# Rule Change Proposal No. 17

**PURPOSE:** to provide seed analysts with a standardized testing method to check seed for the presence of Roundup Ready® gene in soybean seed, either intentional genetic enhancements or unintentional contamination

**PRESENT RULE:** NEW RULE – if adopted this rule would replace the Section 12 tentative rule – Seed Soak Testing for Roundup Resistance in Soybeans.

**PROPOSED RULE**

3.2 Identification and cultivar determination

d. Seed Soak Test to Determine Roundup® Herbicide Resistance in Soybeans - Definitions, methods and tolerances which are used for conducting the trait evaluations of genetically modified, Roundup Ready® soybeans are provided in AOSA Cultivar Purity Testing Handbook, Contribution No. 33 to the Handbook on Seed Testing.

1) **Herbicide Solution Preparation:** Obtain Roundup® herbicide which has been labeled by the manufacturer to consist of 41% of the active ingredient glyphosate. Prepare a 3600 ppm solution as follows: In a 1000 ml volumetric flask or other suitable container that can be sealed add 8.77ml of the Roundup® herbicide and distilled water to bring volume to 1000 ml. Seal the container and mix solution on a stirring plate for a minimum of five minutes. Properly label container with name and strength of solution and date of preparation. Do not use solution if more than 2 weeks old.

   **NOTE:** If other, lesser concentrations of Roundup® herbicide are used, the amounts of Roundup® herbicide and distilled water must be adjusted so that the final concentration obtained is 3600ppm.

2) **Check Samples** - Before beginning a test for Roundup® herbicide resistance, obtain a known sample of 100% Roundup® Ready soybeans and a known sample of soybeans with 0% Roundup® herbicide resistance. Each sample shall be handled separately throughout the planting and evaluation process

3) **Method for Imbibition** - The seeds from the sample under consideration and the two check samples shall be imbibed separately. Plant 400 seeds from each sample in 50 seed replicates in rolled towels moistened with water. Label each sample with the appropriate identification. Place all three samples in a 25°C germinator for 24 hours.

4) **Application of Herbicide Solution** - After 24 hours of imbibition, remove the seed/seedlings from rolled towels and soak leaving the top of seed/seedlings visible to avoid complete submersion in the prepared herbicide solution at 30°C for a period of two hours. Drain herbicide solution.

5) **Germination** - Plant the seeds/seedlings in rolled towels moistened with water and place in 25°C germinator for 8 days.

6) **Evaluation Of Seedlings -** Evaluate seedlings according to descriptions in the AOSA Seedling Evaluation Handbook, Contribution No. 35 to the Handbook on Seed Testing. Seedlings which have the desirable characteristics of normal seedlings in all the categories to be evaluated will be considered both normal and Roundup® herbicide resistant. Record the number normal and abnormal herbicide resistant seedlings and the number of normal and abnormal non-herbicide resistant seedlings. Abnormal non-herbicide resistant seedlings exhibit such deformities as elongated hypocotyls, stunted roots or other deformities

characteristic of herbicide injury. Deformities caused by herbicide application can be confirmed by comparison with the 0% Roundup herbicide resistance check sample.

7) **When To Retest**
   a. Retest when the range between 400 seed replicates of a given test exceeds the maximum tolerance stated in the table 3.2.9, the labeled standard or the product standard, both of which are assumed to be 100% for Roundup® Ready soybeans.
   b. Retest when there are doubts that the chemical solution was prepared correctly, or the solution date indicates that the materials that have been used were more than two weeks old.
   c. Retest when no two satisfactory tests are within tolerance.
   d. Retest if the sample under consideration exhibits Roundup® resistance but the percent of normal herbicide resistant seedlings does not fall within the AOSA germination tolerances (referee to Sec. 5.5) with the label claim for germination.

8) **Calculations and Reporting Test Results**

The results obtained from testing for Roundup® Ready soybeans shall be calculated using the following formula:

$$\frac{\text{Number of normal Roundup® herbicide resistant seedlings}}{\text{Total normal \& abnormal Roundup® herbicide resistant seedlings}} \text{ X } 100 = \% \text{ Roundup® resistant seedlings}$$

**NOTE:** When more than one test is made in the same lab and the results are found to be within tolerance of one another, the results of such tests, or retests, which fall within tolerance shall be averaged and reported as the percentage of Roundup® resistant seedlings present.

9) **Tolerance Applied To Results**
   a) **Roundup Resistant Soybean Seed -** Tolerance is applied when two independent trials or tests in separate labs have been made on the same, properly handled bulk lot of Roundup® Ready soybeans, using the prescribed method. Based on the research data and Industry standards, the divergence between analyses trials is considered large and is to be recorded as a significant difference when laboratory results are below the expected or labeled claim for Roundup® herbicide resistance. The following table states the conditions when a laboratory analysis result obtained on a sample has significantly fallen below expectations which are stated as the labeled claim and the deficiency found is to be considered real.

| Roundup® Herbicide Resistance Expected in Soybean Seed | Lowest Acceptable Roundup® Herbicide Resistance Level Found by Seed Soak Test |
|---|---|
| 100.0% - 99.00% | 96.70% Roundup® herbicide resistant seedlings present |
| 98.99% - 98.00% | 95.20% Roundup® herbicide resistant seedlings Present |
| 97.99% - 97.00% | 94.40% Roundup® herbicide resistant seedlings present |
| 96.99% - 96.00% | 93.50% Roundup® herbicide resistant seedlings present |
| 95.99% - 95.00% | 88.20% Roundup® herbicide resistant seedlings present |
| Less than 95.00% Roundup® herbicide resistant seedlings present | Less than or equal to 88.19% Roundup® herbicide resistant seedlings present * |

* Roundup® Ready soybean samples that contain less than 95% Roundup® herbicide resistant seed (more than 5% other soybean seed) shall be considered to be a mixture of soybean varieties.

b) **Conventional Soybean Seed -** Tolerance is applied when two independent trials or tests in separate labs have been made on the same, properly handled bulk lot of conventional soybeans, using the prescribed method. Based on the research data and Industry standards, the divergence between analyses trials is considered large and is to be recorded as a significant difference when laboratory results are exceeding the expected or labeled claim for Conventional Seed which may be contaminated with Roundup® herbicide resistant soybean seed. The following table states the conditions when a laboratory analysis result obtained for contamination of a sample has significantly exceeded the expectations, which are stated as the labeled claim, and deficiency found is to be considered real.

