

This protocol is used by the Millennium Seed Bank (MSB) to compare the seed longevity of different species held in the seed bank.

The method aims to generate a single seed survival curve, using a carefully controlled ageing environment. Seeds are first rehydrated and then aged using salt solutions to provide the desired relative humidity (RH) environments inside a sealed container.

The controlled ageing test generates a measure of the longevity of a dried collection that can be compared with the known longevity of 'marker' species under the same conditions. Whilst the method does not allow accurate prediction of seed longevity for test species, comparison with marker species enables ranking into longevity categories. This method can also be used to investigate the effects of factors, such as maturity or post-harvest handling, on seed quality.

Preparation of LiCl solutions

The MSB uses non-saturated solutions of LiCl to control the humidity within plastic boxes which have an air-tight seal.

- To prepare the rehydration solution (47% RH): add 385g LiCl to 1 litre distilled water, transfer to the first plastic box and place in an incubator at 20°C.
- To prepare the ageing solution (60% RH): add 300g LiCl to 1 litre distilled water, transfer to the second plastic box and place in a fan-assisted oven at 45°C.

Check the equilibrium relative humidity (eRH) of both LiCl solutions once a month. See Technical Information Sheet_09 and Hay *et al.* (2008) for the LiCl solution preparation protocol.

Right: Plastic box with an air-tight seal, containing seed samples held above a non-saturated LiCl solution

Right: Seeds in a glass dish, ready for rehydration

Preparation tips

- Prepare LiCl solutions at least 24 hours before required.
- To measure the eRH of LiCl solutions, add a few mls to a hygrometer sample chamber, taking care not to contaminate the sensor.
- Regular eRH measurement of the ageing environment solution is important, as loss of water occurs over time due to evaporation when the box is opened.
- Experience at the MSB has shown that in containers with 1 litre of ageing solution (60% RH), eRH will fall by approximately 2% over a one month period. Adjust the solution by adding approximately 40ml of distilled water to counter this.

Preparation of seed samples

Count 10 samples of 50 seeds and place each sample in a single layer in open glass vials or dishes of suitable size. If seeds require chipping or dehusking for germination, perform these treatments prior to rehydration.



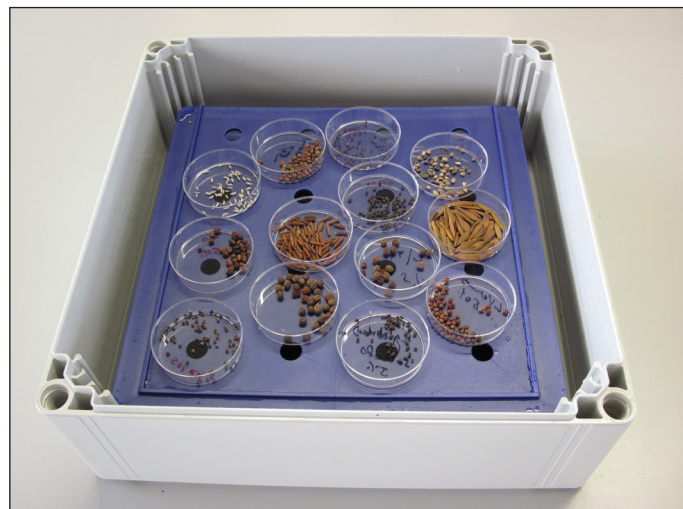
Rehydration: 47% RH, 20°C

The rehydration step minimises the change in seed moisture content when samples are transferred to ageing conditions. Place the vials or dishes containing seeds on a stand inside the rehydration box, so that the seed samples are held above the LiCl solution.

The rehydration period is usually 14 days. However, this is dependent on seed size, so larger seeds may require more time. Check seed eRH using a suitable hygrometer, to ensure that equilibrium has been attained (see Technical Information Sheet_05).

Seed requirements

- For comparative longevity testing of conservation collections: use large collections, from which 500 seeds can be spared.
- Seeds should have a high viability (>85%) and germination requirements must be known.



For small seeds, the eRH reading may be inaccurate because the sample of 50 seeds will not fill the hygrometer sample chamber sufficiently. For such species, rehydrate a larger, surrogate sample of similarly sized seeds, so that the volume is sufficient for accurate eRH measurement. Any suitably sized seeds with a permeable seed coat could be used as the surrogate sample.

Once rehydrated, move the seed samples, in their open dishes or vials, to the ageing box.

Ageing: 60% RH, 45°C

As seeds warm from 20 to 45°C, the eRH of the seeds adjusts to 60%. The storage environment created inside the sealed box ensures that seed samples experience identical ageing conditions.

