AEIC Fall Meeting 2011 Minutes
October 5-6, 2011
Hosted by Pioneer Hi-Bred, a DuPont Company
Des Moines, Iowa

P.L. Hunst, AEIC Secretary

The 2011 AEIC Fall Meeting was hosted by Pioneer Hi-Bred, a DuPont Company, at their headquarters in Des Moines, Iowa on October 5-6. The AEIC group was welcomed by Doane Chilcoat of the Applied Technology Systems Group at Pioneer who gave a brief overview of the company. Pioneer Hi-Bred was founded in 1926 by Henry Wallace as a corn company. In 1973, soybean was added. In 1999, Pioneer merged with DuPont. Pioneer supplied one-third of the sales for DuPont. The challenge in agriculture is to continuously improve productivity since crop production needs to increase by 70% by the year 2050 to meet the needs of the world population. The goals of Pioneer R&D are to a) increase and protect yield through germplasm; b) increase end use value, i.e., the value of the grain; and c) improved input efficiency through the introduction of insect-resistant, herbicide-tolerant plants. Pioneer's approach is to have superior germplasm and use conventional breeding, molecular breeding and transgenics to improve crop productivity. Pioneer today has more than 3000 researchers in 110 research sites in 25 countries. The company has increased its number of R&D employees by 38% over the last three years. In 2010, the company had 1000 technology agreements globally.

AEIC BUSINESS MEETING

2011 AEIC Spring Meeting Secretary's Minutes: A motion was made, seconded and received a positive member vote to approve the minutes as they are posted on the AEIC website.

Treasurer Report (D. Layton):

Item	**Planned**	**Actual**
Beginning Balance (1/1/2011)	$23563.00	$23563.00
2010 Membership Dues	8000.00	5250.00
Interest		17.00
Total Revenue	8000.00	5267.00
Expenditures		
Paper	8000.00	1000.00
Wire transfer		
DE Franchise	25.00	25.00
ANSI/ISO	2900.00	222.00
Board Mtg	100.00	
Spring Mtg	2500.00	
Website	500.00	
Bank Service Charge		
Fall Mtg	2500.00	

Graphic design		
Reprints (brochure)	300.00	
Subscriptions	100.00	
Miscellaneous	100.00	
Total Expenditures	17025.00	1364.00
Projected Balance	14528.00	27476.00
CD	11548.00	11581.00
CD Interest	120.00	
Total Assets	26206.00	39057.00

Due to the low interest rates, the Treasurer elected not to renew the CD and funds were combined into an interest-bearing checking account.

A motion was made, seconded and received a positive member vote to approve the Treasurer's report.

AEIC Membership Update (D. Layton): AEIC membership is as follows:

MEMBERSHIP	Number	Unpaid	Unpaid Amt
Large Companies	12	5	$2500.00
Small Companies	12	4	1000.00
Associate Members	3	1	50.00
Individual Members	3	1	50.00
Total	30	11	3600.00

AEIC Website Update (P. Hunst): The webmaster has done a very good job in meeting all requests to post information. The membership requested that AEIC meeting speakers' slides be posted on the website. It was discussed that if the speakers give their permission, the slides could be posted on the password-protected "Members Only" section of the website. There was also a request to post AEIC meeting registrant lists in the same area of the website. It was also noted that the AEIC brochure file should be accessible on the website.

2012 AEIC Spring Meeting Planning: After much discussion, Bayer CropScience agreed to host the Spring Meeting in Research Triangle Park, North Carolina. This will be the 20th anniversary of the AEIC. Suggested dates were April 18-19 or possibly late March. USDA GIPSA would like to host the 2012 Fall Meeting or the 2013 Spring Meeting. Eurofins GeneScan also offered to host the 2013 Spring Meeting in New Orleans. AOCS also offered to host the 2012 Fall Meeting in Champaign, Illinois.

Suggested topics for the 2012 Spring Meeting are:

- Crop focus: vegetables
- Mycotoxins: comparison of prevalence on Bt transgenic vs non-Bt crops
- Impact of biotech: sustainability
- Regulatory Framework: How does the US system work; how is the data used and what are the bottlenecks?
- Analytical challenges: lectins, endogenous allergens
- History of AEIC

AEIC Vice President Nomination/Election : The AEIC election for the office of Vice President will be held online during the month of November. There is one vote allotted per AEIC member. Nominations will be open until Oct. 31. The following floor nominations were made and accepted:

Yelena Dudin (Monsanto)
Brian Skoczenski (Acadia Labs)

AEIC Goals and Activities (L. Privalle): An update was given on each paper that AEIC members are currently working on.