| **Roundup® Herbicide Resistance Expected in Conventional Soybean Seed** | **Highest Acceptable Roundup® Herbicide Resistance Level Found by Seed Soak Test** |
|---|---|
| Between 0.00% - 1.00% Roundup® herbicide resistant seed contamination | 2.60% Roundup® herbicide resistant seedlings present |
| Between 1.01% - 2.00% Roundup® herbicide resistant seed contamination | 3.80% Roundup® herbicide resistant seedlings present |
| Between 2.01% - 3.00% Roundup® herbicide resistant seed contamination | 7.00% Roundup® herbicide resistant seedlings present |
| Between 3.01% - 4.00% Roundup® herbicide resistant seed contamination | 5.90% Roundup® herbicide resistant seedlings present |
| Between 4.01% - 5.00% Roundup® herbicide resistant seed contamination | 7.20% Roundup® herbicide resistant seedlings present |
| Greater than 5.00% Roundup® herbicide resistant seed contamination * | Greater than or Equal to 7.21% Roundup® herbicide resistant seedlings present * |

* Conventional Soybean Seed that contain more than 5% Roundup ® herbicide resistant seed (less than 95% conventional soybean seed) shall be considered to be a mixture of soybean varieties.

**SUPPORTING EVIDENCE**

10/05/01
Subject: GMO RULE PROPOSAL
Dear AOSA Rules Committee members

Attached with this note you will find the latest statistical analysis, the GMO Rule on Seed Soak Testing, the data for additional lab testing and the previous years of referees which were run on the subject of genetic trait testing that have been run in conjunction with this Rule

To bring any new committee members up to date, after review and passage as a tentative rule by the Board last year, the seed soak method for testing Genetically Modified Soybeans was published as a tentative rule. Since that time additional facilities have used the protocol for evaluation and their additional data has been included for the completion of the statistical analysis during this year the Board gave for action to occur.

STATISTICAL ANALYSIS - SUMMARY OF REPORT PRESENTED

The purpose of doing a statistical analysis is to tell us what happens during the evaluation process of a seed product when a specific method is being used. If the proposed analytical procedure will conceivably be used for work in a number of facilities, then the work completed as a referee must establish the necessary facts that the protocol works,

that the protocol is repeatable, and that the results obtain from the protocol can be evaluated with tolerance so that the final result obtained is true, correct, and statistically sound. In this instance we have learned several things concerning the referee, which was conducted on the Seed soak method.

1. Seed Soak Testing has an acceptable level of repeatability for analysts with various experience levels.
2. Because of repeatability, seed soak protocols can be used to establish that sample deficiency is real.
3. The AOSA 95% confidence levels, or tolerance, are stated for this protocol within the limits of the referee.

TOLERANCE

The end goal after the analysis of any referee is completed are that the tolerance's are incorporating testing differences, lab to lab variance, and within lab variance. Tolerance prepared in this manner insures that the final result may represent the lot being evaluated. The table and statements shown in the summary prepared by Kirk Remund establish 95% tolerance (confidence) levels for the Seed Soak Protocol to represent the sample quality. The summary indirectly states that due to the referee design incorporating spiked samples rather then using seed samples as they existed in the environment where they were produced and sold, the tolerance figures given can not incorporate any sampling variances which arises when product samples are drawn in the field. The statistical analysis does show that the sample analysis outside a 95% level will represent a real deficiency. It may be that a further analysis of the data presented can establish sample variance for this protocol by use of Mile's method of incorporating sample variance through calculations. If not, other means may be capable to serve the same purpose.

I do feel that without Miles formula being run, one statement is based on an incomplete assumption when it is said that " tolerance limits only reflect the lab variability and do not reflect sampling variability". The statement would be better understood if it was said after first completing further calculations via Miles formula or using the data shown. The 100% and 0% check samples utilized for this referee do represent the actual field samples of product. The products were split and subsampled for this referee from the product bags as they existed in nature. The 100% sample utilized was a Roundup Resistant Soybean seed product produced by Garst Seed Company. The 0% sample utilized a conventional soybean seed product also sold by Garst Seed Company. Both products and the results found in this referee for the 100% and 0% sampled represent a seed product as it exists in the environment. An analysis of the 0% and 100% data could give the sample variance for the Seed Soak Protocol if it agreed that those samples are using the products after sampling. I would ask that the Rules committee also take into account that in the environment it is not possible to find the instances of contamination represented in the referee where they can readily be sampled and split for use. The statement implies a sampling process of contaminated products could be done in order to evaluate the problems as they exist and determine all variance including the sampling from the results obtained. The instances of contaminated Roundup or Conventional soybean seed are, to this point, rare. Finding contaminated seed products for use in samples is a dilemma with out any solution Other areas of seed testing take the approach which has been taken with this referee, to spike the samples. Pathology testing referee can not readily find the levels of contamination in the environment which they wish to evaluate. Hence spike samples are prepared. The ISTA Pathology committee under the direction of Jim Sheperd, Canada is presently evaluating a procedure for detecting the presence of Alternaria in Flax seed. The procedure and study of the procedure can be found on the ISTA site. Go to the internet address I have shown, www.seedtest.org/pdc/linnseed/linnseed.cfm. The referee at this site shows results on various levels of Alternaria contamination. Those levels of contamination were artificially created by blending or "spiking" samples of flax to represent each level because they did not exist naturally in nature. Other flax samples which exhibited the highest and lowest contamination levels of Flax were also provided to the participants to analyze as the standards or check samples with the Alternaria protocol. The conditions Dr. Sheperd created in his referee are a duplication of what has occurred within the referee on GMO Seed Soak methodology you are considering. The same process of moving the Flax Pathology test into ISTA Rules is also underway as is being done with the Seed Soak Rule you have before you for consideration.

Thank you,

Jim Lair, Seed Lab Manager
Illinois Department of Agriculture

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Date: September 27, 2001
From: Kirk Remund (314-694-6673)
To: Jim Lair
Subject: RR Soybean Seed Soak Test Referee Statistical Results

I have finally completed the statistical analysis of the referee results. I refer to graphs when I discuss the results and they are in the PowerPoint file that accompanied this memo file. I tried to put the graphs in this memo but had much difficulty.

This analysis is based on the following assumptions:

1. The labs that participated in this referee represent a random sample of all labs that we wish to make inferences to. This assumption may be in question given that only 10 out of the 20 labs participated.

2. The samples used in this referee were prepared without error. We assume that each lab received sets of samples that truly have resistance levels at the stated levels.

