Germination tests are used by the Millennium Seed Bank (MSB) for two reasons:
1. To monitor the viability of seed collections.
2. To develop protocols for turning seeds into plants.
Initial viability is tested once collections have been stored at -20°C for at least 7 days, and ideally within 3 months of banking. Viability is then monitored at least every 10 years.

Germination testing is often the most reliable way of assessing viability. It is important to monitor viability as non-viable seeds may not be apparent at other stages of processing. Optimal germination conditions should allow all viable seeds to germinate using the simplest method possible. For more information on choosing appropriate germination conditions, see Technical Information Sheet_13b.

Preparing for germination testing

Sample size
It is important to know the total number of seeds available in the collection, including the portion expected to be empty or infested. This portion should be assessed after the cleaning process using an x-ray or cut test, see Technical Information Sheet_14. You need to sow enough full seeds to allow proper analysis, without depleting an important collection (see table to the right).

Germination medium
Choose a germination medium such as 1% agar, sand (particularly useful for large seeds) or germination paper. Agar is highly suitable as it is clear and so easy to see the seeds, retains moisture, and can be combined with chemical compounds such as gibberellic acid (GA₃). If using a medium other than agar, take care not to add too much water (it should not pool on the sand / paper) or to let it dry out.

Germination containers
Choose a germination container (Petri dish, box, etc.) that allows light to reach the seeds, and which is large enough to contain all the seeds without overcrowding. Seal containers inside a plastic bag to prevent moisture loss and reduce contamination.

How to make 1 litre of 1% agar
• Weigh out 10g of agar powder into a large jug and mix to a smooth paste by adding 100ml of cold distilled water.
• Add 900ml of boiling water to the agar paste and stir well.
• Gently heat the solution, stirring continuously until boiling.
• Allow to cool to approximately 50°C before pouring.
1 litre of agar yields approximately 33 x 9cm Petri dishes (30ml per plate)

To add gibberellic acid (GA₃) to agar:
• Add 5ml of stock solution (see below) to 445ml of boiled agar (cooled to 50°C) and pour plates as before.

To prepare stock solutions of GA₃ (250mg/l)
• Add 4.5g of GA₃ to 200ml* cool deionised water.
• Adjust pH to 6.5 using sodium hydroxide / hydrochloric acid.
• Filter the solutions using a sterile Nalgene filter and syringe in a laminar flow cabinet.

The stock can be stored for 3 months at 5°C in the dark.

* To ensure final volume is not >200ml, start with 180ml and adjust after setting pH.

Over-sowing
When germination testing collections with empty or infested seed, sow extra seed to compensate for the incompetent portion.

E.g. if 45 / 50 seeds full: 50 ÷ (45 / 50) = sow 56 seeds.
Setting germination tests
Prepare a germination test sheet for each collection (see example test sheet on back page).
Label each germination container and test sheet clearly, including:
- date started
- collection number
- species
- number of seeds sown
- germination temperature
- any additional treatments

Sowing
Place seeds on fresh substrate as necessary (e.g. to control fungal contamination or remove inhibitors leached from the seed coat) noting this on the test sheet each time.

Chemical additives (such as GA₃) should be renewed every four weeks, as these degrade over time.

Test duration
Continue tests until germination stops or all seeds have germinated.
If there is no germination after 42 days, or germination has stopped for more than four weeks, decide whether to continue the test (e.g. slow germination expected), apply a dormancy breaking treatment (see Technical Information Sheet 13b) or end the test. This decision may depend on the family, embryo size, seed health, etc. Seeds of some wild species can take years to germinate!

Cut testing
It is important to dissect any seeds that have not germinated at the end of the test. This will allow proper evaluation and interpretation of the result. Paying special attention to the embryo, record each seed as fresh, mouldy, empty, insect-infested or abnormal. Enter the results on the germination test sheet.

Incubators
Suitable incubators for germination have low energy, cool white fluorescent tubes. Incandescent lamps should be avoided as they produce too much far red light and heat. Photoperiod should coincide with the thermoperiod, with a cycle of 8/16 or 12/12 (light / dark) hours.

Rehydration
Rehydration (above) can reduce risk of imbibition damage, especially where a soaking treatment is applied (e.g. sanitisation). Suspend dry seed over water in a sealed container for 24 hours before sowing.

