Seed Manual

Standard Operating Procedure

Deependra Dhakal

2021-06-27

Contents

Chapter 1

Administrative System

1.1 Scope and Purpose

The administrative division is the main support unit among all remaining technical units of CSTL. It provides human resources along with material and financial aid to all divisions and units of the organization. In coupled with them it provides secretarial services, staff management, store facilities, procurement as well as repair and maintenance of the equipment and other machineries.

Seed samples are submitted to Sample Reception Unit of Administration Division along with the official request letter and are registered here.

1.2 Procedure

1. Each sample along with all the set of Forms that has been prepared in the sample reception area is passed on to the Chief of Administration Division.
2. The chief of the Division examines the sample and the request letter. If the client has provided required information adequate quantity of the seed sample, the client will not be made to provide further clarification otherwise the clear information is generally sought before proceeding. The sample is coded and distributed along with the respective forms to the concerned laboratory unit or units for requested test data.
3. The covering letter accompanied with the submitted sample is retained with the administration unit in a covered folder.
4. The laboratory units, on the other hand, submit the test results with checking by units incharge to the administration unit as soon as the forms/ cards have been duly filled with the test results and comments. The administration unit files them in the respective folder for further action.
5. Results are reported in the percentage and recalculation is done if necessary. Such recalculations are generally carried in the respective laboratory unit.
6. The purity test results are generally converted into percentage by weight. The duplicate percentages are averaged and checked against the respective tolerance tables. It, they are not

in consistent with the tolerance level, additional duplicate tests are performed.

7. Germination test results are expressed in percentage by weight based on number of seeds. If the result is found to be out of tolerance level, the administration division asks the germination laboratory unit to repeat the tests by providing a new set of Pure Seed and Seed Analysis Card (Annex-I)
8. As soon as all data are furnished with the aid of concerned laboratories, a final check is made for the completeness and correctness of data and only then the test result or the test certificate in the Standard Report Format (Annex-II) is dispatched to the client.
9. A copy of the report along with all other relevant analysis cards are filed in the respective cover and is stored in a filing cabinet under "completed tests". These test results are preserved at least for a season or for a period as required under Seed Act and Rules before they are disposed off.
10. The administration unit does also take the responsibility of duly maintenance and servicing of the testing equipment and quality management system of the laboratory unit.
11. The administration unit offers a best possible service to the client and looks for to avail the results to the client whenever requested.
12. For the smooth and effective operation of the laboratory, proper routing of the sample from the registration through reporting becomes very important and prevailing flow diagram is attached.

Chapter 2

Seed Sampling

2.1 Scope

Various attributes of seed such as analytic, physiological, pathological and to some extent variety characteristics are determined in the laboratory based on different tests. Such determinations can only be an estimate unless the whole quantity of seed lot is tested. This is practically impossible, as a lot usually contains a large quantity of seed. Under such circumstances, to give as accurate an estimate as possible representative samples are prepared.

A composite sample is obtained from the seed lot by drawing out small portions at random from different positions in the lot and thoroughly mixing them together. At each stage, thorough mixing is followed either by progressive sub-division or by the abstraction and combination of small portions at random.

2.2 Object

The objective of sampling is to obtain a sample of a size suitable for tests, in which probability of a constituent being present is determined only by its level of occurrence in the seed lot.

2.3 Definitions

2.3.1 Seed Lot

A Seed lot is a specified quantity of seed that is physically and uniquely identifiable.

2.3.2 Primary Sample

A primary sample is a small portion taken from the seed lot during one single sampling action.

2.3.3 Composite Sample

The composite sample is formed by combining and mixing all the primary samples to taken from the lot.

2.3.4 Working Sample

The working sample is a sub-sample taken from the submitted sample in the laboratory, or a sub-sample thereof, on which one of the quality tests described in these ISTA Rules is made and must be at least the weight prescribed by the ISTA Rules for the particular test.

2.3.5 Submitted Sample

A submitted sample is a sample that is to be submitted to the testing laboratory and may comprise either the whole of the composite sample or a subsample thereof. The submitted sample may be divided into subsamples packed in different material meeting conditions for specific tests e.g. moisture or health.

2.3.6 Duplicate sample

A duplicate sample is another sample obtained for submission from the sample composite and marked 'Duplicate sample'.

2.3.7 Sub-sample

A submitted sample is a portion of a obtained by reducing a sample.

2.3.8 Sealed

Sealed means that the containers or individual container in which the seed is held are closed in such a way that, it cannot be opened to gain access to the seed and closed again, without either destroying the seal or leaving evidence of tempering. This definition refers to the sealing of seed lots, as well as of seed samples.

2.3.9 Self-sealing containers

The 'valve-pack' bag is a specific type of self-sealing container. It is filled through a sleeve-shaped valve which is automatically closed by the completion of filling the bag.

2.3.10 Marked/labeled

A container of a seed lot can be considered as marked or labeled when there is a unique identification mark on the container, which defines the seed lot to which the container belongs. All containers of a seed lot must be marked with the same unique seed lot designation (numbers, characters or combination of both). Marking of samples and sub-samples must ensure that there is always an unambiguous link between the seed lot and the samples and sub-samples.

2.3.11 Treated seed

'Seed treatment' is a generic term which indicates that a seed lot has been subjected to;

a) the application of a compound including chemicals, nutrients or hormones

b) the application of a biological product including micro-organisms,

c) a process including wetting and drying

d) an energy form including heat, radiation, electricity or magnetism but does not specify the application method.

Seed treatment does not significantly change the size, shape or add to the weight of the seeds in the lot.

2.4 Conditions for issuing Orange International Seed Lot Certificates

The sampling methods laid down in the ISTA Rules must be followed when seed samples are drawn for the issue of Orange International Certificates. Further conditions have to be fulfilled as listed below.

2.4.1 Seed lot size

The seed lot must not exceed the quantity indicated in ISTA Rules (Table 2C)

2.4.2 Large herbage seed lots of Poaceae

Seeds may have a maximum size of 25000 Kg (with a 5% tolerance)

2.4.3 Check sampling and testing

After approval, the large seed lots of a production plant must be monitored by check sampling and further heterogeneity testing and as a minimum based on purity and other seed count.

If more than one of the last six consecutive check samples tested shows significant heterogeneity, approval must be withdrawn for the species or species of group and company must be re-apply for approval.

2.4.4 Responsibility

The Seed Quality Control Center in a country is responsible for;

- the decision of approval of the seed company ,
- ensuring that each production plant is approved separately, if a seed company has more the one production plant,
- ensuring that testing is done by an ISTA-accredited laboratory,
- the check sampling program.

2.4.5 Marking /labeling and sealing of containers

The seed lot must be in marked/labeled containers which are sealed or under the control of seed sampler.

Where the seed lot is already marked/labeled and sealed before sampling, the seed sampler must verify marking /labeling and sealing on every container. Otherwise the sampler has to mark/label the containers before seed lot leaves his/her control.

The samplers are personally responsible for seals, labels and bags supplied to them and it is their duty to ensure that primary, composite or submitted samples must never be left in the hands of persons not authorized by the seed testing unless they are sealed in such a way they cannot be tampered with.

2.4.6 Sampling from the seed lot

Sampling form (Annex-IV) the seed lot methods must be used. An orange International Seed Lot Certificate issued on a seed lot is still valid after re-packing the seed lot in new containers provided that,

a) the identity of the seed in the initial seed lot is preserved

b) the seed lot designation is not changed.

c) the moving the seed into the new containers is done under the control of an ISTA seed sampler.

d) there is no processing of the seed during filling of the new containers.

2.4.7 Submitted sample

The minimum sizes of submitted samples are as follows

a) For moisture determination,

100 g for species that must be ground and 50 g for all other species. When moisture meters are to be used for testing, a large sample size may be necessary.

b) For verification of species and variety

c) For all other test, at least the weight as described in ISTA Rule.

2.4.8 Uniformity of the Lot

At the time of sampling the lot should have been subjected to appropriate mixing, blending and processing techniques so that it is as uniform as practicable.

2.4.9 Containers

The lot should be in containers which are sealed in jute bags, metal drum that are sealed or air tight.

