"Further strengthening of capacities of phytosanitary sector in the fields of plant protection products, plant health and seeds and seedlings, including phytosanitary laboratories and phytosanitary inspections"
(TWINNING BA/12/IB/AG 01)

Component 3: Seeds and propagation materials

CALIBRATION AND TESTING MATERIALS

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ISTA Accreditation Standard

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ISTA Accreditation Standard for Seed Testing and Seed Sampling

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Gives details of requirements that need to be met regarding equipment, its management, calibration and reference and testing materials

ISTA Laboratory Accreditation Standard

Provision and maintenance of equipment

5.2. Provision and maintenance of equipment

5.2.1. Laboratory staff and samplers must be furnished with or have access to all items of equipment required for correct performance of sampling and testing for which the laboratory is accredited.

5.2.2. Equipment must be run appropriately by authorised staff. Up-to-date instructions on the use and maintenance of equipment (including any relevant manuals provided by the manufacturer of the equipment) must be readily available for use by the appropriate laboratory staff.

5.2.3. Equipment and its software used for testing and sampling must be capable of achieving the accuracy required and must comply with specifications relevant to the tests concerned.

5.2.4. There must be documented procedures for operating, maintaining, calibrating and monitoring of sampling and testing equipment. Whenever practicable, all equipment under the control of the laboratory and requiring calibration must be labelled, coded or otherwise identified to indicate the status of calibration, including the date when last calibrated and the date or expiration criteria when recalibration is due.

5.2.5. All equipment must be properly maintained to ensure protection from corrosion and other causes of deterioration.

5.2.6. Any equipment which has been subjected to overloading or mishandling, or which gives suspect results, or has been shown by calibration or otherwise to be defective, must be taken out of service and clearly labelled until it has been repaired and then shown by test or calibration to be performing its function satisfactorily again.

5.2.7. Each equipment and its software used for testing and significant to the result must, when practicable, be uniquely identified. Records must be maintained of each major item of equipment and its software. Each record must include:

(a) the name, type identification, and serial number or other unique identification of the item of equipment and its software

(b) details of maintenance and monitoring

(c) the current location, where appropriate

(d) the manufacturer's instructions, if available, or reference to their location

(e) details of any damage, malfunction, modification or repair to the equipment

(f) dates, results and copies of reports and certificates of all calibrations, adjustments, acceptance criteria, and the due date of the next calibration

(g) checks that the equipment complies with the specification

5.2.8. Each record may also include:

(h) the names of the manufacturer, supplier and service agent, date received and date placed in service in current location, as appropriate.

ISTA Laboratory Accreditation Standard

Calibration, reference and testing materials

5.3. Calibration, reference and testing materials

5.3.1. All sampling, measuring and testing equipment, for which this is possible, must be adequately calibrated before being placed into service and regularly afterwards, and a log book kept in which is recorded the results of each calibration, service and repairs (see 5.2.7e and f). Calibration and servicing of equipment must be performed according to an established programme.

5.3.2. The overall programme of calibration of equipment must be designed and operated so as to ensure that, wherever applicable, measurements made in the laboratory are traceable to national and international standards of measurement.

5.3.3. Appropriate calibration samples, reference materials and reference standards of measurement must be held by the laboratory, and be used for calibration and reference purpose only. They should, where possible, be traceable to SI units of measurement, or to certified reference materials. Examples include calibration samples for seed blowers, standard buffer solutions for pH meters, calibration weights for balances, and reference collections of seed.

5.3.4. Calibration samples for the blowers must be provided by arrangement with the ISTA Secretariat.

5.3.5. The laboratory must examine the effect of defective equipment on any previous tests, and withdraw and re-issue certificates where faulty results are suspected.

5.3.6. The laboratory must have procedures for safe handling, transport, storage and use of reference standards and reference materials in order to prevent contamination or deterioration and in order to protect their integrity.

WHAT IS “CALIBRATION”?

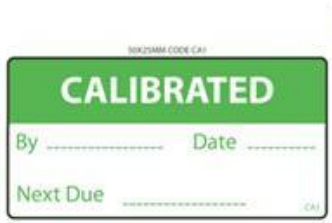
Definitions:

- Oxford English Dictionary:

  “To determine the accuracy of a measuring instrument.”