1) Protein vs DNA methods for biotech (C. Alarcon): Members working on this are D. Layton, C. Alarcon, C. Singsit, T. McFadd, B. Kaufman, R. Shillito, G. Shan. An outline of the paper has been put together and several sections have been drafted.

2) Testing used for commercialization of a biotech event (L. Privalle): Members working on this are L. Privalle, G. Clapper, C. Zhong, J. Cheng and P. Hunst. An outside editor (V. Pantella) has been employed to edit the rough draft put together by the group. The editing is progressing well. The suggested journal for this publication is "GM Crops".

3) Quantification by sub-sampling (B. Kaufman): There is an outline of the sections of the this paper and the goal is to finish the paper by the end of 2011. The paper is meant to address the suitability of semi-quantitative methods.

Member suggestions for new goals/activities:

R. Jenkins suggested that a paper be built around F. Spiegelhalter's talk (given at the AEIC Meeting in Nebraska) about the difficulty in determining true GM content analytically. To bolster the information given in the talk, it was suggested that numbers are generated to go along with the theory. A committee was formed to investigate whether AEIC member labs are interested in pursuing this. The committee members are R. Jenkins, T. Scholdberg, K. Brix-Davis, F. Spiegelhalter, A. Azmy.

L. Privalle also suggested that maybe a workshop would be appropriate to discuss the information around determining true GM content analytically. It was discussed that it would be better to wait until the committee has decided about the study and generated data before hosting a workshop.

UPDATES

Codex/ISO Sampling Activities (R. Shillito): The Codex Committee on Methods/Sampling deals with the principles of sampling/testing in international trade. So far, the committee has not moved the principles to biotech products. ISO Technical Committee 34 SC 16 (Biomarkers) is working on standards for PCR and protein methods. The scope includes food authentication. The standards are for isolation of DNA, qualitative methods, varietal identification, pathogen identification, etc. If AEIC members had not been involved, the standard for testing would have been to test 100 samples (5 groups of 20). Another difficulty is dealing with methods that claim to be event-specific but in actuality identify several events. The next meeting will be in Beltsville, MD on Oct. 25. TC 34 held an international workshop several years ago. A second meeting is scheduled for Beijing, China in late July-early August, 2012.

2012 Global GMO Conference (R. Jenkins): There was a lot of enthusiasm to hold this conference again in 2012, however, site negotiations ran into difficulties and the conference is now indefinitely canceled. A suggestion was made that maybe a South American venue should be suggested instead of an EU venue.

GIPSA Programs Update (P. Wacker): The laboratory proficiency testing program sends out soybean and corn samples semi-annually. The program is voluntary with no associated fee. This year, six corn and four soybean samples are being distributed. The corn events included are T25, CBH351, MON 810, GA21, E176, Bt11, TC1507, MON 863, DAS-59122-7, MIR604, Event 3272, MON 88017 and MON 89034. The soybean events included are Roundup Ready 1, Roundup Ready 2 and LL 2704-12. There are 165 member labs (28 in the US and 137 outside the US). In 2011, the performance was generally good among participants (68 requested samples—65 submitted results). Twenty-four respondents used qualitative DNA tests, seven respondents used quantitative DNA tests and thirty respondents supplied both qualitative and quantitative results. Qualitative results revealed greater than 95% correct on 18 of the 19 events. Quantitative results gave 3 false negatives and 6 false positives. The false positive results for T25 and NK603 may be the result of PAT being present in events Bt11, Herculex I and Herculex RW and EPSPS being present in MON 88017 and GA21. Not all the labs used the same protocol, thus it may be expedient to use event specific analysis. For the next round, events CBH351 and Event 176 will be phased out. More information on the testing program may be found at www.gipsa.usda.gov/fgis/insp_weight/proficprog.html.

Following the GIPSA Programs Update, there were no further updates. A motion was made, seconded and voted positive by the members present to adjourn the business meeting.

