ISTA Seed Quality Assurance

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ISTA Standard Proficiency Test

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CREATED	REVIEWED	APPROVED
28.11.2024	05.12.2024	05.12.2024
PT Coordinator	PTC Committee Head of Accreditation and Technical Department	PTC Chair and Vice-Chair Secretary General

SCOPE

The scope of this document is to define the ISTA Standard Proficiency Test (PT) Programme and to explain the single steps and framework in which it is operated. This procedure is based on principles outlined in ISO/IEC 17043.

The scope is limited to PT rounds for purity analysis, other seed determination, germination testing, moisture content determination, viability (tetrazolium test), vigour and thousand-seed weight test. Seed health proficiency testing, proficiency tests for specific traits (GMO PT) and variety proficiency testing are not described in this document.

RELATED DOCUMENTS

ISTA Accreditation Standard for Seed Testing and Seed Sampling

Acc-D-01-Procedures for Termination, Suspension and Withdrawal of ISTA Accreditation

PT-P-02-Standard Proficiency Test Sample Preparation (internal document, confidential)

Admin-F-27 Complaints and appeals

Acc-D-07-ISTA Accreditation and Scope of Accreditation Policy

ISTA PT Programme Plan

ISTA Rules

ISTA Crop Groups

ISTA List of Stabilised Plant Names

RESPONSIBILITY

PTC: for setting the Standard PT Programme Plan

Statistics Committee: for statistical design elaboration and validation of the PT calculation programme

PT Leader and PTC: for technical preparation of each PT round

PTC Chair or Vice-Chair for validation of selected seed lots, statistical analysis for heterogeneity test, validation of statistical analysis before sending results to participants, validation of the summary PT report

Standard PT Coordinator: for distribution of samples, running of statistical analysis, verifying the results and ratings, sending the summary report to the PTC Chair and sending results to participants

Accreditation and Technical Department: for monitoring of accredited laboratories' performance (including evaluation of follow up actions during the on-site assessment of the laboratories)

Participating Laboratories: for providing seed import permit (if applicable) in due time; for reporting duly checked test results in due time (as it is not possible to modify results after the deadline); for keeping results confidential before the end of each PT; for storing remaining seed samples safely; and for applying follow up corrective actions (as necessary)

ABBREVIATIONS

AWG: Accreditation Working Group

A: excellent performance rating

B: good performance rating

C: minimum performance rating

BMP: below minimum performance rating

PT: Proficiency Test

PTC: Proficiency Test Committee

GMO: genetically modified organisms

PUR, OSD, GER, TZ, VIG, MOI, TSW: purity, other seed determination, germination, tetrazolium, vigour, moisture tests, thousand-seed weight

OIC: ISTA Orange International Certificate

SG: Secretary General

TCOM: Technical Committee

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PROCESS DESCRIPTION

PT rounds are prepared by PT leaders. The PT leaders:

- are defined in the 3-year PT Programme Plan
- are members of the PTC
- belong or have access to a laboratory for technical preparation; this laboratory must be an ISTA accredited laboratory for the PT species, for sampling in the laboratory and for the major expected tests
- are approved by the PTC Chair in consultation with the Accreditation and Technical Department; and
- apply the current version of PT-P-02-Proficiency Test Sample Preparation (confidential document).

SCHEDULE

Regular PT rounds are performed three times per year. Each round is made up of three samples for each test (or group of tests) and for each species. The samples undergo purity analysis, other seed determination, germination testing, moisture content determination, viability testing, vigour testing and/or thousand-seed weight testing, as applicable. PTs for tests other than those mentioned may be organised by the TCOM, but are, for the time being, not yet part of the rating.

PROGRAMME PLAN

The PTC selects the particular species to be used for the Programme over a 3-year period between two ISTA Congresses. The species to be used are chosen to represent the germination type group. They are species listed in the ISTA Rules Table 2C. All species of Table 2C may be part of a laboratory's scope of accreditation. Exception can be made for species requiring blowers for purity analysis. If a laboratory is accredited for one or several of the species mentioned in a crop group, participation in a test round which tests another species of the same crop group is mandatory.

A species for each crop group should be chosen with a frequency of approximately 1.5 times in 3 years for groups 1 to 6 and once in 3 years for groups 7 and 8, with a regular order of species groups as far as possible. The Programme Plan should include at least:

- PUR, OSD, GER – two tests per year
- VIG and TSW – two tests in 3 years
- MOI and TZ – one test per year; and
- OIC completion – one report per year.

PT members volunteer as PT leaders, according to their accreditation scope, expertise and ability to prepare the samples in their accredited laboratories.

At the beginning of each PT period/3 years cycle, generally after an ISTA Congress, the laboratory will receive the Programme Plan.

New member laboratories will receive the Programme Plan together with their membership confirmation.

Non-accredited laboratories that wish to participate as volunteers will be requested to indicate which test round they would like to join by returning the completed form to the ISTA Secretariat.

Accredited laboratories whose scope does not oblige them to participate in every test round may also indicate the rounds they would like to join as volunteers. Results of voluntary test rounds are not included in the overall mean and standard deviation calculation, and they are not included in the laboratory's overall rating.

Completion of the OIC specimen is a voluntary training activity only.

Test rounds are identified by a unique numbering system that is as follows: last two numbers of the year in which the test is performed, a number indicating the round of that year plus an abbreviation of the species tested. Hence, PT24-3 L.sat means that it is the third round of the year 2024 and the species tested is *Lactuca sativa*.

PARTICIPANTS

The list of PT round participants is generated from the database available in the ISTA Secretariat. This list consists of laboratories obliged to participate due to their specific scope of accreditation and laboratories having indicated their interest in voluntary participation. The mandatory status of every laboratory is based on their approved scope of accreditation. The scope of accreditation is defined as described in document Acc-D-07-ISTA Accreditation and Scope of Accreditation Policy.

