The AEIC Spring Meeting was held May 4-5, 2000 in Painesville, Ohio and was hosted by Ricerca, L.L.C. There were 27 attendees representing 20 companies and associations. James A. Scozzie, President of Ricerca gave an overview of Ricerca and its mission. Ricerca serves the pharmaceutical, animal health, agrochemical and specialty chemicals industries by providing chemical and laboratory services.

Chuck Mihaliak (Dow AgroSciences, AEIC President) outlined the objective of the meeting. The objective was to: 1) understand the current status of DNA-based testing for genetically modified crops; 2) understand the need for developing guidelines for validation and use of DNA-based testing methods; and 3) determine if there is a role for AEIC in developing these guidelines. The group of speakers who had been invited were there to help the organization gain more insight into these objectives and to assist the organization in determining its role.

Chuck also gave a brief history of AEIC to assist everyone at the meeting in understanding our organization. AEIC was formed in 1993 to focus on antibody-based detection methods to promote their use in regulatory testing of new products for registration approval. AEIC initially published immunoassay validation guidelines and hosted workshops for EPA-OPP, EPA-OSW and EPA-Office of Water scientists to assist in familiarizing them with the technology. In 2000, AEIC co-hosted with USDA-GIPSA the workshop entitled “Grains Biotechnology Methods Validation”. At the AEIC 1999 Fall Meeting, the organization had incorporated a biotech initiative. The points of this initiative are: 1) develop performance-based method validation guidelines for proteins in genetically modified plants, food commodities and derived products; 2) serve as an educational resource to regulatory bodies and end users (food processors, food companies, seed companies, growers, etc.); 3) engage in an industry-wide effort of cooperation to obtain consensus on guidelines for the proper use of immunoassay methods; and 4) remain abreast of industry developments of other methods and technologies for the quantitative and qualitative detection of genetically modified crops. Through the organization’s working groups, a publication on method validation guidelines will soon be published in the Journal of Food and Agricultural Immunology. AEIC also organized a roundtable discussion at the last Society of Food and Agricultural Immunology Meeting in the UK.

Don Kendall (USDA-GIPSA) presented a summary of the USDA-AEIC workshop held in February, 2000. The objectives of the workshop were to discuss the validation and application of detection methods for genetically modified (GM) grains. A synergistic approach is required for the application of detection methods for GM grains because: 1) no single method can detect all GM events and 2) no single method can quantitate all GM events. The polymerase chain reaction (PCR) detects DNA and is highly sensitive, however, it is also a slow and expensive method. ELISA detects specific proteins and is fast and relatively inexpensive. However, it is not sensitive to all target proteins and very few kits are available for the GM events currently on the market. The feedback from workshop participants was that 1) working groups should be created to deal with issues; 2) a strategic plan should be developed for testing GM grains; 3) communication must be maintained between all groups; 4) harmonize the efforts of organizations such as GIPSA, AACC, JRC, etc.; and 5) address the need for rapid action on the standardization of testing. The goals of the GIPSA GM detection lab are to: 1) establish sampling procedures for grains; 2) evaluate and accredit independent labs for PCR testing; and 3) make third party verification of quick test kits. Accreditation of independent labs is important because the grain markets are seeking testing to minimize their risks. GIPSA has developed a protocol for accreditation of PCR labs for grains. The basics of this plan are: 1) the labs must demonstrate the capability to differentiate between conventional and biotech derived grains; 2) performance testing will be done through the use of unknown samples (quantitative non-
specific and qualitative specific); and 3) lab reviews will be conducted to audit quality control measures and analytical processes. Admission and maintenance fees will be charged for accreditation. GIPSA has established a working relationship with NIST to characterize standard reference materials. They are also working with the EU Joint Research Council (JRC) on methods and reference materials. GIPSA will, as they have done with mycotoxin kits, verify the claims of test kit manufacturers. There will also be fees charged for this performance verification.

Q&A:

Question: It is impractical for one agency to accredit labs. Why not go to a GLP-like self-regulating system?

