

## 2016 AOSA Rules Change Proposal 3

**Purpose:** To clarify the process of dividing the submitted sample to obtain the working sample.

### Present and Proposed Rule:

#### 2.2 Obtaining the working sample

The working sample on which the actual analysis is performed shall be taken from the submitted sample in such a manner that it will be representative. A suitable type of mechanical divider (conical, centrifugal, riffle, etc.) should be used. To avoid damage when dividing large-seeded crop kinds such as beans, peas, etc., prevent the seeds from falling great distances onto hard surfaces. When dividing coated, encrusted, and pelleted seeds, mechanical dividers may be used only if the distance of the fall does not damage the applied materials. When more than one working sample must be obtained from the same submitted sample, all portions remaining after the preparation of the first working sample shall be combined together in to a single bulk sample before preparation of the next working sample.

For seed moisture determination, sub-samples must be drawn quickly to avoid exposing the seeds to the ambient air. Mechanical dividers are not appropriate for this purpose. Refer to section 2.2 b (3).

- a. **Mechanical dividers.** – This method is suitable for most kinds of seeds. The apparatus divides a sample into two approximately equal parts. The submitted sample is mixed by passing it through the divider, recombining the two parts and passing the whole sample through a second time and similarly a third time. After mixing, the sample shall be reduced by passing the seed through the divider repeatedly, removing half the sample on each occasion. This process of successive halving and subsequent combining of random half-portions is continued until a working sample of approximately, but not less than the minimum weight(s) stated in Table 2A is obtained.

Use of compressed air or a vacuum is highly recommended for cleaning mechanical dividers.

- (1) **Centrifugal divider (Gamet type):** This divider is suitable for all kinds of seed though it is not recommended for oilseeds (such as rapeseed, canola, mustards, flax) and kinds susceptible to damage (such as peas, soybeans, etc) and the extremely chaffy types.

The divider makes use of centrifugal force to mix and scatter seeds over the dividing surface. The seed flows downward through a hopper onto a shallow rubber cup or spinner. Upon rotation of 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 a 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 when not carefully operated, and therefore the following procedure must be used:

- (a) Preparation of the apparatus:
  - (i) Level the divider using the adjustable feet.
  - (ii) Check the divider and four containers for cleanliness. Note that seeds can be trapped under the spinner and become a source of contamination.
- (b) Sample mixing:

- (i) Place a container under each spout.
- (ii) Feed the whole sample into the hopper; when filling the hopper, the seed must always be poured centrally.
- (iii) After the sample has been poured into the hopper, the spinner is operated and the seed passes into the two containers. Turn off spinner.
- (iv) Full containers are replaced by empty containers. The contents of the two full containers are fed centrally into the hopper together, the seed being allowed to blend as it flows in. The spinner is operated.
- (v) The sample mixing procedure is repeated at least once more.

(c) Sample reduction:

- (i) Full containers are replaced by empty containers. The contents of one full container are set aside and the contents of the other container are fed into the hopper. The spinner is operated.
- (ii) The successive halving process and subsequent combining of random half-portions is continued until the working sample(s) of not less than the minimum weight(s) required stated in Table 2A are obtained.
- (iii) Ensure that the divider and containers are clean after each mixing operation.

- (2) **Soil/Riffle divider:** This divider is suitable for most kinds of seed. For round-seeded kinds such as *Brassica* species, the collection containers should be covered to prevent the seeds from bouncing out.

This divider consists of a hopper with attached channels or ducts, a frame to hold the hopper, four collection containers and a pouring pan. Ducts or channels lead from the hopper to the collection containers, alternate ones leading to opposite sides. Riffle dividers are available in different sizes for different sizes of seed. The width and number of channels and spaces are important. The minimum width of the channels must be at least two times the largest diameter of the seed or any possible contaminants being mixed.

This apparatus, similar to the centrifugal divider, divides the sample into approximately equal parts.

(a) Preparation of the apparatus:

- (i) Place the riffle divider on a firm, level clean surface. Ensure the divider is level.
- (ii) Ensure that the divider and the four sample collection containers are clean. Check all channels, joints and seams of the divider and collection containers to ensure there are no seeds or other plant matter present before each use.
- (iii) Two clean empty collection containers shall be placed under the channels to receive the mixed seed.