Receiving the Programme Plan at the beginning of the PT period, each participating laboratory must carefully check requirements for seed import from Switzerland. If an import permit or phytosanitary certificate are required the laboratory must immediately inform the ISTA Secretariat, to allow sample dispatch. It is the laboratory's responsibility to ensure safe receipt of the samples according to phytosanitary requirements.

SAMPLE PREPARATION AND DISPATCH TO THE ISTA SECRETARIAT

The PT leader will select the seed lots. Preliminary analysis is done by the PT leader and the PTC Chair.

The ISTA Secretariat informs the PT leader of the number of participants to determine the number of samples needed. The PT leader prepares the samples according to the PT-P-02–Standard Proficiency Test Sample Preparation document, provided by the ISTA Secretariat. The PT leader prepares samples from each of the three separate seed lots of the selected species.

The seed lots are of commercial value, meaning that the purity (before spiking with other seeds) and germination levels meet normal international marketing requirements. The PT leader ensures that the seed is free of living insects and mites. For other seed determination, other seed species are added to the samples to evaluate retrieval and identification of those species. The added other seeds are heat treated in order to render them non-viable.

Twenty samples are selected randomly: ten samples are subjected to a heterogeneity test to ensure the lots chosen are suitable for a PT and another ten are stored under suitable conditions (according to the ISTA Rules on storage after testing) in the PT leader's laboratory for quality assurance. The remaining samples are sent to the ISTA Secretariat for distribution to participants. Samples are randomly assigned to the participating laboratories and an allocated random number is used to maintain the anonymity of the participants.

Note: The purity samples are prepared according to the ISTA Rules, on the basis of a weight containing 2'500 seeds. Thus, the sample weight may be different to the standard weight prescribed in the ISTA Rules Table 2C. The moisture sample weight may be less than required by the ISTA Rules, but the sample will contain enough material that the test can be repeated.

SAMPLE DISPATCH

Samples are dispatched from the ISTA Secretariat at the beginning of April, August and December each year. Where needed, the import permit and phytosanitary certificate are enclosed. Laboratories are notified by email upon sample dispatch.

Laboratories not having received the samples within 3 weeks of the dispatch date are required to contact the ISTA Secretariat.

Each sample package is accompanied by an instruction letter. The deadline for reporting of results is 90 calendar days after the PT round package has been dispatched from the ISTA Secretariat.

Laboratories having persistent problems with delivery of the sample packages will be required to take immediate action to improve the situation.

TEST COMPLETION

Participants are expected to initiate the tests required for a round as soon as possible after the samples have been received. Before testing, the samples must be stored under suitable conditions (according to the ISTA Rules for sample storage before testing). If the laboratory receives samples that are not acceptable (e.g. opened/damaged seed packets, leakage of seeds, etc.), the ISTA Secretariat must be informed and asked for replacement samples.

Except in cases of specific instruction from the PTC, the laboratory must test the samples using the same ISTA method it usually uses when testing seed for ISTA Certificate purposes, and to report the method on the results forms. Samples contain enough seed material to allow the participant to repeat the test if needed. The participants are responsible for the correctness of all results/information reported. Results cannot be corrected after the reporting deadline of the test round.

For some test rounds the laboratory will be asked to also report the results on an ISTA Certificate, specimen which will be provided by the ISTA Secretariat.

Unless special instructions from the phytosanitary authorities have been given, the seed remaining after the test(s) must be carefully stored for possible follow up action. Remaining seed can also be used for laboratory staff training or as a reference material after the PT round is closed. Remaining seed must not be used other than for laboratory purposes.

REPORTING OF RESULTS

Data report forms will be provided to participants by email. The participants must complete the forms reporting the raw data, the means of the test results and the testing method.

If an other seed determination test is involved, the plant names must be reported in alphabetic order and in accordance with the current version of the ISTA Rules Table 2C or the ISTA List of Stabilised Plant Names. Where it is not possible to determine the species with certainty on the basis of seed characteristics, reporting must be done to the most precise taxon possible. **Note: Synonyms will not be accepted as correct.**

Results are submitted to the ISTA Secretariat only by email and exclusively on the forms provided by the ISTA Secretariat. Results must be submitted by the reporting deadline; otherwise, they will not be included in the calculation.

DATA ENTRY

The ISTA Secretariat verifies whether the results are reported correctly. Submitting the results in the appropriate format of the reporting forms is crucial for the subsequent data calculation.

The ISTA Secretariat verifies whether the proper reporting procedures have been followed. The reported data are imported in the calculation database.

STATISTICAL EVALUATION

The purity, germination, moisture, viability, vigour and thousand-seed weight results are statistically analysed. Statistical analysis is developed from the method of Tattersfield (1979 – *Seed Science and Technology* **7**(2), 247–257). The principle of the method is to calculate Z-scores (Z_i) based on reference mean and standard deviation values, estimated after removing apparent outliers from the observations in the set of laboratories with accreditation.

Exception: For the vigour test there is no exclusion of outliers due to the very small number of accredited laboratories.

The ranking evaluation was added in the PT in 2002, following the test round PT02-3 on *Pisum sativum* (see activity report 2003/2004 of the ISTA Committees, page 62: https://www.seedtest.org/en/services-header/documents/proficiency-test-documents.html).

Initial analysis is carried out to identify the outliers within accredited laboratories. Results of the outliers differ considerably and may bias the estimate of the ‘true’ mean.

A second analysis is then made excluding the outliers, to find the overall mean (x) and standard deviation (s) of accredited laboratories.

The laboratory’s mean (x_i) is correlated with this overall mean, with the estimated ‘true value’ of the sample, and with the standard deviation derived from the data of the accredited laboratories only, according to the formula:

$$Z_i = (x_i - x)/s$$

These values are used to generate the tables and graphical presentations on the individual report sheet. On the report sheet, the individual laboratory performance is presented in comparison to the overall performance of other laboratories. Details on the interpretation of the report sheets are given in ***Annex I Explanation and interpretation of PT report sheets*.**

The Z-scores provide a good indication of a laboratory's analytical competence. The probability of a Z-score greater than ±2.0 or ±2.68 for a single test component (example pure seed in the purity test) is less than 5% or 1% respectively.