Answer: This is a good point and the accreditation effort may eclipse GIPSA resources eventually. Audience comment: GIPSA gives credibility in overseas markets to testing. Audience comment: GLP-like guidelines are not enough to satisfy the wants and needs of the supply chain at this time.

Anne Bridges (Chairperson, AACC Committee for GMO Detection) gave an overview of her committee’s efforts. The American Association of Cereal Chemists (AACC) consists of members from the length of the food supply chain. The members have indicated that there is an urgent need to identify biotech events in ingredients. At the advent of biotech crops, it was foreseen that identification of biotech events would be needed to identify “value-added” traits (reduced saturated fats, enhanced amino acids, etc.), however, with Europe’s resistance to the current biotech crops, the need for testing has arisen much quicker. In North America, there are no approved testing methods for biotech products. Many companies in the supply chain are looking to the EU for “experience” in testing. The EU has conducted several ring trials for qualitative PCR and quantitative ELISA. Each of these trials have raised issues and concerns of one kind or another. The challenge for the AACC Committee is to meet the needs of all AACC members. For example, in Europe, PCR analysis is being asked for. At grain elevators, operators want very fast generic tests. The issues for all methods are sampling, the availability and reliability of reference standard materials, and validation of methods. The issues for customers are low cost tests, competent analysts and labs, and timely reporting of results. The issues for testing labs include sample preparation, management of sample flow, cross-contamination (especially in PCR), and having approved/non-approved genetic event information readily available. The AACC approves methods and the timing of committee events is the critical issue for new method approvals. The AACC technical committees determine if a method has the potential of becoming a useful AACC procedure. Collaborative studies are required for all new and original methods as well as for existing methods to which significant revision or modification is proposed. The collaborative trial is proposed and approved by the committee. The trial must be completed and presented 30 days prior to the AACC Annual Meeting for consideration as “First Approval” method. A “First Approval” method is followed for three years before final acceptance is granted. There is a loophole to the system. If a method is deemed essential and requires quick availability, the committee can consider the method scientifically sound and it then goes under the “Proposed Method” category. For biotech methods, the AACC committee is made up of experts and all groups are represented. The market needs for methods is real, however, the timing for approval is critical and it is short (method and data must be submitted prior to September, 2000 for approval).

Q&A:

Question: Does it take 12 months following the AACC annual meeting to be put “on books”?
Answer: Proposed method can go into books in November, 2000. This can only be done once a year (at the annual meeting). The AACC by-laws are cast in stone.

Question: Do current guidelines for method approval encompass these types of methods (PCR)?
Answer: There is no DNA testing in guidelines at this time. The method guidelines define what must be in the method.

Question: How does AACC judge method performance?
Answer: Technical committees are the experts and their vote determines if the method performance is adequate.

Leah Porter (American Crop Protection Association, ACPA) gave an overview of ACPA’s role in biotech crops. The ACPA Biotech Committee is made up of several subcommittees: Government Affairs, International Trade, Law, Public Affairs and Technical/Regulatory. Under the Technical/Regulatory Subcommittee, several work groups have operated. These are the Agricultural Biotechnology Stewardship Working Group (came together for the monarch butterfly issue), the Diagnostic Testing Work Group and the Inter-Industry FDA Task Group. The Diagnostic Testing Work Group provides background on detection methods for biotech derived traits. They support the validation programs for diagnostic testing methods such as that being developed by GIPSA. The group is currently developing a consensus document on standard reference materials. The ACPA Biotech Committee has been dealing with some “thorny” issues. The group has asked the government to set down the definition of for “GM free” and the criterial that must be met to put this on a label. Another issue is the unintended pollen drift/property rights of neighbors and producers—an issue from the proposed USDA organic rule. And labeling—at what stage is labeling to occur—the seed, processed ingredients, final food product? Japan, South Korea and Australia have already put laws on their books for labeling. Efforts in all these areas need to be coordinated through the various industry organizations, commodity groups, academia and regulatory agencies.