INVITED TALKS

Genetically-Engineered Animals (M.B. Wheeler, Univ. of Illinois): The transgenic alteration of animals history began in 1971 with the development of transgenic lab animals. In 1980, pronuclear injection was developed on mice and in 1985 was used in rabbits, sheep and pigs. The technology was first used on cattle in 1991. A transgenic animal is defined as an animal which has a stable incorporation of the gene of interest into the germ-line and is able to pass the gene of interest to its offspring. The goals for producing transgenic animals is to over-express certain genes, modulate gene expression, knock-out gene expression, turn on gene expression and alteration of the primary structure of an endogenous gene product. The methods used to accomplish these goals include a) sperm-mediated DNA transfer, b) recombinant retroviruses, c) pronuclear injection, c) embryonic stem cells, d) germ cell transfer and e) nuclear transfer cloning. Pronuclear injection has been an efficient method in mice but is detrimental to embryos in pigs and cattle. Several applications of transgenic animals were discussed. Modification of milk would be advantageous to increase casein components for cheese, produce pharmaceutical proteins or industrial enzymes, reduce the fat in milk, increase the protein, fat, lactose content in meat cattle to increase their offspring weaning weight (improved growth, survival). Spider silk proteins can be produced in cow milk. The spider silk is 50 times stronger than Kevlar and would be used in the making of auto tires, airbags, surgical sutures, etc. A cow gene has been transferred to pigs which allow the increase in litter size due to milk production.

Another application of transgenic technology in animals is the modification of animal growth via manipulation of known growth factors, growth activator receptors and growth modulators. Alternative loci that alter growth patterns have also been investigated such as the natural "knockout" of the myostatin gene in Belgian blue bulls (increases the muscle mass). By investigating these loci, new insights in the action and control of the genes involved in growth may be obtained. It would also be beneficial to gain insight into resistance of animals to parasitic organisms, viruses and bacteria in order to modify it to be beneficial to more animal species.

Another objective with transgenic animals is to increase reproductive performance. Increasing prolificacy or fecundity involves understanding these multi-gene traits. There is also quite a bit of interest in the production of animal cells, tissues or organs that contain human antigens or proteins for xenografts. This would allow production of additional materials that my be useful for biomedical purposes.

Science-based regulation of genetically-engineered animals and products ensures the safety of products and bolsters public confidence in the technology. FDA announced final regulatory guidance for transgenic animals in January, 2009. Comprehensive coordination for regulations that bridge the divide between food and biomedical uses was required. However, no further progress has been made since 2009 for regulations and thus, transgenic animal technology in the US is now disappearing. Students are not being trained in the US and most research in the area is now conducted outside of the US. It is too expensive to conduct the research in the US due to the regulatory restraints such as incineration of animals. In China, however, the technology is popular and is being used for production purposes. Currently, there are herds of transgenic animals ready to deploy. In the US, the New Animal Drug approach is being developed. This is a mandatory process that provides "approval" and affords a consolidated regulatory review and oversight for animal health, human health and the environment by affording an efficient process with regard to the use of agency expertise and other resources. Process requires answering a lot of questions such as conducting a study to show that genes do not "jump" through the air from one animal to another and another study to show that a female animal bred to a transgenic male animal is not "tainted" for life, i.e., that is the female is then bred to a non-transgenic male, the resulting offspring will not have the transgenic trait of the previous male.

In summary, transgenic animals can deliver substantial improvements in terms of cost, safety, availability of drugs, treatments, etc. The technology holds promise to improve nutritional attributes of animal food products and improve human health. BIO has published a document entitled "GE Animals and Public Health" which may be accessed at www.bio.org/node/2522.

AquAdvantage Salmon (J. Buchanan, AquaBounty Technologies): AquaBounty Technologies provides molecular solutions for problems in the global aquaculture industry. The lead development product is AquAdvantage salmon. The company also has projects in trout, tilapia and shrimp. The projects target performance, fertility,

disease prevention and disease treatment. Currently, there is an increase need for seafood since the oceans cannot keep up with human demand. Domestication of fish is needed. Fish are an efficient source of animal protein and their culture must double by the year 2030 in order to keep up with the human population demand.

The salmon industry is multi-billion industry. The US imports greater than 97% of the Atlantic salmon consumed. Currently salmon are cultured via net pens in the ocean. There is negative publicity concerning escapes, disease transfer to wild salmon, environmental impact of the net pens, feed use and social impacts. The goal of the industry is to move production to land and out of the oceans. The AquAdvantage salmon have been engineered for rapid growth by the insertion of a growth gene from Chinook salmon which helps to improve the productivity and economics for commercial aquaculture.

The AquAdvantage salmon were developed by injected 10,000 salmon eggs with growth gene. The resulting salmon were screened and the positives identified via their rapid growth compared to those that did not have the growth gene. Those with the growth gene reach market size in half the time of conventional Atlantic salmon and improve feed utilization by 10%. The growth phenotype was found to be heritable. A PCR method is available for positive detection of the GE salmon.

