AEIC Fall Meeting 2000
Portland, Maine
September 28-29, 2000

P.L. Hunst, AEIC Secretary

The AEIC Fall Meeting was hosted by Beacon Analytical Systems, Inc. in Portland, Maine on September 28-29, 2000. Forty attendees, representing 25 companies and organizations, attended the meeting which was held at the Eastland Park Hotel in downtown Portland. AEIC was welcomed to Portland by **Brian Skoczenski (**Beacon Analytical) and **Charles Micoleau** (Head of the Maine Biotechnology Assoication).

Chuck Mihaliak (AEIC President) reitereated that AEIC has been talking for the last 6-9 months about detection methods and formulating validation criteria for genetically enhanced (GE) crops, however, the events surrounding the recall of taco shells, pointed to the urgency to address these topics. The objective of the meeting was to frame what AEIC can do and then formulate some defined goals to work towards.

Dean Layton (Envirologix) facilitated the first session of the day on September 28.

The first speaker was **Dirk Reif** (Cargill) who spoke on "Customer Needs". Dirk is the technical resource person within Cargill for GMO issues. Dirk reiterated that Cargill supports agricultural biotechnology and believes that the technology will continue to improve efficiency, productivity and wholesomeness of crops and foods. Ag. biotechnology benefits the farmers (improved weed, disease, pest control; increased yields; healthier plants), the environment (allows zero tillage practices; less use of chemical pesticides), and the food and feed processors (improved productivity, less waste; increased nutrition; more specificity of use). Dirk presented diagrams of the distribution of soybean and corn from the farmer to processor. There are many processes and products which are derived from the grain and a company such as Cargill is being requested to test for the presence of GE crops at each to prove that they are not part of the process. This is a daunting task for these companies. Testing is also challenging due to regulatory overview processes differing between countries; different tolerances in each country; zero tolerances for unapproved events; and the consideration of legal liability as pertains to recalls, back shipping or destroying grain shipments. Dirk also pointed out that DNA-based tests currently rely on general testing, i.e., the detection of common genetic elements such as the 35s promoter and nos terminator. However, these testing methods will not be effective since new products will be coming on the market next year which do not use these promoters. There is also a dire need for certified reference materials to standardize testing results. The tolerance limits are also set at a percentage GMO--the question is: percentage of what? If it is percent weight, how does a company convert units of DNA or protein measurement to percent GMO?

Dave Grothaus (Pioneer/DuPont) presented "Protein-Based Assay Methods and Validation Criteria". Protein-based assays are utilized in all steps in product development for a GE crop. The guidelines for the validation and use of immunoassays in ag. biotechnology have been outlined in a paper authored by AEIC and recently published in the Journal of Food and Agricultural Immunology. The parameters for validation include sensitivity (LOD, LOQ), accuracy (spike/recovery), extraction efficiency, specificity (matrix interferences, cross reactivity), ruggedness (same results produced in different labs with different analysts), stability (evaluation of analyte stability), precision (intra- and inter-assay), and standard curve quality control (use of percent error).

Steve Tanner (USDA GIPSA) gave a brief update of GIPSA's accreditation program. They have received input from all sectors of suggestions for their program. They have also started meetings with NIST to work on standard reference materials. NIST has expertise in the DNA testing area from their forensics scientists so they understand the methodology. GIPSA is moving ahead with a detect and identify program—not a quantification program. They have also had meetings with their European counterparts who have expressed that their greatest concerns are testing methods and reference standards. Steve talked briefly about the StarLink corn, the subject of the taco shell recall. StarLink corn was grown by 2500 producers in the US in 2000 on 300,000 acres total. These producers were dispersed geographically and will produce approximately 45 million bushels of the corn. The total US production of corn is projected to be 10 billion

bushels (7 billion bushels are used in food/feed in the US; 2.2 billion are exported). Therefore, the StarLink corn will make up approximately 0.4% of the total corn produced. The task is to determine what remains of this corn in the system and to make sure it only ends up in animal feed.