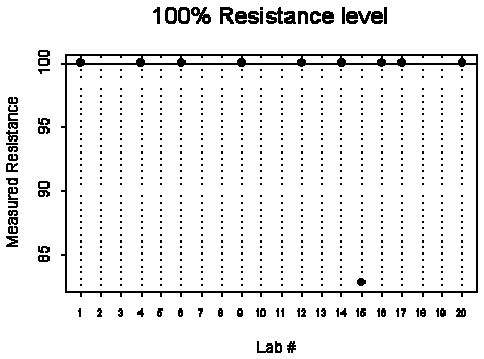
The first 12 plots in the PowerPoint file are data plots for each resistance level. The four replicates are plotted for each lab with a couple of exceptions. A horizontal line is drawn through the points to indicate the known Roundup resistance level. Note in these plots that Lab #15 had much difficulty analyzing these samples and it differs significantly from the other labs. For this reason, I have eliminated Lab #15 results from the analysis that follows.

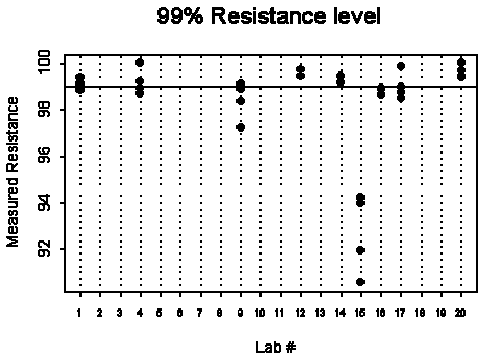
The last 20 plots are data plots for each lab for the low resistance levels and also for the high resistance levels. There are two plots for each lab that reported data. The true resistance levels are plotted against the measured resistance levels. A dotted-line is also plotted to show the agreement line. A couple of other data notes are worth making from these plots. Lab #4 and Lab #20 appear have a slight positive bias for the high resistance level samples while Lab #16 has a slight negative bias. There are also some bias patterns in the low resistance sample results.

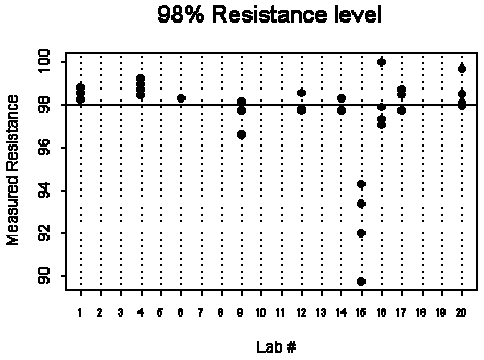
I noted a slight to moderate skew in the data (across labs and reps) at each resistance level. Because of this I took an arcsine square-root transformation on the data to obtain symmetry in the data needed for normality assumptions. The following table contains 95% tolerance intervals (95% confidence level) for each resistance level. We expect that 95% of the future lab samples at the stated resistance levels in the table would have measured resistance levels within these tolerance bounds. For example, we expect that most samples (i.e., 95% of the samples) coming into the labs with 96% resistance levels will have measured resistance values between 93.5% and 98.1%.

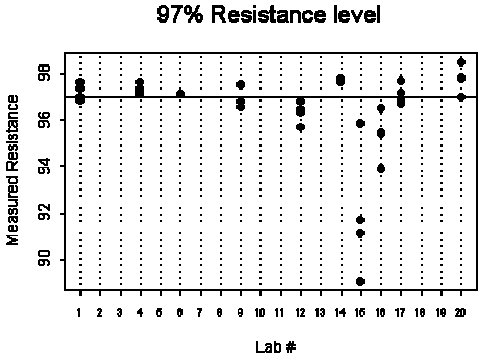
| Resistance Level | Lower 95% limit | Upper 95% Limit | | Resistance Level | Lower 95% Limit | Upper 95% Limit |
|---|---|---|---|---|---|---|
| 1% | 0.1% | 2.6% | | 95% | 88.2% | 98.6% |
| 2% | 0.5% | 3.8% | | 96% | 93.5% | 98.1% |
| 3% | 0.3% | 7.0% | | 97% | 94.4% | 98.8% |
| 4% | 1.8% | 5.9% | | 98% | 95.2% | 99.9% |
| 5% | 2.3% | 7.2% | | 99% | 96.7% | 100% |

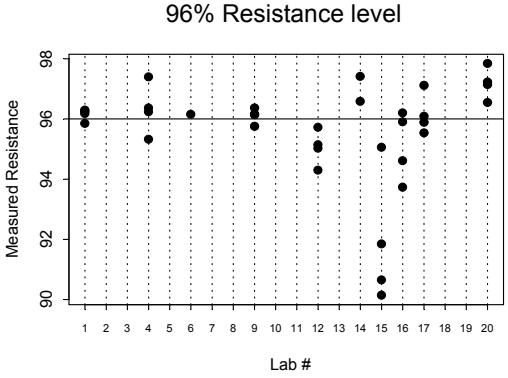
These results are based on the assumptions stated earlier. Note that this tolerance interval width is a function of the number of data points in this study, the lab-to-lab variability and the pooled replicate variability within a lab. I illustrate the use of this table with an example. If a measured sample result is obtained at 92% resistance, we see if any of these tolerance intervals include this resistance level. We note that the tolerance interval for 95% resistance includes this value but all other intervals do not so we can conclude that the true level of resistance is below 96% and very probably could be 95%. If a sample result is obtained at 95%, then by looking at the tolerance intervals we conclude that 95%, 96%, 97% and 98% are all very probably values for the true resistance level in the sample. Please note that these tolerance intervals should not be used to establish published tolerances since these tolerances limits only reflect the lab variability and do not reflect sampling variability. Please let me with any questions about this analysis.