2.4.10 Marking and Sealing the Lot

At the time of sampling, all containers must be sealed, labeled or marked to show lot identification.

2.5 Procedures for Sampling the Lot

Sampling is done in two stages:

1. A submitted sample is taken in the warehouse of field and sent to the seed-testing laboratory. This sample is usually ten times bigger than the quantity required for testing.
2. A working sample is prepared from the submitted sample in the laboratory for quality test.
3. Before the warehouse sampling, the seed lot shall be so arranged that each individual container of the lot is conveniently accessible. If the nature of the stacking of the lot or type of the container makes it impossible to apply procedures adequately, this sampling shall not be undertaken and when there is definite evidence of heterogeneity either physical or documentary, sampling shall be refused.

2.6 Sampling Intensity

2.6.1 General principles

The number of primary samples to be taken depends on the size of a lot and type of containers. A composite sample is obtained from the seed lot by taking primary samples from different positions in the whole seed lot and combining them. From this composite sample, four subsamples are obtained by sample reduction procedures at one or more stages forming the submitted sample and finally the working samples for testing. For issuing ISTA Certifications, specific requirements have to be fulfilled.

2.6.2 Apparatus

Sampling and sample reduction must be performed using appropriate techniques and equipment that is clean and in good condition. Containers (metal) used to collect primary samples, composite sample and during mixing and dividing must be static free to avoid chaff or small seeds adhering to the inside of the containers.

2.6.3 Procedures

1. Procedures for sampling a seed lot

1.1 Preparation of a seed lot and conditions for sampling

At the time of sampling, the seed lot must be as uniform as practicable. If the seed lot is found to be obviously heterogeneous, sampling must be refused or stopped. In cases of doubt heterogeneity can be determined.

Seed may be sampled in containers or when it enters containers. The containers must be fit for purpose, i.e. must not damage the seed, and must be clean to avoid cross contamination. The

containers must be labeled or marked before or just after sampling is completed. The seed lot must be so arranged that each part of the seed lot is conveniently accessible.

2.6.4 Sampling intensity

A. For seed lot in containers of 15 kg to 100 kg capacity (inclusively), the sampling intensity according to Table 2A must be regarded as the minimum requirements.

B. For seed lots in containers smaller than 15 kg capacity, containers must be combined into sampling units not exceeding 100 kg, e.g. 20 containers of 5 kg, 33 containers of 3 kg or 100 containers of 1 kg. For seed mats and tapes, small packets or reels may be combined to sampling units of not exceeding 2,000,000 seeds. The sampling units must be regarded containers as in Table 2A.

When sampling seed in containers of more than 100 kg, or from streams of seed entering containers, the sampling intensity according Table 2B must be regarded as the minimum requirement.

When sampling a seed lot of up to 15 containers, regard less of their size, the same number of primary samples must be taken from each container.

2.7 Sampling Instruments and Methods

2.7.1 Taking primary samples

When defining the number and/or the size of primary samples, the seed sampler needs to ensure (besides meeting the minimum sampling intensity) that the minimum amount of seed required for the requested test(s) is sent to the testing laboratory and enough seed remains available for obtaining duplicate samples if requested.

Primary sample of approximately equal size must be taken from a seed lot, irrespective of where in the lot or container the primary sample is taken.

When the seed lot is in containers, the containers to be sampled must be selected at random or according to a systemic plan throughout the seed lot. Primary samples must be drawn from the top, middle and bottom of containers, but not necessarily from more than one position in any container, unless so specified in Table 2A and Table 2B.

When the seed is in bulk or in large containers, the primary samples must be drawn from random position. Containers must be opened or pierced for abstraction of primary samples. The sampled containers must then be closed or the contents transferred to new containers. When seed is to be packed in special types of containers (e.g. small, not penetrable, or moisture-proof containers), it should be sampled, if possible, either before or during the filling of the containers.

The instruments being used must neither damage the seed nor select according to seed size, shape, density, chaffiness or any other quality trait. All sampling apparatus must be clean before use to prevent cross contamination. Trier must be long enough so that the opening at the tip reaches at least half of the diameter of the container. When the container is not accessible from opposite sides, the Trier must be long enough to reach the opposite side. Sampling seed lots may be done by one of the methods listed below.

a. **Manual sampling from a seed stream**. Seed streams may also be sampled by using manual instruments when fulfilling the requirements listed under "a".

b. **Sampling Stick**. The sampling stick (e.g. stick Trier, sleeve type Trier, spiral Trier) consist of two parts, one of which fits loosely inside the other, but tightly enough so that seed or impurities do not slip between them. The outer part has a solid pointed end. Both parts have slots in their walls so that the cavity of the inner part can be opened and closed by moving the two parts against each other by either a twisting or a push pull motion. The sampling stick may be used horizontally, diagonally or vertically. The spiral Trier has slots in a spiral arrangement for their subsequent opening from the tip to the handle and may only be used for seeds of a size smaller than *Triticum aestivum*.

However, when used vertically or diagonally downwards the sampling stick must either have partitions dividing the instrument into a number of compartments or have slots in a spiral arrangement. The minimum inside diameter should be about 25 mm for all species. When using the sampling stick, insert it in closed position into the container, gently push it so that the point reaches the required position, open the sampling stick, agitate it slightly to allow it to fill completely, gently close and withdraw it and empty the primary sample into a container. Care should be exercised in closing the sampling stick so that seeds are not damaged.

c. **Nobbe trier**. The Nobbe trier (Dynamic spear) is a pointed tube with an opening near the pointed end. Seed passed through the tube and is collected in a container. The minimum internal diameter of the Nobbe trier should be about 10 mm for clovers and similar seeds, about 14 mm for cereals and about 20 mm for maize.

When using the Nobbe trier, insert it at an angle of about 30^{o} to the horizontal plane with the opening facing down, push the trier until it reaches required position and revolve it through 180^{o}. Withdraw it with decreasing speed from the container, gently agitating the trier to help maintain an even flow of seed, and collect the seed sample coming from the tier in a suitable container.

d. **Sampling by hand**. This method can be used for all species and may be the most suitable method for seed that may be damaged by the use of triers, seeds with wings, seeds with low moisture content, seed tapes and seed mats.

For hand sampling seed in containers, all positions inside the containers must be accessible. Containers with layers which are not accessible from the regular opening may have to be cut open, sampled and repackaged. Containers may be partially or completely emptied during the sampling process to gain access to all positions in the containers. For sampling by hand, clean the hand and roll the sleeve up if necessary, insert the open hand into the container to the required position, close and withdraw the hand, taking great care that the fingers remain tightly closed about the seeds so none may escape, and empty the hand into a receiving pan.

2.7.2 Obtaining the composite sample

When possible, the primary samples are compared with each other during sampling. The primary samples can only be combined to form the composite sample if they appear to be uniform. If not, the sampling procedure must be stopped. When primary samples are collected directly into one container, the content of this container may be regarded as the composite sample only if it appears uniform. If not, must not be used for obtaining a submitted sample.

2.7.3 Obtaining the submitted sample

The submitted sample must be obtained by reducing the composite sample to an appropriate size by one of the methods referred to in sample reduction. Obtaining subsamples such as for moisture testing must be carried out in such a way that changes in moisture content are minimal. The composite sample can be submitted to the seed testing laboratory if it is of appropriate size or if it is difficult to mix and reduce the composite sample properly under warehouse conditions.

Duplicate samples, which were requested not later than at the time of sampling, must be prepared in the same way as the submitted sample.

2.7.4 Dispatch of the submitted sample

The submitted sample must be marked with the same identification as the seed lot. For an Orange International Seed Lot Certificate, the sample must be sealed. Submitted samples must be packed so as to prevent damage during transit. Submitted samples should be packed in breathable containers.

Subsamples for moisture testing, and samples from seed lots which have been dried to low moisture content, must be packed in moisture-proof containers which contain as little air as possible. Submitted samples for germination tests, viability tests and health tests may only be packed in moisture-proof containers if suitable storage conditions can be assured. Submitted samples must be dispatched to the seed testing laboratory without delay.

2.7.5 Storage of submitted samples before testing

Every effort must be made to start testing a submitted ample on the day receipt. Storage of orthodox seeds, when necessary, should be in a cool, well-ventilated room.

