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1. Introduction & Purpose

Samples in storage at NLGRP should have their viability monitored at intervals throughout storage. The purpose of the monitor test is to assess viability of samples that have been stored for some length of time (this is variable). The monitor test results are used to determine if seed in storage is deteriorating.

This SOP defines monitor tests and explains how the test procedures differ from viability tests conducted on newly received samples. Readers should cross reference this SOP with the viability test SOP.


2. Scope

Monitor tests are a category of viability test. It is assumed that the reader is already familiar with the methods for conducting a viability test. Monitor tests differ from viability tests on new samples in several ways as explained in this SOP.

3. Definitions & Abbreviations

The following definitions and key terms are pertinent to this SOP:

Base sample	The sample already in storage
Cryogenic sample	Storage in or over liquid nitrogen
Germination Card	Large index-type card containing germination and viability data about a sample.
GRIN-Global	Germplasm Resources Information Network
Monitor test	Viability test conducted on samples that have already been checked in, packaged and stored in either the cryo vault or the conventional vault. A monitor test should not be confused with a retest (see definition below).
Replicate	A replicate is any set of seeds used in a viability test (e.g. one replicate of 50 seeds, 2 replicates of 25 seeds, etc.)
Retest	A retest is any viability test conducted immediately subsequent to another viability test of the same sample. There can be many reasons for conducting a retest. Usually a retest is conducted to confirm the result of the previous test or because the previous test conditions were suspect or problematic. A retest should not be confused with a monitor test (see definition above).

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Tetrazolium Test	Chemical viability test for seeds using the compound 2,3,5 triphenyl tetrazolium chloride.
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.

All laboratory works must wear closed toe shoes.


Seed may be treated with hazardous chemicals.

(See the SOP for handling samples with seeds that have been treated with hazardous substances.)

5. Materials and Equipment

Materials:


- Germination Cards
- Coin Envelopes (Storage: **Room 207**)
- Laminated Foil Seed Pouches
- Black Seed Storage Tray
- Germination Blotters
- Germination Towels
- Filter Paper
- Creped Cellulose Paper
- 4x4 square plastic boxes
- Red plastic trays
- 10% Bleach Solution
- Crispers: 8"X10 3/4" clear plastic boxes
- White trays for germination carts
- Disinfected (H2O2) rubber bands
- **Lab Chemicals**
 - Stored in **Room 206**:
 - 0.2% Potassium nitrate
 - Gibberellic acid (and 500 ppm solutions)
 - Ethephon

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- 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% H₂O₂ (Hydrogen Peroxide)
 - glycerol

Equipment:

- Computer with GRIN-Global
- Walk-in germinators
- Percival germinators (**Room 210**)
- Single edge razor blades (two types: regular and extra-keen)
- Scalpels

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6. Procedure

6.1 Definition of Monitor test:

6.1.1 Monitor tests are viability tests (see viability testing SOP) done on base samples at intervals after the sample has initially been placed into storage. Monitor tests are different from retests. Retests are subsequent viability tests conducted by an analyst because of problems with test conditions or difficulty with test interpretation. Retests are done immediately after the suspect test.


6.2 Recognizing and Handling Treated Seed

6.2.1 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 equipment: When working with treated seed, lab coats, gloves and dust masks should be worn.

6.2.2 Record keeping:

- 6.2.2.1** If it is discovered that seed is treated, contact the donor to determine the chemical name of the treatment.
- 6.2.2.2** Go to the IT Group and request labels saying: "Treated Seed" to be put on final storage bags at time of packaging.
- 6.2.2.3** Write name of treatment on the Germination card and label all unpacking trays (if there is only one sample, label individual sample).
- 6.2.2.4** 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)

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6.2.3 Handling samples:

6.2.3.1 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.

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

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

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


6.3 Procedures for Monitor Testing:

6.3.1 The seed lab monitor test coordinator (Mike Bricker) has lists of seed samples that need monitor tests. The seed analyst should obtain and sign out a monitor test list from the monitor test coordinator. As samples are removed from the vault and tested, check off and date the completed samples on the list. Trays or samples removed from the cold vaults (**Room 300**) for monitor testing should be kept for short term storage in sub-zero holding **Room 225**. Pull only the trays or samples that will be worked on in one day. Allow half an hour for the seed pouches to warm up before opening them.

6.3.1.1 After opening the pouches and removing the seed for planting, fold the top of the pouch over and paper clip the pouches closed until they can be re-heat-sealed, preferably the same day as planting. If the seed pouches cannot be resealed the same day as opened, they should be resealed as soon as possible thereafter and stored in equilibration **Room 223** until they can be resealed.

6.3.1.2 Re-bagging: Labels on new seed pouches should be placed about halfway up the new pouch. The bottom edge of the location label should be placed just above the level of the storage tray top. Serial number label is placed below the location label. Labels should never be higher than the pouch fold.

6.3.1.3 All sealed pouches should be stored in the sub-zero holding **Room 225** when they are not being worked on and at night until returned to the vault.

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6.3.2 Cryogenic samples pulled for monitor tests will be pulled by Greg Holman. The seeds needed will be planted or counted out that day, the sample given to Greg to be resealed and returned to the tank.

6.3.3 It is not necessary to change seed count numbers unless the sample is cleaned for the monitor test.

6.3.4 Try to keep from cleaning the sample if at all possible. A small portion of the sample can be cleaned, 100-150 seeds, to get a representative sample for testing. Place clean seeds in a paper coin envelope and mark "clean seeds," date, and initial. Place envelope into the sample's pouch.

6.3.5 If 100-150 seeds are cleaned, determine an estimated percentage of cleanout and note the estimated percentage of cleanout on the germination card, date, and initial. Also, load the cleanout percentage on the Viability Program for the sample and make a note of cleanout in the 'Comments' field.

