

The proportion is expressed as a percentage of the total number of units which have produced at least one normal seedling.

1.5.2.7 Germination of coated seeds

The result of a germination test on coated seeds must be reported as follows:

- Following the species name, the words ‘seed pellets’, ‘encrusted seeds’, ‘seed granules’, ‘seed tapes’ or ‘seed mats’, as applicable, must be clearly entered in the space provided.
- The percentage of pellets or seed in tapes with normal seedlings, with abnormal seedlings and without seedlings.
- The duration of the test.

The following additional information must also be reported under ‘Other determinations’:

- The method used for the germination test.
- For seed tapes or mats: the number of normal seedlings per metre of tape or square metre of mat.

Seedlings that are obviously not of the species stated by the applicant, even if otherwise normal, must not be included in the germination result, but their number must be reported separately.

1.5.2.8 Tetrazolium test

The result of a tetrazolium test must be reported under ‘Other determinations’ as follows:

- The statement ‘Tetrazolium test: ...% of seeds were viable’ must be entered.
- In cases where the testing procedure deviates from that prescribed in Table 6A, any deviating procedure must also be reported.

The only variations permitted from procedures given in Table 6A are for premoistening time, tetrazolium concentration, staining temperature or staining time. Precise prescriptions about the limitation of the variations are given in 6.5.

- If individual seeds are tested at the end of the germination test, the result must be reported in accordance with 1.5.2.6 and 5.9.

In addition, in the case of species of Fabaceae, one of the following, and only one, must be reported:

- either** (in cases where the viability percentage of the hard seed is not determined) ‘Tetrazolium test: ...% of seeds were viable, ...% of hard seeds found in the test.’
or (in cases where the viability percentage of the hard seed is determined) ‘Tetrazolium test: ...% of seeds were viable, ...% of hard seeds included in the percentage of viable seed’

At the discretion of the seed testing laboratory, further information may be reported, e.g. percentage of seeds that were empty, with larvae, broken or decayed.

1.5.2.9 Tetrazolium test on coated seeds

The result of a tetrazolium test on coated seeds must be reported as follows:

- Following the species name, the words ‘seed pellets’, ‘encrusted seeds’, ‘seed granules’, ‘seed tapes’ or ‘seed mats’, as applicable, must be clearly entered.

The following additional information must be reported under ‘Other determinations’:

- The statement ‘Number of seeds (of the species stated by the applicant) included in 100 seed pellets’ (or ‘encrusted seeds’, or ‘seed granules’);
- or the statement ‘Number of seeds (of the species stated by the applicant) included in one metre of seed tape’;
- or the statement ‘Number of seeds (of the species stated by the applicant) included in one seed mat or in one square metre of seed mat’.
- The statement ‘Tetrazolium test: ...% were viable’ must be entered.
- In cases where the testing procedure deviates from that prescribed in Table 6A, any deviating procedure must also be reported. The only areas where variations from procedures given in Table 6A are permitted are for premoistening time, tetrazolium concentration, staining temperature and staining time. For precise guidance about the limitation of the variations permitted, see 6.5.
- If individual seeds are tested at the end of the germination test, the result must be reported in accordance with 5.9.

In addition, in the case of species of Fabaceae, one of the following, and only one, must be reported:

- either** (in cases where the percentage of the viability of hard seed is not determined) ‘Tetrazolium test: ...% of seeds were viable, ...% of hard seeds found in the test’
or (in cases where the percentage of the viability of hard seed is determined) ‘Tetrazolium test: ...% of seeds were viable, ...% of hard seeds included in the percentage of viable seed’

1.5.2.10 Seed health test

The results of a test for seed health must be reported under ‘Other determinations’ as follows:

- either qualitative or quantitative results, as specified in the individual methods;
- negative and positive results, as specified in the individual methods;
- the scientific name of the pathogen detected;
- the percentage of infected seeds;
- the method used, including any pretreatment (7.2.2);
- the size of the sample or fraction examined;
- any additional permitted procedure used.

The absence of a statement concerning the health condition of the seed does not necessarily imply that the health condition is satisfactory.

1.5.2.11 Species and variety testing

The results of species and variety testing must be reported under ‘Other determinations’, and in addition the following information must be given:

- a. the request of the applicant;
- b. the trait(s) and the method(s) used;
- c. 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);
- d. whether an authentic standard sample or a standard reference was used; if a standard reference was used, its origin must be indicated;
- e. 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.

1.5.2.11.1 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 to indicate that the species (and/or variety) 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 variety) 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 variety) stated by the applicant.'
- if the total working sample was found to be of a species and/or variety other than that stated by the applicant: 'The sample does not conform to the authentic standard sample of the species (and/or variety) stated by the applicant.'

