AEIC Spring Meeting 2004 Minutes
The Peabody Hotel
Memphis, TN
April 15-16, 2004

by P.L. Hunst, Secretary

Vice President Randy Giroux (Cargill) convened the meeting on April 15. Jennifer Wells (Horizon Ag) welcomed everyone to Memphis on behalf of Horizon Ag and Eurofins, site organizers of the meeting. There were 30+ attendees representing16 companies, 1 organization, 1 university and 2 federal agencies.

AEIC Business Meeting

Secretary's 2003 Fall Meeting Minutes: A motion was made, seconded and approved that the minutes be accepted.

Treasurer's Report: A motion was made, seconded and approved to accept the 2003 final budget as reported by Dean Layton. For 2004, the budget is projected as follows:

Beginning balance (Jan04)		31978
Interest (thru 2/27/04)	18	
Projected interest	90	
Dues (paid to date)	1800	
Projected dues	6550	
Total income	8458	
Expenditures		
CLA Consultancy	4038	
Wire Transfer	30	
Delaware Tax	25	
ANSI/ISO	2500	
	6593	
Projected Expenses		
Spring Meeting	1500	
Fall Meeting	1500	
Protein Paper	4000	
Educational Materials	1000	
Reprints	600	
Website	1500	
Subscriptions	1500	
Misc	150	
Total Projected Expenses	11750	
Total Expenses	18343	
Projected New Balance	22093	

Dean brought up the question as to the objective of the budget—is it to grow it or maintain it? If expenses continue in this trend (expenses exceeding income), the budget will eventually be depleted or the balance would be too low to respond to any initiative needs. Several suggestions were made: 1) initiate a meeting registration fee or 2) raise the membership dues. It was decided to table this discussion until the Fall 2004 meeting. A motion was made, seconded and approved to accept the 2004 budget.

For the AEIC Powerpoint presentation, Medallion Labs proposed that $1000 be paid to them in the form of AEIC dues (Anne Bridges" time) for finishing the editing of the presentation. Medallion proposed to finish the presentation by the end of June, with a first review draft by the end of May.

Member Update:

2003

Large Companies	13
Small Companies	11
Associates	3
Affiliates	11
Individuals	2

2004

Large Companies	14
Small Companies	10
Associates	3
Individuals	2

DNA Validation Paper: The paper was submitted and reviewed by the Journal of AOAC. The journal review was completed and comments were received by the authors. The authors are currently working on the changes. After the changes are made, the paper can be distributed to the AEIC membership.

Fall Meeting 2004: The Fall Meeting will be held on October 21-22 in Champaign, IL and will be hosted by AOCS (G. Clapper). Suggested agenda items were:

Real world issues for implementation of exisiting methods
Landscape for greater global acceptance of protein methods—role of AEIC?
Biology of grain development & genetics—how it impacts testing
Nutrition research on a cellular level (U. of IL speaker?)
Challenging situations of low expressing traits and/or similar traits
Kits and diagnoistic methods—stability of tests; validation of stability; critical control points
Other regulatory follow-ons:
--implementation of EU regs—where are we?
--Swiss regs—update from researcher at Agdia
--issue in Mexico—CIMMYT

Spring 2005 Meeting: The USDA AMS will host the spring meeting in Gastonia, NC (Charlotte, NC area) sometime in March-April, 2005.

The Business Meeting was adjourned.

Updates:

EPA Ft. Meade Lab (M. Rindal): The Ft. Meade lab is in charge of the method validations for PIP registrations. The lab is now up and running after 2.5 years of preparations. The lab is part of the Biological & Economic Analysis Division of EPA. Besides doing PIP method validations, the lab also works on antimicrobial testing of disinfectants, ORD research, CDC LRN (lab reporting network for anthrax decontamination) and GLP. The validation process for PIP methods is as follows: EPA BPPD prioritizes the methods; the MRID is sent to the lab; paper review of method is conducted; and those that pass paper review are scheduled for lab validation. The paper review consists of evaluating the ILV; equipment needed and its availability for the method; work with EPA BPPD to obtain any reagents that are needed from the registrants. All feedback on

methods goes back to EPA BPPD and a report is also sent to the registrant. The lab is currently working on their first validation for a PIP.