Dirk Reif (Cargill) stated that Cargill supports agricultural biotech even though there have been numerous reports to the contrary. There is public confusion over terms commodity grains and specialty grains. Commodity grains handle large volumes efficiently and safely. It is recognized that biotech corn and soybean are substantially equivalent to conventional corn and soybean. Specialty grains handle small volumes efficiently and safely at a higher cost than commodity grains. For specialty grains, there is a complete system approach, i.e., audit trail is necessary. Training and education are musts for the entire system from planting to processing. Currently, specialty grains are a higher cost due to the additional handling, cleaning and testing required. Segregation of grains (biotech from conventional) implies a partial and non-comprehensive system. This is very high risk. For grain handlers, the problems of testing are enormous. Not only must grain in bins be tested, but products from wet milling, dry milling and soybean crushing and grinding are all being requested to be tested for GM content. The EU directive EC49/2000 which was effective April, 2000, indicates that food ingredients must be tested for GM content. The product must be labeled if >1% adventitious contamination is present. To establish if GM contamination is adventitious requires an audit trail. The issues/challenges that are facing handlers and processing companies are: 1) labeling regulations are not unified but are country specific; 2) testing methods are not comprehensive; 3) not all biotech events commercialized in the US are approved in all other countries; 4) tests are needed for non-approved events when in the presence of approved events; 5) need validated test methods for milling, refining and crushing products; 6) there are no standards at the food ingredient level; 7) need quantitative analysis to guarantee thresholds; 7) need to know total testing method variability to evaluate risk; 8) cost of analysis/value of products—low margin for commodity products; and 9) need clear definitions and terminology.

Jim Stitzlein (Consolidated Grain & Barge, CGB) is in marketing developing for CGB. CGB is a moderate size grain company located in the midwest. The company deals with import and export markets. CGB is owned by two Japanese companies who wanted more insight and input into the grain they are buying. Japanese customers asking for non-GM grain (starting with harvest of 1997) which requires an identity-preserved program (IP). CGB does not invite every grower into its IP program because training is essential and there are needs and limitations on every farm. Also, with IP, elevators do not accept delivery of harvested crop every day. It is a buyer’s call situation—synchronization with the buyer. After Japan announced its labeling requirements, 15% of the corn imported to Japan is on non-GM or IP program. This only represents 1% of the corn produced in the US so this demand does not require all of the US corn market to segregate corn to satisfy the market. All US corn producers have had requests as to whether segregation could be done. It is a certificate-based program, i.e., consumers are asking for tests and tests are being done. CGB has had some tests performed on corn with variable results and difficult to quantify. The bottom line for elevators is that tests must be quick, cheap, repeatable and reliable. Lateral flow immunoassay tests will have to be flexible to be adjusted due to customers’ tolerance levels. This has been managed so far by adjusting the sample size tested, however, CGB is not comfortable with the results.
Also, the elevator lab people are not trained to deal with any sort of complexity in testing. Elevators do not want to deal with multiple testing procedures—they only want one. In August, CGB had first experience with PCR testing. CGB submitted samples from three barges of corn. The test results came back as 19-20% GM present. When the vessel was tested (carrying the same corn), the same lab gave results that there was <2% GM. CGB sees that issue is commercial acceptance. If the consumer is a given choices, they will more readily accept the choices presented.

Mike Russell (Central Hanse) talked about issues with PCR testing. The main issue is the lack of standardization which results in high variability within and between labs. The sources of variation include sampling, sample preparation, sample extraction and the PCR method. Variability in sampling is due to the way the sample was taken and the number of samples taken. Sample preparation variability results from different methods of splitting samples, different methods of grinding, variation in particle size and cross contamination. Extraction is dependent upon which procedure used and its efficiency. The PCR method variability is dependent upon the instrument variability, the number of cycles, presence of inhibiting chemicals and the specificity, validity of primer sets. So what can AEIC do to help with all of this? AEIC could provide a science-based source of information to meet the technical needs of groups working to standardize testing. AEIC could provide position papers and technical information such as testing guidelines. AEIC could also be a public voice for technical information, i.e., be a non-biased source of public information on biotech concerns.

Q&A:

Question: If AEIC opened it doors to PCR labs for discussion forum, would they participate.
Answer: They would be foolish not to. All labs need to work towards stabilizing the market in order to survive. We all need to make the status of the data more reliable.

