1. Introduction & Purpose

Newly received NPGS seed samples that have more than 300 seeds require tests to determine viability prior to placement into storage at NLGRP.

2. Scope

The following procedures explain how seed analysts test seed samples for viability.

This SOP does not apply to quarantine samples, critical backup samples, black box samples, or Svalbard samples.

3. Definitions & Abbreviations

The following definitions and key terms are pertinent to this SOP (Knowledge of basic seed anatomy terms is assumed):

AOSA	Association of Official Seed Analysts
Canadian Rules	https://d3n8a8pro7vhmx.cloudfront.net/scst/pages/47/attachments/original/1446838122/2015_Edition__EN__M_P.pdf?1446838122
Dormant	Dormant seeds are viable seeds that did not germinate during the prescribed germination period. The viability of ungerminated seeds remaining at the end of the germination period is determined by a tetrazolium test or if seeds have germinated after application of a dormancy breaking treatment (e.g. scarification/clipping).
GA3	Gibberellic acid
Germs	Germinations
GRIN-Global	Germplasm Resources Information Network
H2O2	Hydrogen Peroxide
Imbibition	Water uptake by seeds
ISTA	International Seed Testing Association
LN2	Nitrogen in a liquid state (-196°C, -320°F).
Moisture Ready	Samples that have been tested for their moisture content and are available for viability testing
SCST	Society of Commercial Seed Technologists
Scarification	Mechanical abrasion or chipping of the seed coat
TZ	Abbreviation for the Tetrazolium test or tetrazolium solution
Viability	Seed with the capacity to grow and produce a normal plant as determined by the germination and/or tetrazolium test.

4. Occupational Health & Safety

No Health & Safety issues have been identified for this procedure.

Seed may be treated with hazardous chemicals. See Section 6.4 of this SOP.

Liquid nitrogen is a very cold, colorless, clear liquid with a boiling point of -196° Centigrade (-350° Fahrenheit). It can cause rapid freezing on contact with living tissue, which can result in cold burns or blindness. Liquid nitrogen as a gas can result in extremely low oxygen levels which can cause unconsciousness or death. Adherence to all safety regulations is required. See SOP 10 LN2 Tank Filling for safety precautions. Also found in Appendix K and D, NLGRP Chemical Hygiene, Hazard and Communication Plan (CARR Fort Collins Safety Site (SharePoint).

5. Materials and Equipment

Materials:

- Seed Tray (black trays that hold seed samples)
- Germination Card
- 10% bleach water
- Germination Blotters
- Germination Towels
- Filter Paper
- Creped Cellulose paper
- 4x4 square plastic boxes
- Red plastic trays
- Glassine and coin envelopes (storage: **Room 207**)
- Crispers: 8” X 10 3/4” clear plastic boxes
- White trays for germination carts
- Disinfected (H202) rubber bands
- Lab chemicals:
  - Stored in **Room 206**:
    - 0.2% Potassium nitrate
    - Gibberellic acid (and 500 ppm solutions)
    - Ethephon
    - 2,3,5 triphenyl tetrazolium chloride (and solutions)
    - Potassium phosphate, sodium phosphate (buffer chemicals for tetrazolium solutions)
    - Litmus paper
  - Stored in hood area, **Room 232**:
    - Fungicides: SD205, Captan (hood area, **Room 232**)
  - Stored in Bay 1 cabinet near sink
    - 3% H202 (Hydrogen Peroxide)
    - glycerol

Equipment:

- Walk-in germinators
- Percival germinators (**Room 210**)
- Air-driven mechanical scarifier
- Single edge razor blades (two types: regular and extra-keen)
- Scalpels
- Scarification kit (**Room 207**) Miscellaneous scarification tools: diamond files, mini-chisels, ring-clamp, saw blades, rubber grip tool, transverse end-cut pliers, plizer, curved snip tool

6. Procedure

6.1 Choosing a Germination Method

6.1.1 The NLGRP preferred method comes primarily from the AOSA Rules for testing seed. AOSA also has an online database for testing of species for which there are no official rules. The analyst can also check the viability program using the reports function to generate a spreadsheet of past tests conducted on similar or same species. Samples which lack method references and NLGRP experience should be tested as one would test the next closest relative for which there is information. If the analyst can't find a suitable close relative, start with the simplest method first (e.g. H2O, 20, P). For species with >20% hard seeds after one week, clip, and allow to germinate.

6.1.2 Online references (www.ars-grin.gov: navigate to taxonomic queries) can help the analyst learn about the synonyms which cause confusion when looking up species. Other uses of online references include learning about the species, learning how the taxa are related and connecting with other analysts via discussion groups and e-mail.

6.2 References for Germination Testing

AOSA Rules for Testing Seed, Association of Official Seed Analysts.

