"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

Temperature measurement and control

Rita Zecchinelli

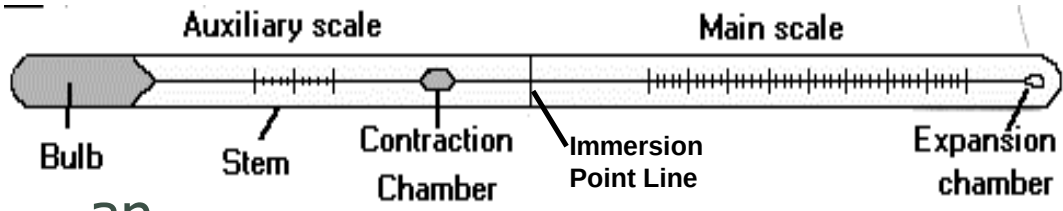
The Measurement of Temperature

Temperature is one of the most commonly measured physical quantities but its basis is not widely understood:

different people will have different perceptions of what is hot and what is cold!

To make objective measurements, we must use a device, such as thermometer in which some physical property of a substance changes with temperature in a reliable and reproducible way.

Thermometers



A thermometer is an instrument that measures the temperature of a system in a quantitative way. The most direct 'regular' way is a linear one.

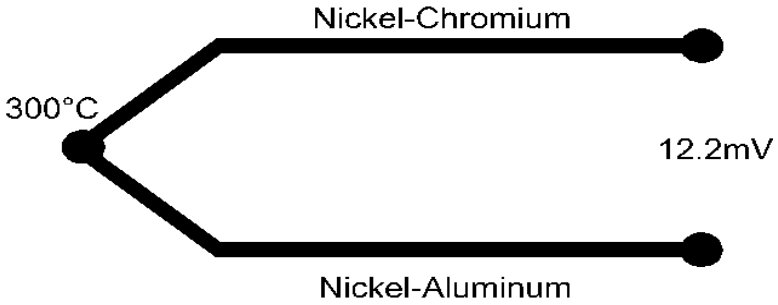
For example, mercury is liquid from -38.9°C to 356.7°C. As a liquid, it expands linearly as it gets warmer and its expansion rate can be accurately calibrated.

The mercury-in-glass thermometer contains a bulb filled with mercury that is allowed to expand into a capillary. Its rate of expansion is calibrated on a scale marked on the glass.

Thermocouples

THERMOCOUPLES

When wires of different metals are fused at one end and heated, a current flows from one to the other. The electromotive force generated can be quantitatively related to the temperature and hence, the system can be used as a thermometer - known as a thermocouple.

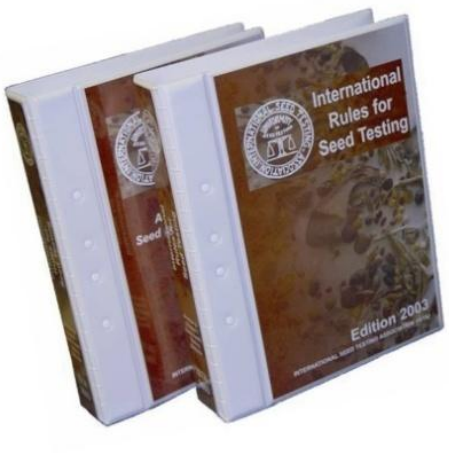


The thermocouple is used in many electronic/digital thermometers and many different metals are used, for example - platinum and platinum/rhodium; nickel-chromium and nickel-aluminium.

Temperature measurement in Laboratories

A variety of different equipment is used to measure temperature in laboratories. Some use thermometers/ temperature probes with accuracy of ± 0.1ºC whereas others use thermometers where it would be difficult to achieve a reading with an accuracy of ± 2º C.

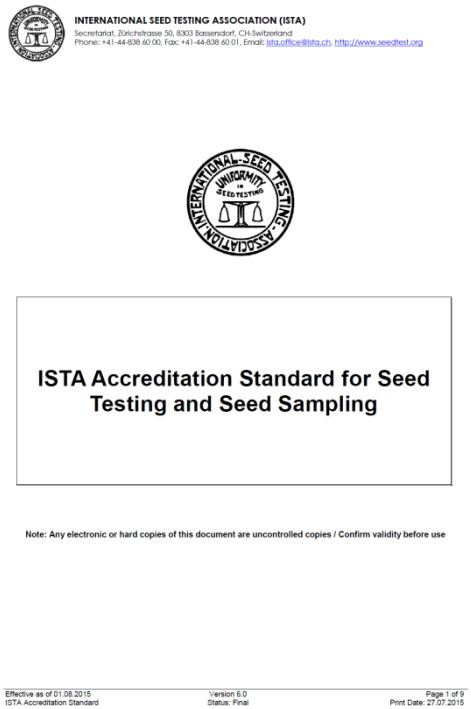
Seed testing and temperature



Temperature plays a critical role in the outcome of many tests.

The ISTA Rules prescribe specific temperature(s) for many procedures. In some case variations that are permitted from prescribed temperature(s) are also set in the Rules.

Temperature measurement, calibration and the ISTA Accreditation Standard



INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)
Secretariat, Zürichstrasse 50, 8303 Bassersdorf, CH-Switzerland
Phone: +41-44-838 60 00, Fax: +41-44-838 60 01, Email: ista.office@ista.ch, http://www.seedtest.org

ISTA Accreditation Standard for Seed Testing and Seed Sampling

Note: Any electronic or hard copies of this document are uncontrolled copies / Confirm validity before use

Effective as of 01.08.2015 | Version 6.0 | Page 1 of 9
ISTA Accreditation Standard | Status: Final | Print Date: 27.07.2015

The ISTA Accreditation Standard for Seed Testing and Seed Sampling states that sampling, measuring and testing equipment shall be calibrated where appropriate before being put into service and thereafter according to an established programme.