1.5.2.11.2 Results of a field plot examination

The result of a field plot examination must, whenever possible, be reported as a percentage of each other species, other variety 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 to indicate that the species (and/or variety) stated by the sender is (are) incorrect.'

1.5.2.12 Moisture content

This Rule is applicable to both the oven method (9.2.7) and the moisture meter method (9.3.2.7).

The result of a moisture content test must be reported in the space provided to the nearest 0.1 %.

The following additional information must also be reported under 'Other determinations':

- For the oven method (9.2.7), the method (i.e. duration and temperature) must be reported.
- For the moisture meter method (9.3.2.7), the statement: 'A moisture meter was used' must be entered.
- If germinating seeds were present in the sample, the following statement must be entered: 'Germinating seeds were found in the submitted moisture sample.'

- If mouldy seeds were present in the sample, the following statement must be entered: 'Mouldy seeds were found in the submitted moisture sample.'
- In the case of pelleted seeds (see Chapter 11), the following statement must be entered: 'The seeds of the submitted moisture sample were pelleted, and the moisture content reported is the average of seed and pelleting materials.'
- For *Arachis hypogaea*, one of the following statements must be entered: 'The submitted sample for moisture determination consisted of seeds in their pod' or 'The submitted sample for moisture determination consisted of seeds with the pod removed (shelled seeds)'.

1.5.2.13 Thousand-seed weight

The result of the thousand-seed weight (TSW) test must be reported under 'Other determinations' with the number of decimal places given in Table 10A, column 4.

The method used ('Counting the whole pure seed fraction' or 'Counting replicates') must also be reported under 'Other determinations', e.g. 'Thousand-seed weight (counting replicates): 20.27 g'.

1.5.2.14 Excised embryo

The result of an excised embryo test must be reported under 'Other determinations' as follows: 'Excised embryo test:% of seeds had viable embryos'

Further details may be given at the discretion of the seed testing laboratory, e.g. percentages of seeds that were empty, insect-damaged or physically damaged.

1.5.2.15 Weighed replicates

The result of a weighed replicates test must be reported in the space provided as follows:

- The result of the purity test (if requested), in the spaces provided for purity tests.
- 'N' must be entered in all the spaces provided for reporting the percentages of the components of the germination tests.

The following additional information must also be reported under 'Other determinations':

- average weight of four replicates;
- average number of normal seedlings in four replicates;
- number of normal seedlings per kilogram;
- other information as specified in 1.5.2.6 and 5.9.

Upon request, other seeds found to be present in the weighed replicates may be reported, giving the scientific name(s) and number(s) of seeds found.

1.5.2.16 X-ray test

The results of an X-ray test must be reported under 'Other determinations' as percentages of filled, empty, insect-damaged or physically damaged seeds, as follows:

X-ray test results

-% filled
-% empty
-% insect-damaged
-% physically damaged

1.5.2.17 Seed vigour test

1.5.2.17.1 Conductivity test

The result of a seed vigour test using the conductivity test method must be reported under 'Other determinations' as follows:

- The result must be expressed in $\mu\text{S cm}^{-1} \text{g}^{-1}$ to the nearest $0.1 \mu\text{S cm}^{-1} \text{g}^{-1}$.
- The seed moisture content before the test must be reported. Where the moisture content has been adjusted before the test, both the initial moisture content and the calculated moisture content after adjustment must be reported.
- The results must be accompanied by a statement of the specific variables used in the test (soaking time and temperature)

1.5.2.17.2 Accelerated ageing test

The result of a seed vigour test using the accelerated ageing (AA) method must be reported under 'Other determinations' as follows:

- Results are expressed as a percentage, calculated to the nearest whole number (see 5.8.2) of normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds. If the result for any of these categories is found to be zero, it must be reported as '0'.
- The seed moisture content before the test must be reported. Where the moisture content has been adjusted before the test, both the initial moisture content and the calculated moisture content after adjustment must be reported.

- The results must be accompanied by a statement of the specific variables used in the test (seed weight per AA box both before and after ageing, ageing time and temperature).

1.5.2.17.3 Controlled deterioration test

The result of a seed vigour test using the controlled deterioration test method must be reported under 'Other determinations' as follows for the two alternative methods of assessing deterioration in 15.8.3.4.3:

a. CD germination test

- Results are expressed as a percentage, calculated to the nearest whole number (5.8.2), and stated as 'Total germinated seeds (normal plus abnormal seedlings) ... %' and 'Normal seedlings ... %'. If the result for either of these is found to be zero, it must be reported as '0'.
- The results must be accompanied by a statement of the specific variables used in the test (method used to raise seed moisture content, raised seed moisture content, deterioration period and temperature).

b. Conductivity test after deterioration

- The result must be expressed in $\mu\text{S cm}^{-1} \text{g}^{-1}$ to the nearest $0.1 \mu\text{S cm}^{-1} \text{g}^{-1}$.
- The results must be accompanied by a statement of the specific variables used during deterioration (method used to raise seed moisture content, raised seed moisture content, deterioration period and temperature) and in the conductivity test (soaking time and temperature).

