"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

Check of germination substrates

Rita Zecchinelli

Goal of this presentation

- Describe ISTA germination media specifications
- Provide guidance on how requirements for ISTA germination media specifications can be measured

ISTA Requirements Germination Media

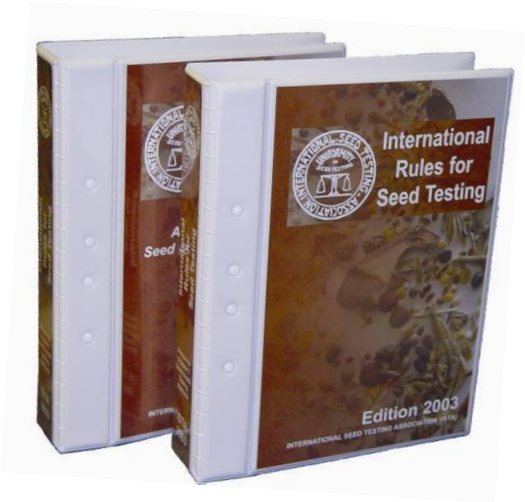
Growing Media used for germination tests are paper, sand and organic growing media.

The media must provide:

- ✓ sufficient pore space for air and water;
- ✓ anchorage of seedlings root system; and
- ✓ solutions (water) needed for the seedlings growth

ISTA Rule 5.4.1

The ISTA Rules: Growing Media



The ISTA Rules provide analysts with definitions and specifications for growing media in terms of:

- Composition
- Water retention characteristics
- pH
- Conductivity
- Cleanliness and freedom from toxicity

ISTA Rules: Growing Media composition

The growing medium can be paper, pure sand or mixtures of organic compounds with added mineral particles

ISTA Rule 5.4.2

Paper: Composition and characteristics

The paper should be of wood, cotton or other purified vegetable cellulose and may take the form of filter pads, blotters or towels.

The paper should be such that:

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

ISTA Rule 5.4.3.1




Sand: Composition and characteristics 1/2

The sand should be reasonably uniform and free from very small and large particles. Round particles are preferable and it is recommended that sand with sharp particles, that may impair seedling development, is avoided.

ISTA Rule 5.4.3.2

Sand: Composition and characteristics 2/2

At least 90% of the particles must pass through a sieve with holes or meshes of 2.0 mm width. If the particle size characteristics given by the supplier are in accordance with these specifications then the laboratory does not need to perform a quality check of the sand particle size. In the absence of a supplier's specification sheet, the laboratory must check the particle size for each batch of sand received.

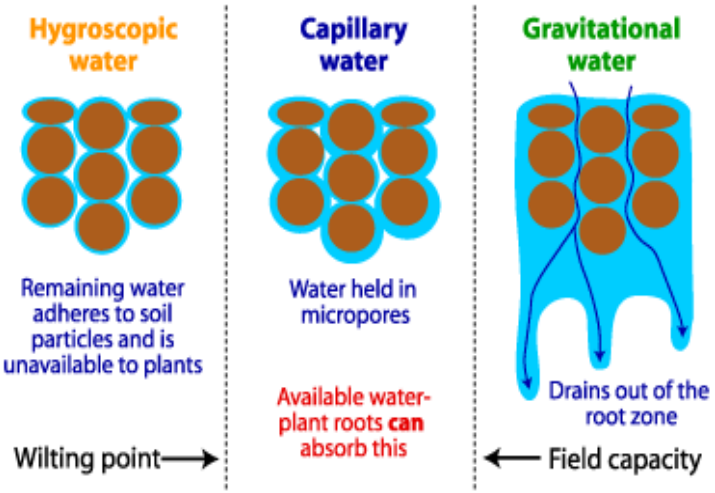
Organic Growing Media: Composition and characteristics

Organic Growing Media is a mixture of organic compounds such as peat, coconut fibres and wood fibres, with a recommended size of less than 5 mm, and mineral particles.

Mineral particles such as sand, perlite, dolomite and vermiculite. The proportion should be between 15 and 20% in volume. It is recommended that 90% of the mineral particles should pass through a sieve having holes or meshes of 3 mm width. **ISTA Rule 5.4.3.3**




ISTA Rules: Water Retention



Available water for plant growth

The media should have the capacity to hold sufficient water to provide continuous movement of water to the seeds and seedlings but also provide sufficient pore space for aeration required for optimal germination and root growth.

