Chapter 10: Testing Coated, Encrusted and Pelleted Seed

Procedures for testing coated and pelleted seeds developed by the AOSA Coated/Pelleted Seed Committee were first approved as tentative rules by the AOSA Executive Board in 1980 (Danielson, 1981). The tentative status provided opportunity for seed analysts to try out the proposed procedures as a means to increase uniformity in testing and determine if the tentative methods were practical and easy to apply. After a few years of practical application, modifications for improvement of the methods were made and were officially adopted to the AOSA Rules effective October 1986 (Hurst, 1986; Maxon, 1986). Since then, the procedures for testing coated and pelleted seeds have continued to evolve, and several changes have been adopted over the years (Allen et al., 2018; Davidson et al., 2010; Erickson et al., 2020; Meyer, 2008; Meyer et al., 2015; Meyer and Patel 2023; Patel et al., 2019). Procedures for testing coated, encrusted, and pelleted seed are given in Section 3.8 of the AOSA Rules.

Definitions for coated, encrusted, and pelleted seed units can be found in Sections 2.1.d of the AOSA Rules (AOSA, 2023a) and in Chapter 2 of this handbook. For simplicity in this chapter, the term 'coated' will include seeds that are coated, encrusted, or pelleted, unless the discussion is specific to one of these types of seed products. Seed quality testing of coated seed is dependent upon several factors: (1) the homogeneity of the seed lot, (2) proper sampling of the seed lot, (3) the quality of the working sample obtained from the submitted sample, (4) proper calibration of equipment, such as balances and mechanical dividers, and (5) proper interpretation and application of the testing procedures by the seed analyst. As mentioned in Chapter 7, the first two factors are usually not under the control of the seed testing laboratory, but it is the responsibility of the seed analyst to recognize if a sample appears to have been correctly drawn and is of sufficient size to be representative of the seed lot. When a sample of coated seed is submitted to the laboratory for purity analysis and other examinations, the size of the submitted sample is based on numbers of seed units rather than on a specific sample weight. For purity analysis a minimum of 7,500 coated units are required, and for noxious weed seed and bulk examinations a minimum of 30,000 coated units are required up to a maximum of 2,000 grams (AOSA, 2023a). It is important to recognize if the sample was received by the laboratory in good condition (e.g., sample container sealed and not leaking seeds; shipping container in good shape; no excessive shipping delays, etc.). Additionally, the sample must be submitted in a firmly packed, crush-proof, and moisture-proof container (AOSA, 2023a).

Equipment

The importance of divider and balance calibration is discussed in Chapters 3 and 7 of this handbook. Mechanical dividers may be used to obtain the working sample for coated seed only when the distance of the fall through the divider does not damage the materials applied to the seed. The Uniform Blowing Procedure (UBP) is not applied to kinds that normally require testing using this procedure when in raw seed format because the coating process and subsequent removal of such coating materials during testing can adversely affect how the seed units behave in the seed blower. Testing of such kinds without the use of the UBP will be discussed later in this chapter. Although the UBP is not used, the blower can be employed as a tool for removing light weight material from the purity working sample.

Depending of the kind of seed to be tested, sieves of various sizes will be needed when washing off coating material. The smaller the seeds the smaller the mesh size of the sieve needed to

retain the seeds while allowing the dissolved coating material to pass through. Also, depending on local laws and regulations the laboratory may need appropriate containers to capture the effluent from the washing process for proper disposal. A drying-oven can be useful for quickly drying washed seeds destined for noxious weed seed or bulk seed examinations, however, a drying oven cannot be used for drying the purity working sample as this must be air dried overnight at room temperature [AOSA, 2023a, Section 3.8].

Removal of Coating Material

The removal of coating material may be necessary for certain parts of the purity analysis and is required for the noxious weed seed and bulk examinations of coated seed samples. The coating material can usually be removed by washing the coated units with water, dilute sodium hydroxide, or other solvents (Figure 10.1). Some coating materials dissolve easily when coated units are placed in the solvent, while other types of coating materials may require considerable soaking time and gentle agitation to achieve complete removal. A magnetic stir plate and magnetic stir bar can be used to provide gentle agitation in a beaker. The important thing to remember is to not damage the seeds in the process of removing the coating material as this can affect the outcome of the test being performed.

Figure 10.1. Removing coating material can be accomplished by soaking and rinsing the coated seed units in water or other solvents and pouring the seed containing solution over a sieve with an appropriate mesh size that retains the seeds while allowing the coating material to pass through (i.e., the smaller the seed inside the coating material the smaller the mesh size used).

Working Sample Size

Working sample weights listed in AOSA Rules, Table 2A, must be adjusted to account for the weight of the materials applied to the seeds (AOSA, 2023a). For the purpose of determining the working sample weights use the most completely coated seed units available in the submitted sample. Due to variation in the types of applied materials, the weight of the working sample must be determined independently for each seed lot tested.

When the seed lot consists of a single kind of seed, the weight of the working sample is determined by counting and weighing 100 coated seed units taken from the submitted sample and multiplying that weight by 25 to calculate the weight of 2,500 seed units required for the purity analysis. Multiply the weight of 100 seed units by 250 to calculate the weight of 25,000 seed units required for noxious weed seed and bulk seed examinations. In all cases, the working sample weight does not need to exceed 1,000 grams for kinds in AOSA Rules, Table 2A, for which the working sample weight of raw seed is 500 grams (AOSA, 2023a). Refer to Chapter 7 for historical information regarding the number of seed units to use for each type of test.

For mixtures of kinds in which all components are coated, first calculate the weight of the working sample as if the sample was raw seed [refer to Section 2.3.b(4)(a) and (b); AOSA 2023a]. Then count and record the weight of 100 coated units. Remove the coating material and air-dry seeds overnight at room temperature. Weigh the dried seeds (de-coated units). Calculate the percentage of coating material and the weight of the working sample using the following formulas:

Formula 1

$$\text{Percentage of coating material} = \frac{(\text{Weight of 100 coated units} - \text{Weight of 100 de-coated units})}{\text{Weight of 100 coated units}} \times 100\%$$

Formula 2

$$\text{Weight of coated purity working sample} = \frac{(\text{Weight of working sample for mixture if raw seed} \times 100\%)}{(100\% - \text{Percentage of coating material})}$$

Refer to Section 2.3.b(4)(b) on rounding procedure for working weights of mixtures (AOSA 2023a).

Example

Coated mixture of perennial ryegrass, red fescue, and red clover. First calculate the weight of the working sample as if the mixture is raw seed.