Jim Stave (Strategic Diagnostics, SDI) was a last minute substitute speaker. He presented his talk that he gave at the USDA-GIPSA workshop in February. Please refer to the GIPSA Workshop meeting notes.

Doris Dixon (Monsanto) gave a talk on the International Seed Federation Initiative (FIS). FIS wants to implement a system approach for measuring the genetic purity of seed. Their intent is to utilize existing OECD certification and is asking for voluntary participation by the seed industry. The initiative involves many groups including ISTA, ASTA, OECD, AOSA and biotech trait providers. FIS is the principal administrator. There are three committees involved in the initiative. The Policy Committee manages communications, negotiations and issues. The Process Committee introduced enhanced process methodology for testing seed into the producer and regulatory testing schemes. The Technical Committee is validating PCR methods that demonstrate robustness/reliability across labs; establish acceptable process standardization of the PCR methods; enhance acceptance of biotech by demonstrating industry’s ability to produce/distribute conventional seed; and to ultimately lead the incorporation of information on the OECD tag for hybrid corn. OECD does not currently have a threshold but a 1% threshold has been proposed to OECD on an experimental basis for the next 2-3 years. Twenty-two labs will provide statistically relevant data. The objective is validate the method—not the labs. The ring test is a combination of gene-specific PCR and event specific (35s, GA21). Qualitative analysis, using pooled samples, will be conducted to achieve 95% confidence that a conventional seed bag contains <1% biotech seed. The initial activity will focus on biotech traits within corn sold in 1999. The status of the initiative is that they are close to finalizing the first contract (transfer of confidential information). The seed samples have been prepared and the PCR protocols are finalized. The targeted completion date for the ring test is June in order to present the results to the OECD Meeting in Rome.

Q&A:

Question: If the variables are controlled, do you have confidence in the results?
Answer: We have confidence in our own PCR—event specific only. There are a lot of internal controls in the experiment.

Question: If the labs have high variability, what will you do?
We have controlled some variability by varying sample size. It is easier to troubleshoot within an organization.

Question: Is threshold too high?
Answer: The internal standards are set well below 1%. We are not delivering seed at 1%. The threshold is set for the seed industry—not the food industry.

Sharon Weiss (International Life Sciences Institute, ILSI) talked about ILSI’s biotech efforts. ILSI started working in biotech in the late 1980’s. They have numerous publications addressing consumer acceptance and allergenicity. The International Food Biotech Committee is a resource for ILSI branches worldwide. The committee collaborates with global and regional organizations. It also monitors, globally, scientific and regulatory developments related to food biotech. The committee has had an extensive outreach effort the last two years with its branches to sponsor workshops (China, Thailand, India, Korea, Mexico, Argentina, Europe). ILSI in Japan sponsored the FAO/WHO Symposium on Biotech and Food Safety which was held in March, 2000 to discuss food safety: hazards, risks and perceptions. In 1998, the committee sponsored the workshop on Detection Methods for Novel Foods Derived from Genetically Modified Organisms. The recommendations from this workshop included: 1) production of appropriate reference materials; 2) ELISA appeared to be method of choice for screening raw materials and basic ingredients; 3) PCR methods are available for qualitative screening and quantitation of DNA in all DNA-containing matrices; 4) DNA and protein methods can be expected to yield false positive findings; 5) there is a need for internally validated detection methods; 6) further development and validation of methods will be contingent upon establishment of EU thresholds; 7) there is value to establish a negative list for analysis; 8) there is a need for a database to provide information on genetic modification; and 9) further research is needed. There will be a follow-up workshop in Brussels, Belgium (possibly December, 2000). The workshop will further explore the issues of sampling and detection methods as applied to different food matrices and their validation. The workshop will be held jointly with the EU JRC.

