**AEIC Spring Meeting 2003 Minutes**

**April 16-17, 2003**

**Wilmington, Delaware**

**P.L. Hunst, AEIC Secretary**

The 2003 AEIC Spring Meeting was held April 16-17 in Wilmington, Delaware and was hosted by Agilent Technologies, Inc. There were 14 companies, 3 associations and 2 government agencies represented.

The meeting was opened by Stacy Charlton (AEIC President, Syngenta) and Scott Harrison (Agilent). Mike McMullen (Vice President Agilent) gave an introductory overview of Agilent. Agilent originally dates back to the early days of Hewlett-Packard but it has been independent of HP since 2000. It is a $6.5 billion company specializing in test & measurement products, semi-conductor products, life sciences products and chemical analysis products. The life sciences division supplies products for the pharmaceutical, biotech, academic research and bio-agriculture markets. These products include GC, LC, GC-LC mass spectroscopy, ICP mass spectroscopy, microfluidics and QA/QC instruments.

**AEIC Business Meeting:**

**Secretary's Minutes of 2002 Fall Meeting:** Motion made and seconded to approve.

**Treasurer's Report:**
The balance was about $37000 in January and the current balance is $32900. Actual expenses to date are $9984. The 2003 budget projections are $21973 which would leave a balance of $8966.

Dues: As of this meeting, AEIC has 28 members (12 large companies, 12 small companies, 3 associate members and 2 individual members). The net revenue from dues is ~$8000 of which $5000 has already been received. Outstanding dues: FMC, BASF, Dow, Medallion Labs, Biogenetic Systems, University of Nebraska, Michigan Dept. of Agriculture and J. Sharp.

**EPA Workshop:** A workshop was held at the EPA facility at Ft. Meade, Maryland. Dave Grothaus, Randy Giroux, Tim Laurik, Anne Bridges and Carl Adams participated as presenters. The EPA lab will do validations of pesticidal GM crop methods, both ELISA and PCR. Scientists from Ft. Meade as well as from EPA BPPD participated. EPA indicated that it was the first time they had ever had a training session with industry. They were afraid of industry "corruption" but were comfortable with AEIC's training since it dealt with the facts of methods. EPA feels that they would come to AEIC if they need further technical support. Dave G. will work with A. Bridges to make the booklet used in the training available to AEIC members.

**Update on AEIC papers:**

DNA Paper: There had to be a change in consultants which took the time between the fall meeting and this one. Markus Lipp will be re-contacting the original team to help finish the paper once the consultant is on board. The cost of the consultant will be split between AEIC and CropLife America. The subject of the paper is validation of PCR methods, i.e., difficulties, pitfalls, etc. and will be published in a peer-reviewed journal.

Protein Paper: The work on this paper has stalled also due to the lack of a consultant. Dave G. will talk with Markus about using the same consultant as the DNA team.

**Powerpoint Presentation:** This is a basic presentation on testing. A. Bridges (Medallion Labs) has been contracted to work on this. As of the last AEIC meeting, Anne's graphics person had received a number of technical comments which Anne has to review and requests our patience. R. Jenkins (USDA) mentioned that U. of Nebraska (Deana Namath) has also put together a basic educational slide set which will be available on their website. Frank Klein will follow up with Deana.

**AEIC Officer Nominations:** Nominations will be called for the offices of President, Vice President and Secretary. A call for nominations will be sent out later in the summer/fall.

**Fall Meeting 2003:** The Fall Meeting will be hosted by Neogen in East Lansing, Michigan. The projected dates of the meeting are October 9 – 10.

The following are possible themes:

- Reference standard materials: update from NIST workshop in June
  - Presentation from IRMM (H. Schimmel)
  - CFIA and CGC: What Canada is doing (R. Giroux will contact)
- Protein assays for single amino acid differences (Markus will contact possible speakers)
- Sampling for GM crop testing: Implications for DNA/protein methods (R. Giroux will coordinate)
- Peptide nucleic acid assay (P. Arbault will contact a speaker)
- Emerging technologies for protein equivalency: Padlock probes (U. Muller will contact speaker)

**Updates:**

Check Sample Program: The program is currently on the 6th round (3rd year). Some participants are doing well and others are not. Participants can analyze samples using either DNA or proteins methods and the participants do not receive the same samples every time.

TAG update (G. Clapper): The TAG is going over methods of GMO detection. The protein methods are at the CEN level which is turning them into a final draft. The draft will be discussed at the next CEN meeting in May at Berlin. Every document is reviewed every 5 years. The qualitative nucleic acid document just went for a vote and will be discussed at the ISO meeting in June. The quantitative nucleic acid and sampling documents have been voted on and have been sent for translation into 4 languages. At the AOCS meeting in Kansas City, the ISO/TAG will meet on May 5. Currently, all the TAG work is on schedule to complete by the end of 2004. CEN has started a working group on allergens, i.e., standardization of testing methods. AEIC could start a working group. The information is contained in the Powerpoint file sent with the meeting minutes.

