Quality Assurance in Germination Testing

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Agenda

Control of equipment

• Germinator

Control of supplies

• Gowing media

• Water

Monitoring
Temperature control of Equipment:

- Where to measure
- Temperature distribution
- Daily temperature check
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)
Why temperature distribution in germinator

- 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 my germinator which are not suitable and that might need to be excluded?
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”
Temperature control - Germinator

Constant temperature equipment

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

However...

If records show temperature is stable in terms of temperature with variations of less than 1.0 °C 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.
Temperature control - Germinator

Alternating temperature equipment

- At least 3 readings should be recorded per day
- At regular pre-set times
- Monitoring of both temperature phases

E.g. alternating temperature of 20 <= 30:

- 20°C for 16h, e.g. two readings
- 30°C for 8h, e.g. one reading
Temperature control - Germinator

Alternating temperature equipment

Change over time between high and low temperature phase should not be more than 3 hours

➢ Measure the temperature versus time
Temperature control - Germinator

Use a temperature measurement instrument with a scale range divided into units of at least 0.5°C

Different types of temperature measuring equipment

Usage of datalogger or min/max devices
Control of Equipment

In general all equipment used which might have an influence on the germination test result, should be controlled and verified, e.g.:

- Water dispenser
- Balances
- Vacuum counter
Control of Equipment

Vacuum counter

• Do not select seeds
• Count seeds by hand 10 times and use the vacuum counter 10 times
• Germinate samples
• Compare the outcome of both results
Control of Supplies

E.g. paper, sand, organic growing media

1. Water retention characteristics
2. pH Value *)
3. Conductivity *)
4. Cleanliness and Innocuity

*) can be replaced by biological tests (ISTA Rules 5.4.5)

Examples given in ISTA Handbook on Seedling Evaluation
1. Water retention

- The maximum amount of water held by the growing media is expressed as a percentage of its dry weight.

- The amount of water added to the medium should be expressed as a percentage of the maximum water holding capacity.
The moisture content of the germination medium must be determined.

A defined weight of substrate is placed in a waterproof container that allows excess water to drain without loss of substrate.
- Water is added till the substrate becomes saturated and excess water is allowed to filter from the container over a 12 hour period during which measures are taken to prevent evaporation

- Weight of saturated media
### Moisture Content Measurement of Germination Media

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<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<tbody>
<tr>
<td>1</td>
<td>Weight of empty moisture container (g)</td>
<td>Replicate 1</td>
<td>Replicate 2</td>
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<td>2</td>
<td>Weight of moisture container with germination media (g)</td>
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<td>3</td>
<td>Weight of moisture container with germination media after drying (g)</td>
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#### Water Retention Measurements

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</thead>
<tbody>
<tr>
<td>5</td>
<td>B. Weight of germination media (g)</td>
<td>Replicate 1</td>
<td>Replicate 2</td>
<td>Replicate 3</td>
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<td>6</td>
<td>C. Weight of germination media after saturation (g)</td>
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<td>7</td>
<td>D. Amount of water in germination media before saturation (g)</td>
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<td>8</td>
<td>E. Dry weight of the germination media (g)</td>
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<tr>
<td>9</td>
<td>F. Maximum amount of water held in the germination media (g)</td>
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<td>10</td>
<td>Maximum amount of water held in germination media as a % of the dry weight</td>
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<td>11</td>
<td>Maximum amount of water held in germination media as a % of the dry weight</td>
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2. pH Value

- pH between the range 6.0 - 7.5
- If pH outside this range: evidence that pH has no negative influence on the germination test results
For Sand and Organic Growing Media, one volume of media are mixed with 5 volumes of water that is to be used for germination tests.

For Paper Media samples are moistened, with water that is to be used for germination tests and the pH is measured on the surface of the paper.
3. Conductivity

- Salinity must be as low as possible and no more than 40 milliSiemens per meter

- Measurement of Conductivity can be replaced by biological tests.
• 20g of media are mixed with 100ml of water, which is used for germination tests, at 20°C ± 1°C.

• Mix for 30 min

• Filter

• The conductivity is measured using a calibrated conductivity meter
4. Cleanness an Innocuity

- Freedom from phytotoxic effects and freedom from negative effects due to micro-organisms

- Use of seeds of species known to be sensitive to toxic substances:
  
  *Lepidium sativum, Phleum pratense, Agrostis gigantea, Eragrostis curvula, Festuca rubra var commutata, Petunia sp.*
Germinate sensitive species on substrate

Observations:
- % of germinated seed at a first count
- % of normal seedlings, abnormal seedlings and non germinated seeds
- Description of visible symptoms

Calculation:
- Presence of visible symptoms or statistical analysis
Water Quality

- Cleanliness (reasonably free from organic or inorganic impurities)
- pH: 6.0 – 7.5 when checked in substrate (unless there is evidence that the pH outside this range does not have negative influence on the germination test results)
Monitoring

• Tool to give you confidence that you always obtain the same result regardless of:
  ➢ Who prepared the sample
  ➢ Planted
  ➢ Evaluated
  ➢ Which equipment was used
Control of Equipment

- Retesting samples covering different analysts and equipment
- Split germination replicates among analysts
- Evaluation of selected seedlings
- Check for differences and trends (visual, statistical)
- Investigate if differences are available
Day, 13 June 2011
ISTA Seminar on Germination

Thank you for your attention