The overall programme of calibration of equipment shall be designed and operated to ensure that, wherever applicable, measurements made are traceable to national and international standards of measurement.

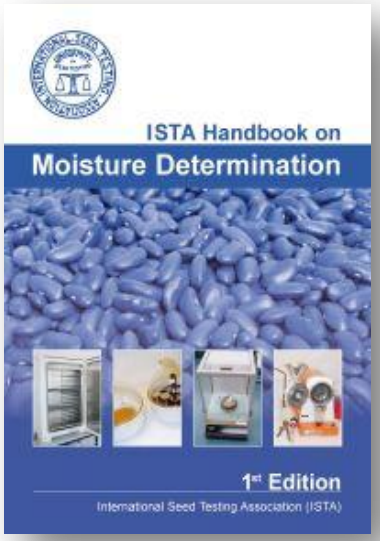
ISTA Temperature Measurement Guidelines

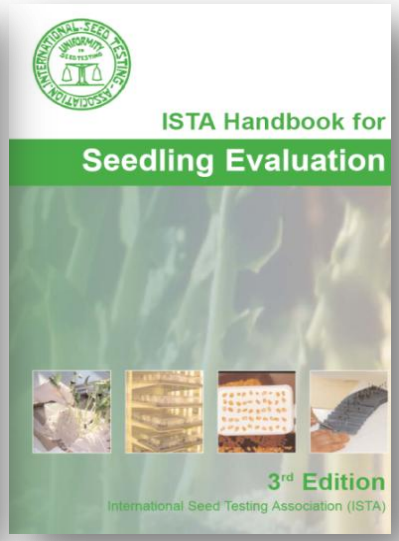
ISTA has produced guidelines to give laboratories and auditors advice and information as to what technical experts from the ISTA Technical Committees expect of laboratories in terms of temperature monitoring and control.

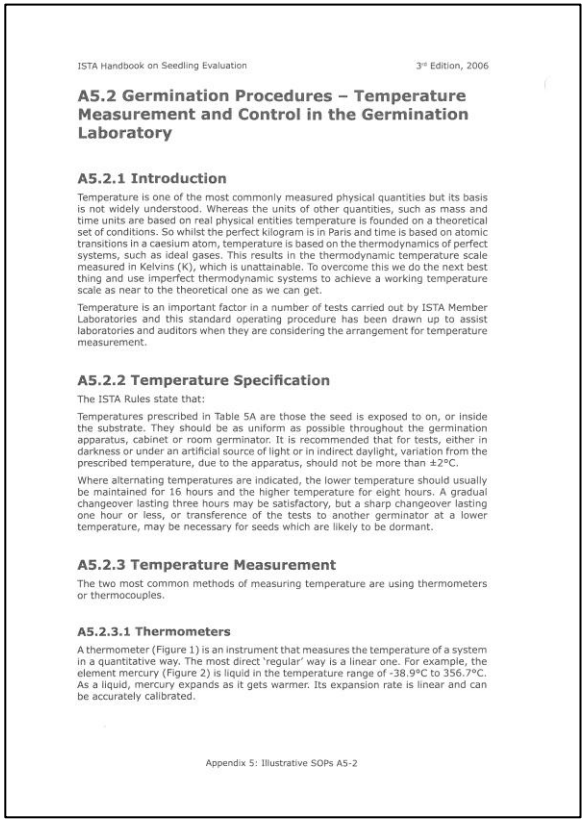
Seed Testing International No 125 (2003)

Publication of ISTA Temperature Measurement Guidelines

Temperature measurement guidelines are also published in ISTA Handbooks.







ISTA Handbook on Seedling Evaluation 3rd Edition, 2006

A5.2 Germination Procedures – Temperature Measurement and Control in the Germination Laboratory

A5.2.1 Introduction

Temperature is one of the most commonly measured physical quantities but its basis is not widely understood. Whereas the units of other quantities, such as mass and time units are based on real physical entities temperature is founded on a theoretical set of conditions. So whilst the perfect kilogram is in Paris and time is based on atomic transitions in a caesium atom, temperature is based on the thermodynamics of perfect systems, such as ideal gases. This results in the thermodynamic temperature scale measured in Kelvins (K), which is unattainable. To overcome this we do the next best thing and use imperfect thermodynamic systems to achieve a working temperature scale as near to the theoretical one as we can get.

Temperature is an important factor in a number of tests carried out by ISTA Member Laboratories and this standard operating procedure has been drawn up to assist laboratories and auditors when they are considering the arrangement for temperature measurement.

A5.2.2 Temperature Specification

The ISTA Rules state that:

Temperatures prescribed in Table 5A are those the seed is exposed to on, or inside the substrate. They should be as uniform as possible throughout the germination apparatus, cabinet or room germinator. It is recommended that for tests, either in darkness or under an artificial source of light or in indirect daylight, variation from the prescribed temperature, due to the apparatus, should not be more than ±2°C.

Where alternating temperatures are indicated, the lower temperature should usually be maintained for 16 hours and the higher temperature for eight hours. A gradual changeover lasting three hours may be satisfactory, but a sharp changeover lasting one hour or less, or transference of the tests to another germinator at a lower temperature, may be necessary for seeds which are likely to be dormant.

