AEIC Fall Meeting 2004
October 20-21, 2004
Champaign, Illinois

Secretary's Minutes
By
P.L. Hunst

The Fall Meeting of AEIC was convened at 9am on October 20. AOCS (American Oil Chemists Society) hosted the meeting at their facility in Champaign, IL. Markus Lipp, President of AEIC, and Randy Giroux, Vice President of AEIC, welcomed everyone to the meeting.

R. Cantrill (AOCS) gave a brief overview of the organization. AOCS consists of its membership, technical services, meetings/exhibits and the AOCS Press. It provides official methods of analysis for fats, oils and oilseeds and also provides a laboratory certification program. AOCS is a liaison with other international organizations such as Codex, IUPAC, CENT/TC and ISO TAG as well as with national organizations such as AOAC, AACC, NCPA, NOPA, ANSI, ISO and AG9000. Forty percent of the AOCS membership is outside of North America.

AEIC Business Meeting

Minutes of Spring 2004 Meeting: A motion was made and seconded to approve the minutes of the meetings. The vote was taken and approved the motion.

Treasurer Report:

Revenue	$		7888
Expenditures	$	11280	
Anticipated Expenditures	$	10700	
Total	$	21980	
Projected New Balance	$	17886	

The 2004 Spring Meeting exceed the amount originally budgeted. The Board will work on guidelines for hosting companies to follow when planning subsequent meetings.

D. Grothaus has requested $8000 to finish the updated protein validation paper. The vision for the paper is to include validation steps, applications of immunoassays that are valid and practical limitations of immunoassays. A motion was made and seconded to fund the $8000 request. The vote was taken and the motion was approved.

Membership/Dues: The membership consists of the following companies/organizations:

Large Companies

			Potential Dues
Agilent	Mark Jensen	L	$ 500
BASF	Dave Bolin	L	$ 500
Bayer Crop Science	Ray Shillito	L	$ 500
Cargill	Randy Giroux	L	$

			500	
Diffchamb	Patrice Arbault	L	$ 500	
Dow AgroSciences	Guomin Shan	L	$ 500	
DuPont/Pioneer	Dave Grothaus	L	$ 500	
Eurofins	Bert Popping	L	$ 500	
FMC	Audrey Chen	L	$ 500	
Medallion Labs	Carl Adams	L	$ 500	
Monsanto	Markus Lipp	L	$ 500	
Neogen	Frank Klein	L	$ 500	
SDI	Jim Stave	L	$ 500	
Syngenta	Jim Brady	L	$ 500	$ 7,000
Small Companies				
Abraxis	Fernando Rubio	S	$ 250	
Agdia, Inc.	Sarah Hindman	S	$ 250	
Beacon Analytical	Brian Skoczenski	S	$ 250	
Biocode	Jim Rittenburg	S	$ 250	
Biogenetic Systems	Alex Kahler	S	$ 250	
Envirologix	Bruce Ferguson	S	$ 250	
GeneScan USA, Inc.	Frank Spieglehalter	S	$ 250	
Genetic-ID	John Fagan	S	$ 250	
Horizon Ag	Jennifer Wells	S	$ 250	
Midwest Seeds	Kalyn Brix-Davis	S	$ 250	
UNE	Susan Hefle	S	$ 250	$ 2,750
Associate Members				
AOCS	Gina Clapper	A	$ 50	
GSF	Petra M. Kramer	A	$ 50	
Michigan Dept. of Ag.	Kevin Worden	A	$ 50	$ 150
Individual Members				

	Jan Sharp	I	$ 100	
	Alice DeLisle	I	$ 100	$ 200
				$ 10,100

A discussion topic was membership reachout. A suggestion was made to invite food companies, third party auditor companies, food testing labs (Silliker, Covance) and possibly animal health companies. In order to raise awareness of AEIC and attract new members, it was suggested that a handout sheet be available for the Midwest AOAC meeting in 2005. The Board will take up this consideration. It was also proposed that maybe some topic areas be identified for the next AEIC meeting which could be addressed by invited speakers from potential member companies.