The AquAdvantage salmon are regulated under the Food, Feed, Drugs and Cosmetics Act (FFDCA) and are expected to be the first transgenic food animal approved by FDA. An exhaustive amount of data has been generated for this approval process. The salmon will have a label since they are considered a "drug". FDA has issued an assessment that the AquAdvantage salmon are as safe as other Atlantic salmon. All parameters measured showed no difference in comparison to conventional Atlantic salmon. Thus, AquAdvantage salmon are safe and sustainable, increase productivity and address the increasing demand for seafood. However, there has been opposition from non-governmental organizations (NGOs) directed to FDA and Congress. The NGOs are vocalizing that the FDA regulatory process is insufficient and have generated a lot of negative press stories. Due to all of this, a bill has been introduced into the House of Representatives to bar genetically-engineered salmon. AquaBounty has received support from CAST and letters have been sent to Congress from various agriculture groups in support of approving the salmon by allowing the regulatory process to proceed to a decision without political intervention. The FDA process is a reasonable science-based process, however, political intervention could stop all future agriculture biotech technologies.

Homogeneous time-resolved fluorescence technology: Theory and application (E. N'garwate, CISBIO): CISBIO has headquarters in Marcoule, France and a site in Bedford, MA. The company supplies assay kits and reagents based on immunofluorescence. The HTRF technology uses FRET (fluorescence resonance energy transfer) between compatible donor and acceptor and their proximity. The technology works by the excitation of the donor molecule and energy transfer to the acceptor molecule. Long emission half-lives are used in comparison to traditional flurophores.

This allows time resolution, eliminates autofluorescence, removes compound background. No washing steps are needed in the assay. In the mix-read assay, cells are added, then the compounds followed by the antibody with the donor molecule and then the antibody with the acceptor molecule. Both antibodies must bind to see fluorescence. The free and bound species remain in the same well. The signals are specific to the target of interest. The assay is amenable to high throughput plate assay format.

In the competitive format assay, the antigen has the acceptor molecule attached and the antibody has the donor molecule. The fluorescence signal decreases with the amount of analyte detected. In the sandwich format, the antigen is sandwiched between two antibodies—one with the donor molecule and one with the acceptor molecule.

There are endless applications for HTRF including protein-protein interaction, immunoassays, GPCR investigation, enzyme activity determination. GPCR binding and functional assays use the tag-lite platform. This platform utilizes a receptor-ligand binding without radioactivity. The binding can be done on live cells. The cAMP assay is a competition assay which works with both Gs and Gi GBPCRs. The signal is table for 7 days and works with with suspension and adherent cells. The inositol-1-phosphate is an accumulation assay to screen all classes of compounds such as agonists, antagonists, allosteric modulators, inverse agonists, etc. The assay correlates with FLIPR (calcium-flux assay) data. Biomarker assays are alternatives to ELISA. The HTRF requires no washing so the assay is fast. Sandwich and immunocompetitive formats available.

CISBIO also offers custom services and custom labeling of a broad range of molecules such as antibodies, peptides, oligos, etc. They also offer multiple labeling options and have consistent batch-to-batch quality. Custom assay development is also available.

Homogeneous non-radiometric immunoassays for improved sensitivity, dynamic range and robustness (P. Roby/S. Anderson, Perkin-Elmer): Perkin-Elmer offers ELISA conversions using their Alpha technologies. This is a bead-based proximity assay, i.e., donor bead and receptor bead are brought into proximity through biomolecular interactions. The donors are excited with a laser (680nm) resulting in the release of singlet oxygen. The singlet oxygen initiates an amplified fluorescence signal cascade in the acceptor bead producing an emission of light between 520 and 620nm. The donor and acceptor beads must be within 200nm of each other.

The comparison of Alpha with FRET is as follows:

Alpha	FRET
Distance: 200nm	Distance: 7nm
Excitation: 680nm	Excitation: 340nm
Emission: 520-620nm	Emission: 665nm

The AlphaScreen assay uses TAR acceptor beads (thioxene, anthacene, rubrene) and is useful for complex matrices. The AlphaLISA uses Europium acceptor beads. Bead

conjugation is easy and clean, reproducible, is done under mild conditions (physiological pH) and there is no need for column purification or dialysis.

The basic protocol for assays is: a) add analyte, b) add biotinylated antibody and antibody acceptor beads, incubate; c) add streptavidin donor beads, incubate; d) excite donor bead; e) donor beads release singlet oxygen. The sandwich format sandwiches the analyte between the donor and acceptor beads. In the competition assay, the analyte binds to the donor bead. A decrease in signal means more analyte is present. The AlphaLISA can be used in biochemical, cells and complex matrices (blood, tissue homogenates). AlphaLISA uses smaller sample volumes so users can move from 96-well formats to 1536-well formats.

The AlphaScreen SureFire is a western blot replacement technology. It affords more reproducibility with less steps and a detection of 0.25 ug protein/well. Molecular weight cannot be determined, however.

