Testing Methods | Seed Laboratory



# **Testing Methods**



# Accurate Testing, Exceptional Service

ISU Seed Laboratory services include: Standard Germination Testing (AOSA, ISTA), Purity Testing and Noxious Weed Exams, Seed Health Testing (NSHS-Accredited), Trait/AP Testing, Vigor Testing (Cold, Saturated Cold, and AA), Tetrazolium Testing (Viability and Vigor), and Fast Green and Hypochlorite Soak Tests.

### ISU Seed Laboratory Testing Methods



Warm Standard Germination Duration: Typically 7-10 days

Warm germination tests are used for labeling purposes and give a reasonable idea of field emergence under favorable conditions. A minimum of 400 seeds is required for an AOSA, NSHS, or ISTA official test. Tests can be conducted in rolled brown paper towels (8 reps of 50 seeds), on blue blotter paper, on crepe cellulose paper (Kimpak®), and Kimpak® covered with sand. Corn and soybeans are normally tested at 25°C (77°F) for 7 days. A test on any species can be ended at the required germination period if an analyst feels maximum germination has been reached. A test can be extended 2 days for slow-growing seedlings.



Germination Test on Carryover RIB Corn Duration: Typically 7 days

For carryover RIB corn samples, we recommend sending 5-8 pounds of seed (for a 95/5 blend). We will conduct a 400 seed germination test & a 200 seed cold test on the blend, and a separate 200 germination test on refuge seeds. Running these three tests generates a germination result for labeling, a cold test on the blended product to relate to the field, and a good estimate of the germination level of the refuge portion.



Sand Germination Duration: Typically 7-10 days

Sand germ tests are done the same as Warm germination, except that a layer of moist sand is placed over the seeds and Kimpak®. This test is useful in suppressing some fungi. It also aids in uniform uptake of water, especially in low moisture soybean seed. For soybean samples, sand germination results are the same or slightly higher than warm germination results. In the

instances of fungal problems or extremely dry seed, the sand germination can be markedly higher. We find that the higher the germination of the seed lot, the less positive effect the sand has on the results.



## Cold Germination: The Iowa Cold Test Duration: Typically 12-14 days

The cold germination test gives a reasonable idea of emergence under less than ideal conditions. Two replications of 100 seed are placed on Kimpak® that has been moistened and chilled overnight at 10C. The seeds are covered with a non-sterile sand/soil mixture and returned to 10C for seven days without light. The cart is then moved to 25C for five to seven additional days. Seedlings that emerge through the sand/soil mixture are evaluated according to AOSA Rules. An extended cold test is also available which exposes seed to two weeks in the cold instead of one.



#### Saturated Cold Germination Duration: Typically 9 or 10 days

The saturated cold germination test is another way of determining how well a seed lot will do under unfavorable conditions. Some consider it to be a more severe test than the tray method. Seeds are placed on a thin layer of saturated soil on paper towels over a special tray. Seeds are turned so that the side of the corn kernel closest to the embryo is down against the soil. The cart is placed in 10°C for seven days without light and then moved to 25°C for two to three more days. The number of normal seedlings, abnormal seedlings, and dead seed are recorded.



Accelerated Aging

## **Duration: Approximately 10 days**

Accelerated aging is another vigor test that can be used to estimate field emergence. Its advantage is that virtually any type of seed can be tested using the AA test. The test consists of placing a species specific mass of seed (not a certain number) on an elevated screen. The screen is placed into an acrylic box that contains 40ml of water. When the box is covered with a tight-fitting lid and placed into the AA chamber, the seeds are exposed to high temperature and humidity. The aging period ranges from 48 to 96 hours depending on type of seed (corn and soybeans: 72 hours). The seeds are removed from the chamber and promptly planted on blotters, paper towels, or Kimpak®. Seeds planted on Kimpak® are covered with a layer of moist soil. The seeds are grown a period of time similar to a warm germination test and the number of normal seedlings are reported.



Tetrazolium (TZ) Test Duration: 2 days

The tetrazolium test gives a rapid estimation of warm germination results. It can also be used for small-seeded species in determining the viability of ungerminated seed at the end of the germination period. Two reps of 100 seeds (typically) are placed between moist brown paper towels or blotter paper overnight. The next day the seeds are pierced, cut in half, or left whole (depending on species) and placed in tetrazolium solution. After a short period of time, the seeds are examined for staining patterns. The tetrazolium test can also be used to detect frost damage, estimate vigor, or diagnose seed lot problems.