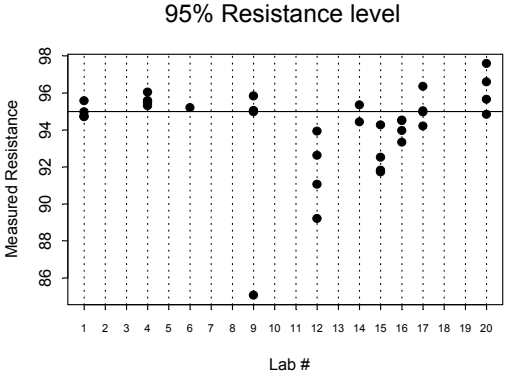

100% Resistance level
Measured Resistance
Lab #

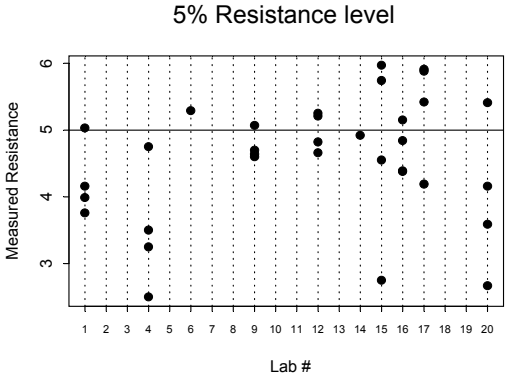

99% Resistance level
Measured Resistance
Lab #

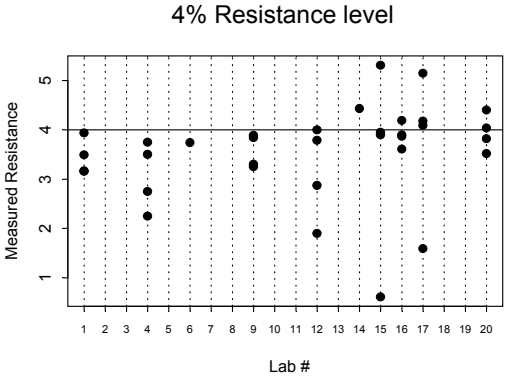

98% Resistance level
Measured Resistance
Lab #

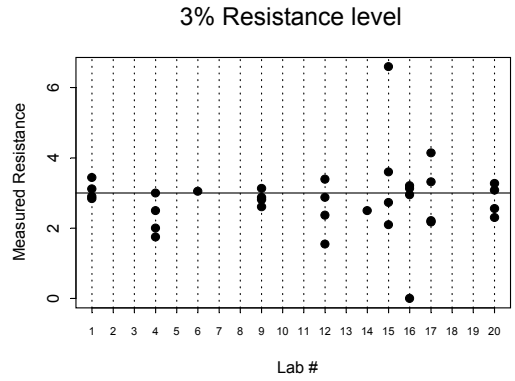

97% Resistance level
Measured Resistance
Lab #

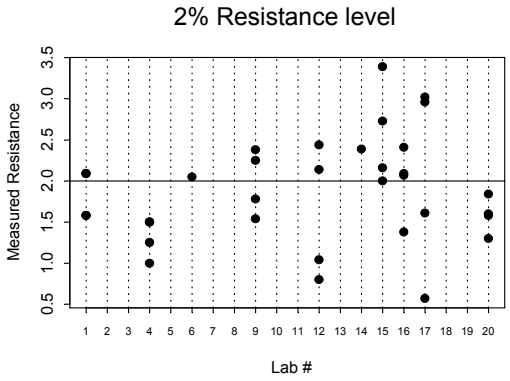

96% Resistance level
Measured Resistance
Lab #

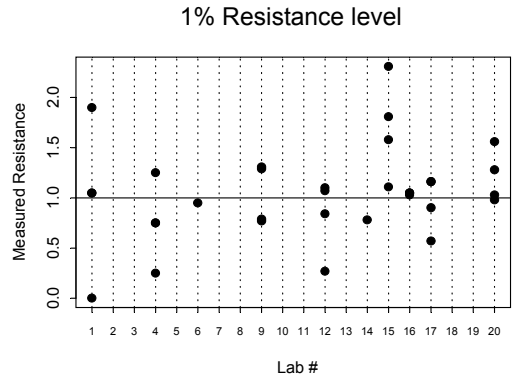

95% Resistance level
Measured Resistance
Lab #

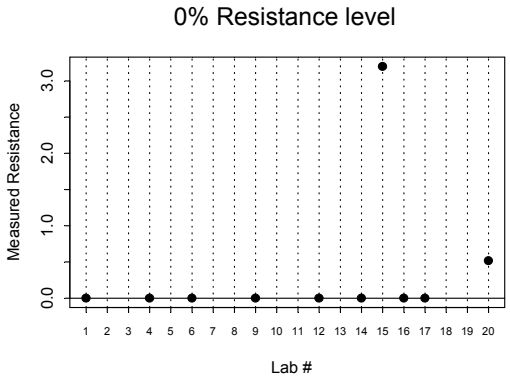

5% Resistance level
Measured Resistance
Lab #

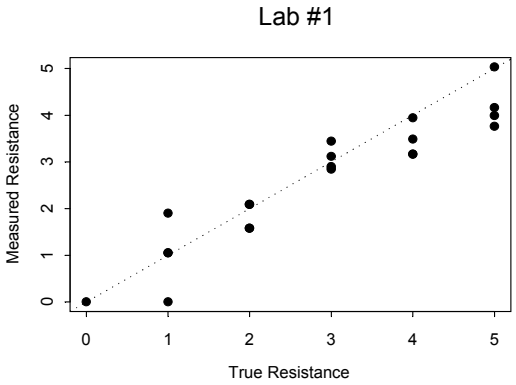

4% Resistance level
Measured Resistance
Lab #

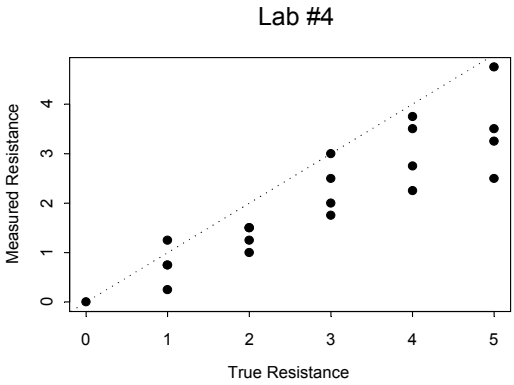

3% Resistance level
Measured Resistance
Lab #


2% Resistance level
Measured Resistance
Lab #


1% Resistance level
Measured Resistance
Lab #


0% Resistance level
Measured Resistance
Lab #


Lab #1
Measured Resistance