Don Kendall (USDA GIPSA) gave a more in-depth view of GIPSA's accreditation program. The GIPSA facility provides sampling information and guidelines, verifies rapid tests, and accredits laboratories. For the labs involved in the PCR accreditation, a letter of intent to participate has been received from these labs. GIPSA will now ask the labs for information on management structure, facilities, SOPs, and staff qualifications. They will also want to know about their procedures for qualitative non-event specific tests and qualitative event specific tests. There are currently 25 labs interested in participating in the program. Industry has expressed concern to GIPSA that qualitative tests will not meet the needs for zero tolerances, however, GIPSA is not sure there is really quantitative test methods out there. The process for accreditation will include the analysis of challenge/performance samples (50 samples; all commercially available events in US); criteria for acceptance/rejection of results; on-site review by GIPSA staff; and a monitoring program. For rapid test performance (ELISAs), manufacturers must request admission of their kit. The performance data is submitted by the manufacturer. GIPSA evaluates the data and performs an in-house verification. These criteria cover both lateral flow and microtiter plate-based tests. GIPSA has worked with sampling theory, contamination control theory, sampling for qualitative vs. quantitative, sample preparation, and risk management for the buyer and the seller of grain. The schedule for GIPSA's program is:

Confidentiality agreements with Life Science Organizations	Completed
Facility completed	late October
Reference materials prepared	October/November
Initiation of accreditation	late October
Labs accredited	late December/early January

New developments that have arisen include requests to broaden lab accreditation to include methods for Southern and Western analyses; requests to recognize other accreditation such as from ISO; develop sampling guidelines specific for biotechnology; use reference materials from JRC IRMM; and re-evaluate the challenge/performance portion of the process.

Leah Porter (American Crop Protection Association; ACPA) gave a brief overview of ACPA's activities via their Diagnostic Methods Working Group. She passed out copies of their consensus document and executive summary.

John Fagan (Genetic ID) gave a brief overview of their testing methods. Their methods include: real-time quantitative PCR, triple check semi-quantitative PCR; a threshold screening method; and varietal ID. The varietal ID method is used by Genetic ID to identify GE soybean and corn varieties, i.e., BT11 vs Mon810 vs Bt176 vs StarLink, etc. The method does not refer to distinguishing conventional corn varieties. According to Genetic ID, the varietal ID method can be either "semi-quantitative" (based on assumptions regarding the starting amount of template molecules and an initial estimation of the amplicon band that is formed) or "threshold" (positive at or above a certain threshold % GMO). The method has been accredited by the UKAS (United Kingdom Accreditation Service). Since it was Genetic ID's results that triggered the taco shell recall by Kraft Foods, John went over briefly how the results were obtained. Their client supplied them with 7 boxes of taco shells from 3 production lots. The taco shells from all 7 boxes (all three production lots) were ground together and then DNA was extracted from the resulting powder. They analyzed duplicate samples of the powder and used their varietal ID method. They used an internal control, several primer sets for StarLink (designed by Genetic ID to bridge sequence elements in the transgene; transgene information was taken from available public literature) and for a species specific reference gene for corn. They also tested for the 35s promoter. Their results (based on the "semi-quantitative" assumptions of the method) indicated that StarLink corn was present at 1% weight based on their calculation of copy number ratio of gene of interest to reference gene copy number. They confirmed their results 3 times with duplicate samples.

James Jennings (Monsanto) gave a brief statement on producing reference materials. This task is not as easy as it was originally thought to be. The seed for the reference material is tested for the trait, event and purity. It is relatively easy to produce the transgenic seed but it is difficult to prove that a conventional

non-GE lot is truly non-GE. This requires thousands of analyses using line specific PCR methods. However, these methods do not identify other potential contaminants such as other GE events. Along with the PCR analyses, all necessary phytosanitary requirements for shipment of the seed around the world must be met.

Following the lunch, the attendees split up into three groups to discuss: 1) PCR validation guidelines; 2) education materials—what do we communicate, how and to what audience; and 3) how do we link our efforts to other organizations. After the mid-afternoon break, the attendees then split into two groups to discuss 1) units of expression—how to convert DNA or protein test results into % GMO; and 2) production of standard reference materials. The results of these breakout sessions were reported on the Friday morning.

The AEIC Business Meeting was held on Friday morning and was presided over by Chuck Mihaliak. The Secretary's minutes form the AEIC Spring Meeting were approved. Kim Magin, AEIC Treasurer, was unable to attend the meeting, therefore, no treasurer's report was given. Penny Hunst reiterated that the "new" AEIC website was launched on Sept. 26 (www.immunochem.org). If anyone has any changes, recommendations, or additional materials to be added to the site, they should contact Penny. It was also announced that the AEIC by-laws are now available on the website under the "Members Only" area. It was pointed out by Penny that there are two committees specified in the by-laws which AEIC has not appointed in the last couple of years. These are the Finance Committee and the Communications Committee. Following a discussion of each, it was voted to amend the by-laws to remove the clause concerning the Finance Committee and to amend the Communications Committee clause to read "when needed, the President shall appoint".