- International Rules for Seed Testing, Seed Science and Technology, International Seed Testing Association (ISTA.
- Handbook of Seed Technology for Genebanks, Vol. II: Compendium of Specific Germination Information and Test Recommendations, International Board for Plant Genetic Resources, 1985
- Seeds of Woody Plants of the United States, Agriculture Handbook No. 450
- Handbook on Seeds of Browse-Shrubs and Forbs, Technical Publication R8-TP8, USDA/Forest Service
- Species Without AOSA Testing Procedures: Newsletter of the AOSA, Vol. 60, No. 2, February 1986 (available online at AOSAseed.com)
- Growing Colorado Plants from Seed: A State of Art. USDA/Forest Service General Technical Report: INT-103
  - Vol. I: Shrubs
  - Vol. II: Grasses and Grass-like Plants

  - Vol. III: Forbs
- Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination, Carol and Jerry Baskin, 1998
- A Summary of Range Plant Seed Germination Research, International Center for Arid and Semi-Arid Land Studies
- Seeds of Wildland Plants, James A. and Cheryl G. Young
- Native Plant Propagation Techniques for National Parks, USDA Soil Conservation Service/U.S. Department of Interior, 1993
- Summary of Germination Methods for Garden and Flower Seeds, N.V. Sluis En Groot, Enkhuizen, Holland, 1948
- See network folder of electronic references: N:\PGRPP\AOSA Rules and Germ References.

6.3 Using public GRIN-Global on the internet:

6.3.1 On the internet, go to: www.ars-grin.gov/npgs/ Search GRIN-Global using the Search tab at the top, you will see the following choices:

- NPGS Collections: Collections Map with links to sites and summary statistics
- Crop Germplasm Committees
- Repository Home Pages PI station contact information, NLGRP is here too.
- Links: related websites

6.3.2 Find taxonomic information

- Look up synonyms; choose “Search GRIN-Global” and then “Taxonomic queries.” Then choose “complex queries.” You can also go here to look up the family of an unfamiliar genus.
- It’s easiest to just put in a genus and then search through the list of species that come up. You don’t need to fill in all the blanks. Click on “submit.” You won’t get anything if you just hit enter.
- It’s easy to make a spelling mistake and not find anything when in fact it really is there. When you get a list of species you can either click on that entry or choose one of the following helpful hints:
  - List of Scientific Names (gives synonyms)
  - Complete Taxonomic Data (gives citations, synonyms, uses, common names and other information)
  - Contact Information (PI stations and Crop experts). Choose “Repository home pages.” Click on the station and work through their web page to find who you need.

- Sometimes we get calls from the public about particular crops. The "crop germplasm committees" is a good place to get contact information about crop experts.

6.4 Recognizing and Handling Treated Seed

6.4.1 Recognizing:

- Pesticide treated seed has a dye, usually pink. A white powdery coating is generally diatomaceous earth but this could also be a pesticide. Green-dyed seed has been treated with potassium nitrate and is not harmful to handle in the seed quality lab.
- Unpack seed in Room 232 or any lab that is vented to the outside.
- If there is visible residue from the treated seed on the outside of the bags, line the unpacking trays with plastic bags. This will make cleaning the trays easier.
- Personal Protective gear: When working with treated seed, lab coats, gloves and dust masks should be worn.

6.4.2 Record keeping:

6.4.2.1 If you are the first person to discover that seed is treated, determine the chemical name of the treatment. Contact the donor, if necessary.

6.4.2.2 Go to the IT Group and request labels saying: "Treated Seed" to be put on final storage bags at time of packaging.

6.4.2.3 Write name of treatment on the Germination card and label all unpacking trays (if there is only one sample, label individual sample).

6.4.2.4 If this is a new chemical to our lab, obtain an SDS (Safety Data Sheet – formerly MSDS) from one of the following sources:

- The donor
- The chemical company that manufactures the treatment
- Computer references: examples
- http://siri.uvm.edu/msds/
- http://www.labsafety.org/
- CSU Environmental Health (phone: 491-4830 or 491-6745)

6.4.3 Handling samples:

6.4.3.1 Samples should be handled under the hood (Room 232) as much as possible. If sample bags need to be individually opened and debagged, do so under the hood. Seed handling for moisture tests and planting should also be done under the hood. Work 6 inches in from the front of the hood and keep materials and clutter away from the intake vents. Wear lab coats and gloves.

6.4.3.2 Cleanup: Inside-out gloves, dust masks and bags that contained treated seed should be discarded in regular trash.

6.4.3.3 Wash lab coats in the lab washing machine (Room 104). Do not take lab coats home to be laundered.

6.4.4 Copies of the MSDS should be added to the Chemical Hygiene Plan reference materials on SharePoint.

6.5 Number of Seeds to Test

6.5.1 Standard Samples stored at -18C:

Seeds in Sample	Reps x Number of Seeds to Test
2000+	4x50
1000-1999	2x50
500-999	50
425-499	25
301-424	10
<300	No Test

- Exception: for SOS samples, test 2x50 seeds or fewer.

6.5.2 Cryogenic Samples:

Seeds in sample	Control	LN2
2000+	2 x 50	2 x 50
1000-1999	2 x 25	2 x 25
<1000	See Standard table. Sample will be placed into Standard -18C storage.	

6.5.3 Monitor Tests:

Seeds in the Sample	Seeds to Test
>500	50
425-499	25
301-424	10
<300	no test

6.6 Cryo paired testing procedures

6.6.1 Seeds should be labeled and packaged in coin envelopes for exposure tests.

6.6.2 Exposure Test Procedures

6.6.2.1 Tank location: **Room 210**. Eye protection and gloves are required when opening the tanks.

6.6.2.2 When removing a section from the tank, place the section on top of the other sections so any liquid nitrogen spilling from the section drains back into the tank.

