

**OFFICIAL SEED TESTING STATION
SEED TESTING LABORATORY, KEDL0100**

Standard Operating Procedure:

Germination Test

SOP/MET/04

Revision number 16

Issue date: 16th April, 2024

**STANDARD OPERATING PROCEDURE FOR
GERMINATION TESTS**

SOP/MET/04

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Quality Assurance Manager

Date:.....

Date:.....



OFFICIAL SEED TESTING STATION SEED TESTING LABORATORY, KEDL0100

Standard Operating Procedure:

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TABLE OF CONTENTS	Page
Purpose	4
Scope	4
References	4
Definitions and Acronyms	4
Responsibilities	4
Procedure/activities	5
Records	7
Flow chart	8
Flow chart for Germination analysis	8

Appendices

Appendix G1: Procedure for germination media preparation.

Appendix G2: Testing new supplies of sand and paper media

Appendix G3: Procedure for Three Shelf Level Temperature & Humidity Check in Germination Rooms

Appendix G4: Procedure for monitoring temperature and humidity in germination equipment

Appendix G5: Boiler operation

Appendix G6: PH Meter operation and calibration

Appendix G7: Germination planting boards

Appendix G8: Vacuum pump & accessories

Appendix G9: Operating Instructions for automatic water distiller

Appendix G10: Scope of Accreditation

Appendix G11: List of Equipment at germination testing

Appendix G12: Procedure for Growing media specification checks



**OFFICIAL SEED TESTING STATION
SEED TESTING LABORATORY, KEDL0100**

Standard Operating Procedure:

Germination Test

SOP/MET/04

Revision number 16

Issue date: 16th April, 2024

Appendix G13: Forms in Germination analysis

- i) Equipment register: temperature monitoring record (FRM/STL/10B)
- ii) Temperature and humidity records for germination(FRM/STL/11)
- iii) Temperature monitoring record for incubation room (FRM/STL/12)
- iv)Temperature records for three shelf levels (top, middle & bottom) in germination rooms. (FRM/STL/33)
- v) Calibration form for PH meter(FRM/STL/32)
- vi) Testing new supplies of germination paper and sand (FRM/STL/28A)
- vii) Water retention capacity Form(FRM/STL/35)

Appendix G14; Temperature Mapping for Germination Rooms

Appendix G15; Sand Growing Media Particle Size

Appendix G16; Testo 174T work instruction



OFFICIAL SEED TESTING STATION SEED TESTING LABORATORY, KEDL0100

Standard Operating Procedure:

Germination Test

SOP/MET/04

Revision number 16

Issue date: 16th April, 2024

1.0 Purpose

- 1.1 This procedure describes the process of germination testing at the Seed Testing Laboratory.

2.0 Scope

- 2.1 From preparation of samples and media to planting, evaluation, calculation and recording of results.

3.0 References

- 3.1 International Rules for Seed Testing (ISTA Rules)
3.2 Seedling Evaluation handbook
3.3 Quality Assurance Manual

4.0 Definitions and Acronyms

- 4.1 KEPHIS: Kenya Plant Health Inspectorate Service (Seed Certifying Agent-SCA)
- 4.2.1 Seed Testing Laboratory (STL) – The Laboratory for seed testing with capability to conduct seed sampling, analyze seed samples for various tests and to report the analysis results.
- 4.3 ISTA – International Seed Testing Association.
- 4.4 Seed Analyst – Person qualified and approved/authorized by Managing Director KEPHIS to analyze seed samples at the Seed Testing Laboratory.
- 4.5 Official Seed Tester (OST) – Person in charge of the Seed Testing Laboratory.

5.0 Responsibilities

- 5.1 STL – Official Seed Tester (OST) - owner of the process
5.2 Analyst – Seed Analyst/In-charge – planting, evaluation, and recording of



OFFICIAL SEED TESTING STATION SEED TESTING LABORATORY, KEDL0100

Standard Operating Procedure:

Germination Test

SOP/MET/04

Revision number 16

Issue date: 16th April, 2024

- Germination results.
5.3 STL – Publishing of final certificates.