- International Standards Organisation:

  “The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values of a quantity realised by a reference standard.”



WHAT IS “CALIBRATION”?

Definitions:

- Wikipedia:

“It is a comparison between measurements – one of known magnitude or correctness made or set with one device and another measurement made in as similar way as possible with a second device. The device with the known or assigned correctness is called the standard. The second device is the unit under test, test instrument, or any of several other names for the device being calibrated.”

WHAT IS “CALIBRATION”?

Final aim:

- To confirm that your measurements are “true”. But..

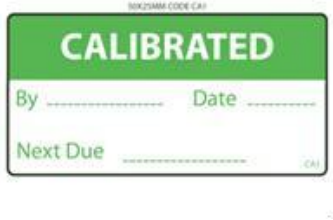
… can we define what “true” means?

Example: calibration of a scale.

A standard weight is known to be 10.00 g. The weight shown by the scale is 10.05 g. The error is 0.05 g. Is it OK? It depends on the required accuracy :

Tolerance of +/- 0.02 g → the scale has not passed calibration.

Tolerance of +/- 0.10 g → the scale has passed calibration.





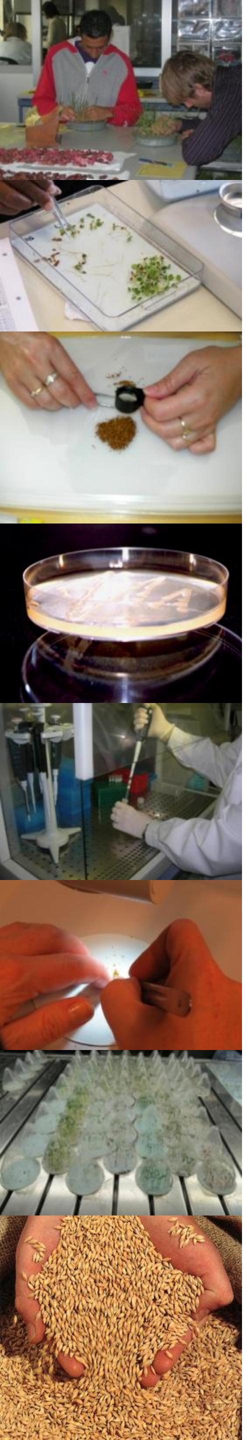





CALIBRATION IN THE SEED TESTING LABORATORY

What has to be calibrated:

- Seed Blowers used for the purity analysis of *Poa pratensis*, *Poa trivialis*, and *Dactylis glomerata*
- Sampling Equipment
- Balances
- Grinding mills
- Thermometers and temperature probes
- pH meters
- Electro-conductivity meters



SEED BLOWERS

For the purity analysis of *Poa pratensis*, *Poa trivialis*, and *Dactylis glomerata* the Uniform Blowing Method must be used.

The blower used must be calibrated at regular intervals: the optimum setting is obtained with the use of the ISTA uniform calibration samples or the use of an anemometer (ISTA Rules 2013).

Use of the ISTA uniform calibration samples: “guidelines for blower calibration and use of the uniform blowing procedure for *Poa Pratensis* and *Dactylis glomerata*” (see https://www.seedtest.org/upload/cms/user/Guidelinesforblowercalibrationanduseoftheuniformblowingprocedure)

Use of an anemometer (to determine the equivalent air velocity - EAV): blower calibration by using the calibration samples → determination of the optimum blowing point (setting of air gate opening) → measurement of the EAV using the anemometer (see ISTA Rule 3.4.2.).

SAMPLING EQUIPM

Samples submitted for testing are mixed and divided to give working samples using mechanical dividers (e.g. Boerner type, riffle divider) as well as hand and spoon methods. The equipment and methods must be capable of producing representative samples from the submitted sample. They must satisfy 2 criteria:

- They must be capable of dividing a sample into two halves; and
- They must not segregate the sample in any way.

Calibration is carried out in-house using specially prepared samples containing a mix of different seed species.

BALANCES

Balances are used in the seed testing laboratory to weigh:

- seed samples;
- seed sample components;
- chemicals used in the preparation of solutions.

Weighing is one laboratory operation where measurements can be made traceable to national and international standards of measurement.