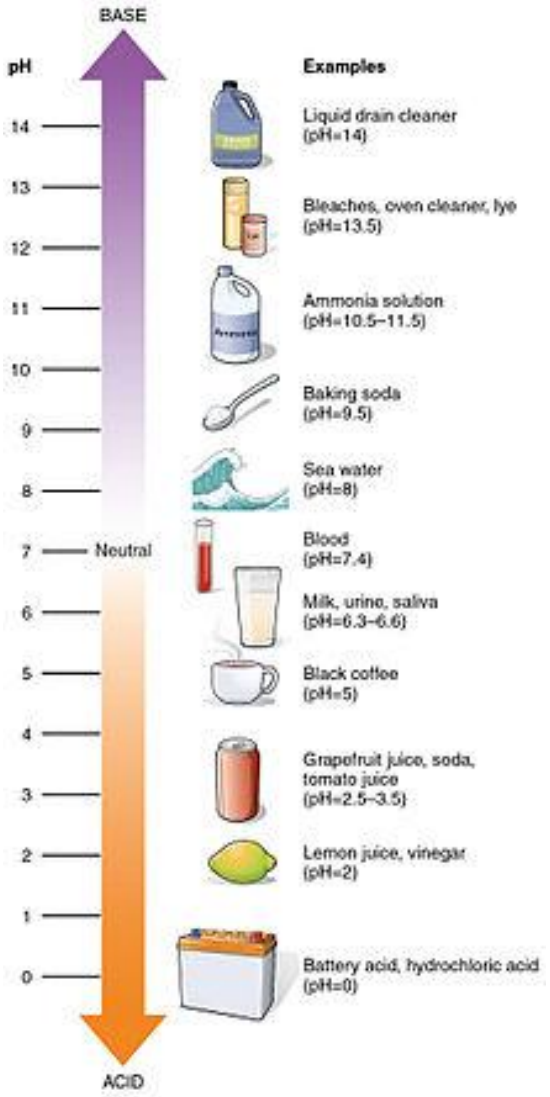
The water content of the growing media should be adjusted to correspond to the need of the species, based on the maximum water holding capacity and shall then be expressed as a percentage of the maximum retention.

ISTA Rule 5.4.2

ISTA Rules: pH

The growing media must have a pH value within the range 6.0–7.5 when checked in the substrate.

ISTA Rule 5.4.2

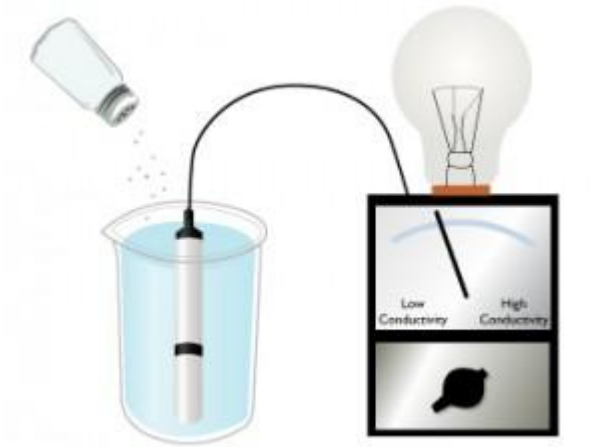


http://en.wikipedia.org/wiki/PH

ISTA Rules: Conductivity

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

ISTA Rule 5.4.2



Conductivity is a measure of the water's ability to conduct electricity.

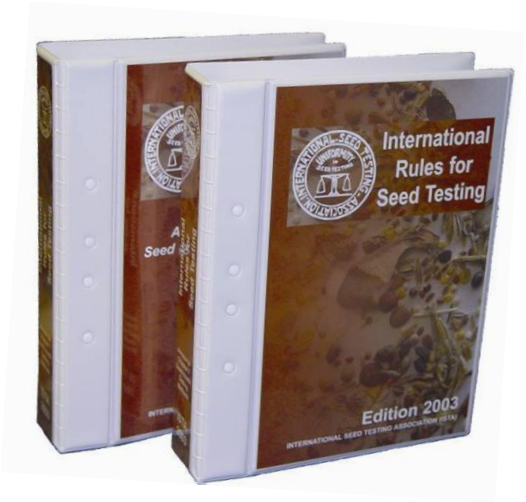
Salinity and conductivity measure the water's ability to conduct electricity, which provides a measure of what is dissolved in water. A higher conductivity value indicates that there are more ions dissolved in the water.