Kind	Percentage in sample as determined by label, test report, or estimate	Percentage of kinds of different size (rounded to nearest whole percent)		Weight of purity working sample (Table 2A)		Results of percentage × weight of purity sample
Perennial ryegrass	35.50	36	×	5	=	180
Red fescue	31.75	32	×	3	=	96
Red clover	30.25	30	×	5	=	150
Totals		98				426

Weighted average = 426 ÷ 98 = 4.347 grams
Working sample for raw seed mixture = 4.3 grams (round to nearest one-tenth gram)

Count 100 coated units and record the weight of the 100 units. The weight of 100 coated units is 0.260 grams. Wash off the coating material and air-dry overnight at room temperature. The weight of the washed seed is 0.173 grams. Calculate the percentage of coating material using Formula 1 and calculate the weight of the coated purity working sample using Formula 2.

$$\text{Percentage of coating material} = \frac{(0.260 \text{ grams} - 0.173 \text{ grams})}{0.260 \text{ grams}} \times 100\% = 33.46\%$$

$$\text{Weight of coated purity working sample} = \frac{(4.3 \text{ g} \times 100\%)}{(100\% - 33.46\%)} = \frac{(4.3 \text{ g} \times 100\%)}{66.54\%} = \frac{4.3 \text{ grams}}{0.6654} = 6.462 \text{ grams}$$

Working sample for coated seed mixture = 6 grams (round to nearest whole gram).

For mixtures of kinds in which some components are coated and others are not, first determine the weight and percentage of coating material for each coated kind in the mixture. Separate 100 coated units of each coated kind. Separately record the weight of 100 coated units of each kind. Remove the coating material and air-dry the seeds of each kind overnight at room temperature. Weigh each kind of dried seeds (de-coated units) and calculate the percentage of coating material for each using Formula 1 above. Adjust the weight given in AOSA Rules, Table 2A, for each coated component of the mixture using the following formula:

Formula 3

$$\text{Adjusted working weight of coated kind} = \frac{(\text{Weight of kind given in Table 2A} \times 100\%)}{(100\% - \text{Percentage of coating material})}$$

Calculate the weight of the purity working sample using the weights of each coated kind so calculated and weights given in Table 2A for the non-coated components of the mixture [refer to Section 2.3.b(4)(a) and (b); AOSA, 2023a].

Example

Mixture of non-coated tall fescue, coated Kentucky bluegrass, and coated white clover. For this example, assume the percentage of coating material on the Kentucky bluegrass is 50% and the percentage of coating material on the white clover is 30%. The purity working weights given in Table 2A for Kentucky bluegrass and white clover as 1-gram and 2-grams, respectively.

The working sample weights for the two coated components are adjusted using Formula 3 above.

$$\text{Adjusted weight of coated Kentucky bluegrass} = \frac{(1 \text{ gram} \times 100\%)}{(100\% - 50\%)} = \frac{(1 \text{ gram} \times 100\%)}{50\%} = \frac{1 \text{ gram}}{0.5} = 2 \text{ grams}$$

$$\text{Adjusted weight of coated white clover} = \frac{(2 \text{ grams} \times 100\%)}{(100\% - 30\%)} = \frac{(2 \text{ grams} \times 100\%)}{70\%} = \frac{2 \text{ grams}}{0.7} = 2.9 \text{ grams}$$

Substitute the adjusted working sample weights calculated above for the weights found in Table 2A.

Kind	Percentage in sample as determined by label, test report, or estimate	Percentage of kinds of different size (rounded to nearest whole percent)		Weight of purity working sample (Table 2A)		Results of percentage × weight of purity sample
Tall fescue (non-coated)	35.25	35	×	5	=	175
KY bluegrass (coated)	31.75	32	×	2	=	64
White clover (coated)	30.00	30	×	2.9	=	87
Totals		97				326

Weighted average of coated mixture = 326 ÷ 97 = 3.36 grams
Working sample weight for coated mixture = 3.4 grams (round to nearest one-tenth gram)

The working sample weight for the noxious weed seed and/or bulk seed examinations is calculated by multiplying the weight of the coated purity working sample by 10 (approximate weight of 25,000 coated units) up to a maximum of 1000 grams (for kinds in Table 2A for which the raw seed working sample weight is 500 grams).

Purity Analysis

There are two types of purity analyses that can be conducted on coated seed: (1) purity analysis on coated units in which the coated unit serves as the pure seed unit, and (2) purity analysis of de-coated units where the pure seed unit is that to which the kind being tested is assigned and the coating material becomes a separate component (refer to AOSA Rules Section 3.8). The purity analysis of coated units may be used on all single kinds of seed except for members of the grass family (Poaceae). Purity analyses of de-coated units must be used for all mixtures of kinds and for all single kinds of the grass family. The de-coated purity analysis can also be used on any single kind of seed at customer request or to meet regulatory labeling and testing requirements.

The purity analysis of coated units consists of the separation of the working sample into four component categories: (1) pure coated units; (2) inert matter; (3) uncoated crop seed; and (4) uncoated weed seed. Details of the items included under each of these categories are shown in Table 10.1. When the purity analysis is conducted on coated units, the identification of the kind of seed must be verified using the method described in Section 3.8. (AOSA, 2023a) and later in this chapter.

Table 10.1. Components of a purity analysis of coated units (AOSA, 2023a).

Pure coated units	Inert matter	Uncoated crop seed	Uncoated weed seed
Entire (intact) coated units. Broken or damaged coated units where more than one-half the seed surface is covered by coating material, except if it is obvious no seed is present or if the seed is not of the kind of seed under test.	Broken coated units if it is obvious no seed is present. Loose coating material. Other material defined as inert matter under section 3.5.	Uncoated seeds of any crop species including the kind being tested. Broken coated units containing a crop seed not of the kind under test. Broken coated units of the kind under test if the coating material covers one-half or less of the seed surface.	Uncoated seeds of weed species. Broken coated units that obviously contain a weed seed.

The purity analysis of de-coated seed units consists of the separation of the working sample into five component categories: (1) pure seed; (2) other crop seed; (3) weed seed; (4) inert matter; and (5) coating material. In this case, the pure seed definitions as described in the AOSA Rules Volume 1, Section 3 are applied, except the Multiple Unit Procedure is not applied to kinds assigned to PSU 22, and the Uniform Blowing Procedure is not applied to kinds assigned to PSU 23 and 24 (AOSA, 2023a). The original working sample weight is recorded because this value will be required to determine the percentage of coating material. The coating material is removed from the entire working sample and the sample must be allowed to air-dry completely overnight at room temperature before continuing on with the separation of the sample into its components. Once the components are separated and weighed, the weight of the coating material is determined by subtracting the sum of the other four components from the original working sample weight. The percentages of the five components are based on the original working sample weight.

Kind and Cultivar Determination

When the purity analysis does not require removal of the coating material, 100 seed units must be de-coated to verify the identity of the kind under test. When cultivar determination is required, a minimum of 400 seed units must be de-coated and examined.

For confirmation of seed kind under test, the 100 coated units are taken from the pure coated unit component from the purity analysis. The coating material is removed and the name and number of each type of seed found is reported under the other determinations section on the Report of Analysis.

For cultivar identification, 400 coated units are taken from the pure coated unit component of the purity analysis, the coating material is removed, and the appropriate cultivar test is performed. The cultivar test results are also reported under the other determinations section.