The next meeting of AEIC will be in the spring of 2001 and will be hosted by Dow AgroSciences in Indianapolis. Chuck suggested having the meeting during the week of **May 14 – 18** so that participants could take in one of the practice sessions for the Indianapolis 500 race held the weekend of Memorial Day. More information will follow on the exact dates but members should keep this week open as a possibility.

A discussion was then started concerning new members. The question was posed as to whether we should invite those companies that participated in this meeting who are not members to join. It was stated that if AEIC wants to become an authority on DNA-based, as well as protein-based methods, we need to have members involved that would provide a consensus voice. It was voted to invite those companies that had participated in this meeting and to ask the member companies to pay for their DNA testing people who participated as affiliate members.

The next question was the AEIC name—should it be changed? It was suggested that the "AEIC" should be retained because it has recognition among the regulatory agencies and companies. One suggestion was to change "Immunochemical" to "Industry". Another suggestion was to add "Biotech" after AEIC. Joe Dautlick (SDI) and Dean Layton (Envirologix) agreed to work on suggestions and present them at the spring meeting. It was also suggested that the web address (immunochem.org) be changed to "aeicbiotech.org". Penny and Chuck will consult with the webmaster about this.

Dave Grothaus (Pioneer/DuPont) suggested that AEIC author a short technical white paper on "what can be done and what cannot done" in testing for GE crops and products. The paper would draw comparisons to using the DNA and protein testing methods in the product development vs. using the methods for labeling compliance. A conclusion might be that what should be hoped for is a consensus decision among US and EU authorities as to what testing methods/criteria are acceptable. It was suggested that this paper could be distributed to a wide audience through other organizations such as ACPA, BIO, AACC, etc. Stuart Reeves (Diamond V Mills) also suggested that it could be published in Food Quality Analysis. Further discussion of this was tabled until the breakout sessions reports were given.

The results of the AEIC election were: Dave Grothaus (Pioneer)—President; Jim Stave (SDI)—Vice President; and Cindy Lipton (Zeneca)—Treasurer. Chuck Mihaliak will be the Immediate Past President and Penny Hunst will continue as Secretary through 2001. The business meeting was then adjourned.

Follow-up on breakout sessions:

PCR Validation Guidelines:

The group had split into 2 sub-groups since they were 19 participants. Each sub-group had a brainstorming session and formulated many ideas for starting a document on this subject. Cindy Lipton suggested that a smaller group undertake drafting such a document as was done with AEIC's last document on immunoassay validation. Volunteers to assist Cindy are Dave Hondred (Pioneer), John Fagan (Genetic ID), April Ernest (Dow AgroSciences), Randal Giroux (Cargill), Stacy Charlton (Novartis), Lori Artim-Moore (Novartis), James Jennings (Monsanto), Kevin Worden (Michigan Dept. of Ag.), Ben Kaufman (Centre Analytical Labs), Alex Kahler (Biogenetics). Once finished, it was suggested to try to publish the article in journals such as Molecular Diagnostics, Biotechniques or Nature Biotechnology.

Education Materials:

This group dealt with what type of education materials might be useful to communicate information on DNA and protein testing. AEIC could produce presentations/seminars, position papers, guidance documents and provide web links to other informative sites. The group suggested that AEIC should start by identifying target audiences and open the communication channels; identify other websites and sources of information and create a basic PowerPoint presentation on validation guidelines to be used by the membership. The group that will undertake the preparation of this PowerPoint presentation will be: Joe Dautlick (SDI), Jim Stave (SDI), John Beeby (Genetic ID), Lori Artim-Moore (Novartis), Chuck Mihaliak (Dow AgroSciences), Stuart Reeves (Diamond V Mills).

The white paper which Dave Grothaus had brought up during the business meeting was also proposed to be written. The paper will focus on information for technical individuals in the food industry. The participants who agreed to assist Dave included: Randy Giroux (Cargill), John Fagan (Genetic ID), Stacy Charlton (Novartis), Jim Rittenburg (Biocode), Ben Kaufman (Centre Analytical Labs), Dave Hondred (Pioneer/DuPont), Stuart Reeves (Diamond V Mills).