ISTA Rules:
Cleanliness and freedom from toxicity

The growing media must be free from seeds, fungi, bacteria or toxic substances which may interfere with the germination of seeds, the growth of seedlings or their evaluation.

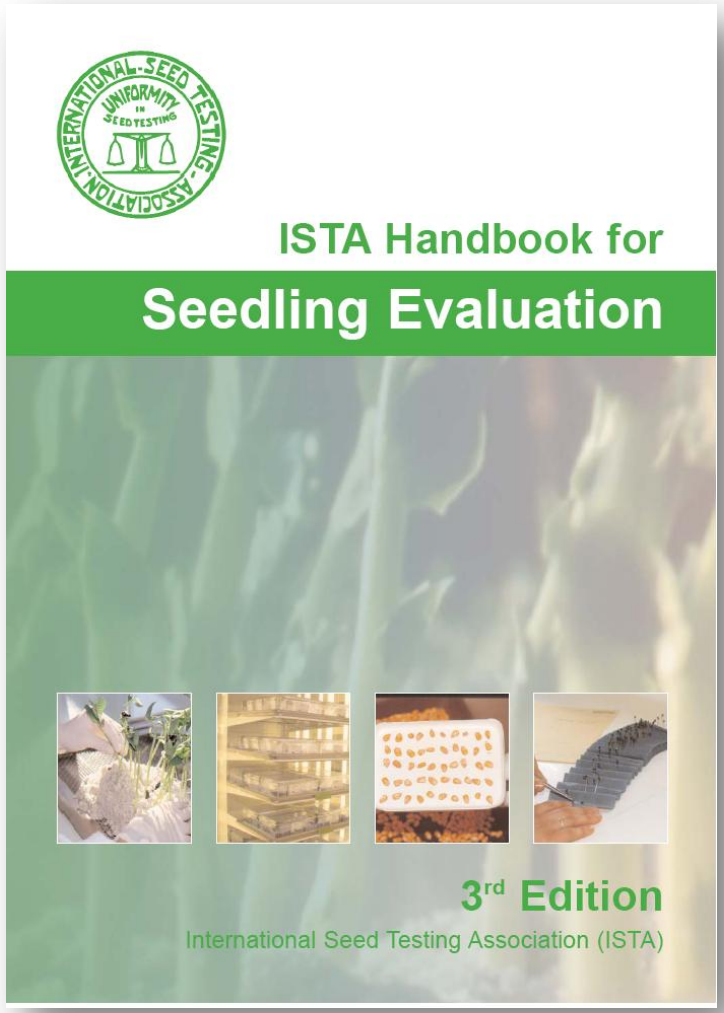
ISTA Rule 5.4.2

Growing media specifications: How does a laboratory ensure compliance?



The ISTA Rules gives the specifications but does not give procedures for the measurement of the different attributes

ISTA Handbook on Seedling Evaluation



The ISTA Handbook on Seedling Evaluation provides the analysts with illustrative Standard Operating Procedures (SOPs); they are guidelines showing how the following attributes of growing media can be measured:

- Water Retention
- pH
- Conductivity
- Cleanliness and Innocuity

ISTA Handbook on Seedling Evaluation: A5.3 Water Retention








A5.3 Germination Procedur
Specification Checks – Wate

A5.3.1 Specification

The ISTA Rules give the specification for water r
media.

When the appropriate amount of water is added,
should have the capacity to hold sufficient wate
of water to the seeds and seedlings. It should al
aeration required for optimal germination and roo
of the growing media shall be adjusted to the
When necessary, for certain species, it can be adj
a particular species. The water retention shall the
the maximum retention.

A5.3.2 Measurement Principle

The benchmark for the measurement of the wa
ISO method 11274 (1998) (as updated): 'Soil qu
determination of water retention characteristic.

The general principle is to measure the maxim
substrate and express this as a percentage of the

A5.3.3 Procedure

The moisture content (MC) of the substrate is mea
according to the ISTA Rules Chapter 9. The hig
used (Figure 1a and b).

The water present in the substrate is equal to MC

Weights are measured to one decimal place.

A defined weight of substrate (W_s) is placed in a
holes that are covered by a filter, which allows e
substrate (Figure 2).