Things to Consider Before Beginning a Purity Analysis, Noxious Weed Seed Examination, or Bulk Seed Examination on Coated Seed

Every sample received in the laboratory must be evaluated as a unique individual. Beyond consideration of how the submitted sample was drawn and shipped to the laboratory there are many other things to consider before beginning the purity analysis, noxious weed seed examination, or bulk seed examination. Here are just a few:

- What type of seed product does the sample represent (coated seed, encrusted seed, pelleted seed, etc.)?
- Is the sample a single kind of seed or a mixture of kinds?
- If the sample is a mixture of kinds are all components coated, encrusted, or pelleted?
- Is the kind(s) of seed to be tested listed in Volume 1, Table 2A, of the AOSA Rules?
- Which coated seed purity analysis method is appropriate for the submitted sample?
- What type of noxious weed seed exam is requested? Note: testing for dust-like noxious weed seeds is not appropriate for coated seed samples and such samples should be rejected by the laboratory for this type of testing.
- What type of bulk seed examination is requested? Note: testing for soil particles is not appropriate for coated seed samples and such samples should be rejected by the laboratory for this type of testing.
- If the sample is required to be de-coated, what is the PSU number for the kind or kinds of seed to be tested?
- Is the kind of seed submitted for testing correctly identified by the sample submitter? If the sample consists of a single kind, is a 100 seed morphological identification of de-coated seed necessary?
- Assuming the sample is a mixture of kinds, are the kinds in the mixture difficult to separate physically and/or morphologically after de-coating?
- What is the purity working sample weight for the kind of seed to be tested?

- What is the appropriate method to use for dividing the submitted sample to obtain the working sample?
- Is the kind of seed to be tested listed in Volume 3 of the Rules? If so, what is the species class? If not, what should the species class be when based on intended use?
- What is the origin of the seed sample? Was it grown locally, in the United States, in some other country and if so where?
- Does the laboratory have the proper personal protective equipment (PPE) for handling the sample?
- What are the local regulations regarding disposal of wash water?
- Does the laboratory have the appropriate equipment to conduct the purity analysis on this kind of seed (e.g., sieves of various sizes, magnetic stir plate and magnetic stir bar, microscope for small-seeded species, diaphanoscope for viewing internal structures of seed units, etc.)?
- Have the individuals performing tests on coated seed samples been well trained on the appropriate methods?

In the next section, four examples of coated seed testing for single seed kinds and mixtures of kinds under test will be discussed in detail.

Example 1: Single Kind Pelleted Seed – Working Weight Greater Than 25 Grams

Step 1: Look up needed information for kind of seed under test.

Kind of seed	PSU	Chaffy; Super Chaffy; Non-Chaffy	Purity working weight from Table 2A
Beta vulgaris, sugar beet, monogerm-type	41	Chaffy*	30 grams (raw seed)

*Note: because the sample is pelleted the chaffy classification is converted to non-chaffy (refer to AOSA, 2023a, Appendix 3) in the event purity tolerances need to be applied.

Step 2: Determine the appropriate purity test method and noxious weed seed examination type.

Customer requests a test where the pellet is the seed unit. The noxious weed seed examination is for All States/Federal Noxious Weed Seed Exam.

Step 3: Determine the weights of the working samples.

Count 100 pelleted seed units and record the weight. Multiply this weight by 25 to determine the purity working sample weight and multiply by 250 to determine the noxious weed seed examination weight.

Weight of 100 pellets = 2.105 grams
Weight of purity working sample = 2.105g × 25 = 52.625g; round to whole number = 53 grams
Weight of noxious exam working sample = 2.105g × 250 = 526.25g round to whole number = 526 grams

Step 4: Divide submitted sample to obtain purity working sample.

Recommend using the riffle divider to limit damage to pellets.

Original purity working weight: 53.57 grams (weigh to two decimal places).

Original noxious exam working sample weight: 530.2 grams (weigh to one decimal place).

Note: in this case the purity sample is not de-coated; therefore, it is necessary to de-coat 25,000 pelleted seed units to complete the noxious weed seed examination (i.e., the purity working sample consisting of pelleted seed cannot be considered part of the noxious working sample).

Step 5: Separate working sample into pure coated units, inert matter, and seed contaminants.

Inert matter: loose coating material
Seed contaminants found in purity analysis: none found
Majority of sample consists of intact pelleted units

Step 6: Calculate the percentage by weight of each component.

Because the working sample size is 25 grams or greater, weigh only the inert matter (note, no uncoated crop seed or weed seed found). The weight of the inert matter component is recorded to two decimal places. Calculate the percentage of inert matter based on the weight of the original working sample (53.57 grams). Round the results to two decimal places. The percentage of inert matter is subtracted from 100.00 to determine the percentage of pure coated units.

Component	Component & total weight in grams	Percentage by weight	Final analysis result (after rounding adjustment)
Pure coated units (pellets)	-	-	99.01%
Uncoated crop seed	0.00	0.00	0.00%
Inert matter	0.53	0.99	0.99%
Uncoated weed seed	0.00	0.00	0.00%
Totals	53.57		100.00%

Step 7: Confirm Identification of the Kind of Seed.

Use the procedure described in Section 3.8 (AOSA, 2023a).
Count 100 pelleted units from the pure coated units component of the purity analysis. Remove the coating. Count and name each type of seed found.

In this example, 100 coated units are washed, and the following types of seed and inert matter are found.
99 *Beta vulgaris*, sugar beet
1 empty beet ball

The results of this separation are reported under the other determinations section of the Report of Analysis.

Step 8: Conduct the All States/Federal Noxious Weed Seed Exam.

Wash off the coating material from the entire noxious weed seed exam working sample (530.2 grams) and allow to dry. In the case of the noxious weed seed exam, it is permissible to dry the sample in a laboratory oven, rather than air-dry overnight, to speed up the process because we are not concerned with testing the viability of the seeds from this test.

Found in the sample are nine seeds of *Galium* sp., bedstraw (Figure 10.2). *Galium* spp., is considered a prohibited noxious weed seed in Connecticut, New Hampshire, and Vermont and is considered a restricted noxious weed seed in Massachusetts, Pennsylvania, and Oregon (*Galium tricorne* only) (USDA-AMS, 2020). Also note, *Galium* spp. is a restricted noxious in Washington only when found in alfalfa, so it is of no concern in this case.

Figure 10.2. Example of washed sugar beet sample containing noxious weed seeds. Top row, beet balls of mono-germ sugar beet after coating removed. Middle row, *Galium* sp., bedstraw, seeds after coating removed. Bottom row, pelleted units of presumed mono-germ sugar beet.