6.6.2.3 Sections are color coded and tanks are labeled 1 and 2.

6.6.2.4 Note the location of your samples and the time placed into the liquid nitrogen tank.

6.6.2.5 Lower the section slowly back into the tank, as dropping the section too fast can cause splashing.

6.6.2.6 Make sure the section handles are down before replacing the tank cover.

6.6.2.7 Exposure time is 24 hours for all samples. Do NOT expose samples on Friday.

6.6.2.8 Take samples out of LN2, allow to reach room temperature (about 30 minutes). Plant the same day.

6.6.2.9 Plant LN2 and corresponding control samples at the same time, following any NLGRP preferred methods first, then AOSA Rules for Testing Seeds. Take special care to use approximately the same moisture content on the media for both LN2 and control, place in the same tray, in close proximity. Try to make all growing conditions the same for LN2 and controls so that results will be comparable. Following any prescribed pre-chill, allow a maximum germination time of 14 days, then TZ any remaining firm seed.

6.6.2.10 Evaluate germination and determine if there is a difference between the control and the LN2 exposed germination test. If the control result is >10% higher than the LN2 result, place the sample into standard storage at -18C. If the LN2 test result is greater than the control result and equal to or higher than 85%, place the sample in LN2 storage.

Retest only the samples which have seed numbers exceeding 1000. See Germination Supervisor regarding questions about germination test.

6.6.2.11 When evaluation is completed, put germination cards back into tray with samples and mark the tray with a dated note "Ready to Package." If a sample will be moved to standard -18C storage because it is out of tolerance, the LN2 germination is less than 85%, or has less than 1000 seed before testing, pull sample and card out of tray. Place these cards and samples in a separate tray and mark with OPR# and a dated note "Standard -18C Samples, Ready to Package". Place tray in **Room 223**.

6.7 Planting procedures and helpful tips (see also: pictorial planting guide: N:\PGRPP\Lab Guide\Planting Guide and templates)

6.7.1 Samples for viability (germination) testing will be located in **Room 223**.

6.7.2 Select a tray for germination testing by pulling any PVPO trays marked "Moisture Ready" first, then select trays with the oldest "Moisture Ready" date. Technicians will be notified of special seed shipments with a higher priority.

6.7.3 Pull germination cards from the tray to be tested. Note if the samples are to be conventionally stored or cryogenically stored. Keep germination cards at your workstation. DO NOT place cards back into the tray.

6.7.4 Tape a note to the front of the tray (over the moisture ready note) with the following information:

- Print: Germs in Progress with date started
- Initials
- May wish to either letter or number the note if working on more than one tray; good reference for tray locating in **Room 223** when germination of samples for the tray are completed.

6.7.5 Determine the germination procedures for the testing. Print the germination method on the germination card in the sub: moist: C column. Also include the count days for the test at the end of the column.

6.7.6 Prior to planting, clean the work surface with a 10% solution of bleach water. Spray the surface and wipe dry with a clean cloth or paper towels. Clean seed boats, tweezers, and planting boards with a cloth or paper towel spayed with 10% bleach water, and dry thoroughly.

6.7.7 Media preparation:

6.7.7.1 Substrate choices on the viability program (See AOSA Rules for more details.)

- B = between blotters (with or without a box)

- T = towels, vertical or horizontal (paper toweling, used either as folded towel tests or as rolled towel tests in horizontal or vertical position)
- P = top of blotters or filter paper in 4x4 boxes with one or two layers of blotters, OR three thicknesses of filter paper (AOSA "(c) top of sand"; not included in our definition for P)
- PP = pleated filter paper or pleated towels
- RB = blotters with raised blotter covers
- O= other: (If you choose O, provide more details in the memo field not going to GRIN-Global. O can include PT, C, TC, TCS and TB.)
- RB = blotters and raised covers, prepared by folding up the edges of the blotter to form a good support for the upper fold which serves as a cover, preventing the top from making direct contact with the seeds;

6.7.7.2 Other Substrate Choices (see AOSA Rules for more details):

- C = creped cellulose paper wadding (0.3-inch thick Kimpak or equivalent) covered with a single thickness of blotter through which holes are punched for the seed which are pressed for about one-half their thickness into the paper wadding.
- TC = on top of creped cellulose paper without a blotter.
- TCS = on top of creped cellulose paper without a blotter and covered with ½ to 3/4 inch layer of sand.
- TB = At NLGRP TB means uncovered top of blotters if the blotters are open and placed directly on the trays with no cover).

6.7.7.3 There are two weights of towels available.

- Heavy weight 76# 10x30, use 1 towel per replicate (or 2 for fava beans and other very large seeds)
- Medium weight 38# 10x30 and 10x15, use 2 towels per replicate.

6.7.7.4 Prior to wetting all towels, keep back 1 dry towel for each 15 to 20 wetted towels.

6.7.7.5 Germination towels should be fully submerged to adequately soak the towels thoroughly. A large quantity of towels may be wetted if doing a large number of tests or a specific number of towels may be counted out for the exact number of tests being done.