A5.2.3 Temperature Measurement

The two most common methods of measuring temperature are using thermometers or thermocouples.

A5.2.3.1 Thermometers

A thermometer (Figure 1) is an instrument that measures the temperature of a system in a quantitative way. The most direct 'regular' way is a linear one. For example, the element mercury (Figure 2) is liquid in the temperature range of -38.9°C to 356.7°C. As a liquid, mercury expands as it gets warmer. Its expansion rate is linear and can be accurately calibrated.

Appendix 5: Illustrative SOPs A5-2

Appendix A5.2

Sensitivity/accuracy of thermometers and probes

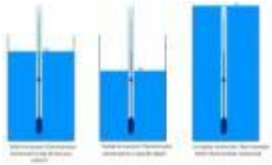
Laboratories should use temperature measurement instruments that are accurate to at least 0.5°C and conform to ISO 386 (*Liquid-in-glass laboratory thermometers-Principles of design, construction and use*).

Measurement uncertainties of thermometers and probes

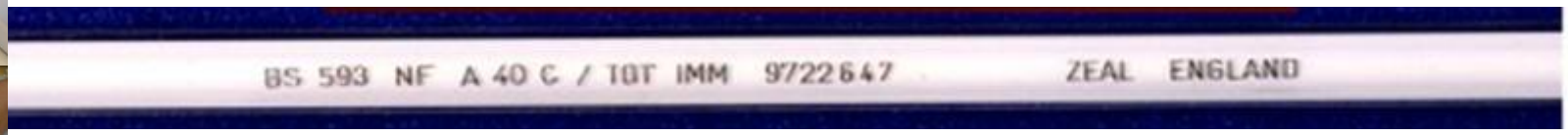
Measurement uncertainties of total and partial immersion thermometers that conform to ISO 386

	Uncertainties of thermometers (in degrees Celsius)							
Thermometer	Scale Range							
scale divided into	Total Immersion				Partial Immersion			
(in degree Celsius)	-25°C to 100°C	-10°C to 50°C	-10°C to 100°C	-10°C to 200°C	-25°C to 100°C	-10°C to 50°C	-10°C to 100°C	-10°C to 200°C
0.01	-	±0.005	±0.01	-	-	±0.005	±0.01	-
0.02	±0.02	±0.01	±0.02	-	±0.02	±0.01	±0.02	-
0.05	±0.05	±0.02	±0.02	±0.05	±0.05	±0.05	±0.05	±0.05
0.1	±0.1	±0.02	±0.02	±0.05	±0.1	±0.05	±0.05	±0.1
0.2	±0.1	±0.05	±0.05	±0.05	±0.1	±0.05	±0.05	±0.1
0.5	±0.2	±0.05	±0.05	±0.1	±0.5	±0.1	±0.1	±0.1

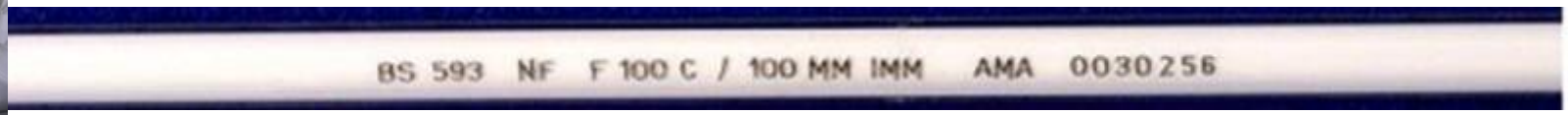
Thermometer immersion



http://www.amrl.net/amrlsitefinity/default/Resources/newsletter/Spring2011/8.aspx



Total immersion thermometers are designed to be totally submerged in the media whose temperature is being measured or at least to the depth equivalent to the level of the thermometer liquid. They are distinguished by the engraved suffix '/ TOTAL' or '/ TOT IMM'.



http://dnr.wi.gov/regulations/labcert/BODEquipment.html

Partial immersion thermometers should only be immersed to the indicated depth. They have a suffix that indicates the immersion depth (e.g. '/ 100 MM IMM' indicates a depth of 10 cm). The depth may also be indicated by an engraved ring on the stem.

Use of total and partial immersion thermometers

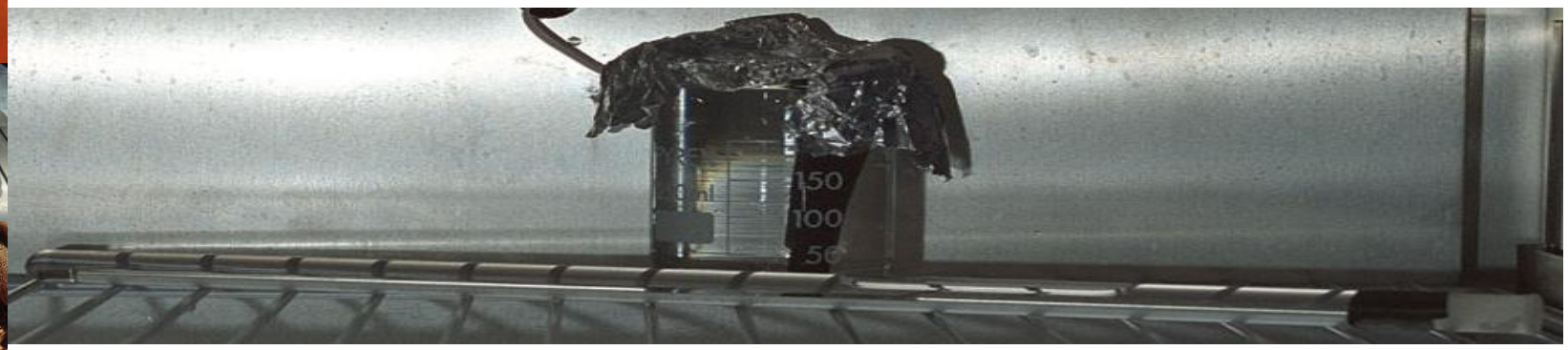
Total immersion thermometers and thermocouples can be used to monitor the temperature of incubators, germination cabinets, walk in germinators, ovens and fridges.