Water is added till the substrate becomes satura
filter from the container over a 12 hour period
to prevent evaporation. At this level of moisture
Capacity and its weight is equal to W_{FC} (Figure 3)

The amount of water present in the substrate bef

$$(H_2O)_S = W_s \times MC$$

The dry weight of the substrate used = $(DW)_S$

$$(DW)_S = W_s - (H_2O)_s$$

The amount of water present when the substrate

$$(H_2O)_{FC} = W_{FC} - W_s + (H_2O)_s$$

The maximum amount of water held in growing media as percentage of its dry
= $(H_2O)_{MAX}$

$$(H_2O)_{MAX} = [(H_2O)_{FC} / DW_S] \times 100$$

For each batch of media at least three measurements should be made on r
samples of the batch. The average of the measurements is used to check comp
with the specification for the media.

Table 1: Example Calculations for Paper, Organic Growing Media and Sand.

	Paper Media	Organic growing media	S
Moisture Content determined using High Constant Temperature Oven Method (130°C for 1 hour) **(MC)**	**7.1%**	**32.0%**	**1**
Weight of Substrate used to determine water retention **(W_s)**	**144.5 g**	**257.2 g**	**6**
Weight of saturated substrate **(W_{FC})**	**602.0 g**	**508.4 g**	**74**
$(H_2O)_S = W_s \times MC$	= 144.5 x 0.071 = **10.3 g**	= 257.2 x 0.32 = **82.3 g**	= 620 = **5**
$(DW)_S = W_s - (H_2O)_s$	= 144.5 – 10.3 = **134.2 g**	= 257.2 – 82.3 = **174.9 g**	= 62 = **5**
$(H_2O)_{FC} = W_{FC} - W_s + (H_2O)_s$	= 602.0 – 144.5 + 10.3 = **467.8 g**	= 508.4 – 257.2 + 82.3 = **333.5 g**	= 740.5 = **2**
$(H_2O)_{MAX} = [(H_2O)_{FC} / DW_S] \times 100$	= (467.8/134.2) x 100 = **348.6%**	= (333.5/174.9) x 100 = **190.7%**	= (216.6/ =

A5.3.4 Illustrated Procedure


Figure 1a and b: The Moisture Content of the Germination media is measured using the ISTA Constant Temperature Oven method at 130°C.

Figure 2a-c: The germination media is weighed. For sand and organic growing media a waterproof container with drainage is required. The drainage holes are covered using a material, such as filter paper, that allows water drainage but prevents the loss of material.

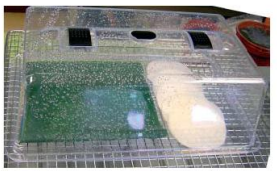



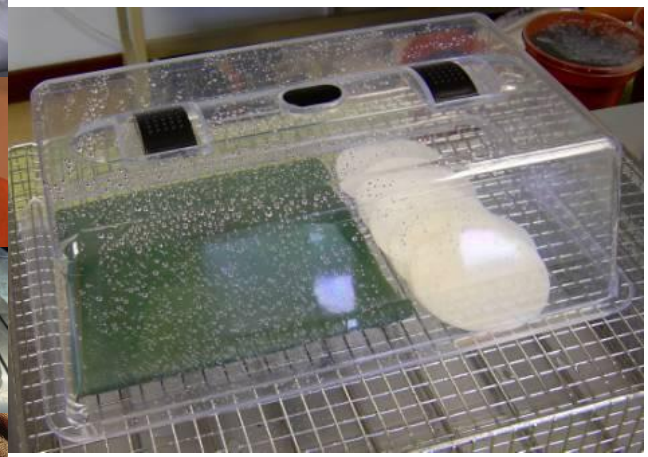
Figure 3a and b: The Germination media is saturated with water and allowed to freely drain for 12 hours with measures being taken to prevent evaporation. The saturated media is then weighed and the maximum amount of water held in the growing media as percentage of its dry weight is calculated.

Water Retention: illustrated procedure

The Moisture Content of the Germination media is measured using the ISTA Constant Temperature Oven method at 130°C.

The germination media is weighed.

Water Retention: illustrated procedure



The Germination media is saturated with water and allowed to freely drain for 12 hours with measures being taken to prevent evaporation.

The saturated media is then weighed and the maximum amount of water held in the growing media as percentage of its dry weight is calculated.