PowerPoint Presentation of AEIC: The slides have all been made to look consistent as far as background, colors, etc. The Board will organize the slides into 5 groups: introductory slides, PCR slides, immunoassay slides, application of methods slides and validation of methods slides. The proposal is to have each group of slides available on the AEIC website via viewable thumbnail pictures which would allow people to browse and then download the set of slides they are interested in.

AEIC Board Election: Nominations for the offices of President and Vice President are now open through Nov. 15. Nominations are to be sent via e-mail to the AEIC Secretary (PLHunst@dow.com). Electronic ballots will be distributed after Nov. 15 and the intent is to complete the voting process by Dec. 1.

Spring Meeting 2005: USDA AMS, located in Gastonia, NC (near Charlotte) will host the meeting. Suggested agenda topics were:
1) AMS, APHIS and FAS speakers to talk about policies that affect the ag industry and the global impacts;
2) seed morphogenesis talk;
3) challenges of combined trait products (“stacked traits”) for testing;
4) how food companies use immunoassay/PCR testing;
5) reference materials;
6) availability of PCR tests via the EU website

AMS is currently checking dates in March for the meeting.

Fall Meeting 2005: Several possible venues were mentioned which included Agdia (South Bend, IN), Envirologix (Portland, ME) and USDA (Washington, DC). No decision was made.

Updates:

EPA (Ft. Meade Lab): M. Rindal gave a brief overview on what the lab is currently doing. The lab will be reporting out on the first method validation soon and it will also be moving into newly renovated Molecular Lab. The second immunoassay method validation (Cry1F ELISA) is underway and 2-3 other validations are scheduled for the fiscal year. The first method validation was a lateral flow test strip for Cry1Ab. Sixty blind samples (30 positive; 30 negative) were used. It was found that the negative material from the sponsor company was not completely negative, therefore, some supplemental testing is being conducted, however, the test strip performed adequately.

USDA AMS: M. Sussman indicated that the AMS lab has expanded their work and now use more real-time PCR. The lab is also using pulse field gel electrophoresis to separate large DNA molecules. AMS will soon have a lab accreditation program for labs performing biotech testing. J. Falk (USDA) is in charge of this program and the program will soon be announced in the Federal Register under proposed rule making. AMS and GIPSA will be working together. AMS will produce reference materials for non-grain commodities such as cotton, bentgrass, alfalfa, etc. A brief overview was given on avocado varietal identification using DNA fragment analysis (microsatellite analysis). The procedure requires 6 hours and results are useful since they stand up in court when a shipment of avocados is condemned from entering the US.

Codex: R. Shillito gave an update on the Committee on Methods and Sampling. The next meeting of the committee is scheduled for April, 2005 in Budapest, Hungary. The committee is a government body which is led by FDA. The US comments on the method/sampling document were that the document should include protein-based methods as well as DNA methods; a convention for expressing % GMO should be included; reference materials should be defined; and the terms food and feed for biotechnology should be used. The document will undergo a second round of drafting to add the protein methods. Any further comments on the document need to be submitted through a government to Roger Wood (FDA) as soon as possible.

ISO: R. Shillito also talked briefly about the participation of the TAG at the CEN meeting in Paris. The discussion was around the quantitative PCR document and the sampling document. For the sampling document, it was originally put together by CEN and thus it is Euro-centric. The ISO comments were rejected and the document passed and became the final draft standard. Then a request was voiced to change the document since it conflicted with certain country laws. A compromise was reached that for GMOs, the existing sampling plans can be used. Extra sampling may also be done for the measure of uncertainty.

OSTP AP Policy: S. Charlton updated the progress on the 2002 OSTP proposal to deal with AP. The proposal was for the conducting of early food safety assessments on those proteins/molecules that have not previously been in a product. FDA has not yet issued a response. It is hoped that when they do respond, it will contain an outline for a system for assessment.