6.7.7.6 Once towels are thoroughly wetted, press out as much of the free water in the towel as possible. An aid in removing free water is to place the towels between two red plastic trays and press down onto the top tray or use rolling pins.

6.7.7.7 Interlace the dry towels in with the wet towels every 15 to 20 towels to help absorb extra moisture.

6.7.7.8 When planting from a large stack of towels, flip the stack over periodically and blot off any excess moisture. Continue planting samples.

6.7.7.9 Use the "Thumb Method" to determine if towels have the proper moisture. Press your thumb into the stack of wetted towels for a few seconds. If a pool of water forms around your thumb, more water needs to be removed. Blot the towels with additional dry towels to remove extra water. The towels have the correct level of water when removing your thumb a light colored area appears at the point where the thumb pressed.

6.7.7.10 Another way to determine the moisture level in rolled towels in using the Canadian Rules method for moistening towels: "Paper toweling should be moistened until its wet weight is three times that of its dry weight".

- Example: If towels weigh 200 grams dry weight, the wet weight should be no greater than 600 grams. One milliliter (ml) of water is equal to 1 gram (g). For a stack of towels weighing 200 g dry weight, add 400 ml of water for a total of 600 g. Allow towels to soak up all the water prior to planting.

6.7.7.11 Blue blotters should be thoroughly wetted by submerging. Blot excess moisture from the blotter to remove the shininess from the blotter. Apply the "Thumb Method" to determine if the blotter is correctly wetted, or use the Canadian Rule for moistening towels. If moistening one or two blotters, submerge for 1 second.

Blotters may be wetted with:

- Tap water
- A solution of 0.2% Potassium nitrate
- A solution of 500ppm GA3

6.7.8 General guidelines for insuring representativeness when planting:

- Pour the entire sample out into a pan before planting.
- When hand counting, count from different parts of the pile as poured on your work surface.
- Pour the sample evenly on the planter head or board before turning on the vacuum and pouring off the excess. Pour the excess towards the back of the pan so that the portion poured onto the head or board is a previously unsampled part of the sample.
- **Never just take seeds off the top of the packet**.

6.7.9 Planting tips:

- Use one blue blotter per 4x4 square plastic box. Use 2 blue blotters in plastic box when using portable germinators in **Room 210**.
- When using folded blotters for a test, and the seed is round, fold the outer edges of the blotter over to eliminate the seeds from rolling off the blotter.
- Do not stack folded blotters over 4 high.
- Do not stack 4x4 square plastic boxes over 2 high.
- Use new or disinfected (H2O2) rubber bands for rolled towels.
- When using the 10X30 rolled towels, rubber band only 2 replicates together. Doing so reduces crowding of the seeds and reduces the possibility of inducing abnormal seedling growth during the test.
- When placing rolled towels in the red plastic trays, leave room in each row for expansion of the seeds during imbibition. Compressed towels can lead to abnormal seedling growth.
- When using the portable germinators, rolled towels and folded blotters need to be placed inside a plastic bag and sealed with a tie or tape to conserve moisture.
- Maintain the moisture content of the rolled towels, blue blotters, and folded blotters throughout the test, however, avoid over-watering. A totally dried out test can result in the seeds going into dormancy or ruin the test, leading to retesting.

6.7.10 Prechilling prior to germination:

- The prechilling period should not exceed 14 days.
- Normal prechilling temperature is 5 degrees C. (**Room 206**)

6.8 Special viability procedures

6.8.1 Hard seeds:

6.8.1.1 Definition: Hard-seededness is a condition where imbibition is prevented due to an impermeable seed coat. Seeds remain “hard” at the end of the test period and look exactly as they did when planting the test. Hard seeds are common in many species, but most often found in species of the following families: Fabaceae, Malvaceae, Convolvulaceae, Geraniaceae.

- Do not confuse hard seeds with rigid seeds which also have seeds that do not swell. Unlike hard seeds, rigid seeds imbibe water and the embryonic tissue inside is hydrated.

6.8.1.2 If hard seededness exceeds 20%, scarify and evaluate the seedlings that emerge.

- Exceptions: Medicago spp., Trifolium spp., Lotus spp., Melilotus spp. and all monitor tests.

6.8.1.3 Methods for Breaking Hard-seededness:

- Objective: Scarification may be performed in a variety of ways. The main objective is to allow water uptake. This must be accomplished without creating abnormalities so that proper evaluation of the germination will occur. Of the above methods, clipping of the seed at the cotyledonary lobe or the removal of the "lens" in legumes, tend to serve the function of water imbibition without causing abnormalities with the least effort. It should be noted that both of these methods allow for easy access of fungi to the embryo and fungal infection may increase with scarification by these means causing inaccurate assessment of the germination potential. If the analyst is worried about fungal problems, the hot water scarification method can sometimes break hard-seededness without allowing undue fungal disease.
- Mechanical scarification - Seeds may be clipped, filed, or pierced opposite the radicle end at the tip of the cotyledons or rubbed with an abrasive material such as sand paper to allow water uptake into the seed.
    - For legumes, the strophiolar lens may be removed by "popping" the lens off with a razorblade or scalpel.
    - Clip hard seeds at the end of the 'first' count. (If there are swollen seeds or seeds just beginning to germinate, keep them separated from the clipped hard seeds. AOSA rules allow extending the test an additional 5 days to allow swollen seeds to germinate).
- Hot water scarification - Fill a small beaker ½ full of water. Bring water to a boil. Remove water from the heat source and place seed in the hot water. Allow the beaker of water to cool to room temperature. All seeds should have taken up water and be swollen when the water has cooled. If any seeds remain "hard", repeat the procedure using only the remaining hard seeds.
- Acid scarification - This method is NOT recommended for use at the NLGRP. Acid scarification usually creates seedling abnormalities which would interfere with the proper evaluation of the seed lot and give misleading germination information.