Online Learning Modules (D. Namuth, U. of NE): The purpose of the modules is for extension coordinators, field researchers, etc. The basis of the modules is to train on how a GM plant is produced and there are now 58 modules on this. The latest module added is on RT PCR. An outline for oil chemistry has also been done with G. Clapper (AOCS). The module for protein detection in plants needs feedback from AEIC before it can be put online. Also, is AEIC interested in doing other modules such as a sampling module or one on PMPs (plant made pharmaceuticals)? It was decided that the U. of NE website (www.croptechnology.unl.edu) should have a link on the AEIC website.

USDA AMS: The AMS lab in Gastonia, NC administers the national organic program, has oversight for seed regulation and provides testing services and grants intellectual property rights for certain plant varieties. AMS now has a memorandum of understanding with GIPSA. The memorandum is to increase efficiencies between the two and reduce duplication of effort as well as defining responsibilities and shared roles. Within the USDA Marketing/Reg. Programs, there are now clear contacts for biotech needs. AMS' responsibilities include cotton, dairy, fruit, vegetables, livestock, seed, poultry and tobacco. GIPSA's responsibilities are grains and oilseeds, intellectual property and obtaining reference materials. The shared responsibilities between AMS and GIPSA include exchanging information, determining areas of cooperation, sharing program resources, sharing of staff for educational purposes and sharing direct responsibilities. Last year AMS and GIPSA sponsored a joint meeting on ag biotech issues. The meeting was attended USDA and state ag people as well as representatives from the biotech technology providers. The issues that were identified were testing, rules and regulations, marketing, education, funding and miscellaneous. For testing, USDA will take leading role in validating methods and will be involved in obtaining reference materials and establishing reference labs and administering proficiency tests and accreditation. For rules and regulations, it was felt that there should be more clearly defined rules for biotech, uniform terminology, better communication between agencies, control genetic drift. For marketing, certification programs are needed and AMS will continue to have a role in marketing assistance. Education is necessary at the state level. More information is needed on potential allergens. The states also want assistance for promoting biotech derived food/fiber and outreach to show that biotech food is value added. The states indicated that they have no funds for the activities and would need funding. For pollen drift, it was felt that more studies are needed and that national insurance policies for farmers that might suffer losses due to corn rootworm attacking forage of Bt crops. California's Mendocino County representatives were very active at this meeting.

ISO TAG Update (R. Shillito, Bayer): The TAG is the US expert group that deals with detection of GMOs and derived products, development of standards for Ag Biotech products for seed, grain, feed and food (ISO/TC 34/WG 7); which is working on standards prepared by CEN TC 275 WG11 per the Vienna agreement. The Vienna agreement governs the parallel development of standards by CEN and ISO. The US is represented by ANSI. In ISO, there are 137 countries that have membership as either a participant or an observer. Not all countries are members of each TC (technical committee).

Standards that are currently under review include: general requirements/conditions; protein-based methods (published); nucleic acid extraction methods; qualitative nucleic acid-based methods (awaiting action by CEN on ISO comments); quantitative nucleic acid-based methods (new technical vote requested) and sampling (was a final standard but was withdrawn by CEN). The last Working Group meeting was held in South Korea in February and was chaired by France. Delegates were present from the US, China, S. Korea, Japan, Iran, France, Norway, Argentina, Italy and Germany. The ISO board requires that committees make sure that standards are globally relevant. However, the standards as proposed are Eurocentric and we were able to bring up this issue whenever an item was not sufficiently global in its scope. A new work item proposal was for setting criteria for what kind of information is needed for methods to be added to

the standards as annexes. The work item was voted on by the Working group countries and adopted. The sampling standard is now a final draft standard, however, CEN is concerned that it will conflict with labeling and traceability. US proposed that a joint working group of ISO and CEN be formed to write a new draft.

The next meeting will be in the US and the 2006 meeting will be either in Iran or Japan.

USDA GIPSA (R. Jenkins): Ron will be chairing the Midwest Section of the AOAC in 2005 in Kansas City. The proposed title of the session is "GMOs: Seeds of salvation or seeds of deception". Topics may include BSE testing and screening, allergens, methods of validation, other topics and posters.

The DNA quantitation project between NIST/Bayer/GIPSA has been stalled due to lack of equipment and budget constraints. A luminometer was recently acquired.

For reference materials, GIPSA was awareded $75000 and is currently in the process of advertising a position. The target is Dec04 to have reference materials available that IRMM does not have.

GIPSA is still compiling the Jan04 results for the proficiency program. Seventy-eight organizations reported results with 30 using qualitative methods. Participants used a combination of DNA and protein-based tests. The results will be posted on the GIPSA websites (www.usda.gov/gipsa). The preliminary results are looking encouraging.