(b) Sample mixing:

- (i) Pour the whole sample into the divider by running the seed backwards and forwards along the edge of the divider so that all the channels and spaces of the divider receive an equal amount of seed, or pour the entire submitted sample into one or more of the sample collection pans and then pour the sample into the divider by holding the long edge of the pan against the long edge of the riffle hopper and then rotating the bottom up so that the seeds pour across all channels at the same time, followed by any additional containers using the same procedure.
- (ii) The two full containers shall be replaced with two clean empty containers.

(iii) The contents of one full container shall be poured into the divider by holding the long edge of the pan against the long edge of the riffle hopper and then rotating the bottom up so that the seeds pour across all channels at the same time, followed by the other full container using the same procedure.

(iv) This process of mixing the entire submitted sample shall be repeated at least one more time before successive halving begins.

(c) Sample reduction:

(i) The contents of one full container are set aside. Empty containers are placed under each channel, and the contents of the other container is poured into the hopper by holding the long edge of the pan against the long edge of the riffle hopper and then rotating the bottom up so that the seeds pour across all channels at the same time.

(ii) The successive halving process and subsequent combining of random half-portions is continued until the working sample(s) of not less than the minimum weight(s) required stated in Table 2A are obtained.

(iii) Ensure that the divider and collection containers are clean after each mixing operation. Check all channels of the divider, the joints and seams.

(3) **Boerner divider:** This divider is suitable for most kinds of seed, including chaffy species, peas, beans, soybeans, etc.

This divider consists of a hopper, a cone, and a series of baffles which direct the seed into two spouts. The baffles are arranged in a circle at the top and form equal width alternate channels and spaces. The channels lead to one spout, the spaces to the other. The width and number of channels and spaces are important. Five channels and spaces should be regarded as a minimum. The more channels the better but the minimum width of the channels must be at least two times the largest diameter of the seed or any possible contaminants being mixed.

(a) Preparation of the apparatus: Ensure that the divider and the two sample collecting pans are clean.

(b) Sample mixing:

(i) Place a collecting pan under each spout.

(ii) Close the valve at the bottom of the divider.

(iii) Pour the seed centrally into the hopper.

(iv) Quickly open the valve. Gravity will distribute the seed evenly through the channels and spaces.

(v) To mix the seed, repeat the steps at least twice for free flowing seed and three times for chaffy grasses.

(c) Sample reduction: The contents of one full collection pan are set aside. Repeat steps in 2 “sampling mixing”. To improve the randomness of reduction, choose collection pans from alternate sides for the successive halving process. The successive halving process and subsequent combining of random half-portions is continued until the working sample(s) of not less than the minimum weight(s) required stated in Table 2A are obtained.

#### b. Non-mechanical methods. -

(1) **Hand mixing/spoon method:** This method should only be used for samples of a single small-seeded species that is smaller than *Triticum* spp., very chaffy species or uncleaned seed

where it is demonstrated that one of the mechanical dividers will not produce a representative working sample(s).

- (a) Preparation of the apparatus: Ensure that two trays, spatula and spoon are clean.
- (b) Sample mixing:
  - (i) The sample is poured uniformly over a tray with a side to side swinging motion.
  - (ii) The receiving pan should be kept level.
  - (iii) This mixing procedure is repeated a minimum of three times.
- (c) Sample reduction:
  - (i) A tray, a spatula and a spoon with a straight edge are required. After the preliminary mixing, pour the seed evenly over the tray with a side-to-side swing, alternately in one direction and at right angles to it. The depth of the seed in the pan shall not exceed the height of the vertical sides of the spoon. Do not shake the tray thereafter.
  - (ii) With the spoon in one hand, the spatula in the other, and using both, remove small portions of seed from not less than five random places on the tray.
  - (iii) Sufficient portions of seed are taken and subsequently combined until the working sample(s) of not less than the minimum weight(s) required stated in Table 2A are obtained.

- (2) **Hand-halving method:** This method can be used when a proper mechanical divider is not available.