GIPSA Verfication Program (R. Jenkins): The biotech program is a proficiency-based program and is voluntary (no fee). There is quarterly dissemination of samples (12 corn/12 soybean). The corn events include T25, CBH351, MON810, GA21, E176, BT11, NK603 and soybean event is CP4 EPSP. The program was implemented in Feb02 and the 5th round of samples were distributed in Feb03. The program will expand to include new events on the market (1507, MON863). There are currently 23 participants in the US and 48 outside of the US. Fifty percent of the participants have the capability to test for all events. The majority of the participants use DNA tests.

USDA GIPSA currently has a research collaboration with NIST and will co-sponsor a workshop on certified reference materials. The collaboration is also looking at the quantification of genomic DNA from plants (different methods) to investigate if methods agree. They have also evaluated extraction methods for corn DNA and this is soon to be published in the Journal of Agriculture & Food Chemistry (the article is currently available on the web).

ILSI Training Program (D. Grothaus): There was supposed to be a training program in India in Feb03, however, it was cancelled due to the world political situation. The program will be re-scheduled since the government scientists are very interested in the training.

OSTP Adventitious Presence Policy (S. Charlton): This was a paper issued by OSTP for the earlier assessment of material going into field trials. The acreage trigger for this assessment has not yet been decided. There is no adventitious presence mentioned in the paper, rather it is called "intermittent low levels (ILL)". The policy is not exclusive to transgenic materials.

**Invited Presentations**

**Uwe Muller (Nanosphere, Inc.)** gave a talk on nanoparticle probes. Nanosphere Inc. deals mostly with human genome work and is not yet in agriculture. The company was spun off from Northwestern University and currently has 45 employees. Their market is mainly *in vitro* diagnostics. The technology is based on nanospheres which have been around since 1973 and were originally used in the colloidal gold staining method for transmission electron microscopy. For human genome work, the particles can be coated with DNA using covalent bonding to increase $T_m$ thus resulting in sharper transitions and higher stringency in binding. Labels may also be incorporated (such as silver amplification and Raman labels). Detection of the coated nanoparticles is accomplished through several methods. The first is through the detection of a simple color change due to particle aggregation in solution. This requires no instrumentation. The second method is resonance light scattering which is due to light being scattered due to the vibrations of the molecules. Multiple colors are a result of the size, shape and composition of the particles. Two very different sized particles are required to generate the different colors. The third method utilizes silver amplification which causes Rayleigh scatter due to the presence of the silver. This method requires a light scattering instrument. The nanoparticle actually participates in the hybridization reaction versus other technologies where a molecule is used to detect hybridization. The detection limits for light scattering are 7 molecules Cy3/$um^2$. For single nucleotide polymorphism (SNP) detection, a fluorescently labeled target is enhanced with nanoparticle probes. There is so little DNA present that there is no signal over background. The signal is enhanced 50X by silver staining. For a 250bp PCR product, SNP detection and discrimination is at about 100 aM (attomole) sensitivity in preliminary tests. Nanosphere's new research activities include: 1) 2D array with nanoparticle SERS (surface enhanced Raman) probes to generate multiple colors (will allow many analyses on one chip); 2) colorimetric detection formats; and 3) electrical detection & miniaturization (use of electrons on integrated chip) which has resulted in 10 femtomole sensitivities on a prototype chip.

**Mark Jensen (Agilent)** gave a presentation on microfluidics in agriculture. Microfluidics is a microscopic form of capillary electrophoresis. The chip is the size of a postage stamp and contains large wells which contain the electrophoresis apparatus and small channels which hold the samples. The samples move on the chip via electrodriven flow. For proteins, the sample is injected into a well containing SDS and intercalating dye. There is a destaining step which removes the SDS micelles that are not bound with the protein. The system allows for rapid sizing and analysis of molecules from 14 – 200 kDa in size. The resolution is 10% and the linear dynamic range is 100X. The sensitivity of the system is comparable to non-colloidal Coomassie staining. The DNA chip uses DNA fluorescent intercalating dye. Detection is via a red laser or blue LED detection. There are different chips for different size ranges with the sample volume being 1 microliter.

