MINUTES OF THE 2002 AEIC SPRING MEETING
May 9-10, 2002
Raleigh, North Carolina

The Spring Meeting of AEIC was held at the Doubletree Guest Suites Hotel in Raleigh-Durham, North Carolina on May 9-10, 2002. The meeting was hosted by Aventis CropSciences and Syngenta. There were approximately 35 attendees representing 15 companies and organizations.

Don Kendall (USDA GIPSA) gave an update on the performance verification program for rapid tests of grain. The program initially focused on those tests for Cry9C, however, the program is now open to all proteins in commercialized biotech events. Performance verification of qualitative tests is not an issue but quantitative test verification are presenting a challenge. No one can explain how protein expression in ug/g relates to GM % by weight. There is also an issue with the variability in expression between kernels—in the qualitative tests, pools of seeds/grain can be tested. Currently, the quantitative plate tests are being evaluated as qualitative tests since this is what industry is looking for. The Proficiency Program was initiated in February and was originally intended for PCR tests. However, participants can use any method—DNA or protein—in the program. The program is intended to assess the variability in the capabilities of labs to test for GM traits. There are not many labs that can test for all the traits in the program (T25, Mon810, BT11, GA21). All the results are qualitative and can be accessed via the GIPSA website. Another group of samples for the Proficiency Program will be sent out in May. There are currently 27-28 participant labs, mostly outside the US. Samples will be sent out on a quarterly basis. The current results report does not distinguish which methods were used and it was suggested to Don that results of methods would be very valuable. Another program that is being developed within USDA (not GIPSA) is a Process Verification Program. The purpose is to have USDA certify a process, i.e., have USDA be a third party reviewer of the process. This will be more than just a paper exercise since the process will be sent out for review. This program will probably not be rolled out until late summer. And finally, Don brought up concerns about false positives with the Cry9C lateral flow test strips. He feels that all lateral flow test strips may be affected since the problem seems to be associated with the use of tap water (especially containing high concentrations of sulfates). This can cause a false positive result. The easiest solution is for all test kit manufacturers to specify the use of distilled and/or deionized water for the tests.

Marc Rindal (EPA OPP Microbiology Lab, Ft. Meade) gave a brief overview of his lab's functions and how it will be used for method validation for PIPs (plant-incorporated-protectants; transgenic pesticidal plants). The EPA OPP Microbiology Lab is part of the Biological and Economic Analysis Division and is not involved with the registration process of pesticides. The majority of the lab's time, since last fall, has been spent on testing sporicidal agents for anthrax. The lab has just recently been able to turn its attention to its other initiatives which includes the PIP method validation program. For a PIP to be registered by EPA, the registrant must submit protein and DNA methods for independent lab validation. These methods will also be submitted to the EPA lab to test the method. The lab is currently putting together their process for conducting these validations which will be done GLP (EPA is exempt from conducting GLP but the lab has chosen to follow GLP guidelines). The lab is currently in the process of setting up equipment for PCR and ELISA methods. This fall, AEIC/AACC will conduct a workshop with the lab which will cover topics such as 1) building a DNA/protein detection method validation lab program, 2) equipment

validation, 3) DNA detection methods, 4) protein detection methods, 5) new techniques that are emerging.

Jim Stave (on behalf of AACC) gave a short presentation on the AACC Sample Check Program. The program involves the checking of GM grains—corn and soybeans. There are six rounds of samples sent/year (each round includes eight corn samples and four soybean samples) and subscribers may use any method—DNA or protein. Subscribers send their results (anonymously) to AACC and then they are tabulated. The samples are all ground grain and are sieved for particle uniformity. They are placed in identical glass vials and contamination is guaranteed to be <0.01% of samples at <10% moisture. The first set of samples were sent out in September, 2001. The qualitative results are fairly consistent, however, the quantitative results were not close to the GM amounts AACC claimed were in the samples.

