

SEMIS

Sample Reduction & Purity Analysis

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Object of Purity Analysis

The object of the purity analysis is to determine:

- (1) The percentage composition by weight of the sample being tested and by inference the composition of the seed lot, and
- (2) The identity of various species of seeds and inert particles constituting the sample.

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Purity Exam vs Noxious Weed Exam (AOSA)

Purity Exam (2.5)

~ **2,500 seeds**

- Separate into 4 fractions:
 - 1) Pure seed
 - 2) Other crop seed
 - 3) Weed seed
 - 4) Inert matter

Noxious Weed Exam (3.1)

~ **25,000 seeds**

- Primary (prohibited)
- Secondary (restricted)



Purity Sample Requirements and Information

| | Max. wt. of lot (kg) | Min. submitted sample (g) | Min. Purity Sample (g) | Other seeds by number | PSD # | Chaff-iness | Substrate | Temp. (°C) | First Count | Final Count | Additional directions | Seedling Evaluation (seedling group) |
|-----------------------------------|----------------------|---------------------------|------------------------|-----------------------|-------|-------------|---------------|------------------|-------------|-------------|---|--------------------------------------|
| Maize Zea mays | 40,000 | 1,000 | 900 | 1,000 | 40 | | BP; TPS; S | 20-30, 25, 20 | 4 | 7 | | Poaceae (D) A-1-2-3-2 |
| Sorghum Sorghum bicolor | 30,000 | 900 | 90 | 900 | 42 | C | TP;BP | 20-30 25 | 4 | 10 | Prechill | Poaceae (D) A-1-2-3-2 |
| Field Beans Phaseolus vulgaris | 30,000 | 1,000 | 700 | 1,000 | 11 | | BP; TPS; S | 20-30, 25, 20 | 5 | 9 | | Fabaceae(F) A-2-1-2-2 |
| Soybeans Glycine max | 30,000 | 1,000 | 500 | 1,000 | 11 | | BP; TPS; S | 20-30, 25 | 5 | 8 | | Fabaceae(F) A-2-1-2-2 |
| Cowpea Vigna unguiculata | 30,000 | 1,000 | 400 | 1,000 | 11 | | BP; S | 20-30, 25 | 5 | 8 | | Fabaceae(F) A-2-1-2-2 |
| Broadbean Vicia faba | 30,000 | 1,000 | 1,000 | 1,000 | 11 | | BP; S; O | 20 | 4 | 14 | Prechill | Fabaceae (G) A-2-2-2-2 |
| Rice Oryza sativa | 30,000 | 700 | 70 | 700 | 38 | C | TP, BP, S | 20-30, 25 | 5 | 14 | Preheat; soak in H ₂ O or KNO ₃ | Poaceae (D) A-1-2-3-2 |
| Wheat Triticum aestivum | 30,000 | 1,000 | 120 | 1,000 | 33 | C | TP, BP, S | 20 | 4 | 8 | Preheat; Prechill; GA ₃ | Poaceae (D) A-1-2-3-3 |

Calculation and Expression of Results (3.6)

3.6.1.3 Rounding:

- Add together the percentages of all fractions.
- Trace fractions are excluded from calculations. Other fractions shall together total 100.0%.
- If the sum does not equal 100.0%, then add or subtract 0.1% from the largest value (normally the pure seed fraction). If a correction of more than 0.1% is necessary, check for a calculation error.
- The scientific name of the species of pure seed must be reported on the analysis certificate.
- The kind of inert matter and the scientific name of each species of other seed must be reported. Scientific names must be in accordance with Table 2A, or where applicable, with the current ISTA List of Stabilised Plant Names.

Table 7.1. Principal differences among procedures for reporting final purity analysis results.

| Rules | AOSA | USDA | CFIA | ISTA |
|--|--|--|--|--------------------------------|
| Purity components | Pure Other crop Inert Weed seed | Pure Other crop Inert Weed seed | Pure Other crop Inert Weed seed | Pure Inert Other species |
| Number of decimal places to report results | 2 | 2 | 1 | 1 |
| Report of components < .005% | .01 | .01 | Trace | Trace |
| Report of kind or variety name | Common name | Common name | Common name | Scientific name |
| Report of contaminating species name | Common or scientific name | Not required | Common or scientific name | Scientific name |
| Other examinations | Bulk | Noxious weed seed | Grade table evaluation | Other species |

Reporting Purity Analysis

Reporting requirements can be the same for organizations, but also vary somewhat.

A significant difference between North America and ISTA is the four-part purity versus ISTA's three-part purity.