Scott Fritschel (DuPont Qualicon) gave an overview of DuPont Qualicon. The company was originally set up to address testing needs in the food industry 10 years ago. Their initial work has focused on microbial diagnostics. Qualicon has a lot of expertise in working with the food industry and genetic expertise in food testing. They use a homogeneous fluorescent detection PCR which is their own technology. It is a closed tube method with a high sensitivity. The closed tube avoids the problem of the amplicon in the lab which is a possible contamination source. The method has a broad linear range and can quantify between 0.05% and 100% GM. A unique feature of their method is the tableted PCR reagents. Each tablet contains every reagent except the template DNA. There is only one pipetting step which is the addition of the template DNA. The tablets eliminate reagent preparation. They have done multiplex with up to three primer pairs. They also have primer-less tablets which are useful for quick method development. Results are usually available within 6 hours after receiving sample and are reproducible. In addition to providing testing services, Qualicon will also have a qualitative PCR kit and a quantitative PCR kit. These kits will be available probably by 3rd quarter of 2000.

Q&A:

Question: How does Qualicon technology compare to what is currently on the market?
Answer: It is an intercalating dye technology. The way the technology is implemented allows us to tease out fluorescence due to amplicon. Essentially, we are analyzing the melting curve of the amplicon.

Question: What are the specific components going to be in the kits?
Answer: The tablets will be in the tubes. The tablets contain specific primers, Taq, etc. A DNA extraction method is not included but we do provide recommendations based on what has worked in our lab. We can provide tablets for 35s and lectin for soy sample analysis. For corn, we supply tablets for 35s and invertase. We have no problem with the assumption that lectin is a single copy gene—it seems to be consistent across varieties. The sample testing we do is for samples farther down the food chain so homogenization has already occurred between GM and non-GM. This is different from testing seed or grain.

Question: For corn, would you make an adjustment for the number of copies of 35s?
Answer: We are currently struggling with this. There are no reference materials available. We are currently comparing the unknown sample to a calibration curve of a reference standard made from what is currently ‘in commerce’. This may tighten the variability.

Question: Have you made any decisions about what matrices you will be testing?
Answer: We are currently doing ingredient testing. We have not ruled out food products. We are waiting for market indications to proceed.

Question: What are you reporting?
Answer: We report back % GM/total DNA in the sample.

Question: Have you looked at the amplicon size for refined products?
Answer: We have not yet looked at this.

The day ended with a facilitated discussion among the speakers and AEIC members. Chuck asked the group to list the broad issues. The list that was derived contained:

a) Standard reference materials: protein and DNA; need to be certified to a % of a particular trait.
b) Specify the genetic element to target to determine GM content: 35s, unique identifier, copy number.
c) Existing vs. future products: what to do when current testing methods no longer viable.
d) Bridge gap between policy and reality.
e) PCR validation guidelines needed—quantitative, threshold, event specific.
f) PCR endproduct performance should be emphasized rather than method performance.
g) Correlation between all detection methods (protein & DNA)—agreed on conversion factor for each to % GM.
h) Education for customer asking for testing and education of persons selling grain and food ingredients—what are the testing methodologies, their limitations, which is best for each purpose.
i) Formulate a realistic picture of strengths and limitations of protein and DNA methods—clearinghouse of information.
j) Development of peer reviewed vehicles of testing methods—performance claims, correlation issues, amplicon size.
k) Availability of target proteins for assay development by kit manufacturers.
l) Availability of ELISA kits for all products.
m) Availability of technology providers’ internal testing methods developed for their products.
n) Application of testing methods and their use (in identity preservation, etc.).
o) Linkage among industry associations to avoid duplication.

An attempt was made to narrow this list down to the top five that AEIC may want to consider. These were:

1) Education and educational materials
   application of methods
   interacting with government agencies, the media, etc.
   communication of strengths and limitations of testing methods
2) Guidelines for standard reference materials
   work with GIPSA
   help determine what is necessary to characterize these standards
3) Validation criteria/guidelines for PCR
4) Performance-based evaluation of PCR methods
5) Provide linkage among industry associations

The prioritization and discussion of these was tabled for the AEIC business meeting.

