



Department of Environment and Natural Resources
Ecosystems Research and Development Bureau

12 November 2021

MEMORANDUM FOR THE SECRETARY

THRU : The Undersecretary
Policy, Planning and Project Management

FROM : The Director
Ecosystems Research and Development Bureau

SUBJECT : **ERDB MEMORANDUM CIRCULAR ADOPTING THE
MANUAL ON THE SEED LABORATORY OPERATIONS
OF THE ECOSYSTEMS RESEARCH AND
DEVELOPMENT BUREAU (ERDB)**

The undersigned is respectfully furnishing the Office of the Secretary a copy of the Manual on the Seed Laboratory Operations of ERDB. The Seed Laboratory Operations Manual contains all the laboratory services provided such as seed testing of different tree species specifically the viability test, and the seed health test. These include the seed collection, seed count, and Moisture Content (MC) determination. It also contains the general precautions in the operation of different equipment/instruments, appropriate handling of chemicals and glasswares, personal protective equipment, proper waste labeling, storage and disposal and other measures that laboratory personnel and clients must observe to ensure the safety and cleanliness of the laboratory set-ups and promises.

The Laboratory and Experimental Services Division (ERDB) of ERDB shall periodically review this manual for updating and for the introduction of other safety and maintenance measures based on the general accepted standards for laboratories.

**FOR YOUR INFORMATION AND FURTHER INSTRUCTIONS,
PLEASE.**


HENRY A. ADORNADO, Ph.D.

College, 4031, Laguna, Philippines

Tel Nos: (6349)5363628; 5362229; 5362269 Fax No. (6349)5362850



Department of Environment and Natural Resources
Ecosystems Research and Development Bureau

ERDB MEMORANDUM CIRCULAR

No. 2021 - 10


**SUBJECT : ADOPTION OF THE GUIDELINES IN THE MANUAL ON
THE SEED LABORATORY OPERATIONS OF THE
ECOSYSTEMS RESEARCH AND DEVELOPMENT BUREAU
(ERDB)**

In the interest of the service and in line with the ERDB's effort to provide quality and reliable laboratory test results in support to the different Research, Development and Extension Programs, Projects, and Activities (RDE PPAs), the guidelines stipulated in the Manual on the Seed Laboratory Operations are hereby adopted for the information and guidance of all concerned.

The Manual on the Seed Laboratory Operations contains the entire laboratory services provided such as seed testing of different tree species specifically the viability test, and the seed health test. These include the seed collection, seed count, and Moisture Content (MC) determination. The seed processing and testing activities are very essential to ensure the quality of the seeds collected prior to germination and planting.

The Laboratory and Experimental Services Division (LESD) of ERDB shall periodically review this manual for updating of Standard Operating Procedures (SOPs) according to the availability of equipment and processes based on the generally accepted standards for laboratories.

This Memorandum Circular takes effect immediately.


HENRY A. ADORNADO, Ph.D.
Director



Department of Environment and Natural Resources
Ecosystems Research and Development Bureau

SEED LABORATORY OPERATIONS MANUAL

2021

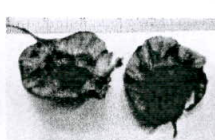
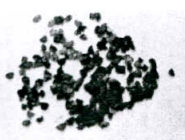
Laboratory and
Experimental
Services
Division



Department of Environment and Natural Resources
Ecosystems Research and Development Bureau

FOREST TREE SEED CENTER

SEED LABORATORY OPERATIONS MANUAL



Laboratory Service Section (LSS)
Laboratory and Experimental Services Division (LESD)