Laboratories should engage a competent organisation to carry out calibration, preventative maintenance, servicing and any necessary repairs to balances. In addition they should use check weights to monitor the performance of balances at regular interval.

GRINDING MILLS

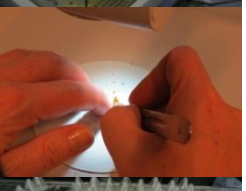
The ISTA Rules give a list of species for which grinding is obligatory when carrying out Moisture Content Determination.

In addition the rules give a specification for the dimensions of the ground material.

To test that grinders are operating to the required specification a checking procedure must be developed using sieves that are traceable to national and international standards of measurement.





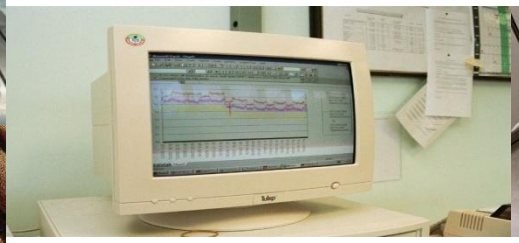
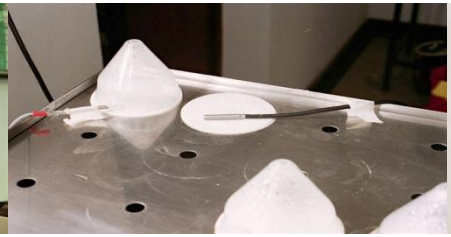
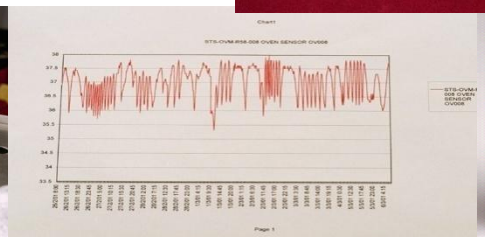



TEMPERATURE MEASUREMENT

The ISTA Rules indicate specific temperatures for:

- Germination tests;
- Dormancy breaking pre-treatments in fridge and oven;
- Moisture Content tests;
- Tetrazolium tests;
- Vigour tests;
- Seed Health tests;
- … other tests.



TEMPERATURE CALIBRATION

Temperature measurement is a laboratory operation where measurements can be made traceable to national and international standards of measurement.

Working **thermometers** and **temperature probes** are checked against calibrated thermometers or probes at a temperature within the normal working range. The frequency is at least once a year.

A competent organisation is engaged to carry out calibration of the calibrated thermometers and probes at least every 5 years. In addition, ice point determinations are used to check the accuracy of probes and thermometers on an annual basis.



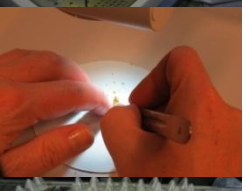

TESTING MATERIALS

The ISTA Rules give specifications for materials used in the seed testing laboratories. Where materials can have an influence on the results of tests there must be quality checks. The following materials can have an effect on the outcome of tests:

- Germination Substrates;
- Chemical Solutions, e.g. KNO_3, Gibberellic Acid, Buffer Solutions, Tetrazolium Solution;
- Water.





GERMINATION SUBSTRATES

Quality control procedures involves the following steps:

1. Assigning each new batch a number and recording the amount and date of receipt in a substrate log book.
2. Initiating substrate quality tests as prescribed in the ISTA Rules (before putting into use):
   - Water retention
   - pH
   - Conductivity
   - Innocuity
   - Particle size (for sand and organic growing media only)
3. Recording results of tests and filing them in substrate log book. If the new batch meets requirements set in ISTA Rules and there is no evidence of any detrimental characteristic, the new batch is acceptable and can be used for testing.

CHEMICAL SOLUTIONS,

e.g. KNO_3, Gibberellic Acid, Buffer Solutions, Tetrazolium Solution

The laboratory should hold a Chemical Register that gives details of all chemicals held and utilised. The manufacturers' batch number for the chemical is also detailed in the register with the date of receipt. Details of any expiry dates are also held.

When solutions of chemicals are prepared details are recorded.

CHEMICAL SOLUTIONS,

An example of procedure when preparing Gibberellic Acid Solution

A solution of 500 ppm Gibberellic acid to break dormancy in some species is prepared by dissolving the following 500 mg GA_3 in 1 litre of water.