6.0 Procedure/Activities

- 6.1 Take sample for testing from Purity Section and sign sample movement register (R/STL/17)
- 6.2 Select germination test method depending on the species under test i.e. either germination by weighed replicate or on 400 seeds. Check Appendix G10 of this SOP or the current ISTA Rules for germination test method according to species.
- 6.3 a) For germination on 400 seeds, mix sample well and count four replicates of 100 seeds – using vacuum counter, counting boards or manual counting.
b) For Weighed replicate germination method, weigh 4 replicates of approximately the weight stated in ISTA Rules Table 13A and 13B. Use ISTA Rules clause 13.5 for further information
- 6.4 Choose the substrates to use: Sand, paper or soil (if phytotoxicity is suspected). Check appendix G10 of this SOP for the laboratory preferred method.
- 6.5 Prepare the substrates and pre-treat the samples if necessary as per ISTA Rules Table 5A.
- 6.6 Plant the sample and take the remnant sample to the sample store.
- 6.7 Pre-chill at 5⁰C -10⁰C Cold room, SL 006 if necessary as per ISTA Rules Table 5A. Duration of pre-chilling is specified in appendix G10 of this SOP.
- 6.8 For Beta vulgaris, pre-washing must be done in running water at a temperature of 25⁰C ±2⁰C and dried at a temperature of 20⁰C to 25⁰C.
- 6.9 Incubate the sample in the respective germination room/incubator temperature for the specified number of days as specified in ISTA Rules Table 5A.
- 6.10 Examine and evaluate the seedlings, categorizing them into Normal seedlings (N), Abnormal seedlings (A), Hard seeds (H), Fresh and Dead seeds (D).
- 6.11 Record the number of seeds lost or found and if there are more than 5 seeds found or lost in the test, the test must be repeated.
- 6.12 Duration of tests**
- a) Duration of the test may be shortened if the maximum germination of a sample has been obtained.
- b) Duration of the test may be extended by 7 days for species with a prescribed period of 14days or less, or up to half the prescribed period for species with a prescribed period of 14 or more days.



OFFICIAL SEED TESTING STATION SEED TESTING LABORATORY, KEDL0100

Standard Operating Procedure:

Germination Test

SOP/MET/04

Revision number 16

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- c) If the germination test period ends on a non-working day (Weekend or public holiday), the final count can be done the day before or after this date,
- d) Tests in sand lasting not more than 14 days, the first count may be omitted.
- e) Upon request by the client, the test can be terminated before the final count, but only the percentage of normal seedlings will be reported. The results of other categories (Abnormal seedlings, Hard, Fresh, and dead seeds) shall be reported as 'N'. If the germination period was extended beyond the period in table 5A, the statement 'After the prescribed period of days, there were% normal seedlings' shall be added

6.12 Record the results on the germination card (WC/STL/GERM).

6.13 Calculation of results

6.13.1 Germination on 400 seeds

6.13.1.1 If there are upto 5 seeds lost or found as extra in the test, then each replicate must be adjusted to 100 by calculation e.g. a replicate with 80 normal seedlings, 10 abnormal seedlings, and 9 dead seeds with one seed missing, adjust to 100 as follows;

- i) Normal seedlings = $\frac{80 \times 100}{99}$
- ii) Normal seedlings = $\frac{10 \times 100}{99}$
- iii) Dead seeds = $\frac{9 \times 100}{99}$

6.13.1.2 Calculate the average for each category of normal, abnormal seedlings, hard, fresh and dead seeds in the four 100-seed replicates. The sum of normal, abnormal seedlings and un-germinated seeds must be 100.

6.13.1.3 Follow the rounding procedure in clause 5.8.2 of the current edition of ISTA Rules.

6.13.1.4 To check for tolerances, compare the average percentages with Table 5B of the ISTA Rules. The result is considered reliable if the difference between the highest and the lowest replicate does not exceed the tolerance indicated. Otherwise repeat the test.