Results between ±2.0 or ±2.68 provide an indication to the laboratory that it may have an analytical problem and it should verify its state of readiness to perform the test in question. Individual Z-scores between 2.00 and 2.68 (or −2.00 and −2.68) indicate a possible problem which the laboratory should investigate.

Results greater than 2.68 are considered unsatisfactory and the laboratory should actively investigate the cause of the discrepancy. The Z-scores for each component are also averaged over the three samples to further clarify the laboratory's performance represented by its accumulated deviation from the expected mean.

The analysis of performance for other seed determination is based on a retrieval and identification rate of the added seeds. The performance in other seed determination is reported as a percentage of retrieved and identified seeds to genus level of seeds added to a round's sample. The relative difficulty of retrieving a species from the sample under test is considered, as explained below.

THE RATING SYSTEM

The laboratory is given an in-round rating for each particular test. An in-round rating applies for all participants. In addition, the accredited laboratories receive an overall rating for each test they have performed as obligatory participant. The overall rating is derived from the performance over the last six PT rounds. A laboratory may have an overall A rating for germination but an overall BMP rating for other seed determination, for instance.

In-round rating for PUR, GER, MOI, TZ, VIG and TSW

The in-round rating systems for purity analysis, germination testing, moisture content determination, viability testing (TZ), vigour testing and thousand-seed weight (TSW) are based on the sum of absolute Z-scores. Only normal seedlings and pure seeds are taken into consideration for germination and purity. Z-scores for abnormal seedlings and non-germinated seeds are also reported but do not affect the in-round rating (the category of non-germinated seeds includes dead, fresh and hard seed). A, B, C and BMP ratings are given. The in-round rating system for these tests is shown in Table 1. Examples of possible ratings are given in Table 2.

Table 1. In-round rating system

Score	Sum of absolute Z-scores
A	Sum of absolute Z-scores ≤ 3.5
B	3.5 < Sum of absolute Z-scores ≤ 5.3
C	5.3 < Sum of absolute Z-scores ≤ 7.0
BMP	Sum of absolute Z-scores > 7.0

Table 2. Example of in-round ratings for germination

	Normal			**Abnormal**			**Non-germinated**		
	L1	**L2**	**L3**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
Your %	88.00	84.75	95.75	06.75	11.25	03.25	05.25	04.00	01.00
Mean %	90.87	88.90	96.26	4.23	6.05	2.34	5.08	4.58	1.29
Z-scores	**-1.44**	**-1.50**	**-0.31**	**1.30**	**2.20**	**0.72**	**0.12**	**-0.30**	**-0.30**
Σ of absolute Z-scores			**3.25**			**4.22**			**0.72**

✓ **Sum of absolute Z-scores for normal seedlings ≤ 3.5**

⇒ **In-round: A**

	Normal			Abnormal			Non-germinated		
	L1	**L2**	**L3**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
Your %	90.75	90.00	86.75	7.75	9.75	11.00	1.5	0.25	2.25
Mean %	93.23	94.46	89.35	4.25	4.76	9.12	2.38	0.83	1.53
Z-scores	**-1.51**	**-2.12**	**-0.87**	**2.34**	**2.58**	**0.63**	**-0.93**	**-0.78**	**1.09**
Σ of absolute Z-scores			**4.5**			**5.55**			**2.80**

✓ **3.5 < Sum of absolute Z-scores for normal seedlings ≤ 5.3**

⇒ **In-round: B**

	Normal			Abnormal			Non-germinated		
	L1	**L2**	**L3**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
Your %	89.25	89.50	86.75	4.25	4.76	9.12	2.38	0.83	1.53
Mean %	93.23	94.46	89.35	4.25	4.76	9.12	2.38	0.83	1.53
Z-scores	**-2.42**	**-2.36**	**-0.87**	**1.84**	**2.71**	**0.79**	**1.47**	**-0.44**	**0.33**
Σ of absolute Z-scores			**5.65**			**5.34**			**2.24**

✓ **5.3 < Sum of absolute Z-scores for normal seedlings ≤ 7.0**

⇒ **In-round: C**

	Normal			Abnormal			Non-germinated		
	L1	**L2**	**L3**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
Your %	85.75	88.00	90.75	12.50	10.25	8.75	2.38	0.83	1.53
Mean %	93.23	94.46	89.35	4.25	4.76	9.12	2.38	0.83	1.53
Z-scores	**-4.55**	**-3.07**	**0.47**	**5.51**	**2.84**	**-0.12**	**-0.67**	**1.24**	**-1.56**
Σ of absolute Z-scores			**8.09**			**8.47**			**3.47**

✓ **Sum of absolute Z-scores for normal seedlings >7.0**

⇒ **In-round: BMP**

In-round rating for other seed determination

The performance in other seed determination is reported as a percentage of retrieved and identified seeds to genus level of seeds added to a round's sample. Identification to species level is expected. The species names must be reported in accordance with the current version of the *ISTA List of Stabilised Plant Names*. Synonyms will not be accepted.

Samples sent to the participants will not have been purified before adding the other seed inclusions. Therefore, seeds other than those that were added can also be found but they are not considered in the rating. The in-round rating is based on the actual retrieval rate of a distinct species. The percentage of retrieved and identified seeds for each species among all participants is calculated (Table 3). Based on the actual retrieval and identification rate, a factor is assigned to each added species to take the relative difficulty into account. This factor is multiplied by the total number of seeds added for this species. The factor is then multiplied by the number of seeds found by the laboratory. The percentage of retrieved and identified seeds determines the in-round rating. The thresholds for assigning a factor are given in Table 3. The thresholds for the other seed determination ratings are given in Table 4. An example for this rating system is presented in Table 5.