The AEIC business meeting was held on Friday morning. The Secretary’s minutes of the 1999 Fall Meeting were approved. Kim Magin gave the Treasurer’s Report. AEIC currently has $12453 in the treasury. Dues announcements will be sent in May-June for 2000. Kim did not have the exact number but she believes they are currently 27 member companies.
Beacon Analytical had supervised the making of the plaques for Pioneer and Ricerca (hosts of the 1999 Fall Meeting and 2000 Spring Meeting, respectively). Beacon will oversee this for the 2000 Fall Meeting.

The AEIC website has had a few updates, however, more recent information is needed. Penny is asking for suggestions and welcomes anyone who would like to work with her and the webmaster to update it. Meeting registrations for the GIPSA workshop and the AEIC meeting were handled online through the site which was very convenient for the meeting organizers. Slides from the speakers at this meeting will posted on the site as well as Cindy Lipton’s AEIC talk at the ACS meeting.

Cindy Lipton gave an update on her talk at the ACS Meeting in San Francisco at which she presented AEIC’s paper on validation guidelines. The talk was presented during the Immunochemistry Summit hosted by Jeanette von Eamon (EPA). Most of the talks at the summit were technical. Cindy did receive a lot of requests for reprints of the paper.

Jim Rittenburg asked for clarification of AEIC’s policy on using slides of previous presentations since he has been asked to give a talk at a local college. Chuck indicated that it has generally been agreed that members can use the slides that are posted on the website.

Chuck will ask Dave Grothaus to mention AEIC at the end of his talk at an upcoming meeting in Munich.

Chuck then facilitated the discussion on “What is the future of AEIC? What will we do?” With the biotech initiative adopted by the membership at the last meeting, AEIC has changed course. There is some work yet to do with immunoassays but the organization should also consider taking on some of the broader issues brought out on the previous day. A suggestion was made to broaden AEIC’s scope to include “other bioanalytical methodologies” besides immunoassays. This would possibly expand the membership to include more DNA detection type organizations and contract labs. It would allow AEIC to bridge protein and DNA technologies to address the needs of industry and would give AEIC the opportunity to provide direction to the government agencies on diagnostics.

The discussion then turned to how we would launch this expansion. After much discussion, it was decided that we would write a letter to the membership explaining the broadened scope. The letter would also include initiatives developed at this meeting that AEIC would work on. The letter would also be sent to the speakers who participated in this meeting and ask all (members and speakers) to pass it on to other interested parties who may want to join the organization. The AEIC Secretary will draft the letter and submit it for Board review and approval before sending it out. The 2000 Fall Meeting will be a working session meeting, i.e., as before, split into groups the first day and discuss action items for each initiative and formulate timelines. There will also be two speakers—possibly Dirk Reif from Cargill and someone from the pharmaceutical industry who has dealt with PCR validation for drug products. The initiatives would be:

1) PCR Method Validation Standards—performance-base evaluation of PCR; validation guidelines/criteria.
2) Education Materials
   communication of strengths/limitations of protein and DNA methods
   appropriate applications of the methods
3) Linkage among industry organizations/associations to avoid duplication
   provide a clearinghouse of information
4) Definition of units for expressed protein or DNA
   correlation to % GM
5) Standard Reference Materials
   provide input to GIPSA for their efforts in this area

It was also proposed to change the wording of the AEIC mission to:

“To support and advance **diagnostic** testing technologies, the AEIC will:
- provide a consensus voice for applications to the agricultural, biotechnology and environmental industries;
- develop educational programs;
furnish scientific expertise; and
establish performance standards for bioanalytical methods.”

There was also a short discussion of changing the name. It was felt that this would have to be discussed at the next meeting. However, it was also stated that the acronym “AEIC” now has recognition so any name change should incorporate these letters.

The planning committee for the next meeting is: Dwight Denham (SDI), Fernando Rubio (Abraxis), Suzan Woodhead (Ricerca), Kim Magin (Monsanto), Dean Layton (Envirologix) and Dave Grothaus (Pioneer). The 2000 AEIC Fall Meeting will be hosted by Beacon Analytical in Portland, Maine on September 28-29, 2000.

Spring 2000 Attendee List:

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<td>Gene Peters</td>
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<td>April Ernest</td>
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<td>Jim Brady</td>
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<td>Fernando Rubio</td>
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<td>Leah Porter</td>
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