Perkin-Elmer has many "toolbox beads" available for making assays as well as optimization kits and kits for biomarker targets.

In conclusion, Alpha is a time proven and widely adopted technology. It has high sensitivity, versatile and affords straightforward immunoassay development. It is fully automatable and cost effective.

Multiplexed protein quantification by LC-MS/MS: Case studies in transgene protein analysis (T. Hu, Pioneer Hi-Bred): For transgenic events, RT-PCR has been traditionally relied upon for expression of transgenes although it is not always indicative of protein expression. Western analysis and ELISA are also widely used, however, high quality antibodies are not always available, especially in early event selection. It takes many months to product antibodies and for some proteins, such as membrane-bound proteins, it may be difficult to produce antibodies. This has brought forward the need for a new technology with a short turnaround time and is efficient. Mass spectroscopy (MS) has been explored to fulfill this need. Target proteins are digested with trypsin. One or more signature peptides of the target proteins can then be quantified using LC-MS/MS. LC-MS/MS employs mass analyzers in tandem. The LC-MS/MS is extremely specific and sensitive and has been used widely in the pharmaceutical industry for PK/PD studies for 30+ years to quantify small analytes, including peptides. For peptides, they are separated via LC (retention time) and then via MS for parent mass separation (size) and daughter mass separation (ions). Pioneer has used LC-MS/MS to look at 3 proteins in corn leaf – GAT, PAT zmHRA. ELISAs for each of the proteins have been developed, however, two different extraction buffers are used. One of the extraction buffers contains 1% BSA which makes it difficult to normalize samples by total protein. For LC-MS/MS, the extracted proteins are normalized (via total protein) and then digested with trypsin. The trypsinized proteins are injected into the HPLC column. Recombinant proteins are spiked into null matrix to make standard curves. Internal standards (isotope-labeled proteins) are used for normalization only. Microwave-assisted digestion was used to speed up the trypsin step, i.e., trypsin digestion could be completed in 30 min.

Approximately 200 samples/day/instrument can be analyzed. For the GAT protein, 4 peptides were chosen as the signature peptides but only 3 of these were used to quantify the protein content. Quantification results were comparable to results obtained with ELISA. The summary of this work has been published in J. Agric. & Food Chemistry. In summary, quantification of low abundant proteins is feasible and the method is highly selective. The selection and optimization of peptides is critical for both sensitivity and selectivity of the method. Cross validation with multiple peptides increases confidence. Method development can be done in days/weeks instead of months.

GIPSA rapid test kit evaluation program update (G. Giese, USDA GIPSA): The rapid test kit evaluation program was shutdown in July, 2009. Temporary certificates were issued to 13 test kits during 2009. Three test kits were submitted in 2010. Updated criteria documents were put into place in September, 2010 and a program manager was hired in December, 2010. There was a notice of restart published in late February, 2011. Currently 31 kits have been evaluated and processed. These included:

10 quantitative mycotoxin kits
19 qualitative mycotoxin kits
1 qualitative biotech kit
1 falling number kit

Two certificates of certification (COC) have been issued for quantitative kits and 17 certificates of performance (COP) have been issued for qualitative kits. The backlog of kits was officially finished on September 1, 2011.

There have been several program changes. For quantitative kits, there is no LOD requirement. DON kits will include naturally contaminated corn and the %RSD for aflatoxin, DON and fumonisin have been tightened. For qualitative kits, temperature sensitivity data is required for all kits. Biotech kits can be protein-specific. The time requirement for kits failing the evaluation has been moved from 6 months to 3 months. Kit manufacturers are now responsible for obtaining reference material from the life science companies.

In the future, no exceptions for the deadline for extensions of all expired certificates will be granted. Updates to the criteria documents will include wording on the number of tests and concentration. The USDA website will be more user-friendly and will allow test kit submissions online. The test kit monitoring program will be assured to function during the lifetime of the certification. A check sample proficiency program is also being proposed for sites that use the kits to insure proper training.

More information may be obtained from the GIPSA website:
www.gipsa.usda.gov/GIPSA/webapp?area=home&subject=gropi&topic=iws-rtk
or by contacting Greg at Gregory.J.Giese@usda.gov.