2021

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INTRODUCTION

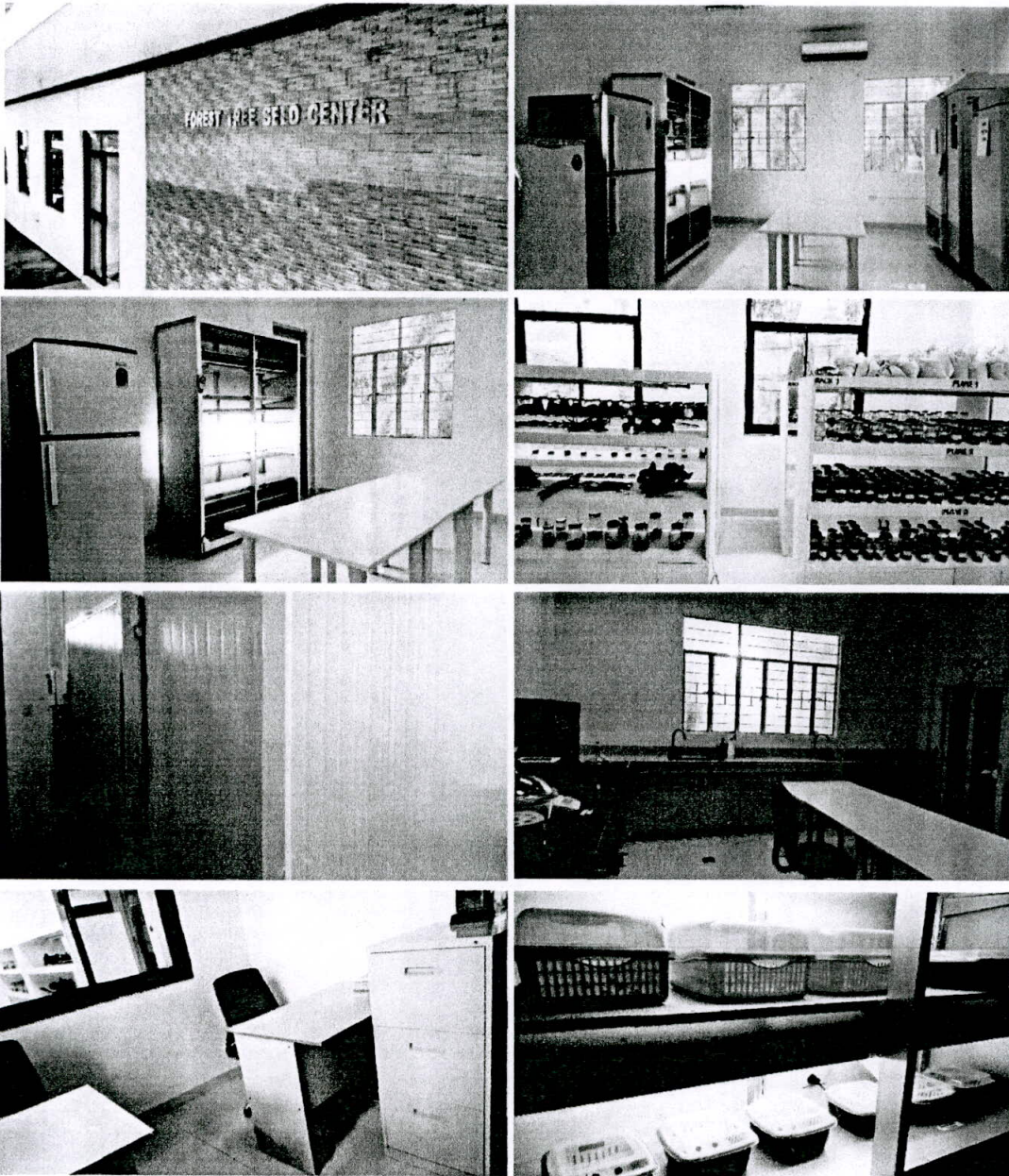
The Ecosystems Research and Development Bureau (ERDB) is the principal research arm of the Department of Environment and Natural Resources (DENR) organized through Executive Order No. 192 in June 1987. It merged the functions of the former Forest Research Institute (FORI) and the National Mangrove Committee (<http://erdb.denr.gov.ph>). ERDB has three (3) Technical Divisions and three (3) Support/Service Divisions.

The Laboratory and Experimental Services Division (LESD) is one of the service divisions responsible in the coordination and monitoring of the provisions of laboratory services being performed by the Research, Development and Extension Centers (RDECs), and the collaboration with other national and international institutions regarding researches on the Environment and Natural Resources (ENR). LESD also serves as the repository of the wildlife flora and fauna specimens, quality seeds, and other needed services in support to Research and Development (R&D) Programs, Projects and Activities of the ERDB Main Office (ERDB MO) and Research, Development and Extension Centers (RDECs) (<http://erdb.denr.gov.ph>).