To determine the rate of occurrence per pound, per ounce, and per kilogram of bedstraw based on the number of seeds found use the working sample weight (530.2 grams):

a. To determine the rate of occurrence of bedstraw per pound of seed use this formula. The rate per pound is rounded to the nearest whole number.

$$\frac{\text{Number of seeds found}}{\text{Weight of working sample in grams}} \times \frac{453.6 \text{ grams}}{\text{Pound}} = \text{Number of seeds per pound}$$

$$\frac{9}{530.2 \text{ grams}} \times \frac{453.6 \text{ grams}}{\text{Pound}} = 8 \text{ bedstraw per pound}$$

b. To determine the rate of occurrence of bedstraw per ounce of seed use this formula. The rate per ounce is rounded to one decimal place.

$$\frac{\text{Number of seeds found}}{\text{Weight of working sample in grams}} \times \frac{28.35 \text{ grams}}{\text{ounce}} = \text{Number of seeds per ounce}$$

$$\frac{9}{530.2 \text{ grams}} \times \frac{28.35 \text{ grams}}{\text{ounce}} = 0.5 \text{ bedstraw per ounce}$$

c. To determine the rate of occurrence of bedstraw per kilogram of seed use this formula. The rate per kilogram is rounded to the nearest whole number.

$$\frac{\text{Number of seeds found}}{\text{Weight of working sample in grams}} \times \frac{1{,}000 \text{ grams}}{\text{kilogram}} = \text{Number of seeds per kilogram}$$

$$\frac{9}{530.2 \text{ grams}} \times \frac{1000 \text{ grams}}{\text{kilogram}} = 17 \text{ bedstraw per kilogram}$$

The following items must appear under the noxious weed seed examination section on the Report of Analysis in accordance with Section 15 (AOSA, 2023a). For this example, assume the customer wants to know the rate per pound of the noxious weed seed found.

Weight of the noxious weed seed exam working sample: 530.2 grams

Scientific name, or common name, or both of the noxious weed seed found, the number of each type found and rate of occurrence per unit weight: 9 *Galium* sp., bedstraw, 8 seeds per pound

The type of examination conducted, including the name of the region and examination exclusions as appropriate: All States/Federal Noxious Weed Seed Examination

Note: although it should be understood that this examination does not include detection of dust-like noxious weed seeds such as *Orobanche*, *Striga*, *Alectra*, *Aeginetia*, etc., seed analysts should make this clear to their customers.

Example 2: Single Kind Coated Seed – Working Weight Less Than 25 Grams

Step 1: Look up needed information for kind of seed under test.

Kind of seed	PSU	Chaffy; Super Chaffy; Non-Chaffy	Purity working weight from Table 2A
Trifolium repens, ladino clover and white clover	2	Non-chaffy	2 grams

Step 2: Determine the appropriate purity test method and noxious weed seed examination type.

Customer requests a test where the coated seed is the seed unit. No noxious weed seed examination is requested.

Step 3: Determine the weights of the working sample.

Count 100 coated seed units and record the weight. Multiply this weight by 25 to determine the purity working sample weight.

Weight or 100 coated units = 0.1302 grams
Weight of purity working sample = 0.1302g × 25 = 3.255g; round to one decimal place = 3.3 grams

Step 4: Divide submitted sample to obtain purity working sample.

In this case the coating material appears to crumble easily, so recommend using the hand halving method to limit damage to coated units.

Original purity working weight: 3.389 grams (weigh to three decimal places).

Step 5: Separate working sample into pure coated units, inert matter, and seed contaminants.

Inert matter: loose coating material
Seed contaminants found in purity analysis: 2 uncoated *Trifolium hybridum*, alsike clover; 10 uncoated *Trifolium repens*, ladino clover

Note: Before seed of contaminating species can be determined as seeds to be counted or as inert matter they must first be assigned as other crop seed or weed seed.

Step 6: Classify seed contaminants based on ladino clover = <u>A</u> under spp. class; refer only to Column A.

Excerpt from AOSA Rules, Volume 3 (AOSA, 2023b)

NOMEN #	SCIENTIFIC NAME	COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
300618	*Trifolium hybridum* L.	clover, alsike	Fabaceae	A	C	C	C	C	C	C	C
300625	*Trifolium repens* L.	clover, ladino; clover, white	Fabaceae	A	C	C	C	C	C	C	C

Uncoated crop seed
2 *Trifolium hybridum* (alsike clover) 10 *Trifolium repens* (white clover)

Once the determination of whether the contaminating species are classified as uncoated crop or weed seeds, they must also meet the criteria of these categories versus being classified as inert matter. Refer to AOSA Rules, Volume 1, Sections 3.3, 3.4, 3.5, and 3.8.f(3) to make these determinations. For this example, assume the criteria for uncoated crop seed are met.

Step 7: Calculate the percentage by weight of each component.

Because the working sample size is less than 25 grams, weigh the pure coated units, uncoated crop seed, inert matter and uncoated weed seed. The weight of each component is recorded to three decimal places. Calculate the percentage of each based on the sum of these weights (i.e., not on the original working weight). Round the results to two decimal places. The sum of four components must equal 100.00 percent. Note the slight drop in weight of the total weight of all the components compared to the original weight. This loss could be due to seed moisture loss or loss of material during the purity separation. The seed analyst should verify this was not due to loss of material. In this case the sum of the components equal 100.01 percent; therefore, the percentage of pure coated units is adjusted down by 0.01 percent [refer to AOSA Rules, Volume 1, Section 3.1.b(4) and (5)].

Component	Component & total weight in grams	Percentage by weight to demonstrate rounding	Final analysis result (after rounding adjustment)
Pure coated units	3.295	97.255	97.26% adjusted to 97.25%
Uncoated crop seed	0.010	0.295	0.30%
Inert matter	0.083	2.450	2.45%
Uncoated weed seed	0.000	0.000	0.00%
Totals	3.388		100.01% adjusted to 100.00%

Step 8: Confirm Identification of the Kind of Seed.

Use the procedure described in Section 3.8.i (AOSA, 2023a).
Count 100 coated units from the pure coated units component of the purity analysis. Remove the coating. Count and name each type of seed found.

In this example, 100 coated units are washed, and the following types of seed found.

97 *Trifolium repens*, ladino clover
2 *Trifolium hybridum*, alsike clover
1 *Polygonum aviculare*, prostrate knotweed

The results are reported under the other determinations section of the Report of Analysis.

Note: if the customer is concerned with the contaminants found in the 100 seed identification, the seed analyst can suggest a retest using the de-coated purity procedure.

Example 3: Mixture of Kinds – All Components Coated

Step 1: Look up needed information for kind of seed under test.

In this example we will look at how to determine the appropriate working sample weight based on information available from the production records. In a case like this the laboratory test results may ultimately be used to establish the label information for the seed lot.

Kind of seed	PSU	Chaffy; Super Chaffy; Non-Chaffy	Purity working weight from Table 2A	Mixture composition based on production records
Festuca rubra, red fescue	22	Chaffy	3 grams	38%
Lolium perenne, perennial ryegrass	21	Chaffy	5 grams	37%
Festuca arundinacea, tall fescue	21	Chaffy	5 grams	25%

Step 2: Determine the appropriate purity test method and noxious weed seed examination type.

