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Seed Academy

Adventitious Presence (AP) Testing of Seed or Grain

INTRODUCTION

Adventitious presence (AP)-is the unintended presence of biotech traits in conventional or non-GMO seed. To test for the absence of herbicide and insecticide traits, SoDak Labs, Inc. offers three AP tests; AP-Seed, AP-Grain, and PCR GMO Screen testing.

DEFINITIONS AND TESTS

AP-Seed: use on seed lots intended for planting purposes.

AP-Grain: use on commercially harvested grain.

Herbicide Bioassay: seedling growth test which uses positive and negative check seeds, and the test sample imbibes the herbicide solution. Non-trait seedlings are stunted while trait seedlings grow normally, allowing for a "visual expression" of tolerance to the herbicide and provides a quantitative result as % positive for the respective herbicide trait.

ELISA Lateral Flow Strip (LFS) method: protein is extracted from ground up seeds or grain. If biotech protein is present a color change occurs on the lateral flow strip if the protein is present. AP% is estimated using Seed Calc 8 or QuickScan system.

PCR – GMO: DNA is extracted from finely ground grain, test detects presence of 35S promoter and NOS terminator used when biotech traits are inserted into plants. Extremely sensitive test, grain dust may test positive along with ground seed. AP% is estimated using Seed Calc 8.

Pools: protein and DNA extraction tests use seed pools of 300 to 1000 kernels which are ground into flour. Pool size is determined by the ability of the test to detect one biotech grain in the entire pool. For example, if four 300 seed pools each contain one biotech kernel, all pools will be positive and true level of AP can not be estimated as the total number of biotech grains is unknown.

NON-GMO SEED OR CONVENTIONAL SEED

AP-SEED – This test is recommended for seed used for planting purposes and involves three (3) separate tests, two separate herbicide bioassays and an ELISA protein assay. AP-Seed is completed on either 1200 or 2400 seeds. Herbicide bioassays for the detection of either Glyphosate, 2,4-Dichlorophenoxyacetic (2,4-D; Enlist ™) and Glufosinate (LibertyLink®) are conducted on seeds and result is reported as %AP (trait positive) for that herbicide (Figure 1.). Three ground pools of 400 to 800 seeds (Figure 2.) are screened for insect traits (YieldGard® RW, YieldGard® CB, Agrisure® CB, Herculex® CB, Viptera® CB, Herculex® RW, Agrisure® RW) using a Lateral Flow test (Figure 3.). Based on the number of positive pools and total number of seeds, Seed Calc 8 (www.seedtest.org/en/statistical-tools-_content---1--1486.html) estimates the impurity or GMO level of the submitted sample.

FIGURE 1. Herbicide bioassay showing 2 trait positive seedlings in a 100 seed subset of a 1200 seed test.

FIGURE 2. Ground 800 seed pools to be extracted for protein.

FIGURE 3. ELISA Lateral Flow strip comb showing positive reactions (top marks) and negative reactions for specific traits, as well as control reactions (bottom marks).

GRAIN FOR FEED OR FOOD

AP-GRAIN – Protein is extracted from ground material of 800 seeds and a lateral flow strip comb that detects YieldGard® Corn Borer, Roundup Ready™, YieldGard® Rootworm, Herculex® I, Liberty Link™, Herculex® RW, Agrisure® RW, Cry2A in SmartStax™, Viptera®, and Duracade® is used. The results are interpreted with the QuickScan system that has been calibrated to known concentrations of each protein reaction on the lateral flow strip.

PCR GMO SCREEN TESTING – Submitted samples are pooled into groups of 1000 kernels or less, for a total of 3000 (Figure 4.) or 10,000 kernels. DNA extraction is completed on ground pools and tested for specific DNA sequences utilized in transgenic insertion (35S and TNOS, optional -PAT, FMV). Based on the number of positive pools and total number of kernels, Seed Calc 8 estimates (Figure 5.) the impurity of the submitted sample for the primers utilized in testing. The 10,000 kernel test provides a highly sensitive result when absence of biotech traits is required.

FIGURE 4. Three pools of 1000 kernels.

Impurity Estimation & Confidence Intervals (Assay measure impurity characteristic)
(Number of kernels sampled should not exceed 10% of total number in population)

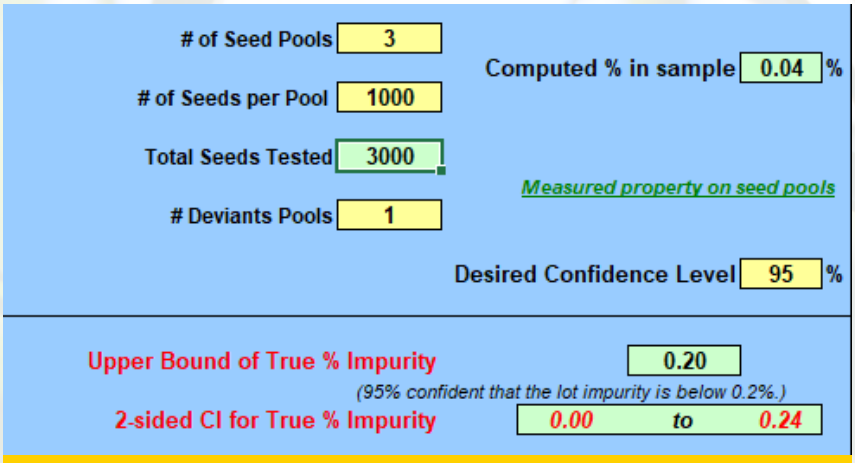

FIGURE 5. Calculation example using Seed Calc 8.

TABLE 1. Reference guide for selection of AP-Tests

Test	Sample Type	Size of Test	Method	Detection Level	Report
AP - Seed	Viable Seeds	2400 or 1200 seeds	Herbicide Bioassays and ELISA screening for insecticide traits	0.12-0.25% or less	Estimated impurity is calculated based on the percentage of trait tolerant seedlings, number of positive pools, the number of total seeds tested and the number of proteins tested.
AP-Grain	Grain	800 kernels	ELISA LFS comb for both Herbicide and Insecticide Traits	0.9% or less	Estimated impurity is interpreted quantitatively with the QuickScan system based on the development of the test line of the lateral flow strip compared to known results.
PCR Testing	Viable Seeds or Grain	Seeds are grouped into equal pools for a total of either 10,000 or 3000 seed	Detection of specific DNA sequences utilized in transgenic insertions	0.03-0.10%	Estimated impurity is calculated based on the number of positive pools, the number of total seeds and the primers used.