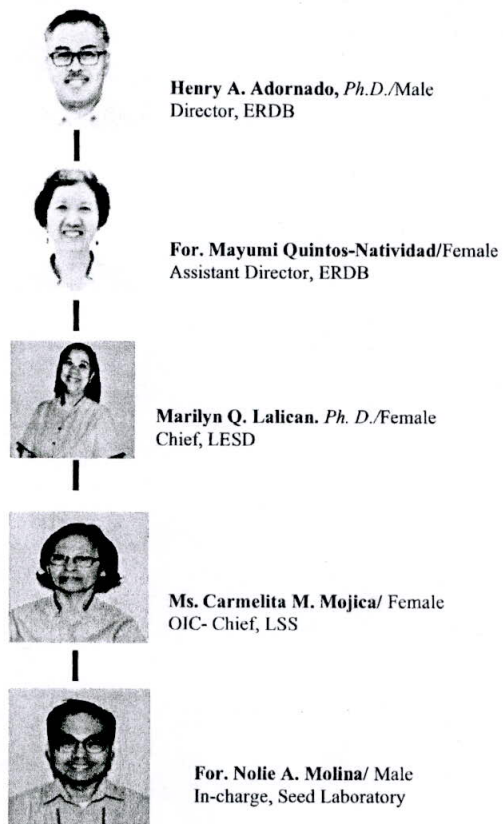
The Laboratory Service Section (LSS) is one of the 2 Sections of LESD with ten (10) different laboratories, which include the Seed Laboratory. The Seed Laboratory is housed in the Forest Tree Seed Center and serves as the facility for processing and investigating of fruits and seeds of major and minor forest products collected from various field sources. The Seed Laboratory also provides venue or workplace for various research studies, programs and projects of ERDB MO, RDECs as well as non-ERDB clientele that handle seed identification, testing and seed health tests.

This Manual contains the entire laboratory services provided such as seed testing of different tree species specifically the viability test, and the seed health test. These include the seed collection, seed count, and Moisture Content (MC) determination. The seed processing and testing activities are very essential to ensure the quality of the seeds collected prior to germination and planting.

THE ERDB MO SEED LABORATORY/FTSC



ORGANIZATIONAL STRUCTURE OF ERDB MO SEED LABORATORY



SEED PROCESSING AND TESTING

After passing the requirements stipulated in the DENR Administrative Order (DAO) No. 2021-21 dated June 30, 2021 also known as “Adopting the Field Manual for Tree Seed Collection, Processing and Certification of Quality Planting Materials and Providing Mechanisms on its Adoption”, the seeds collected are ready for transport to the Seed Laboratory for Seed Processing and Testing.

Seed Processing (DAO 2021-21)

Seed Extraction

This process involves the removal of debris and chaff contaminating the seeds. Be noted that the activities depend on the selected species. Basically, the process involves the following:

- Extracting the seeds from the fruits (drupe and other species with hard endocarp);
- Extracting from fleshy fruits (berries);
- Extracting from pods and cones; and
- Dewinging or removal of wings (e.g. in the seeds of dipterocarps)

Seed Drying

Proper drying of newly extracted seeds from fleshy fruits shall be done to protect the seed from deteriorating. This can be done by either by sun or air drying. In sun drying, the seeds shall be scattered in a mat or in a clean, cemented area in sieve or wire mesh. The length of drying shall be determined for each tree species.

Seed Cleaning/Upgrading

Seeds shall be cleaned after drying to remove further the impurities particularly for small seeded species. The common method of cleaning shall be done (e.g. winnowing or blowing of air to separate seeds from debris).

1. Seed Sorting

Segregation of seeds shall be done depending on the weight and size of the seeds.

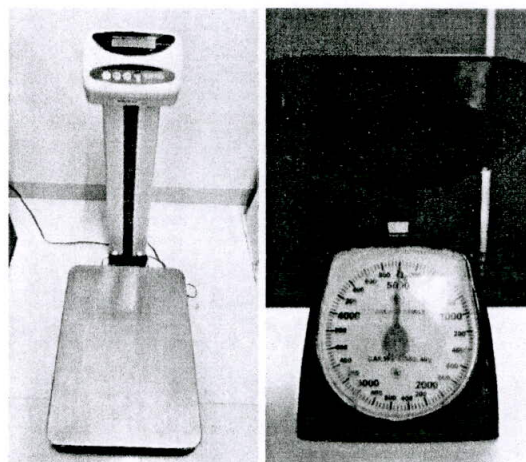
2. Purity Test- it is the determination of the purity of seeds.

Purity Test is applicable only to small seeded species (e.g. agoho, malapapaya and bagras) wherein impurities i.e. fruit debris, dried leaf and stem is being removed from the seed lot. This test determines the “pure seed” which contains both viable and non-viable seeds. The % Purity is computed as follows:

$$\% \text{ Purity} = \frac{\text{weight of the sample} - \text{weight of impurities}}{\text{weight of the seed sample}} \times 100$$

Seed Count

It is the determination of the number or quantity of seeds per kilogram or per gram (g). For big seeds (e.g. lumbang, bitaog); the actual counting of seeds per kg is done to determine the seed count. For medium seeds (e.g. kupang, raintree) - a 100 g to 250 g of sample is counted then compute for the estimated count per kg. For small seeds (e.g. agoho, bagras) – weigh 5-10 g per sample and compute the estimated seed count. The commonly used equipment is the weighing scale.