Steve Tanner (USDA GIPSA) also recommended that AEIC become involved with the ad hoc committee for methods analysis for the Codex Alimentarius. The ad hoc committee members are from government only, however, they are looking for input from the private sector. Steve will provide an introduction to Bob Lake (FDA) who is heading up the US delegation through either Chuck Mihaliak or Dave Grothaus for AEIC.

Linkages Between Organizations:

This group first determined what other organizations are doing in the area of GMO detection. These included ring tests, preparation of reference materials, accreditation, and policy making. AEIC should not do any these. Instead, AEIC should provide industry consensus on detection and validation of methods. AEIC also should provide educational opportunities such as presentations and seminars. The audience for these activities would be ourselves (use internally in our companies and with our customers), the grain and food industry, and the regulatory agencies. Outreach would include disseminating information via other organizations (ACPA, AACC, BIO, AOAC, USDA, FDA, ILSI, etc.). We should also look for information and technical expertise in other source groups such as those involved in food microbe detection, clinical diagnostics, forensic scientists, those involved in quantitative PCR (viral load investigators, etc.).

Definitions and Units:

The group had discussed how to correlate protein or DNA units to % GMO. The analyses can be done but the correlation is difficult. There is a need for a document to put the analysis and the conversions into perspective. The document would help explain molecular percent which is derived from DNA testing and the factors which affect it such as ploidy levels, presence of stacked events, homogeneity of samples, possible DNA degradation. The same would be true for protein testing where the variables are variable expression levels among events, extraction efficiency issues, degradation of protein in samples, etc.

Standard Reference Materials:

This group's consensus was that uniformity between testing sites and technologies needs to exist. The ideal reference materials would be pure DNA and/or protein. However, the quantity required and the purification issues make this unreasonable. If spiked reference materials would be used, the matrix influence would have to be assessed; what matrix fractions and the number to be used; and whether there is a true negative matrix available. If seed is used, does this really reflect grain since grain is what is being regulated? Currently, USDA is working with NIST to come up with standard reference materials. These would become the primary reference materials and then companies could develop working standard reference materials based on the primary reference materials. GIPSA is currently asking companies for check samples for their program. Joe Dautlick suggested that a scenario for standards used in medical diagnostics for enzymes might be a solution. In medicine, there are no pure standards for enzymes of medical importance. This created a problem in medical testing because completely different units would be reported from every lab doing an analysis. The medical diagnostics community arbitrarily set standards and christened them the "gold standards" for these medical enzymes. These were then multiplied out to create secondary standards for use by testing labs.

Chuck Mihaliak closed the meeting by reiterating that this had been an incredibly valuable exercise and that we had made real progress in establishing some goals. These brainstorming sessions are often the easiest step and now the follow-up activities of writing papers must be continued.

Attendees:

Name		**Company**
Artim-Moore, Lori		Novartis
Magram, Jonas		GeneticID
Brady, Jim		Novartis
Bridges, Anne		AACC
Brix-Davis, Kalyn		Midwest Seeds
Charlton, Stacy		Novartis
Chen, Audrey		FMC
Dautlick, Joe		SDI
Denham, Dwight		SDI
Dinsmore, Andrew		Zeneca (UK)
Ernest, April		Dow AgroSciences
Fagan, John		GeneticID
Fan, Titan		Beacon Analytical
Ferguson, Bruce		Envirologix
Freedlander, Dick		Atofina (Elf Atochem)
Giroux, Randal		Cargill
Grace, Tom		Biacore
Grothaus, Dave		Pioneer/DD
Harrison, Scott		Centre Analytical Labs
Hondred, David		Pioneer/DD
Hunst, Penny		Dow AgroSciences
Jennings, James		Monsanto
Kahler, Alex		Biogenetic Services, Inc.
Kaufman, Benjamin		Centre Analytical Labs
Kendall, Don		USDA-GIPSA (Speaker)
Klein, Frank		Neogen

Layton, Dean		Envirologix
Lipton, Cynthia		Zeneca
Mihaliak, Chuck		Dow AgroSciences
Porter, Leah		ACPA
Reeves, Stuart		Diamond V Mills
Remund, Kirk		Monsanto
Reif, Dirk		Cargill (Speaker)
Rittenburg, Jim		Biocode
Rubio, Fernando		Abraxis
Stave, Jim		SDI
Stewart, Bonnie		Beacon Analytical
Toth, John		Atofina (Elf Atochem)
Walters, Donald		DuPont Agricultural Biotech
Worden, Kevin		Michigan Ag. Dept.