Method Code: SOP 2.21
Subject: Methodology for epiphytic orchid seed viability test using Triphenyl Tetrazolium Chloride (TZ)
Section: All sections

1). Introduction and Principle.

2). Equipment and Materials.

1. 2mL Eppendorf tubes (one per species or seed treatment).
2. 5cm diameter filter papers.
3. 5cm cellulose acetate water filters with 3mm grid lines.
4. 1% triphenyl tetrazolium chloride (TZ) solution in phosphate buffer.
5. Tween 20 surfactant
6. Shott bottle
7. Forceps
8. Microscope slide
9. Gel plate scanner
10. Laptop with Umax imaging software
11. Gloves
12. Buchner funnel and vacuum pump (for counting suspended seeds with a microscope)

3). Procedure

Tz using seeds directly from a collection	**Tz using seeds from the imbition test**
1. Fold a piece of filter paper into a square packet (fold the filter paper in half then fold the 3 rounded edges in towards the middle of the packet) 2. Place seeds in the packet. ≤ 300 seeds and staple the top fold 3. Label the packet using pencil 4. Suspend the packet in 1% TZ solution in a Shott bottle wrapped in a double layer of aluminium foil. 5. Place in the 30°C incubator for 24h <u>Counting (using the gel scanner)</u> 1. Remove the packet from the TZ solution using forceps. The TZ solution in the Shott bottle can then be discarded. 2. Open the packet and 'Tip' the seeds onto a microscope slide – try and get the seeds suspended in one drop – use a spatula to scrape off excess seeds from the filter paper if you haven't got enough seeds suspended (wear gloves for this step). If the droplet is too big some of the seeds will be out of focus, to get round this wipe the slide with Tween 20 before you put the drop on it. 6. Place the slide onto the gel plate scanner	3. Using the 1% TZ solution rinse the seeds directly into a 2mL Eppendorf tube off the acetate filter paper. 4. Label the tube and place into a test tube rack. 5. Wrap the test tube wrack in a double layer of aluminium foil. 6. Place in the 30°C incubator for 24h <u>Counting (using the gel scanner)</u> 7. Pour out the TZ solution into a weighing boat until you have about 0.5-1ml solution left in the Eppendorf with most of the seeds, remove the seeds from the Eppendorf using a pipette and place into one drop of TZ solution on a microscope slide. (If the droplet is too big some of the seeds will be out of focus to get round this wipe the slide with Tween 20 before you put the drop on it.) 8. Place the slide onto the gel plate scanner with white card taped to lid and click 'preview' 9. Once you have an image on the screen drag the black rectangle over the droplet on the slide **10. Save the image before scanning** 11. Click 'scan' once the scan is complete

with white card taped to lid and click 'preview'

7. Once you have an image on the screen drag the black rectangle over the droplet on the slide
8. **Save the image before scanning**
9. Click 'scan', once the scan is complete check your image is suitable for assessment.
10. Count the number of full and red seeds on the scan – work out the % and this is your viability score (you could enhance the saturation and contrast of the image on Photoshop here as it makes it easier to count – you can also overlay a fixed grid on Photoshop which makes the seeds easier to count)

Counting using a microscope

11. Open packet and spread seeds out if they are clumped together.
12. Place a Petri dish lid with a grid drawn on it over the filter paper
13. Count the number of full and red seeds on the filter paper – work out the % and this is your viability score

check your image is suitable for assessment.

12. Count the number of full and red seeds on the scan – work out the % and this is your viability score (you could enhance the saturation and contrast of the image on Photoshop here as it makes it easier to count – you can also overlay a fixed grid on Photoshop which makes the seeds easier to count)

Counting using a microscope

13. Place a cellulose acetate filter marked with a grid on top of a Buchner funnel vacuum staging (turn the vacuum on). Pour off seeds and TZ onto the grid in one go. This ensures an even distribution of seeds within each grid cell.
14. Count the number of full and red seeds on the filter paper – work out the % and this is your viability score

Timing

Stage	Time (h)	Accumulated time (h)	Day (e.g. start)
1. Stain in TZ (in the dark at 30°)	24	24	1 (Mon)
2. Scan image	10mins	24 10mins	2 (Tues)
3. Observe results	10mins	24 20mins	2 (Tues)

4). Health and Safety.

- **Only authorised and trained staff should carry out this procedure**
- **Good laboratory practice must be followed.**

5). References

Hosomi, S.T., Santos, R.B., Custodio, C.C., Seaton, P.T., Marks, T.R. and Machado-Neto, N.B. (2011). Preconditioning *Cattleya* seeds to improve the efficacy of the tetrazolium test for viability. *Seed Sci. & Technol.,* **39**, 178-189.