Withdraw one sample of 50 seeds at random on the following days: 1, 2, 5, 9, 20, 30, 50, 75, 100, and 125. Sow each sample as a germination test, under appropriate conditions for that species. Run each test for at least 42 days, and until there have been 14 days without any germination. Perform a 'cut test' at the end of each germination test, to identify any non-germinated seeds that are incompetent (empty or infested). The number of fresh and mouldy seeds should also be recorded. This is an important part of assessing seed viability. Exclude incompetent seeds from the germination percentage calculation. Note any abnormal seedlings, but do not score them as germinated.

Analysis and interpretation

Plot seed viability (percentage germination) against the ageing period (days) to create a seed survival curve. This is usually analysed using probit analysis (a type of regression analysis) to fit the viability equation (Ellis & Roberts, 1980):

$$v = K_i - p/\sigma$$

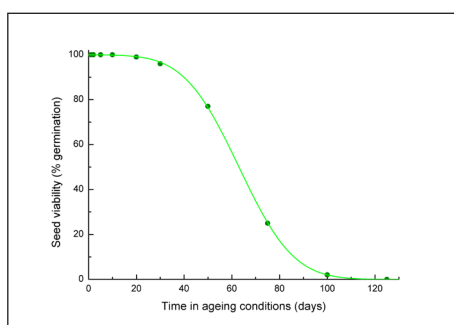
where v is the viability (in probits) of the collection after p days in the ageing environment. K_i is the y-intercept and a measure of the initial seed viability (in probits), and σ (sigma) is the time for viability to fall by 1 probit.

The time for viability to decline to 50% (p_{50}) can be read off the seed survival curve or calculated using the equation:

$$p_{50} = K_i \times \sigma$$

p_{50} values are used to rank species, allowing longevity comparisons between species and with the marker species in the screen.

Below: A typical seed survival curve



Practical tips

- Starting a comparative longevity experiment on a Wednesday will allow for samples to be withdrawn on week days.
- Using glass vials and Petri dishes rather than plastic containers will minimise static electricity.
- Two marker species with known viability constants, used at the MSB, are *Ranunculus sceleratus* (short-lived) and *Brassica napus* (long-lived). The longevity of test species is compared with these.
- The sampling schedule shown is designed for species of unknown longevity. For species expected to be short- or long-lived, adjust the schedule accordingly. For example, if the germination data indicates that the seeds are showing little sign of viability loss, you could increase the sampling intervals during the test.

Further reading

Ellis R.H. and Roberts E.H. (1980). Improved equations for the prediction of seed longevity. *Annals of Botany* 45: 13-30.

Liu, K., Eastwood, R.J., Flynn, S., Turner, R.M. and Stuppy, W.H. (2008). Seed Information Database (release 7.1, May 2008) <http://www.kew.org/data/sid>.

Hay F.R., Adams J., Manger K. and Probert R. (2008). The use of non-saturated lithium chloride solutions for experimental control of seed water content. *Seed Science and Technology* 36: 737-746.

Probert R.J., Daws M.I. and Hay F.R. (2009). Ecological correlates of *ex situ* seed longevity: a comparative study on 195 species. *Annals of Botany* 104: 57-69.

Equipment specifications

Description	Model/Product	Supplier
Seed containers	<ul style="list-style-type: none"> • 2 ml clear Wheaton-style vial - VGA-220-012C • 5 ml clear Wheaton-style vial - VGA-220-121U • Glass Petri dishes 60 x 12 mm - PDS-100-011U 	Fisher Scientific Ltd: www.fisher.co.uk
Sealable box	Electrical Enclosure Box Cubo 0 (conforming to IP67): 300 x 300 x 132 mm <ul style="list-style-type: none"> • ABS base - OABP303010B • Clear lid - OPCT303003L 	Ensto UK Ltd: www.ensto.com
Stand to hold seed samples above LiCl solution inside box	Fisherbrand incubation tray in polypropylene blue: 250mm x 240 mm - FB55681	Fisher Scientific Ltd: www.fisher.co.uk
Fan-assited oven capable of reaching 60°C	LEEC KIF Compact	Jencons-PLS: www.jencons.co.uk
Lab-based hygrometer	HC2-AW sensor with USB interface, connected to laptop/PC running HW4-E software. Range: 0 to 100% RH, -40 to 85 °C.	Rotronic Instruments (UK) Ltd: www.rotrotron.com
Statistical analysis software	<ul style="list-style-type: none"> • Genstat version 12.1 • Origin version 8 	VSN International: www.vsn.co.uk Origin Lab: www.originlab.com

Please note that the above equipment is used by the Millennium Seed Bank 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.