Partial immersion thermometers can be used to monitor the temperature of incubators, ovens and fridges through an external aperture on the apparatus provided that they can be immersed to the required depth within the apparatus.

http://dnr.wi.gov/regulations/labcert/BODEquipment.html

Stabilizing Readings

For ease of taking measurements and to reduce dramatic temperature fluctuations that can result from the opening of doors of apparatus, thermometers and probes should be submerged in glycerol or sand.



Where to measure

ISTA Rules 5.6.2.3

Temperatures (...) are those to which the seed is exposed on or inside the substrate

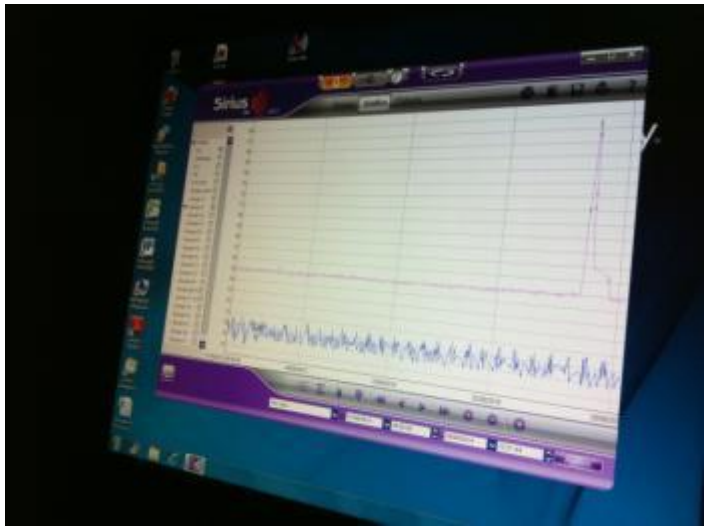
→ Probes: placed in condition the seeds are exposed to (on the seed bed)

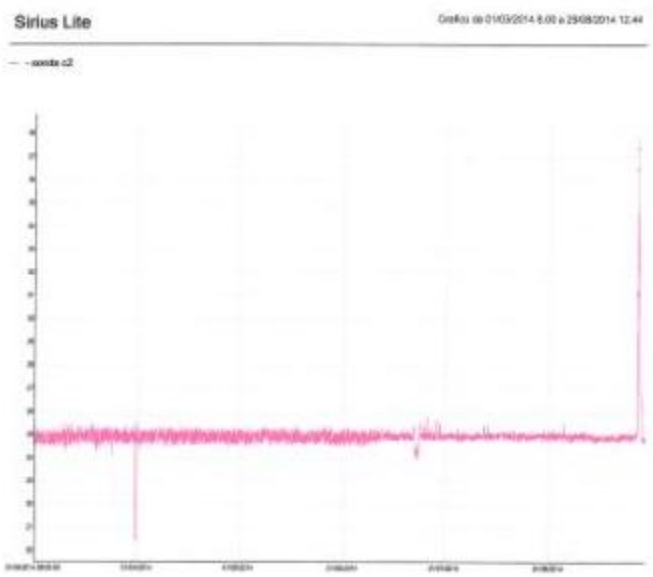
Total and Partial immersion thermometers can NOT be used to monitor the temperature of Copenhagen Tanks or Germinators since it is not possible to meet immersion requirements.

Thermocouples with contact probes must be used.

Frequency of temperature measurement: data-loggers

Many laboratories use data-loggers or other temperature monitoring equipment that constantly monitors temperature and records of readings are available on an hourly basis or less.












Manual temperature recording: frequency

Constant temperature equipment

At least 3 readings per day, at regular pre-set times

However…

If records show temperature is stable with variations of less than 1ºC between readings the recording frequency can be reduced to once per day.

But…

If there is any indication of a change in performance recording frequency must be increased.

Manual temperature recording: frequency

Alternating temperature equipment

- At least 3 readings must be recorded per day, at regular pre-set times
- The timing of these readings must be such that both temperature phases are monitored
- Change over time between high and low temperature phase should not be more than 3 hours.

Measure the temperature versus time

Number of temperature measuring points

For Germination/Temperature Rooms or Cabinets with an area of:

- less than 10m^2 there must be at least one measuring point;
- between 10m^2 and 20m^2 at least two measuring points are recommended ;
- 20m^2 or more at least three measuring points are recommended .

Where there is more than one measuring point the difference in temperature between measuring points should be no more than ±2°C. When comparing the temperature of the room against the specified temperature, the mean of the temperatures recorded at the measuring points is used.

Adjusting readings obtained from thermometers and temperature probes to take account of calibration data.

How to read and use calibration certificates

Example of Thermometer and probe certificate








CERTIFICATE OF CALIBRATION

Issued by: Electrical Mechanical Instrument Services (UK) Ltd.