**Virginia Pantella (AOAC)** talked about validation of kits by AOAC. AOAC is a nonprofit organization founded in 1874 to review and validate analytical methods. The AOAC Institute was incorporated in 1991 as a subsidiary of AOAC International. The institute is a rapid entry point into the AOAC validation and approval process. Once a kit is approved, the company is licensed to use the certification mark in advertisements and packaging for one year periods. AOAC reviews annually to insure the quality is still present. About 50% of the approved performance tested methods are from the US and 50% are from outside the US (mainly EU and Japan). About

62% of the expert reviewers are from the US and 38% are from outside the US. The AOAC Institute has 10 years experience in validating test kits and completes method reviews in less than 6 months. The validation procedure includes 5 steps: consulting (1 month), application (1 month), data collection (1.5 months), review (1 month) and publication. The cost is $15000 for non-members (members receive a $500 rebate) and this excludes the cost of the outside laboratory. The consulting step is optional and usually costs about $2000. Once approved, the cost is $3000/year to maintain. Depending on the type of test, the cost for the outside lab varies between $5000 - $30000. AOAC maintains a database of test kits (www.aoac.org/testkits/TKDATA2.htm). The common problems encountered in test kits include: incomplete instructions, method not fully adapted to new matrices, rely on existing enrichment procedures for recovering bacteria and assume the procedures will work, market-driven desire to shorten incubation/development times, and "just not ready for primetime" (manufacturing/QC problems). The good news is that the general level of sophistication has increased and the majority of the kit manufacturers want to do the right thing overall. The AOAC Institute plans to expand into new areas such as food allergens, total plate counts, equipment certification, pharmaceuticals, veterinary diagnostic kits and validation against non-US reference methods.

**Marcia Holden (NIST)** talked about the programs at NIST. NIST stands for the National Institute of Standards and Technology (www.cstl.nist.gov/biotech) and is a "non-regulatory" federal agency residing in the Department of Commerce. NIST was formerly known as the National Bureau of Standards. There are 4 programs within NIST: measurement and standards program, advanced technology program, manufacturing extension partnership program, and national quality program. NIST's biotech is located in the Chemical and Technology Lab. For biotech, NIST's interests are in DNA technologies, bioprocess engineering, biomolecular materials and structural biology & computational biology. NIST's outputs are standard reference materials and measurements (databases, etc.). For biotech, the databases include the protein data bank, biomacromolecule cystallization database and short tandem repeat database. NIST has standard reference materials for human identification and forensics, DNA diagnostics, DNA damage and repair, fluorescence and peptides. For the future, NIST would like to add work in proteomics, tissue engineering, microbial forensics, DNA diagnostics, gene expression and GMO's.

NIST and USDA-GIPSA are sponsoring a workshop on standard reference materials for GM crops on June 27, 2003. The purpose of the workshop is to define protein and DNA standards and their matrices. The discussion will center on the scientific and technical challenges of standard reference materials for protein and DNA; agreement on specific standard types; ranking of the standards needs; and planning the outline of a future workshop and/or international meeting. The major driver is coordination between world agencies on standard reference materials, i.e., NIST will not produce the same materials as IRMM. Canada is interested in producing canola standard reference materials and Australia in interested in producing cotton standard reference materials. Currently, there is no formal way for agencies to coordinate.

**Katrin Schroeder (GeneScan)** talked on identity preservation (IP). For IP, the analytical considerations for non-GM are threefold: 1) contains absolutely no GM; 2) no GM detected and 3) GM is detected but is below a threshold level. Protein testing is generally conducted in the primary stages of the supply chain (grain). PCR has the widest application to testing the supply chain but it is also the most expensive of the testing methods. Considerations for an IP program are a) recognizing a lab for testing, b) sensitivity of DNA analysis (nature/size of sample, DNA extraction, specifics of amplification method), c) sampling (sampling early in supply will have the least homogeneity but further processing results in loss of DNA), d) sampling strategy (dependent on anticipated %GM present and/or threshold of GM), e) control (non-GM monitoring), f) segregation (prevent mixing of controlled product with uncontrolled product), g) traceability (documentation system), and h) manage control (classical ISO measures). IP is the ultimate control and traces its origin back to the wine and beer industry. The new aspect for IP is food safety which is a response to traceability.

**Patrice Arbault (Diffchamb S.A.)** gave us an overview of the newest member of AEIC. Diffchamb S.A. is mainly an EU-based company with 110 employees. It has one main focus: food diagnostics. Diffchamb was originally founded in Sweden in 1987 and acquired French (Transia in Lyon), Italian, British and Dutch companies in 1991. In 2000, they opened their Australian subsidiary and in 2002 opened subsidiaries in the US (Chicago and Napa Valley, CA). As of April 17, 2003, Diffchamb now belongs to the Raiso Group from Finland. Diffchamb's technologies include immunoassasys for food safety and differential ph-metry for food quality. In R&D, Diffchamb is working on PCR, PNA, nicogreen probes and cell culture. For food safety, Diffchamb has tests for foodborne pathogens, bacterial toxins, mycotoxins, food allergens, antibiotics, hormones, anabolics, corticosteroids and food freshness (measure of ATP). They also have recently signed an agreement with Envirologix to sell test kits for GMOs. For food quality, they have wine applications (glucose, fructose, sucrose, acetic acid, malic acid, lactic acid, glycerol, acetaldehyde, alcohol) and milk/dairy applications (urea, ammonia, glucose/lactose, lactic acid, citric acid, lactulose and the instruments to do the tests). In 2002, Diffchamb's performance was 14 million euros. More information about Diffchamb is available on their website at www.diffchamb.com.