Water Retention: illustrated procedure

Details of the calculation are in the SOP

The amount of water present in the substrate before it is saturated = $(H_2O)_S$

$(H_2O)_S = W_s \times MC$

The dry weight of the substrate used = $(DW)_S$

$(DW)_S = W_s - H_2O_s$

The amount of water present when the substrate is at Field Capacity = $(H_2O)_{FC}$

$(H_2O)_{FC} = W_{FC} - W_s + (H_2O)_s$

The maximum amount of water held a growing media as percentage of its dry weight = $(H_2O)_{MAX}$

$(H_2O)_{MAX} = (H_2O_{FC} / DW_S) \times 100$

Germination Growing Media Specification Checks
Water Retention

Measure the moisture content of the growing media using the ISTA high constant oven method (130°C for 2 hours).
The moisture content is expressed as the percentage of water in the growing media divided by the weight of the media.

Moisture Content =
A

A defined weight of growing media is saturated with water and excess water is allowed to freely drain over a 12 hour period during which measures are taken to prevent evaporation

Weight of growing media =
B

Weight of growing media after saturation =
C

Calculations

1. Multiply the weight of growing media by the Moisture Content.
A x B = **D**
This is the amount of water in the growing media before wetting

2. Subtract the amount of water in the growing media before wetting from the weight of the growing media
B - D = **E**
This is the dry weight of the media

3. Subtract the dry weight of the growing media from its weight after saturation
C - E = **F**
This is the maximum amount of water held in the growing media

4. Express the maximum amount of water held in the growing media as a percentage of the dry weight
(F/E)100

There is a:
Water retention flow chart that gives step by step instructions.

ISTA Handbook on Seedling Evaluation:
A5.3 Water Retention
A5.3.5 calculation flow chart








An excel sheet has been developed to help in the calculation:

Moisture Content Measurement of Growing Media

		Replicate 1	Replicate 2
1.	Weight of empty moisture container		
2.	Weight of moisture container with germination media		
3.	Weight of moisture container with germination media after drying		
4.	Moisture content	#DIV/0!	#DIV/0!

A.	***Moisture Content of Growing Media***	***#DIV/0!***

Water Retention Measurements

		Replicate 1	Replicate 2	Replicate 3
B.	Weight of Growing Media			
C.	Weight of Growing Media after Saturation			
D.	Amount of water in growing media before saturation			
E.	Dry weight of the growing media	-	-	-
F.	Maximum amount of water held in the growing media	-	-	-

Maximum amount of water held in growing media as a % of the dry weight	#DIV/0!	#DIV/0!	#DIV/0!

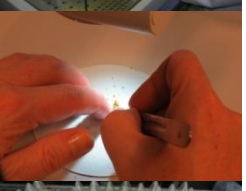
Maximum amount of water held in growing media as a % of the dry weight	***#DIV/0!***

The Excel sheet is downloadable at ISTA Website:
http://seedtest.org/en/tool-box-_content---1--1191.html

ISTA Handbook on Seedling Evaluation: A5.4 pH





A5.4 Germination Procedures - Growing Specification Checks – pH

A5.4.1 Specification

The ISTA Rules give the specification for the pH of growing media.

The pH value should be within the range 6.0-7.5 when checked in the

A5.4.2 Measurement Principle

The benchmark for the measurement of the pH is the ISO method 103 (updated): 'Soil quality – Determination of pH'.

The general principle is to measure the pH of the water available fo when checked within the substrate.

A.5.4.3 Procedure

The preparation for measurement varies according to the germination

Organic Growing Media and Sand

Samples of 5 ml or more of the organic growing media or sand a 5 volumes of water that is to be used for germination tests[1]. T stirred for 5 min and then allowed to stand for a minimum of maximum of 24 hours. After standing the mixture is stirred and pH value of the suspension solution measured (Figure 1).

Paper Media

Samples of germination paper are moistened with water that is germination tests[1] and the pH is measured on the surface of the

The pH can be measured using pH paper with an appropriate ra and 3) or using a calibrated pH meter (Figure 4). For paper med a pH meter a specific probe manufactured for measuring the pH of paper must be used (Figure 5 and 6).

For each batch of media at least three measurements should random samples of the batch.

Should the three measurements differ by more than 0.5 the batch heterogeneous and should be rejected.

The average of the measurements is used to check compliance specification.

[1] It is recommended that the conductivity of the water should be < 0.2 r and its pH should be > 5.6 at 25°C.