1.5.2.17.4 Radicle emergence test

The result of a seed vigour test using the radicle emergence test must be reported under 'Other determinations' as follows:

- Results are reported as a percentage of seeds with emerged radicles calculated to the nearest whole number (5.8.2). If the result is found to be nil, it must be entered as '0'.
- The results must be accompanied by a statement of the temperature used for the test and the time of the radicle emergence counts in hours, e.g. 'Radicle emergence test 90 % with emerged radicles after 66 h at 20 °C.'

1.5.2.17.5 Tetrazolium vigour test

The result of a seed vigour test using the TZ method must be reported under ‘Other determinations’. Results are expressed as a percentage, calculated to the nearest whole number of vigorous seeds, e.g. ‘Tetrazolium vigour tests using 0.1 % TZ solution for 3 h at 35 °C: 90 % vigorous seeds’.

1.5.2.18 Size and grading of seeds

The result of a screening analysis test for size and grading of seeds must be reported under ‘Other determinations’ as the average of two screening analyses falling within the permitted tolerance limits.

1.5.2.19 Weighted average test for seed lots transported loose in bulk containers

The result of a weighted average test performed on seed lots, as described in Chapter 17, must be reported in the normal way, except that:

- a. Across the date of sampling, date sample received, date test concluded and test number boxes insert the statement: ‘Seed loose in bulk container(s) – see under Other determinations.’
- b. Under ‘Other determinations’, list the test number, date of sampling and date test concluded of all constituent lots together with the statement: ‘The test results reported represent the weighted average of the results reported on these certificates which were not significantly different from each other.’

1.5.2.20 Seed mixtures

The results of tests on seed mixtures can only be reported on a Blue International Seed Sample Certificate (see 1.2.2).

For the species tested, ‘Seed mixture’ must be entered. The composition of the mixture, determined during testing, is listed under ‘Other determinations’.

1.5.2.20.1 Purity and component analysis

The results of the purity analysis are reported according to Chapter 3.

The actual weight of sample examined to the minimum number of decimal places indicated in 4.7 must be reported under ‘Other determinations’, i.e. ‘Purity and composition analysis: ... g of seed examined.’

The mixture composition is reported under ‘Other determinations’ in one of the following formats, as requested by the applicant:

1. The percentage by weight of pure seed (if applicable, including declared inert material); inert matter and other seeds must be given to one decimal place and entered in the spaces for purity. The pure seed percentage is calculated using the total weight of the pure seed of all mixture components.
2. The percentage by weight of the pure seeds of the mixture components using the total weight of the pure seed fraction. In addition, if applicable, the percentage by weight of the ‘inert material according to declaration’ referred to the sum of the weights of all mixture components (pure seeds and inert material according to declaration) must be given to one decimal place under ‘Other determinations’.
3. The percentage by weight of mixture components, pure seeds or inert material according to declaration using the sum of the weights of the pure seed fraction and the declared inert material.
4. The percentage by number of the pure seeds of the mixture components using the total number of seeds of the pure seed fraction.

In addition, if applicable, the percentage by weight of the ‘inert material according to declaration’ using the sum of the weights of all mixture components must be given to one decimal place under ‘Other determinations’.

1.5.2.20.2 Determination of other seeds by number

The results of a determination of other seeds by number on a seed mixture must be reported according to 4.7.

1.5.2.20.3 Germination, seed viability, seed vigour and other tests using replicates of 100 seeds

Test results are reported only for those species for which methods are given in the appropriate Chapter of the ISTA Rules. The results of these tests must be reported under 'Other determinations'.

Germination test results are not reported in the 'Germination' section of the certificate (an 'N' must be entered there), but under 'Other determinations'. When 100 or more seeds are tested, the percentage results of the test for each mixture component tested are reported to the nearest whole number. The number of seeds tested is also reported. Tolerances as described in the appropriate Chapters are applied to tests of 400, 300, 200 and 100 seeds.

When fewer than 100 seeds are tested, the actual number of seeds in each category (e.g. normal seedlings or viable seeds) is reported, together with the total number of seeds tested.

The method used in the test must be reported on the certificate according to the appropriate Chapter for each component species tested.

1.5.2.20.4 Thousand-seed weight

The results as calculated according to 18.7 must be reported under 'Other determinations'.