Customer requests a purity analysis and a state noxious weed seed examination for California in order to determine the percentage of coating material for proper labeling and whether the seed lot contains any California noxious weed seeds. In this case the purity analysis must be based on de-coated seed because the sample contains members of the Poaceae or grass family.

Step 3: Determine the raw seed working sample weight based on the production records.

The production records indicate that red fescue is the predominant kind of seed in the mixture; however perennial ryegrass and tall fescue have similar individual purity working weights, and in this case these two kinds combined comprise greater than 50 percent of the mixture (62%). There are two options for determining the weight of the purity working sample for this mixture: (1) use the weight given in Table 2A (AOSA Rules, Volume 1) for the components that comprise greater than 50 percent of the mixture; or (2) calculate the weighted average of the working weights given in Table 2A for all components in the mixture.

Under option 1: the raw seed purity working sample weight = 5 grams.

Under option 2: the following calculation is used to determine the raw seed purity working sample weight.

Kind of Seed	Percentage in sample as determined by label, test report, or estimate		Weight of purity working sample (Table 2A)		Results of percentage × weight of purity sample
Red fescue	38	×	3	=	114
Perennial ryegrass	37	×	5	=	185
Tall fescue	25	×	5	=	125
Totals	100				424

Weighted average = 424 ÷ 100 = 4.24 grams

Round answer to the nearest one-tenth gram: working sample for mixture applying option 2 = 4.2 grams

Either purity working sample weight from option 1 or option 2 may be used. For this example, use the 5-gram purity working sample weight and ten times this amount for the noxious weed seed exam working weight (i.e., 50 grams) for the next step.

Step 4: Determine the percentage of coating material.

Count 100 coated seed units and record the weight. Wash off coating material and air-dry overnight at room temperature. Weigh the de-coated seed units and record the weight.
Weight of 100 coated units = 0.0072 grams
Weight of 100 de-coated units = 0.0053 grams

Calculate percentage of coating material using Formula 1

Formula 1

$$\text{Percentage of coating material} = \frac{(\text{Weight of 100 coated units} - \text{Weight of 100 de-coated units})}{\text{Weight of 100 coated units}} \times 100\%$$

$$\text{Percentage of coating material} = \frac{(0.0072 \text{ grams} - 0.0053 \text{ grams})}{0.0072 \text{ grams}} \times 100\% = 26.39\%$$

Step 5: Calculate the coated seed purity and noxious working sample weights and divide submitted sample to obtain the purity and noxious weed seed working samples.

Calculate the weights of the coated purity and noxious working samples using Formula 2.

Formula 2

$$\text{Weight of coated purity working sample} = \frac{(\text{Weight of working sample for mixture if raw seed} \times 100\%)}{(100\% - \text{Percentage of coating material})}$$

Calculation of purity working weight:

$$\text{Weight of coated purity working sample} = \frac{(5 \text{ grams} \times 100\%)}{(100\% - 26.39\%)} = \frac{(5 \text{ grams} \times 100\%)}{73.61\%} = \frac{5 \text{ grams}}{0.7361} = 6.793 \text{ grams}$$

Purity working sample weight for coated seed mixture = 7 grams (round to nearest whole gram).

Calculation of noxious working weight:

$$\text{Weight of coated purity working sample} = \frac{(50 \text{ grams} \times 100\%)}{(100\% - 26.39\%)} = \frac{(50 \text{ grams} \times 100\%)}{73.61\%} = \frac{50 \text{ grams}}{0.7361} = 67.93 \text{ grams}$$

Noxious weed seed working weight for coated seed mixture = 68 grams (round to nearest whole gram).

Once the appropriate working sample weights are established the sample is divided into the purity and noxious weed seed exam working samples. Record the weight of each working sample to the appropriate number of decimal places.

Purity working sample = 7.105 grams
Noxious weed seed exam working sample = 68.25 grams

Step 6: Remove coating material and air-dry overnight at room temperature.

Wash off the coating material from each working sample using method described earlier in this chapter. Spread the de-coated seed out in a single layer over blotter paper, paper towels, or similar material and allow to air-dry completely overnight at room temperature.

Step 7: Separate the working sample into pure seed for each kind in the mixture, seed contaminants, inert matter, and residual coating material.

Seed contaminants found in purity analysis: 12 *Dactylis glomerata*, orchardgrass; 4 *Poa pratensis*, Kentucky bluegrass; 2 *Alopecurus pratensis*, meadow foxtail; 1 *Taraxacum officinale*, dandelion

Note: Before seed of contaminating species can be determined as seeds to be counted or as inert matter they must first be assigned as other crop seed or weed seed (see Step 8 below).

Inert matter: chaff, empty florets, broken seed units, and stem fragments

Coating material remaining in sample after washing and drying should be considered part of the total amount of coating material calculated in Step 9 below.

Step 8: Classify seed contaminants based on a combination of the species class for all the kinds in the mixture.

In this case, tall fescue is R and T under spp. class, red fescue is T and perennial ryegrass is A and T; therefore, the overall combined spp. class for the mixture is A, R, T. To determine if the contaminant species are other crop or weed seeds refer to Columns A, R, and T. Meadow foxtail, orchardgrass, and Kentucky bluegrass are classified as C under all three columns. Dandelion is classified as W under all three columns.

Excerpt from AOSA Rules, Volume 3 (AOSA, 2023b)

NOMEN #	SCIENTIFIC NAME	COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
2651	*Alopecurus pratensis* L.	foxtail, meadow	Poaceae	A, R	C	W	W	C	W	C	W
13114	*Dactylis glomerata* L.	orchardgrass	Poaceae	A, R	C	C	C	C	C	C	C
16631	*Festuca arundinacea* Schreb.	fescue, tall	Poaceae	R, T	C	C	C	C	C	C	C
300215	*Festuca rubra* L. subsp. *rubra*	fescue, creeping red; fescue, red	Poaceae	T	C	C	C	C	C	C	C
22494	*Lolium perenne* L.	ryegrass, perennial	Poaceae	A, T	C	C	C	C	C	C	C
28996	*Poa pratensis* L.	bluegrass, Kentucky	Poaceae	T	C	C	C	C	C	C	C
80051	*Taraxacum officinale* F. H. Wigg. aggr.	dandelion	Asteraceae	V	W	W	W	W	W	W	W

Other crop seed	Weed seed
12 *Dactylis glomerata* (orchardgrass) 4 *Poa pratensis* (Kentucky bluegrass) 2 *Alopecurus pratensis* (meadow foxtail)	1 *Taraxacum officinale* (dandelion)

Once the determination of whether the contaminating species are classified as other crop seed or weed seed, they must also meet the criteria of these categories versus being classified as inert matter. Refer to AOSA Rules, Volume 1, Sections 3.3, 3.4, and 3.5 to make these determinations. In this case, orchardgrass and Kentucky bluegrass have a requirement for use of the UBP that may be disregarded for times when occasional seed units of these species are found as contaminants in a sample. Instead, the florets must be examined to determine if they contain caryopses.