OFFICIAL SEED TESTING STATION SEED TESTING LABORATORY, KEDL0100

Standard Operating Procedure:

Germination Test

SOP/MET/04

Revision number 16

Issue date: 16th April, 2024

6.13.1.5 Use Table 5C of current edition of ISTA Rules to check tolerances between two tests of the same sample

6.13.2 Germination by weighed replicate

- 6.13.2.1.1 Add together the weight of the four individual replicates and calculate the average weight of replicates.
 - 6.13.2.1.2 Add the number of normal seedlings and calculate the average.
 - 6.13.2.1.3 Calculate the number of Normal seedlings per kg
 - 6.13.2.1.4 To check for tolerances, compare the total number of normal seedlings with Table 13C of the current edition of ISTA Rules. The result is considered reliable if the difference between the highest and the lowest replicate does not exceed the tolerance indicated. Otherwise repeat the test.
- 6.14 Repeat the test if :the replicates exceeds the maximum tolerated range, there are evidence of errors in test conditions, seedling evaluation or counting and if the results of germination tests are not reliable due to phototoxic or spread of fungi, or bacteria. Refer to the current edition of ISTA Rules Section 5.7.
- 6.15 Take the weight replicate card (WC/STL/WGT.REP) to sample reception.

7.0 Germination media specifications

- i. Water retention- When water is added, should hold sufficient water to allow the seedling grow for the entire test duration.
- ii. Paper media-Roots must grow on and not into the paper
- iii. PH: 6.0-7.5
- iv. Conductivity: not more than 40µs/m
- v. Cleanliness-Free from fungi, bacteria or toxicity.

8.0 Records

- 8.1 Germination test card (WC/STL/GERM)
- 8.2 Germination register (R/STL/03)
- 8.3 Kephis Seed Database
- 8.4 Test Certificates
- 8.5 Archives
- 8.6 Sample movement register (R/STL/17)
- 8.7 Germination retest register (R/STL/15)



**OFFICIAL SEED TESTING STATION
SEED TESTING LABORATORY, KEDL0100**

Standard Operating Procedure:

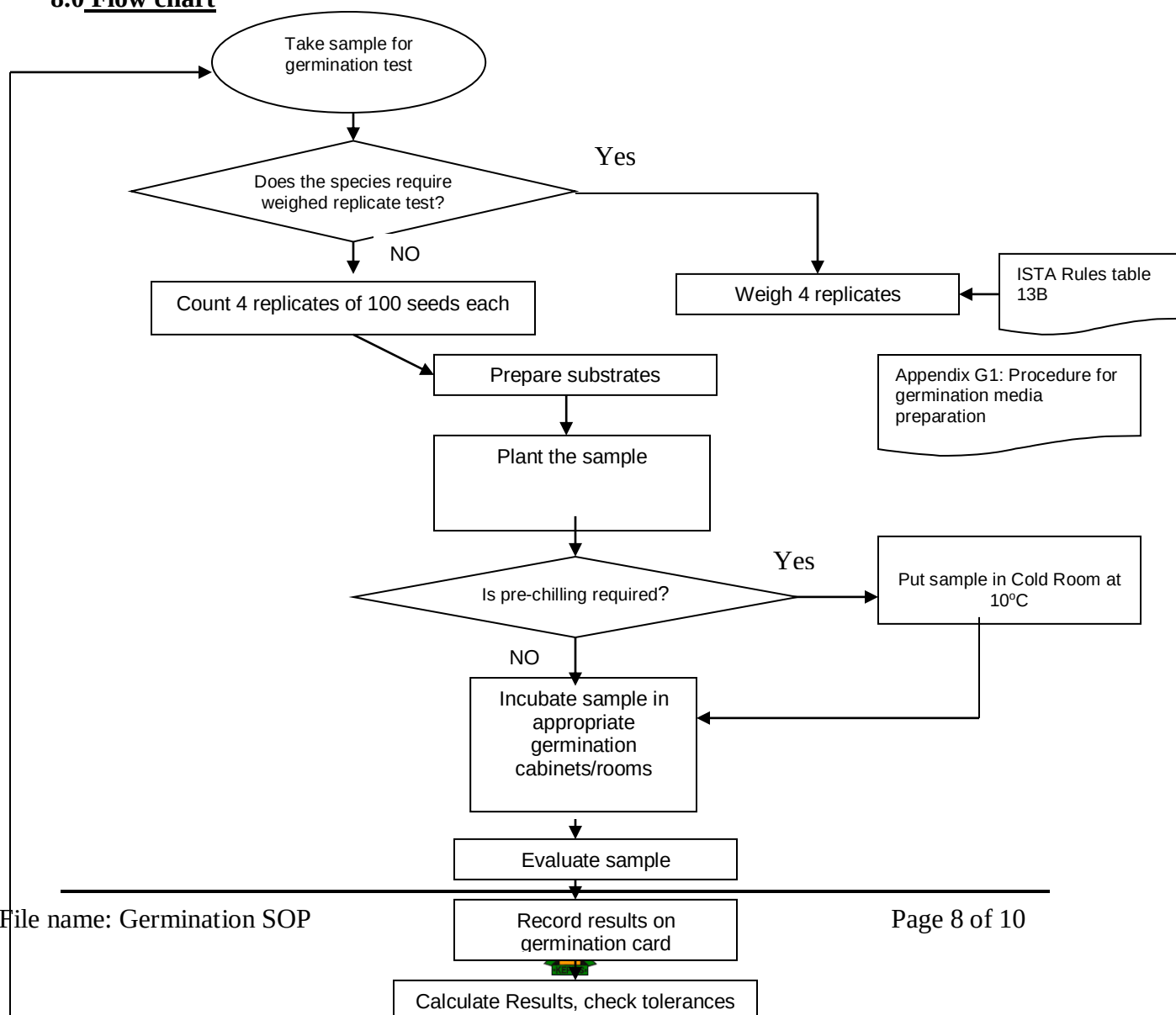
Germination Test

SOP/MET/04

Revision number 16

Issue date: 16th April, 2024

8.0 Flow chart



OFFICIAL SEED TESTING STATION SEED TESTING LABORATORY, KEDL0100

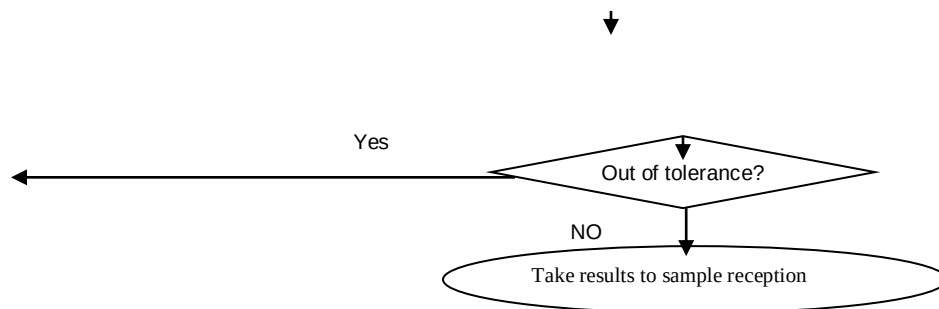
Standard Operating Procedure:

Germination Test

SOP/MET/04

Revision number 16

Issue date: 16th April, 2024



9.0 Appendices

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- e. Calibration form for PH meter(FRM/STL/32)
- f. Testing new supplies of germination paper and sand (FRM/STL/28A)
- g. Water retention capacity Form(FRM/STL/35)



**OFFICIAL SEED TESTING STATION
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Germination Test

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10.0 Revision history

Revision Number	Clause	Changes
16	7.0	Specifications for germination media added.