Date of Issue: 29 June 2008

Certificate Number: 306875

UKAS CALIBRATION

0123

EMIS
Central Equipment Base
Greenwell Road
East Tullos
Aberdeen
AB12 3AX

Tel: 01224 894494
Fax: 01224 894929
email: info@emis-uk.com
web: www.emis-uk.com

emis

Approved Signatory

Approved Signatories : G.Robertson ( )

Equipment Description	: Digital Thermometer c/w Type K Probe & Thermocouple Wire
Manufacturer	: Hanna
Type	: HI93530
Indicator Serial Number	: 54126
Asset Number	: Not Specified
Probe Serial Numbers	: 54126-TW1 & 54126-P
Calibrated Range	: +3°C to +40°C
Date Received	: 18 June 2008
Date Calibrated	: 19 June 2008
Customer	: Scottish Agricultural Sciences
Address	: Edinburgh EH12 9FJ
Job Reference	: 302879/1
Calibration Procedure	: 011
Measurement Method	: Calibration was carried out by comparison against two reference standards within a stirred liquid bath.
Traceability	: All measurements reported in this certificate are traceable to recognised National Standards. The temperature scale used in this Laboratory is the International Temperature Scale of 1990.

Comments

No adjustments were carried out.

Cal. Eng. : D. Coburn

CERTIFICATE OF CALIBRATION

Issued by:
Electrical Mechanical Instrument Services (UK) Ltd.

emis

Applied Standard	Indicated on UUT probe s/n 54126-TW1	Indicated on UUT probe s/n 54126-P
°C	°C	°C
3.01	3.2	2.9 / 3.0
7.03	7.2	7.0
20.04	20.3	20.1
30.06	30.5	30.5
40.09	40.5	40.5 / 40.6

Notes * / * denotes flashing least significant digit.

Immersion Depth : 90 mm

The uncertainty of measurement is

Measured Value of Applied Standard : ±0.25°C

Indication on UUT : ±1 digit

The reported expanded uncertainty is based on a standard uncertainty multiplied by a coverage factor k=2, providing a level of confidence of approximately 95%. The uncertainty evaluation has been carried out in accordance with UKAS requirements.

The uncertainties given in this certificate relate only to the measured values obtained at the time of test, and carry no implication regarding the long term stability of the unit under test.

Error, true value, correction/adjustments of a measured temperature

The calibration certificate of a thermometer/temperature probe gives the following data

Standard Temp	Probe s/n 54126-TW1 (Observed Temp)	Correction
3.01°C	3.2	-0.19
7.03°C	7.2	-0.17
20.04°C	20.3	-0.26
30.06°C	30.5	-0.44
40.09°C	40.5	-0.41

- Standard temperature = reference temperature "true value"
- Observed temperature : temperature read on the thermometer/probe checked
- Error / Correction : difference between the true temperature and the observed temperature. Apply the correction to get the true temperature

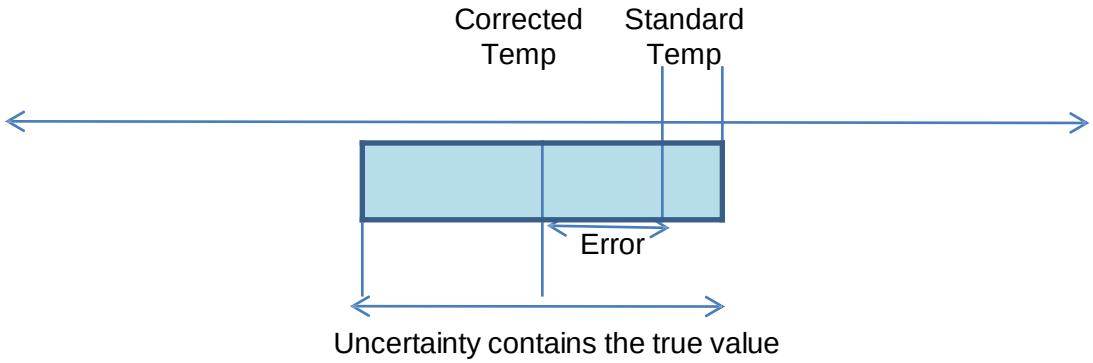
NB : this applies to both reference calibrated thermometers and working thermometers.

Uncertainty of measurement of temperature

After, applying the correction (= (the error)), there is no certainty that the temperature is accurate

Definition: "the uncertainty of measurement (U of M) is the dispersion of the values given to the temperature"

There is a high probability that the true value is within a range of data, illustrated as below.



Uncertainty of measurements

The factors involved in the uncertainty of measurements are:

- Sampling
- Environment (light, RH, ...)
- Precision of reading on the equipment (thermometers)
- Variation between replicates in the same conditions (e.g.: reading 1: 20.0°C; reading 2: 19.9°C ; reading 3: 20.1°C...)

"NF ENV13005-Guide pour l'expression de l'incertitude de mesure"

Tolerances and uncertainty of measurement applied to the check of temperature of incubators, germinators, ovens ….

In practice, in seed testing what do you do with uncertainty of measurement and tolerance?