6.8.1.4 Supplies needed for breaking hard-seededness:

- Tweezers or forceps for holding seed. Specialized tweezers for holding a variety of seed sizes and shapes are located in the box for scarification tools.
- Razor blades or scalpel (located in cleaning room, East cabinet, 2nd right drawer).
- Miscellaneous scarification tools: diamond files, mini-chisels, ring-clamp, saw blades, rubber grip tool, transverse end-cut pliers, plizer,

curved snip tool (located in cleaning room, East cabinet, 2nd right drawer).

- Air-driven, mechanical scarifier (located in cleaning room, south cabinet top). This is not recommended for routine use. This device does not uniformly scarify seeds and can severely damage seeds.

6.8.1.5 Evaluating Scarified Seeds:

- Clipping sometimes causes abnormalities that are not inherent in the seed (artifacts).
- If it is clear that the clipping caused the artifact, and there are no other inherent defects, consider the seedling normal.
- If it is not clear whether the clippings have caused the abnormality and if infection complicates the evaluation, conduct a separate TZ test on a separate replicate.

6.8.1.6 Reporting Hard Seeds and the Results of Breaking Hard-seededness.

- Hard seeds found in a proportion less than 20% of a sample are reported on the card and viability program as hard seeds. The percentage of hard seeds is added to the percentage of normal seedlings to obtain the total viability.
- For samples that have more than 20% hard seeds:
  - NLGRP Germination Card: If the germination test is an initial test (first test of the sample), the open area under 'No. SEEDS' to the left-hand side of card can be used for recording the number of "Hard Seeds Clipped" for each replicate, and "Clipped Seeds' Normal Seedlings" for each replicate. If the germination test is a Monitor Test, place the "Hard Seeds Clipped" and "Clipped Seeds' Normal Seedlings" data within the 'date' and 'replicates' area for the test. All "Clipped Seeds' Normal Seedlings" are to be recorded as "Dormant". Any abnormal seedlings from the "Clipped Seeds" record in "Abnormal". Any swollen seeds from either 'Clipped Hard Seed' or non-clipped seeds are to be TZ tested and recorded in "Dormant". Make appropriate notes in the "Remarks" area regarding the swollen seeds.
  - NLGRP Viability Program: Record the number of hard seeds clipped for each replicate in the "Clipped Seed" field. To open the "Clipped Seed" field, click on the "Dormant" box. Highlight 'Clip Hard Seed Coat', and click. "Clipped Seed" field will open directly above the "Normal" column and directly below the "6th Count" column. In addition to the "Clipped Seed" field, there

are three additional fields: "Clipped Normal Seedling" Record normal seedlings from clipped seeds. If there are multiple counts, add the number of newly germinated seedlings to the number already recorded in the clipped normal seedling field. If there are no counts at the end of the test, record the last date of the test by entering 0 in the last row of the count area. This tells the program the end date of the test. "Clipped Swollen TZ" Swollen seeds should be separated into those that were clipped and those that were not clipped. "Non-Clipped Swollen TZ" Record swollen seeds (from the non-clipped portion) determined to be viable by TZ. These are considered dormant. The "Dormant" field will be a sum of the "Clipped Normal Seedling", "Clipped Swollen TZ", and "Non Clipped Swollen TZ" fields, which is automatically calculated in the NLGRP Viability Program. Hard Seed data generated will be used for future NLGRP research.

6.8.2 Fungicide use (when & what)

Application of fungicide treatment to seed is generally not part of the seed testing routine at the NLGRP but is recommended for the following species:

- Peanuts (If fungicides are not used for peanuts, tests are to be inspected daily.)
- Cotton (only when retesting because of disease impairing proper evaluation on the initial germ test)
- Any species where there is a need to reduce the effects of fungi on seed germination and seedling development during the germination test. This would be applicable during retesting or paired testing.

6.8.3 Beta sp.

6.8.3.1 Soak Beta sp. seed before planting

6.8.3.2 NLGRP does not have a running water bath apparatus. Substitute a 4-6 hour soak for the 2 hour running water bath.