Invited Talks:

Tools for compliance and enforcement (EU) (K. Fatmi, Eurofins): A brief introduction of Eurofins was first given. Eurofins originally started in France and now has a network of labs around the world. There are two centers of competence in the US (California and Iowa). Testing is done for food, environment and pharmaceuticals.

The global area of GM crops was 70 million hectares in 2003. There was more adoption in the industrialized countries with the US, Canada, Argentina, Brazil and China being the leaders. GM adoption is the greatest in soybeans, followed by cotton, canola and corn. In the EU, there were 2 regulations in 1997-98. These were replaced by the 2000 regulations which established thresholds and the inclusion of additives/flavorings. Under these regulations, GM contaminants (under 1%) did not have to be labeled and those over 1% had to be labeled. In 2002, new regulations appeared for food/feed and traceability of food/feed products. As of April, 2004,food/feed precuts which contain GM or exist of GM and GM derived products must be labeled. The control of GM is through analysis and traceability. If a product is produced from and with ingredients containing GM, then it is labeled. All steps in the process of production (from the GM crop to the finished product) must be labeled. Food/feed produced with GM products do not have to be labeled. Also, food made with GM enzymes (such as cheese) does not have to be labeled. The threshold is 0.9% for an approved GM event, 0.5% for a positively assessed GM event (assessed by EFSA) and 0% for non-approved events. The aim of the regulations is to protect human health and the environment. Due diligence will be done through analysis and documentation with PCR being major analysis tool. If the analysis is negative, full documentation will be needed.

The regulations present an analytical challenge for GM events such as global or specific screening, matching of ELISA results at the beginning of the food processing chain with RT PCR at the end of the chain, stacked vs. unstacked events, detection of 35S and nos may not be sufficient to detect all events, will need event specific systems, and sampling schemes of raw vs. finished products. How do the regulations work? As an example, if a cow is fed GM feed and given GM produced drugs, the meat/milk from the cow do not have to be labeled. If the cow itself is GM and produces milk, then the traceability/labeling requirements are active.

For the labeling of food allergens, ingredients constituting less than 25% of the product do not need to be labeled. This presents a health and liability issue. The new directives abolish the 25% rule and will include alcoholic beverages. The allergens cannot be hidden in the ingredients of a food and ingredients such as cereals with gluten, crustraceans, eggs, fish, peanuts, soybeans, milk, nuts, seasame seeds and celery must be clearly labeled.

Practical considerations and issues encountered during PCR method development and analysis of biotech products (F. Spiegelhalter, Eurofins): DNA testing strategies are implemented for screening (yes/no), identification (which type of GMO in sample or yes/no) and quantification (GM content, threshold). A concern is that regulators believe that 1% can really be distinguished from 0.9% analytically. As an example, if a 5'-3' exonuclease fluorescent PCR method is used, the taqman measures when the amplification occurred but not how much occurred. The following equation can be used to determine % biotech DNA:

$$\frac{\text{Biotech DNA}}{\text{Species DNA}} \times 100 = \% \text{ biotech DNA}$$

The units of the equation do not matter since they will disappear. The assay design is relative from the beginning since the decision is what DNA is going to be used for the standards. What really matters is the w/w %. The typical contributors to the equation are biotech corn which contributes to both the numerator and denominator and conventional corn which contributes only to the denominator. This results in % biotech corn/corn DNA.

Another consideration is the use of taxon specific nucleic acid sequences which is defined as reference nucleic acid sequence native to the corresponding taxon. The nucleic acid sequence should be present in a constant copy number and not showing allelic variation among cultivars of the target taxon. For example, two kits were compared. The 35S primers were identical and the study was to compare 3 reference systems for corn DNA—2 hmgA and invertase. The reference DNA should be preferably specific for the target taxon with no undesirable cross-reaction with the nucleic acid sequence from other taxa. The results of the study showed that the primer systems looked different but not enough to be of concern. It was expected that the CT values should all look alike, however, 4 fold more or less DNA was measured. This resulted in not using the invertase system.

Another example is the measurement of 35S. All commercialized corn events contain 35S, except GA21. Different GM events have different amounts of 35S. Results will differ by a certain factor, i.e., Bt176 has 2X the amount that MON810 has. The 35S can be calibrated quantitatively to give accurate results for reference samples. However, in the real world, GM events that contribute 35S are unknown in the sample. The Japan standard method always gives higher results dependent on the reference material used. If Bt176 used, will derive an amount that is 2.5X higher for MON810. If use MON810 as the reference, this will result in 10X for Bt176. If GA21 is used as the reference material, MON810 and Bt176 will not be detected. Therefore, there are many factors in the analyses that can skew the % GM results.