Non-orthodox (i.e. recalcitrant or intermediate) seeds should be tested as soon as possible after obtaining the submitted sample from composite sample and, necessary, storage should be done under species specific optimum conditions.

2.8 Procedures for obtaining the submitted and working sample

2.8.1 Minimum size of working sample

Minimum sizes of working samples are prescribed in the appropriate chapter for each test. The working sample weights for purity analyses given in Table 2C are calculated to contain at least 2500 seeds. These weights are recommended for normal use in purity tests.

The sample weights in column 5 of Table 2C, part 1, for counts of other species are 10 times the weights in column 4, subject to a maximum of 1000 g.

2.8.2 Sample reduction methods

If the seed sample needs to be reduced to a size equal to or greater than the size prescribed, the seed sample must first be thoroughly mixed. The submitted/working sample must then be obtained

either by repeated halving or by abstracting and subsequently combining small random portions. The apparatus and methods for sample reduction are described in 8.2.1 to 8.2.4. One, two or more of these methods may be used in one sample reduction procedure. When using 0ne of the dividers described for seed pellets the distance of fall must not exceed 250 mm.

Except in the case of seed health, the method of hand halving must be restricted to contain genera listed in 8.2.4. Only the spoon method and the hand halving method may be used in the laboratory to obtain working samples for seed health testing where other samples or equipment may be contaminated by spores or other propagating material.

For seed tapes and mats take pieces of tape or mat at random, to provide sufficient seeds for the test.

After obtaining a working sample or half-working sample the remainder must be re-mixed before a second working sample or half working sample is obtained.

To obtain the submitted sample for moisture content determination (8.4.4 a), sub-samples must be taken in the following way: first, mix the composite sample. Then take a minimum of three samples from different positions and combine them to create the sub-sample for moisture of the required size. The sub-sample for moisture must be taken as soon as possible to avoid changes in moisture content.

To obtain the working sample for moisture content determination sub-samples must be taken in the following way: before taking the sub-sample, mix the sample by either stirring the sample in its container with a spoon or by placing the opening of the original container against the opening of the similar container and pour the seed back and forth between the two containers. Take a minimum of three sub-samples with a spoon from different positions and the combine them to create the sub-sample of the required size. The seed must not be exposed to the air during sample reduction for more than 30 s.

2.8.2.1 Mechanical divider method

This method is suitable for all kinds of seeds except some very chaffy seeds. The apparatus divides a sample passed through it into two or more approximately equal parts. The submitted sample can be mixed by passing it through the divider, recombining the parts and passing the whole sample through a second time, and similarly, a third time if necessary. The sample is reduced by passing the seed through repeatedly and removing parts on each occasion. This process of reduction is continued until a working sample of approximately, but not less than, the required size is obtained.

The dividers described below are examples of suitable equipment.

a. **Conical divider**. The conical (Boerner type) consist of a hopper, cone, and series of baffles directing the seed into two spouts. The baffles from alternate channels and space of equal width. They are arranged in a circle and are directed inward and downward, the channels leading to one spout and the spaces to as opposite spout. A valve or gate at the base of the hopper retails the seed. When the valve is opened the seed falls by gravity over the cone where it is evenly distributed to the channels and spaces, then passes through the spouts into the seed pans.

The following dimensions are suitable: About 38 channels, each about 25 mm wide for large seeds and about 44 channels each about 8 mm wide for small free- flowing seeds.

b. **Soil divider**. The soil divider (riffle divider) consists of a hopper with about 18 attached channels or ducts alternately leading to opposite sides. A channel width of about 13 mm is suitable. In using the divider the seed is placed evenly into a pouring pan and then poured in the hopper at approximately equal rates along the entire length. The seed passes through the channels and is collected in two receiving pans.

c. **Centrifugal divider**. In the centrifugal divider (Gamet type) the seed flows downward through a hopper onto a shallow cup or spinner. Upon rotation to the spinner by an electric motor the seeds are thrown out by centrifugal force and fall downward. The circle or area where the seeds fall is equally divided into two parts by stationary baffle so that approximately half the seeds fall in one spout and half in the other spout. The centrifugal divider tends to give variable results unless the spinner is operated after having poured the seed centrally into the hopper.

d. **Rotary divider**. The rotary divider comprises a rotating crown unit with 6 to 10 attached subsample containers, a vibration chute and a hopper. In using the divider the seed is poured into the hopper and the rotary divider is switched on so that the crown unit with the containers rotates with approx. 100 rpm and the vibration chute starts to feed the seed into the inlet cylinder of the rotating crown. The feeding rate and therefore the duration of the dividing operation can be adjusted by the distance between the funnel of the hopper and the chute and the vibration intensity of the chute. There are two principles: (i) The inlet cylinder feeds the seed centrally onto a distributor within the rotating crown distributing the seed to all containers simultaneously. (ii) The inlet cylinder feeds the seed de-centrally into the inlet of the containers rotating underneath the inlet cylinder so that the seed stream is subdivided into a lot of subsamples.

e. **Variable sample divider**. The variable sample divider consists of a pouring hopper and tube underneath that rotates with about 40 rpm. The tube distributes the seed stream from the pouring hopper onto the inner surface of a further hopper, which is well fitted into a third hopper all being concentric. In the second and the third hopper there are slots that comprise 50% of the perimeter of the hoppers. 50% of the seed will pass through the two hopes into a collecting pan. The other 50% will stay within the hoppers and will then go into a second collecting pan. The two hoppers can be twisted against each other resulting in more narrow slots. The effect is that a smaller percentage will pass through the slots. Either the smaller sample outside the hoppers or the bigger sample inside the hoppers can be used as the required sample. The position of the two hoppers in relation on each other can be adjusted accurately, resulting in pre-determined subsample sizes.

2.8.2.2 Modified halving method

The apparatus comprises a tray into which fits a grid of equal-sized cubical cells, open at the top and every alternate one having no bottom. After preliminary mixing the seed is poured evenly over the grid. When the grid is lifted, approximately half the sample remains on the tray. The submitted sample is successively halved in this way until a working sample, of approximately but not less than the required six, is obtained.

2.8.2.3 Spoon method

The spoon method is recommended for sample reduction for seed health testing. For other test is it restricted to species with seeds smaller then *Triticum spp.*, to the genera *Arachis*, *Glycine* and *Phaseolus*, and to tree genera *Abies*, *Cedrus* and *Pseudotsuga*. A tray, a spatula and a spoon with a straight edge are required. After preliminary mixing, pour the seed evenly over the tray: do not shake the tray thereafter. With the spoon in the one hand, the spatula in the other, and using both remove small portion of seed from not less than five random places. Sufficient portions of seed are taken to constitute a subsample of the required size.

Test results must be reported in accordance with the rules for calculating, expressing and reporting results in the appropriate chapter of the ISTA Rules. If there is a space on the certificate for certain determinations which are not made applicable, 'N' for not tested' must be placed in the space.

2.8.2.4 Hand halving method

To genera of chaffy seeds, easily damaged fragile seed, seed of trees and shrubs, seed health tests.

2.9 Heterogeneity testing for seed lots in multiple containers

The object of heterogeneity testing is to detect the presence of heterogeneity which makes the seed lot technically unacceptable for sampling.

2.9.1 The H value test

2.9.1.1 Definitions of terms and symbols

The testing of predominantly in - range heterogeneity of an attribute adopted as an indicator involves a comparison between the observed variance and the acceptable variance of the attribute. The container-samples of a seed lot are samples drawn independently of each other from different containers. The examinations of container - samples for the indicating attribute must also be mutually independent. Since there is only one source of information for each container, heterogeneity within containers is not directly involved. The acceptable variance is calculated by multiplying the theoretical variance caused by random variation with a factor f for additional variation, taking into account the level of heterogeneity which is achievable in good seed production practice. The theoretical variance can be calculated from the respective probability distributions, which is the binomial distribution in the case of purity of the other seed count.