Genomics-assisted breeding for sunflower hybrid development (P. Venkatramana, BioDiagnostics): A sunflower consortium was initiated approximately 1 year ago to do high throughput genotyping, R&D and NGS (SNP discovery). The consortium-based

projects are cost effective, efficient, rapidly attain goals and are collaborative. SNP markers are effective tools for breeding since single nucleotide changes are detected. These SNPs are co-dominant, highly abundant and transferable across populations. There are 3 phases to the SNP project: a) Phase I: SNP identification; b) Phase II: mapping; and c) Phase III: alleles, QTL mapping. The SNP genotyping platforms are BeadXpress, Beadlab/Bead Station and Genome Analyses (all by Illumina). The bead technology uses a universal array bead. The veraCode bead is cylindrical, glass micro-bead which has embedded holographic diffractive element codes and is used for broad multiplexing range.

Sunflower has a complex genome of about 3.5 Gb. The criteria for choosing a panel of lines was based on the identification for the sequence should not be redundant with earlier publically available sequences. Restriction action DNA (RAD) cuts at different sites in the sequence. The contiguous assembly was validated. Through this process approximately 233,000 SNPs were identified. For the Infinium assay, 10,000 SNPs based on single bead assays. The manufactured SNP chip for sunflower has 8723 SNPs. The chip was used to genotype 2 panels – diversity and mapping. A characteristic pattern of genetic diversity was found. Mapping of trait specific markers is being done using association mapping and 2-parent mapping. A SSR-SNP-based genetic map has been developed.

In the future, a standard panel of 384 markers for routine usage across a range of breeding projects will be identified. Association mapping will be used to identify markers linked to various traits of interest and validate/implement them in MAS programs. Decisions can be made on genome wide marker data to identify individuals. Marker-assisted breeding also is advantageous for complex traits and reduces advance stage testing. In the breeding population, there is good potential to produce exceptional lines since the data collected is from the existing lines. Plants most likely to have phenotype of interest are picked via selection of plants with best marker profile.

Overview of ILSI IFBiC workshops (D. Grothaus, Monsanto): ILSI is a neutral forum for discussing/advancing scientific issues that impact public health. Academic, industry and government scientists participate on the ILSI taskforces to address key scientific issues with good science. The goal is always to develop and publish papers on the issues. ILSI IFBiC focuses on science, partnerships, outreach, harmonization, building capacity to ensure scientific information regarding food/feed safety of biotech crops. ILSI has conducted sampling/detection workshops beginning in 2002 in Brazil and Argentina. The workshops have been presented globally since then and some key geographical areas are being re-visited such as India, Brazil and Japan due to personnel changes within those government agencies. The goals of the workshops are to a) provide an understanding of scientific principles of sampling and analysis; b) provide knowledge of what sampling can do and does not do; c) share the latest information and discuss how to bridge scientific and communication gaps; d) help the development of a road map for harmonized, science-based approach to testing. More information on the workshops can be found at www.ilsi.org.

ISTA's biotech trait detection workshops: Past/future (B. Kaufman, Pioneer Hi-Bred): ISTA was founded in 1924 as an independent organization free from economic interest and political influence. The goal of the organization was to develop, adopt and publish standard procedures for sampling/testing of seeds; promote the uniform application of procedures for evaluation of seeds; and promote research in all areas of seed science and technology. ISTA has 201 member labs from 79 countries. Approximately 120 of ISTA member labs are accredited by ISTA. ISTA operates via technical committees. The GMO committee was established as a taskforce in 2001 and in 2010 became a committee.

The GMO Committee establishes ISTA rules chapter for GMO detection, oversees GMO testing lab accreditations, organizes proficiency tests on GMO tesing, promotes exchange of information by offering training programs, workshops and online information portal. Industry representatives are on the committee, however, in the past it was closed to industry representation. ISTA uses the proficiency-based approach, i.e., does not matter how you do it—as long as you are getting the correct results. This approach is inclusive to allow participation of any lab regardless of the lab's technological level or budget. Workshops are done with the assistance of the Statistics Committee. The workshops were started in 2003 in Thailand and have been held globally in countries such as Egypt, Slovenia, Turkey, Jamaica, China, etc. There have been 12 total workshops with a total of 250 participants from over 30 countries. ISTA has collaborated with many entities on these workshops such as FAO, Iowa State University, Institute of Seed/Seedlings Croatia, etc. The workshop focuses on PCR methods giving the participants both theory and hands-on lab experience. A typical workshop program will include testing plans (qualitative/quantitative; use of Seedcalc), PCR introduction and lab, assay and process validation, detection of stacked traits and sharing of participants' experiences. Participants receive a certificate of participation and a CD containing the proceedings of the workshop. In the future, the plans are to diversify the workshops to include basic GMO testing, statistical aspects of GMO testing and advanced GMO testing (uncertainty calculations, stacked traits on Seedcalc). The next workshop is scheduled for Shanghai in December, 2011. More information can be obtained on ISTA from their website (www.seedtest.org).