The Weighing Scale

For orthodox species, seed count is determined after drying of the seeds while for recalcitrant seeds (e.g. dipterocarps), seed count shall be done right after %MC determination.

Seed Testing (DAO 2021-21)

Percent Moisture Content (% MC) Determination

The determination of Moisture Content (MC) is very vital since by % MC determination we will know if the seeds are needed to be dried further or not. This is also needed to determine the storage physiology of the particular seed sample.

The equipment needed are analytical balance, oven and the aluminum containers. For small sized seeds (e.g. bagras and agohe), 0.1 g should be weighed for 4 replicates, for medium sized seeds (e.g. kupang and rain tree) 10-20 seeds per replicate while for big sized seeds (bitaog and white lauan), 3-5 seeds per replicate. Oven dry the container and the seeds at $103 \pm 2^\circ\text{C}$ for 17 hours (4pm – 9am next day). Place the seeds in a dessicator to cool off then compute the % MC. The % MC can be computed using the formula:

$$\% \text{ MC} = \frac{M2 - M3}{M2 - M1} \times 100$$

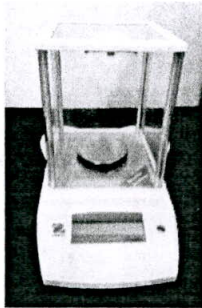
Wherein:

M1= weight of the container

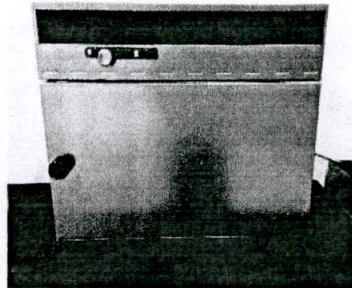
M2= weight of the container + seeds (green weight)

M3= weight of the container + seeds (oven dry weight)

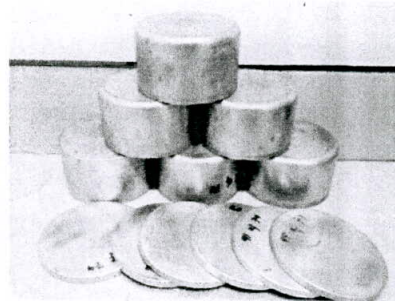
For seeds orthodox species, the determination of MC will be done to determine how many days are needed to dry the seeds. Likewise, for recalcitrant species, the determination of MC will be done to determine if the initial MC is still within the critical limit whether sowing of the seeds can be delayed for a few days with a minimal reduction in percent germination.



Analytical Balance



Mechanical Oven



Aluminum Containers

Viability/Germination Test

It is done to determine the percentage of seeds that can be expected to produce normal plants under the favorable condition. Results of the germination test shall be properly recorded.

Methods of Viability/Germination Testing:

- a) Direct Testing – done through actual germination test using dormant seeds. Seeds are sown immediately without any pre-treatments.

For dormant seeds, treatments like: (a) mechanical scarification or removal of seed coat, pericarp, nicking or cutting of the seed coat or depulping; (b) use of chemicals such as H_2SO_4 , H_2O_2 and HCL to soften the seed coat; and (c) physical (e.g. hot water or hot and cold treatment) shall be tested to determine which one is the most appropriate for a particular seed.

- b) Indirect Testing – done through cutting test, floatation test, and by physical observation such as normal color and other deformity of seeds.
 - Cutting Test- from the seedlot, select 100 seeds. Cut the seeds into halves and count the number of empty and full seeds (viable embryo, greenish-white and fresh appearance).
 - Soaking Test- Randomly select 100 seeds. Soak the seeds in tap water for 12-24 hours. Examine the seeds after soaking, most viable seeds remain submerged, some are swollen. None viable seed floats. Count the number of viable seeds.
 - Physical Observation Test- Randomly select 100 seeds. Examine the seeds. Viable seeds have normal shape and appearance, uniform

seedcoat, glossy and healthy in appearance. No-viable seeds are smaller, dull in color or discoloured, deformed and with crack seed coat.