Step 9: Calculate the percentage by weight of each component.

Weigh each kind of pure seed, other crop seed, inert matter, and weed seed. The weight of each component is recorded to three decimal places. The sum of these weights is subtracted from the original working sample weight (i.e., 7.105 grams) to determine weight of the coating material. Calculate the percentage of each component based on the original working sample weight. Round the results to two decimal places. The sum of all components must equal 100.00 percent. In this case the sum of the components equal 100.01 percent; therefore, the percentage of coating material is adjusted down by 0.01 percent [refer to AOSA Rules, Volume 1, Section 3.1.b(4) and (5)].

Component	Component & total weight in grams
Red fescue	1.889
Perennial ryegrass	1.813
Tall fescue	1.235
Other crop seed	0.021
Inert matter	0.053
Weed seed	0.001
Total weight of components other than coating material	5.012

Weight of coating material = original working sample weight – sum of components other than coating material

Weight of coating material = 7.105 grams – 5.012 grams = 2.093 grams

Component	Component & total weight in grams	Percentage by weight to demonstrate rounding	Final analysis result (after rounding adjustment)
Red fescue	1.889	26.587	26.59%
Perennial ryegrass	1.813	25.517	25.52%
Tall fescue	1.235	17.382	17.38%
Other crop seed	0.021	0.296	0.30%
Inert matter	0.053	0.746	0.75%
Weed seed	0.001	0.014	0.01%
Coating material	2.093	29.458	29.46% adjusted to 29.45%
Totals	7.105		100.01% adjusted to 100.00%

Step 10: Examine the de-coated noxious weed seed examination working sample for California noxious weed seeds.

Refer to the most current version of *State Noxious-Weed Seed Requirements Recognized in the Administration of the Federal Seed Act* to determine what species are classified as noxious weed seed in California. The seed analyst should explain to the customer that the state noxious weed seed examination does not include the search for dust-like noxious weed seeds (e.g., *Orobanche ramosa* or *Striga* spp., both are considered noxious weed seeds in California).

During the examination one achene of *Cirsium vulgare* (bull thistle) is found. This species is classified as a restricted noxious weed seed in California. Assume the customer wants to know the rate per pound of bull thistle in the seed lot.

To determine the rate of occurrence per pound of bull thistle based on the number of seeds found use the working sample weight (68.25 grams):

$$\frac{\text{Number of seeds found}}{\text{Weight of working sample in grams}} \times \frac{453.6 \text{ grams}}{\text{Pound}} = \text{Number of seeds per pound}$$

$$\frac{1}{68.25 \text{ grams}} \times \frac{453.6 \text{ grams}}{\text{Pound}} = 6.6 \text{ bull thistle seeds per pound}$$

Round to a whole number = 7 bull thistle seeds per pound.

The following items must appear under the noxious weed seed examination section of the Report of Analysis in accordance with Section 15 (AOSA, 2023a).

- Weight of the noxious weed working sample: 68.25 grams
- Scientific name, or common name, or both, of the noxious weed seed found, the number of each type found and rate of occurrence per unit weight: 1 *Cirsium vulgare*, bull thistle, 7 per pound; a restricted noxious weed seed in California.
- The type of examination conducted, including the name of the region and examination exclusions, as appropriate: State Noxious Weed Seed Exam – California (excluding *Orobanche* spp. and *Striga* spp.)

Example 4: Mixture of Kinds – Two of Three Components Coated

Step 1: Look up needed information for kind of seed under test.

Kind of seed	PSU	Chaffy; Super Chaffy; Non-Chaffy	Purity working weight from Table 2A	Mixture composition based on production records
Festuca arundinacea, tall fescue	21	Chaffy	5 grams	47.50%
Dactylis glomerata, orchardgrass (coated)	24	Chaffy	3 grams	33.25%
Trifolium repens, white clover (coated)	2	Non-chaffy	2 grams	14.25%

Note: Since greater than 33% of the mixture is composed of the chaffy uncoated kind of tall fescue, the entire mixture is treated as chaffy when comparison of test results to label claim is required in application of purity tolerances. Refer to AOSA Rules, Volume 1, Section 14.2.a for the application of purity tolerances for chaffy seeds.

Step 2: Determine the appropriate purity test method.

Customer requests only a purity analysis in order to determine the percentage of coating material in the mixture for proper labeling. In this case the purity analysis must be based on de-coated seed because the sample contains members of the Poaceae or grass family.

Step 3: Determine the percentage of coating material for each coated kind in the mixture.

Count 100 coated seed units for each coated kind (i.e., orchardgrass and white clover) and record the weights.

Weight of 100 coated units for orchardgrass = 0.1809 grams
Weight of 100 coated units for white clover = 0.1382 grams

Wash off coating material from each coated kind and air-dry overnight at room temperature. Weigh the de-coated seed units for each kind and record the weights.

Weight of 100 de-coated units for orchardgrass = 0.1206 grams
Weight of 100 de-coated units for white clover = 0.0813 grams

Calculate with percentage of coating material for each coated kind using Formula 1

Formula 1

$$\text{Percentage of coating material} = \frac{(\text{Weight of 100 coated units} - \text{Weight of 100 de-coated units})}{\text{Weight of 100 coated units}} \times 100\%$$

$$\text{Percentage of coating material for orchardgrass} = \frac{(0.1809 \text{ grams} - 0.1206 \text{ grams})}{0.1809 \text{ grams}} \times 100\% = 33.33\%$$

$$\text{Percentage of coating material for white clover} = \frac{(0.1382 \text{ grams} - 0.0813 \text{ grams})}{0.1382 \text{ grams}} \times 100\% = 41.17\%$$

Step 4: Calculate the adjusted purity working sample weight for each coated kind.

Calculate the adjusted weight of each coated kind using Formula 2.

Formula 2

$$\text{Weight of coated purity working sample} = \frac{\text{(Weight of working sample from Table 2A} \times 100\%)}{\text{(100\% - Percentage of coating material)}}$$

$$\text{Weight of coated purity working sample for orchardgrass} = \frac{(3 \text{ grams} \times 100\%)}{(100\% - 33.33\%)} = \frac{(3 \text{ grams} \times 100\%)}{66.67\%} = \frac{3 \text{ grams}}{0.6667} = 4.5 \text{ grams}$$

$$\text{Weight of coated purity working sample for white clover} = \frac{(2 \text{ grams} \times 100\%)}{(100\% - 41.17\%)} = \frac{(2 \text{ grams} \times 100\%)}{58.83\%} = \frac{2 \text{ grams}}{0.5883} = 3.4 \text{ grams}$$

Step 5: Calculate the adjusted purity working sample weight for the mixture, then mix and divide the purity working sample.