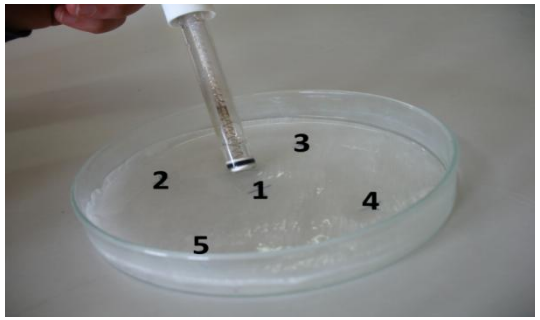
The stock solution is stored in a fridge at 5 ± 2^oC for uo to 6 months. Details of the date of preparation, the manufacturers' batch numbers of the chemicals used are recorded. The analyst preparing the solution is also responsible for recording the batch number and expiry date on the stock solution bottle/container.

WATER

ISTA specification outlined in the ISTA Rules:

- Cleanness. Water used to moisten the substrates should be reasonably free from organic or inorganic impurities.
- pH. When checked in the substrate, the pH must be within the range 6.0-7.5 (unless there is evidence that an outside value doesn't have negative influence on the germination test results).

It is recommended that water is analysed by an accredited laboratory (e.g. every two years).



EQUIPMENT

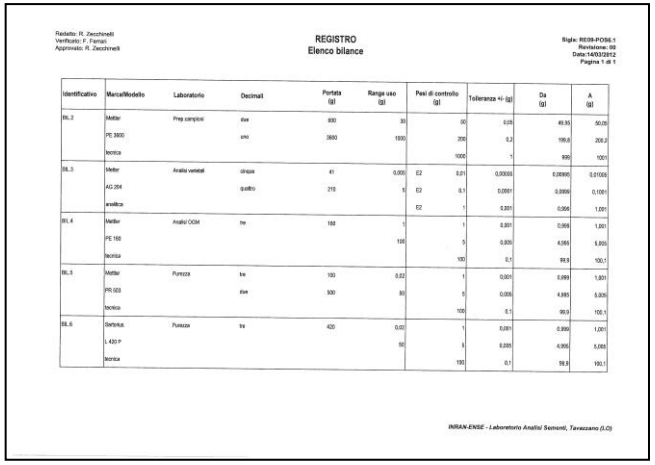
Each piece of equipment whose proper functioning is relevant to the test results must be properly maintained, calibrated, and operated by authorized personnel. For each piece of equipment:

- criteria and specifications for the purchase must be defined
- equipment must be checked, before placing into use
- a responsible person (and a deputy) must be identified
- a preventive maintenance programme (including calibration and checks) is defined and implemented
- relevant records must be retained (concerning purchasing, characteristics, maintenance, calibration, checks, responsibilities)

EQUIPMENT

There will be an equipment master list which is an inventory of all equipment in the laboratory. It is usually held by the Laboratory Manager.

There will be one or more Equipment Register (e.g. a register for a specific kind of equipment such as a Balance Register and/or a general register for all or the other pieces of equipment).



Redatto: R. Zecchinelli
Verificato: F. Ferrari
Approvato: R. Zecchinelli

REGISTRO
Elenco bilance

Sigla: REG9-POS6.1
Revisione: 00
Data:14/03/2012
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Identificativo	Marca/Modello	Laboratorio	Decimali	Portata (g)	Range uso (g)	Pesi di controllo (g)	Tolleranza +/- (g)	Da (g)	A (g)
BIL.2	Mettler	Prep.campioni	due	600	30	50	0,05	49,95	50,05
	PC 3600		uno	3600	1000	200	0,2	199,8	200,2
	tecnica					1000	1	999	1001
BIL.3	Mettler	Analisi varietali	cinque	41	0,005	02	0,01	0,00005	0,01005
	AG 204		quattro	210	1	02	0,1	0,0001	0,1001
	analitica					02	1	0,001	1,001
BIL.4	Mettler	Analisi OGM	tre	160	1	1	0,001	0,999	1,001
	PE 160				100	5	0,005	4,995	5,005
	tecnica					100	0,1	99,9	100,1
BIL.5	Mettler	Purezza	tre	100	0,02	1	0,001	0,999	1,001
	PR 503		due	500	50	5	0,005	4,995	5,005
	tecnica					100	0,1	99,9	100,1
BIL.6	Sartorius	Purezza	tre	420	0,02	1	0,001	0,999	1,001
	L 420 P				50	5	0,005	4,995	5,005
	tecnica					100	0,1	99,9	100,1