Table 3. Thresholds defining the factor assigned to each species based on the actual retrieval and identification rate of all seeds added of a distinct species

Retrieval rate [%]	Assigned factor
≥ 90	3
≥ 85	2
< 85	1

Table 4. In-round rating for other seed determination based on retrieval and identification rate

In-round rating	Percentage of retrieved and identified seeds
A	≥ 90.0
B	≥ 80.0
C	≥ 70.0
BMP	< 70.0

Table 5. Example laboratory results (test round 04-1 B.nap, *Brassica napus*)

Species	# of seeds added	Retrieval rate [%]*	Factor	# of seeds found	# of seeds added × factor	# of seeds found × factor
Galeopsis tetrahit	2	88	2	2	4	4
Galium aparine	3	93	3	3	9	9
Sinapis alba	4	83	1	4	4	4
Chenopodium sp.	3	84	1	3	3	3
Polygonum aviculare	3	91	3	3	9	9
Sinapis arvensis	3	61	1	3	3	3
Lotus corniculatus	4	72	1	0	4	0
Raphanus sativus	4	81	1	4	4	4
Thlaspi arvense	2	79	1	0	2	0
Sum	**28**			**22**	**42**	**36**
Percentage						**86**
In-round rating						**B**

* The retrieval rate [%] is the mean for all participants

Overall rating

The determination of the overall rating applies for accredited laboratories with obligatory participation in the particular crop group/test combination. Numerical values are attributed to the different in-round ratings. The values are then added for each test over the last six rounds as given in Table 6.

Table 6: Overall rating for all tests based on the in-round ratings over the last six rounds

In-round rating	Attributed value
A	5
B	4
C	3
BMP	0

Range	Overall rating
28-30	A
21-27	B
16-20	C
below 16	BMP

HETEROGENEITY RESULTS

These results represent the mean results of ten randomly selected samples, tested in the PT leader laboratory. The purpose of the test is to decide whether the seed lots are sufficiently homogenous and suitable for the PT purpose. As the heterogeneity results are obtained in one laboratory only, they are not expected true results, and this value is not used for calculating the rating of the participants' performance. The only expected/known data are related to the other seeds spiked in the samples. These data are provided to participants when reporting the preliminary results.

Reporting Results and Confidentiality

After calculation of results, individual reports with in-round results and overall rating are produced. Validation of results is done by comparing rates of in-round results with rates of overall rating for accredited laboratories. A check for outliers is also performed. Provisional results and final results are issued and sent to the laboratories.

All individual results are treated confidentially by all parties who have access to them.

To answer complaints and remarks, for corrective actions validation, or for internal data investigation, the confidentiality policy is also respected. For disclosing the results, the participating laboratory must be asked for permission. However, in the case of a BMP overall rating which will have a negative impact on the laboratory accreditation scope, the AWG will be informed.

Reporting In-Round Results

Preliminary in-round results are validated by the PTC Chair, respecting data confidentiality. The following is verified:

- frequency of A, B, C, BMP ratings for accredited laboratories
- any segregation of the results per method used and
- number of outliers.

Once approved, the laboratory will receive a report sheet for each test component. These provisional results are sent electronically.

As the results are imported into the calculation database exactly as participants report them, the possibility of making transcription errors is minimised. Nevertheless, if an error occurs on the side of the ISTA Secretariat, the laboratory shall notify the ISTA Secretariat immediately after receipt of the results. Please note that transcription errors made on the side of the laboratory cannot be corrected by the Secretariat, if reported after the results submission deadline.

Reporting Overall Rating

On the report sheets, the overall rating for each test will also be indicated. A laboratory obtaining an overall C rating will receive an alert as the laboratory runs the risk of obtaining an overall BMP rating if the level of performance does not increase.

A laboratory with poor PT performance (with a BMP overall rating) might be subject to suspension or withdrawal of ISTA accreditation, according to the ISTA directive Acc-D-01-Procedures for Termination, Suspension and Withdrawal of ISTA Accreditation for that test and related tests.

Summary Report

After provision of the individual results to participants, the summary report for the PT round is published on the ISTA website. It displays the overall means, standard deviation and the overall Z-scores distribution, without referring to any individual result of any particular laboratory. It also gives an overview of the method used for respective tests and gives general information about the performance of accredited and non-accredited laboratories.

Corrective Action

Corrective actions must be initiated by accredited laboratories when the in-round rating is C or BMP.

The laboratory is expected to carry out self-initiated investigations and develop a corrective action plan to address the problem. This action plan could include a request for help from the ISTA Secretariat who may ask advice from the PTC Chair, PT leader, TCOM Chairs or other relevant experts for such help or validation of action plans. On request of the laboratory, sharing of information is done with anonymised data.

The corrective action taken will be assessed by the ISTA auditors at the time of the next audit or earlier if deemed necessary.

COMPLAINTS AND REMARKS

Participants are invited to place any complaint, remark or question to the ISTA Secretariat about the PT round, its organisation or regarding their personal results, no later than 1 month after the final results have been provided. The analysis of the request is carried out by the ISTA Secretariat and if needed with the help of the PTC Chair. For technical topics, the ISTA Secretariat and PTC Chair may ask analysis of the request by PT leader, TCOM Chairs or other relevant experts. On request of the laboratory, sharing of information with technical experts is done with anonymised data.

Late claims cannot be taken into consideration. Complaints or appeals against suspensions due to PT performances with a BMP rating can be submitted by the laboratory by email or using the complaints form (Admin-F-27 Complaints and appeals).