Since none of the components of the mixture represents greater than 50 percent of the mixture, the purity working weight is determined by calculating the weighted average of the working weights given in Table 2A for non-coated kinds and using the adjusted Table 2A weights calculated in Step 4 for the coated kinds as follows:

Kind	Percentage in sample as determined by label, test report, or estimate	Percentage of kinds of different size (rounded to nearest whole percent)		Weight of purity working sample (Table 2A & Step 4 above)		Results of percentage × weight of purity sample
Tall fescue (non-coated)	47.50	48	×	5	=	240
Orchardgrass (coated)	33.25	33	×	4.5	=	148.5
White clover (coated)	14.25	14	×	3.4	=	47.6
Totals		95				436.1

Adjusted weighted average = 436.1 ÷ 95 = 4.59 grams
Purity working sample weight for coated seed mixture = 4.6 grams (round to nearest one-tenth gram)

Once the appropriate working sample weight is established the sample is divided to obtain the purity working sample. Record this weight to the appropriate number of decimal places.

Purity working sample = 4.658 grams

Step 6: Remove coating material and air-dry overnight at room temperature.

Wash off the coating material from the working sample using method described earlier in this chapter. Spread the de-coated seed out in a single layer over blotter paper, paper towels, or similar material and allow to air-dry completely overnight at room temperature.

Step 7: Separate the working sample into pure seed for each kind in the mixture, seed contaminants, inert matter, and residual coating material.

Because the orchardgrass component is coated, the Uniform Blowing Procedure (UBP) is not applied (refer to AOSA Rules, Volume 1, Table 3A, PSU 24). In this case, the seed units for orchardgrass must contain at least one caryopsis to be classified as pure seed.

Seed contaminants found in purity analysis: 78 *Phleum pratense*, timothy; 82 *Poa pratensis*, Kentucky bluegrass; 57 *Festuca rubra*, red fescue; 1 *Poa annua*, annual bluegrass; 4 *Trifolium* sp., clover

Note: Before seed of contaminating species can be determined as seeds to be counted or as inert matter they must first be assigned as other crop seed or weed seed (see Step 8 below).

Inert matter: empty florets; chaff; stem fragments, broken seeds

Coating material remaining in sample after washing and drying should be considered part of the total amount of coating material calculated in Step 9 below.

Step 8: Classify seed contaminants based on a combination of the species class for all the kinds in the mixture.

In this case, tall fescue is R and T under spp. class, orchardgrass is A and R and white clover is A; therefore, the overall combined spp. class for the mixture is A R T. To determine if the contaminant species are other crop or weed seeds refer to Columns A, R, and T. Red fescue, timothy, and Kentucky bluegrass are classified as C under all three columns. Annual bluegrass is classified as W under all three columns. *Trifolium* spp. is classified as C under Columns A and T and W under Column R; therefore, according to item #7 under the Cautions section of Volume 3 (AOSA, 2023b), this species is classified as C when such a conflict occurs.

Excerpt from AOSA Rules, Volume 3 (AOSA, 2023b)

NOMEN #	SCIENTIFIC NAME	COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
13114	*Dactylis glomerata* L.	orchardgrass	Poaceae	A, R	C	C	C	C	C	C	C
16631	*Festuca arundinacea* Schreb.	fescue, tall	Poaceae	R, T	C	C	C	C	C	C	C
300215	*Festuca rubra* L. subsp. *rubra*	fescue, creeping red; fescue, red	Poaceae	T	C	C	C	C	C	C	C
28019	*Phleum pratense* L.	timothy	Poaceae	A	C	C	C	C	C	C	C
28904	*Poa annua* L.	bluegrass, annual	Poaceae	T	W	W	W	W	W	W	W
28996	*Poa pratensis* L.	bluegrass, Kentucky	Poaceae	T	C	C	C	C	C	C	C
300625	*Trifolium repens* L.	clover, ladino; clover, white	Fabaceae	A	C	C	C	C	C	C	C
300627	Trifolium spp.	clover	Fabaceae	A	C	W	W	W	W	C	W

Other crop seed	Weed seed
82 *Poa pratensis* (Kentucky bluegrass) 78 *Phleum pratense* (timothy) 57 *Festuca rubra* (red fescue) 4 *Trifolium* sp. (clover)	1 *Poa annua* (annual bluegrass)

Once the determination of whether the contaminating species are classified as other crop seed or weed seed, they must also meet the criteria of these categories versus being classified as inert matter. Refer to AOSA Rules, Volume 1, Sections 3.3, 3.4, and 3.5 to make these determinations. In this case, Kentucky bluegrass has a requirement for use of the UBP that may be disregarded for times when an occasional seed unit is found as a contaminant is a sample and is also disregarded when the sample is coated seed (refer to AOSA Rules, Volume 1, Table 3A, PSU 23).

Step 9: Calculate the percentage by weight of each component.

Weigh each kind of pure seed, other crop seed, inert matter and weed seed. The weight of each component is recorded to three decimal places. The sum of these weights is subtracted from the original working sample weight (i.e., 4.658 grams) to determine weight of the coating material. Calculate the percentage of each component based

on the original working sample weight. Round the results to two decimal places. The sum of all components must equal 100.00 percent. In this case the sum of the components equal 100.01 percent; therefore, the percentage of tall fescue is adjusted down by 0.01 percent [refer to AOSA Rules, Volume 1, Section 3.1.b(4) and (5)].

Component	Component & total weight in grams
Tall fescue	1.743
Orchardgrass (de-coated)	1.313
White clover (de-coated)	0.505
Other crop seed	0.195
Inert matter	0.052
Weed seed	0.004
Totals	3.812

Weight of coating material = original working sample weight – sum of components other than coating material

Weight of coating material = 4.658 grams – 3.812 grams = 0.846 grams

Component	Component & total weight in grams	Percentage by weight to demonstrate rounding	Final analysis result (after rounding adjustment)
Tall fescue	1.743	37.419	37.42 % adjusted to 37.41%
Orchardgrass (de-coated)	1.313	28.188	28.19%
White clover (de-coated)	0.505	10.842	10.84%
Other crop seed	0.195	4.186	4.19%
Inert matter	0.052	1.116	1.12%
Weed seed	0.004	0.086	0.09%
Coating material	0.846	18.162	18.16%
Totals	4.658		100.01% adjusted to 100.00%

Example 5: Mixture of Kinds – Two of Three Components Coated and Indistinguishable in Coated Format

Step 1: Look up needed information for kind of seed under test.

Kind of seed	PSU	Chaffy; Super Chaffy; Non-Chaffy	Purity working weight from Table 2A	Mixture composition based on production records
Medicago sativa, alfalfa	2	Non-chaffy	5 grams	40.00%
Trifolium pratense, red clover (coated)	9	Non-chaffy	5 grams	34.00%
Trifolium hirtum, rose clover (coated)	9	Non-chaffy	7 grams	26.00%

Step 2: Determine the appropriate purity test method.

Customer requests only a purity analysis in order to determine the percentage of coating material in the mixture for proper labeling. To make this determination, the purity analysis must be based on de-coated seed.