Marcus Lipp (Monsanto) gave an update on the ANSI/ISO initiative. Under WTO, there are the SPS (sanitary/phytosanitary) and TBT (trade barrier treaty) which define health protection for food, plants and animals. The SPS and TBT cannot specify conditions that are unnecessary, arbitrary, scientifically unjustifiable or disguised as trade barriers. Both of these respect and identify CODEX and ISO as relevant for defining methods for detection. Continued involvement of AEIC in ISO will ensure that appropriate methods will be available for settling any trade disputes and will also mean that the US will have a voice in what methods are chosen.

Marc van de Bulcke (Aventis CropSciences) gave a presentation on testing and registration of GMOs in the EU. The EU regulations for GMOs are 90/220 for deliberate release and 97/258 for novel foods. The 90/220 is being replaced by 2002/18 (applicable in October, 2002). For this registrants will have to supply control samples—both negative and positive. The control samples may be any tissue, seed, grain or DNA. They must also supply a DNA detection method for the purposes of monitoring in the environment, along with reference materials for the tests. For 97/258 novel food, corn (2001) 425 proposal covers GM food/feed and the final text will be adopted in 2003. Corn (2001) 182 proposal covers GM traceability and the final text will be adopted at the end of 2003. For food /feed, detection tools can be either DNA- or protein-detection. Samples will be classified as either positive or negative, however, there is discussion as to what a 'negative' control is. The governments realize that a negative control is not possible but they will need to find a way to reconcile it. For GM traceability, the spirit of the proposal is to provide a paper trail. Technical guidance on sampling and testing is anticipated to published by the EU Commission with the JRC as the technical lead. It is not clear where the 'paper trail' will start—at field, at elevator, at port of entry, etc. For adventitious presence of GM events, the last word was the 0.5% would be the tolerance level. The discussion is now turning to EU approved versus unapproved. The European network of GMO labs (ENGL) is headed by the JRC in Ispra, Italy. The JRC in Italy will coordinate method assessments, ring trials and information storage/distribution. The JRC in Belgium will coordinate reference material production (flour, DNA, protein) and global reference material distribution. The JRC has a consortium agreement with assigned governmental labs of the member states. They also have a joint agreement proposal between industry and themselves to focus on the approved EU events. The purpose is to continue to develop standardized methods and materials for worldwide distribution in order to attain global acceptance of methods.

Stacy Charlton (Syngenta) gave a presentation on the drivers for biotech diagnostics in North America. The demand for diagnostics has come from the commodities sector for seed production quality control, closed loop production of plant made pharmaceuticals (PMPs) and to meet the legal requirements in export markets. For commodities, the demand is for methods that are fast, cheap and easy. Rapid immunoassay formats such as lateral flow strips have proved to be the most useful. The major biotech providers have committed to making sure that such tests are commercially available at the time the biotech crop is grown on a commercial basis. For seed purity quality control, the herbicide tolerance bioassay, lateral flow strips and PCR have all been used. For PMPs, the BIO has committed to the appropriate stewardship measures for the commercial production of these products. IP systems used to produce these crops will include

the use of rapid diagnostics. To meet legal requirements in export markets, adventitious presence of biotech seed in conventional seed must be ascertained. The restriction may be zero tolerance for biotech seed or a non-zero threshold. In both cases, the testing regime will target a specific threshold and confidence level.

Matt Lorenc (Applied Biosystems) presented the Taq-man PCR system. The system provides quantitative results. Taq-man differs from conventional PCR through the addition of fluorogenic probe between the primers. This allows greater specificity and a closed system which avoids contamination. The system is amenable for high throughput because it is gel-free. It also utilizes internal positive controls to control false negative responses. The system will also accommodate multiplex assays by the use of two reporter dyes.

Glen Donald (Third Wave Agbio) talked on the Invader technology. The Invader assay reagents are custom made to order and are validated on a panel of customer provided samples. The reagents can be dispensed onto 96 or 384 well plates. The plates are provided with the kits since they contain (in dried down format) the reaction reagents. All assays are set up and run using the same protocol. The system is in an isothermal format. The denatured genomic DNA is added and then the target specific probes to the microtiter plate containing the dried down reaction reagents. The Invader reaction incubation takes 3-4 hours and then the plate is read with a fluorescent reader. The features of the system are 1) homogeneous, 2) direct signal amplification, and 3) scalable. The cost is $1-2 per well.