INRAN-ENSE - Laboratorio Analisi Sementi, Tavazzano (LO)

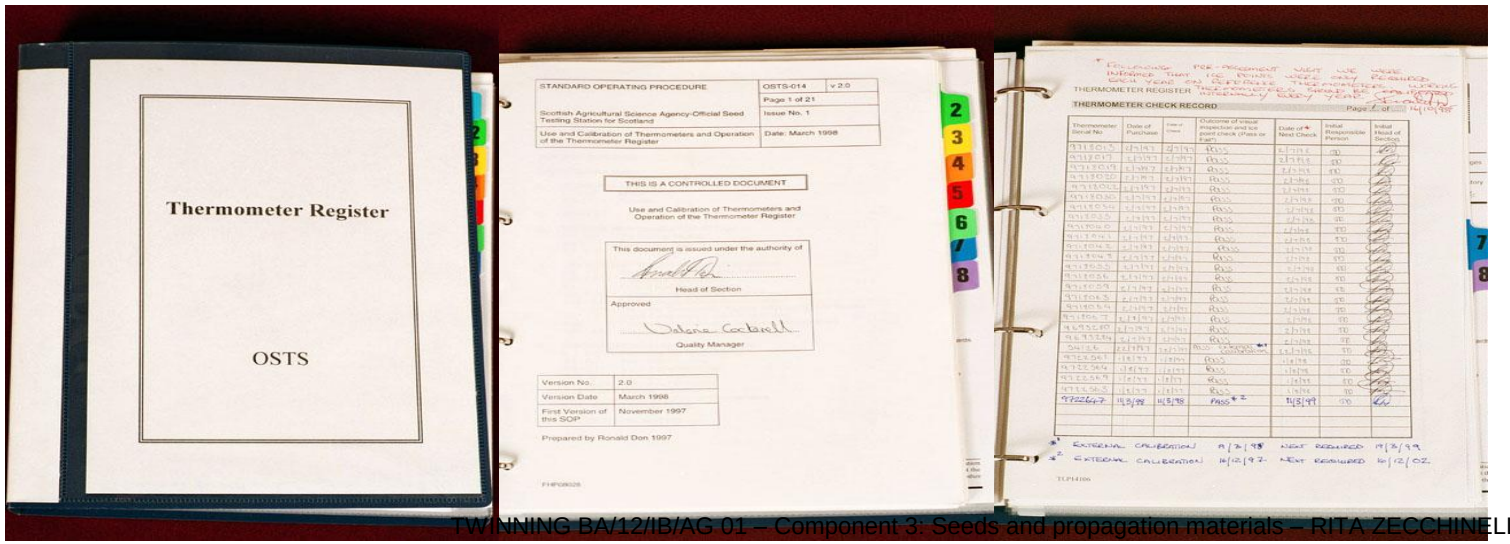
PURPOSE OF EQUIPMENT REGISTERS

Equipment registers ensure that:

- Specifications for equipment are drawn up before they are acquired;
- Equipment is checked against required performance before being placed into service;
- Each piece of equipment is entered into the appropriate register and assigned a unique reference number which is clearly displayed on the equipment;
- Procedures are put into place to ensure that equipment is properly maintained, checked regularly for correct functioning and, if required, properly calibrated;
- Procedures are put in place to ensure that records of the performance and maintenance of all equipment are kept.

OPERATION OF REGISTERS

The day to day operation of each register as regards maintenance records and other entries is under the supervision of the person designated as responsible for the equipment involved and designated "*The Responsible Person*". Each such person has a deputy.



CONTENT OF AN EQUIPMENT REGISTER

An equipment register will contain:

- A list of equipment with their unique reference numbers
- Record of Responsible Persons
- Acceptance Record
- Amendments to Acceptance Record
- Maintenance records
- Calibration Records (where required)
- Daily Check Sheets and Control Charts (where appropriate)

Thank to
Ronald Don
ISTA Secretariat
And to you for your attention