Sanitisation
Seeds can be sanitised by soaking in a 0.5% sodium hypochlorite (NaOCl) solution, containing a 1% surfactant (Tween 20) for 10 minutes and then rinsing under running water for 1 minute.

Germination test monitoring
Score germination weekly, removing seedlings when the radicle is at least 2mm long (for very small seeds, shorter radicles are acceptable). Record the date, days after sowing, and number germinated on the test sheet. Record abnormal seedlings (e.g. those with cotyledon growth only) separately.

Sanitisation
Seeds can be sanitised by soaking in a 0.5% sodium hypochlorite (NaOCl) solution, containing a 1% surfactant (Tween 20) for 10 minutes and then rinsing under running water for 1 minute.

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Germination test evaluation

Calculating percentage germination and viability

Empty and insect-infested seeds are excluded from the calculations as they could never germinate. This fraction (unlike viable / non-viable seeds) does not change over time and so does not need monitoring in a germination test. Abnormal seedlings are recorded as fresh but not germinated. To calculate percentage germination and viability use the following equations:

Germination (%) = G / X * 100
Viability (%) = (G+F+A) / X * 100

where:
G = number of seed germinated
X = number of seed sown (excluding empty and infested)
F = number of fresh seed
A = number of abnormal seedlings

Interpreting germination results

High viability collections should achieve a germination result above 85%, meeting the international regeneration standard (FAO, 2014). However, statistical analysis can also be used to make decisions on collections of any viability.

The most successful test (achieving highest germination with the simplest method) can then be ‘accepted’ and those same conditions used for any future testing.

Cut test reliability

It is important to remember that viability calculated from a cut test is only an estimate. Assessing viability across all tests and including controls should increase the reliability of tests assessed in this way.

Germination test non-germinated seed
Perform a binomial test on raw data

Most seeds mouldy on cut test
Viability is not significantly different to germination

Most seeds fresh on cut test
Viability is significantly greater than germination

Germination requirements known
If viability = 0%
then seeds are likely dead

Germination requirements not known
Retest using different conditions

As with other statistical tests, sample size is important. Tests of less than 10 seeds are too small for meaningful statistical analysis.

Recording data and databases

If you want to analyse or share your germination data, it is important that you record all raw data in an appropriate format. Use a data management system with recognised seed bank data standards such as BRAHMS software (BRAHMS, 2015), which is capable of exporting data in a standard format.

Monitoring collections over time

Management decisions (e.g. to collect again or regenerate) should be implemented if / when collection quality drops to <85% of initial viability. To begin with, viability should be monitored at least every 10 years (MSBP Seed Conservation Standards, 2015).

Test intervals should be reduced (e.g. from 10 to 5 years) when the first significant decline in viability is detected. Where possible, duplicate short-lived collections to cryo-storage. Where no decline is shown after at least three retests, intervals can be extended (e.g. from 10 to 20 years). This can help to reduce staff costs and conserve small seed collections.

Analyse retest data using a Z-test (Ellis et al., 1985) or by probit analysis where possible. See Technical Information Sheet_01 in this series for further details on Probit analysis.

Health and safety

- Scoring of tests should be carried out in a dust containment hood (above) to prevent inhalation of fungal and bacterial spores.
- Take care when handling chemicals; safety glasses and disposable gloves should be worn.
- Dispose of used scalp blades in a sharps container.
- Clean all surfaces after use with disinfectant.
- Wash hands with biocide soap after working with seeds.
- Treat all seeds as though poisonous.
- Wear a lab coat.

Further reading

Refer to Technical Information Sheet_13b for references.
## Equipment specifications