DuPont Qualicon helps protect the food supply (D. DeMarco, DuPont): For DuPont, 34% of its revenue comes from the Agriculture-Food Division. The trends for food safety include increasing regulations, tests with speed/simplicity, increasing public cases of recalls and brand damage, consumer and media pressure, evolution of human diets (convenience, fresh, 'natural'), high acceptance of molecular technology and consolidation of industrial companies which brings a need for consistency. Food safety is the #1 application for Qualicon. It is estimated that there are 76 million cases of food poisoning in the U.S. every year. There is a push to move to rapid food testing to avoid product/brand recall and still be cost effective. The ideal is to produce automated results that require no interpretation.

The business of DuPont's Nutrition and Agriculture is 16 years old. In 1994, PCR was introduced to food testing. In 2000, the automated BAX system to amplify/detect in one system was introduced and in 2005 the BAX system Q7 (RT-PCR, speciation) was

introduced. Since 2009, the company has continued innovation looking at new targets and new technologies. The key market segments are poultry, beef, produce, ready-to-eat (deli meats/cheeses) and regulatory bodies. The company has a global scope with its headquarters in Wilmington, Delaware.

The BAX system tells whether a pathogen is present. The RiboPrinter identifies it and assists in answering where, when and where the pathogen is coming from. Tests utilized include end point PCR, RT-PCR (taq-man), RT-PCR (Scorpions probes). PCR chemistries include SYBR green for the end point assays and RT for taq-man probes and Scorpion probes.

Qualicon is a development-focuses organization and does not do much research itself. Rather, Qualicon has collaborators for research such as DuPont Corporate, government, universities and other companies. Some of these collaborations include a USDA CRADA for the STEC suite and a FDA CRADA for shigella, China CDC/JSCIQ for shigella and campylobacter, Fed. University of Sao Paulo for RiboPrinter mycobacterium tuberculosis and University of Zurich for cronobacter. In the future, Qualicon wants to convert legacy assays to RT PCR, work on automated sample preparation/concentration system for foods and develop a high level multiplex technology for advanced detection.

The STEC is a diverse group of pathogens that cause disease in humans/animals. Illnesses have been on the rise globally (*E.coli* O157). There have been 30 outbreaks in the U.S. in the last 25 years due to this group of pathogens in milk, punch, lettuce, etc. USDA considers O157 as an adulterant in ground beef but does not consider the other STEC pathogens as adulterants. In 2009, the U.S. Senate moved to declare all STEC serotypes as adulterants. The bill has been referred to Committee. In 2011, there was the *E. coli* O104 outbreak in Germany which put increased pressure on USDA to move on their regulations. Meat producers are not happy, however, there was a 2010 outbreak in ground beef which caused a recall by Cargill. On Sept. 13, 2011, the public comment was announced for the proposed regulations. If passed, testing would begin March 5, 2012 in meat plants. In 2010, Qualicon entered into a CRADA with USDA to develop enrichment/confirmation using PCR. The difficulty in selectively culturing organisms is still unsolved. The design requirements for the PCR include a) a run of 1 hour, b) run on current Q7 platform, c) 3 panel assays, d) simple sample preparation (crude lysis with heat). Enrichment protocols are still evolving. Organisms have differential susceptibilities to antibiotics. Sensitivity has to be one organism/ large batch. Organisms are regulated at the 0% tolerance level.

Authenticity testing in food products (L. Reimann, Eurofins): Food adulteration occurs for economical gains. For example, "pure orange juice" may have high fructose corn syrup added for sweetness, "extra virgin olive oil" has been found to contain hazelnut oil, "all-natural juice drinks" are augmented with synthetic vitamin C, "American catfish" are actually catfish from Viet Nam, "wild salmon" may actually be farmed salmon and "chondroitin from shark" has been found to contain chondroitin from beef. Food adulteration is defined as the fraudulent addition of non-authentic substances or removal or replacement of authentic substances without informing the consumer. Most of these