6.8.3.3 Place the replicates in labeled boxes with warm (not hot) water. Spraying the floating seeds helps to submerge them. At the end of the soak, rinse the seeds using a small strainer (available near the sinks). Seeds may be blotted and planted or allowed to dry (can dry overnight but do not wait longer than one day to plant). Use the long towels folded up twice for planting. Plant replicates of 25 seeds in a single line towards the top of the towel. Check the samples beginning at 3 days. With the seed units planted in one line, it is ok to allow the test to progress further. Final count is 14 days (10 for sugar beets). When evaluating, take care to match the seedlings with their corresponding seed units. A seed unit may have more than one seedling. At least one normal seedling must come from a unit to consider it normal.

6.8.3.4 Pop open or cut ungerminated seed balls to test for dormancy. Further reference: AOSA rules – sec. 6.8 c

6.8.4 Helianthus (sunflower)

For sunflowers with high dormancy, TZ rep 1, delete reps not tested.

6.8.5 Rhus - TZ only

6.8.6 Cuphea (some are sensitive to freezing temperatures) New samples are being stored in **Room 206**. Protocol for monitor tests and distributions:

- Allow sample to come to room temperature. Count out monitor test replicate or distribution seeds.
- Place in coin envelope.
- Place envelope in TZ oven (35°C) for a 1 hour period prior to germination planting or packaging for distribution.

6.9 Laboratory chemicals: solution preparation, use and safety 5/26/1999

MSDS information for all chemicals is in the Chemical Hygiene Plan located on SharePoint. For all solutions, record the date prepared, your initials, and store in the amber bottles in **Room 206**.

6.9.1 KNO3, Potassium Nitrate, Stored in **Room 206**

6.9.1.1 Use AOSA table 3 for species requiring KNO3 (also see AOSA rules.)

6.9.1.2 Use a 0.2% solution to moisten blotters at the beginning of the test.

6.9.1.3 Do not re-wet the blotters with KNO3 during the germination test, re-wet the blotters with water.

6.9.1.4 To prepare a solution, dissolve 2 grams of KNO3 in 1000 ml of DI water.

6.9.1.5 Beakers, mechanical stirrer, stir bars, weigh paper and weigh boats are available at the weigh station in Bay 3 of **Room 222**). A supply of DI or distilled water is available in **Room 231**.

6.9.1.6 Safety precautions

- Avoid eye and skin contact
- Avoid inhalation
- Labware can be washed in the sink
- Towels used for spill cleanup may be disposed of in ordinary trash cans.

6.9.2 GA3, Gibberellic Acid, Stored in **Room 206**

6.9.2.1 Use AOSA Rules for instructions. The AOSA rules only give a range of concentrations that may be used (200-500ppm). The analyst has the option to choose what concentration to use. Other references may be more specific.

6.9.2.2 Solution preparation:

- 0.2 g. GA3 dissolved in 1 liter of water = 200 ppm
- 0.5 g, GA3 dissolved in 1 liter of water = 500 ppm

6.9.2.3 Beakers, mechanical stirrer, stir bars, weigh paper and weigh boats are available at the weigh station in Bay 3 of **Room 222**. A supply of DI or distilled water is available in **Room 231**.

6.9.2.4 Safety precautions

- Avoid eye and skin contact.
- Avoid inhalation.
- Labware can be washed in the sink.
- Towels used for minor spill cleanup may be disposed of in ordinary trash cans.

6.9.3 TZ, 2, 3, 5 Triphenyl tetrazolium chloride, Stored in **Room 206**

6.9.3.1 See 3.1 for use and references.

6.9.3.2 Two concentrations are commonly used: 0.1% and 1%.

- 1% TZ solution = 1 g TZ salt dissolved in 100 ml of buffered solution
- % TZ solutionn = 0.1g TZ salt dissolved in 100 ml of buffered solution

6.9.3.3 Buffer solutions, Stored in **Room 231**. Use 2 solutions:

- Dissolve 9.078 g. KH_2PO_4 in 1000 ml. H_2O
- Dissolve 9.472 g. Na_2HPO_4 in 1000 ml H_2O (*WARNING* labeled as an irritant)
- Mix two parts of solution 1 to three parts solution 2. This is the buffered solution and should be at pH 7.
- Buffer chemical safety precautions:
- Avoid eye and skin contact
    - Avoid inhalation. Labware can be washed in the sink. Towels used for spill cleanup may be disposed of in ordinary trash cans.

6.9.3.4 TZ Safety precautions

- Avoid eye and skin contact. If TZ solution is accidentally splashed on the skin or eyes, wash promptly.

- Avoid inhalation.
- Best practices for measuring TTC powder, limiting the possibility for spills or inhalation: Open both side glass doors of the scale. Place a tissue on the counter nearby. With hands reaching into both sides of the scale, measure out the powder by holding the bottle directly over the weigh boat and scooping out the powder with the spatula directly to the weigh boat below. When the weight is reached, place the spatula down on the tissue and replace the cap on the bottle while your hand is still holding the bottle in the glass scale enclosure. Use the tissue to wipe the spatula and any powder that may have spilled on the scale or counter. Wash the spatula with water and discard the weigh boat and tissue in the trash.
- Labware can be washed in the sink.
- Towels used for small spill (<250 ml of 1% TZ) or dust cleanup can be disposed of in ordinary trash.
- Towels or kitty litter (available under the sink in Bay 2) for large spills (>250 ml of 1% TZ) should be placed in a plastic bag (bags are in **Room 211** in the cabinet with the planting boards), with the contents labeled and the bag given to the trained hazardous waste generator for the Seed Quality Lab, Annette Miller, for proper disposal with CSU hazardous waste.
- Liquid TZ waste should be poured into the collection container located under the sink in the middle bay of **Room 222** to be disposed of by the trained hazardous waste generator for the Seed Quality Lab.
- See the Chemical Hygiene plan for further spill SOP guidance.