A5.4.4 Illustrated Procedure


Figure 1a and b: For sand and organic growing media, one volume of media is mix with 5 volumes of water that is to be used for germination tests. The mixture is stirr for 5 min and then allowed to stand for a minimum of 2 hours and a maximum of hours. After standing the mixture is stirred and the stabilised pH value of the suspensi solution measured.

Figure 2a and b: Paper media samples are moistened with water that is to be used for germination tests[1] and the pH is measured on the surface of the paper. The pH is measured using a calibrated pH meter or pH paper.

Figure 3: Using pH paper to measure t pH of paper germination media.

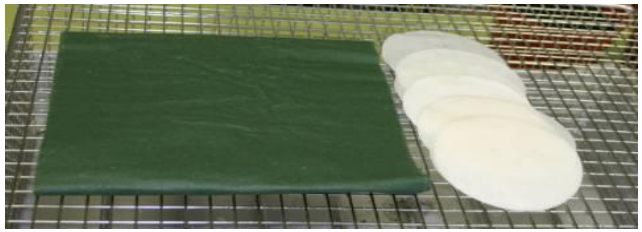
[1] It is recommended that the conductivity of the water should be < 0.2 milliSiemens/ and its pH should be > 5.6 at 25°C.

Figure 4: A pH meter with a specific probe manufactured for measuring the pH on the surface of paper must be used for paper media.

Figure 5: Surface (left) and dip (right) probes for pH meter.

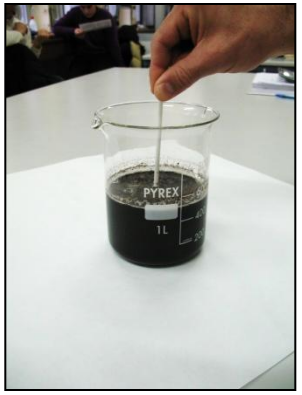
Figure 6: Surface probe for measuring the pH of paper.

pH: illustrated procedure

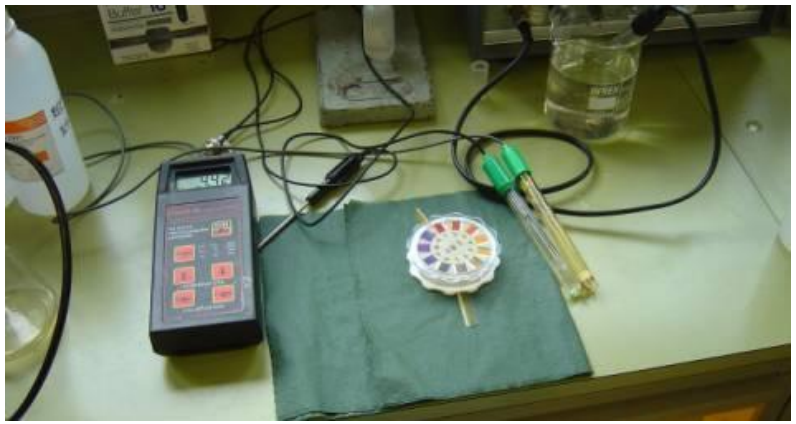


Paper Media samples are moistened with water used for germination tests and the pH is measured on the surface of the paper.

For Sand and Organic Growing Media, one volume of media is mixed with 5 volumes of water used for germination tests. The mixture is stirred for 5 min and then allowed to stand for a minimum of 2 hours and a maximum of 24 hours. After standing the mixture is stirred and the stabilised pH value of the suspension solution measured.



pH: illustrated procedure



The pH is measured using a calibrated pH meter or pH paper.

Using pH paper to measure the pH of paper germination media.

Surface (left) and dip (right) probes for pH meter.

pH: illustrated procedure

A pH meter with a surface probe must be used for paper media.

At least three random samples from each batch of media should be checked. Five readings are recommended for each sample.

Tipo di carta: *dischi Ø 14*			**Data:** *gg/mm/aaaa*		**Operatore:** *A.A.*		
Campione 1	pH 6,24	pH 6,18	pH 6,14	pH 6,27	pH 6,05	media	6,2
Campione 2	pH 6,37	pH 6,13	pH 6,34	pH 6,15	pH 6,17	media	6,2
Campione 3	pH 6,24	pH 6,23	pH 6,19	pH 6,25	pH 6,08	media	6,2
						pH	**6,2**

pH: illustrated procedure

A pH meter with a dip probe must be used for sand and organic media.