additions are done at the percent level since it is too much trouble to add parts per million. The tools used to detect adulteration include visual or microscopic exams, spectroscopic techniques and targeted analysis. Visual/microscopic exams are low cost, quick, able to be performed onsite and require educated system or operator. The adulterant may not be able to be identified. Spectroscopic techniques include NIR, mid-IR and Raman IR. For example, IR was used to separate authentic honey from non-authentic honey. Targeted analyses include the use of chemical analysis for specific components, DNA analysis, ELISA for specific proteins and major component analysis. The advantages of targeted analysis are that the adulterants are normally identified, the techniques have high sensitivity and do not require matric matched comparison. Some of the specific techniques include chiral analysis, oligosaccharide profiling and anthocyanin profiling. Stable isotope analysis has been used to detect additions of sugar, ethanol, dilution with tap water, artificial substances and mislabeling. An example of isotope analysis is the use of SNIF-NMR which can measure deuterium and ^{13}C distribution within a given molecule. With this technique, vanillin was able to be identified containing vanilla bean, lignin and synthetic vanillin. For ascorbic acid, the technique can discern between C3 or C4 plant sources and other adulterations. Another example is identifying tequila and "tequila misto". There is more tequila sold than available agave to make it. Most of the tequila which is sold is "tequila misto" which is 50% agave-derived and 50% corn or cane-derived. The challenges for using isotope analysis is the make sure that the authentic database covers all possible authentic samples and to improve discrimination between authentic and non-authentic samples. If adulteration is below 20%, it is usually not detected. And even if methods improve, those that are adulterating adjust to the new detection methods capabilities.

Introducing Solae (A. Azmy, Solae): Solae is a food ingredient supplier with a focus on protein from soybeans. Solae is a joint venture of DuPont and Bunge and is headquartered in St. Louis. It possesses 11 manufacturing plants in North America, South America, Europe and Asia. Currently, Solae has 2000 employees and sales of $1.4 billion. Solae's core values are safety and health, environmental stewardship, high ethics and respect for people. The company's vision is to advance global nutrition through food ingredient innovation. The goal of the company is to be a preferred partner in developing and delivering nutritional and functional solutions. Solae contracts about 800,000 acres of soybeans—60% are non-GM identity-preserved and 40% are commodity soybeans. Identity preservation complies with the EU regulations. Identity preservation is tested at every stage from farming protocol through processing. Soy protein is a high quality protein which is low in fat and saturated fat. Solae is the biggest supplier of soy-based infant formula as wells as emulsifiers, meat analogs, confectionary, etc. Other products include textured/functional concentrates (used in processed meats), soy isolates and lecithin. A big challenge for Solae is the absence of thresholds for GM content in various countries.

Species authenticity by DNA testing (E.P. Smith, Eurofins GeneScan): Identification of species is important to the food industry to avoid fraud, for health and safety reasons and for consumer preference. In the early stages of the supply chain, species identification is relatively easy, i.e., identify by visually seeing the whole fish. Later in the supply chain,

it is not so easy (i.e., a piece of fish). Processed products lose morphological characteristics that may make it easy to identify the species. For instance, rainbow trout have been substituted for "lox" salmon. For species identification, several approaches may be employed which include high throughput sequencing, marker analysis, RFLP analysis and RT-PCR species specific tests. The Barcode of Life project is a project to barcode every living creature. The information is publicly available. The target is the mitochondrial gene cytochrome oxidase. Mitochondrial DNA comes from the maternal side and is relatively stable in species. Marker analysis has been used to determine coffee varieties. 100% Arabica coffee commands a premium price so it is of interest to determine if a coffee actually contains 100% Arabica. For rice, there are 15 strains of rice that may be marketed as basmati. Again, marker analysis is useful to distinguish these varieties. There are also single species tests that have been developed to distinguish species such as elk, buffalo, deer, etc.

Development and validation of canola A-genome specific endogenous reference system for RT-PCR assay (N. Henderson, DuPont): The requirements for a reliable endogenous reference system for RT-PCR is that it must be very specific for the taxon, stable Ct values across the species and single copy. Canola is from the Cruciferae/Brassicae family and was defined in 1979. Edible oilseed rape (double low) contains <2% erucic acid. Three species are identified: *B. juncea* (brown mustard), *B. napus* (Argentine canola), *B. rapa* (Polish canola). All share the characteristic of the A-genome. Many closely related weeds such as *Arabidopsis* and *Sinapis* contaminate canola grain. There are five RT-PCR methods listed for canola but none are demonstrated as specific to canola. The desire was to find endogenous specificity to the A-genome. Regions were sequenced from several genes from multiple varieties over many geographic regions. The sequences were assigned to different genomes. For one sequenced region, the A-genome fell into three contigs, the B-genome fell into one contig and C-genome fell into two contigs. The A-genomes were found to be closely related. An assay (AGSA) was designed to be specific to the A-genome. Probes and primers were designed to use regions distinct from the B- and C-genomes. The assay was compared to the other 5 assays. The AGSA assay only identified *B. napus*, *B. juncea*, *B. rapa*. The other 5 assays all exhibited cross reactivity. In summary, the AGSA assay is specific to the A-genome of canola and does not cross-react with other *Brassica* species. The assay performs stably across A-genome varieties and the specificity and stability has been tested across varieties and regions.