Ralph McDade (Luminex) talked on their technology for multi-analyte profiling using suspension arrays. The suspension is a fluid drop so it is a 3-D system and consists of 1) tests (protein, DNA), 2) microspheres (100 colors), 3) fluidic technology (accelerates microspheres in front of lasers), and 4) high speed digital processing. The system is called the Luminex xMap. The microspheres are polypropylene and are colored by their taking up a mixture of two hydrophobic fluorophores. Each color of microsphere has an assay built on it. There are no washing steps in the assays therefore, the system is relatively fast. The procedure is: 1) Luminex provides the dyed microspheres, 2) the customer builds the assay on the spheres; 3) the assay spheres are mixed with the sample; 4) the reporter molecule is added; and 5) the microspheres are passed by the lasers. The machine uses the signal from 100 beads with the same assay to determine the outcome. The benefits to the system are speed, fast reaction format, low cost (using less reagents), less waste, reproducible, flexible, simple, can have many internal controls. The system was originally designed for immunoassays, however, it has also been used for ligand receptor assays, enzymatic assays and nucleic acid hybridizations.

The AEIC Business Meeting was held on Friday morning (May 10). There was a relatively low attendance (14 people total). The Secretary's minutes of the Fall Meeting were accepted. Dean Layton gave an updated Treasurer's report. As of May 8, the balance was $31,256.78. The outstanding dues for 2002 are $4250 and for 2001 they are $1450. Expenses have been estimated to be approximately $19,555.22 so the new balance (with the outstanding dues) would be $17,401.56. It was noted by Aventis that Bayer will be the member once their merger is complete. Other companies with outstanding 2002 dues are Cerexagri, FMC, Neogen, Agdia, Beacon Analytical, Biogenetic Systems, EPL Analytical and Genetic ID.

There was discussion on promoting new memberships. The following suggestions were made:

Medaillon Labs (J. Stave will contact)
Nestle (R. Shillito will contact)
Kraft (R. Shillito will contact)
Hershey (D. Layton will contact)
Biodiagnostics (A. DeLisle will contact)
Trilogy (S. Charlton will contact)
Luminex (D. Grothaus will contact)
Third Wave Agbio (J. Stave will contact)
Prodigene (R. Shillito will contact)

Horizon (A. Scott will contact)
Qualicon (D. Grothaus will contact)

AEIC Initiatives

1) PowerPoint Presentation

Marcus Lipp was the only person to send comments back to Dean. It was suggested that a contractor be utilized to finish the organizing and editing. J. Stave indicated that he will contact A. Bridges (Medaillon Labs) who had indicated previously that they would be able to do the editig and organizing for a fee. Dean and Marcus volunteered to work with the contractor to finish the project.

2) DNA paper

Alice DeLisle (DeLisle Biotechnology Consultants Inc.) has been contracted to coordinate the development of the paper. P. Song (Dow AgroSciences), S. Charlton (Syngenta), R. Giroux (Cargill), M. Lipp (Monsanto), R. Shillito (Aventis) and F. Speigelhalter (GeneScan) are assisting Alice. The focus of the paper is PCR validation for both qualitative and quantitative testing. It will encompass reference materials, documentation, parameters, applications, summary/checklist and an overview of US and international guidelines for testing.

3) Protein paper

Cindy Lipton has been contracted to coordinate the development of the paper. D. Grothaus (Pioneer), S. Hefle (UNE), J. Stave (SDI) and D. Layton (Envirologix) are assisting Cindy. The focus of the paper is the testing of processed materials such as feed/food and for allergencity. The paper will include: validation of matrices, Cry9C food testing, a feed testing example. The first outline has been completed but a new timeline for completion needs to be determined by the coordinating committee.

4) Sampling paper

Stacy Charlton (Syngenta) had written a draft paper and sent it to several AEIC members for review last year but has not received comments back. Since sampling has become more of an important issue since StarLink, there is value in reassessing the focus of the draft paper and then decide if the focus needs to be readjusted or just refined. T. Currier (Aventis), M. Lipp (Monsanto), D. Kendall (USDA), L. Friese (USDA) will assist Stacy on the paper.