6.3.6 If the sample is cleaned, prior to monitor testing, take cleaned sample to Andy or Greg to be weighed. A new label with updated seed count will be created and the information will be updated on the computer program and sent to GRIN.

6.3.7 Test the number of seeds as follows:

Seeds in Sample	Seeds to Test
≥500	50
425-499	25
301-424	10
≤300	No test

6.3.8 Plant the seeds for a viability test using AOSA test conditions and use AOSA evaluation criteria or if none exist, use conditions in the prior test conducted on the sample. Record the results on the germination card and in the viability program.


6.3.9 Hard seeds do not need to be clipped.

6.3.10 Reporting Unfilled Seeds (*empties*)

6.2.10.1 Germination results are based on number of seeds planted.

6.2.10.2 Keep track and determine the percent of unfilled seeds.

6.2.10.3 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" and note the presence and percent of empties in the GRIN comments section.

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6.3.11 Check and update (if needed) the sample's storage location on the germination card. GRIN Global can provide the storage location for the sample.

6.3.12 Completed monitor tested germination cards are to be refiled into the germination card files in **Room 217**.

6.3.13 Special procedures for *Cuphea*

6.2.12.1 Allow sample to come to room temperature.

6.2.12.2 Count out monitor test replicate or distribution seeds.

6.2.12.3 Place in coin envelope.

6.2.12.4 Place envelope in TZ oven (35C) for a 1 hour period prior to germination planting or packaging for distribution.

6.3.14 Monitor tested samples will not be moved from cryogenic storage to conventional storage if the samples tested are below the 85% viability standard.

6.4 Missing samples, cards and card barcodes.

6.4.1 Missing Cards:

6.4.1.1 Check the 'Discard' file in **Room 210** to see if the sample has been discarded. If card is not in the Discard File, follow the steps below.

6.4.1.2 Send e-mail to Seed Analyst Group, IT Group with serial number and genus species.

6.4.1.3 If no response (roughly 1 week maximum) from e-mail go to IT Group to have a duplicate card printed.

6.4.1.4 In red ink, write "Duplicate" on top right hand corner of new card.

6.4.1.5 If original card is eventually found, give the original card to the IT group.

6.4.2 Missing Samples:


6.4.2.1 Check the *Discard* file in **Room 210** to see if the sample has been discarded. If card is not in the Discard File, follow the steps below.

6.4.2.2 Thoroughly inspect Seed Storage Tray as it may be out of order.

6.4.2.3 Send e-mail to Seed Analyst Group, IT Group with serial number and genus species. Explain that the seed sample was not in its location and explain the steps that have been taken so far to locate the missing sample.

6.3.2.4 If sample cannot be located, give the card to the IT group and Seed Quality Lab Supervisor for follow-up.

6.4.3 When there is no barcode label on the card, make a label with printer in **Room 208**. In the labels program, select donor label. Place label on lower right corner.

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6.5 Outsourcing of Monitor Tests to Colorado Seed Laboratory

6.5.1 Mike Bricker at NLGRP is the liaison between NLGRP and the Colorado Seed Laboratory's manager for administration of the cooperative agreement.

6.5.2 Responsibilities of NLGRP

6.5.2.1 Provide a Monitor Tests pulling list, developed by IT group, from which the Colorado Seed Laboratory pulls seed for germination testing

6.5.2.2 Pulling of seed samples from cold storage in the vault

6.5.2.3 Sealing of seed samples' seed pouches prior to return to cold storage

6.5.2.4 Return of seed samples to cold storage

6.5.2.5 Pulling germination cards for seed samples to be tested, transfer of seed germination test results from worksheets provided by Colorado Seed Laboratory to germination cards (at this time the sample's location in cold storage is updated).

6.5.2.6 Downloading of germination test results to NLGRP Viability Program

6.5.2.7 Downloading of germination tests results to GRIN-Global

6.5.2.8 Re-filing of germination cards

6.5.2.9 Providing a report of completed Monitor Tests at end of fiscal year to IT group for year ending reports

6.5.3 Responsibilities of Colorado Seed Laboratory:

6.5.3.1 Pulling of seed from storage pouches for the Monitor Testing of samples listed on the pulling list provided by NLGRP (seed samples remain at NLGRP at all times)

6.5.3.2 Conducting the germination test at the Colorado Seed Laboratory, utilizing germination test procedures from AOSA Rules for Testing Seeds, Table 3, or from specific germination test procedures provided by NLGRP


6.5.3.3 Providing the germination test results, in worksheet form, to NLGRP

6.4.3.4 Providing a memo on 'number of samples tested' at end of fiscal year or by a specified date agreed to by NLGRP and the Colorado Seed Laboratory's manager (currently the requested memo date is July 1 of the current fiscal year)

6.5.4 Handling of special testing conditions:

6.5.4.1 Seed samples requiring germination testing with fungicide will be tested by NLGRP personnel

6.5.4.2 Samples requiring treatment with fungicide at time of testing are samples formerly tested with fungicide at time of receipt by NLGRP

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due to germination problems. (As of Feb. 2016—Arachis testing procedure no longer uses fungicide treatment. MBricker)

6.5.4.3 The Colorado Seed Lab will not conduct any TZ tests (dormancy or stand-alone TZ tests) on NLGRP samples. (March 17, 2016, samples high in dormancy are on the NO-Test List for CSL and those species are tested by NLGRP staff—MBricker)

7. Related Documents, flowcharts and Links

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

8. Staff Training and Competency

Seed Analyst –Position Description

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

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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

12. Revision History

Effective Date of the SOP	Version #	Description	Reviewed By
March 17, 2016	1.0	Section 6.5	Mike Bricker

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