6.9.4 Glycerol, Stored at room temperature, **Room 222**, bay 1, glass door cabinet above the sink.

6.9.4.1 Use Clearing solution for dark seeded TZ's. See AOSA Rules for references.

6.9.4.2 Safety

- Labeled as irritant
- Avoid skin and eye contact
- Labware can be washed in the sink
- Towels for spills can be disposed of in ordinary trash.

6.9.5 SD-205 and Captan Fungicide Application (how)

6.9.5.1 Fungicide application to seed is to be performed under the fume hood located in **Room 232**.

- Fungicide should **ALWAYS** be applied under the fume hood and should never be used in the viability lab where the analyst is exposed to the chemical. If a spill of fungicide occurs, handle the spill as a hazardous waste spill. Contact your supervisor and/or a member of the safety committee.

6.9.5.2 Personal Protective Equipment (PPE) should be used whenever in the presence of fungicidal chemicals.

- Respirator/dusk mask
- Rubber gloves
- Lab coat

6.9.5.3 Treating and planting large seeds

- In the Seed Quality Lab, count replicates and place in envelopes. Prepare germination towels.
- Wear PPE: dust mask, gloves, lab coat
- Turn on hood (**Room 232**), pull hood sash down as far as possible. Work 6 inches from the front of the hood.
- Place one replicate of seeds in one of the green-labeled jars
- Use glass rod/scoop to transfer a tiny amount of fungicide to the jar
- Place top on jar, roll jar gently, evenly coating seeds. Treatment will be barely visible on the seeds. More is not better!!
- Plant treated seeds.
- When finished, wipe down hood counter, place gloves and paper wipe in regular waste container.

6.9.5.4 Treating and planting small seeds

- In the Seed Quality lab, count replicates and place in coin envelopes. Prepare germination media.
- Wear PPE: dust mask, gloves, lab coat
- Turn on hood, pull hood sash down as far as possible. Work 6 inches in from the front of the hood.
- Pour one replicate of seeds into one coin envelope
- Use glass rod/scoop to transfer a tiny amount of fungicide to the envelope or small screw top glass jars.
- Shake the envelope, evenly coating seeds. Treatment will be barely visible on the seeds. More is not better!!
- Plant treated seeds.

- Re-use the envelope to treat next sample, and to reduce contaminated waste. There may be sufficient fungicide left in the envelope to treat the next replicate.
- When finished, wipe down hood and counter, place gloves, paper wipe and contaminated envelope in regular trash.

6.10 Viability Program

- Current version was released 4/2014. Bugs encountered should be reported to IT programmer.

6.11 Evaluation

6.11.1 Evaluation follows AOSA Seedling Evaluation Handbook.

6.11.2 Other helpful references:

- Iowa State University Seedling Evaluation Manual for Corn and Soybeans
- ISTA Seedling Evaluation Handbook

6.11.3 Check unfamiliar species every few days.

6.11.4 For excessively moldy samples, transfer ungerminated seeds onto clean substrata.

6.11.5 Remove obviously dead seeds. Remove and TZ ungerminated seeds after 14 days of germination.

6.11.6 If you suspect test condition problems, retest or do a fresh TZ.

6.11.7 At the end of evaluating samples in towels, rubber bands can be disinfected and reused. Soak overnight in 3% H2O2 (Hydrogen Peroxide). (Soak rubber bands overnight?)

6.11.8 At the end of evaluating samples in boxes, place dirty boxes in the bin in **Room 212**

6.12 TZ testing

6.12.1 Purpose

6.12.1.1 TZ tests are performed to assess viability of ungerminated seeds at the end of a germination test. Viable seeds (as determined by TZ test) are recorded as "dormant."

6.12.1.2 Stand-alone TZ tests are tests not done in conjunction with a germination test. Results from the following tests should not be recorded as dormant. Results from these tests are simply normal "viable".

- Backup viability test for situations where test conditions may be adversely affecting the germination test.

- Substitute viability test for some species that have very long germination times or species that are particularly difficult to germinate in a laboratory setting (e.g. Rhus).

6.12.2 Methods. Any of the following references may be used:

- AOSA/SCST Tetrazolium Testing Handbook, 2010 edition.
- ISTA Handbook on Tetrazolium Testing, International Seed Testing Association, 1985.
- Use of the Tetrazolium Chloride Method for Determining the Viability of a Range of Flower Seed Species. Preprint No. 25, P.R. Leadley and M.J. Hill, 16th ISTA Congress, 1971.
- Grabe, D. and J. Peters. 1998. Lactic Acid Clearing of Grass Seeds in Tetrazolium Tests. Seed Technology. V 20, no. 1. 106-108.