Fall Meeting 2002

The AEIC Fall Meeting will be held in Minneapolis, MN on October 3-4, 2002. The meeting will be hosted by Medaillon Labs (A. Bridges) and Cargill (R. Giroux). The program for the meeting was discussed and a tentative agenda was put together:

First day:
- Updates (GIPSA, AACC, NIST, JRC, AOAC, etc.)
- Reference Material Talks/Discussion (NIST, JRC)
- AEIC Business Meeting (following lunch)
- Peptide Antibodies (Talk)

Second day:
- Microbial food safety assays (3 talks)

A motion was made and seconded to adjourn the meeting at 11am EDT.

Welcome to USDA/GIPSA's Technical Services Division

Kansas City, Missouri

USDA

USDA/GIPSA Biotechnology Program Update

AEIC: Spring Meeting

Donald C. Kendall
Biotechnology Program Manager
Technical Services Division
Kansas City, MO
May 9, 2002

GIPSA's Biotechnology Program

- **Public Input (ANPR)**
- **Performance Verification Program**
- **Proficiency Program**
- **Process Verification**
- **USDA/GIPSA Web Site**

USDA

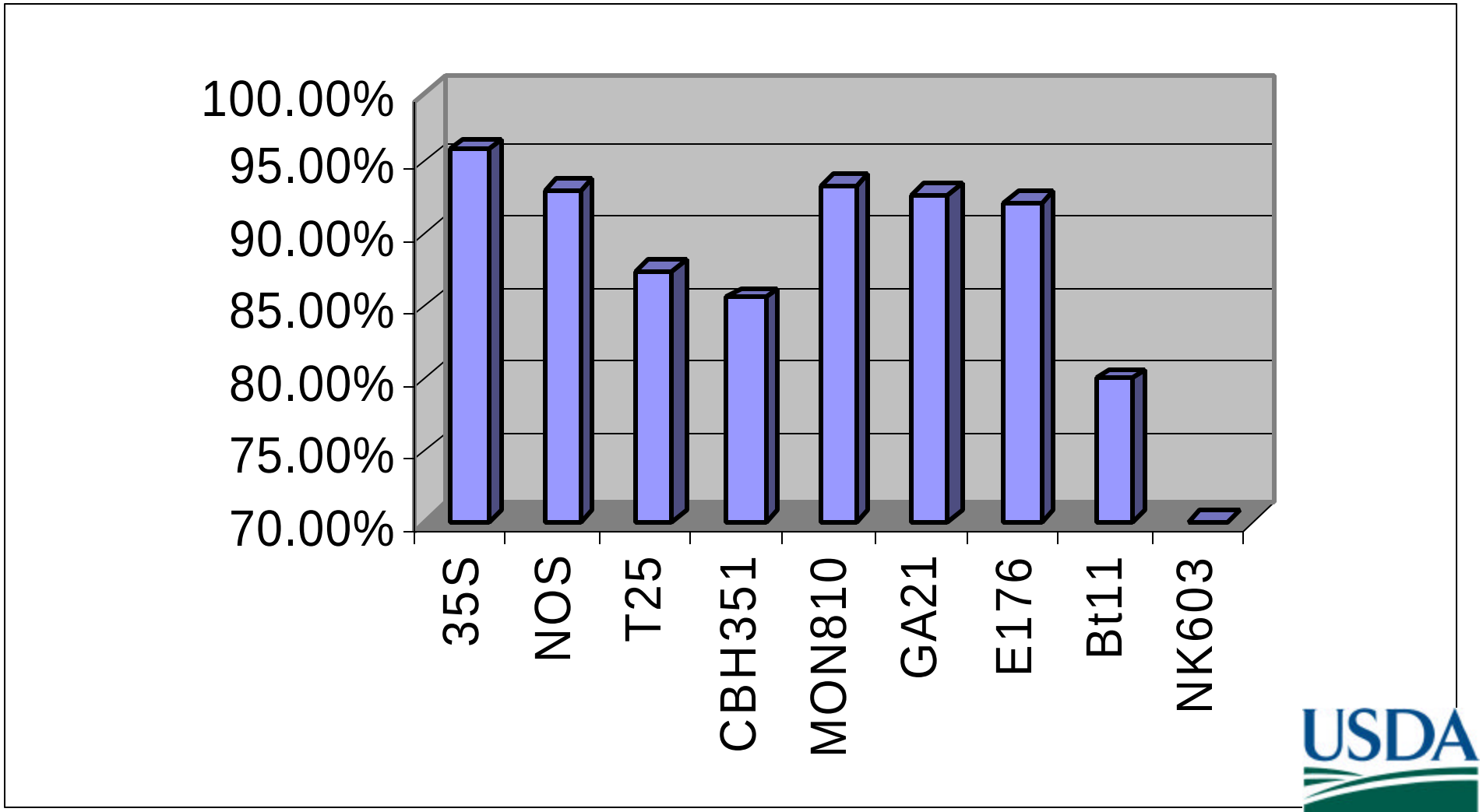
ANPR Message to USDA

- **Develop reliable, practical methods to sample and measure end-use quality attributes**
- **Promote international standardization of methods**
- **Provide assistance in the development of quality assurance processes where product testing is impractical**

Performance Verification Program

- **Expanded to other events**
- **Performance verified tests for corn (NK603) and soybeans (glyphosate tolerant)**
- **Establish fee for program (target 10/2002)**

GIPSA Proficiency Study Results: % Correct



GIPSA Proficiency Program

- **Voluntary participation, anonymity preserved**
- **No fee at this time**
- **Quarterly dissemination of samples**
- **12 corn samples potentially containing various combinations of U.S. approved events (T25, CBH351, GA21, MON810, E176, Bt11, NK603)**
- **Three soybean samples: CP4 EPSPS**

USDA

GIPSA Proficiency Program

- **Program implementation: February 2002**
- **National and International organizations**
- **Qualitative results only**
- **DNA- and Protein-based testing**
- **Summary report to participants, and posted on GIPSA Biotechnology web page**