At least three replicates from each batch of media should be checked.

Substrato: *organico (tipo XXX)*	**Data:** *gg/mm/aaaa*	**Operatore:** *A.A.*
Campione 1		pH 7,0
Campione 2		pH 7,1
Campione 3		pH 7,3
MEDIA		**pH 7,1**

The measurements of random samples or replicates shouldn't differ by more than 0,5.

The average of the measurements is used to check compliance with the specification.

ISTA Handbook on Seedling Evaluation: A5.5. Conductivity

A5.5 Germination Procedures - Growing Media Specification Checks – Conductivity

A5.5.1 Specifiation

The ISTA Rules give the specification for the conductivity of the growing media.

Conductivity: the salinity must be as low as possible and no more than 40 milliSiemens per metre.

A5.5.2 Measurement Principle

The benchmark for the measurement of conductivity is the ISO method 11265 (1994) (as updated): 'Soil quality: determination of specific electrical conductivity'.

The general principle is to measure the conductivity of solutes in the media.

A5.5.3 Procedure

For paper, sand and organic growing media, 20 g are mixed with 100 ml of water, which is used for germination tests[1], at 20°C ± 1°C. This is stirred for 30 min before obtaining the solute by passing the mixture through a filter paper (Figure 1).

The solute conductivity is measured using a calibrated conductivity meter employing a dip cell (Figure 2).

For each batch of media at least three measurements should be made on random samples of the batch.

If the difference between replicates is greater than 5 milliSiemens per metre the batch of media should be rejected.

The average of the measurements is used to check compliance with the specification.

[1] It is recommended that the conductivity of the water should be < 0.2 milliSiemens/m and its pH should be > 5.6 at 25°C.

A5.5.4 Illustrated Procedure

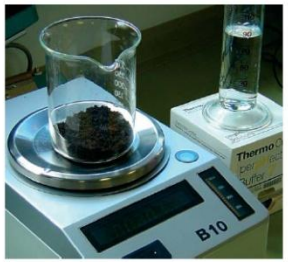


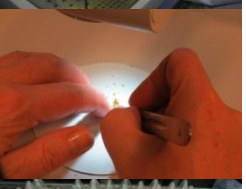
Figure 1a and b: 20 g of media are mixed with 100 ml of water, which is used for germination tests[1], at 20°C ± 1°C (a). This is mixed and left for 30 min before filtering (b).

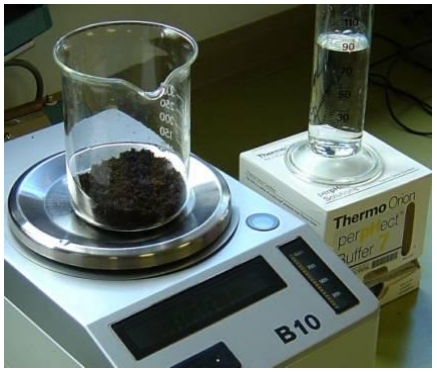
Figure 2: The conductivity of the filtrate is measured using a calibrated conductivity meter using a dip cell

Conductivity: illustrated procedure







20g of media are mixed with 100ml of water, which is used for germination tests, at 20° C ± 1° C. This is stirred for 30 minutes before filtering.

The conductivity of the filtrate is measured using a calibrated conductivity meter using a dip cell.

At least three replicates from each batch of media should be checked. The measurements of the replicates shouldn't differ by more than 5 milliSiemens. The average of the measurements is used to check compliance with the specification.

ISTA Handbook on Seedling Evaluation: A5.6 Cleanliness and Innocuity








A5.6 Germination Proce Media Specification Che Innocuity

A.5.6.1 Specification

The ISTA Rules give the specification for t media.

The growing media must be free from s which may interfere with the germination evaluation.

A substrate which shows statistical eviden normal seedlings is decreased compared due to toxic materials in the substrate is symptoms can be sufficient to declare a su not be used for germination tests.

The presence in the substrate of micro-o can affect the germination or the developr statistical evidence that the number of decreased compared to a reference substra must not be used for germination tests use. Disinfection should be carried out in suppress or kill seed borne disease orga In addition, disinfection must not render phytotoxicity and micro-organisms should

A.5.6.2 Measurement

Cleanliness and innocuity of media are de

A.5.6.3 Procedure

Tests for Phytotoxicity

Germination tests are carried out: with th substrate (media acceptance test).