True Resistance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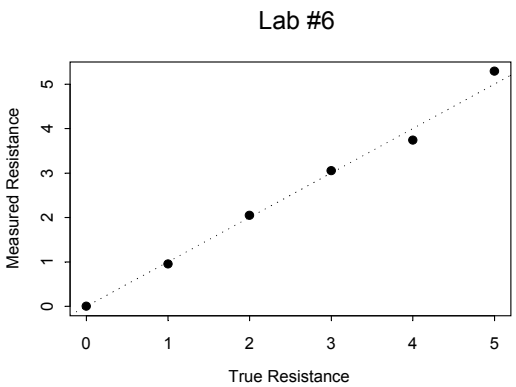
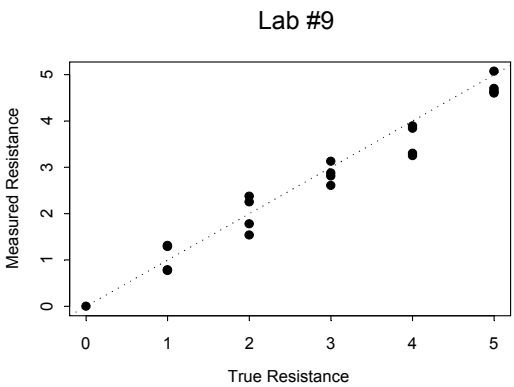
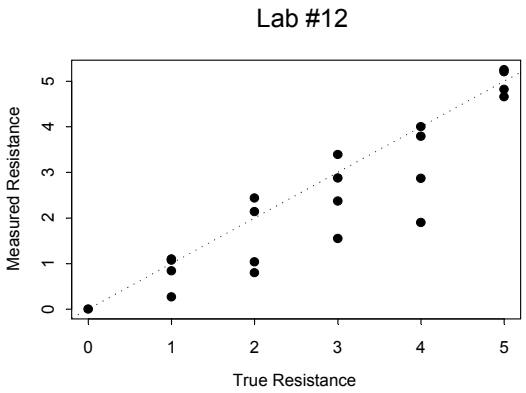
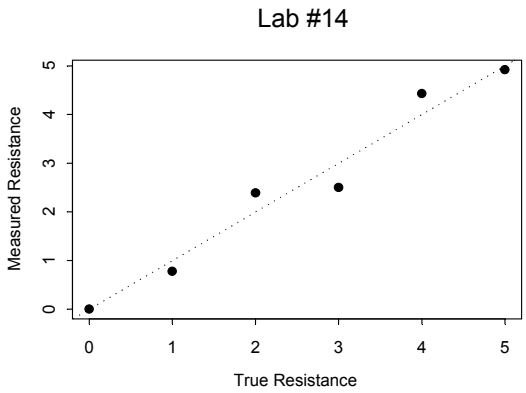
True Resistance

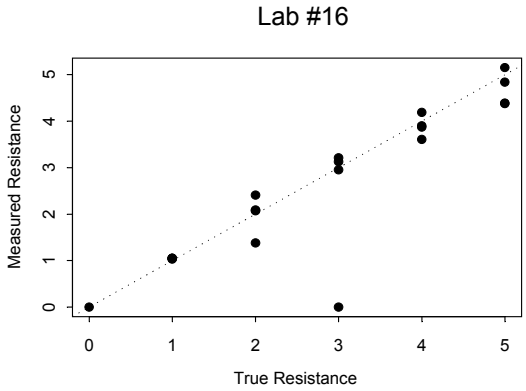

Lab #6
Measured Resistance
True Resistance

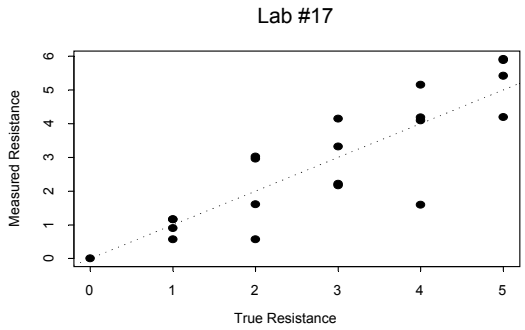

Lab #9
Measured Resistance
True Resistance


Lab #12
Measured Resistance
True Resistance
0
1
2
3
4
5


Lab #14
Measured Resistance
True Resistance
0
1
2
3
4
5


Lab #15
Measured Resistance
True Resistance
0
1
2
3
4
5
6


Lab #16
Measured Resistance
True Resistance
0
1
2
3
4
5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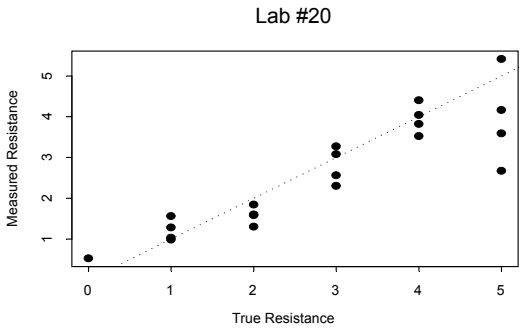
Measured Resistance
True Resistance
0
1
2
3
4
5
6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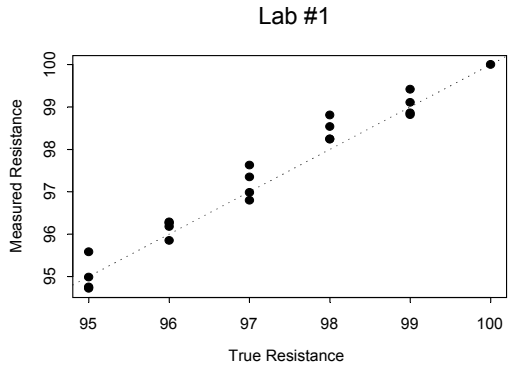
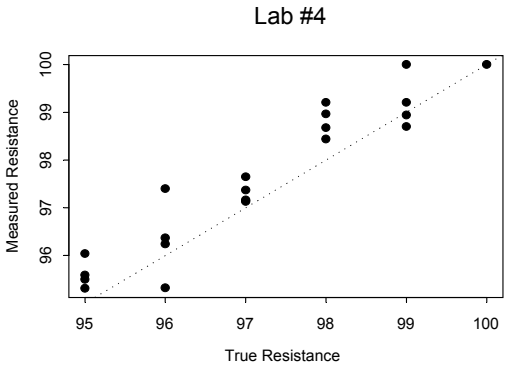
Measured Resistance
True Resistance
1
2
3
4
5