ANNEX

Annex 1 Explanation and Interpretation of PT Report Sheets

DISTRIBUTION LIST

Any party interested in participation in the ISTA Proficiency Test

REVISION HISTORY

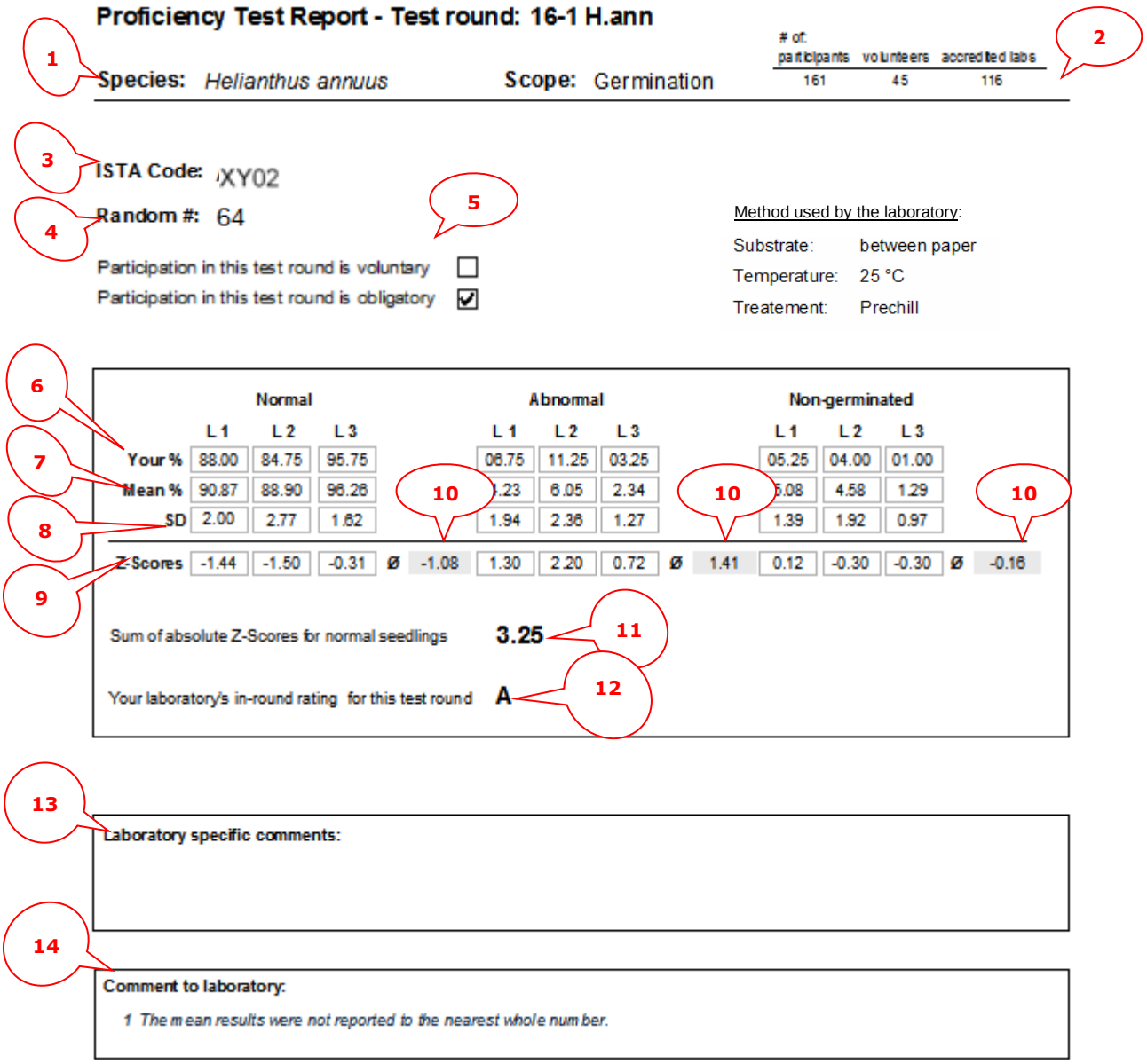
Version #	Changes
3.5	New layout of the document Definitions of ABC ratings Change of the samples shipping timetable Explanation that the sample weight might be different than in the ISTA Rules Explanation that heterogeneity results can differ from the provisional results Removal of the paper reporting Enclosure of automatic data import for calculation Updated report forms and corresponding explanation Removal of the PT Programme Plan and link to the website
3.6	Update on the Accreditation Standard name Website links update for the new PT plan Detailed definition of responsibilities Defining responsibility for the seed import requirement Explanation that the samples weight is minimum 2500 seeds Description of the statistical evaluation history Improved description of the outlier's exclusion from the calculation Improved description of the overall ratings assignment Explanation on the transcription errors corrections Addition of the section 'Complaints and remarks' New GER histogram example Removal of the Purity histograms explanation
3.7	Added reference to the 'standard' PT, replacing term 'basic' PT Excluding other PT types Vigour test statistics explained Importance of the appropriate reporting forms use is emphasized Seed must be frozen if insects are suspected
3.8	Adding information about the thousand-seed weight Updating responsibilities Explaining the selection of the PT leaders Specifying the Programme Plan species frequency Results confidentiality defined Corrective action specified
4.0	Table 2A renamed to 2C Thousand-seed weight tests included Crop group list Annex removed and linked with the website posted document List of Stabilised Plant Names included in references Examples of the basic reporting errors excluded Linking reference procedures with the website published documents Annex on germination ratings incorporated in the text Chapter of summary report included Frequency Bin and Frequency Range included Method used by the laboratory included in the report

5.0	Layout changed Admin-P-05-Appeals and Complaints Procedure replaced with Admin-F-27 Complaints and appeals Editorial changes
6.0	Annex 2_Flowchart removed Hyperlinks updated Heterogeneity results chapter updated Annex 1, point 5 updated

ANNEX 1 EXPLANATION AND INTERPRETATION OF PT REPORT SHEETS

The numbers in the following explanations refer to the circled numbers on the example report pages. The example is for germination, but the same explanations also apply for other tests.