6.13 Test Results

6.13.1 Card Notation Guide

6.13.1.1 For cryo samples, circle the best germination result. If the control result is >10% higher than the LN2 result, place the sample into standard -18C storage. If the LN2 test result is greater than the control result and higher than 85%, place the sample in LN2 storage.

6.13.1.2 Make any notation in the remark field (at the bottom of germination card) concerned with the sample. Note anything that would be helpful in monitoring the sample's viability while in storage such as: possible LN2 damage, a trend in abnormal germinations, extra treatments used like SD205, Captan, or adding GA3.

6.13.1.3 Cleaned Samples

- Entire sample cleaned: record the percentage of material removed in the "% cleanout" space at the bottom of the card
- Partial sample cleaned: do not record a percentage in "% cleanout", but still list the partial cleanout information on the card as a comment.
- See Chapter 3, E. 5. for calculation of inert that has not been cleaned out.

6.13.1.4 After the data from the card has been entered into the viability program, place a check mark next to the viability result indicating it has been sent to GRIN-Global.

6.13.1.5 No Test:

- Samples that have less than 300 seeds are not tested.

- In the first germination column write with red ink “No Test, <300 seeds, date and initials.”

6.13.2 Load “No Test” samples in the Viability Program:

6.13.2.1 Students can load a “No Test” sample to the Viability Program by downloading data from GRIN-Global in the same manner as downloading data for a germination test.

6.13.2.2 When downloading a “No Test” sample, at the data window for the ‘number of replicates for the test’, scroll to the bottom of the selections to ‘No test (-1 germination)’ and highlight. Continue the download.

6.13.2.3 When the sample is loaded to the Viability Program, and the sample’s working field is opened, note there is a “-1” in both the ‘Final Viability’ and ‘Final Normal’ columns. This “-1” is the GRIN-Global designation for a “No Test” sample.

6.13.2.4 Additional comments can be made while the sample is in the ‘Pending’ mode

6.13.2.5 The test is now ready to be sent to GRIN-Global

- Only a Technician can send a test to GRIN-Global
- Students are to give the “No Test” sample’s germination card to the Technician they are planting for, once the sample is loaded to the Viability Program
- Keep cards of “No Test” samples separate

6.13.2.6 Use the same procedure for downloading “No Test” samples to GRIN-Global as used for germination tested samples.

6.13.2.7 Check to see if the data was transferred to GRIN-Global using GRIN-Global Curator Tool.

6.13.3 When to Retest

6.13.3.1 A sample has at least 1000 seeds and results are questionable.

6.13.3.2 There is evidence that the test results are not reliable due to any of the following:

- errors in seedling evaluations
- presence of fungi or bacteria
- improper test conditions
- inaccuracies in counting and recording results.
- After retesting, circle the test result that will be recorded as official viability

6.13.4 When to Change from Cryo to Standard -18C and How

6.13.4.1 If a sample is out of tolerance (difference between LN2 reps and control reps greater than or equal to 10%)

6.13.4.2 Evidence that LN2 exposure caused damage.

6.13.4.3 LN2 result is less than 85%.

6.13.4.4 If sample has less than 1000 seeds before germination test.

6.13.4.5 Pull sample, card, and label(s) from tray and separate them into another tray and mark “standard -18C storage”. When there are one or two samples to change, separate them from the cryo samples in the same tray, marking them with "changed from cryo to conventional". With a large number of samples to change, us a new tray or crisper box for all samples to be changed.

6.13.5 Reporting Unfilled Seeds, “empties”

6.13.5.1 Germination results are based on number of seeds used.

6.13.5.2 Keep track and determine the percent of unfilled seeds.

6.13.5.3 Make a notation of those findings in the remark field at bottom of germination card. (Percentage of empties found in the number of seeds planted). Example: Empties: 15% (included in dead). In the viability program GRIN-Global comments section, state the empty comment first before all other comments.

6.13.5.4 Determine if the sample needs to be cleaned and re-tested.

7. Related Documents, flowcharts and Links

The following **flowchart** (provide links) are pertinent to this SOP:

- Link to a procedural flowchart

8. Staff Training and Competency

9. Infrastructure & Work Environment (I&WE)

Light, temperature and humidity are similar to other office environments. Growth chambers and seed storage areas have strict environmental control devices.

Access is not restricted to the Seed Analyst work area room **222** but the Vault and other seed storage areas are secured and restricted.

No open-toed shoes are allowed in the Laboratories. Seed is not pest and pathogen free.

10. Proactive Management

11. References

AOSA Rules for Testing Seeds Volumes 1-4:

Volume 1: Principles and Procedures
Volume 2: Uniform Blowing Procedure
Volume 3: Uniform Classification of Weed and Crop Species
Volume 4 Seedling Evaluation

AOSA Rules handbooks are updated yearly and a copy is kept on the network. It is illegal to download and copy the electronic Rules Handbook to individual computers without an individual membership.

AOSA/SCST Tetrazolium Testing Handbook, 2010 edition Hortus Third, Agricultural Handbook 30 - Located in **Room 210 bookshelf**.

Each analyst has their own collection of various seed ID books & Manuals.

12. Revision History

Effective Date of the SOP	**Version #**	**Description**	**Reviewed By**
03/01/2016	1.0		Stephanie Greene

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