Lab #1
Measured Resistance
True Resistance
95
96
97
98
99
100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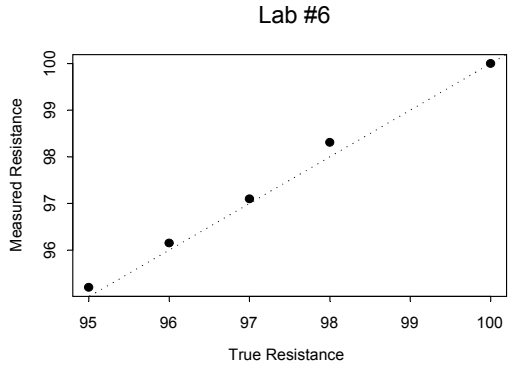
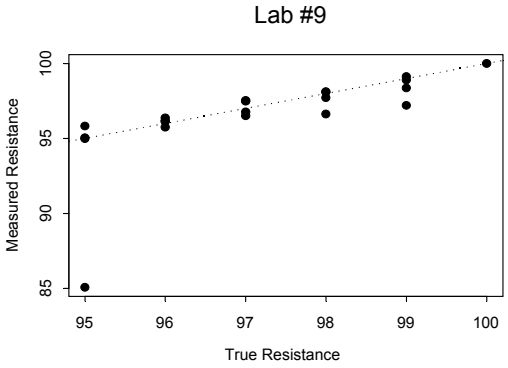
Measured Resistance
True Resistance
96
97
98
99
100


Lab #6
Measured Resistance
True Resistance
96
97
98
99
100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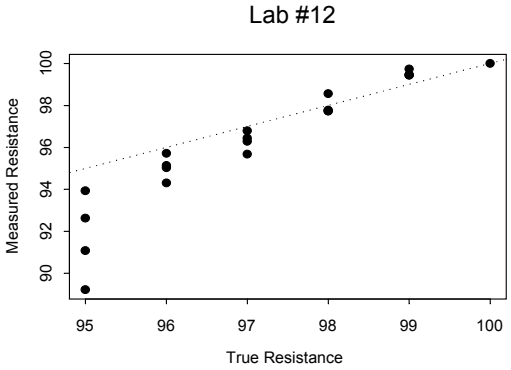
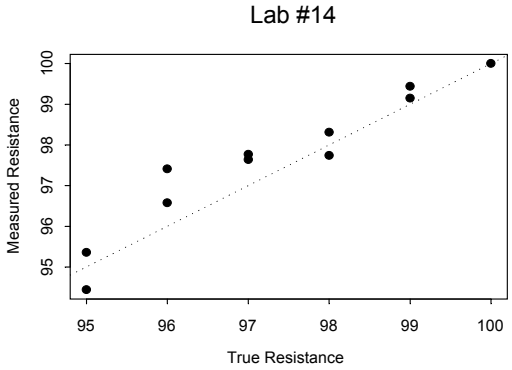
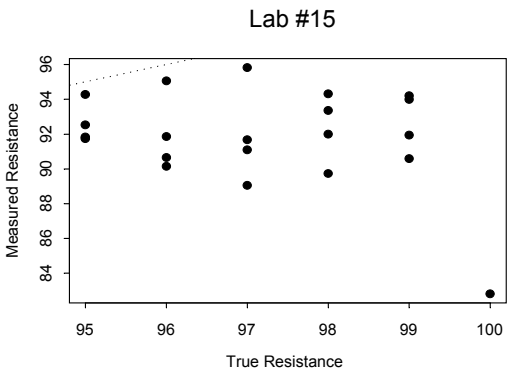
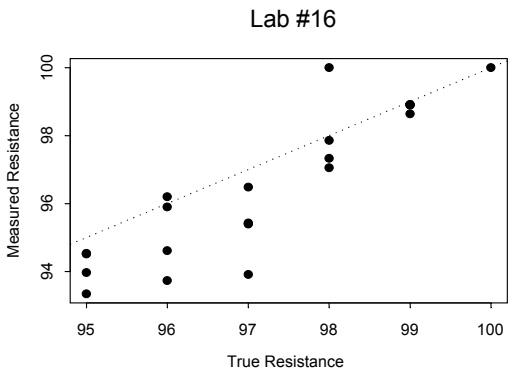
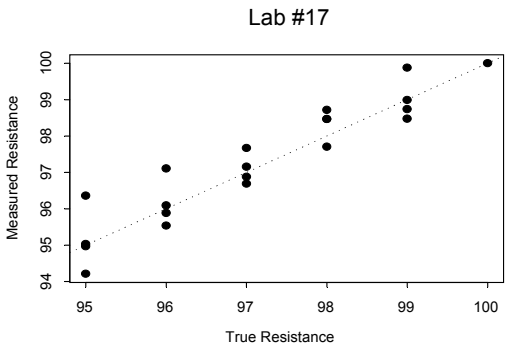
Measured Resistance
True Resistance
85
90
95
100

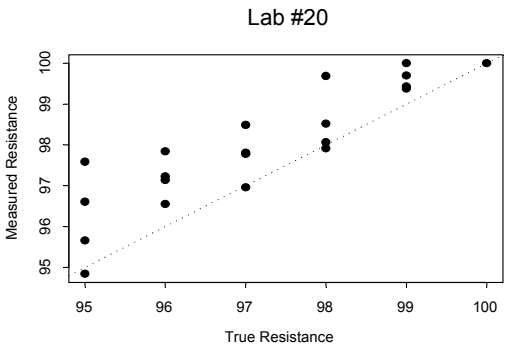

Lab #12
Measured Resistance
True Resistance
90
92
94
96
98
100


Lab #14
Measured Resistance
True Resistance
95
96
97
98
99
100


Lab #15
Measured Resistance
True Resistance
84
86
88
90
92
94
96


Lab #16
Measured Resistance
True Resistance
94
96
98
100


Lab #17
Measured Resistance
True Resistance
94
95
96
97
98
99
100


Lab #20
Measured Resistance
True Resistance
95
96
97
98
99
100

**Illinois Referee Summary**
**Testing of Genetically Modified Seed Products**

**BACKGROUND:** At the conclusion of the AOSA meetings in Ames, IA last year a consensus was not reached within the regional meeting participants that a project would be run for this year. As a result a project was selected by the chair which was of concern to the management in Illinois and participants for the Illinois referee were obtained by contacting labs.

**PURPOSE:** The purposes of the Illinois referee were to evaluate a seed soak testing protocol for dual uses it labs. Seed soak protocols would be used to determine between, and within, lab accuracy for resistance levels in genetically modified seed and conventional seed. The problem to evaluate a each level would be the unwanted presence of contaminants these two evaluation processes at not presently addressed in the testing arena with any standard, recognized protocols. Even so, the research had shown that seed soak protocol tests and methodology were already being utilized in the states of North Carolina, Arkansas, Illinois, Iowa by both commercial and state facilities. The conventional seed product evaluations for roundup seed contamination was a new step or use incorporated in this referee to determine if it was feasible to adapt the protocol to this purpose.