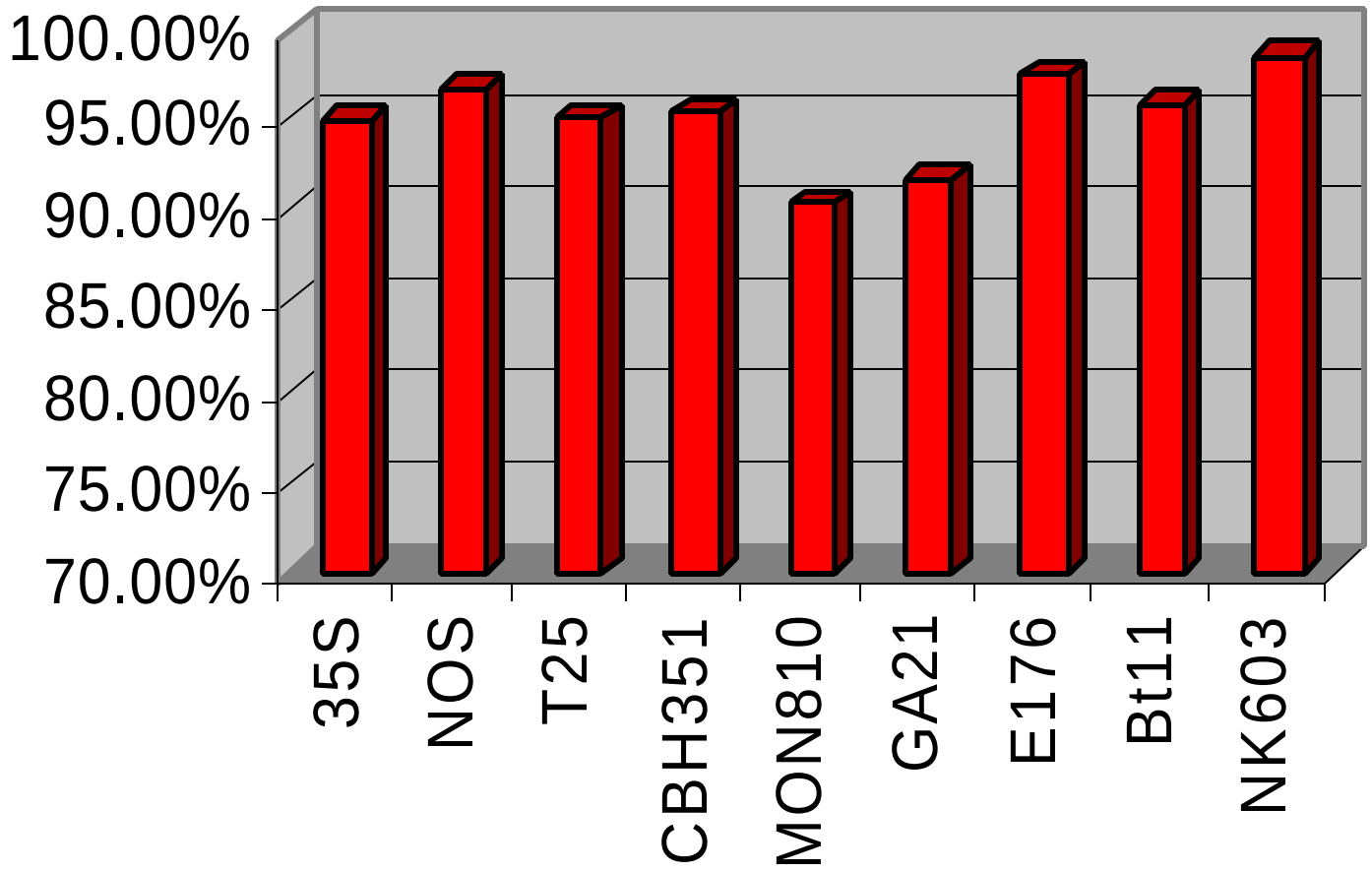
USDA

GIPSA Proficiency Program Results

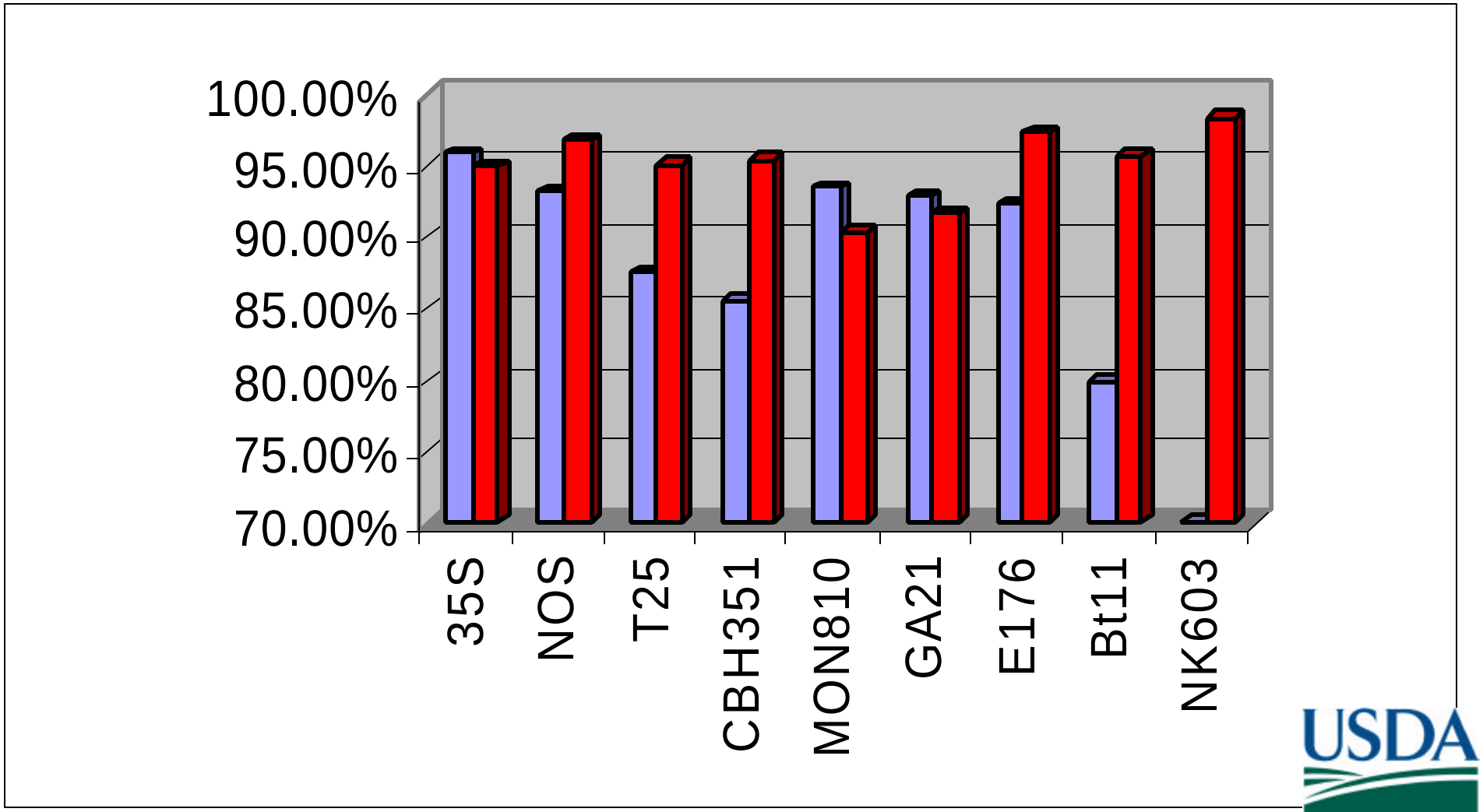
- 22 participants; 18 reported results
- Variability in capability and ability (DNA- and Protein-based testing used)
- 8 participants reported results for all events
- 10 participants were 100 %
- Next sample distribution: May 2002