Herbicide Bioassay Duration: Typically 6 - 7 days

Herbicide Bioassay (HB) tests can be used to determine the percentage of seeds in a lot that are tolerant to a herbicide (genetic purity) or it can be used to detect the absence of tolerance (adventitious presence). We recommend using immunoassay or PCR tests to determine whether seeds are free of biotech traits. HB tests are available for corn (Roundup Ready®, Liberty Link®, Clearfield®), soybeans (Roundup Ready®, STS®), cotton (Roundup Ready®), and canola (Roundup Ready®). Herbicides (active ingredients) used in testing are glyphosate

(WeatherMax  $\$ ), glufosinate (Liberty  $\$ ), Imazethapyr (Lightning  $\$ ), and chlorsulfuron (Glean  $\$ ).



Fast Green Duration: One day

The fast green test is conducted on corn and sorghum seeds. Briefly exposing seeds to fast green solution and then rinsing off excess solution results in damage to the surface of seeds becoming apparent. Damage is classified as light (damage to base of seed or small areas away from the embryo), medium (damage extending along either side of the embryo) or severe (damage to seed over the embryo). The test is especially useful in setting equipment in seed conditioning facilities so as to maximize output while minimizing damage from machinery.



Immunoassay Duration: Seeds - One to three hours, Leaves - 6 to 8 days

Immunoassay tests (ELISA plates and Lateral Flow Strips) utilize antibodies of what is being tested for (biotech traits) to determine if protein for that trait is present (yes/no or qualitative) or how much is present (quantitative). Genetic purity testing requires checking individual seeds or leaves. Generally, leaves contain more protein than seeds and therefore are very useful for detecting traits that don't necessarily produce much protein in seeds (i.e. Bts). ELISA plates are a natural for this (YieldGard Corn Borer®, YieldGard Rootworm®, YieldGard Plus®, or Herculex®). To test for the absence of biotech traits, combs containing strips for each trait can be used to check for all traits simultaneously. An alternative is to use a single strip that detects stacked traits. A critical factor is knowing the capabilities of the ELISA plate or lateral flow strip. Detection limits range from 1 trait seed in 70 conventional seeds to 1 trait seed in 1000 conventional seeds.



PCR Duration: Variable

Polymerase Chain Reaction (PCR) is a high-tech method to extract, increase, detect and identify DNA from biotech traits (if present). It is highly sensitive and especially good in determining absence of biotech traits. PCR can give qualitative or quantitative results. Attention to detail and in depth knowledge of molecular biology and available biotech traits are critical to success in using PCR.



# **Seed Health Testing**

The Iowa State University Seed Health Testing Laboratory tests for more than 350 viral, bacterial, and fungal pathogens on most crops, including corn, soybeans, vegetables, and flowers using a variety of methods. Tests are available to address nearly all phytosanitary and quality assurance concerns. All phytosanitary certification is performed in accordance with National Seed Health System (NSHS) standards. The Seed Health Testing Lab is NSHS accredited, in accordance with USDA-APHIS regulations.

# **Price Lists**

Current price lists are available on our Online Services Webpage (secure web lockers) or copies can be requested by contacting ISU Seed Lab Customer Service at 515-294-6826 or seedlab@iastate.edu.

# For more in-depth information on ISU Seed Lab testing methods, visit: Testing Q&As

## Follow us!



# Read about Seed Lab events and activities on the ISU Seed Science Center Facebook and Twitter pages @ISUSeedSci!

#### Iowa State University Seed Laboratory | Michael G. Stahr, Manager

109 Seed Science Center, 2115 Osborn Drive, Iowa State University, Ames, Iowa 50011-1050. Ph. (515) 294-6826, Fax (515) 294-8303. Email: seedlab@iastate.edu

Homepage: www.seedlab.iastate.edu

Seed Lab Hours: Monday-Friday 8:00-5:00 p.m. Summer Hours: 7:30 a.m.- 4:00 p.m.

Seed Science Center: www.seeds.iastate.edu

© Copyright 2009-2017. Iowa State University of Science and Technology. All rights reserved.