- N_o - Number of containers in the lot
- N - Number of independent container-samples
- n - Number of seeds tested from each container-samples (1000 for purity, 100 for germination and 10000 for other seed count)
- x - Test result of the adopted in a container-samples
- $\sum$ - Symbol for sum of all values
- f - Factor for multiplying the theoretical variance to obtain the acceptable variance to obtain the acceptable Variance

Mean of all X values determined for the lot in respect of the adopted attribute:

Table 2.1: Factors for additional variation in seed lot to be used for calculating W and finally the H-value

Attributes	Non-chaffy seeds	Chaffy seeds
Purity	1.1	1.2
Other seed count	1.4	2.2
Germination	1.1	1.2

$$\bar{X} = \frac{\sum x}{N}$$

Acceptable variance of independent container-samples in respect of purity or germination percentages:

$$W = \frac{\bar{X}(100 - \bar{X})}{n} \times f$$

Acceptable variance of independent container-samples in respect of other seeds"

$$W = \bar{X} f$$

Observed variance of independent container-samples based on all x values in respect of the adopted attribute:

$$V = \frac{N \sum x^2 - (\sum x)^2}{N(N-1)}$$

H-value:

$$\mathrm{H}_{\mathrm{calculated}} = \frac{V}{W} - f$$

Negative H-values are reported as zero.

Remarks:

- For purity and germination calculate to two decimal places if N is less than 10 and three decimal places if N is 10 or more.
- For the number of other seeds, calculate to one decimal place if N is less than 10 and to two decimal places if N is 10 or more. For definition of non-chaffy and chaffy seeds see Chapter 3 of the ISTA Rules.

2.9.1.2 Sampling the lot

The number of independent container samples must be not less than presented in Table 2D. Sampling intensity has been chosen such that in a lot containing about 10% deviating containers, at least one deviating container is selected with a probability of p=90% Since the detection of a deviating container is conditional on Selection, the power of both tests to detect heterogeneity is at best close to equal, but usually lower than the chosen selection probability. The containers to be sampled are chosen strictly at random. The sample taken from the container must adequately represent the whole containers, e.g. the top, middle and bottom of a bag. The weight of each container-sample must be not less than half that specified in the Table 2A.

2.9.1.3 Testing procedure

The attribute adopted to indicate heterogeneity may be:

a) Percentage by weight of any purity component,

b) Percentage of any germination test component or

c) The total number of seeds or the number of any single species in the determination of other seeds by number.

In the laboratory, a working sample is drawn from each container-sample and tested independently of any other sample for the chosen attribute.

The Percentage by weight of any component may be used, provided it can be separated as in the purity analysis, e.g. pure seed, other seeds, or empty seeds of grasses. The working sample should be of such weight as is estimated to contain 1000 seeds counted from each container- sample. Each working sample is separated into two fractions: the selected component and the remainder.

Any kind of seed or seedling determinable in a standard germination test may be used, e.g. normal seedling, abnormal seedling or hard seeds. From each container- sample a germination test of 100 seeds is set up simultaneously and completed in accordance with conditions specified in ISTA Rules (Table 5A).

The seed count may be of any component that can be counted e.g. a specified seed species, or all other seeds together. Each working sample must be of a weight estimated in it of the number of seeds of the kind selected (i.e. other seed count).

2.9.1.4 Use of Sampling intensity and critical value.

Sampling intensity and critical value shows the critical H values which would be exceeded in only 1% of tests from seed lots with an acceptable distribution of the attribute adopted as indicator. If the calculated H value exceeds the critical H value belonging to the sample number N, the attribute and the chaffiness in Table 2D, then the lot is considered to show significant heterogeneity in the in-range, or possibly also the off- range sense. If, however, the calculated H value is less than or equal to the tabulated critical H value, then the lot is considered to show no heterogeneity in the in-range, or possibly off-range sense with respect to the attribute being tested.

2.9.1.5 Reporting results

The result of the H value heterogeneity test for seed lots in multiple containers must be reported under 'Other determinations' as follows;

- $\bar{X}$: mean of all x values determined for the lot in respect of the adopted attribute;
- N : number of independent container samples;
- N_o : number of containers in the lot;
- The calculated H value;
- The statement: This H value does/does not indicate significant heterogeneity

Note: the H value must not be calculated or reported if $\bar{X}$ is outside the following limits:

- Purity components: above 99.8% or below 0.2%
- Germination: above 99.0% or below 1.0% and Number of specified seeds: below two per sample.

2.9.2 The R value test

The object of this test is to detect off-range heterogeneity of the seed lot using the attribute adopted as an indicator. The test for off-range heterogeneity involves comparing the maximum difference found between samples of similar size drawn from the lot with a tolerated range. This tolerated range is based on the acceptable standard deviation, which is achievable in good seed production practice.

Each independent container-sample is taken from a different container, so that heterogeneity within containers is not directly involved. Information about heterogeneity with containers is contained, however, in the acceptable standard deviation which is in fact incorporated into the tabulation of tolerated ranges. The acceptable standard deviation was calculated by the standard deviation due to random variation according to the binomial distribution in the case of purity and germination, and to the Poisson distribution in the case of the other seed count, multiplied by the square root of the factor f given in Table 2E, respectively. The spread between containers is characterized by the calculated range to be compared with the corresponding tolerated range.

2.9.2.1 Definitions of terms and symbols

N_o: Number of containers in the lot N: Number of independent container-samples n: Number of seed tested from each container-sample (1000 for purity, 100 for germination and 2500 for other seed count, see Testing procedure under 'The H value test') x: Test result of the adopted attribute in a container-sample $\sum$: Symbol for sum of all values

Mean of all x values determined for the lot in respect of the adopted attribute[1]:

$$\bar{X} = \frac{\sum x}{N}$$

Range found as maximum difference between independent container samples of the lot in respect of the adopted attribute:

[1] for precision of X for the R-value test, see 2.9.1.1 'Remarks' to the H value test (ISTA Rules)

$$R = x_{min} - x_{max}$$

2.9.2.2 Sampling the lot

Sampling for the R value test is the same as for the H value test (see 2.9.1.2); the same samples must be used.

2.9.2.3 Testing procedure

The same testing procedure of purity, germination and the other seed count are used for the R value test as are used for the H value test (see 2.9.1.3). For calculation, the same set of data must be used.

2.9.2.4 Use of tables

Seed lot off-range heterogeneity is tested by using the appropriate table for tolerated, i.e. critical rane:

- Table 2G for components of pure seed analyses,
- Table 2H for germination determinations, and
- Table 2I for number of other seeds.

For higher other seed counts (in non-chaffy seeds), tolerances (R) are calculated by using the following formula and rounding up to the next whole number.

- For $N = 5 - 9$: $R = \sqrt{\text{average count of other seed}} \times 5.44$
- For $N = 10 - 19$: $R = \sqrt{\text{average count of other seed}} \times 6.11$
- For $N = 20$: $R = \sqrt{\text{average count of other seed}} \times 6.69$

For higher other seed counts (in chaffy seeds), tolerances (R) are calculated by using the following formula and rounding up to the next whole number.

- For $N = 5 - 9$: $R = \sqrt{\text{average count of other seed}} \times 6.82$
- For $N = 10 - 19$: $R = \sqrt{\text{average count of other seed}} \times 7.65$
- For $N = 20$: $R = \sqrt{\text{average count of other seed}} \times 8..38$

Find the value $\bar{X}$ in the "Average" columns of the appropriate table. When entering the table, round averages following the usual procedure; read off the tolerated range which would be exceeded in only 1% of tests from seed lots with an acceptable distribution of the attribute:

- In column 5-9 for cases when N = 5 to 9,
- In column 10-19 for cases when N = 10 to 19, or
- In column 20 when N = 20

If the calculated R value exceeds this tolerated range, then the lot is considered to show significant heterogeneity in the off-range sense. If however, the calculated R-value is less than or equal to tabulated tolerated rage, then the lot is considered to show no heterogeneity in the off-range sense with respect to the attribute being tested.

When using the tables, round averages to the next tabulated value (if in the middle, then downwards).

2.9.2.5 Reporting results

The result of the R value heterogeneity test for seed lots in multiple containers must be reported under "Other determinations", as follows:

- $\bar{X}$: mean of all x values determined for the lot in respect of the adopted attribute;
- N: number of independent container samples;
- N_0: number of containers in the lot;
- the calculated R-value;
- the statement: 'This R value does/does not indicate significant heterogeneity.'