AEIC name: The AEIC Board is offering a $100 prize for alternate words for “AEIC” (currently, the letters stand for Analytical Environmental Immunochemical Consortium). The alternate words must fit the mission of the group. There is no guarantee that any of the suggestions will be used. Suggestions are to be submitted to Dean Layton (Envirologix).

Presentations:

J. Stave (SDI) talked on ground and spiked matrices as protein standards. Reference materials form the basis for harmonization of methods and standards in detection kits must be calibrated to the reference materials. Standards are used in quantitative methods to construct standard curves in order to interpolate concentrations of target analytes in samples. In qualitative methods, standards are used as negative or positive controls. Standards should: correlate to the analyte concentration in the sample under analysis; should be homogeneous; should be stable over time; should be reproducible; should be verifiable; and must account for extraction efficiency, matrix effects, the form of the protein in the sample. When preparing standards, either purified protein or a ground matrix, positive and negative raw materials must be obtained. The raw materials need to be mixed at the desired concentrations and then the homogeneity must be determined, followed by the development a sample preparation procedure. The sample preparation procedure should be applicable to both the samples to be analyzed and standards. For standards, matrix effects need to be taken into account, i.e., when a sample is extrarcted, many substances are extracted which may interfere and affect the assay performance. This interference will shift the standard curve. One option to remove matrix effects is dilution, however, the sensitivity of the assay will decrease. The extraction efficiency is the % of the target

protein that is extracted by the method protocol. Most methods do not rely on 100% extraction since it is not cost effective (requires repeated extractions). Extraction efficiency is dependent on particle size, i.e., the smaller the particles in the sample, the better the extraction. The choice of the type of standard (liquid, lyophilized powder, ground matrix) is based on practical considerations (form, availability, homogeneity, stability, convenience).

R. Cantrill (AOCS) updated the group on ISO and the ISO process. Countries are represented by a single national standards organization which is often a government body or a similar organization such as ANSI. ISO has 148 member countries that are either at the participating (P) level or the observer (O) level. Not all countries are members of each technical committee (TC) or sub-committee (SC). ANSI is the participating organization of ISO/TC 34 food products and ISO/TC 34/SC2 and SC11. ANSI receives all notifications of TC34 activities and also administers a few individual participating memberships. Most participating memberships are administered by other US organizations which form TAGs (technical advisory group). A TAG consists of an administrator, a chairperson and members. It is open to all applicants and usually has a balance between industry, government and academia including those who are consultants and those who are retired. There is a currently a push to have consumer groups involved on the TAGs. TAG members are expected to be active participants in reviewing all documents and should be available to provide a consensus position on issues brought before the TAG. Participation in both the US TAG and the ISO committee meeting is encouraged. ANSI is the "P" for ISO/TC 34. To participate in a WG, the US must have a P membership of TC 34 and form a TAG. The organization wishing to host the TAG must belong to ANSI and should be a source of experts willing to join US TAG and participate in generating a consensus position. The administering organization is the AOCS and the chairperson is Kim Magin (Monsanto). Kim was the prime motivator in participating in the TC 34 WG. ANSI charges an annual fee for organization membership. Participation give an international voice; access to all ISO versions of standards under development; and ISO is building a strategic partnership with the WTO (Codex). The ISO/TC 34 develops standards in field of human and animal foodstuffs. For the ISO/TC 34 WG7, a consideration is to use standards to increase the quality and reliability of testing, promote the use of validated methods and resolve disputes. The stages of standard development include: 1) development of new work item proposal; 2) working draft; 3) committee draft; 4) draft international standard; 5) final draft of international standard and 6) publication of international standard. Five years after a standard is published, it comes up for a review. CEN is the EU standards committee and how it works with ISO is laid out in the Vienna Agreement (all projects are led by ISO except those pertaining to GMOs). The only chance ISO has to see CEN GMO proposals is at the draft international standard step and all comments come out of the TAG. ISO standards development is summarized below:

a) detection of GMOs and derived products – sampling
b) detection of GMOs and derived products – quantitative nucleic acid based methods
c) detection of GMOs and derived products – qualitative nucleic acid based methods
   (document is lost at CEN)
d) nucleic acid extraction document
   (document lost at CEN)
e) protein based methods
   (published but needs to be revised)
f) general requirements and definitions
   (document lost at CEN)
g) GMO in oleaginous seeds
   (reissued; vote by 12/31/04)
h) acceptability criteria for methods to annexed to ISO standards
   (final draft to be submitted for single vote)
i) QN annexes from China
   (to be routed through ISO)

M. Abouzied (Neogen Corp.) gave a presentation on immunoassay analyses of allergenic residues in food and food ingredients. Food allergens are those molecules/ingredients that trigger an immune response in sensitive individuals. Food intolerance is very different since it does not affect the immune system. Food allergens are introduced into food by a) inadequate cleaning of shared equipment, b) use of re-work materials, c) switching of ingredients, d) formulation mistakes, e) wrong labels on packages, and f) labeling terms. Products are tested to ensure that unlabeled and potentially dangerous ingredients do not make their way into food products; to decrease waste; to protect companies from staggering cost in case of product recall; and to protect the company's reputation. It is most efficacious to catch a problem at the source rather than in the finished product. The current detection methods used are chromatographic (GC, HPLC-MS), immunochemical (RAST, ELISA, immunoblotting, lateral flow devices), and genomic (PCR). Immunoassay testing is widely used since raw materials, equipment, product can be tested at any point and on-site testing can be conducted. Immunoassays are also low cost, fast, highly sensitive, need minimal staff training, and high output. Currently, there are no allergen standards available for immunoassays. For standard reference materials, it must be decided as to what should be tested—raw materials, processed materials or different forms of the commodity. For example, whole egg—raw or dried? The IRMM reference materials are certified for other purposes such as for trace elements, nutritional properties and mycotoxins.

M. Bandla (Envirologix) gave an update on emerging trends and applications of lateral flow device testing and what is happening in the marketplace. Gold is the most popular for probes, however, polystyrene or paramagnetic beads may also be used. Gold is the most sensitive and the easiest to QC. The formats for lateral flow strips can be single analyte/strip or multi-analyte (on same strip or by binding individual analyte strips together). The markets for lateral flow tests include seed companies (expression, seed lot purity), grain handlers and processors, identity preservation (IP). For IP, the lateral flow strip tests can be retained as documentation of testing, although it is recommended that the strips should be scanned and the scan retained for records. Envirologix has done some preliminary work on cutting the pad and using for PCR analysis. For GMOs, lateral flow strips can provide semi-quantitative data, rapid results, multianalyte testing, appropriate sensitivity, robustness. Robustness of the strip tests is affected by humidity which can cause hydrolysis of the antibodies, crystallization of sugars in the conjugate pads, impact the adhesive holding the strips together and the immobilization ability of the membrane. Another problem is that the strip tests will fail if the strip is dipped too deepling into the sample beyond the sample line. A new format to counteract this problem is needed. Strip tests must also be amenable to different extraction procedures and must be tolerant to brief exposures to high temperatures. There are currently no event specific strips, i.e., no Cry1Ab strip tests can distinguish between the Cry1Ab events of MON810, Event 176 and BT11.

R. Jenkins (USDA GIPSA) gave an update on reference materials for biotech-derived grains/crops. There are two testing technologies for grains/crops—protein-based and DNA-based. There are no reference materials for proteins. The units (%w/w) need to be comparable between protein tests and DNA tests. Protein reference materials must be immunoreactive, adequate purity, good extraction efficiency and appropriate conformation of the protein. DNA methods are sensitive, event specific, quantitative and standard methods exist. However, DNA tests are also expensive, time-consuming, dependent on lab environment, require a trained staff and expensive equipment. Reference materials for DNA require suitable raw materials, be homogeneous, exclude cross-contaminants and be traceable. The IRMM is the current source of reference materials. NIST and GIPSA are not producing any reference materials. NIST and GIPSA do have a joint project on plant DNA as standards and quantifying the DNA by traceable means. The primary quantification methods that will be looked at include measuring the phosphorus content using ICP-OES with HPLC, nucleoside content in enzyme-digested DNA using LC-MS, determination of base content in formic acid-digested DNA, gravimetric determination, and fragment size analysis. The concordance of the common lab methods for DNA quantification (spectroscopy, luciferase enzyme assay, intercalating dyes) are being investigated and it has been found that the methods do not agree with each other.