ILSI Taskforce on Nutritional Safety Assessments of Food/Feed (R. Shillito, Bayer): The ILSI taskforce has a publication on the nutritional assessment of a crop which has intentionally been changed for composition. A workshop was held in Paris and the participants included regulators from various countries. The purpose of the workshop was to review the chapters of the paper, discuss and solicit comments. The conclusions of the paper included: 1) the existing comprehensive safety and nutritional assessment processes used with agronomic traits are appropriate; 2) safety and nutritional assessments should begin with compositional analyses; 3) safety assessments should center on the comparison of the new food/feed with an appropriate comparator that has a history of safe use; 4) additional studies may be needed on a case-by-case basis; 5) the assessment of the novel food should not depend on the technology that developed it. The document "Nutritional and Safety Assessments of Foods and Feeds

Nutritionally Improved through Biotechnology" is now posted and available free to the public on the CRFSFS website at: http://www.ift.org/cms/?pid=1000362

Molecular Approaches to Antibody Engineering (A. Porter, Haptogen): Haptogen is a spin-off company from the U. of Aberdeen in Scotland. Aberdeen is a technology dominated city and has the largest assemblage of scientists within the EU. Haptogen is a drug development company dealing with therapeutic antibody technology, anti-infectives and CNS (obesity) and strong and developing intellectual property.

Haptens are molecular signatures of larger molecules and can consist of polymers, sugars, peptides, modified state proteins, cell surface antigens. The fv is the simplest part of a monoclonal antibody but falls apart. The scfv, scFv disulfide and StAb are more stable. Using the monoclonal antibody for atrazine, the binding site of the MAb (called the scAb) for the atrazine was cloned. The scAb has increased sensitivity for the antigen. The stAb can be used in non-physiological conditions and can be used in solvent extracted soils.

Haptens are too small to be seen by the immune system and must be conjugated to a carrier. The goal is have antibodies to the hapten but not to the carrier (also called interface binders=hapten + carrier antibodies). To avoid the interface binders, selected a single chain Fv (antibody binding site). With this, antibodies can be made in humans, rodents, sheep, camels, etc. The Fv can also be displayed on a phage particle to generate large libraries in order to amplify and select for the appropriate monoclonal antibodies. Sheep make antibodies constantly and keep making better ones as more exposed to the antigen. If you can select for free hapten binders, the sheep antibodies show good stability in non-physiological conditions.

Haptogen now has a joint venture with Mologic to exploit the sheep antibody repertoire with antibody engineering. This gives new levels of performance, higher affininity and compatibility with phage display. Antibody engineering allows the antibody to be precisely engineered for a particular application. Also making antibodies in sharks—specifically the nurse shark. It has a 12 kDa antibody binding domain which is the smallest domain. The antibodies are robust in non-physiological conditions and have stability at 37C for 8days and probably longer.

Proteomics: A tool to investigate effects due to genetic engineering in the context of natural variability: A model study using Arabidopsis thaliana (M. Ruebelt, Monsanto): A proteome is the complete set of proteins present in a cell at a given time. Proteomics is accomplished through 2-D gel electrophoresis. The method was optimized for *Arabidopsis thaliana* seeds. The proteins are first separated by their isoelectric points and then are separated by pI and molecular weight. A computer program measures the spots on the blots and quantifies and thus, allows comparisons to be made between gels. N-terminal sequences can be used on the protein in a particular spot to identify it. To use to look at unintended effects, it is necessary to realize that we never have the real isogenic parental line to compare. Therefore, it is important to know the the natural background. For this experiment, the natural background of 12 ecotypes was assessed. The results showed the polymorphism between the ecotypes and that there was a broad range of variability. Quantitatively, the spot quantities varied between 1 and 53 fold among ecotypes. Seventy-five percent of the spots varied between 1 and 3 fold between ecotypes. Qualitative and quantitative differences in seed protein of various ecotypes could be easily detected. Also, there was variation within ecotypes.

Transgenic lines were assessed. The six lines had different expression of the GUS gene. There were 413 reproducible spots in all lines. There was one additional spot in all the transgenic lines which was not present in the isogenic line. The additional spot was sequenced and found to be GUS.

Proteomics is a powerful tool for detecting qualitative and quantitative differences. No differences, other than the intended difference, were detected between the transgenic and non-transgenic lines.