2.9.3 Interpretation of results

Whenever either of the two tests, the H value test or the R value test, indicates significant heterogeneity, then the lot must be declared heterogeneous. When, however, neither of the two tests indicates significant heterogeneity, then the lot must be adopted as non- heterogeneity, having a non- significant level of heterogeneity.

Chapter 3

Pro-forma for equipment register

3.1 Example of Pro-forma for Equipment Register

3.1.1 Scope and Purpose

1.1 Scope

1.1.1 This gives details of the equipment that are used in the laboratory and describes the care and maintenance procedure.

1.2 Purpose

It is to give details on following:

1.2.1 Details of all the equipment available in the laboratory 1.2.2 Policy concerning purchase of equipment 1.2.3 Procedure for checking and commissioning new equipment and maintenance of existing ones

3.1.2 Operation Procedure

2.1 One master copy of the equipment Register is maintained with accession record e.g. Lab No., Type of instrument, Model No., Name of the manufacturer, Date of receipt, Serial No. and the prevailing condition. 2.2 The document is not issued or circulated to any person outside CSTL but anyone could consult with reasonable reason to look at it with the permission from the Chief of the Laboratory. 2.3 Chief of the Laboratory authorizes any additions or deletions of equipment as well as any calibration before it is endorsed in the register. 2.4 The maintenance record sheets are checked and authorized by the person currently responsible for the equipment and when completed, it is added into the register. 2.5 In the absence of the CSTL Chief or currently appointed responsible person a designated deputy may act in his or her behalf. 2.6 The operation of the Equipment Register and the maintenance of individual pieces of the said equipment are covered by standard procedures contained in the Procedures Manual, which should be consulted before any action is taken.

Operation of Staff Register

Section 1: Qualification and Experience at

Entry to Register ACCESSION RECORD

Staff Number……. Employment
Family Name……. Contract ☐ Permanent ☐
(Tick as appropriate)
Given Names…… Duration of
Contract Appointment Date of First Appointment…….
Post……
……
Date of Entry to Register……

Academic / Professional Qualifications at Entry to Register

Record of Relevant Experience at Entry to Register			
From	To	Employer	Duties and Experience Gained

Accepted by ……. Date……..

Confirmed by ……Date …….
Chief of Laboratory

STAFF REGISTER Section 2: Training at Entry to Register

Staff Number … .. ……
Family Name ……
Given Name …….

In-house Training Record at Entry to Register		
Date	Details of Training	Documents Attached

External Training Record at Entry to Register		
Date	Details of Training	Documents Attached

Where appropriate, documents that support training such as copies of any certificates of achievement or attendance should be attached and listed in column 3 of the table

Accepted by …… Date …….
(Employee)

Confirmed by …….Date .....
Chief of the Laboratory

Chapter 4

Operation of staff register

Chapter 5

Operation and calibration of blowers

5.1 Scope and Purposes

5.1.1 Scope

- Blower's aids are often useful in seed testing laboratory to separate light weight material such as chaff and empty florets in grasses from the heavier seeds.

5.1.2 Purpose

- Use of blowers saves at least 50 % of the time spent on the purity test.

5.2 Principle

2.1 A good blower provides a uniform flow of air and is capable of standardization as well as of retaining all the particles which it separates.

5.3 Operation Mechanism

- Blowing apparatus essentially consists of a centrifugal blower, the outlet of which is connected to the bottom end of a vertical tube of a few centimeters internal diameter and about half a meter length. A fine wire retains the sample before it is blown and also, holds the resultant heavy fraction. Different arrangements exist to catch the light fraction. A valve allows the wind velocity to be set a rate that has been found optimal for the kind of seed.
- For certain species ISTA has devised an alternative - the Uniform Blowing Method. To this end ISTA Secretariat has distributed calibration samples and instructions to operate. The blower is set with the sample and after blowing the samples to be tested, the heavy

fraction is considered to be full and the light fraction is considered to be empty. The method is compulsory for Poa pratensis and Dactylis glomerata and it is recommended for Chloris gayana as an alternative to the hand method.

5.4 Calibration of the Blower

- Carry out calibration of the blower using specially prepared samples of the relevant species in which the light and heavy fractions are distinctively stained.
- Keep these samples normally in dust and moisture proof containers.
- Regularly check the calibration of the blower as the setting will be affected by atmospheric pressure, humidity, temperature, draughts and even variations in electric voltage of power supply.
- Analyst In-charge normally takes the responsibility of calibration of a blower and carries it out according to a strict procedure.

Chapter 6

Use and calibration of balances

6.1 Scope and purposes

6.1.1 Scope

- The essential piece of equipment for a seed analyst is a balance. The sample submitted for testing should contain sufficient seed in quantity and should be compatible to minimum sample weights for each kind of seed as specified in Seed Regulations. This situation demands a precision balance.
- Purpose
- This SOP 6 intends to give a general understanding on the basic kinds of balance available for seed analysis together with guidance on the use of these balances in general.

6.2 Principle

- Balances to be used in the laboratory must be checked for its performance each day that they are calibrated with the standard weight of the appropriate mass.

6.3 Kinds of Balances

- There are three basic kinds of balances in common usage. They are conveniently described as mechanical, electromechanical and electronic. If they are properly used, there should be no difference between them in quality of results.
- Mechanical balances are the most basic type. They are true balances in that the object to be weighed is balanced against a fixed known mass. They require no electricity.
- Electromechanical balance is an advanced form of the basic type. They do balance an object against a known mass but use a light and system of moving mirrors to indicate the weight on a moving scale. Counter weights are normally selected by turning one or more knobs connected to a system of levers, which release a series of weights in turn on to the balance beam. They are faster to use than mechanical balances, but the degree of precision and complication in

their construction makes them expensive to build and they need regular skilled attention if accuracy is not to be deteriorated. This type of balance is being rapidly replaced by the electronic type.

- In principle, Electronic type balance is entirely different from either mechanical or electromechanical types. The basic idea is quite simple. The balance pan is fixed to an electromagnetic core, around which are electric coils forming what is called a force motor. In very simple terms, what happens is that the weight of the sample tries to push the core down and this is resisted by the balance feeding more power into the coils to keep it in place. As the required power is proportional to the weight on the pan, it is possible for the electronic brain of the balance to measure the power and to display it in terms of the weight on the pan. Electronic balances have the advantages of being very quick and easy to use and they need no regular maintenance.

6.4 Operating Procedure for Calibration

- The laboratory should be equipped with two standard weights for this purpose.
- CSTL has one digit- two electronic balance, two digits -one electronic balance, three digit-one electronic balance and four digits -one electronic balance. One is of 20 grams for the analytical balance with torsion scale. Another is of 500 grams for the other balances that are used in the CSTL.
- Keep these standard weights in a very safe place and handle them using the correct procedures to ensure that their mass remains accurate.
- Always use forceps or a gloved hand while handling these weights.
- Place the weight on top of a tarred piece of tissue paper or lens cloth on the balance pan
- Determine the weights of these standards in the analytical balance as a check according to the need.
- Record the results of these checks on a “Balance Control Chart” (Appendix XVII)
- The allowable range on this chart is ± half the minimum significant reading required, for example, if the need were to weigh to the nearest gram then the allowable range would be ± 0.5 g and if the need were to weigh to 0.01 g then the allowable range would be ± 0.005 g.
- If a balance reading goes outside the allowable range, withdraw it from the service and send it for maintenance. Do not put it in use until it again gives satisfactory calibration readings. Detail procedure is given in working instruction.

Chapter 7

Use and calibration of thermometer

7.1 Scope and Purpose

7.1.1 Scope

- Environment (Temperature, Relative Humidity etc.) plays a vital role in different seed testing methods in the laboratory. The temperature provided by incubators, germination cabinets, room germinators, pre-chilling, pre-heating, constant temperature, alternate temperature etc. has crucial role in testing role in testing. Temperatures must be checked using thermometers that are properly calibrated and whereas the daily records of temperature checks have to be maintained.