USDA

GIPSA Proficiency Program Results: % Correct



Proficiency Study and Program: Study in Blue/Program in Red



Process Verification

- Response to ANPR
- Final product testing is expensive
- Testing cannot answer all questions
- Quality processes are effective
- Meet customer needs

USDA

Process Verification: GIPSA's Plan

- Export and domestic sales
- Processes from seed to consumer product
- Verify the process, not the product
- Apply ISO 9001 principles
- Use certified auditors

USDA

Process Verification: GIPSA's Plan

- **Authority is the Agricultural Marketing Act**
- **Fee supported**
- **Applicant name and process posted on GIPSA web site**
- **Applicant can claim USDA/GIPSA process verified for product**

Cry9C Lateral Flow Strip Test

- False positives with well water
- GIPSA reproduced with Ferrous sulfate
- Prepared solutions at various concentrations
- False positives with water solutions (SDI and ENV)
- False positive with corn extract at high concentrations (NEO)

Cry9C Lateral Flow Strip Test

Iron Conc.	Env	Neo	SDI
0.3 mg/l (H_20)	Neg	Neg	Neg
3.0 mg/l (H_20)	Neg	Neg	Neg
50 mg/l (H_20)	Pos	Neg	Pos
500 mg/l (H_20)	NCL*	Neg	Pos
0.3 mg/l (corn)	Neg	Neg	Neg
3.0 mg/l (corn)	Neg	Neg	Neg
50 mg/l (corn)	Neg	Neg	Neg
500 mg/l (corn)	NCL	Pos	Neg

*NCL means No Control Line Developed

USDA

USDA/GIPSA Biotechnology Web Site

- Programs
- Documents related to export
- Sampling information and guidance
- ANPR
- Educational information
- Other USDA biotechnology links

Web Site: www.usda.gov/gipsa

- GIPSA Biotechnology Backgrounder
- Rapid Test Performance Evaluation Program EXPANDED
- Proficiency Program
  - Proficiency Program Results: February Distribution of Samples NEW!
  - DNA-Based Laboratory Proficiency Study Final Report
- GIPSA StarLink™ (Cry9C) Testing Program
- Export Documents
- Sampling for Biotechnology-Derived Grains and Oilseeds
- **Advance Notice of Proposed Rulemaking:** Request for public comments on how USDA can best facilitate the marketing of grains, oilseeds, fruits, vegetables, and nuts in today's evolving marketplace.
- Publications
- USDA Biotechnology Web Site

USDA

USDA/GIPSA
Biotechnology Program