- Tolerance is an ISTA requirement

"The tolerance is the range of value within which the temperature measured is acceptable"

- uncertainty of measurement gives the accuracy of the probe

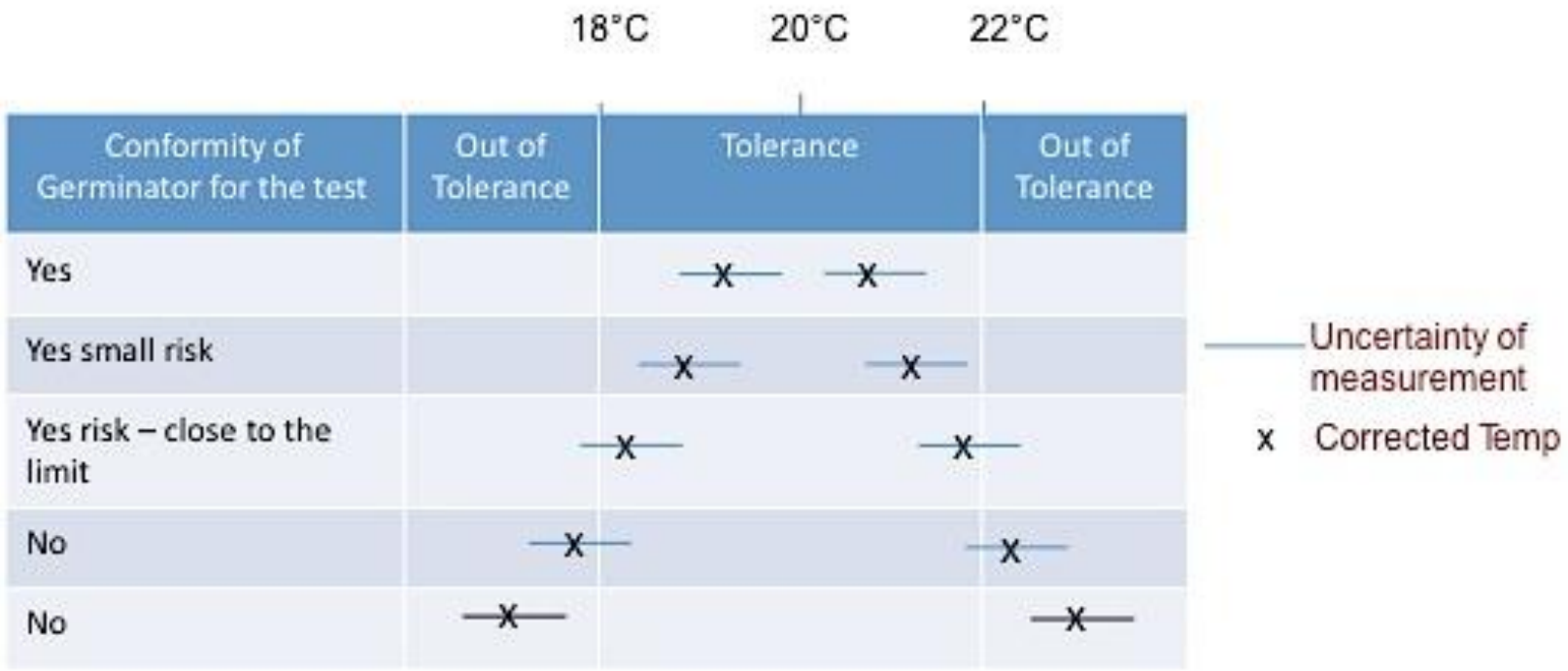
"The uncertainty of measurement must be small compared to the tolerance "

It is generally accepted that uncertainty of measurement/tolerance must be ≤ 1/10 the value of the tolerance

Equipment is considered conform when the corrected check temperature is within the limits of temperature (tolerance) and the uncertainty of measurement is *≤ 1/10*

Equipment is considered conform when the error corrected check temperature is within the tolerance

Example of application of this rule to germination testing:



Tolerances, error and uncertainty of measurement applied to the check of temperature of incubators, germinators, ovens ….

Conclusions

- The error is applied as a correction to the observed temperature to get the “true temperature”
- The tolerance is checked after the correction of the Observed Temperature → gives the conformity of the equipments acceptance of the analyses results
- Uncertainty of measurement is used for the acceptance of the thermometer/probe. As a general rule, the uncertainty of measurement must be ≤ 1/10 (0.2 °C*).
- In ISTA seed testing tests, uncertainty of measurement is **not** used on a daily basis to decide on the temperature conformity of equipment.

*in the case of germination testing

Frequency of External Calibration of Thermometers and probes

External calibration of Reference thermometers should be carried out at least every 5 years and and this should be supplemented by annual in-house ice point calibration.

Any probe whose temperature reading differs from the standard by more or less than ±0.5°C should be removed from service.

Calibration using steam/boiling water should not be attempted.

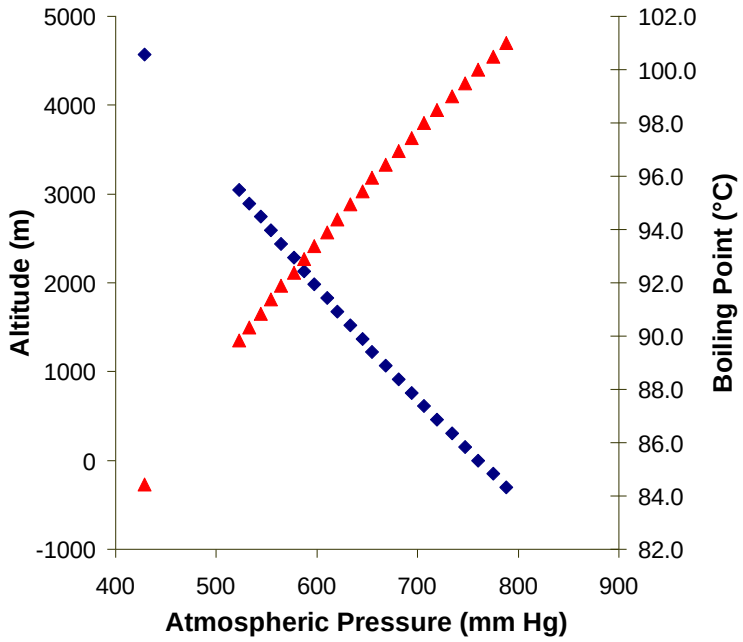
Calibration of thermometers international ice point method

International methodology should be followed.