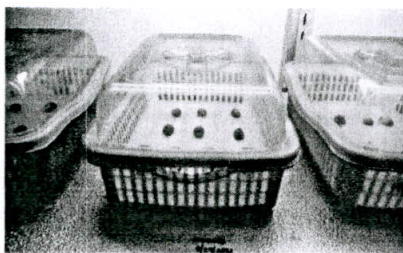
Seed Health Test

- a. Blotter Test- For small sized seeds (e.g. bagras and agoho), place 50 seeds in a petri dish lined with 3 layers of filter paper, moistened with distilled water. This will be replicated 4 times. For medium to large seeds (gmelina, kupang, supa and mahogany), place the seeds in plastic germination tray with 3 layers of paper towel moistened with 150 ml distilled water. This will be also replicated 4 times. The replicates will be incubated with light for 5-7 days to enhance fungi sporulation. After that, the seeds will be examined using a compound microscope for the presence of fungi and other microorganisms. All structures observed were recorded, picked up the structures and observed using pointed transfer needle. The isolate was fixed using plain lactophenol for further examination up to species level.

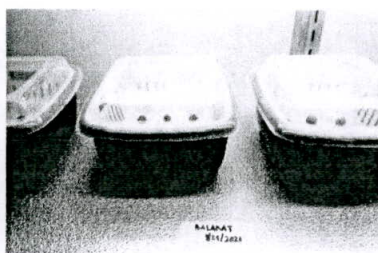
The percent infection of each isolate together with the % germination of the species tested were also computed.

$$\% \text{ germination} = \frac{\text{No. of seeds germinated}}{\text{Total No. Of seeds sown}} \times 100$$

$$\% \text{ Infection} = \frac{\text{No. Of seeds infected}}{\text{Total no. Of seeds}} \times 100$$



The Plastic Germination Trays



The Compound Microscope

- b. Dry Inspection Test – put 200 seed samples in a petridish and observe under the microscope
- c. Agar Plate Test- Use of potato dextrose agar (PDA). Disinfect the seeds with 10% NAOCL for 5 minutes and wash thoroughly with distilled water. Blot the dry seeds and aseptically transfer in plated PDA.

For both blotter and agar plate test, the petri-dishes and trays are incubated in an incubation chamber with light for 5-7 days to enhance fungi sporulation. The seeds are examined in a microscope for the presence of fungi and other microorganisms. All the structures are to be picked-up and observed using the pointed transfer needle. Fix the isolate using plain lactophenol for further examination up to the species level. Record all the microfungi observed and identified, record in the seed health testing form and compute the percent infection of each isolate together with the % germination of the seeds tested.

LABORATORY SAFETY GUIDELINES

Personnel In-charge

Name	Position/Designation
For. Nolie A. Molina	Science Research Specialist II/In-Charge, Seed Laboratory

Personal Protective Equipment (PPE)

List of Personal Protective Equipment in the Seed Laboratory

- 1 Laboratory Gown
- 2 Face Mask (disposable)
- 3 Latex gloved

Safe Handling of Samples

Seed Samples

- Samples are stored in sterilized containers and kept in rooms with controlled temperature and relative humidity. In the case of the ERDB Seed Laboratory/FTSC, the seeds can be stored in the walk-in-refrigerator wherein the temperature can be manipulated depending on the requirement of the seeds to be stored.

Safe Handling of Equipment

- Cleaned or sterilize glass wares (e.g. test tubes, flasks, beakers, petri-dishes and plates) are be stored in designated cabinets. Used glasswares not needed to be sterilized are being cleaned immediately as possible.
- Laboratory equipment and appliances such as growth chamber, laboratory oven, autoclave, incubator, and refrigerator among others are be cleaned weekly.

- Launder protective clothing on a regular basis and especially after being contaminated due to the spill or relative incident. Do not launder protective clothing at home.

Safe handling of chemicals

- Chemicals and reagents are kept in designated storage with proper labels.

Laboratory Waste Management

- The Laboratory only produces waste from plant materials such as seeds and plant tissues.

Analysis	Waste Generated	Waste Container
Non-Hazardous Wastes		
Seed Germination Testing/ Seed Viability Testing	Seeds	Bins labeled as “biodegradable solid wastes”
	Plant Tissue	
Other Wastes		
	Used Gloves	Scaled Bag labeled as “biohazard waste”
	Face Masks	Bins labeled as “special wastes”

REFERENCES

DENR. 2021. Department Administrative Order (DAO) No. 2021-21. Adopting the Field Manual for Tree Seed Collection, Processing and Certification of Quality Planting Materials and Providing Mechanisms on Its Adoption. pp.22-23.

ERDB-DENR. 2019. Laboratory Safety and Waste Management Manual. pp.72-73.

ERDB. Lifted from <http://erdb.denr.gov.ph> on October 19, 2021.

LESD. Lifted from <http://erdb.denr.gov.ph> on October 19, 2021.