Germination, in-round ratings report



Proficiency Test Report - Test round: 16-1 H.ann

1 **Species:** *Helianthus annuus* **Scope:** Germination

2

# of: participants	volunteers	accredited labs
161	45	116

3 **ISTA Code:** XY02

4 **Random #:** 64

5

Participation in this test round is voluntary ☐

Participation in this test round is obligatory ☑

Method used by the laboratory:

Substrate: between paper

Temperature: 25 °C

Treatement: Prechill

	Normal				Abnormal				Non-germinated			
	L 1	L 2	L 3		L 1	L 2	L 3		L 1	L 2	L 3	
6 Your %	88.00	84.75	95.75		06.75	11.25	03.25		05.25	04.00	01.00	
7 Mean %	90.87	88.90	96.26	10	.23	6.05	2.34	10	5.08	4.58	1.29	10
8 SD	2.00	2.77	1.62		1.94	2.36	1.27		1.39	1.92	0.97	
9 Z-Scores	-1.44	-1.50	-0.31	Ø -1.08	1.30	2.20	0.72	Ø 1.41	0.12	-0.30	-0.30	Ø -0.16

Sum of absolute Z-Scores for normal seedlings **3.25** 11

Your laboratory's in-round rating for this test round **A** 12

13 **Laboratory specific comments:**

14 **Comment to laboratory:**

1 The mean results were not reported to the nearest whole number.

Germination, overall ratings report

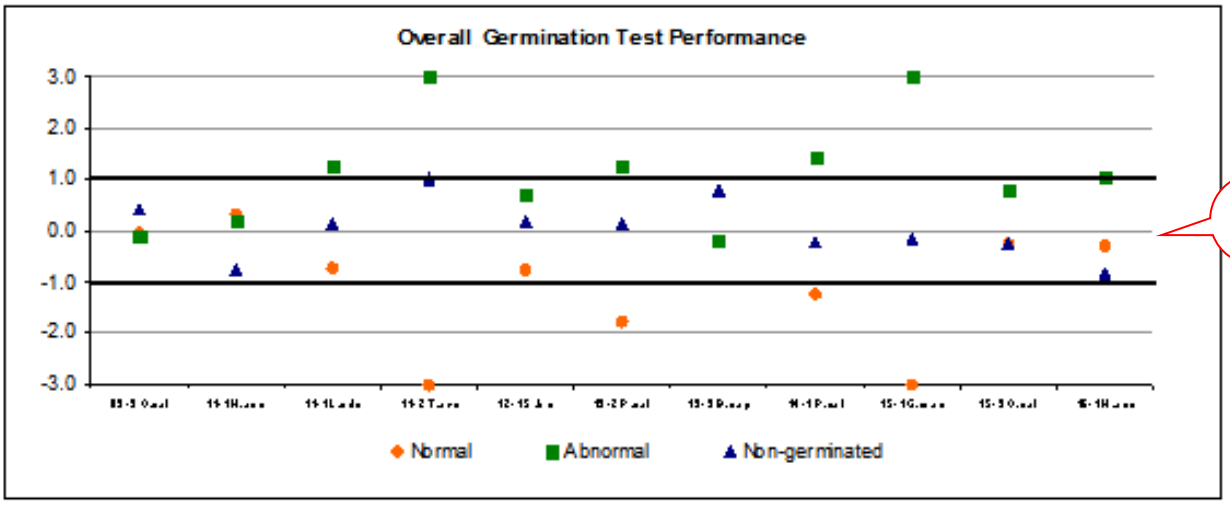
Overview - PT Overall Performance (Germination)

ISTA lab code	PT test round	Accredited?*	Volunteer**	Obligatory	Rating
XY02					
	09-3 O.sat	☑	○	◉	A
	11-1 H.ann	☑	○	◉	A
	11-1 L.odo	☑	◉	○	A
	11-2 T.aes	☑	○	◉	BM P
15	12-1 S.bic	☑	○	◉	A
	13-2 P.sat	☑	◉	○	C
	13-3 B.nap	☑	○	◉	A
	14-1 P.sat	☑	◉	○	B
	15-1 G.max	☑	◉	○	BM P
	15-3 O.sat	☑	○	◉	A
	16-1 H.ann	☑	○	◉	A

Overall rating***

Total sum of ratings*****	25
Number of rounds	6
Overall rating	**B**

16



17

* Refers to the laboratory's status at the time of sample dispatch.

** Depending on the laboratory's scope of accreditation, participation may be voluntary in spite of existing accreditation.

*** An overall rating will only be given after a minimum of six test rounds for which participation was mandatory. Rounds which were not mandatory are not included in the calculation of the overall rating.

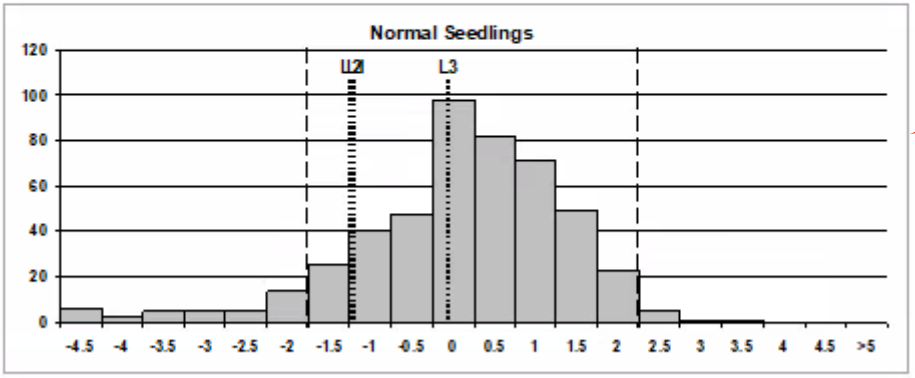
***** Values attributed to each in-round score are given in PT-P-01-ISTA Proficiency Test Programme.