R. Jenkins (USDA GIPSA) also gave an update on GIPSA's proficiency testing. The results of the proficiency testing are posted on GIPSA's website. The qualitative results show no unusual aspects. The quantitative results show, however, some differences. For example, NK603 was under reported in North American labs and over-reported in EU labs. There was also huge variability in the reported numbers. In summary, the qualitative data suggests a low probability of discordance between continents when screening for specific biotech events. For the quantititave testing, the data suggests a high probability of discordance between continents when identifying thresholds, especially at lower concentrations. GIPSA proficiency samples are not reference materials, however, labs sometimes use the GIPSA samples when the IRMM samples are not available. An endogenous control gene needs to be identified and standardized so that proficiency samples can be compared to IRMM materials. Ron also reminded the group that the Midwest AOAC Meeting will be held on May 23-26 in Kansas City and more details can be accessed at the website (www.midwestaoac.org).

C. Haldemann (ISTA GMO Task Force) gave an update on the ISTA proficiency tests on GM seeds. The objective of the testing is to check the ability of individual labs to detect the presence or absence of GM seeds in samples of conventional seeds. The final data analysis will be to compile performance of labs and provide the data for each lab's internal performance database. For proficiency test 1, 30 samples (300 seeds each) were provided to each lab. The samples contained 12 negative samples 6 positive samples (1% MON810), 6 positive samples (1% T25), 6 positive samples (MON810+T25: 1 seed : 2 seeds). In proficiency test 2, 10 samples/lab were provided (3 negative samples and rest positive) and for proficiency test 3, 12 samples/lab were provided (3 negative samples, 3 positive samples each of MON810+T25 at 0.2, 2 and 4%). In proficiency test 1, 66% of the labs were from the EU, 30% from the Americas, 2% from Asia and 2% from Africa. Of these labs, 62% were government labs, 33% were seed companies and 5% were other labs. The results from the labs indicated that 70% reported the correct results and 30% reported false positives or false negatives for the samples. For proficiency test 2, 85% of the labs reported the correct results and 15% reported false negatives or false positives. For proficiency test 3, 81% reported the correct results and 19% reported false positives or false negatives. Each lab uses its own method to fit the type of samples—either protein or DNA-based. Most of the labs used PCR methods. The lab rating system is still being determined by ISTA and will be based on 6 proficiency tests. The proficiency testing is free for ISTA members and non-members must pay a fee to participate. In proficiency test 4, soybean samples will be given out (12 samples/lab; 3000 seeds/sample). For further information on the testing, go to www.seedtest.org.

D. Pinero (Eurofins) gave an overview of the AEIC paper on PCR validation. The paper is expected to publish in the Journal of the AOAC in Jan/Feb05. The paper describes the fundamental elements of PCR analyses and the application to grain products. There is a detailed description of the issues that need to be considered when sampling and testing. The discussion of method validation includes the in-house validation as well as interlaboratory validation. The parameters of validation that are common to qualitative and quantitative PCR methods include 1) specificity and 2) applicability. Qualitative methods need to be evaluated for false negative and false positive results as well as ruggedness of the method. For quantitative PCR methods, validation parameters include 1) determining the LOD, LOQ and ROQ (range of quantitation), 2) the precision and accuracy, 3) sensitivity, and 4) ruggedness. The paper also includes a discussion of reference materials in regards to type and sources.