Procedure:

- (a) Seed is poured evenly onto a clean smooth surface.
- (b) The sample shall be thoroughly mixed using a flat-edged spatula and placed into a pile.
- (c) The pile shall be divided in half using a straight edge or ruler.
- (d) Each half portion is divided in half.
- (e) Each of the portions is divided into half again. There are now eight portions.
- (f) Arrange the eight portions into two rows of four.
- (g) Alternate portions should be combined to obtain two halves e.g. combine the first and third portions from row 1 one with the second and fourth portions from row 2 two. Remove the remaining four portions.
- (h) Repeat steps (a) to (g) until sufficient portions of seed are taken and subsequently combined to constitute a working sample(s) of not less than the minimum weight(s) required stated in Table 2A are obtained.

- (3) **For seed moisture determination,** mix the submitted sample by tumbling or shaking the submitted sample bag, then open bag and use a spoon to remove portions from several random locations within the bag and combine to obtain the appropriate working weight for one replicate. Place seeds in a moisture testing container. Repeat the procedure of mixing and sampling for the second replicate. Do not expose the sample to ambient air for more than one minute.

### **Harmonization and Impact Statement:**

The current AOSA Rules instructions for repeated halving the submitted sample to obtain the appropriate working sample size are most similar to those found in the Canadian Methods and Procedures (CM&P). The instructions found in the Federal Seed Act Regulations are a bit vague and only require the sample to be repeatedly divided until reaching the desired weight, but they do not specify the sample be divided repeatedly in half. The proposed clarification will bring the AOSA Rules more in line with the ISTA Rules by clarifying that it is acceptable to combine together random portions from the halving process to constitute a working sample. Impact of this clarification should

be negligible since it is often necessary to combine portions together to achieve the desired working sample weight (refer to example under supporting evidence).

**Supporting Evidence:** To demonstrate the repeated halving process of the submitted sample and the need for combining together the subsamples to obtain the appropriate working sample weight, the following example is provided:

Kind of seed: Corn

Submitted sample size: 1,300 grams

Minimum purity working sample size: 500 grams

After thorough mixing of the sample through the divider (as described per divider type), the sample is repeatedly split in half yielding two subsamples as follows:

Run through divider	Size of sample to be split in half (grams)	Subsample 1 (grams) (seed to move to next run through divider)	Subsample 2 (grams) (excess seed)	Working sample
1	1300	650	650	-
2	650	325	-	325
3	325	162.5	-	162.5
4	162.5	81.2	81.3	-
5	81.2	40.6	40.6	-
6	40.6	20.3	20.3	-
7	20.3	10.2	-	10.1
8	10.2	-	5.1	5.1
<b>Total weight</b>	-	-	797.3	502.7

In this example it is impossible to obtain the appropriate working sample weight by repeatedly dividing the sample in half without combining subsamples of various sizes together. Although the intention of the procedure in the AOSA Rules is probably to combine subsamples together, the current wording was called into question through communication with the Purity Subcommittee Chair.

Additional information:

Wording in the Canadian M&P for halving the sample until obtaining the appropriate working weight is similar to that in the current AOSA Rules.

Excerpt from ISTA Rules

**2.5.2.2 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 2.5.2.2.1 to 2.5.2.2.4. One, two or more of these methods may be used in one sample reduction procedure. When using one 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 certain genera listed in 2.5.2.2.4. 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 (2.5.4.4 a), subsamples 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 subsample for moisture of the required size. The subsample for moisture must be taken as soon as possible to avoid changes in moisture content.

To obtain the working sample for moisture content determination (9.1.5.2) subsamples must be taken in the following way: before taking the subsample, 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 a similar container and pour the seed back and forth between the two containers. Take a minimum of three subsamples with a spoon from different positions and combine them to create the subsample of the required size. The seed must not be exposed to the air during sample reduction for more than 30 s.

#### Excerpt from Federal Seed Act Regulations

##### **§ 201.45 Obtaining the working sample.**

(a) The working sample on which the actual analysis is made shall be taken from the submitted sample in such a manner that it will be representative.

(b) The sample shall be repeatedly divided to the weight to be used for the working sample.

Some form of efficient mechanical divider should be used. To avoid damaging large seeds and coated seeds, a divider should be used which will prevent the seeds from falling great distances onto hard surfaces. In case the proper mechanical divider cannot be used or is not available, the sample shall be thoroughly mixed and placed in a pile and the pile shall be repeatedly divided into halves until a sample of the desired weight remains.

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