A tolerance was to be applied at the completion of testing to the results obtained from calculations used for the roundup resistant products or conventional seed products. The tolerance would determine if the protocol had found a significant difference with the claim stated. If no claim is stated then the implied use is utilized as the claim.

**METHOD SELECTIONS FOR REFEREE:** The seed soak protocol had been sent to the Rules committee of the Association of Official See Analysts for adoption on a tentative basis. The action occurred in 1999-2000 when the protocol was initially sent to the AOSA for consideration. The proposal was rejected by the Rule committee. The basis for the rejection stated at that time was not having sufficient data within and between labs.

In the 2001 referee, the state of Illinois prepared r research samples required for evaluation at each participating facility. The model of running forty-four samples in each lab for the referee utilized was obtained from the Chair of the AOSA Statistics committee. A conventional seed product and a genetically modified seed product were obtained in order to prepare the referee samples. Referee samples were prepared by counting out and hand inserting seeds from each of the source products which would result in representative samples that had the appropriate number of conventional or genetic off types at each level of resistance evaluated. The forty-four samples per lab were then packaged and sent out to $D_1$ laboratories working in state government, certification and the seed industry. Due to the number of samples which were required, seed was not ready to be mailed until January of 2001. In total 924 samples were mailed out.

**RESULTS:** See the attached summary of the data which was completed and returned by the due date To date 308 samples were completed and returned by seven of the twenty-one laboratories who were sent seed. Four hundred seed replicates within each category of resistance were evaluated at each laboratory. Four samples represented each level of resistance The total number of tests evaluated was 28 samples at each level of resistance shown on the summary. The average of four replicates at each laboratory is reported in the data summary.

The concern expressed last year by the Rules chair that there was a need to evaluate sufficient numbers of samples both within and between laboratories at the high and low end of resistance has been met.

The data obtained in the 2001 Illinois referee indicates that repeatable results can be obtained within laboratories as well as between facilities. The results showed consistent, repeatable data at most laboratories for the genetically modified seed products as well as the conventional seed products which had genetic contamination. One laboratory was consistently outside of tolerance The results were still incorporated since people need to be able to evaluate all data obtained if action is taken to incorporate these types of protocols into daily routines. Based on comments which were solicited from the out of tolerance laboratory, their staff believed that interpretation error on their part appeared to be the main factor leading to the problems which are reported in this referee summary.

**FY2001 IL REFEREE – GENETICALLY MODIFIED SEED REPLICATE TESTS USING SPIKED SAMPLES**

| LAB# | A EXPECTED 100% RESISTANCE | B EXPECTED 99% RESISTNACE | C EXPECTED 98% RESISTANCE | D EXPECTED 97% RESISTANCE | E EXPECTED 96% RESISTANCE | F EXPECTED 95% RESISTANCE |
|---|---|---|---|---|---|---|
| 1 | 100.00 | 99.05 | 98.46 | 97.19 | 96.14 | 95.01 |
| 3 | 95.75 | 99.21 | 98.82 | 97.33 | 96.33 | 95.61 |
| 9 | 100.00 | 98.4 | 97.65 | 97.08 | 96.11 | 92.74 |
| 12 | 100.00 | 99.52 | 97.75 | 96.3 | 95.05 | 91.71 |
| 14 | 100.00 | 99.3 | 98.03 | 97.71 | 96.99 | 94.9 |
| 15 | 97.18 | 92.68 | 92.35 | 91.91 | 91.93 | 92.59 |
| 16 | 100.00 | 94.95 | 95.37 | 92.61 | 95.11 | 94.08 |
| Std Dev | 1.64 | 2.48 | 2.13 | 2.24 | 1.54 | 1.36 |
| 95% High | 102.59 | 102.03 | 101.01 | 99.93 | 98.88 | 97.13 |
| 95% Low | 99.31 | 97.06 | 96.75 | 95.45 | 95.80 | 94.40 |
| Average | 98.99 | 97.59 | 96.92 | 95.73 | 95.38 | 93.81 |
| Highest = | 100.00 | 99.52 | 98.82 | 97.71 | 96.99 | 95.61 |
| Lowest = | 95.75 | 92.68 | 92.35 | 91.91 | 91.93 | 91.71 |
| Expected | A=100% | B=99% | C=98% | D=-97% | E= 96% | F=95% |

| LAB# | A EXPECTED 5% RESISTANCE | B EXPECTED 4% RESISTNACE | C EXPECTED 3% RESISTANCE | D EXPECTED 2% RESISTANCE | E EXPECTED 1% RESISTANCE | F EXPECTED 0% RESISTANCE |
|---|---|---|---|---|---|---|
| 1 | 4.92 | 4.43 | 2.58 | 2.39 | 0.78 | 0 |
| 3 | 3.50 | 3.06 | 2.31 | 1.31 | 0.75 | 0 |
| 9 | 4.7 | 3.6 | 2.86 | 2.02 | 1.04 | 0 |
| 12 | 4.99 | 3.14 | 2.55 | 1.61 | 0.82 | 0 |
| 14 | 4.92 | 4.43 | 2.58 | 2.39 | 0.78 | 0 |
| 15 | 4.75 | 3.44 | 3.76 | 2.57 | 1.7 | 3.2 |
| 16 | 4.68 | 3.89 | 2.37 | 2.0 | 1.05 | 0 |
| Std Dev | 0.48 | 0.52 | 0.46 | 0.42 | 0.31 | 1.12 |
| 95% High | 7.08 | 6.19 | 5.13 | 4.42 | 3.26 | 3.54 |
| 95% Low | 6.13 | -7.22 | -7.72 | -7.96 | -8.59 | -8.76 |
| Average | 4.64 | 3.71 | 2.72 | 2.04 | 0.99 | 0.46 |
| Highest = | 4.99 | 4.43 | 3.76 | 2.57 | 1.70 | 3.20 |
| Lowest = | 3.50 | 3.06 | 2.31 | 1.31 | 0.75 | 0.00 |
| Expected | G=5% | H=4% | I=3% | J=2% | K=1% | L=0% |