7.1.2 Purpose

- The purpose of calibration of thermometers is to take into consideration that the temperature provided by incubators, germination cabinets and room germinators must be within ± 20 °C. Pre-chilling and pre-heating should be carried out within a range of 50 °C and the oven temperatures used in the determination of seed moisture contents should be carried out within a temperature range of 30 °C.

7.2 Principle

- In all cases, temperatures should be as uniform as possible throughout the equipment and should not vary by more than half the range permitted for the mean.
- Temperatures must be checked using thermometers that are calibrated and daily records of temperature checks must be maintained.

7.3 Operating Procedure for Calibration

- Check the performance of temperature controlled equipment data logger.
- Monitor the temperature of each piece of equipment over a period of at least seven days at half an hour intervals.
- The temperature profiles of apparatus should then be derived by plating probes on top, middle and bottom shelves and at three positions on the middle shelf at the front, middle and back.
- The temperature profile for any piece of apparatus should have a range of less than one half the range permitted for the mean temperature.
- Temperature profiles can be derived over a period of 24 hours with readings taken at half hourly intervals.
- During all temperature monitoring operations the apparatus should not be opened or otherwise disturbed.
- Above-mentioned temperature monitoring exercise is repeated on an annual basis.
- Records of temperature monitoring exercises are filed in the Equipment Register.
- Apparatus is replaced if maintenance and adjustment fails to ensure that the temperature specifications are met.
- For future orders of any temperature-controlled equipment, the required temperature specification should be quoted as a condition for the acceptance of the equipment.
- The new equipment is installed and is used only after its temperature is monitored and profiles are derived.
- Equipment that fails to meet the required specification is returned to the manufacturer and no payment is made.

7.4 Monitoring Procedure

- Temperature controlled items of equipment that are in service and conform to temperature specifications are routinely monitored.
- When these are in use the temperature reading is recorded each day and is charted.
- At least two readings are taken each day.
- In the case of alternating temperature equipment, at least one reading is taken in the high temperature phase and one in the low temperature phase. An example of a temperature-monitoring chart that could be attached to an incubator is given in Appendix XVI. Completed temperature charts are filed in the Equipment Register. They are required of be retained for five years and be presented for inspection by auditors if required.
- If the temperature of a piece of apparatus goes outside the required limits, it is withdrawn from the service.
- Before accepting back to the service after any maintenance, the apparatus is checked as outlined above. Details of such checks are filed in the Equipment Register.
- In freezers, temperature measurements are made with A20C/ Total Thermometer, which is a total immersion thermometer covering the range $-20\,^{\circ}\mathrm{C}$ to $20\,^{\circ}\mathrm{C}$ marked in $0.1\,^{\circ}\mathrm{C}$ intervals.
- In Refrigerators, Incubators, Germinators and germination room, the temperature measurements are made with a calibrated A 40C/ Total Thermometer, which is a total immersion thermometer covering the range of $0.5\,^{\circ}\mathrm{C}$ to $40.5\,^{\circ}\mathrm{C}$ marked in $0.1\,^{\circ}\mathrm{C}$ intervals.
- Thermometers for the above and freezers are placed in a clear glass tube (length: 431mm, OD: 14mm, ID: 12mm), which is then filled with glycerol and sealed at the open and with

plastic film and adhesive tape. The tube containing the thermometer is placed as near the middle of the working area as possible avoiding newly introduced items that have not yet had time to reach the correct temperature. The tube is left in place for at least one hour and then a reading is taken as soon as the door is opened. The temperature is recorded and expressed to one decimal place.

- Measurement in ovens and drying cabinets, which are provided with a port of insertion for the thermometer, is made with a partial immersion thermometer with 100mm immersion.
- Care should be taken to site the thermometer so that the immersion length indication is just visible when it is viewed from inside the piece of equipment.
- Appropriate thermometers are B60C/100 type. They are a partial immersion thermometer covering a range of 0 °C to 60 °C marked in 20 °C intervals.
- A B105 C/100 that is a partial immersion thermometer covering the range of 45 °C to 105 °C marked in 0.2 °C intervals.
- A 160C/100 is a partial immersion thermometer covering the range of 99 °C to 160 °C marked in 0.2 °C intervals. The immersion length of all three types is 100 mm. The thermometer actually used should be the one that places the nominal temperature of the apparatus nearest the middle of the scale range.
- The thermometer is placed as near as possible to the middle of the unit.
- If the appliance has a transparent door, the thermometer readings should be taken through the door without opening it.
- However, care should be taken to ensure that the thermometer can be read properly without distortion of the view by the door material.
- The two consecutive readings must not differ by more that one scale division on the thermometer.
- Thermometers in drying cabinets and ovens are positioned and left to equilibrate for at least five minutes before any reading is observed.
- All thermometers are calibrated on an annual basis and a record of calibrations are filed for at least five years.
- For thermometers with a 00 C marking, ice point temperature readings are required. Where the scale of the thermometer does not go through 00 C, the thermometer is calibrated against a calibrated K type thermocouple attached to a portable temperature meter

Chapter 8

Seed registration system

8.1 Scope and Purpose

- The main task of sample reception in administration unit is to register the submitted samples as in Appendix XVII of SOP8, provide them with identification number, decide what kinds of tests are required, and to prepare working sheets (analysis forms) for each test.

8.2 Procedure

- Most of the samples receipt in the laboratory is by post, through peon and through other personnel.
- Place these samples in the incoming containers until they are registered. Verify, before unpacking the sample, which both the sample and test desired meet the conditions regarding identification, marking, sealing, packing and weight etc.
- Depending on the type of sample, sometimes, irregularities may come across such as
  - The species or cultivars name on the request form is not the same as on the label of the sample.
  - A moisture test has been requested but a special moisture test sample has not been submitted.
  - Lot of time appears to be elapsed since the sample was drawn from the lot.
  - Weight of the submitted sample was not adequate.
- Record such irregularities on the request form.
- Ask the applicant or the sampler to provide additional information.
- Record all relevant details on the form.
- Enter the information on the sample slip as in Appendix XVIII 0f SOP8 for reference in case of seriously damaged package and sent the sample back to the sender or sampler.
- In other cases, communicate with the sender for required information immediately.
- Do not enter the identity of the applicant or client in the analysis form in order to avoid the biasness of analyst in analytical performance.
- Stamp dates of receipt and sample registration number in the analysis forms, the request

form and in the label of the sample. (The use of a numbering machine and rubber stamp is recommended not only for numbering and for dating but also for other items that appear frequently such as code number and species name.)

- Arrange safe storage of blank forms, rubber stamps, scissors, glue etc. and make them readily available for the use in the sample reception section.
- Arrange enough space for temporary storage of incoming samples so as to leave working surfaces free for safe handling of each sample and the preparation of the appropriate document.

Chapter 9

Sample mixing, calibration of dividers and operation of seed divider register

9.1 Scope and purpose

9.1.1 Scope

- For all tests to be carried out in the Central Seed Testing Laboratory it is necessary to prepare representative sample of submitted seed lot for examination. To obtain such representative sample equipment called SEED DIVIDER is used.
- Seed Dividers that are used to generate representative seed sample are required to be calibrated and be traceable to international standard.

9.1.2 Purpose

The result of all seed tests are dependent on the seed sample that is on test and the purpose of this procedure is to ensure

- That all seed dividers that are used in the CSTL are regularly checked to make it certain that they deliver representative sample of the submitted sample lot under examination within their performance capabilities and operational requirements.
- Those seed dividers, which are used to obtain representative working sample, and the result of which are reported by the section on its test certificates, are traceable to international standard.
- That trends in the performance of seed dividers are monitored to anticipate potential out of calibration situations.

9.1.3 Safety

- All those personnel who are engaged in seed analysis must wear a fastened laboratory coat.
- Seed is mixed and divided mechanically in a room. If seed is dressed with chemical, if it is chemically treated, or if it is dusty, the operation is done in a ventilated cupboard with the dust extraction system switched on.
- If seed is chemically dressed or treated, protection gloves and facemask must be worn during the mixing and dividing process.
- The electrical integrity of all centrifugal dividers must be checked regularly.
- Staff members, using seed dividers, must also bring to attention of the responsible person of any concerns they have about the safety of any procedures involving the use of any seed divider.