The ice point may be realised in an insulated flask or vessel containing an ice-water melting mixture. The ice particles should be no more than a few millimetres in diameter and the water and ice should be pure or prepared from de-ionised water, which is air saturated. For high precision the thermometer should be maintained in the mixture for 10 minutes prior to reading. In theory accuracies of ±0.001°C may be achieved but in practice ±0.005°C is more likely.

Calibration of thermometers steam/boiling water method

The boiling point of water varies with atmospheric pressure and this is dependant on both weather conditions and altitude. At sea level with an atmospheric pressure of 760mm Hg the boiling point may be 100°C but in Kathmandu in Nepal



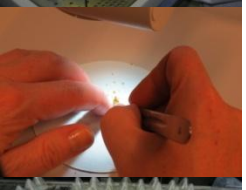
at an altitude of 1310m and atmospheric pressure of 650mm Hg the boiling point is 95.5°C.

Temperature probes should not Calibrated using steam/boiling water.

What about working thermometers and probes?





Working thermometers and probes

These do not need to be externally calibrated but an internal check must be carried out and corrections made on the basis of this check.

The internal check is carried out by comparing them with an externally calibrated thermometer/probe. An ice point check may also be undertaken.

Checks using steam/boiling water are not appropriate

Calibration of thermometers against an external standard

When Calibrating a batch of thermometers against an externally calibrated probe the calibration should be carried at temperatures within the normal working range (e.g. 10°C, 15°C, 20°C,25°C, 30°C) and in homogenous, stable conditions where fluctuation in temperature between readings is limited.

Where working probes are calibrated against an externally calibrated probe this should take place at least once a year.

Any probe whose temperature reading differs from the standard by more or less than $\pm 0.5^{o}C$ should be removed from service.

Error and correction of working thermometers and probes

The same applies for working probes as for externally calibrated reference probes. To estimate the standard temperature (“True temperature”), two corrections need to be made:

- The correction of the externally calibrated reference probe (if ≠ 0)
- The correction of the working probe

The corrections are additive, taking in consideration the positive, or negative signs of the corrections

Question

- An externally calibrated reference probe has a correction of +0.3°C
- A working probe calibrated against the above has a correction of -0.1°C
- In your incubator you read with the working probe 20.5°C what is the “true temperature” ?

Solution

The temperature is 20.5 -0.1 +0.3 = 20.3°C

The correction applied to the working probe is:

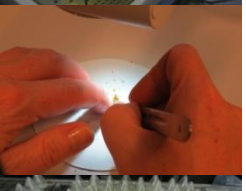
0.3 – 0.1 = **0.2°C**

Conclusions

- The correction of the working probe is used to adjust temperature readings
- The uncertainty of measurement of the working probe is **not** required. A series of measures may indicate whether a probe is reliable or not, in case of doubt, get it calibrated externally or replace it.

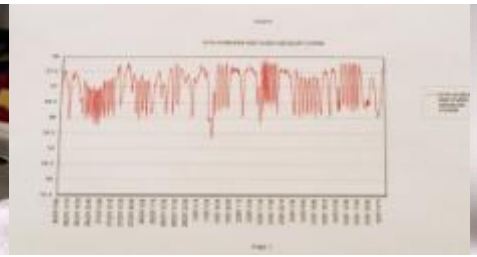





Calibration of Thermocouples, Electronic Meters and Temperature Data loggers






The procedures for the calibration of thermocouples and temperature probes of electronic meters and data loggers should be same as that adopted for thermometers. In other words they can be calibrated externally, or in-house:

- against a externally calibrated probe or thermometer; or
- using the ice point method.

As with thermometers, any probe that differs by more than ±0.5°C from the standard should be removed from service.

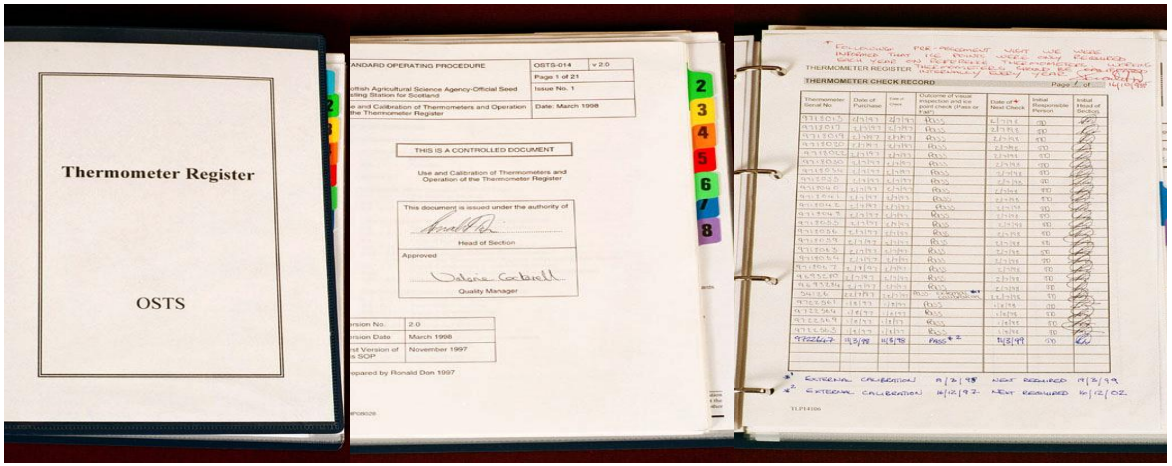



Records of Calibration Checks

ISTA Accreditation Standard:

"*9.9 All records of results, calibration, maintenance and repair of equipment and reference materials must be kept for a minimum of six years*"

Calibration Checks should be recorded and be available for inspection. The easiest way to do this is in a Thermometer/Probe Register. Calibration and temperature records for individual pieces of apparatus should be archived for a period of at least six years.



Temperature control

Temperature profiles

- Does it make a difference where my sample is located (on which shelve, on which spot)?
- Will it always be in the required temperature range?
- Are there areas in the equipment which are not suitable and that might need to be excluded?

Temperature Profiles

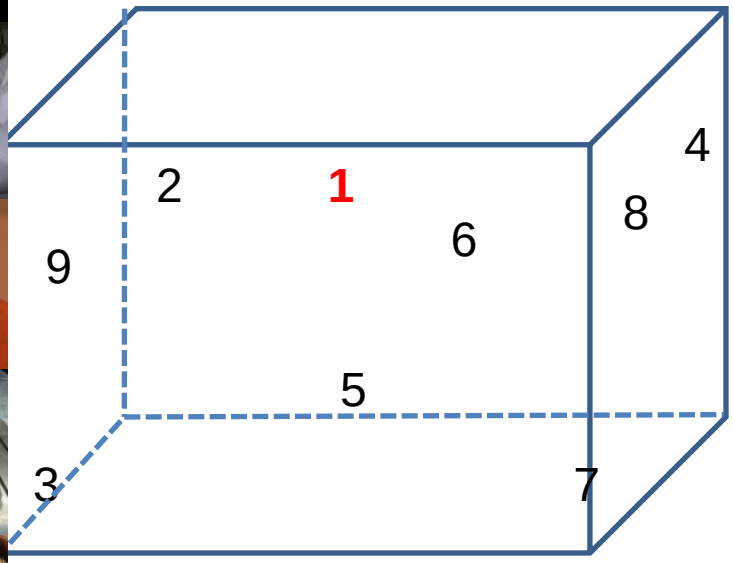
Before accepting a piece of temperature controlled equipment (ovens, incubators, fridges, germination rooms, etc.) into service a laboratory must ascertain the temperature profile of the equipment to ensure that it is fit for purpose.

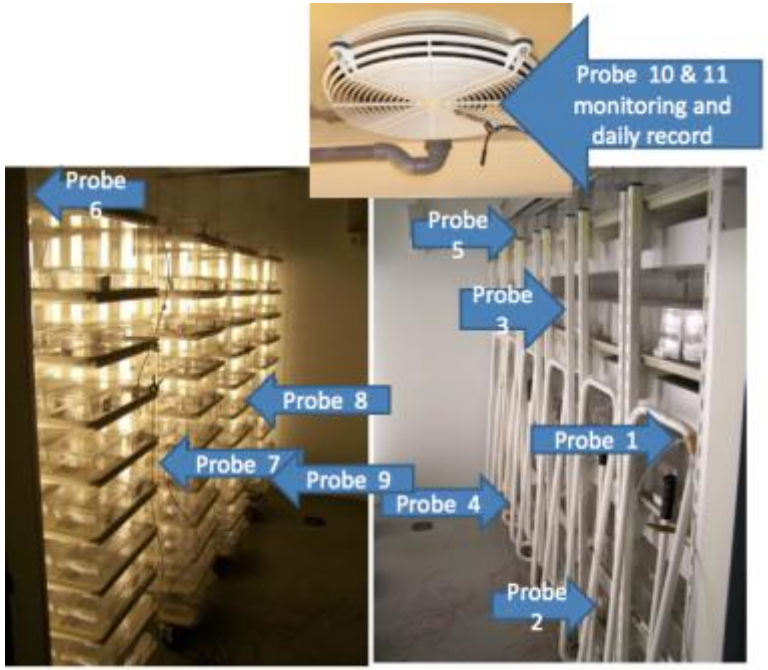
There should be a minimum of 9 check points covering different combinations of height, depth and breadth, as appropriate. For example, for incubators, fridges and ovens temperature should be recorded on top, middle and bottom shelves and on each shelve the temperature should be measured at 3 points; front (next door), middle and back. The temperatures of all points measured must be within $\pm$ 2^{o}C before a piece of equipment is accepted into service.

Once in service profiles need only measured again if a piece of equipment undergoes repair or major service.

Temperature control - Germinator

- 8 spots to record the temperature profile (will be removed later)
- 1 spot for the "monitoring probe" (will remain)





Temperature control - Germinator

From the records obtained:

- Define which areas in the germinator are suitable
- Adjust the temperature setting to shift more toward the middle of the temperature range (new readings for profile would be required)
- Calculate the acceptable temperature of the "monitoring probe"

THANK YOU TO:

RONALD DON

ISTA SECRETARIAT

AND YOU FOR YOUR ATTENTION!