# SUMMARY OF GENETIC TESTING PROGRAMS IN THE STATE OF ILLINOIS FISCAL YEAR 2001

## ROUNDUP SOYBEAN EVALUATIONS & VIOLATIONS FOUND

### 2000-01 Conventional Soybeans

### Roundup Positive

| | Sample | Variety | Lot # | Date Tested | + or - | soak % res. |
|---|---|---|---|---|---|---|
| 1 | DP33 | novartis S23-Z3 | TU0043 | 11/20/00 | P | 0% |
| 2 | JZ92 | asgrow A3244 | 1759EXWAY | 1/3/01 | P | 0% |
| 3 | JR11 | hisoy HS3591 | SCFEP4C01 | 1/5/01 | P | 0% |
| 4 | JR33 | pioneer 93B11 | B3LER11033-00-0306 | 1/19/01 | P | 0% |
| 5 | MD76 | asgrow A2247 | 5643EX141 | 1/24/01 | P | 0% |
| 6 | JB68 | remington NB200 | ROB | 1/24/01 | P | 0% |
| 7 | JZ129 | stine 3398-8 | SL1006 | 1/24/01 | P | 1.85% |
| 8 | DP86 | trisler 2880 | S020-1 | 1/31/01 | P | 0% |
| 9 | JR49 | hisoy HS2361 | SCFS63201 | 1/31/01 | P | .25% |
| 10 | PB129 | hisoyHS3391 | SCFEP2B01 | 2/6/01 | P | 0% |
| 11 | JB86 | LG C3545 | 2730005 | 2/6/01 | P | .50% |
| 12 | JB87 | LG C9244 | 1220006 | 2/6/01 | P | .25% |
| 13 | JB90 | LG C3663N | 2730043 | 2/6/01 | P | .75% |
| 14 | JB92 | LG C9288 | 1110047 | 2/6/01 | P | 0% |
| 15 | LV66 | hisoyHS3071 | SCFS01803 | 2/14/01 | P | 0% |
| 16 | JB106 | stine 2788 | TSI010 | 2/14/01 | P | 1% |
| 17 | JB107 | stine 398-8 | TSI004 | 2/14/01 | P | 0% |
| 18 | DP120 | hisoy HS3391 | SCFEP3B01 | 2/14/01 | P | 0% |
| 19 | PB149 | garst D358 | 4T4U | 3/6/01 | P | .75% |
| 20 | DP173 | novartis S46-44 | TU0232 | 3/6/01 | P | .50% |
| 21 | DP177 | novartis S30-J2 | TU0009 | 3/6/01 | P | 0% |
| 22 | DP178 | novartis S19-90 | TU0363 | 3/6/01 | P | 0% |
| 23 | JB135 | novarits S32-Z3 | TU0292B | 3/6/01 | P | 0% |
| 24 | PB184 | patriot 391 | F0T1 | 3/15/01 | P | 0% |
| 25 | JZ232 | maverick | 2130 | 3/15/01 | P | 0% |
| 26 | JZ243 | diener DB337NGB | 02800A | 3/15/01 | P | .25% |
| 27 | JZ260 | hoblit HB322 | 121 | 3/15/01 | P | 0% |
| 28 | JR78 | asgrow A3244 | 1259EP1D1 | 3/15/01 | P | 0% |
| 29 | JR83 | stine 2700-0 | GRI015 | 3/15/01 | P | .25% |
| 30 | JR84 | stine 2972-2 | GR1021 | 3/15/01 | P | 0% |
| 31 | DP218 | stine 4302-2 | MMI003 | 3/15/01 | P | 0% |
| 32 | DP220 | stine 3950-0 | MMI011 | 3/15/01 | P | 0% |

### 2000-01 Roundup Seed Soak Testing

### Samples Testing Below 98% Resistance

| | Sample | Variety | Lot # | Test Date | % Normal | % Resist. |
|---|---|---|---|---|---|---|
| 1 | JB 13 | stine S3183-4 | WWI004 | 1/4/01 | 69 | 97.18 |
| 2 | JB 13 Retest | stine S3183-4 | WWI004 | 1/12/01 | 81 | 97.29 |
| 3 | PB113 | DG 3463NRR | 1195S | 2/9/01 | 73 | 96.69 |
| 4 | PB113 Retest | DG 3463NRR | 1195S | 2/21/01 | 74 | 100 |
| 5 | JZ322 | stine 3522-4 | SWI171 | 4/24/01 | 87 | 94.57 |
| 6 | JZ 332 Retest | stine 3522-4 | SWI171 | 5/2/01 | 84 | 93.84 |
| 7 | PB242 | hisoy RT4495 | SFRC48624 | 4/24/01 | 62 | 96.87 |
| 8 | PB242 Retest | hisoy RT4495 | SFRC48624 | 5/2/01 | 54 | 99.08 |

Summary of Percentage Abnormals Found in Samples
FY2001 Data

GMO Testing versus Warm Germination Evaluations

| GMO Abnormals Reading vs Warm Germ Abnormals | #Evaluation in FY 2001 | #that Passed the GMO Testing | # that Failed the GMO Testing |
|---|---|---|---|
| GMO abnormals < or = warm germ | 527 (77.74% of all tests) | 526 | 1 |
| GMO abnormals 1-3% > warm germ | 95 (13.95% of all tests) | 94 | 1 |
| GMO abnormals 4-6% > warm germ | 36 (5.28% of all tests) | 36 | 0 |
| GMO abnormals 7-9% > warm germ | 12 (1.76% of all tests) | 12 | 0 |
| GMO abnormals 10% + > warm germ | 11 (1.61% of all tests) | 11 | 0 |
| Subtotal of Category | 682 | 680 | 2 |
| Data of Failed GMOs | 2 | n/a | 2 (See JB13, JZ322) |
| Total of All Testing | 682 Evaluated | 680 Passed (99.71% of all tests) | 2 Failed (0.29% of all tests) |

Data Source: Illinois Testing Program – FY2001 Data

## Additional supporting evidence **

1. Letters of Recommendation Sent to AOSA Board of Directors - FY2001. Prepared by Association of Official Seed Analyst Members. **
2. Statistical Analysis - Region II Referee Summary - FY 2000. Prepared by Kirk M. Remund, Statistician Monsanto, Inc. **
3. AOSA Region II Referee Data Summary - FY 2000. **
4. AOSA Region II Referee Data Summary - FY 1999. **
5. AOSA Region II Referee Data Summary & Survey - FY 1998. **

** **NOTE -** Other documentation supporting the Rule proposed in the FY2001 Referee had already been submitted to the Rules Committee and AOSA Board when the Rule for Seed Soak Testing of Roundup Resistant Products had first been considered. Due to the volume of the documents, they are available to the members of the Rules Committee by review of publications or can be mailed to your attention on request. Please forward mail requests to James N. Lair, Seed Lab Manager, Illinois Department of Agriculture, 801 Sangamon Avenue, Springfield, IL 62794-9281. These pieces of documentation were previously submitted:

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**Date of Proposal**: October 9, 2001 Revised December 11, 2001