There is a move to change to a three-part purity in the U.S., but some say it is a simple matter to combine Other Crop and Weed Seed for international shipment.

A nearly constant chore is to keep up with changes to common and scientific names. Common names may vary greatly by region.

Weighing (3.5.1.A)

The minimum number of decimal places necessary for weighing, in order to calculate percentages to one decimal places, is indicated below:

| <u>Weight of working sample in grams</u> | <u># of decimal places</u> |
|--|----------------------------|
| Less than 1.000 | 4 |
| 1.000 to 9.999 | 3 |
| 10.00 to 99.99 | 2 |
| 100.0 to 999.9 | 1 |
| 1000 or more | 0 |

Component parts shall be weighed in grams to the same number of decimal places as the working sample (AOSA).

The percentage of each part shall be determined to two decimal places.

Calculation and Expression of Results (3.6)

One Whole Working Sample (3.6.1)

3.6.1.1 Test for weight gain or loss during analysis

- Add together weights of all fractions from working sample.
- Compare to original weight as a check against gain or loss.
- If discrepancy of more than 5% of the initial weight, retest.

3.6.1.2 Calculation of component percentages

- The percentage by weight of each component part shall be given to one decimal place.
- Percentages must be based on the sum of the weights of the components, not on the original weight of the working sample.
- The percentage of seed of any particular species other than pure seed, or any particular kind of inert matter need not be calculated except as required by 3.7 (Reporting Results).

The Purity Analysis

Seed Unit: structure usually regarded as a seed in planting practices and in commercial channels (AOSA).

3.2.1 Pure seed: The pure seed shall refer to the species stated by the applicant, or found to predominate in the test, and shall include all botanical varieties and cultivars of that species including:

The following structures (even if immature, undersized, shriveled, diseased or germinated, provided they can definitely identified as being of that species) unless transformed into visible fungal sclerotia, etc.:

- Intact seed units as defined for each genus or species in the Pure Seed Definitions in the current 3.2.1.A.
- Pieces of seed units larger than one-half their original size.

Purity Analysis (AOSA)

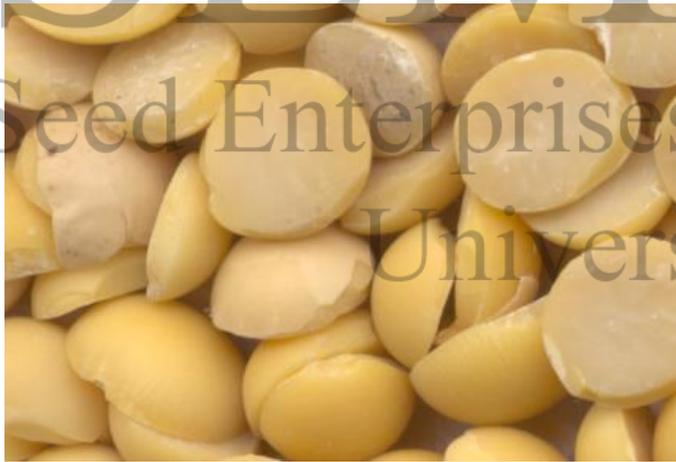
2.7 Pure seed descriptions below apply unless otherwise stated in 2.7e.

- a.** Immature or shriveled seed units, and seeds units that are cracked or otherwise damaged.
 - b.** Insect damaged seeds, provided damage is entirely internal, or that the opening in the seed coat is not sufficiently large to determine the amount of tissue remaining.
 - c.** Seeds that have started to germinate.
 - d.** Seed units with nematode galls, fungus bodies (i.e. ergot, smut, etc.) and spongy or corky caryopses, which are entirely enclosed within the seed unit.
 - e.** Seed units described in definitions 1- 48 shall be considered pure seed.
- Structures not specifically described as part of the PSU shall be removed and classified as inert matter.
 - PSU numbers in table 1 correspond to definition numbers 1-48.
 - For species not listed in Table 1, use the PSU definition that best describes the species concerned.

Inert Material



ergot



Determination of Other Seeds by Number

- Object: Estimate the number of seeds of other species stated by the applicant either generally (e.g. all other species) or by reference to one category of seeds (e.g. species scheduled as noxious in a certain country) or specifically (e.g. *Elytrigia repens*).
- In determining the number of other seeds , the definitions in 3.2 shall be observed. The extent of the determination shall be reported as:
 - Complete Test: one in which the whole working sample (4.5.1a) is searched for all other seeds present.
 - Limited Test: one in which the search is restricted to stated species in the whole working sample (4.5.1a)
 - Reduced Test: one in which only part of the working sample is examined (4.5.1b)
 - Reduced-Limited Test: one in which less than the prescribed weight of seed for a working sample is examined for stated species only.