<table>
<thead>
<tr>
<th>Description</th>
<th>Model/Product</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plastic germination containers</strong></td>
<td>• Disposable Petri dishes SLS Select 90mm triple vent (SLS2002)</td>
<td>Scientific Laboratory Supplies <a href="http://www.scientific-labs.com">www.scientific-labs.com</a></td>
</tr>
<tr>
<td></td>
<td>• Box clear PS 174x115x60mm (6145)</td>
<td></td>
</tr>
<tr>
<td><strong>Germination substrates</strong></td>
<td>• Agar powder Fisher Bioreagents (BPE2641-1)</td>
<td>Fisher Scientific UK Ltd <a href="http://www.fisher.co.uk">www.fisher.co.uk</a></td>
</tr>
<tr>
<td></td>
<td>• White seed germination blotter 2” circle (WDB2), 3.25” circle (WDB3.25) and 9x6” rectangle (custom size)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Sand (meets ISTA guidelines)</td>
<td>Anchor Paper Company Seed Solutions <a href="http://www.anchorpaper.com/index.php/seed-solutions">www.anchorpaper.com/index.php/seed-solutions</a></td>
</tr>
<tr>
<td><strong>Chemical additives to germination substrates</strong></td>
<td>• Gibberellic acid BioReagent (G7645)</td>
<td>Sigma-Aldrich Company Ltd. <a href="http://www.sigmaaldrich.com">www.sigmaaldrich.com</a></td>
</tr>
<tr>
<td></td>
<td>• Potassium nitrate 99.5+% for analysis Certified AR (P/6120/53)</td>
<td>Fisher Scientific UK Ltd <a href="http://www.fisher.co.uk">www.fisher.co.uk</a></td>
</tr>
<tr>
<td><strong>Germination incubators with appropriate lighting and temperature regimes</strong></td>
<td>LMS cooled incubators • 280 cyclic with timed lights, autodefrost Series 1A available in various sizes</td>
<td>LMS Ltd <a href="http://www.lms.ltd.uk">www.lms.ltd.uk</a> or <a href="http://www.ecomcat.com">www.ecomcat.com</a></td>
</tr>
<tr>
<td><strong>Containment hood for scoring germination tests</strong></td>
<td>Powder handling workstation with HEPA filtration (XIT Plus 804)</td>
<td>Bigneat Ltd <a href="http://www.bigneat.com">www.bigneat.com</a></td>
</tr>
<tr>
<td><strong>Dissecting microscopes</strong></td>
<td>• Nikon binocular stereo zoom microscope (SMZ2445) with Photonic cold lightsource with goose neck (PL1000)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Lynx stereo microscope</td>
<td>Jencons Scientific Limited <a href="http://www.ecomcat.com">www.ecomcat.com</a></td>
</tr>
<tr>
<td></td>
<td>• Distel/Trigene Advance Disinfectant SL Green (1% solution made up)</td>
<td>Vision Engineering <a href="http://www.visioneng.com">www.visioneng.com</a></td>
</tr>
<tr>
<td></td>
<td>• Ethanol 99.8+% (GLC) 0.7897g/ml absolute duty free (70% solution made up)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Tween 20 or Polysorbate 20 nonionic detergent</td>
<td></td>
</tr>
<tr>
<td><strong>Laboratory coat and disposable gloves</strong></td>
<td>Locally available</td>
<td></td>
</tr>
<tr>
<td><strong>Permanent waterproof marker pens</strong></td>
<td>Stabilo Write-4-all</td>
<td>Locally available</td>
</tr>
<tr>
<td><strong>Dissection kits</strong></td>
<td>Kit no. 2 (AGT5242)</td>
<td>Agar Scientific Ltd <a href="http://www.agarscientific.com">www.agarscientific.com</a></td>
</tr>
<tr>
<td><strong>Disinfectant (for bench and equipment)</strong></td>
<td>• Distel/Trigene Advance Disinfectant SL Green (1% solution made up)</td>
<td>Scientific Laboratory Supplies <a href="http://www.scientific-labs.com">www.scientific-labs.com</a></td>
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<td><strong>Disposable paper towel</strong></td>
<td>Locally available</td>
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<tr>
<td><strong>Aluminium foil for dark germination</strong></td>
<td>Locally available</td>
<td></td>
</tr>
<tr>
<td><strong>Surface sterilising solution</strong></td>
<td>Sodium hypochlorite and Tween in de-ionised water</td>
<td></td>
</tr>
</tbody>
</table>

Please note that the above equipment is used by the Millennium Seed Bank Partnership and has been chosen carefully using our many years’ experience. The list of suppliers is for guidance only and does not represent an endorsement by the Royal Botanic Gardens, Kew. The manufacturer’s instructions must be followed when using any of the equipment referred to in this Information Sheet.