Step 3: Determine the percentage of coating material for the coated kinds in the mixture.

In this case the coated red clover and rose clover are indistinguishable in the coated format; therefore, it is permissible to determine the percentage of coating material for these two kinds combined and then adjust with working weights of each kind from Table 2A separately (Erickson and Tatum, 2020, personal communication).

Count 100 coated seed units and record the weight.

Weight of 100 coated units = 0.3241 grams

Wash off coating material from each coated kind and air-dry overnight at room temperature. Weigh the de-coated seed units and record the weight.

Weight of 100 de-coated units = 0.2405 grams

Calculate with percentage of coating material using Formula 1

Formula 1

$$\text{Percentage of coating material} = \frac{(\text{Weight of 100 coated units} - \text{Weight of 100 de-coated units})}{\text{Weight of 100 coated units}} \times 100\%$$

$$\text{Percentage of coating material} = \frac{(0.3241 \text{ grams} - 0.2405 \text{ grams})}{0.3241 \text{grams}} \times 100\% = 25.79\%$$

Step 4: Calculate the adjusted purity working sample weight for each coated kind.

Calculate the adjusted weight of each coated kind using Formula 2.

Formula 2

$$\text{Weight of coated purity working sample} = \frac{(\text{Weight of working sample from Table 2A} \times 100\%)}{(100\% - \text{Percentage of coating material})}$$

$$\text{Weight of coated purity working sample for red clover} = \frac{(5 \text{ grams} \times 100\%)}{(100\% - 25.79\%)} = \frac{(5 \text{ grams} \times 100\%)}{74.21\%} = \frac{5 \text{ grams}}{0.7421} = 6.7 \text{ grams}$$

$$\text{Weight of coated purity working sample for rose clover} = \frac{(7 \text{ grams} \times 100\%)}{(100\% - 25.79\%)} = \frac{(7 \text{ grams} \times 100\%)}{74.21\%} = \frac{7 \text{ grams}}{0.7421} = 9.4 \text{ grams}$$

Step 5: Calculate the adjusted purity working sample weight for the mixture, then mix and divide the purity working sample.

Since none of the components of the mixture represents greater than 50 percent of the mixture, the purity working weight is determined by calculating the weighted average of the working weights given in Table 2A for non-coated kinds and using the adjusted Table 2A weights calculated in Step 4 for the coated kinds as follows:

Kind	Percentage in sample as determined by label, test report, or estimate	Percentage of kinds of different size (rounded to nearest whole percent)		Weight of purity working sample (Table 2A & Step 4 above)		Results of percentage × weight of purity sample
Alfalfa (non-coated)	40.00	40	×	5	=	200
Red clover (coated)	34.00	34	×	6.7	=	227.8
Rose clover (coated)	26.00	26	×	9.4	=	244.4
Totals		100				672.2

Adjusted weighted average = 672.2 ÷ 100 = 6.722 grams
Purity working sample weight for coated seed mixture = 7 grams (round to nearest whole gram)

Once the appropriate working sample weight is established the sample is divided to obtain the purity working sample. Record this weight to the appropriate number of decimal places.

Purity working sample = 7.025 grams

Step 6: Remove coating material and air-dry overnight at room temperature.

Wash off the coating material from the working sample using method described earlier in this chapter. Spread the de-coated seed out in a single layer over blotter paper, paper towels, or similar material and allow to air-dry completely overnight at room temperature.

Step 7: Separate the working sample into pure seed for each kind in the mixture, seed contaminants, inert matter, and residual coating material.

Seed contaminants found in purity analysis: 10 *Trifolium repens*, white clover; 2 *Trifolium dubium*, small hop clover

Note: Before seed of contaminating species can be determined as seeds to be counted or as inert matter they must first be assigned as other crop seed or weed seed (see Step 8 below).

Inert matter: broken seeds

Coating material remaining in sample after washing and drying should be considered part of the total amount of coating material calculated in Step 9 below.

Step 8: Classify seed contaminants based on a combination of the species class for all the kinds in the mixture.

In this case, alfalfa, rose clover and red clover are A under spp. class; therefore, the overall combined spp. class for the mixture is A. To determine if the contaminant species are other crop or weed seeds refer to Column A. White clover is classified as C under A. Small hop clover is classified as W under A.

Excerpt from AOSA Rules, Volume 3 (AOSA, 2023b)

NOMEN #	SCIENTIFIC NAME	COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
300359	*Medicago sativa* L. subsp. *sativa*	alfalfa; lucerne	Fabaceae	A	C	C	C	C	C	C	C
40219	*Trifolium dubium* Sibth.	Hop clover, small; clover, suckling; shamrock, Irish	Fabaceae	A, F	W	C	W	W	W	W	W
40241	*Trifolium hirtum* All.	clover, rose	Fabaceae	A	C	C	C	C	C	C	C
300623	*Trifolium pratense* L.	clover, red	Fabaceae	A	C	C	C	C	C	C	C
300625	*Trifolium repens* L.	clover, ladino; clover, white	Fabaceae	A	C	C	C	C	C	C	C

Other crop seed	Weed seed
10 *Trifolium repens* (white clover)	2 *Trifolium dubium* (small hop clover)

Once the determination of whether the contaminating species are classified as other crop seed or weed seed, they must also meet the criteria of these categories versus being classified as inert matter. Refer to AOSA Rules, Volume 1, Sections 3.3, 3.4, and 3.5 to make these determinations.

Step 9: Calculate the percentage by weight of each component.

Weigh each kind of pure seed, other crop seed, inert matter and weed seed. The weight of each component is recorded to three decimal places. The sum of these weights is subtracted from the original working sample weight (i.e., 7.025 grams) to determine weight of the coating material. Calculate the percentage of each component based on the original working sample weight. Round the results to two decimal places. The sum of all components must equal 100.00 percent. In this case the sum of the components equal 100.01 percent; therefore, the percentage of alfalfa is adjusted up by 0.01 percent [refer to AOSA Rules, Volume 1, Section 3.1.b(4) and (5)].

Component	Component & total weight in grams
Alfalfa	2.758
Red clover (de-coated)	1.519
Rose clover (de-coated)	1.202
Other crop seed	0.008
Inert matter	0.013
Weed seed	0.001
Totals	5.501

Weight of coating material = original working sample weight – sum of components other than coating material

Weight of coating material = 7.025 grams – 5.501 grams = 1.524 grams

Component	Component & total weight in grams	Percentage by weight to demonstrate rounding	Final analysis result (after rounding adjustment)
Alfalfa	2.758	39.260	39.26% adjusted to 39.27%
Red clover (de-coated)	1.519	21.623	21.62%
Rose clover (de-coated)	1.202	17.110	17.11%
Other crop seed	0.008	0.114	0.11%
Inert matter	0.013	0.185	0.19%
Weed seed	0.001	0.014	0.01%
Coating material	1.524	21.694	21.69%
Totals	7.025		99.99% adjusted to 100.00%

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