Determination of Other Seeds by Number

4.5 Procedure

4.5.1 Working Sample

- The size of the working sample shall be either a weight estimated to contain at least 25,000 seed units or not less than the weight prescribed in Table 2a, Part 1.
- If a species stated by the applicant is difficult to identify, a minimum of one fifth of the prescribed working sample weight only need be examined for that particular species.

4.5.2 Determination: The working sample is searched either for seeds of all other species or of certain stated species, as required by the applicant. The number of seeds found of each species is counted.

If the search is limited to certain species, the exam may be stopped when one or more seeds of one or all the stated species has been found.

Aids for Purity Analysis

- Hands lens and binocular microscopes are often necessary for accurate Identification and separation of small seed units and fragments.
- Reflected light is very useful for separating sterile florets of grasses from fertile ones.
- Sieves can be used in separating trash, soil, and other small particles from the working sample.
- Seed blowers can be used to separate light-weight material such as chaff and empty florets in grasses from the heavier seeds.

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Blowers

- Blowers that will give the most accurate separations handle only small samples (up to five grams).
- A good blower should provide a uniform flow of air, be capable of standardization, and retain all the particles which it separates.

Uniform Blowing Method (3.5.2.A.5):

- Obligatory for *Poa pratensis*, *P. trivialis*, & *Dactylis glomerata*.
- Material blown out is considered to be inert matter.
- Blowing pressure for *P. pratensis* & *D. glomerata* is determined by means of calibration samples issued under authority of ISTA.
- A (Blowing), B (Separation of the heavy fraction), C (Separation of the light fraction), D (Alt. procedure), E (Procedure for chemically treated seeds).

Purity Analysis

(2.12) Multiple Unit Procedures (ISTA 3.5.2.A.6)

- Multiple units are grass structures (spikelets, etc.) that contain more than one floret.
- The multiple unit procedure estimate the amount of inert matter contained in these grass structures.
- This procedure can only be used on species listed in section 2.12.

(2.13) Pelleted, coated or encrusted seed purity procedures

- There are special procedures for seeds that have been pelleted, coated, or encrusted.
- Purity analysis of coated seed
- Purity analysis of de-coated seed (performed on request or because sample is a mixture).
- Noxious weed exam shall be made on de-coated seeds.
- 100 coated units shall be examined for verification of the kind of seed present. Determination of cultivar shall be made on 400 coated units.

Facilities for Purity Analysis

- Purity analysis (including noxious weed examinations) is typically tedious and therefore has requirements (and suggested arrangements) for the physical organization of a laboratory.
- It is considered beneficial to have natural light available, typically from the south.
- Temperature and humidity control are important for use of blowers and their calibration samples.
- Workstations may have a raised platform with a glass or acrylic top on which to examine seeds and separate into fractions. The largest fraction may be pulled into a pan underneath the platform.
- Alternatives are a vibratory station that moves seeds past the analyst or a belt that does the same.
- Magnification used varies from hand lens, to fixed magnifying fixtures, to dissecting scopes.



Table 3.2. Comparison of seed characters of five *Trifolium* species.

| Species | <i>T. fragiferum</i> L. | <i>T. hybridum</i> L. | <i>T. pratense</i> L. | <i>T. repens</i> L. | <i>T. vesiculosum</i> Savi |
|---------------------------------------|--|--|---|---|----------------------------|
| General Shape | broadly ovate | oval to heart-shaped | triangular to mitten-shaped | oval to heart-shaped | round to oval |
| Radicle Compared to Cotyledon Lobe | equal to or longer | equal or slightly shorter | >½ the length of the cotyledon lobe | equal to or slightly shorter | equal to or longer |
| Radicle Divergent from Cotyledon Lobe | no | yes | yes | yes | slightly |
| Surface Texture | smooth | smooth | smooth | smooth | tuberculate |
| Color | yellow to terra cotta with dark mottling | yellow to green with purple, blue-green, or black mottling | yellow with red and purple tinge to entirely purple | yellow to terra cotta some with green tinge | terra cotta to red |
| Luster | lustrous | dull | dull | dull to lustrous | dull |



References like the Seed Images web page or the table above are useful in identifying seeds. Seed Images has word descriptions and full color images of many species of seed.

Sieves may be used to separate pure seed from other fractions.

Analysts taking the Purity exam for SCST are required to have their own seed herbarium. The availability of seeds for herbariums varies greatly.