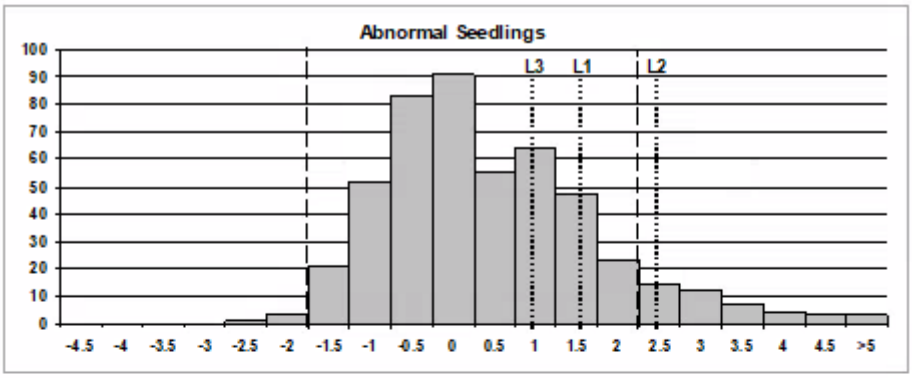
Germination, frequency distribution report

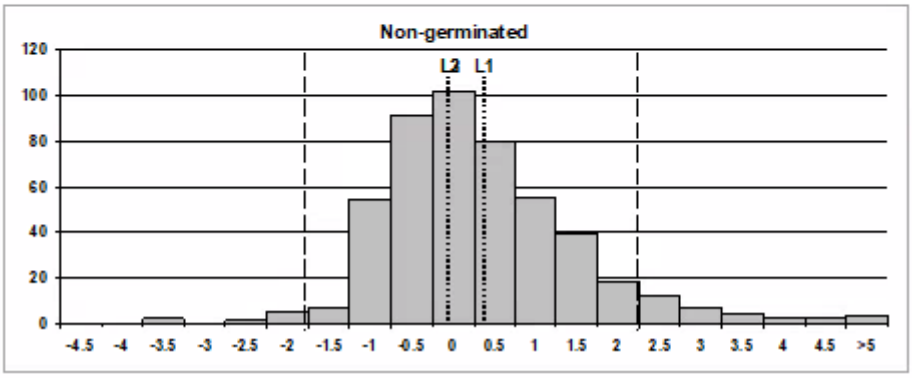
Proficiency Test Report – Test Round 16-1 H.ann

Species : Helianthus annuus **Scope:** Germination

The following histograms showthe frequency distributions of all participants Z-Scores for the relevant components, i.e. **Normal** and **Abnormal Seedlings** and **Non-germinated Seeds**. The Z-Scores from all three samples are included in each histogram. For further explanations, please refer to the document 'The ISTA Proficiency Test Programme'







18

L1, L2, L3 – samples of the first second and third lot

Frequency Bin (x-axis)	Frequency Range
<-4.5	<= -4.5
-4.0	>-4.5 to <= -4.0
-3.5	>-4.0 to <= -3.5
-3.0	> -3.5 to <= -3.0
-2.5	> -3.0 to <= -2.5
-2.0	> -2.5 to <= -2.0
-1.5	> -2.0 to <= -1.5
-1.0	> -1.5 to <= -1.0
-0.5	> -1.0 to <= -0.5
0	> -0.5 to <= 0
0.5	> 0 to <= 0.5
1.0	>0.5 to <=1.0
1.5	>1.0 to <=1.5
2.0	>1.5 to <=2.0
2.5	>2.0 to <=2.5
3.0	>2.5 to <=3.0
3.5	>3.0 to <=3.5
4.0	>3.5 to <=4.0
4.5	>4.0 to <=4.5
>4.5	>4.5

Note: Each Frequency Bin (bar on the graph) contains a range of Frequencies for example, Frequency Bin **'0'** contains frequencies from **>-0.5 to <=0**

1. The PT round being reported.
2. The number of participants having returned results for this round distinguished in total number of participants, number of volunteers and number of accredited laboratories. Only results from the accredited laboratories are used in calculation of the overall mean and standard deviation.
3. The ISTA member laboratory code, identifying the laboratory to which this report applies.
4. The random number assigned to the samples analysed by this laboratory. The laboratory receives three samples for the round, each with the same number.
5. Indicates whether the laboratory has ISTA accreditation and whether participation is mandatory if the test is included in the scope of accreditation, or voluntary if the test is not part of the laboratory's scope of accreditation.
6. The mean of the results of the replicates submitted by the laboratory for each of the three samples (L1, L2 and L3) for each attribute in the test, e.g. percentage normal, abnormal seedlings and non-germinated seeds for germination, or percentage pure seeds, inert matter and other seeds for purity. **Note** that dead, hard and fresh seeds are combined into one category 'non-germinated' for this purpose. However, laboratories are requested to report dead, hard and fresh as appropriate to assist in identifying the cause of deviating results.
7. The overall mean calculated using only the accredited laboratory results and only after outliers have been excluded. Outliers are results which are significantly different from most other results and if included in the calculations would distort the overall mean and standard deviation.
8. The standard deviation calculated using only the accredited laboratory results. The standard deviation is a measure of how widely values are dispersed from the mean. A very low standard deviation indicates most laboratories have reported results relatively close to the mean. A large standard deviation indicates more variation among laboratories.
9. Z-scores, also known as normalized scores. Use of Z-scores allows results from unrelated samples to be combined. They are calculated by subtracting the overall mean (x) from the laboratory's mean (x_i) and dividing by the standard deviation (s), i.e. $Z_i = (x_i – x)/s$. The further a laboratory's result is from the overall mean, the larger the Z-score will be. A Z-score of 0.00 is obtained when the laboratory's result exactly equals the overall mean. A negative Z-score indicates the result was lower than the overall mean, while a positive result indicates it was higher. Individual Z-scores between 2.00 and 2.68 (or –2.00 and –2.68) indicate a possible problem which the laboratory should investigate. Results greater than 2.68 are considered unsatisfactory and the laboratory should actively investigate the cause of the discrepancy.
10. The average Z-score for each attribute. This is a measure of bias. An average Z-score of 0.0 indicates no bias. A high positive value indicates bias toward high results for this attribute while a high negative value indicates bias toward low results for this attribute. These values are plotted in the chart (see 17 and 18).
11. The total sum of absolute Z-scores for normal seedlings (or pure seeds) determine the in-round rating. For this example, the sum of absolute Z-scores is 1.44 + 1.50 + 0.31 = 3.25 for normal seedlings.
12. The rating achieved by the laboratory for this round. The rating is calculated using the Z-scores from the normal seedlings when germination is tested and pure seed when purity is tested. There is no link between these tests, i.e. the rating for a germination test has no effect on the rating of the purity test. For this example, the sum of absolute Z-scores for normal seedlings is 3.25. The sum is □ 3.5 so the rating is A.
13. Comments the laboratory have provided in addition to its results.
14. Comments on items on which the laboratory is requested to pay attention. This field will normally be empty if the laboratory did not experience any testing difficulties.
15. The in-round ratings for previous rounds. A missing rating indicates the laboratory did not participate in the indicated round. This might occur, for example, with non-accredited laboratories which do not volunteer to participate in all rounds, or for accredited laboratories when the crop group represented in the round was not in the laboratory's scope of accreditation.
16. The overall rating based on the results of the last six rounds for the test being reported. A rating of C is a warning that there is a potentially serious testing problem which the laboratory should immediately investigate. A BMP rating is unsatisfactory, and the laboratory will be subject to suspension of their accreditation for this test.
17. Chart plotting the average Z-scores for each attribute over time. Values below -3 and above +3 are represented as ±3 at the border line of the chart. The test rounds are indicated on the x-axis by the test round code. The