9.2 Principle and Methodology

9.2.1 Principle

- All seed dividers operate on the same principle although the mechanical methods used may be quite different. A seed sample is thoroughly mixed before being divided into two equal portions.
- The weight of the working sample required is achieved by a succession of mixing and halving operations.

9.3 Methodology

Seed dividers in operation are of three different design types such as a) Centrifugal Divider (Gamet type divider), b) Boerner Divider and c) Riffle Divider.

- **Boerner Divider** consists of a hopper, a cone with numerous ports closely spaced around its circumference. Alternate ports lead through ducts to a common outlet on one side, and the others lead similarly to an outlet on the opposite side. A valve or gate at the base of the hopper regulates the retention flow of the seed through outlets into the seed pan. This divider is convenient to handle but it is difficult to check for cleanliness. From time to time it needs to be opened and cleaned with the use of blower.
- **Riffle Type Divider** (Multi-slot divider) has rectangular ports that are held in straight row in a frame with alternate ports leading to right and left. When the seed is poured evenly over the frame, it flows down the ports with half of it to each side.
- **Gamet Type Divider** (Centrifugal): This divider makes use of centrifugal force to mix and scatter seeds over the dividing surface. In this divider, the seeds fall onto rapidly rotating disc, which throws half of it out to each side. This divider tends to give variable results, if it is not properly operated. Care should be taken that the divider is leveled by means of the adjustable foot knobs and sample is poured centrally into the hopper.

9.4 Persons Responsible for Action

The Chief of the laboratory unit or the Manager who is responsible for:

- To make it certain that all seed dividers, purchased for the purpose, meet the criteria required for the intended application and that they are checked against those criteria before being placed in service.
- To arrange for initial calibration, preventative maintenance, servicing and any necessary repairs.
- To ensure that all new seed dividers are endorsed in the " Seed Divider Register" in Annex – XIX and are removed from the register when withdrawn from service.
- To provide suitable calibration samples to check the performance of seed dividers
- To appoint "Responsible Person" in Appendix–XX and a deputy for seed dividers.
- The Responsible persons are required to maintain a general watching in brief of seed dividers and to ensure that any concerns regarding their operation are acted upon.
- They must maintain the Seed Divider Register. It is also to ensure that all required checks on seed dividers and any maintenance or repairs that were carried out are properly recorded.
- The responsible persons take the charge to ensure that immediately after servicing of any seed divider for any reason a calibration sample is used to check performance of the unit and the result so obtained is recorded.
- The Responsible Person should
  - Ensure that pro-forma of Section 2, 3 and 4 (see Appendix XXI, XXII, XXIII) are displayed near each seed divider.
  - Replace fully completed Section 2, 3 and 4 forms timorously and number them numerically starting from one.
  - Sign completed section 2, 3 and 4 forms and file them in the Seed Divider Register.
  - File details of any maintenance or repairs in the Seed Divider Register.
- All Staff Members 3.3.1 The first person to use a seed divider on any given month is required to carry out the monthly calibration check on the Seed Divider and to record the results on the appropriate forms. Appendix XXI, XXII, XXIII3.3.2 All staff members are required to ensure that the seed divider, which they are using has had a monthly calibration check. They must also bring to the attention of the Responsible Person about the correct functioning of any seed divider.
- All staff are responsible for ensuring tat they do not use any seed divider for species for which it is unsuitable.
- Staff members using seed dividers are responsible for ensuring that they are clean and properly leveled before commencing mixing and dividing operations.
- Concerned user takes responsibility of cleaning dividers after use and ensures that they are properly cleaned.

9.5 Operation of the Seed Divider Register

9.5.1 Copies of relevant pro-forma for compilation of the Seed Divider Register. Chief of the laboratory unit holds the master copy.

- Appointment of Responsible Persons
- The chief of the laboratory unit chooses Responsible Person and the Deputy among the staff members who should be familiar with the operation of the full range of seed dividers that are in use in the section.
- As a rule, the Responsible Person should be of at least a Seed Analyst grade.

- Chief of the laboratory unit records Current Responsible Person and the Deputy in the Responsible Person Section of the Seed Divider Register and then initials the entry.
- If somebody replaces the current Responsible Person else the chief of the laboratory unit cancels the previous appointment by completing the "Date Replaced" column and initials the change.
- Once the Responsible Person has been appointed the chief of the laboratory unit hands over the Seed Divider Register into her/his custody.

9.5.2 Entry of New Seed Dividers on the Register and Monthly Calibration checks.

- This is the responsibility of the chief of the laboratory unit and is subject to the same procedures for purchase and performance checks as other equipment.
- The chief of laboratory initiates a register entry for the seed divider by adding it to the index of the seed divider register and completing the appropriate parts of pro-forma for section 1 of the Register.
- The chief of laboratory unit specifies the calibration sample to be used and the range of accuracy in terms of precision of sample and component division.
- The chief of laboratory unit specifies the calibration sample to be used and the range of accuracy in terms of precision of sample and component division.
- Persons carrying out checks and calibrations of seed dividers records the weight of components to the nearest of 0.1 gram.
- Responsible Person put his or her initial in Sections 2, 3 and 4 of the Seed Divider Register, which records the monthly checks of a Seed Divider. He or she also fills details of Seed Divider in the blank pro-forma for section 2, 3 and 4.
- To obtain the monthly sample and component division value, the Responsible Person takes the weight of the appropriate calibration sample and records it in the "Initial Weight Column". (Section 4)

Section 2: - The Responsible Person enters the "Allowed Range" in Section 2 of the Register. It should normally be ± 5% the accuracy specified by the chief of the laboratory unit and defines the range within which the sample division must fall to confirm that the seed divider is still within the calibration range. - The graphical part of Section 2 should have its scale defined. In general, the scale should be set in such a way that the "Allowed Range" represents a displacement of between 50% and 75% of full scale on the graph on either side of the nominal setting. - The responsible person mixes and divides the calibration sample into two portions e.g. A and B using the new seed divider and weighs two portions and records it in section 2. In the meantime, addition of A and B is also recorded in the sheet. Percentage of the total weight of portions A and B is calculated and is recorded in the appropriate columns. The deviation of the percentage portions A form 50% on the chart is calculated. The Responsible Person puts initials with date in each entry.

Section 3: - The calibration sample is a mixture of two components a) seed and b) Admixture. The weight of one of the components "the admixture" is less than 42%. Both portions A and B are weighed and are recorded. - For portion A the components are separated and weighed, the weight is recorded. Same is repeated for portion B. - The "calibration sample" part of the form is now completed by

???

- The percentage admixture in the calibration sample is now calculated and recorded.
- The proportion admixture in both portion A and B is calculated as

???

The tolerance for component separation is checked against the table given in this SOP 9. To check the tolerance, the percentage admixture in the calibration sample is located in the tolerance table and the percentage of the admixture found in the two portions compared to the values in the table opposite the total admixture value. If the actual percentage of the admixture values obtained is greater than the table values, component division is out of the limits required. The entry should now be dated and initialed.

Section 4:

All components are combined, weighed and returned to the calibration sample container. The initial weight of the calibration sample and the final weight after mixing, division and component separation are recorded on the section 4 form. The 'allowed weight change' is entered in the section 4 of the Register. It will normally be +/- a value specified by the Chief of the laboratory unit and defines the range within which any change in weight at any division must fall. Values falling within this range are indicative of the calibration process having been carried out without any excess gain or loss in sample weight.

The entry should now be dated and initialed.

- The Responsible Person presents Section 1 to the Chief of Laboratory Unit for scrutiny and signature and places Section2, 3 and 4 near to the seed divider in a plastic pocket
- Provided that the Chief is satisfied, the new seed divider meets satisfactory performance criteria and has gone through correct calibration then she or he may accept it into the seed divider register by signing section 1.1 for the seed divider accepting it for service.
- The completed forms for section 1 of the seed divider register must be added as a new part of the register relevant to seed divider and the seed divider must be added to the contents section of the register. This must be done by the chief of the laboratory unit who must put his initial in the new entry.
- The Chief of Laboratory Unit then informs. The Responsible Person that the seed divider is now accepted for use.
- The Responsible Person must now arrange for the seed divider to have a label affixed to it showing its number.