1.5.2.21 Genetically modified organisms

The result of a genetically modified organism test must be reported under 'Other determinations' as follows:

- the request of the applicant;
- the name and scope (with reference to the target) of the method(s) used;
- a description of the working sample (e.g. pure seed fraction, inert matter present, other seeds present, washed seed);
- the number of seeds in the working sample;
- a description and the source of the reference material used (e.g. certified reference material, provider);
- the limit of detection of the method (when testing seed groups or seed bulk) according to the value verified by the laboratory;
- the limit of quantification of the method (when testing seed bulk with a quantitative method) according to the value verified by the laboratory.

1.5.2.21.1 Qualitative test results

Suggested phrases for reporting the detection of test targets depending upon the result are as follows:

- If the test target(s) was(were) not detected: 'The test target was not detected.'
- If the test target(s) was (were) detected: 'The test target was detected.'

1.5.2.21.2 Quantitative results obtained by multiple qualitative tests of individuals or groups of seeds or seedlings

Results should be reported relative to the percentage of seeds or seedlings showing the test target specified by the applicant. The total number of seeds tested, the number of groups, and the number of seeds per group must be reported. Suggested phrases for reporting such results depending upon the result are as follows:

- If the test target(s) was (were) not detected: 'The test target(s) was (were) not detected.'
- If the test target(s) was (were) detected: 'The % of seeds in the lot with the test target(s) was determined to be ...%, with a 95 % confidence interval of [...%, ...%].'

or

'For the test target(s) specified by the applicant, the seed lot meets the specification of ...% (maximum or minimum) with ...% confidence.'

If the results do not show evidence that the seed lot meets a given specification at the desired confidence, then the estimated percentage of seed with the 95 % confidence interval will be reported.

1.5.2.21.3 Quantitative measurements of GMO in bulk samples

Results should be reported relative to the percentage of the test target specified by the applicant by mass or number of DNA copies. 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) must be indicated.



Required phrases for reporting depending upon the results are as follows:

- a. If the test target was not detected (no signal or below the limit of detection): ‘The test target was not detected at a level above the limit of detection.’
- b. If the test target was detected at a level above the limit of detection and below the limit of quantification: ‘The test target was detected at a level below the limit of quantification of the method used.’
- c. If seeds showing the test target were found at a level above the limit of quantification: ‘The test target(s) percentage in the seed lot was determined to be ...% by mass or number of copies, with a 95 % confidence interval of [...%, ...%]’
or
‘For the test target(s) specified by the applicant, the seed lot meets the specification of ...% (maximum or minimum) by mass or number of copies with ...% confidence.’

If the results do not show evidence that the seed lot meets a given specification at the desired confidence, then the estimated percentage by mass or number of copies with the 95 % confidence interval will be reported.

1.5.2.22 Reporting of results of tests not covered by the Rules

Results must be reported under ‘Other determinations’. The test method must be reported and followed by: ‘(This method is not covered by the *International Rules for Seed Testing*).’

1.5.3 Reporting of uncertainty of measurement on ISTA Certificates

Uncertainties of measurements associated with test results are accessible through the tolerance tables in the ISTA Rules and are not reported on the ISTA Certificates.

1.5.4 Statement referring to compliance with legislative requirements

In addition to results of tests carried out, it is permissible, at the issuing laboratory’s own risk, to make a statement that the seed lot tested meets particular legislative standards. ISTA takes no responsibility for such statements.

1.6 Validity of ISTA Certificates

The results on an original ISTA Certificate are valid until superseded, or partly superseded, by new results on another valid original ISTA Certificate, issued, for the same particular test(s).

If an original certificate is re-issued because of new or amended test results, it must carry a statement indicating that the new results replace previous results, and referring to the Reg. No. of the superseded certificate. In this case, the date entered on the certificate is the new date of issuing.

If an original, duplicate or provisional certificate is lost, a replacement certificate can be issued. In this case, the date entered on the certificate is the same as on the lost certificate.

A new original Orange International Seed Lot Certificate may be issued to supersede a previous certificate for the same seed lot or subplot under the same reference (i.e. seed lot seal and identification) and the same particular test(s), provided that a new submitted sample from that lot or subplot is taken and tested. The new certificate is only valid for the particular lot or subplot that was re-sampled. If a subplot is re-sampled it becomes a new seed lot and must be given a new seed lot identification mark.

A new original Blue International Seed Sample Certificate may be issued to supersede a previous certificate for the same sample and the same particular test(s), provided that the same sample is re-tested. If a new sample is submitted and tested, it must be regarded as independent from the previous sample, and the results on the previous certificate are not superseded.

Previously issued certificates do not need to be returned to the issuing laboratory.

The reference dates are (in order of priority) the date of sampling, the date the test was concluded, and the date of issuing the certificate.

1.7 Disputed results

If the results reported on an ISTA Certificate are contradicted by subsequent test results at another accredited laboratory and the problem cannot readily be resolved, the laboratory issuing the certificate should contact the ISTA Secretariat to determine the correct course of action.