To verify that a bach of media is suitab species evaluated in the laboratory that ar are used: *Agrostis gigantea, Eragrostis Lepidium sativum, Petunia* sp. and *Phleu*

At least 400 seeds each of two sensitive media taken at random from the batch of

Evaluating the tests requires the assessm

- The percentage of germinated seed specified in Table 5A of the ISTA R the test).
- The occurance of normal and abnor

substances, non-germinat

- Specific symptoms amor tips, roots raised from tl hypocotyls. In Poaceae s and shortened.

When the media is to be used *Hordeum vulgare* or *Zea mays*, species rather than two sensitiv

For those unfamiliar with phytot set up. This is the reference sut phytotoxic effects, is added, for of 14 mg/litre.

Analysis of the results should be

Visual evidence of the absence significant difference between th are required to declare a substr

Tests for Freedom from the

The biological test used to ass observation of the number of de

A.5.6.4 Acceptable carrying out Analysis

General rule, when carrying out the new media gives a lower re obtaining such a result by chan the media should be rejected. It can be taken to alleviate its d probability is close to the limit, decision as to wether reject the

A.5.6.5 Examples of Biological Tests for C

Laboratories should analyse the Analysis of Variance (ANOVA) undertaken.

Example 1 – Media is Pl the Germination of Indi

Observations

In test media stunted root growtl the germination media. (Photog effects as a result of harmful su Section A.5.5.6).

[1] Germination results are the mea

Results of Germination

Table 1: Results[1] of germina the reference media.

Sample
1
2
3
4
Mean

Table 2: Single Factor Analy

Source of Variance	Degrees of Freedom
Total	7
Media	1
Error	6

The germination of the in and the ANOVA shows tha (82.5%) is significantly lo The probability of obtainir should be rejected and mu

Example 2 – Media Species

Observations

In test media there wa development.

Results of Germination

Table 3: Results[1] of germina the reference media.

Sample
1
2
3
4
Mean

[1] Germination results are th

Table 4: Single Factor A

Source of Variance	Degree Freedo
Total	7
Media	1
Error	6

Although there appears The ANOVA shows tha (88.5%) is significantl The probability of obta need to be undertake increase in the numbe media? This batch of m tests unless action can

Example 3 – Medi Species

Observations

In test media there development.

Results of Germinati

Table 5: Results[1] of gern the reference media.

Sample
1
2
3
4
Mean

Table 6: Single Factor A

Source of Variance	Degree Freedo
Total	7
Media	1
Error	6

There appears to be ANOVA shows that the not significantly differe

[1] Germination results ar

of media can be ac obtained using ano

A.5.6.6 Pho phytotoxic e

Figure 1: *Hordeum* s 2,4 D in the germina

Figure 2: *Lepidium* s the blotter pad. See to normal seedlings.

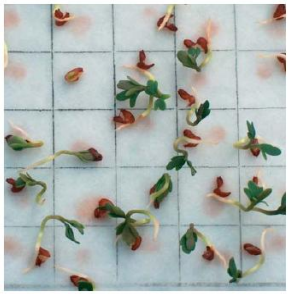



Figure 3: *Lepidium seedlings:* those on the right are normal having been affected by high levels of salinity in the germination medium whilst those on the left are abnormal.

Cleanliness and Innocuity: Step by step procedure 1/2

- Samples of the new media are tested alongside samples of media currently in use
- Indicator species are used if the media is to be used for a wide range of species
- If only one of two species will be tested using the medium then they are used to evaluate the media
- Signs of phytotoxicity, disease or other problems are noted. Specific symptoms are:
  - shortened roots
  - roots raised from the substrate
  - discoloured tips
  - short and thick hypocotyls
  - reduced growth

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- Germination results obtained using the new batch of media are compared (ANOVA TEST) to those obtained using the current media:
  - the occurrence of abnormal seedlings with symptoms of phytoxicity (easier at an early stage)
  - the occurrence of normal, abnormal seedlings, no germinated seeds (final count)

    (optimum: 400 seeds/2 species/4 samples)
- If there are no problems and no significant differences between the germinations obtained using the old and new batches of media the new batch is accepted for use in the laboratory.

Thank to:
Ronald Don
ISTA Secretariat
and to you for your attention

Any Questions?