9.6 Regular Calibration of Seed Divider

9.6.1 Calibration Frequency

- Traceable Seed Dividers are calibrated monthly by the first person to use the divider within any given month.
- A seed divider does not need to be checked in month if it is not used.
- If a seed divider is returned to use after any kind of maintenance or repairs, under no circumstances it is placed for use without undergoing a calibration.

9.6.2 Appropriate Calibration Samples

- The Chief of Laboratory prepares calibration samples to be used for the S purpose of checking the operation of seed dividers.
- They are composed of seeds of two species, one of whose presence is less than 42% on weight basis means the portion of admixture weight.
- The species of seed taken into use and the precise compositions are a matter of concern to the chief of the laboratory unit.
- Calibration samples are labeled with a number and details of the type of seed dividers that are checked with.
- The chief of the laboratory keeps a logbook, which details the weight and composition of each calibration sample he prepares.
- The chief of the laboratory unit issues new calibration samples as and when required.

9.6.3 Care of Calibration Samples

- Calibration samples are only removed from their containers for as long as it is necessary and are returned immediately after the use.
- It is important that every effort is made to avoid unnecessary contamination and or loss of sample during the mixing, dividing and separation procedures.
- The chief of laboratory is informed when the total weight of the calibration sample is less than
  - 900 grams if it is to be used on centrifugal and Boerner Dividers.
  - 500 grams if it is to be used on the Riffle type divider.

9.6.4 Monthly Check Procedure

The procedure being used is described in Appendix-XXI, XXII, XXIII.

9.6.5 Recording of Calibrations

- All calibrations and checks are recorded on appropriate pro-forma.5.5.2 The person carrying out the check puts on his or her initial on the entries on the forms. When a form is full it should be returned to the Responsible Person who will check it, enter the end date, and the sign and puts the new date before filling it in the Seed Divider Register under the appropriate Seed Divider.
- The Responsible Person issues a new form having completed the header with the details of the seed divider and check parameters. The page number, the next in numerical sequence for that seed divider and the start date of the chart is also filled in.
- A seed divider fails the test if the indicated value differs from the allowed range and / or the Allowed Weight change and/ or if component separation is out of tolerance with anticipated values.

9.7 Action on Failure

- In the event that any seed divider fails any calibration or any person suspects that it is not functioning correctly for any reason, then the concerned person immediately labels the seed

divider as "OUT OF CALIBERATION, DO NOT USE" and informs the person responsible for seed dividers.

- The responsible person will investigate. Should the fault be confirmed then the Responsible Person informs the Chief of Laboratory and the later will arrange for the necessary repairs and recalibration.
- If the chief of Laboratory determines that this is possible after inspection, then he or she institutes checks on suspect data and also institutes a "Quality Failure Report"
- The quality Manager and the chief of Laboratory consult among each other after having investigated the situation and determines whether the exists to notify clients of possible problems with reported data resulting from the seed divider being out of calibration.

Chapter 10

Operation and maintenance of the seed reference collection

10.1 Scope and Purpose

10.1.1 Scope

1.1.1 This procedure applies to all seed reference collections that are used in CSTL. It is narrated as follows.

1.1.2 Main seed Reference Collection. This is the Master Collection containing seed specimens of different species. It is located in the office of the Head of Section of CSTL and is used as a means of identifying seed, which are not in either of the other collections and verifying additions to other collections.

1.1.3 Purity Laboratory Seed Reference Collection. This collection contains seed specimens of species likely to be encountered in the purity laboratory. It is used as a means of identifying seeds, which are not in the Seed Analyst's Seed Reference Collections.

1.1.4 Seed Analyst's individual Seed Reference Collection

1.1.5 These are the Seed Analyst's own Individual Seed Reference Collections.

10.1.2 Purpose

The purpose of this procedure is to ensure that

1.2.1 All identifications made on seeds found in the course of the Purity and Determination of other Seeds by number Test are traceable to reference seed samples.

1.2.2 All seed reference collections are maintained so as to ensure that seed identification can be made accurately and efficiently.

10.2 Principle

In the purity tests and determination of other seed by number tests, Seed Analysts are required to identify contaminant seeds that are present in the sample lot. This collection of seeds is facilitated in identification, which contains authentic samples of seeds of different species.

10.3 Persons Responsible for Action

10.3.1 Head of Section

- The Head of Section is responsible for ensuring that the seed reference collections are maintained in such a way that seed identifications can be made accurately and efficiently.
- The Head of Section is responsible for appointing "Responsible Person" and Deputy for the seed reference collections. In practice, they are usually the Laboratory Manager and the Senior Seed Analyst in-charge of the Purity Laboratory respectively. 3.2 Responsible Person
- The Responsible Person and Deputy Responsible Person are recorded in the Responsible Person Record Sheet by the Head of Section who initials the entries and hold records in the file in Appendix XIV. If the appointments replace existing Responsible persons, the Head of Section cancels the previous appointment by completing the "date replaced" column and initialing the change.
- The Responsible Persons are required to maintain a general watching brief on the Seed Reference Collections and to ensure that any concerns regarding their operation are acted upon.

10.3.2 Seed Analyst

- Seed Analysts are required to ensure that their individual Seed Reference Collections are maintained in such a way that identifications can be carried out accurately and efficiently.
- Seed Analysts are to ensure that they refer any doubtful identifications to the Responsible Person or Deputy Responsible Person.

10.4 Nomenclature and Classification

- The Linnaean binominal system of nomenclature is used for the collections of naming of seeds. For each species, a botanical or "Latin" name is used.
- Classification can help greatly in the identification of unknown seeds and uses the following groupings starting from species as the basic unit.

Genus - A group of closely related species Family - A group of closely related genera Order - A group of closely related families Class - A group of closely related orders Phylum - A group of closely related classes

- The particular names used for the species in each of the collections follow the ISTA "List of Stabilized Names" 6th edition, 2013.

10.5 Main Seed Reference Collection

10.5.1 Location and structures

The main Seed Reference Collection is situated in the office of the Head of Section.

The arrangement of The Main Seed Reference Collection is such that it is convenient and easy to use. The seeds are filed by family first, then by genus and then by species. Seeds from the same botanical family are grouped together.

10.5.2 Source and Authentication of Seed

In a seed reference collection, it is vital that all specimens are correctly identified before they are included. Seeds contained in the Main Seed Reference Collection have been obtained from botanical gardens and gene banks throughout the different continents. These seeds have been obtained from authentic sources and the identity of each species guaranteed by the supplier.

10.5.3 Containers and labels

Specimens are kept in clear glass tubes, plastic boxes or cellophane packets. The genus and species' botanical names are either enclosed or attached on a label along with the index reference number. Details of the country and place of origin where available, are also noted on the label.

When an Analyst finds a seed in an analysis with which she or he is unfamiliar, she or he firstly refers to his/her own individual seed reference collection. If no positive identification is made, the larger purity laboratory reference collection is referred to. If he/ she is still unable to identify the seed or seeds with the aid of these collections, the Main Seed Reference Collection is then finally referred.

10.5.4 Maintenance

Name changes take place in all areas of taxonomy. Botanical family names, genus names and species names can be changed following work completed by taxonomists. When this happens, the new names are listed in ISTA publications such as the Newsletter or Updates to the ISTA List of stabilized plant Names. It is then the job of the Responsible Person to update the relevant family, genus or species names in the Main Seed Reference Collection. The Responsible Person also has to ensure that these name changes are carried out in the purity laboratory reference collection and the Analyst's own individual seed reference collections with verified references. This task is usually delegated to the Deputy Responsible Person.

Chapter 11

Physical purity test

Chapter 12

Germination test

Chapter 13

Determination of seed moisture content

Chapter 14

Determination of other seed number

Chapter 15

Determination of thousand seed weight

Chapter 16

Tetrazolium test

Chapter 17

Variation in seed testing and the use of tolerances and limits of variation

Chapter 18

Production and control of media

Chapter 19

Guard sample storage

Chapter 20

Procedure for check on germination substrate

Chapter 21

Issuing of ISTA certificates

Chapter 22

Proficiency sample testing