average Z-scores are expected to be randomly distributed over time as positive and negative values above and below the zero line. The results should also fall between ±1 most of the time. If the results are dispersed over a wide range the laboratory should investigate why this is occurring and take corrective action, as it indicates the laboratory may not be in an adequate state of readiness to perform analyses according to ISTA Rules.

18. Histograms of the frequency distribution of Z-scores. For each attribute (e.g. normal seedlings, abnormal seedlings, non-germinated seeds) the three Z-scores for all participants are plotted on one chart. Each laboratory can compare its performance to the overall results by noting their Z-scores and observing their location on the x-axis of the histogram (see Frequency Bin/Frequency Range table in the *Germination, frequency distribution report* section above).
19. Names of the species that the PT leader added to the seed samples:
    - The number of seeds added ('# seeds added') per species, the retrieval rate ('Retrieval rate [%]') based on the total number of seeds found by all participants and the number of seeds reported ('# seeds found') by the laboratory.
    - The factor ('Factor') which is multiplied by the number of seeds added and the number of seeds found (i.e. '# seeds added × factor' and '# seeds found × factor') based on the retrieval rate.
20. There are three different levels of identification which are all counted as reported correctly:
    - *'Correct species?'* Species or lower taxon (e.g. subsp.) reported correctly at species level (e.g. *Triticum aestivum* or *Triticum aestivum* subsp. *aestivum*) and following the accepted name from the ISTA Rules or *ISTA List of Stabilised Plant Names*.
    - *'Correct genus?'* Species reported correctly to genus level. Synonyms with a genus name different from the correct genus name are counted as a non-correct report.
    - *'Different species?'* Correct genus but wrong species. Synonyms with a genus name different from the correct genus name are counted as a non-correct report.

    Note: misspelling of the scientific name is indicated under *'Misspelled?'*.
21. Total sum of seeds added, and seeds found; the total sum of seeds found, and seeds added multiplied by the factor.
22. The percentage of '# seeds added × factor' and '# seeds found × factor' are the basis for the in-round rating.
23. The laboratory's in-round rating for this round.
24. Name and number of seeds exactly as they were reported by the laboratory.

Other seed determination, in-round ratings report

Proficiency Test Report - Test round: 19-3 H.vul

Species: *Hordeum vulgare* **Scope:** Other Seed Determination

This laboratory is accredited ☑
Participation in this test round is obligatory ☑

ISTA Code: XY02 **Random #:** 129

CALCULATED RESULT

Lot #	Species name	# seeds added	Retrieval rate [%]	Factor	# seeds found	# seeds added x factor	# seeds found x factor	Misspelled?	Correct Species?	Correct Genus?	Different Species?
Lot 1											
	Avena sativa	3	87.5	2	3	6	6	○	◉	○	○
	Medicago sativa	4	84.3	1	4	4	4	○	◉	○	○
	Sorghum bicolor subsp. bicolor	4	85.9	2	4	8	8	○	◉	○	○
Lot 2											
	Bassia scoparia	2	55.0	1	2	2	2	○	○	◉	○
	Fagopyrum esculentum	2	87.1	2	2	4	4	○	◉	○	○
	Phleum pratense	4	72.9	1	4	4	4	○	○	◉	○
	Setaria pumila	3	85.8	2	3	6	6	○	◉	○	○
Lot 3											
	Echinochloa esculenta	4	84.3	1	4	4	4	○	○	◉	○
	Persicaria lapathifolia	2	82.7	1	2	2	2	○	◉	○	○
	Thlaspi arvense	3	88.0	2	3	6	6	○	◉	○	○
	Sum	**31**			**31**	**46**	**46**	**Percentage**	**100.0%**		

In-round rating: A

Data reported by the laboratory:

Lot #	Species name	# of seeds
Lot 1		
	Avena sativa	3
	Medicago sativa	4
	Sorghum bicolor subsp. bicolor	4
Lot 2		
	Bassia sp.	2
	Fagopyrum esculentum	2
	Phleum sp.	4
	Setaria pumila	3
Lot 3		
	Echinochloa sp.	4
	Persicaria lapathifolia	2
	Thlaspi arvense	3